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ARTICLE
Healthcare and Sports from the Perspective of Qi, Fascia, and Taiji-quan

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1. Background

Traditionally, Taijiquan (Tai Chi Chuan) is presented as a practice to nurture Qi (Chi) energy guided by the principles of Yin and Yang. It is difficult to articulate what Taijiquan is as the underlying concepts of Yin and Yang and Qi are foreign to the West. While one may not relate to the theory, there is nothing burdensome about the art in practice—the body relates to it readily. Anyone, young or old, can pick up the art with no prerequisites. The practice is as it appears, without strenuous physical demands besides that of moving in balance. And interestingly, before long, practitioners enjoy clear health benefits, which keeps the practice going. It is this simple efficacy of health welfare that is driving the worldwide popularity and acceptance of the seemingly alien art. The United Nations body, UNESCO, recently honored Taijiquan, listing it as an Intangible Cultural Heritage of Humanity.

The practice of Qi nurturing, referred to generally as Qi energetics or Qigong, goes back to ancient times. Qi theory holds that good health is a measure of robust Qi coursing through the body interconnecting the internal (Zangfu) organs in harmony. The health benefits of Taijiquan are thus presumed in the art as a Qi nurturing practice, and they have been studied extensively in scores of research papers.

However, the traditional theory does not present Taijiquan as a health exercise. Rather, the classical literature touts it as a martial arts par excellence. Incongruous as the slow-motion

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practice may be to the speed and power in combat and boxing, Taijiquan bills itself as a kungfu art of the highest order. Taiji kungfu skills are underscored by the principle of “using softness to overcome hardness” (yi rou ke gang). These skills are characterized by softness, such as “guiding an in-coming force away to emptiness” (yin jin luo kong) and “using four ounces to repel a thousand-pound force” (si liang bo qian jin). They beguilingly indicate that the strength used in the kungfu techniques is not borne necessarily of phenomenal musculature. Taijiquan's kungfu prowess finds its basis in neijin or “internal strength.”

Qualifying neijin strength as “internal” is to distinguish it from li, the strength of normal physical training. The cultivation of Qi in Taijiquan practice is primarily to develop neijin. This highly refined strength is built by infusing Qi into li in the training. For practical convenience, one can think of neijin as equal to Qi plus Li or as Qi-inspired Li. The aesthetics of the Taijiquan form are grounded on the expression of neijin.

The long cherished belief of Taijiquan’s martial superiority was unceremoniously shattered in a fight in April 2017, when a Chinese Mixed Martial Artist (MMA) fighter thrashed a self-styled Taijiquan grandmaster in 10 seconds, and harangued that Taijiquan combat was mostly fake. The sacrosanct neijin was desecrated as bogus. In one fell swoop, the cultural heritage of Taijiquan was shaken at its core, and it whipped up such an uproar in the Chinese cyberspace that it shut down the internet portal.

Subsequently, vying for fame and fortune, some other self-proclaimed Taijiquan and Wing Chun masters who challenged and fought MMA fighters, were handily defeated as well. The venerable Chinese martial arts kept being thrust in the public eye as being ineffectual. The old and secretive kungfu theories in the classics that had served to inspire the arts were ringing hollow. A good part of the debate lamely pushed aside the combat issues by emphasizing the culture (wen) of the art. But the martial elements are in the Taiji DNA, which is what sets it apart from being a dance form.

It is therefore imperative that the role of Qi in neijin and Taijiquan be resolved at the fundamental level. Neijin can be unpacked in terms of the change in momentum, the principle of levers, and the balance of bipedal functionality to elucidate the highly lauded kungfu skills of Taijiquan. These and other combat issues are addressed in the author's papers, Taijiquan's Enigma [1] and Is Taijiquan a martial art or a dance? [2]. However, the discussion skirted the core concept of Qi itself, taking it as given in Chinese Medicine.

Qi is a foundational concept in Traditional Chinese Medicine (TCM), but neither Qi nor TCM are built on scientific constructs; they predate science. Although it may serve to think of Qi as a bioenergy, it remains undefined in terms of Science. Thus the discourse of neijin and Taijiquan often becomes stymied.

The significance of Qi is in its functional forms, not as a bioenergy per se. Though it is not measurable as a scientific quantity, Qi is cognizable as a sensation. Music is not appreciated by the study of the physics of harmonics and tones. The crux lies in the perception of Qi as a bioenergy coupled with the efficacy of its functionality. What is crucial is that one can cultivate cognitive perception of Qi, making it pragmatic and decipherable without measuring instruments. This is the defining feature of Qi, which is explicated more fully in the author's recent paper, Science in Qi [3].

The present work may be regarded as a supplement to this paper as an application to Taijiquan and neijin. The phenomenon of neijin is manifested in the musculoskeletal framework, thus subject to biomechanics and the laws of physics. The paper studies fascial tension in Qi’s key functional role in the Taiji discipline of body motion to develop neijin. In the function, we see the fascial tensional network of the body as providing a medium for Qi, and thus a concrete representation of Qi. More consequentially, the science in Qi can help one reach the rarefied heights in the art of Taijiquan.

2. Qi and Fascia

Fascia is a continuum of connective tissue that wraps around muscles and envelops internal organs and other structures, providing a body-wide web of physical connectivity with viscoelastic properties. Although the components that make up fascia are the same, namely, protein fibers (collagen and elastin), ground substance (fluid content), and fibroblasts (cells), they vary in composition and form depending on its function.

The deep fascia that surrounds individual or groups of muscles, separating them into fascial compartments, is a dense connective tissue with a higher proportion of collagen fibers. The fascia of loose connective tissues which envelop and support organs is more fluid with a larger content of ground substance and lesser protein fibers; it spreads throughout the body, filling the spaces between structures and surrounding the blood and lymph vessels.

The ground substance is usually a fluid consisting of water, polysaccharides (hyaluronic acid), and proteins. The protein fibers are embedded in the ground substance, which together with the fibroblasts form the extracellular matrix that makes up the fascia. When muscles move, the fascial sheath allows them to glide over one another with no friction. The innocuous-sounding ground substance
magically offers no shearing force in the fasic function of gliding. Its structure changes at the microscopic level and adapts organically to its singular function of gliding, as captured in Guimberteau’s video, a most remarkable representation of differential equations in motion [4]. The gliding, made friction-free by the structural changes, guides the collagen and elastic fibers of the fascia to align in the direction of muscle extension, producing tensile force—the fascial tension.

2.1 Fascial Manipulation

Massage therapists have long known that applying proper physical pressure on the body can bring relief to muscle aches, soreness, and tightness and improve the range of movements caused by fascial dysfunction and restriction. Luigi Stecco, a physiotherapist, found that there was a functional link between the fascia and the muscles and joints it connects. Stecco’s insight was that muscle ailments could be caused by impediments to the gliding function of the fascial planes. He developed a fascial manipulation technique that applied deep manual frictional pressure on the muscle fascia at a series of therapeutic points referred to as centers of coordination, some of which he noted, coincided with acupuncture points. The mechanical action of friction within the layers helps to release the fascia, making it more fluid and thus improving its function. This is the gist of the method of the Stecco Fascial Manipulation method [5].

Earlier and separate from Stecco, Dr. Ira Rolf had also introduced her fascial manipulation system (Rolfing Structural Integration). Together they and others have forged a new field of musculoskeletal therapy, finally giving fascia its rightful place in medical science as more than just a packing material [6].

Fascial manipulation techniques are preceded by the TCM therapy of cupping. The world was set abuzz by the prominent reddish round marks on Michael Phelps’ shoulders during the swim meets at the Rio 2016 Olympics (Figure 1). He had been treated with TCM’s cupping therapy which relaxed his muscles and eased his joints and movements. He credited the therapy for helping him achieve his athletic best again at the ripe old age of 31, adding 4 more golds to his unimaginable career total of 23 Olympic golds.

The cupping therapy creates a vacuum that pulls a portion of the body’s flesh into a suction cup as a mini mount, which effectively manipulates the fascia. TCM explains it as pulling blood-Qi to improve circulation. There are other TCM modalities that can be viewed as manipulation techniques of the fascia as well, namely, moxibustion, scraping (Guasha), acupuncture, and tuina massage [7]. The body-wide network

Figure 1. The Cupping Therapy manipulates the fascia by pulling the flesh into the suction cups

of fascia offers a physiological framework for Qi flow in the meridian system. It is found that in acupuncture treatment, the fascia grabs onto the acupuncture needle as it is twirled (a micro-level example of the Weissenberg Effect of viscoelasticity), with the pull measurably stronger at some acupoints, which led Langevin to propose that Qi pathways reside in fascial folds [8]. That manipulation of the fascia by acupuncture needles could stimulate Qi flow in the body has been observed earlier (1992 Kimura, et al. [9]).

2.2 Fascia’s Role in Generating Strength

Fascia and Qi are linked more directly in their roles of generating strength. Although muscles and fascia are of two different tissue types, they are integrated in the function of body movements. When a muscle contracts it extends the tendon attached to the bone, creating a tension. To ensure the safe and smooth transmission of force and motion, the stretch and speed are monitored by the proprioceptors of muscle spindles, and the tension, by the Golgi tendon organs. Coupled with the action of an agonist-antagonist pair, there is a constant exchange of muscle contractile force and fascial tension, which is the “aliveness” activity that maintains the toning tension for force transmission in the muscle-tendon unit (Figure 2).
The muscle and tendon work in synergy to sustain the oscillation between active muscle force and passive fascial tension. The metabolic energy fires the muscle contraction, loading the viscoelastic fascia, which then recoils at muscle relaxation, regulated by nerve receptors. However, because of anatomical constraints and the limitations of viscoelasticity, the muscle and tendon-fascia cannot be weaponized as a catapult or a bow that stores potential energy on extension ready to launch a projectile or an arrow upon release. The body’s powerful action is propelled by contractile muscles, primed and harmonized in conjunction with the fascial tension.

However, sports training is not devoid of fascial conditioning, as it is found in warm-up and stretching routines, as well as in footwork and agility drills.

The slow-motion methodology of Taijiquan of developing internal strength (*neijin*) eschews muscle development. Indeed, permeating the training culture is the guiding mantra of “using mind-intent, not using force” (*yong yi bu yong li*). The training is focused on nurturing Qi energy associated with balance and harmony, and the harnessing of Qi to discipline body motion to develop *neijin*. That is, by operational default, Taijiquan training is harnessing the fascial tension to discipline body motion.

First of all, there must be awareness of the sensation of fascial tension for it to be cognizable in its functional efficacy. The fascia is rich with mechanoreceptors; the periosteum has nociceptive nerve endings, and the tendon, proprioceptors, all of which are sensitive to manipulation. The awareness is induced by the attentiveness to the breath and movements and the slow-motion practice—the meditative component of the practice.

A cognitive perception of the fascial tension develops in the awareness, coupled with the functional effects of the discipline. This represents the nurturing of Qi that cultivates the Qi-cognition of the fascial tensional network, which is harnessed to guide and harmonize body motion. This leads to the Fascia-Qi Hypothesis in Taijiquan training.

The art of Qi nurturing in Taijiquan cultivates the cognitive perception of the fascial tensional network in the discipline of body motion.

Cognition of the functional effects of fascial tension elevates it to Qi (as force or energy), called fascia-Qi. Taijiquan practice cultivates this Qi-cognition as it is being harnessed to discipline muscle actions.

### Fascia-Qi Hypothesis
Fascial tension gives rise to Qi when it is harnessed in the discipline of muscle actions. Implicit in Qi is a functional dimension that is accessed through cognitive development via a combination of sensory receptors. Cognition of fascial tension is a cultivated perceptual sensation that captures the functional effects of the discipline of body motion, specifically that of the balance factors. This bypasses mathematical analysis and leads to the art in science—the Fascia-Qi Hypothesis in Taijiquan.

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### 2.4 Bridging the Gap between Command and Action
In Taijiquan, the practice mantra of *yong yi bu yong li* (“using mind-intent not using force”) is both perplexing...
and gratifying at the same time. It is bewildering because the admonition of “not to use li” is directed at an appearance of physical exertion of muscular force in practice as error. It is frustrating because motion is not possible without the contractile forces of muscles. Yet it is gratifying because the body responds and relates to the mantra in delightful comprehension. The methodology induces a restraint of reactive muscle activations that is conducive to the discipline of body motion.

The command to execute an action triggers the motor-neurons to send signals to the muscles to contract, moving the body segments that sum up to the action. The action follows our command, but there is a gap between command and action—we have no control over how the muscles are recruited or activated. Thus we find that the performance of the action may often not be satisfactory, as in a golf-drive, due to the gap of control of the multiple pathways between the command and the final action [1]. The issue is to make sure that the action executed is tapping the force potential that the body is capable of in the performance. The strength depends not only on the active contractile forces of the muscles but crucially on the reactive tensile forces of the tendons and aponeuroses. The slow-motion methodology of Taijiquan operationally appeals to the role of the fascia to bridge the gap.

The operation of Qi in Taijiquan that bridges this gap in the discipline of body motion is described by the principle of “the mind-intent conducts Qi, and the Qi drives body motion” (Yi yi dao qi yi qi yun shen). The mind-intent of yi conducting Qi encompasses the command of the action and the motor-neuronal signals to the muscles; and the Qi driving the motion consists of the contractile muscle force and the fascial tension, namely, the fascia-Qi by the Fascia-Qi Hypothesis. With the proposition of fascia-Qi, the Yi-Qi-Motion paradigm of Taiji theory finds a physiological basis in the neural cortico-spinal pathway of muscle activation.

Importantly, what must not be overlooked is the cognition of the functional effects of Qi in the pragmatic guidance of body motion. As it turns out, because balance is paramount in bipedal functionality, generating strength can be reduced to the discipline of balance. Taijiquan develops the cognition of Qi by its functional factors of balance and harmony, which is pursued next.

3. Qi and Yin-Yang Balance

What are we doing in the practice of Taijiquan? The traditional answer, couched in metaphysics, is that Taijiquan is a practice to nurture Qi by disciplining body motion to be in accord with the Principle of Yin-Yang Balance. This goal is the raison d'être of the slow-motion methodology that characterizes the practice, described poetically as a meditation in motion. What never ceases to tantalize and beguile is that the easy-going practice promises to deliver kungfu skills of the highest order. The theory is that the Qi cultivation in Taijiquan develops neijin as the body’s core strength. And neijin, the stuff of physical strength (Li) plus Qi, forms the basis of Taijiquan’s much touted phenomenal kungfu.

Martial prowess does not operate outside of science. Balance and a prerequisite of core strength are necessary ingredients, without which combat skills no matter how great cannot be executed with effectiveness. The biomechanics of kungfu skills is not just about strength but the agility of change in application, executed in spontaneous response with the right force direction and magnitude. This is the consummate force of neijin that ensues from the ideal Taiji motion, imbued by the Principle of Yin-Yang Balance.

3.1 Functional Efficacy of Balance

We can think of Yin-Yang Balance as “internal balance” and add quickly that its foundational basis is the balance in body motion, between the internal forces of muscle actions within and the external forces, primarily, of gravity. It is the principle of balance that gives us our bipedal functionality in range, versatility, strength, and precision. However, balance does not accord the same functional efficacy.

For example, you are in balance in a standing posture and you take in a deep breath, as when asked to by a physician with a stethoscope on your chest. Inadvertently, you heave up your chest, causing your abdomen to hollow and your body to be top-heavy. While still in balance, you will topple easily under a gentle nudge. Your balance becomes functionally less stable. The science of balance governs the art of body motion.

The action of balancing an arm stretched out to the side may just be a balance between the muscle forces adjusting to gravity. But working the levers of the arm in balance are the fascial tension and muscle forces of a varying combination of muscles of the shoulder, chest and arm—the deltoid, trapezius, pectoralis, rhomboids, rotator cuffs, biceps and triceps, and many more.

Holding the arm in balance without moving, the muscles may seem to be at rest. But certain muscles might be acting excessively, requiring adjustment from countering muscles. The balance support can often be made by lesser muscle actions overall internally. Indeed, we have a sense that certain combinations of muscles are preferred, namely, those that give us less stress in the support. This gives us cognition that there is an error in
balance and a differentiation of a better balance as well.

Although we have voluntary command of skeletal muscles, we cannot allocate muscle forces, so much here and so much there, to find a preferred combination of a better balance. However, abiding by the mantra of “not using li-force,” the innervation of muscles becomes less associated with the action. This induces the relaxation mode of the muscle loading cycle to let the fascial tension attend more positively to find a better balance, like in tuning a stringed musical instrument.

We learn to cognize stress and associate it with the errors of muscle actions—too excessive or too lax—at the joints by sensory receptors in the tendons and fascia. Not only can we cultivate this cognition, we can also respond to adjust to a preferred combination of less stress by simply “letting-go” of the muscle actions for them to resettle. Taijiquan takes this rudimentary functional response and develops it into a sophisticated organic tool called fangsong (relaxation by “letting go”) to resolve the stress of muscle-action imbalances at a joint.

### 3.2 Relaxation, Fingsong and Qi

The fangsong effect can be simulated by enlisting someone to hold a finger and letting the arm hang like a cable. This works to restrain the dominating muscle actions, and brings about a resettling of the muscle forces and fascial tensions that adjust the excessiveness or deficiency in the support; it thereby reduces the errors of the tensile forces of the arm and induces a sensation of ease by a lesser stress. Concomitantly, one gains a body comprehension of the joints and the weight of the arm, which builds up to a fascial-tensional connectivity, cognized as Qi. In practice, fangsong is also simulated by another practice mantra to “sink the shoulder and drop the elbow” (chen jian zhui zhou).

The fangsong tool works by a simple pragmatic rule to reduce the errors of muscle actions: relax by letting-go (namely, fangsong) at the perception of excessiveness (stress of the dominance of muscle forces), or when the arm is lax or droopy (the perception of deficiency) stretch internally by fascial tension to connect to the fingers. The attentiveness of the practice cultivates cognition of less stress in the arm balance and the associated fascial tension as Qi. The process of Qi cultivation also serves to refine the tool of fangsong organically to further reduce the margins of error better, carving the practice path that leads to the goal of internal balance. In short, the Qi nurturing associated with the fangsong reduction of errors in the discipline of internal balance is a tuning to harmonize the internal dynamics of fascial tension and muscle forces against the external force of gravity. This enlivening of fascial tension gives cognition of fascia-Qi, and realizes the Fascia-Qi Hypothesis.

### 3.3 Fingsong and State of Muscle Rest

The muscle retracts at rest when the contraction ends. At the micro level, the basic contractile unit is a sarcomere, which lines up one by one in a microfibril of a muscle cell-fiber. The sarcomere contracts when its thick filaments (myosins) pull in the thin ones (actins) in a crossbridge action, with the expenditure of adenosine triphosphate (ATP) energy. Alongside this array is a third filament, titin, a polypeptide, that functions as a spring that resists the shortening and lengthening of the sarcomere unit. The titin filaments restore the sarcomere at rest, thus engendering muscle retraction with no further energy cost (Figure 3).

The operation of fangsong, a relaxation of letting-go, induces the muscles more to a rest state in the resettling of the muscle actions. Fingsong manipulates the cycles of muscle contraction and retraction together with the fascial tension to bring the muscles in the balancing function to a more restful state. Also, the contractile force of the sarcomere unit is at the maximum at the length from about 2 micron to 2.35 micron, the optimal fiber length, which range is around its resting length (see graph in Figure 3). The fangsong process of relaxation not only engenders a more restful state for muscles in the support of balance but enhances the functional strength as well. These qualities accrue to the core strength of the neijin being developed. The restful state is cultivated in standing meditation, the practice of standing in stationary postures, for example in Figure 5.

The electromyography (EMG) readings cannot distinguish the different standing postures because the muscles are at rest state. However, a standing posture in balance may not have the same functional efficacy, as discussed earlier. Fangsong elicits muscle rest, but the state of muscle rest does not imply the function of fangsong. That is, fangsong brings about a better state of muscle rest. While the postural muscles may be EMG silent, Qi-cognition is nurtured in Taijiquan practice to differentiate the functional differences towards a better balance.

### 3.4 Fingsong in the Whole Body

Extending the fangsong discussion from the arm to the whole body, we see that there are multitudes of muscles that can produce the same body action from which we seek the preferred combinations of less stress or disharmony. This entails resolving muscle actions at hundreds of joints. The task is compounded by the tensile integrity of the skeletal frame where resolution at one joint could affect that at the other joints, thus requiring a

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This is where the art of Taiji steps in with a practical solution path to a complex mathematical problem of resolving imbalances of muscle actions at the matrix of joints. The methodology prescribes the solution of simplifying the myriad joints into pairs of three correspondences, which can then be further subdivided. The fangsong resolution is then applied systematically, starting with the major pairings of the shoulder and pelvis, elbows and knees, and hands and feet. This is then extended to the elbows and knees, and then further to the hands and feet. In this way, the fangsong process gradually integrates functionally the major joints in the pairings, thus instilling cognition and body comprehension of the whole body, which is then harnessed to harmonize the tensile forces of the muscles and fascial tension against gravity and other external forces.

Not all joints are created equal. The sacral iliac joint (SIJ) and the pelvic joints that form a triangle of joints are bestowed a preeminent status as they control the movements of the spine and legs via the pelvis. Taijiquan refers to the complex of the pelvis and the triangle of joints as the kua. The kua serves as the hub of force transfer between the upper body and the legs and ground \[^{[13]}\]. The role of the kua is central in generating the force needed for performance in martial arts, sports or work.

### 3.5 Kua, the Complex of the Pelvis, and the Triangle of Joints

Pivotal to the discipline of body motion is the role of the kua. Working as the center of the kua is a point called the dantian, which is located at about three-finger width below the navel and a third of the way inside. Taijiquan's ingenious solution to the unwieldy fangsong resolution of the matrix of joints is to inculcate a role of centrality in the dantian. This is done by applying the fangsong resolution at the joints relative to the dantian and kua, thus developing a Qi-cognition of the fascial tensional connection between the joint and the dantian by the Fascia-Qi Hypothesis.

The constant reference to the dantian and kua means that the fangsong is working each time on the muscles of the waist, groin and abdomen with the fascia and aponeuroses to settle in balance and on the cognition of the cultivated Qi “sinking to the dantian,” \[^{[14]}\] and on the Qi-cognition of the fascial tensional network. This builds a body-wide web of Qi connectivity of the joints centered at the dantian.

The fascial tensional network is thus harnessed as it is being cognitively developed to form the body-wide Qi connectivity, affirming the Fascia-Qi Hypothesis. It is crucial to note that the centrality of the dantian is not just a point of reference but is actualized as a control center operating via the triangle of joints in the discipline of internal balance. The maturing of Qi development represents the refined harnessing of the fascial tensional network, empowering one as a conductor, as described by Stecco. This reduces the discipline of Taiji motion to the play of the kua.

To the question of what we are doing in Taijiquan practice, we can now add that it is about cultivating Qi-
cognition of the fascial tensional network that forms a body-wide web of Qi connectivity centered at the dantian, which provides the basis for the discipline of body motion to be in Yin-Yang Balance (Figure 4).

To recap, Taiji's fangsong practice of constantly settling the body into a state of better balance inspires a most comprehensive balance. The continual refinement of the practice produces precision leading to a consummate balance that keeps the base solidly rooted under any situation, a most advantageous asset in a combat interaction. Qi's role in generating strength and force reveals a lot more of physics in Qi, which is reviewed next.

4. Qi and Force

The body produces two kinds of forces. The first is the contractile force of muscles, which can only do one thing, to produce motion, as in a punching action. The second is the force created when the body's motion is obstructed or resisted. This is the force that inflicts damage and it rides on the quality of the motion. Taijiquan offers that the force that ensues from motion disciplined in accordance with the Principle of Yin-Yang Balance is consummate—of the right force vector in response.

The same punch does not produce the same force. It depends on what is struck. If the fist is directed at a concrete slab at the speed of a trained karate expert, the slab will break. The KO that excites fans, results when it strikes the head squarely. But if it misses, then there would be no force to speak of—it would remain the motion of a punch.

4.1 Sequential Kinetics and Momentum of Body Motion

Force is the phenomenon of a change in motion, or more precisely, a change in momentum (Momentum = Mass x Velocity), which is given by Newton's Second Law of Motion:

\[
\text{Average Force} = \frac{\text{Change in Momentum}}{\text{Time duration of the change}}
\]

To increase the magnitude of the force potential in an action, the first order is to produce a larger momentum in the motion, with more speed or more mass. The more momentum that can be brought to bear, the greater the force potential.

The body is a structure of many segments linked at the joints, which can move independently. For their momenta to align in motion, the segments must not move out of kinetic sequence. However, in the anxiety to throw a fast punch, the muscles closest to the punching action, namely those of the arm and shoulder, tend to dominate, causing the fist to jump ahead in the action. This would cut the muscle power and momentum of the other parts of the body from contributing, reducing greatly the force potential.

We are not wired to prioritize momentum in physical actions. The command to strike does not necessarily lead to body segments moving in the right order of sequential kinetics. Quite to the contrary, the motor-neural circuits often activate muscles that would undermine sequential kinetics and degrade the output of force potential. The physics is easy to understand, but how do we get the segments to move in harmony that aligns momenta?

To induce the right kinetic sequence, Taijiquan training
inculcates a Qi-cognition of action in three sectional functions: “the extremity section leads, the middle section follows/guides, and the root section drives.” For example, in the punching action, the fist as the extremity leads/points, the root section at the shoulder joint drives, and the elbow in the middle follows/guides. This is the Principle of Three Sections (Sanjie), a part of the afore-mentioned Ten Essential Principles. That imposes the discipline of sequential kinetics aligning the momenta of the three sections in an action [15].

4.2 Body’s Rotational Motion

An object’s motion is described by the motion of its center of mass (CM) and the rotation about an axis through its CM. The functionality of the object’s motion depends on the harmony of the two motions. If a football is thrown not spinning in the long axis, it will tumble along in an erratic path. The motion of the human body is the sum of the motions of its many segments, each with a rotational mode to complement the motion of its CM. The hand turning the palm facing up to palm facing down is a self-rotation which complements the arm’s circular motion in tracing an arc.

The rotational mode is fundamental to body motion and is most evident in performance arts. The discipline of harmonizing the rotational modes in body motion is incorporated at the core of Taijiquan training, referred to as “silk-reeling” or chansi gong, so called as it evokes silk being twirled and pulled gently without breaking. This develops the silk-reeling energy, called chansi jin, the gem of the core strength of neijin. Chansi jin is key to the performance of body motion whether it be martial arts, sports or work.

The discipline of silk-reeling instills the rotational mode in body motion. Integrated with the discipline of Yin-Yang Balance, following the pragmatic rule of bu diu bu ding (“neither lax nor resisting”), the principle of silk-reeling underlies the ideal motion of Taijiquan. Chansi jin is discussed more fully in the author’s book [16].

Ode to Chansi Gong

Hidden in the depths of the I-Ching
Mystic energies are said to delve
But to seek the dharma of Taijiquan
Walk the path of chansi gong

4.3 Obstacle to Aligning Momenta

Here we see the paramount importance of the kua borne out again. The kua and shoulder define the torso which represents the largest mass, thus the most crucial component in the body’s momentum. The generation of waist power relies on the torso turning as a whole. However, the torso is not habituated to turn as a whole. In daily bipedal functionality like walking, the chest and the abdominal segment rotate in opposite orientations in zeroing out their angular momenta to stabilize the gait. This is a remarkable energy-saving feature of our bipedal locomotion, which is facilitated by the engineering design of the spinal curvatures. The lordosis causes the spine to twist in opposite directions at the thoracic-lumbar vertebrae whenever the pelvic girdle tilts, the effect of the spinal engine [17].

The inadvertent effect of the spinal engine is the main obstacle to getting the angular momenta of the chest and abdominal region to stay aligned for the torso to turn as a whole.

The discipline of the trunk rides crucially on the discipline of the midsection to maintain the levelness of the kua and keep the lordosis in the sagittal plane, that minimizes the spinal engine action. This involves the abdominal muscles (the external and internal obliques and the transverse abdominis) which wrap around as a corset, attaching to the abdominal aponeurosis on the front and the thoracolumbar fascia at the back. These muscles, unlike those of the biceps and triceps that attach to the bones, are not easy to relate to in discipline. The discipline of the torso must also incorporate the fascial tension harmonizing the muscle tensile forces of the rectus abdominis and the erector spinae, together with the iliopsoas muscles, to keep the trunk erect.

This highlights the crucial role of the thoracolumbar fascia and the abdominal aponeurosis of the fascial tensional network. The muscles of the kua and abdomen are attached either directly or indirectly to the thoracolumbar fascia [18]. The fangsong operation disciplines the chest and the abdominal segment to stay aligned in momentum. This cultivates cognition of the fasciae on the front and back, thus developing a fuller Qi associated with the discipline. The cognition of the fangsong relaxation is perceived as Qi filling the pelvic bowl of the abdominal region, centering at the dantian. This reinforces the Qi “sinking to the dantian,” and is perceived as developing dantian Qi. The dantian Qi is harnessed to discipline the torso to turn as a unit.

4.4 Central Status of the Dantian

The practical import of dantian Qi is that the unwieldy task of balancing the myriad joints is reduced to one of cultivating dantian Qi, and the mastery of the art is reduced to the establishment of “the central status of the dantian” (yi dantian wei hexin), as concisely and...
insightfully articulated by Chen Xiaowang.\textsuperscript{[19]}

Cultivating dantian Qi instills in the dantian the role of asserting control over the trunk's rotational momentum via the triangle of joints, the thoracolumbar fascia, and the aponeuroses. The fangsong tempering of the abdominal and kua muscles in the discipline of balance develops cognition of the lordosis staying in the sagittal plane as it maintains the levelness of the kua movements. The Qi sinking is the disciplining of the midsection, thus the torso, to settle and rest on the kua. This translates to Qi-cognition of the torso mass hanging on the shoulders and resting on the kua support as a column.

The ultimate Qi development in Taijiquan is to achieve the fullness of dantian Qi, which signifies the establishment of the central status of the dantian. This means that the control of the ideal Taiji motion is asserted at the dantian center via the body-wide fascia-Qi network, namely, the actualization of the dantian as the control center. The fullness of Qi means that Qi is extending to the far reaches of the body's extremities, as described in the Principle of Four Extremities (Sishao), yet another one of Chen Changxin’s Ten Essential Principles. With the fullness of dantian Qi, Chen Xiaowang's standing meditation posture exudes the functional jin of balance (peng jin) from the core strength of neijin (Figure 5).

In the posture, the body perceives the shoulder-kua connection of the torso, the anchoring of the feet-base on the ground, and the control at the dantian-kua via the fascial tensional network. This represents the enlivening of the thoracolumbar fascia that disciplines the alignment of momentum in the transference of force between the ground and the upper body. And it elaborates on the role of the fascia in the force distribution between the ground and upper body as noted by Gracotvetesky, “The viscoelastic property of its collagen has a direct impact on the way the muscles are used and forces are channeled from the ground to the upper extremities.”\textsuperscript{[20]}

With the fascial tension guiding the clarity of kinetic sequence, the execution of waist power (dangyao jin) can be articulated as the jin-force action at the kua coiling up the torso to shoulder, through the elbow to the hand, thus the momenta are synchronized. At the same time, the reaction jin-force at the kua is coiling down through the knees to the feet in the opposite orientation to anchor solidly on the ground in support. All the while in the force transmission, the body’s balance is kept intact by the principle of internal balance. Disciplined thus when the motion is accelerated, the power of the action that ensues, called fajin, is explosive and graceful at the same time, as can be enjoyed in the video clip of Chen Xiaowang's Fajin\textsuperscript{[21]}. Zhan Zhuang is a practice of mindful attentiveness to the body settling into the kua in balance, centering at the dantian. The process develops cognitive perception of Qi filling the pelvic bowl concentrating at the dantian. This cultivates the fullness of dantian Qi that establishes the central status of the dantian. This achievement accords unity to the body frame of the three correspondences of the shoulder and kua, the elbows and knees, and the hands and feet, and extends to the far reaches of the body, via the body-wide fascia tensional network, centered at the dantian. In the depth of tranquility, the posture exudes the core strength of neijin, in ever readiness of response in the right force vector.

Figure 5. Grandmaster Chen Xiaowang in Zhan Zhuang Standing Meditation

5. Conclusions

Stripped of the esoteric of Yin and Yang, the discourse has been about strength, balance, and body motion. It is about the theory of the functional relationship:

\[
\text{Strength + Balance} = \text{Functional Efficacy of Body Motion}
\]

Whether it be sports or martial arts, performance lies in the functional expression of body motion in the spontaneous response of the right force vector in competition. The issue in training is how to translate the command of action such as a golf drive to muscle activations that produce the motion of the action that is satisfactory. Taijiquan resorts to the nurturing of Qi that develops cognitive perception to overcome the neural gap between command and action, which is put forth in this paper as the harnessing of the fascial tensional network. This is the cognitive perception of fascia-Qi by the Fascia-Qi Hypothesis, which is an integration of the body senses,
grounded on the efficacy of functionality.

Sports training can draw from the age-old methodology of strength training by the art of Taijiquan. Sports can certainly benefit from a training that produces a spontaneous response of the force of neijin, of precision and timeliness. Certain sports have adopted fascia training. It is hoped that this paper will lead to an integration of Taiji methods in sports training.

While Taijiquan’s methodology is focused on the cultivation of the core strength of neijin, it is based on the Qi energetics of the ancient art of daoyin tuna, which promotes the Qi harmony of the internal organs, and thus health wellbeing and longevity. Indeed, the Ten Essential Principles of Taiji theory refers to the functional harmony of the internal organs in the Principle of Wuzang (“Five Internal Organs”). The permeating harmonizing effects of Qi find its basis in the body-wide fascial tensional network, which envelops all the internal organs. Taiji practice thus is maintaining the homeostasis of the body’s organ systems, the passport to health. [22]

Specifically, the practice of Taijiquan helps maintain a stable and healthy gait. What with harmonizing the muscle forces and fascial tension against gravity and of fangsong constantly settling of the body into a better state of balance, it is no wonder that Taiji practice mitigates fall injuries as the body responds, as a matter of course, to settle into balance. And balance in mobility is most vulnerable in old age. [23]

Finally, back to Taijiquan, the perceptual insight of Qi cognition is instilled by the practice-process of cultivation that entails analyzing the physics of body kinetics, guided by the soft logic of Yin and Yang under the tutelage of neijin, [24] Taijiquan is necessarily a self-cultivation of Yin-Yang Balance. Taijiquan effectuates the right physics in the body’s response via the mastery of Qi—the art of Taijiquan in science. The upshot is that the force that arises from the ideal Taiji motion—one in accordance with the principle of Yin-Yang balance—is consummate and of the right force vector in spontaneous response. The science in Qi brings clarity to the practice which can inspire the body to reach its full potential in the art of Taijiquan and in sports.

References


00155.
[21] Chen Xiaowang’s Fajin action: https://www.youtube.com/watch?v=5LosS2vjmek&t=18s.
A Meta-analysis of Acupuncture Treatment for Irritable Bowel Syndrome

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Meta-analysis
Quality evaluation

ABSTRACT

To assess the efficacy of acupuncture for treatment of irritable bowel syndrome through meta-analysis of randomized controlled trials in recent 20 years. Online databases, including CNKI, VIP, WANFANG, PubMed, Cochrane Library, Web of Science and Embase were searched for randomized controlled trials (RCTs) of acupuncture for IBS. Retrieval time was from January 1, 2000 to January 31, 2021. According to Jadad scoring criteria, the bias risk and quality assessment of each RCT included were evaluated by two researchers. RevMan 5.3 software was used for the meta-analysis. Eight RCTs were selected which include a total of 1181 patients. The control group has 425 patients and the experimental group has 756 patients. The result of meta-analysis indicates that the total effective rate for the experimental group was superior to that of the control group [OR=3.29, 95%CI [2.16~5.03](P<0.01)], and the funnel plot was basically symmetric. Acupuncture therapy is shown to have a good safety and compliance record. However, the number of high-quality trials is small, and there are some deficiencies in the methodology of clinical research. Acupuncture, as a supplementary therapy for irritable bowel syndrome, has positive clinical significance and prospects for application. The methodology of clinical research needs to be further improved.

1. Introduction

Irritable bowel syndrome (IBS) is one of the most common clinical functional gastrointestinal diseases (FGIDs). It is a significant burden to patients in their daily lives, affecting their mental health, ability to work, and general quality of life. Modern medicine has focused its therapies on relieving spasms to improve abdominal pain, stopping diarrhea, anti-inflammatory measures, promoting gastrointestinal motility, antidepressant treatment, anti-anxiety treatment, etc. However, although IBS symptoms can be significantly improved with these treatment modalities, there are also many adverse reactions. Nowadays, acupuncture therapy is considered to be an effective alternative method to the treatment of IBS, and there are a large number of clinical reports [1] to that effect. In order...
to further understand the clinical effects and other aspects of acupuncture for IBS, we have collected relevant clinical research literature from January 2000 to January 2021, from China and other countries, evaluated the quality of the clinical literature relating to acupuncture for IBS, and present the resulting meta-analysis of its efficacy in this paper.

2. Materials and Methods

2.1 Materials

2.1.1 Literature Sources

Online databases, including CNKI, VIP, WANFANG, PubMed, Cochrane Library, Web of Science and Embase were searched for published randomized controlled trials (RCTs) of acupuncture for IBS.

2.2 Literature Retrieval

Key words: irritable bowel syndrome, acupuncture, electroacupuncture, acupoint. Retrieval time was from January 1, 2000 to January 31, 2021.

2.3 Inclusion Criteria

① According to the Rome III criteria revised by the Rome Committee of functional gastrointestinal diseases in 2006, the patient was definitely diagnosed as having irritable bowel syndrome (IBS); ② Randomized controlled trials (RCTs) were described; ③ Acupuncture therapy was used in the experimental group. Traditional Chinese medicine therapy and Western medicine therapy that does not include acupuncture were used in the control group. ④ The course of treatment should be at least once a week. According to the specific situation, if the same or similar research reports were presented in the same group or similar group, we have only chosen one.

2.4 Exclusion Criteria

① Patients who were not diagnosed definitely as having irritable bowel syndrome, and did not meet the Rome III criteria; ② Dissertations, animal experimental literature, review literature, case reports, experience-based reports, and repeated published literature (i.e. additional versions of the same literature/study) were excluded; ③ Retrospective study of cases; ④ Patients who have a severe disease of the intestines or have other serious diseases; ⑤ Acupuncture therapy was not mentioned in the literature or the acupuncture therapy is the complementary therapy; ⑥ The number of cases was fewer than 20 in experimental group or control group; ⑦ Massage, scalp acupuncture, ear acupuncture, abdominal acupuncture and other micro-needle literature. Those who met any one of the above criteria were not included in the study.

2.5 Outcome Measures

① Main outcome measures: clinical efficacy (total effective rate), irritable bowel syndrome severity scale (IBS-SSS); ② Secondary outcome measures: symptom score, irritable bowel syndrome quality of life scale (IBS-QOL), Self-Rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS), Hospital Anxiety and Depression Scale (HADS), Bristol scale, quality of life, sleeping score, security, adverse reactions, etc.

2.6 Data Collection and Quality Evaluation

First of all, we read the title and abstract for the first time, eliminated the conference papers, dissertations and other literature that do not meet the inclusion criterion, and then read the full text carefully for further screening. If there are repetitive published studies in different languages, only the Chinese literature has been reserved. Two researchers screened the literature and extracted relevant data independently, cross-checked their data, and negotiated with the third-party review group for uncertain and fuzzy decisions. Jadad scoring criteria were used to evaluate the quality of the literature. The final included literature was divided into low-quality literature (1-3 points) and high-quality literature (4-7 points). The evaluation contents include: ① whether to use the appropriate random grouping method to generate random sequence; ② whether to use allocation concealment, whether the description is clear and the method is appropriate; ③ whether to use blinding and whether the specific method is appropriate; ④ whether to describe the numbers and reasons of patients who quit the study or where there was loss of follow-up.

2.7 Statistical Method

Meta-analysis: Revman 5.3 software was used for statistical analysis. Odds ratio (OR) and 95% confidence interval (CI) were used for count data, and weighted mean difference (WMD) and 95% confidence interval (CI) were used for measurement data. The heterogeneity of each group was analyzed by x² test and I² test. Studies with I² of 25% to 50% were considered to have low heterogeneity, studies with I² of 50% to 75% and more than 75% were considered to have moderate and high heterogeneity respectively. If the heterogeneity was not significant (heterogeneity < 0.1), the fixed effect model was used to merge the studies. P < 0.05.

Literature quality evaluation: Revman 5.3 software was used to evaluate the methodological quality of the included studies.
3. Results

3.1 Literature Quality Analysis

3.1.1 Literature Retrieval

At first, 4190 articles were retrieved from a Chinese database and 135 articles were retrieved from foreign databases, with a total of 4325 articles. Then, following the inclusion criterion, exclusion criterion, and Jadad scoring criteria strictly, eight high-quality (4-7 points) RCTs were selected from 315 articles [2-12] (Figure 1).

3.1.2 Basic Information of Excluded Literature

According to Jadad scale, 307 articles were excluded for different reasons (Table 1).

Table 1. Relevant Information of Excluded Literature

<table>
<thead>
<tr>
<th>Reasons</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclear generating method of random sequence</td>
<td>279</td>
</tr>
<tr>
<td>Inappropriate generating method of random sequence</td>
<td>21</td>
</tr>
<tr>
<td>Unclear randomization concealment</td>
<td>96</td>
</tr>
<tr>
<td>Inappropriate randomization concealment or not use</td>
<td>203</td>
</tr>
<tr>
<td>Unclear blinding</td>
<td>10</td>
</tr>
<tr>
<td>Inappropriate blinding</td>
<td>294</td>
</tr>
<tr>
<td>Not described the reason of dropouts</td>
<td>290</td>
</tr>
</tbody>
</table>

3.1.3 Basic Information of Included Literature

Among the eight included studies with the theme of acupuncture, five [2,4,6,8] were in Chinese and three [5,7,9] in English. There were 1181 patients, 425 in the control group and 756 in the experimental group. All the studies [2-9] used the method of generating random numbers by computer; six papers [2-3,7-9] used the appropriate randomization concealment method; only two papers [5,9] used blinding; and six papers [2,4,6-9] described the numbers and reasons of dropouts, withdrawals and discontinued cases. There were different degrees of bias in the aspect of allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and selective reporting (Figure 2 and Table 2).

3.1.4 Evaluation of Literature Quality

Although there was a large amount of clinical literature on acupuncture for IBS, the overall proportion of high-quality RCTs was not very high. Even for high-quality RCTs, none focused on the health economics of acupuncture for IBS, and only some literature involved an explanation of safety problems, so there were some deficiencies in methodology.

3.2 Curative Effect Analysis

Among the eight clinical studies, only five [2,4,6,8] reported the total effective rate of the experimental group.
### Table 2. Basic Information and Quality Evaluation Table of Included Literature

<table>
<thead>
<tr>
<th>Trials</th>
<th>Cases Number</th>
<th>Treatment Course</th>
<th>Follow-up</th>
<th>Outcome Measures</th>
<th>Stochastic Method</th>
<th>Allocation concealment</th>
<th>Blinding</th>
<th>Reasons for withdrawal</th>
<th>Jadad Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang Q, et al, (2019)[7]</td>
<td>60</td>
<td>60</td>
<td>4w</td>
<td>Not Done</td>
<td>④ ⑧</td>
<td>Computer</td>
<td>Unknown</td>
<td>Not Done</td>
<td>Described Clearly 4’</td>
</tr>
<tr>
<td>Pei L, et al, (2018)[8]</td>
<td>20</td>
<td>20</td>
<td>12w</td>
<td>Not Done</td>
<td>① ⑨</td>
<td>Computer</td>
<td>Appropriate</td>
<td>Yes</td>
<td>Not Done 6’</td>
</tr>
<tr>
<td>Li J, et al, (2017)[9]</td>
<td>27</td>
<td>54</td>
<td>6w</td>
<td>Not Done</td>
<td>①</td>
<td>Computer</td>
<td>Unknown</td>
<td>Not Done</td>
<td>Described Clearly 4’</td>
</tr>
<tr>
<td>Qian H, et al, (2011)[11]</td>
<td>60</td>
<td>60</td>
<td>4w</td>
<td>Not Done</td>
<td>④ ⑥</td>
<td>Computer</td>
<td>Appropriate</td>
<td>Not Done</td>
<td>Described Clearly 5’</td>
</tr>
</tbody>
</table>

Notes: ① IBS-SSS ② IBS-QOL ③ 5-HTTLPR ④ TCM syndrome scoring ⑤ VIP of serum ⑥ MAPK ⑦ SAS, SDS ⑧ Coliform detection ⑨ Visceral pain scale ⑩ Stool frequency and character ⑪ Life quality ⑫ Adverse reactions ⑬ Bristol scale

**Figure 2. Risk Bias Assessment of Methodological Quality**
and the control group, and the remaining three \cite{5,7,9} did not report the total effective rate. In addition, five trials \cite{4,6-9} contained specific descriptions of the improvement of clinical symptoms, and the remaining three trials \cite{2-3,5} had no specific description of the improvement of symptoms.

### 3.2.1 Heterogeneity Test

χ² test (χ² = 2.89, P > 0.05), I² = 0% < 50%, it can be considered that the five independent similar trials \cite{2-4,6,8} have homogeneity, so the fixed effect model was selected for statistical method (Table 3, Figure 3).

### 3.2.2 Test of Combined Statistics

\[ Z = 5.53, P < 0.01, \] with statistical significance. The funnel plot of the five independent similar trials \cite{1-3,5,8} is shown in Figure 4: the figures are basically symmetrical, so it could be considered that the bias of the five trials is small (Figure 4).

<table>
<thead>
<tr>
<th>Trials</th>
<th>Treatment Method</th>
<th>Experimental Group</th>
<th>Control Group</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guo J, et al 2021</td>
<td>Acupuncture</td>
<td>Baihui, Yintang, Tianshu, Shangjuxu, Zusani, Sanyinjiao, Taichong</td>
<td>Dicetel p.o</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Wang S, et al 2020</td>
<td>Acupuncture and Tongxiyao formula</td>
<td>Shangjuxu, Tianshu, Taichong, Sanyinjiao, Zusani</td>
<td>Dicetel p.o.</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Wang Q, et al 2019</td>
<td>Acupuncture</td>
<td>Tianshu, Zusani, Shangjuxu, Sanyinjiao, Taichong</td>
<td>TrimebutineMaleate p.o.</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Pei L, et al 2018</td>
<td>Acupuncture</td>
<td>Baihui, Yintang, Zusani, Shangjuxu, Sanyinjiao, Tianshu, Taichong</td>
<td>Sham acupuncture</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Li J, et al 2017</td>
<td>Acupuncture</td>
<td>Baihui, Taichong, Shangjuxu, Tianshu, Zusani, Sanyinjiao, Yintang</td>
<td>Dicetel p.o</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Zheng H, et al 2016</td>
<td>Electroacupuncture</td>
<td>Quchi, Shangjuxu, Tianshu, Dachangshu</td>
<td>Loperamide p.o.</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Qian H, et al 2011</td>
<td>Acupuncture</td>
<td>Zhongwan, Tianshu, Shangjuxu, Xiajuxu, Neiguan, Taichong, Zusani, Pishu</td>
<td>Sham acupuncture</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Alastair F, et al 2005</td>
<td>Acupuncture</td>
<td>Acupoints selecting based on syndrome differentiation</td>
<td>Sham acupuncture</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

![Figure 3. Comparison of the Total Effective Rate Between EG and CG of Five Trials](https://example.com/figure3.png)

![Figure 4. Funnel Plot of Clinical Efficacy of Acupuncture for IBS](https://example.com/figure4.png)
3.3 Compliance Analysis

Six trials \[2,4,6-9\] described the specific numbers and reasons for dropouts (Table 4).

**Table 4. The numbers and Ratio of Dropouts**

<table>
<thead>
<tr>
<th>Trials</th>
<th>Control Group</th>
<th>Experimental Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases Dropout numbers Ratio</td>
<td>Cases Dropout numbers Ratio</td>
</tr>
<tr>
<td>Wang Q, et al (2019)</td>
<td>60 5 8.33%</td>
<td>60 4 6.67%</td>
</tr>
<tr>
<td>Li J, et al (2017)</td>
<td>27 1 3.70%</td>
<td>54 3 5.56%</td>
</tr>
<tr>
<td>Qian H, et al (2011)</td>
<td>60 0 0%</td>
<td>60 0 0%</td>
</tr>
<tr>
<td>Alastair F, et al (2005)</td>
<td>28 1 3.57%</td>
<td>31 0 0%</td>
</tr>
<tr>
<td>Total</td>
<td>364 23 6.32%</td>
<td>695 40 5.76%</td>
</tr>
</tbody>
</table>

3.4 Safety Analysis

Among the eight high-quality RCTs, only four trials \[2,5-7\] included a safety analysis and discussion, and the conclusion was consistent: acupuncture for irritable bowel syndrome has a good safety record.

3.5 Health Economics Evaluation

No one discussed the problem of health economics, so we could not compare the economic advantages of acupuncture for IBS.

4. Discussion

A survey of worldwide databases turned up a total of 4,325 studies or reports which were initially screened by reading the title and abstract of each study, resulting in 1181 studies being evaluated further. These 1181 studies were read for content, and then evaluated by Jadad scoring criteria. Among them, 307 so-called RCTs were rated as low-quality literature (1-3 points). The specific performances of the low score are as follows: ① The method of generating random sequence is not correct, such as according to the visiting sequence and the date of admission, or the method of randomization allocation is not described. ② The randomized concealment method is not appropriate. For instance, they did not use computer control or sealed opaque envelopes or other methods that make it impossible for clinicians and subjects to predict the sequence allocation; or the related explanations were not clear enough, they were simply described as using the random number table. ③ There was no blinding or lack of detailed description, such as no placebo group in the trial. ④ The numbers and reasons of dropouts and withdrawals were not clarified. In fact, there were different degrees of deduction among the eight high-quality papers which were rated as 4-7 points: two of them \[4,6\] did not explain the method of generating randomization concealment; six of them \[2,4,6-8\] did not use blinding; and two of them \[3,5\] did not explain the numbers and reasons of dropouts and withdrawals. At the same time, as shown in Table 1, most trials did not have follow-up for a long time, and the final outcome measures and measurement index of each trial were different, which makes it impossible to evaluate the long-term efficacy of acupuncture for irritable bowel syndrome systematically and scientifically in this paper, which will also have influence on the results of this meta-analysis, more or less.

Among the eight high-quality RCTs included in this paper, the treatment method for the experimental group always involved the use of acupuncture. Among them, six trials \[2,5-9\] were treated with acupuncture alone. One \[3\] included a Tongxie prescription, and one \[4\] included moxibustion (prescription and moxibustion were auxiliary therapies). The treatment method for the control group(s) involved Western Medicine (Dicetel tablets, TrimebutineMaleate tablets and Loperamide tablets) and sham acupuncture. Three trials \[5,8-9\] used sham acupuncture for the control group.

Effectiveness is one of the important criteria when evaluating the usefulness of any treatment or modality. We can confirm the effectiveness of acupuncture for irritable bowel syndrome by meta-analysis. Although we recognize problems or inconsistencies in different aspects of the eight high-quality studies, these problems have no decisive impact on the judgment of effectiveness. The results of meta-analysis showed that the overall efficacy for the experimental group was better than the control group, and there were five trials \[2,4,6,8\] detailing that the total effective rate of the experimental group was better than the control group. Acupuncture therapy can significantly improve clinical efficiency, reduce the recurrence rate, and more obviously relieve abdominal pain, diarrhea, and other clinical symptoms of patients with irritable bowel syndrome.

In short, the clinical literature detailing acupuncture treatment for irritable bowel syndrome has gradually increased in the past decade. Although the high-quality
RCTs account for a relatively small proportion of all relevant literature, the meta-analysis of the existing high-quality RCTs shows that acupuncture treatment has definitely demonstrated clinical efficacy, combined with good compliance and safety for irritable bowel syndrome. There are relatively few high-quality RCTs of acupuncture treatment for this disease, which reflects that the methodology of acupuncture clinical research still has a large space for improvement, especially in the evaluation of long-term efficacy, safety and health economics.

References


ARTICLE

An Association of Vitamins A and E with Hyaluronic and Lactobionic Acids may Prevent Molecular Changes Associated with Keratocyte to Myofibroblast Transition

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ABSTRACT

Inflammatory events in the corneal stroma may activate keratocytes and trigger their transition towards myofibroblasts, which now produce different extracellular matrix (ECM) proteins thus causing corneal opacification. Corneal haze is a frequent side effect after photorefractive keratectomy (PRK) to correct high myopia. Currently, a preventive treatment with mitomycin-c can be used to limit the occurrence of this phenomenon. However, mitomycin-c is a toxic drug, not devoid of side effects, which may occasionally involve the corneal endothelium. Therefore, we have searched for a less risky, natural way, to prevent keratocytes transition. To this purpose, we have used as markers of the phenotype switch the proteins lumican (highly expressed by keratocytes and much less by myofibroblasts) and smooth muscle actin (αSMA) (highly expressed by myofibroblasts and poorly found in keratocytes), beside Fibronectin (Fn), the expression of which is also increased by transforming growth factor-beta (TGFβ) treatment. Treatment of human keratocytes with TGFβ was used to induce the protein shift. Among different possible candidates, we have found that vitamins A and E, hyaluronic and lactobionic acids may prevent, either alone, or much better in association, the shift in the ratio between lumican and αSMA and the increased Fn expression. In conclusion, it could be speculated that topical treatment of the ocular surface with an association of these four compounds could be able to prevent or at least limit the occurrence of post-PRK corneal haze, with the additional advantage of lubrication, hydration and antioxidant defense exerted by these molecules.

Keywords: Corneal haze Keratocytes Lumican Vitamins Hyaluronic acid Lactobionic acid

1. Introduction

The cornea is the most external tissue of the eye, and the main lens of the ocular dioptr, accounting for approximately 48 diopters [1]. In order to perform efficiently its role, the cornea must be highly transparent, so that the light arrives undisturbed to the photoreceptors

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in the retina, with no blurring or distortion of the images. Such transparency of the cornea is due to three different characteristics of this tissue. (I) The cornea is avascular. The pathologic presence of blood vessels in the cornea would alter the delicate hydration balance of this tissue, and in case blood vessels should invade the area covering the pupil, they could shield and deflect the light entering the eye. The avascularity of the cornea is maintained through the presence of a VEGF-trap molecule, a soluble, truncated form of the VEGF receptor-1 (sVEGFR-1) the product of the soluble Flt gene \(^2\) (sFlt-1). (II) The hydration state of the corneal stroma is optimized for transparency, and tightly controlled by the ionic pumps of the corneal endothelium \(^3\). Failure of these pumps, for iatrogenic, traumatic or genetic reasons, leads to corneal edema and corneal opacification potentially invalidating for the patient \(^4\). (III) Corneal stroma has a special architecture that results in a transparent matrix. Collagen fibrils are arranged in approximately 200 layers of lamellae, each one arranged regularly almost right angle to each other \(^5\). This arrangement, critical for transparency, is maintained by proteoglycans such as lumican and keratocan \(^6\). In the case of local inflammatory events, such as after PRK, the release by injured epithelial cells of the inflammatory cytokine TGFβ induces the transformation of keratocytes into myofibroblasts, characterized by a decreased production of lumican and an increased expression of the cytoskeletal protein αSMA \(^7,8\).

PRK triggers inflammation and a wound healing response in the corneal stroma. Keratocytes replicate under the influence of TGFβ, and undergo the transition towards myofibroblasts, thus causing corneal haze. Moreover, regenerating nerve endings after the lesion caused by PRK tend to avoid corneal regions populated by myofibroblasts \(^9\), so that corneal haze may be accompanied by delayed nerve regeneration. Mytomycin C is a cytotoxic antibiotic that alkylates DNA and proteins, thus inducing apoptosis of proliferating cells. The intraoperative use of mytomycin C, by killing the replicating keratocytes, has been shown to be effective in reducing the risk of haze development \(^10\) and delayed nerve regeneration \(^9\) after PRK. Such treatment, however, despite being in use since many years with no reports of serious complications, is not devoid of potential side effects. In a preclinical study on rabbits, mitomycin C significantly reduced keratocyte density in the anterior stroma, with an effect lasting at least 6 months \(^10\). Such reduction may cause biomechanical instability, iatrogenic ectasia and increased risk of corneal melting in case of a further delay of keratocyte repopulation \(^11\). Endothelial toxicity and endothelial cell loss are other possible side effects, putting the patient at risk of corneal edema development because of a reduced function of the endothelial pump system \(^12\).

Based on this knowledge, we set out to investigate whether we could identify some different natural, non-toxic compounds, which upon topical administration could prevent the myofibroblast transition of keratocytes, thus reducing the risk of corneal haze development after a traumatic corneal injury. In order to obtain an initial proof of concept, we have used a primary cell culture of human keratocytes, treated with TGFβ to induce the keratocyte to myofibroblast transition, monitored by the expression of the main determinants characterizing the two states: lumican, αSMA and Fn.

2. Materials and Methods

2.1 Cell Culture

Human primary corneal keratocytes (Innoprot, cat. No. P10872) have been used throughout the study. Human keratocytes (HK) were routinely grown in fibroblast medium (Innoprot, cat. No. P60108) with 2% fetal bovine serum (FBS) and 5% horse serum (HS), in a humidified incubator at 37°C, and 5% CO\(_2\). HK from the original vial were expanded to generate freezing lots considered at passage 2. Freshly thawed cultures were normally splitted by trypsinization at 1/3 dilution for no more than 10 passages (roughly 15 population doublings).

2.2 Cell Treatment

Trypsinized cells from semi-confluent cultures were seeded in 6 multiwell plates at a cell density of 5000/cm\(^2\) in growth medium. The next day, wells were rinsed with PBS, and cells shifted 4 hours to serum free fibroblast medium. A further 3-hour pre-incubation followed, with 50 μM vitamin E (Sigma-Aldrich Cat. No.258024), 10 μM retinyl palmitate (Sigma-Aldrich Cat. No. PHR1235), 2% w/v sodium lactobionate (SL) (Glentham Life Science, Wiltshire, United Kingdom Cat. No. GK2515), 0.15% w/v hyaluronic acid (HA) (MW 1.5 MDa) (kindly supplied by SOOFT Italia SpA, Montegiorgio, Italy) prepared at the indicated final concentrations, either alone or in association. Then, TGFβ1 (R&D Systems, United Kingdom Cat. No. 240-B-002) was added at 10 ng/ml, and plates incubated for further 48 hours. At the end of the incubation period, cell monolayers were rinsed three times with PBS and extracted with RIPA (Calbiochem-Merck, Darmstadt, Germany, Cat. No. 20188) in presence of protease inhibitors (Protease Inhibitor Cocktail Set III EDTA-Free, Calbiochem-Merck, Darmstadt, Germany, DOI: https://doi.org/10.30564/jim.v10i2.3382
Cat. No. 539134) for 30 minutes on ice. Extracts were then clarified by centrifugation at 13000 rpm for 20 minutes at 4°C, and proteins in supernatants quantified by the BCA assay (Sigma-Aldrich, Cat. No. 2322).

### 2.3 Western Blotting

Thirty µg of proteins were then loaded on a pre-casted 4-12% SDS-PAGE, and blotted onto a nitrocellulose membrane, which was then saturated with skimmed milk and incubated with antibodies against αSMA (Abcam, cat. No. ab32575), lumican (Abcam, cat. No. ab168348), Fn (Abcam, cat. No. 6328) or GAPDH (Cell Signaling, cat. No. 2118) overnight at 4°C. The next day membranes were incubated with the respective peroxidase-labeled secondary antibodies (Amersham, GE Healthcare, Illinois, USA, Cat. No. NA934V) for 1h at room temperature. The peroxidase signal was then developed by chemiluminescence (ECL SuperSignalTM West Dura Extended Duration Substrate, Thermo Fisher Scientific, Massachusetts, USA, Cat. No. 34075) and revealed with the ChemiDocTM Touch Imaging System (BIORAD, Hercules, California, USA). Signal intensity was quantitated by the Image-J Software.

### 2.4 Immunofluorescence

The expression pattern of Lumican and αSMA was investigated by immunofluorescence staining of HK grown on coverslips coated with collagen and Fn in 24 well plates at a cell density of 5.000 cells/cm². The next day, cells were starved 4 hours in serum free medium (SFM), and then incubated for 3 hours with Mix1 or left in SFM. TGFβ1 (Sigma-Aldrich) was added at 10 ng/ml, and cells incubated for further 48 hours. At the end of the incubation period, cell monolayers were rinsed three times with PBS and fixed with 4% paraformaldehyde for 15 minutes at room temperature. Subsequently, cells were incubated for 30 minutes at room temperature in blocking solution (0.1% Triton in PBS with 0.5% bovine serum albumin (BSA)) and incubated overnight with primary polyclonal antibodies diluted 1:500 in blocking solution: anti-αSMA (Abcam, cat. No. ab32575) or anti-lumican (Abcam, cat. No. ab168348). The next day, cells on coverslips were washed 3 times in PBS before adding the secondary antibody diluted 1:1000 in blocking solution (Alexa Fluor 488 AffiPure Goat Anti-Rabbit IgG, cat. No. 111-545-045) for 1 hour at room temperature. After further washings in PBS, nuclei were counterstained with Hoechst 33342 (Invitrogen, cat. No. H3570) diluted 1:5000 in PBS and incubated for 4 minutes at room temperature. Cell fluorescence was detected with a Leica TCS SP 8 AOBS confocal laser scanning microscope.

### 2.5 Statistics

All experiments have been run in triplicates and repeated at least three times. One-way ANOVA followed by Tukey’s test was used to evaluate the statistical significance of each experimental group. Differences with P value < 0.05 were considered statistically significant. All data are expressed as the mean ± standard deviation. All statistical analyses were conducted with the GraphPad PRISM statistical software package version 5.00 for Windows (GraphPad Software, San Diego, CA USA).

### 3. Results

#### 3.1 Effects of Different Molecules on the Expression of Lumican and αSMA on Human Keratocytes

Human primary keratocytes kept in cell culture with 2% FBS express more lumican than αSMA, as expected by normal keratocytes in a healthy corneal stroma. However, we observed a variable ratio lumican/αSMA between 1.25 (Figure 1C) and 1.8 (Figure 2C) due to an increased expression of αSMA with senescence. Likely, also the presence of FBS - even though at low concentration - during routine tissue culture might have contributed toward this progressive shift, as it is known that the keratocyte phenotype is better preserved under serum-free conditions [14]. The effect of the addition to the culture medium of different molecules chosen after a screening of several more, based on their influence on the ratio lumican/αSMA, is also shown in Figure 1. Vitamins A and E, SL and HA at the concentrations used had no effect on cell viability (not shown). Vitamin E at 50 µM and Vitamin A at 10 µM had opposite effects on the lumican/αSMA ratio since it was slightly decreased by Vitamin E and slightly increased by Vitamin A (Figure 1C). SL 2% enhanced lumican expression, almost doubling its ratio to αSMA, while HA had no detectable effect (Figure 1C). The association of all four (Mix 1) or of vitamins only (Mix 2) had the net result of enhancing lumican expression (Figure 1B) and therefore also the ratio lumican/αSMA (Figure 1C).

#### 3.2 Effects of TGFβ on the Ratio Lumican/αSMA is Countered by Each Molecule, either Alone or in Association

Treatment for 48 hours of HK cells with TGFβ (10 ng/ml) triggered a 50% enhanced expression of αSMA...
and a 70% decreased expression of lumican (Figure 2), characteristic of the transition keratocyte-myofibroblast, as expected [7-8]. The simultaneous presence with TGFβ of any of the molecules illustrated above was able to prevent such transition (Figure 2A), as indicated by the reduced changes observed on αSMA and lumican. Every molecule was able to normalize the levels of αSMA and to prevent the decrease of lumican expression (Figure 2B), so that the ratio lumican/αSMA, although still lower than control cells, was significantly higher than TGFβ-only treated cells (Figure 2C). Both associations (Mix 1 and Mix 2) returned a ratio lumican/αSMA clearly in favor of lumican (Figure 2C), although with a different effect on the single components (Figure 2B). In fact, Mix 1 strongly reduced the effects on αSMA (which was even lower than in control) with a lesser effect on the decrease of lumican, while Mix 2 maintained lumican expression at control levels, while slightly decreasing αSMA expression. However, the overall effect on their ratio was comparable (Figure 2C).

3.3 Immunofluorescence Staining of Human Keratocytes for Lumican and αSMA Indicates a Phenotypic Shift, Countered by the Association of All Four Molecules (Mix1)

Figure 3A shows by immunofluorescence labeling of HK the decreased intensity of lumican after TGFβ treatment, and how Mix 1 (the most effective among the two, including all 4 components) was able to preserve lumican expression. On the contrary, αSMA expression was enhanced by TGFβ (Figure 3B), with HK showing a more elongated phenotype, with long stress fibers of αSMA. The presence of Mix 1 prevented the increase of αSMA and the morphological shift.

3.4 Enhancement of Fn Expression by TGFβ is Prevented by Each Molecule either Alone or in Association

Among the changes induced in keratocytes by TGFβ
Figure 2. Effect of different molecules on the expression of lumican and αSMA in HK (passage 3) treated with TGFβ1. A: HK were pre-treated for 3 hours with each molecule at the indicated concentration; then TGFβ1 (10 ng/ml) was added, and incubation continued for further 48 hours. Thirty μg of proteins were loaded on a 4-20% SDS PAGE, and specific bands revealed by the respective antibodies. B: Densitometry analysis of each band (lumican or αSMA) is reported with respect to GAPDH as arbitrary units (a.u.). C: Ratio of lumican vs. αSMA derived from the densitometric analysis shown in B (actual numbers are shown at the bottom of each bar).

* p < 0.05 vs. control; § p < 0.05 vs. TGFβ
HK: Human Keratocytes; SMA: Smooth Muscle Actin; SFM: Serum Free Medium; SDS-PAGE: Sodium Dodecylsulphate-Polyacrylamide-Gel-Electrophoresis; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; TGF: Transforming Growth Factor.

Figure 3. Immunofluorescence analysis of lumican and αSMA in HK (passage 3) treated with TGFβ1 (10 ng/ml) in the presence or absence of the association of the four compounds (Mix 1). A: lumican expression is decreased by TGFβ treatment, and conserved in the presence of Mix 1; B: αSMA expression is increased by TGFβ1 treatment, where also a morphological change is apparent, while Mix 1 blunted both effects of TGFβ1.

HK: Human Keratocytes; SMA: Smooth Muscle Actin; SFM: Serum Free Medium; TGF: Transforming Growth Factor.
treatment, Fn expression is known to be enhanced [14]. Therefore, we evaluated how Fn expression was modulated in this human keratocyte cell line by the different molecules in the presence or in the absence of TGFβ (Figure 4). In the absence of TGFβ, Fn expression was doubled by HA treatment, while the association of the four components (Mix 1) resulted in a 50% decrease (Figure 4A-C). Addition of TGFβ (10 ng/ml) to the cell culture resulted in a 100% increase of Fn expression (Figure 4B-D). The simultaneous presence of each single molecule was already enough to prevent such increase. The presence of the Mix 1 further decreased Fn expression by 50% despite the presence of TGFβ, while the association of the vitamins A and E alone (Mix 2) only partially prevented the increase of Fn expression, which however remained at levels higher than the control.

4. Discussion

We have shown in this paper a basic proof of concept that an association of natural molecules at nontoxic concentrations may have the ability to counteract the consequences of an inflammatory state of the cornea which, through the action of TGFβ, leads to keratocytes transdifferentiation, alterations of corneal stroma, and finally to corneal opacification (corneal haze).

Corneal stroma keratocytes produce a specific and balanced population of proteoglycans (lumican, keratocan, mimecan, decorin), the arrangement of which is essential for corneal transparency [15]. Among these, lumican seems to be the main orchestrator in order to produce and maintain a regular structure of corneal stroma, which is an essential requisite for transparency. Lumican is a keratan sulfate (KS) proteoglycan belonging to the small leucine rich proteoglycan family (SLRP). SLRPs contain a protein core modified by glycosaminoglycans side chains, which confer them their specific properties in the regulation of tissue-specific matrix assembly, and mediating cell-matrix interactions [16]. Preclinical studies have indicated a role for lumican in the pathogenesis of different ocular diseases such as glaucoma, myopia, inflammatory eye diseases and wound healing [17]. A prominent role of lumican in the corneal stroma is the regulation of collagen fibril assembly [18]. Mice made homozygous for a null mutation in lumican show collagen fibril abnormalities in
the posterior stroma, where collagen is more abundant, finally resulting in a 25% reduction in KS content, a 40% reduction of stromal thickness and bilateral corneal opacification [19]. Differently, null mice for keratocan (another cornea specific KS proteoglycan) show a less severe corneal phenotype, with a thinner, but still transparent cornea [20,21]. Indeed, it is known that mutated keratocan may result in changes of corneal curvature, and thus its refraction ability, but no significant collagen fibril structural defects [22]. On the other hand, lumican overexpression in corneas of wild type mice also resulted in no alteration of collagen organization and corneal transparency; however it decreased corneal keratocan expression, thus indicating a regulatory role of lumican for keratocan expression [23]. Moreover, lumican contains an aminoacid sequence endowed with self-assembling properties, and able to stimulate collagen biosynthesis [24,25].

αSMA is a cytoskeletal protein typical of muscle cells. Expression of αSMA in fibroblasts or keratocytes (during their transition to myofibroblasts) may happen during wound healing since wound closure is facilitated by the contractile properties and higher motility of these myofibroblasts [26,27]. The production of αSMA is linked to the presence of Fn in the basement membrane because its synthesis is enhanced by the interaction of Fn with its integrin receptor [28].

Therefore, in keratocytes, the ratio lumican/αSMA and the amount of Fn produced are indicative of the differentiation state of the cells: a predominance of lumican over αSMA, and low levels of Fn production suggest a well differentiated state, whereas a decrease of lumican/αSMA ratio because of an increased production of αSMA and Fn indicate a degree of transdifferentiation. We report here that primary human keratocytes in tissue culture can indeed maintain a differentiated state with a ratio lumican/αSMA > 1 (Figure 1 and Figure 2), and low levels of Fn production (Figure 4), which can be basically modulated by the addition of some natural compounds, either isolated or mixed together.

Transforming growth factor-β (TGFβ) is a pleiotropic cytokine that regulates a myriad of cellular processes and has important roles in morphogenesis, embryonic development, adult stem cell differentiation, immune regulation, wound healing and inflammation [29]. In the eye, it has been found to be induced after PRK to regulate wound healing and keratocytes transdifferentiation, thus triggering corneal opacification [30]. TGFβ was found to regulate cell phenotype and induce epithelial-mesenchymal transition in cancer cells, thus enhancing their invasive and metastatic ability, through the mechanistic target of rapamycin (mTOR) pathway [30]. The same trigger and pathway is also involved in the transdifferentiation keratocytes-myofibroblasts after PRK [31]. Rapamycin, a known inhibitor of the mTOR pathway [31,32], can thus prevent the transdifferentiation and also the consequent corneal haze [33].

Our data show indeed that TGFβ treatment resulted in decreased lumican expression and a parallel increase of both αSMA and Fn, so that the ratio lumican/αSMA was dramatically decreased (Figure 2).

Different strategies have been published aimed at preventing the keratocyte/myofibroblast transition. The only clinical intervention allowed nowadays consists in topical application of mitomycin C, which, by its genotoxic activity, inhibits the proliferation of myofibroblast progenitor cells, and thereby inhibits mature myofibroblasts generation [31]. It is an effective treatment [34], even though there is no unanimous consensus on the treatment protocol [35]. Among alternative treatments under investigation, rapamycin has been shown to work efficiently in a preclinical setting [36], also after topical application as eye drops [37], but no clinical data have been reported so far. Onion extract, due to its flavonoid content, can also reduce scar formation by inhibiting fibroblasts metabolism [38], and has shown its efficacy also in a dermatologic clinical setting [37]. Topical application as eye drops on canine eyes subjected to PRK were shown to prevent αSMA increase in keratocytes, and significantly reduced corneal haze formation [39].

We now show evidence that an association of vitamins A and E with SL and HA can also prevent TGFβ-induced expression changes of lumican, αSMA and Fn, typical of the transdifferentiation of keratocytes. Vitamin A deficiency in humans (and in mammals in general) can lead to alterations of the corneal epithelium (designated as xerophthalmia) characterized by opacification and keratinization [39] and dependent on the inactivation of the Notch1 pathway [40]. The efficiency of corneal proteinases also depends on a correct supply of vitamin A, the deficiency of which can lead to decreased exfoliation of epithelial cells, increased levels of keratofibrils in corneal keratocytes, increased stromal keratocyte degradation and increased susceptibility towards ulceration [41]. However, a clinical study designed to evaluate the efficacy of a topical perioperative supplementation of vitamin A (250 IU per gram of ointment) in patients undergoing PRK found no significative effects on re-epithelialization time, postoperative pain, corneal haze formation, or visual outcomes after PRK [42]. On the contrary, a high dose oral supplementation of vitamin A and E (25 000 IU retinol palmitate and 230 mg alpha tocopheryl nicotinate) to patients scheduled to receive PRK treatment resulted in
accelerated re-epithelialization time and reduced corneal haze formation \[43\]. These latter results are consistent with our results shown in Figures 2 and 3 in which treatment of HK with vitamins A and E either isolated or in association (Mix 2) in the presence of TGFβ was already able to counteract its effects on the expression of lumican, αSMA and Fn, and likely on the transdifferentiation of keratocytes.

We had already shown that topical instillation of SL, either alone or in association with HA, is able to exert an antiinflammatory effect on the cornea, preventing the increase of TGFβ and matrix metalloproteinase 9 (MMP9) induced by inflammatory agents \[44\]. Consistently, in a rabbit model of PRK the instillation of HA eye drops inhibited subepithelial haze by favoring a more physiologic wound healing \[45\], likely due to the interaction of HA with its cellular receptor CD44, shown to be involved in rabbit corneal epithelial wound healing \[46\]. Our data here illustrated further support a role for HA in corneal healing after the wound caused by the PRK procedure, because just by itself it can limit the changes in lumican, αSMA and Fn expression induced by TGFβ (Figure 2 and 4), and in association with the other compounds shows an even better conservative ability (Figure 2 B). Similar results are also obtained with SL (Figure 2 and 4), showing for the first time its ability to influence keratocyte transdifferentiation and their response to inflammatory events.

In conclusion, we have shown here the respective efficacy of vitamins A and E, HA and SL either alone or in association to contrast the shift in the expression of keratocyte transdifferentiation markers such as lumican, αSMA and Fn induced by the pro-inflammatory cytokine TGFβ, likely the main responsible of keratocyte transdifferentiation and corneal opacification after PRK. Morphological changes induced by TGFβ could also be prevented by the association of the 4 compounds (Figure 3). We are now investigating the molecular pathways involved in this shift, and how the different molecules here tested can interfere with them, and prevent the transdifferentiation and the corneal haze. Also, the efficacy of such formulation in a rabbit model system of corneal haze will be verified.

References


ARTICLE

Energy Level and Success of Internal Defibrillation for Shockable Rhythm during Cardiopulmonary Bypass in Cardiac Surgery: A Retrospective Study

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ABSTRACT

Internal defibrillation is commonly indicated for shockable rhythm following cross-clamp removal in cardiac surgery. Low energy decreases the success rate of defibrillation but high energy can cause myocardial damage. This study aimed to determine the success rate of internal defibrillation for shockable arrhythmias after cardiac surgery. Retrospective data of 1,424 patients who developed shockable rhythms (ventricular fibrillation or ventricular tachycardia), and required internal defibrillation after aortic cross-clamp removal during cardiac surgery, without deep hypothermic circulatory arrest technique, from August 2015 to July 2017, were reviewed.

The overall success rate of internal defibrillation in the first attempt of defibrillation was 61.5%. The success rate of the energy levels at 30, 10, and 7 Jules were 66.7, 64.9, and 61.5%, respectively. The success rate was higher in patients who had a better ejection fraction than those who failed after defibrillation. This was significantly associated with higher pH, higher bicarbonate, lower serum calcium, and lower total cardioplegic volume during cardiopulmonary bypass (CPB). Redo-valve surgery, valvular surgery, and combined coronary artery bypass graft with valvular surgery had a non-significantly lower success rate (p-value = 0.989). Incidence of failure for defibrillate patients in redo-valvular surgery, combined coronary artery bypass graft with valve surgery, adult congenital heart defect, and valvular surgery; requiring four or five shocks was non-significantly increased. Recurrent rate of ventricular fibrillation/ventricular tachycardia was 13.5%.

The success rate of internal defibrillation was not related to the dose of energy used after being weaned off CPB.

1. Introduction

During cardiac surgery, most patients require the cardiopulmonary bypass (CPB) system. For discontinuing CPB, at the end of the surgery, one of the important factors for weaning from CPB is cardiac rhythm. An organized, effective, and stable cardiac rhythm can occur spontaneously after removal of the aortic cross-clamp; however, in some cases cardiac conduction may resume electrical activity with ventricular fibrillation (VF) and/or ventricular tachycardia (VT). If VF occurs in a warm myocardium, it can increase cardiac wall tension, compromising endocardial perfusion,

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and allowing for possible subendocardial infarction.

Defibrillation is only indicated for shockable rhythms (VF, VT). Energy levels of internal defibrillation can start at 5-10 Jules \(^{[1-3]}\), and may be increased up to 50 Jules in some patients. Defibrillation is more effective when the heart is adequately rewarmed to a general body temperature (more than 30 degrees Celsius), with the success rate at approximately 56-64\% \(^{[3]}\). If ventricular fibrillation persists or recurs repeatedly after multiple, unsuccessful attempts of defibrillation, further management is to further warm the heart; so as to correct electrolyte abnormalities, and to begin antiarrhythmic drugs. Although, defibrillation is a life-saving procedure, the delivery of multiple high-energy shocks may be associated with myocardial damage and subsequent hemodynamic impairment \(^{[4]}\). Additionally, large shocks can damage cells and result in post-shock arrhythmias that may reinitiate fibrillation \(^{[1,4]}\).

The primary objective of this study was to determine the success rates of different energy levels of internal defibrillation for VF or VT after CPB. The secondary objective was to determine the factors affecting the success of internal defibrillation.

2. Material and Methods

2.1 Study Design

This study was approved by the Local Research and Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC. 60-360-08-1). By reviewing the retrospective data from the medical records, anesthetic records, and CPB records of cardiac surgical patients in Songklanagarind Hospital, from; February 2014 to July 2017.

2.2 Participants

All patients who developed VF or VT during weaning from CPB, with a body temperature above 30°C were included in the study (Figure 1). The exclusion criteria were patients aged under 18-year-old and/or used the deep hypothermic circulatory arrest (DHCA) technique during surgery. The selection of each energy level and types of antiarrhythmic agents for defibrillation were determined by the decision of the attendant anesthesiologist. Repeated attempts of defibrillations were needed for unsuccessful conversions, or for recurrent episodes of shockable rhythms.

2.3 Measures

Patient demographic data included: age, gender, body mass index (BMI), comorbidities, current medications, preoperative ejection fraction (EF), preoperative congestive heart failure, left ventricular hypertrophy, American Society of Anesthesiologists (ASA) classification, and type of surgery. The intraoperative cardiopulmonary bypass data included: the success rate and level of energy for defibrillation, and the number of internal defibrillations. The factors affecting the success of internal defibrillation; such as, arterial blood gas, electrolytes, body temperature, CPB and aortic cross-clamp time, use of inotropic/vasopressor agents, antiarrhythmic agents, type of operations and recurrent VF/VT, were recorded.

2.4 Sample Size

After reviewing the using of 7 Jules of energy level of internal defibrillation in patients who developed VT/VF during cardiac surgery in Songklanagarind Hospital, the success rate approximately was 60\%. It was then used for the sample size calculation with the accepted maximum error of 0.1, and significant level of 0.05. To cover the 10% dropout rate, the sample size finally was 104 patients.

2.5 Analysis

Statistical analysis was performed using the R program (version 3.14). Patient characteristics are described as the mean, and standard deviation for the normal distribution, as the median and interquartile range (IQR) for the non-normal distribution, or absolute and relative frequencies. The comparisons between the groups were performed with the unpaired t-test for normally distributed data, or with the Mann-Whitney U test for other quantitative data; and with the Chi-squared test or Fisher exact test for qualitative variables. Univariate regression and multivariate analysis were performed to assess the association between the success of energy level and the perioperative variables. Statistical significance was considered if p-value < 0.05.

3. Results

Among a total of 1,424 patients undergoing cardiac
surgery without DHCA technique, a hundred and four patients (7.3%) had VF/VT during weaning from CPB. The majority of patients were men (68.3%), with the mean age ± S.D. being 53.5 ±14.1 years old (range between 18-82 years of age); common current medications before surgery were diuretics (69.2%). The cardiac operations were mainly elective surgery (83.7%), and valvular heart surgery (61.5%). The types of surgeries were similar between the two groups. Baseline cardiac rhythm before surgery was normal sinus rhythm (67.3%), while pre-operative ejection fraction was 53.4 ±15%. Most patients (74%) had left ventricular hypertrophy (LVH); 34.6% of patients had a diagnosis of congestive heart failure and 16.3% patients received inotropic agents before surgery (Table 1). The pre-operative ejection fraction in patients who were successfully converted after shock (56.9 ±13.1%) was higher than those who failed after defibrillation (48.1 ±16.2%), p-value = 0.011).

From a total of 104 patients who had VF/VT, 64 patients (61.5%) were shocked successfully, while 40 patients (38.5%) were failed after the first attempt of defibrillation. The energy used for the first attempt of internal defibrillation started at 5, 7, 10, 20, and 30 Jules (J). Most of the energy levels were 10 J (57.8%), following by 20 J(23.4%), 10 J(12.5%), 5 J(3.1%) then 30 J(3.1%). The success rates of the first attempt defibrillation at 30, 10, and 7 J were 66.7, 64.9, and 61.6%, respectively (Table 2). The use of energy levels at 5 and 20 J had lower success rates (50.0 and 55.6%). With sub-

### Table 1. Demographic data of patients who received first attempt of internal defibrillation for VF/VT

<table>
<thead>
<tr>
<th>Preoperative factors</th>
<th>Total (N = 104)</th>
<th>Success (n = 64)</th>
<th>Fail (n = 40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median ±IQR</td>
<td>53.5 ±14.1*</td>
<td>58 (47.8,65.0)</td>
<td>54 (37.5,59.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>male</td>
<td>71 (68.3)</td>
<td>44 (68.8)</td>
<td>27 (67.5)</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>33 (31.7)</td>
<td>20 (31.2)</td>
<td>13 (32.5)</td>
<td></td>
</tr>
<tr>
<td>BMI, median ±IQR</td>
<td>22.7 ± 4.1*</td>
<td>22.9 (19.8,24.9)</td>
<td>22.9 (20.6,25.4)</td>
<td>0.51</td>
</tr>
<tr>
<td>Comorbid disease, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetes</td>
<td>12 (11.5)</td>
<td>6 (9.4)</td>
<td>6 (15.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>hypertension</td>
<td>30 (28.8)</td>
<td>20 (31.2)</td>
<td>10 (25.0)</td>
<td>0.64</td>
</tr>
<tr>
<td>dyslipidemia</td>
<td>10 (9.6)</td>
<td>7 (10.9)</td>
<td>3 (7.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>Current medication, n (%)</td>
<td>93 (89.4)</td>
<td>58 (90.6)</td>
<td>35 (87.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>ASA physical status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>82 (78.8)</td>
<td>53 (82.8)</td>
<td>29 (72.5)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18 (17.3)</td>
<td>9 (14.1)</td>
<td>9 (22.5)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4 (3.8)</td>
<td>2 (3.1)</td>
<td>2 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Type of surgery, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>Elective</td>
<td>87 (83.7)</td>
<td>55 (85.9)</td>
<td>32 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Emergency</td>
<td>17 (16.3)</td>
<td>9 (14.1)</td>
<td>8 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Preoperative EF, mean ±SD</td>
<td>53.4 ±15</td>
<td>56.9 ± 13.1</td>
<td>48.1 ± 16.2</td>
<td>0.01</td>
</tr>
<tr>
<td>ECG baseline, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Normal sinus</td>
<td>70 (67.3)</td>
<td>39 (60.9)</td>
<td>31 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>27 (26.0)</td>
<td>20 (31.2)</td>
<td>7 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Heart block</td>
<td>1 (1.0)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Bradycardia</td>
<td>6 (5.8)</td>
<td>4 (6.2)</td>
<td>2 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>77 (74.0)</td>
<td>44 (68.8)</td>
<td>33 (82.5)</td>
<td>0.18</td>
</tr>
<tr>
<td>Preoperative congestive heart failure, n (%)</td>
<td>36 (34.6)</td>
<td>18 (28.1)</td>
<td>18 (45)</td>
<td>0.12</td>
</tr>
<tr>
<td>Preoperative use inotropic drug, n (%)</td>
<td>17 (16.3)</td>
<td>9 (14.1)</td>
<td>8 (20.0)</td>
<td>0.60</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2 (1.9)</td>
<td>0 (0)</td>
<td>2 (5.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Dopamine</td>
<td>11 (10.6)</td>
<td>5 (7.8)</td>
<td>6 (15.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>5 (4.8)</td>
<td>4 (6.2)</td>
<td>1 (2.5)</td>
<td>0.65</td>
</tr>
<tr>
<td>Operations, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Coronary bypass graft (CABG)</td>
<td>11 (10.6)</td>
<td>7 (63.6)</td>
<td>4 (36.4)</td>
<td></td>
</tr>
<tr>
<td>Adult congenital heart diseases</td>
<td>3 (2.9)</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Valvular heart surgery</td>
<td>64 (61.5)</td>
<td>39 (60.9)</td>
<td>25 (39.1)</td>
<td></td>
</tr>
<tr>
<td>Combined CABG+ valve surgery</td>
<td>9 (8.7)</td>
<td>5 (55.6)</td>
<td>4 (44.4)</td>
<td></td>
</tr>
<tr>
<td>Redo-valvular surgery</td>
<td>4 (3.8)</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Thoracic aorta surgery without DHCA</td>
<td>8 (7.7)</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Other (pulmonary embolectomy, myxoma removal)</td>
<td>5 (4.8)</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
<td></td>
</tr>
</tbody>
</table>

*= mean ± S.D., ASA = American Society of Anesthesiologists, BMI = body mass index, DHCA = deep hypothermic circulatory arrest, ECG = electrocardiography, EF = ejection fraction, VF = ventricular fibrillation, VT = ventricular tachycardia
analysis from a total of 27 defibrillations of the energy level at 20 J, these occurred mainly in valvular surgery (59.3%). Failure of shock was largely found in coronary artery bypass graft (CABG) and combined CABG with valvular surgery (66.7%). However, the success rates were not significantly different in the first attempt of defibrillation among each energy level (5, 7, 10, 20, and 30 J) (p-value = 0.9).

After the first attempt of defibrillation, forty patients (38.5%) had failure to convert VF/VT into a normal sinus rhythm. These patients required a second defibrillation, with 55% having successful shocks; eighteen patients required a third attempt, with 50% of successful defibrillation. Additionally, nine patients required a fourth attempt, with 44.4% having successful defibrillation. Five patients required a fifth attempt, with 100% having successful defibrillation at the energy levels of 20, 30 and 50 Jules (Table 2). The energy levels in the second, third, and fourth attempt were significantly increased at higher levels than those in the earlier attempt (p-value < 0.001). The energy levels of the success in the third and the fourth attempts were higher than those in the failure of defibrillation; nevertheless, they were not significantly different (p-value 0.51), (Figure 2).

Thirteen patients (0.9%) had recurrent or a second episode of VF/VT, after successful normal rhythm conversion. From the first to the third attempt of defibrillation, for the second episode of VF/VT, the energy levels in the failed attempt were higher than those in successful defibrillation; however, they were not significantly different (Figure 3). The recurrent rates of shockable rhythms were 27.3, 25, 14.1, and 12.5 %; in the CABG, redo-valve, valvular surgery, and thoracic aortic surgeries, respectively. CABG and redo-valvular surgery had recurrent rates more than other surgeries. Third recurrent episodes of VT/VF occurred in six patients (0.4%). All of these required only one attempt of defibrillation at the energy levels of 5, 20, 30, and 50 Jules to successfully convert their rhythms (Table 2).

Table 2. The level of energy, success, and the attempts of internal defibrillation for the first and recurrent shockable rhythms

<table>
<thead>
<tr>
<th>VT/VF</th>
<th>Defibrillation attempt</th>
<th>success n(%)</th>
<th>Energy level (Jules)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>first</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>first</td>
<td>Yes</td>
<td>2(50.0)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>2(50.0)</td>
<td>5(38.5)</td>
</tr>
<tr>
<td></td>
<td>second</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>fourth</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>fifth</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>First (n=104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second (recurrent)</td>
<td>(n=13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>first</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>second</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>fourth</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Third (recurrent)</td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>one</td>
<td>yes</td>
<td>1(100)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

VF = ventricular fibrillation, VT = ventricular tachycardia
Figure 3. The energy levels for the success and failure of defibrillation in first and recurrent VF/VT

The anti-arrhythmic agents were required in 63.5% of the total patients who developed shockable rhythms. These were usually administered during multiple attempts of defibrillation, or for recurrent VT/VF. Lidocaine (43%) was most frequently used, and the least (25%) was amiodarone. Lidocaine combined with magnesium, amiodarone combined with magnesium, and amiodarone combined with lidocaine were given in 15.2, 6.1, and 3.0%, respectively: triple medications were administered in 16.7%. Lidocaine was commonly used in the dose range of 50-120 milligrams, with the dose range of magnesium being 1-2 grams, and amiodarone was administered in the dose of 150-300 milligrams.

During CPB, the patients received inotropic/vasopressor agents, which consisted mainly of epinephrine (68.3%). The CPB and aortic cross-clamp time were 146.2 ±58.6 and 105.8 ±46 minutes, respectively. Both of these were not significantly different between the successful group and the failed group. During CPB, the higher serum bicarbonate (22.4 & 21.7, p-value =0.009), lower serum calcium (1.08 ± 0.06 & 1.11 ± 0.07, p-value = 0.02), and lower total cardioplegic volume (2250 & 3050, p-value = 0.006) significantly increased with the success of the first attempt at defibrillation (Table 3).

Table 3. The cardiopulmonary bypass data of patients who received first attempt of internal defibrillation for their first VF/VT.

<table>
<thead>
<tr>
<th>Perioperative factors</th>
<th>The first attempt of defibrillation</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N = 104)</td>
<td>Success (n = 64)</td>
<td>Fail (n = 40)</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (mins), median ±IQR</td>
<td>146.2 ±58.6*</td>
<td>132 (106.2,166.2)</td>
<td>145.5 (113.2,182.5)</td>
</tr>
<tr>
<td>Aortic cross clamp time (mins), mean ±SD</td>
<td>105.8 ±46</td>
<td>103.4 ± 42.8</td>
<td>109.6 ± 51.2</td>
</tr>
<tr>
<td>Intraoperative inotropic drug, n (%)</td>
<td>103 (99.0)</td>
<td>63 (98.4)</td>
<td>40 (100.0)</td>
</tr>
<tr>
<td>- Epinephrine</td>
<td>71 (68.3)</td>
<td>42 (65.6)</td>
<td>29 (72.5)</td>
</tr>
<tr>
<td>- Norepinephrine</td>
<td>46 (44.2)</td>
<td>27 (42.2)</td>
<td>19 (47.5)</td>
</tr>
<tr>
<td>- Dopamine</td>
<td>18 (17.3)</td>
<td>11 (17.2)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>- Dobutamine</td>
<td>29 (27.9)</td>
<td>18 (28.1)</td>
<td>11 (27.5)</td>
</tr>
<tr>
<td>- Milrinone</td>
<td>16 (15.4)</td>
<td>7 (10.9)</td>
<td>9 (22.5)</td>
</tr>
<tr>
<td>Body temperature, n (%)</td>
<td>36.9 ±1.1*</td>
<td>37.2 (36.7,37.4)</td>
<td>37.3 (36.8,37.6)</td>
</tr>
<tr>
<td>Nasopharyngeal, median ±IQR</td>
<td>36.9 ±1.1*</td>
<td>37.2 (36.7,37.4)</td>
<td>37.3 (36.8,37.6)</td>
</tr>
<tr>
<td>Rectal, median ±IQR</td>
<td>35.8 ±1.3*</td>
<td>36 (35.1,36.4)</td>
<td>36.2 (35.6,36.8)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg), median ±IQR</td>
<td>52.2 ±13*</td>
<td>50.5 (44.62)</td>
<td>50.5 (40.56)</td>
</tr>
<tr>
<td>ABG, median ±IQR</td>
<td>7.4 ±0.1*</td>
<td>7.4 (7.4,7.4)</td>
<td>7.4 (7.3,7.4)</td>
</tr>
<tr>
<td>- pH</td>
<td>7.4 ±0.1*</td>
<td>7.4 (7.4,7.4)</td>
<td>7.4 (7.3,7.4)</td>
</tr>
<tr>
<td>- PaO2</td>
<td>267.8 ±81.2*</td>
<td>270.5 (83.7)</td>
<td>263.4 (77.9)</td>
</tr>
<tr>
<td>- PaCO2</td>
<td>37 ±3.9*</td>
<td>36.9 (4.1)</td>
<td>37.1 (3.5)</td>
</tr>
<tr>
<td>- HCO3-</td>
<td>22 ±2.2*</td>
<td>22.4 (21.2,23.7)</td>
<td>21.7 (20.4,22.6)</td>
</tr>
<tr>
<td>Electrolytes, median ±IQR</td>
<td>135.8 ±5.3*</td>
<td>135.1 (4.9)</td>
<td>136.8 (5.8)</td>
</tr>
<tr>
<td>- Sodium</td>
<td>135.8 ±5.3*</td>
<td>135.1 (4.9)</td>
<td>136.8 (5.8)</td>
</tr>
<tr>
<td>- Potassium</td>
<td>4.4 ±0.6*</td>
<td>4.4 (0.6)</td>
<td>4.3 (0.5)</td>
</tr>
<tr>
<td>- Chloride</td>
<td>106.3 ±4.3*</td>
<td>107 (104,109)</td>
<td>107 (103.8,110.2)</td>
</tr>
<tr>
<td>- Calcium</td>
<td>1.1 ±0.1*</td>
<td>1.1 (0.1)</td>
<td>1.1 (0.1)</td>
</tr>
<tr>
<td>Total cardioplegic volume (mL), median ±IQR</td>
<td>2810.6 ±1387*</td>
<td>2250 (2000,2850)</td>
<td>3050 (2075,4125)</td>
</tr>
<tr>
<td>Time from last cardioplegia to VF/VT (mins), median ±IQR</td>
<td>45.2 ±44.1*</td>
<td>25.5 (17.8,77.8)</td>
<td>24 (15.5,53.5)</td>
</tr>
</tbody>
</table>

*= mean ±S.D., ABG= arterial blood gas

DOI: https://doi.org/10.30564/jim.v10i2.3459
4. Discussion

The energy levels of the internal defibrillation for VF/VT, occurring during the weaning CPB, in this study varied from 5, 7, 10, 20, and 30 J; with a success rate of 50, 61.5, 64.9, 55.6, and 66.7%, respectively, for the first attempt of defibrillation. The success rates of our study showed nonlinear correlation with the level of energy used for defibrillations. Kerber et al, administered 5, 10, and 20 J of shocks, and reported the successes at 56% of 5 J shocks, 70% of 10 J shocks, and 80% of 20 J of shocks [5]. Carol et al, used 5, 7.5, 10, 20, 30 J shocks, and demonstrated that the success rates were 56% of 5 J, 59% of 7.5 J, 64% of 10 J, 85% of 20 J, and 100% of 30 J of shocks [4]. Although, our success rate at the high energy level of 20 J and 30 J were lower than in the previous study [2], the study by Carol et al, showed that an energy level above 2.5 J had a plateau defibrillation success rate of 50-60% [4], similar to our results. Schuder et al also reported a nonlinear correlation for transthoracic defibrillation shock with success [6]. An increasing level of the shock strength, above an optimal range, can decrease the success of defibrillation. Several studies have demonstrated that an increase in post-shock arrhythmias can be a possible cause of unsuccessful defibrillation [1,2]. High energy shocks might produce contractile abnormalities as well as post-shock arrhythmias, caused by ultrastructural damage in the mitochondria of the myocardium [2]. This might explain our lower success rates at 20 and 30 J shocks in the first attempt.

A sub-analysis of the success rate at 20 J, from a total of 27 shocks, which was not better than the lower energy, showed that it was mostly used in valvular surgery (59.3%). However, most of the failed shocks occurred in CABG, and combined valve with CABG surgery (66.7%). A possible etiology for the redevelopment of VF/VT would be a coronary air embolism, which is more likely during open-heart surgery than coronary artery or close heart surgery. Additionally, the presence of coronary artery disease also decreases the VF threshold; especially when a rapid heart rate is associated with coronary occlusion [2].

The incidences of failure of the fourth or fifth defibrillations in redo-valvular surgery, combined CABG with valve surgery, adult CHD, and valvular surgery were greater than in the patients with CABG, thoracic aortic surgery, and other surgeries. Especially, in redo-valvular and adult CHD surgeries, which were less likely to succeed from defibrillation. Carol et al, reported the difficulty to defibrillate patients with valvular heart disease as well [4]. Incidence of failure to defibrillate patients with valvular heart disease with the second or third shock was greater than in patients with coronary artery disease. The favorable effect of valve surgery on left ventricular loading conditions might reduce proarrhythmic stress and stretch as well as being associated with proarrhythmic risk. Coronary artery disease, structural heart disease, and left ventricular dysfunction were among the factors that predisposed malignant ventricular arrhythmia. Also, cardiac surgery exposes the patients with a substrate for ventricular arrhythmia to various arrhythmic triggers; such as, ischemia, reperfusion injury, hemodynamic changes, and electrolyte shifts that could lead to ventricular arrhythmia; especially acute ischemia or reperfusion injury [7].

Chapman et al, demonstrated that left ventricular hypertrophy (LVH) was significantly correlated with the defibrillation threshold [8]. However, Kerber et al, found that the LVH did not elevate defibrillation energy requirements [9]. From our results, the success rates insignificantly correlated with the LVH of the patients. However, ejection fraction (EF) can predict the success of the shock in the first attempt. This was lower in patients with failed shock (48.1%) than in the successful shock group (56.9%) (p-value = 0.011). So, higher EF was significantly associated with higher successful shocks in the first attempt.

Defibrillation during the states of acid-base imbalance has been influenced by the effect of derangements on the ventricular fibrillation threshold. In an animal study, acid-base abnormalities did not elevate defibrillation energy requirements; whereas, hypoxia reduced the energy needed to defibrillate [9]. Therefore, pH and blood gas alterations did not significantly affect the normal defibrillation threshold [10]. Higher bicarbonate levels significantly increased the success rate in the first attempt of shock in our clinical trial, but this different value was very scarce in the clinical setting.

Electrolytes, such as serum potassium, play an important role in the evoked potential generation for cardiac conduction. From the previous study, the success of defibrillation was associated with high serum potassium and coronary perfusion pressure at the first shock, and could correctly predict the outcome in 78% of the first shock [3]. Similar to our results, serum potassium and mean arterial pressure in successful shocks were higher than in the failed shocks, in the first attempt; but they were not significantly different.

VF or VT occasionally persists, and defibrillation needed to be repeated. In this case, the conventional strategy is to administer lidocaine, magnesium, and various anti-arrhythmic agents, and then repeat defibrillation. However, multiple repeated defibrillations are associated with increased risk of complications, such as, ischemia, reperfusion injury, hemodynamic changes, and electrolyte shifts that could lead to ventricular arrhythmia; especially acute ischemia or reperfusion injury [7].
not only increase the risk of myocardial damage and reduce cardiac function, but may also attenuate the fibrillation threshold \[^{11}\]. Lidocaine and magnesium are generally the common drugs of choice in many hospitals; this includes our institute.

There are some limitations in this study. First of all, it is a retrospective design. No uniform protocol of the energy level and antiarrhythmic drugs is applied for internal defibrillation within our hospital. Secondly, the blood gas determinations in the majority of the patients were not obtained at the exact time of developing VF/VT. Instead, they were before the occurrence of VF/VT, and after the therapy (defibrillation) in many cases. Finally, the numbers of patients for each energy level were too small; especially at 5, 7, and 30 J levels, when compared with the level at 10 and 20 J. Future prospective studies of internal defibrillation may resolve these uncertainties.

5. Conclusions

The success rate of internal defibrillation did not significantly correlate to the dose of energy at the levels from 7 to 30 J after weaning off cardiopulmonary bypass. However, it was significantly related to the preoperative ejection fraction, and relatedly converted to intraoperative acidosis, serum calcium, and cardioplegic volume.

References


ARTICLE
Chinese Prescription Kangen-karyu as Potential Anti-Alzheimer’s Disease Therapeutic: Analyses of BACE1 and GSK-3β Inhibitory Activities

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ABSTRACT
Inhibition of β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) or glycogen synthase kinase-3β (GSK-3β) is estimated to be the central therapeutic approach for Alzheimer’s disease (AD). In this study, water extract of Kangen-karyu, its crude drug and chemical composition used in oriental medicine were evaluated regarding their BACE1 and GSK-3β inhibitory activities. Fluorescence resonance energy transfer was used to characterize the BACE1 inhibitory effect of Kangen-karyu, its crude drug and chemical composition. GSK-3β activity was determined using the Kinase-Glo Luminescent Kinase Assay Platform. The water extract of Kangen-karyu inhibited BACE1 and GSK-3β in concentration-dependent manners when compared with reference drugs, quercetin and luteolin. Among six components of Kangen-karyu, the water extracts of Salviae Miltiorrhizae Radix or Cyperi Rhizoma exhibited significant inhibitory effects on BACE1 and GSK-3β. Among the constituents of Salviae Miltiorrhizae Radix extract, salvianolic acid C, salvianolic acid A, rosmarinic acid, and magnesium lithospermate B significantly inhibited BACE1. In addition, they inhibited GSK-3β with an IC50 value range of 6.97 to 135.35 μM. From these results, one of the effectiveness and its mechanisms of action of Kangen-karyu against AD may be the inhibition of BACE1 and GSK-3β, and one of the active ingredients of Kangen-karyu is Salviae Miltiorrhizae Radix and its constituents.

Keywords: Alzheimer’s disease  β-Site amyloid precursor protein-cleaving enzyme 1  Glycogen synthase kinase-3β  Kangen-karyu  Salviae Miltiorrhizae Radix  Salvianolic acid C  Salvianolic acid B

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1. Introduction

Alzheimer’s disease (AD) is the most prevalent neurodegenerative dementia with two major pathological features: extra- and intracellular amyloid plaques and intraneuronal neurofibrillary tangles formed of amyloid β-protein (Aβ) and phosphorylated tau protein, respectively\(^1\). Aβ toxicity is believed to play a major role in the pathogenesis of AD\(^2\). For that reason, anti-amyloid strategies have been a major focus of AD drug development.

Aβ is a cleavage product of amyloid precursor protein (APP) by two proteases: β-site APP cleaving enzyme 1 (BACE1) and the γ-secretase complex. APP is firstly cleaved by BACE1, producing secreted APP-β and a C-terminal fragment (CTF) known as β-CTF. β-CTF is subsequently cleaved by γ-secretase to release Aβ \(^3\). BACE1 is a rate-limiting enzyme for Aβ generation and is considered one of the major therapeutic targets for AD\(^4\). However, the increased production of Aβ peptides derived from APP via the sequential proteolytic cleavages catalyzed by BACE1, has a controversial link to the glycogen synthase kinase 3 (GSK-3) enzyme. In AD, GSK-3β is responsible for the phosphorylation of microtubule-related tau protein, which affects microtubule stability and delocalization of the abnormal tau protein to brain cells and dendrites\(^5\). Due to the aggregation of hyperphosphorylated tau proteins, neurofibrillary tangles form, and trigger synaptic dysfunction and neuronal apoptosis, which lead to cognitive impairment\(^6\). Inhibition of BACE1 or GSK-3β is considered as the central therapeutic approach against AD.

Traditional Chinese medicine has been widely used in China for thousands of years. Traditional Chinese medicine has now established its position in the Western world, and has become a prime source of drug discovery. Kangen-karyu, which consists of six medicinal herbs, has attracted considerable attention due to their biological activities and potential health benefit effects: Salviae Miltiorrhizae Radix, Cnidii Rhizoma, Paeoniae Radix, Carthami Flos, Aucklandiae Radix, and Cyperi Rhizoma (Table 1). Kangen-karyu is a traditional herbal formula slightly modified the composition of Chinese prescription (Guan-xin No.2) that has been used to treat blood circulation-related various symptoms, neuro-degenerative disorders, diabetes, and diabetic complications. The recent clinical trials have reported the potential clinical applications of using Kangen-karyu extract, such as for cognitive dysfunctions in type 2 diabetes symptoms trough improving central cholinergic dysfunction\(^7\), age-related memory deficit by normalizing neuroplasticity-associated neuronal signaling system, and the VEGF signaling system in the brain \(^8\) against oxidative stress mediated tissue injury \(^9\), type 1 and type 2 diabetes and diabetic complications \(^10,11\), a neuroprotective effect \(^12\). However, there are as yet no reports of BACE1 and GSK-3β, regarded as the central therapeutic approach against AD.

### Table 1. Composition of Kangen-karyu.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Botanical name</th>
<th>Family name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salviae Miltiorrhizee Radix</td>
<td><em>Salvia miltiorrhiza</em> BUNGE</td>
<td>Labiatae</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td><em>Cnidium officinale</em> MAKINO</td>
<td>Umbelliferae</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td><em>Paeonia lactiflora</em> PALLAS</td>
<td>Paeoniaceae</td>
</tr>
<tr>
<td>Carthami Flos</td>
<td><em>Carthamus tinctorius</em> L.</td>
<td>Compositae</td>
</tr>
<tr>
<td>Aucklandiae Radix</td>
<td><em>Aucklandia lappa</em> DCNE.</td>
<td>Compositae</td>
</tr>
<tr>
<td>Cyperi Rhizoma</td>
<td><em>Cyperus rotundus</em> L.</td>
<td>Cyperaceae</td>
</tr>
</tbody>
</table>

Therefore, we present the performance evaluation for the in vitro BACE1 and GSK-3β inhibition potential of water extract of Kangen-karyu, its crude drug and chemical composition.

2. BACE1 Inhibition

We performed a comparative study on BACE1 inhibition with a boiled water extract of Kangen-karyu and its components. As shown in Table 2, the boiled water extract of Kangen-karyu demonstrated moderate inhibition. Among the components, moderate inhibition was also observed with a boiled water extract of Salviae Miltiorrhizae Radix and Cyperi Rhizoma followed by mild inhibition by Cnidii Rhizoma, Paeoniae Radix, and Carthami Flos. No inhibition was observed with a boiled water extract of Aucklandiae Radix.

### Table 2. BACE1 inhibitory potentials of water extract of Kangen-karyu and its constituents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC(_{50}) values (\mu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kangen-karyu</td>
<td>77.40 ± 4.58</td>
</tr>
<tr>
<td>Salviae Miltiorrhizae Radix</td>
<td>89.84 ± 1.87</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td>147.09 ± 0.93</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td>143.57 ± 1.79</td>
</tr>
<tr>
<td>Carthami Flos</td>
<td>233.34 ± 0.05</td>
</tr>
<tr>
<td>Aucklandiae Radix</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Cyperi Rhizoma</td>
<td>91.16 ± 2.21</td>
</tr>
<tr>
<td>Quercetin(^*)</td>
<td>10.49 ± 0.28</td>
</tr>
</tbody>
</table>

\(\text{IC}_{50}\) values are expressed as the mean ± SED. \(^*\)Used as positive control.

Furthermore, we studied the compositions of Salviae Miltiorrhizae Radix (Figure 1). Among the six compounds tested, salvianolic acid C exhibited significant inhibition.
of BACE1 with an IC$_{50}$ value of 9.18 ± 0.03 μM, while IC$_{50}$ of the positive control quercetin was 10.49 ± 0.54 μM, as shown in Table 3. Likewise, salvianolic acid A exhibited similar maximum inhibition of BACE1 with marked IC$_{50}$ inhibitory activity, but salvianolic acid B and caffeic acid showed weak inhibitory activities against BACE1. Rosmarinic acid and magnesium lithospermate B displayed moderate to mild activity against BACE1.

Table 3. BACE1 inhibitory potentials of compounds identified in Salviae Miltiorrhizae Radix.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ values$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvianolic acid A</td>
<td>13.01 ± 0.32</td>
</tr>
<tr>
<td>Salvianolic acid B</td>
<td>168.90 ± 0.70</td>
</tr>
<tr>
<td>Salvianolic acid C</td>
<td>9.18 ± 0.03</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>29.77 ± 0.70</td>
</tr>
<tr>
<td>Magnesium lithospermate B</td>
<td>30.35 ± 2.67</td>
</tr>
</tbody>
</table>

$^a$The 50% inhibitory concentrations (IC$_{50}$, μM) are expressed as the mean ± SEM.

3. GSK-3β Inhibition

As shown in Table 4, the boiled water extract of Kangen-karyu potently suppressed GSK-3β with an IC$_{50}$ value of 17.05 ± 1.14 μg/mL. All components of the boiled water extract of Kangen-karyu’s individual components showed potent suppression against GSK-3β with IC$_{50}$ values ranging from 7.77 to 93.61 μg/mL. The extract of Salviae Miltiorrhizae Radix (IC$_{50}$: 7.77 ± 1.38 μg/mL) was the most potent among them, followed by Cyperi Rhizoma (IC$_{50}$: 20.68 ± 2.50 μg/mL). The extracts of other herbal components showed weak and moderate inhibitory activity in GSK-3β assays.

Table 4. GSK-3β inhibitory potentials of water extract of Kangen-karyu and its constituents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ values$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kangen-karyu</td>
<td>17.05 ± 1.14</td>
</tr>
<tr>
<td>Salviae Miltiorrhizae Radix</td>
<td>7.77 ± 1.38</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td>66.74 ± 2.05</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td>62.51 ± 1.89</td>
</tr>
<tr>
<td>Carthami Flos</td>
<td>93.61 ± 3.99</td>
</tr>
<tr>
<td>Aucklandiae Radix</td>
<td>85.04 ± 6.32</td>
</tr>
<tr>
<td>Cyperi Rhizoma</td>
<td>20.68 ± 2.50</td>
</tr>
<tr>
<td>Luteolin$^b$</td>
<td>2.18 ± 0.13$^*$</td>
</tr>
</tbody>
</table>

$^a$The 50% inhibitory concentrations (IC$_{50}$, μg/mL) are expressed as the mean ± SED. $^b$Used as positive control.

Next, we evaluated the constituent compounds of Salviae Miltiorrhizae Radix which showed excellent GSK inhibitory effect. As listed in Table 5, magnesium lithospermate B, salvianolic acid A, salvianolic acid B, and salvianolic acid C displayed strong inhibition against GSK-3β. Especially, salvianolic acid B was the most effective, inhibiting the enzyme by 50% at 6.97 ± 0.96 μM. Magnesium lithospermate B, salvianolic acid
A, and salvianolic acid C showed roughly one-fifth the activity of salvianolic acid B with similar IC₅₀ values of approximately 30 μM. On the other hand, rosmarinic acid (IC₅₀: 135.35 ± 4.69 μM) and caffeic acid (IC₅₀: 425.01 ± 7.61 μM) showed moderate or mild inhibitory activity in GSK-3β assays.

**Table 5.** GSK-3β inhibitory potentials of compounds identified in Salviae Miltiorrhizae Radix.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvianolic acid A</td>
<td>30.21 ± 3.14</td>
</tr>
<tr>
<td>Salvianolic acid B</td>
<td>6.97 ± 0.96</td>
</tr>
<tr>
<td>Salvianolic acid C</td>
<td>31.82 ± 2.08</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>425.01 ± 7.61</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>135.35 ± 4.69</td>
</tr>
<tr>
<td>Magnesium lithospermate B</td>
<td>33.07 ± 3.88</td>
</tr>
<tr>
<td>Luteolin†</td>
<td>2.18 ± 0.13</td>
</tr>
</tbody>
</table>

*The 50% inhibitory concentrations (IC₅₀, μM) are expressed as the mean ± SEM. †Used as positive control.

4. Discussion

Under pathological patterns, the permitted ongoing treatment approaches for AD are acetylcholinesterase inhibitors (AChEIs: donepezil, galanthamine, and rivastigmine, all enhancing acetylcholine levels) and the non-competitive/low affinity N-methyl-D-aspartate receptor antagonist (NMDA antagonist: memantine, activating glutamate neurotransmission) [13]. However, these types of drugs only provide the effect of delaying or relieving symptoms. Since AChEIs (cholinergic) and memantine (glutamaticergic) target two different aspects of AD pathology, combination treatment is beneficial effective in patients with moderate to severe AD, by comparison with the monotherapy [14]. Furthermore, discovery of different targets that are decisive importance in promoting AD pathogenesis is the current main focus. Currently, it has been hypothesized that BACE1 and GSK-3β contribute distinctly to suppressing the development of AD as a linking bridge between Aβ and tau protein.

A number of recent studies demonstrated a correlation between AD and oxidative stress [15-17]. Specifically, AD may be associated with cellular oxidative stress including augmentation of protein oxidation, protein nitration, glycoloxidation, and lipid peroxidation as well as the accumulation of Aβ [15,17,18]. In general, oxidative stress involves the production of superoxide (O₂⁻), and the formation of nitrotyrosine and peroxynitrite (ONOO⁻) derived from nitric oxide (NO), all of which are destructive free radical oxidants [19]. Therefore, drugs that are effective against all destructive free radical oxidants may be important therapies for AD [20-23]. Simultaneous inhibition of nitrotyrosine and ONOO⁻ along with BACE1 and GSK-3β may represent a promising avenue of research for the development of anti-AD agents.

In a previous study, we reported that Kangen-karyu inhibited reactive oxygen species (ROS) production in the presence of high glucose-induced oxidative stress using LLC-PK₁ cells, renal tubular cells, which are the most vulnerable renal tissue to oxidative stress. The intracellular ROS (O₂⁻, NO, and ONOO⁻) induced by high glucose was concentration-dependently inhibited by Kangen-karyu treatment. Kangen-karyu also reduced the overexpression of inducible nitric oxide synthase, cyclooxygenase-2 proteins induced by high glucose. Furthermore, treatment with Kangen-karyu inhibited the nuclear translocation of nuclear factor-kappa B [24]. Moreover, Kangen-karyu had a pleiotropic effect on several oxidative stress-related parameters and exerted a renoprotective effect on the development of diabetic nephropathy in type 2 diabetic db/db mice [11]. These findings indicate that Kangen-karyu is a potential therapeutic agent that will reduce the damage caused by hyperglycemia-induced oxidative stress associated with diabetes. In addition, Kangen-karyu might prevent AD by attenuating the increased oxidative biomarkers, including the generation of ROS.

In the present study, we investigated the anti-AD potential of Kangen-karyu and its components using BACE1 and GSK-3β inhibitory assays. Based on the results shown in Tables 2 and 4, the water extract of Kangen-karyu shows inhibitory potential against BACE1, as well as GSK-3β. Among the six components, Salviae Miltiorrhizae Radix and Cyperi Rhizoma showed moderate inhibition of BACE1, while Salviae Miltiorrhizae Radix exhibited stronger inhibitory activity. In contrast, Cnidii Rhizoma, Paeoniae Radix, Carthami Flos, and Aucklandiae Radix showed weak inhibitory activity against BACE1 and GSK-3β. Focusing on GSK-3β inhibition, Salviae Miltiorrhizae Radix was about 2.7 times more active than Cyperi Rhizoma and, thus, was selected for further study. Therefore, we estimated the potentials of those chemical composition from the active water extract of Salviae Miltiorrhizae Radix.

Phytochemical constituents of Salviae Miltiorrhizae Radix have been extensively studied. Water-soluble fraction of Salviae Miltiorrhizae Radix contains caffeic acid and its derivatives such as rosmarinic acid (dimer), salvianolic acid A (trimer), salvianolic acid B (tetramer), salvianolic acid C (trimer), and magnesium lithospermate B (tetramer). Chief of all, salvianolic acids are the principal water-soluble ingredients in Salviae Miltiorrhizae Radix, among which salvianolic acid A...
(caffeic acid trimer) and salvianolic acid B (caffeic acid tetramer) are the most abundant phytochemicals. A review of the BACE1 and GSK-3β inhibitory activities of our evaluated compounds showed that a monomeric caffeic acid showed weak activity while its dimer, trimer, and tetramer displayed a significant increase in activity. Especially, it exhibited remarkable activity in trimers and tetrathers rather than dimers. This pattern means that the activity is enhanced when caffeic acid is condensed. Simultaneous inhibition of both BACE1 and GSK-3β may provide efficient benefits in the treatment of AD.

In the present study, we evaluated the anti-AD activity of Kangen-karyu and its invididual ingredients via BACE1 and GSK-3β assays. The results demonstrated that Salviae Miltiorrhizae Radix is the main active component of Kangen-karyu against the two enzymes. Furthermore, rosmarinic acid derivatives were found to be marked inhibitors. Among them, salvianolic acid C was a potent mixed inhibitor of BACE1 and showed the lowest IC₅₀ value (9.18 ± 0.03 μM), while salvianolic acid B exhibited the highest inhibitory activity against GSK-3β with an IC₅₀ value of 6.97 ± 0.96 μM. Therefore, one of the mechanisms of action of Kangen-karyu against AD may be the inhibition of BACE1 and GSK-3β, and one of the active components of Kangen-karyu is Salviae Miltiorrhiza Radix and its constituents, salvianolic acids C and B.

5. Conclusions

Alzheimer’s disease drugs generally developed as a single target strategy are not only unsatisfactory for AD treatment, but also have several side effects. Therefore, as a potential effective strategy in the treatment of AD, the choice of the multi-target strategy has been proposed. Therefore, Kangen-karyu and its herbal formula (Salviae Miltiorrhizae Radix) containing caffeic acid derivatives with both BACE1 and GSK-3β inhibitory activities could be a promising herbal medicine for the treatment drug of cognition deficit disorders, such as AD-type dementia.

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Conflict of Interest Statement

The authors declare no conflict of interest.

References


ARTICLE

Vitamin D in Food Supplements: Labeling Survey

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ABSTRACT

An adequate vitamin D (vitD) intake (Recommended Daily Allowance, RDA= 5µg) is crucial for health maintenance and its deficiency is associated with several health problems. The increase in hypovitaminosis D cases and the proliferation of food supplements (FS) that are easily accessible by the population, have led to an unrestrained chronic consumption of FS. VitD may accumulate in the body and originate toxicity (Tolerable Upper Limit, UL=100 µg). The aim of this study was to evaluate if daily vitD doses mentioned in FS labels are in conformity with RDA. 210 solid and liquid FS (for pediatrics and adults) sold in Portuguese pharmacies, supermarkets, health shops and on the internet were examined for indicated daily intake of vitD and compared to RDA and UL values. 51.43% of FS have values higher than RDA, 8.57% higher than UL. The average vitD daily dose in FS is 24.48 µg, with a high variability between samples (0.25 - 250 µg). Majority of FS labels recommend vitD daily doses above RDA and some even above UL, regardless of being for adults or children. Therefore, it is crucial that vitD dose in FS is reviewed to ensure the safety of these products.

1. Introduction

Vitamin D (vitD) is a hormone crucial for the regulation of physiological processes, namely related to bone metabolism, immune system, cardiovascular and insulin synthesis, and has been associated with several pathologies [1-3]. It is a fat-soluble vitamin and is obtained endogenously, mainly from sun exposure where ultraviolet rays hit the skin and initiate vit D synthesis. It is also present in very few foods (yeast, fungi, cod liver oil and oily fish) and available in many food supplements (FS) as well as in fortified foods, such as dairy products.

VitD has two isomers (vitD2 or ergocalciferol and vitD3 or cholecalciferol). VitD itself is biologically inert and must undergo two hydroxylations for activation: in the liver (producing calcidiol or 25-hydroxyvitamin D [25(OH)D = 25(OH)D2 and 25(OH)D3]) and in the kidney (forming calcitriol or 1α, 25-dihydroxyvitamin D [1α, 25(OH)2D2 and 1α, 25(OH)2D3]) [4].

Homeostasis requires a daily plasma concentration of vitD. Human body levels are estimated mainly by the measurement of its major circulating form: 25(OH)D, which is considered an adequate indicator of the level of vitD in individuals [1]. Optimal vitD status is an important health issue and it is generally agreed that plasma or

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serum levels of 25(OH)D should be used to assess vitD status, as they reflect both dietary intake and dermal production. According to the U.S Institute of Medicine (IOM) and the UK National Osteoporosis Society (NOS) [4], 25(OH)D concentrations ≤30 nmol/L are considered deficient and concentrations between 30-50 nmol/L are insufficient. The scientific community consensually assumes that serum 25(OH)D concentrations below 25-30 nmol/L should be prevented and treated. Additionally, values higher or equal to 50 nmol/L are included in several guidelines as an optimal concentration [7,8].

Regarding the consumption of vitamins and other nutrients, there are a number of terms that are used as such: the Recommended Dietary Allowance (RDA) which is the average daily intake, sufficient to meet the nutritional needs of the majority of the healthy population; Adequate Intake (AI), parameter that indicates the value corresponding to adequate nutritional intake, and is only used when there is insufficient scientific evidence to determine RDA, particularly in newborns and infants; and Tolerable Upper Intake Level (UL) that refers to the maximum recommended daily intake that does not cause adverse health effects and can be safely ingested without generating toxicity in the majority of the population [9,10].

In 2010, daily doses of 200 IU were considered by the Institute of Medicine, insufficient to maintain a desirable 25(OH)D level. Hence, doses of 400 IU/ day (10 µg) for infants, 600 IU/ day (15 µg) for children, teenagers and adults, and 800 IU/ day (20 µg) for the elderly (over 70 years) were suggested [19]. Although there are different recommendations for some subgroups of population, in most European countries, including Portugal, the RDA is set at 5 µg for all [11,12].

The dietary reference values (DRV) for vitD have been revised by the European Food Safety Authority (EFSA), based on an assessment carried out between 2013 and 2016. The panel derived as Adequate Intakes (AI) for the EU population, assuming minimal sunshine exposure: infants aged 7-11 months: 10 µg/day and 15 µg/day for all groups aged one year and more (including pregnant/lactating women). In the presence of cutaneous vitD synthesis, the values of intake should be lower or even zero [13].

EFSA also revised the UL for vitD, setting it at 100 µg/day for adults, pregnant women and children over 11 years old. UL values of 50 µg/day and 25 µg/day were proposed, respectively for 1-10 years old group and infants [14].

Considering the threshold values of 25(OH)D concentrations described above, there is a high worldwide prevalence of vitD deficiency, whose negative impact on public health requires public health measures such as fortification of foods with vitD [7].

VitD deficiency is common in Middle East countries, reaching up to 80% of the population. In European countries the values are lower with a prevalence of approximately 20% in Northern Europe and 30-60% in Western, Southern and Eastern Europe, but, more than 10% of Europeans reveal a severe deficiency (serum 25(OH)D <30 nmol/L) [7].

Several serious pathologies seem to be associated with VitD deficiency, such as multiple sclerosis, diabetes, heart disease, cancer, rickets [15-17].

Due to the growing awareness of vitD deficiency and associated health problems, it became a popular food supplement with a significant increase in the consumption of vitD-fortified food products, drugs and nutraceuticals. Given the high popularity of FS and the easy access to them by the general population without medical supervision, together with the growing number of prescriptions of vitD, including very high doses, there might be a greater risk of vitD intoxication, with or without hypervitaminosis [2,16].

Usually, overproduction of vitD following sun exposure, and ultimate toxicity is not likely to happen because it is a process regulated by a feedback loop, leading to photodegradation of the excess vitD produced [18,19]. On the other hand, exogenous forms of vitD may contribute to intoxication, when excessive amounts are consumed for a long time. The concern is greater for vitD3 than for vitD2 due to the higher bioavailability of the former [20,21]. Moreover, different formulations of vitD FS, originate significantly different plasma levels of 25(OH)D [22].

Although vitD toxicity is considered to be rare, the consequences on health can be serious. The initial symptoms include confusion, weakness, fatigue, headache, appetite loss, nausea, vomiting, abdominal pain, polyuria, polydipsia, cardiovascular symptoms, among others [2,23]. Its clinical manifestations include severe hypercalcemia, hypercalciuria and hyperphosphatemia [2,19,24,25].

Although the toxicity of vitD is rarely appreciated, the medical community and health regulators are aware of the fact that it is one of the most toxic fat-soluble vitamins, hence the growing concern about increasing its consumption [7].

The policies and practices regarding voluntary fortification and legislation vary among European countries. The list of vitamin and minerals and their forms that can be added to FS is defined by The Commission Regulation (EC) No 1170/2009 of 30 November 2009 [25]. Commission Directive 2008/100/EC defines the labeling
of foodstuffs with respect to the recommended daily allowance (RDA) [11]. This recommendation assumes that 200 IU (5 g) vitD/day is sufficient to prevent rickets [26]. However, it ignores the physiological benefits of vitD. Currently, in Europe, most multivitamin preparations are labeled as “100% RDA” corresponding to 200 IU of cholecalciferol.

FS are not meant for therapeutic purposes and should be used as a complement of a regular dietary intake of vitD and sunlight exposure, in order to achieve the RDA values. Hence, the goal of our work was to evaluate the information present on the label of vitD FS available on the Portuguese market, in terms of dosage and recommended daily allowance of vitD.

2. Materials and Methods

The daily intake of vitD described on 210 FS labels was analyzed and compared with the RDA. FS were available in Portuguese pharmacies, health shops, supermarkets and internet. Liquid and solid pharmaceutical forms whose label indicates the presence of vitD in their composition (in addition to other ingredients) were considered in the study, regardless of the use of the FS.

For confidentiality issues, the commercial name of the products under study has been omitted and the designation FS has been adopted. Solid FS (SFS) included adult formulations, while liquid FS (LFS) included both pediatric and adult formulations.

A statistical analysis was carried out using Excel and SPSS Statistics (Statistical Package for Social Sciences) version 25.0 software for Windows.

3. Results

The 210 analyzed FS labels presented a vitD mean daily dose of 24.48 µg, with a high variability between samples (coefficient of variation = 178.19%), with a minimum value of 0.25 and a maximum of 250 µg/day (Table 1).

Table 1. VitD label daily dose values

<table>
<thead>
<tr>
<th>Formulation</th>
<th>N</th>
<th>VitD Label dose a (µg/day)</th>
<th>Mean ± SD b (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FS</td>
<td>210</td>
<td>10.00 (0.25; 250.00)</td>
<td>24.48 ± 43.62</td>
</tr>
<tr>
<td>SFS</td>
<td>145</td>
<td>10.00 (1.20; 250.00)</td>
<td>24.56 ± 46.41</td>
</tr>
<tr>
<td>LFS</td>
<td>65</td>
<td>10.00 (0.25; 175.00)</td>
<td>24.31 ± 36.97</td>
</tr>
</tbody>
</table>

a VitD label dose, expressed as medians, with minimum and maximum values given in brackets; b SD-Standard Deviation

Analyzing the solid and liquid formulations separately, both presented similar values. To perform the comparison of vitD daily dose between the SFS and LFS, the non-parametric test of Mann Whitney was applied, since vitD data have a non-normal distribution (p<0.05) in these groups. Following the application of the Mann Whitney test, no significant difference (p>0.05) was found between vitD daily dose in SFS and LFS.

Comparing recommended vitD daily doses indicated on the labels with the RDA established for vitD in Europe (5 µg/day), it was found that the majority of FS, solids and liquids, have values far above RDA, including 12 SFS and 6 LFS samples equal or higher than UL (Graph 1).

Graph 1. VitD label daily doses in solid and liquid FS

SFS with the highest vitD label value are those containing this vitamin as the sole ingredient. In multivitamin preparations, the daily dose of vitD reaches a maximum of 30 µg/day (FS_051). On the other hand, in LFS the highest levels were observed in multivitamins, with values that reach 175 µg/day (LFS_037). In LFS containing only vitD, the maximum value mentioned in the label is 125 µg/day (LFS_040).

Since LFS included pediatric (LFS_P; N=23) and adult (LFS_A; N=42) formulations, a comparison was also made between both sample groups using the Mann Whitney test. LFS_P presented a mean value of 25.05 ± 30.67 µg/day (minimum= 0.25 µg/day, maximum= 100 µg/day); LFS_A presented a mean value of 23.90 ± 40.35 µg/day (minimum= 0.5 µg/day, maximum= 175 µg/day). The test result did not show any significant difference between these groups (p>0.05). The majority of LFS_P and LFS_A labels mentioned values far above RDA, with two pediatric and four adult formulations equal or higher than UL, considering UL=100 µg/day in both groups (Graph 2). The number of LFS_P samples that exceeds UL rises to seven if the EFSA proposed UL value for children is taken into account.
4. Discussion

In Europe, the established daily dose for vitD is 5 µg (RDA = 100%), which includes all sources of vitD (from diet to sun exposure and supplementation). However, there are FS that indicate values 50 times higher, which corresponds to 5000% of RDA and 250% of UL.

Since vitD is fat-soluble, it tends to be distributed in the fat compartment such as adipose tissue resulting in a reduced clearance and accumulation in the body. Thus, regular intake of high doses should be monitored to prevent adverse effects such as kidney and heart disease and musculoskeletal pain. In addition, ingestion of excessive amounts of this vitamin can lead to elevated plasma and urine calcium levels, probably related to the excessive amount of 25(OH)D, not followed by its conversion to 1,25(OH)2D. In fact, plasma concentrations of 25(OH)D above 220 nmol/L can cause hypercalcemia leading to soft tissue calcification and ultimately damaging the heart and kidneys [27]. Babies are particularly sensitive to vitD overdoses due to high bone turnover. A study case was reported of a 4-month-old girl who received daily 50,000 IU of vitD3 in liquid oral supplements, for two months, and suffered severe hypercalcemia, hypercalciuria and nephrocalcinosis [28]. Recently, a cross-sectional study on vitD toxicity was conducted in a pediatric toxicological referral center from Iran, on children younger than 12 with a daily ingestion over 1500 IU of vitD [29]. The acute vitD toxicity in the pediatric population in Iran was found to be benign and probably related to the high prevalence of vitD deficiency in that country. A literature review on the risk of vitD toxicity in pediatrics concluded that although rare, cases of vitD intoxication causing dramatic life-threatening symptoms still occur in children [30].

Nevertheless, in our study, pediatric formulations had daily dose recommendations as high as those for adults, and in both cases, sometimes higher or equal than the UL of 100 µg/day.

5. Conclusions

FS are readily available and are poorly regulated. These conditions contribute to their overuse which, in the case of vitD, can trigger hypercalcemia. It is not understandable that FS labels suggest daily vitD doses higher that the RDA, especially considering that FS are not meant to treat hypovitaminosis and should serve only as a complement of a normal dietary regimen. To safeguard consumer safety, it is essential to have adequate and strict FS labelling legislation, in particular by ensuring compliance with the RDA values.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

References


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The body is observed to function optimally in life in some individuals while others have various problems. In the complexity involved, this paper describes saliently the mechanisms for biological robustness from birth and subsequent neuro-vascular and core matching patterns well-coordinated till adulthood. These mechanisms the individual develops and maintains his core to keep vitality against environmental perturbations and they can be dysfunctional. The three related dimensions of the fascial organization, the co-directed nervous and perfusional elements in the body are emphasized. Re-understanding of these mechanisms in the body-map can be useful to revise the basis for re-defining our therapies.

Keywords: Snug and fit Chinese medicine Core vs Match Neurovascular coupling Qi deficiency

1. The Formation before Birth

Embryogenetic and organogenetic mechanisms of development, highly conserved among species at tissue, cellular, and molecular levels \cite{1,2}, unfailingly prepare for a sustainable body at birth. The body later develops in momentum while well preserving its innate characteristics to overcome living demands.

After conception, the human body at cellular level develop from proliferation, differentiation, acquisition of polarity and cell movement. At tissue level, patterned cell differentiation collectively migrate and form the complex structures of adult organs. Cell migration is essential \cite{3}. Final morphogenesis and integral organization is achieved through physical and chemical cues in the microenvironment \cite{4-6} unto processes highly controlled and coordinated in time and in space.

Concurrently, the neural, vascular and interconnective matrix spread out the contextual assembly over the body as three essential dimensions together. Nervous elements and blood vessels in an entanglement relationship develop in parallel \cite{7}. Following similar differential cues from the same mesenchymal connective tissue environment, they ramify in progressive branching network to reach together every part. While secreted signals mediate information propagation, the coupled neural and perfusional elements embody the ramifying sophistication of body elements in formal order. Mechanics also mediate information propagation between cells \cite{8,9} and biophysical attributes of the associated mesenchymal matrix contribute to instructive sculpting of the embryo topology \cite{10}. The tightly regulated organization gives rise to functional tissues and operational organs that match the physiological needs of the organism.
2. The Body Prepared at Birth

2.1 Biological Robustness with Adaptivity

For primal development, the body at birth is equipped through endowed programs robust enough for species survival and also through varying degrees of adaptations to its environment as windows open during pregnancy. In due course adapting life challenges and environmental demands, the body and whole-body functions would deviate significantly.

Biological robustness\[11\] sustains the individual up the years ahead. Short of robustness, there need be continual good performance recruited to keep the individual well through life over many uncertainties and constraints. Robust living systems have mechanisms doubly assured to make sure that the outcomes of biological processes are stereotypical even when environments vary or perturbations arise\[22\].

Thus formed at birth, most are endowed as being alike, as being made-up through evolutionary conserved genetic networks that pattern cellular changes and shape the organs, and also through cellular-physical mechanisms related to shared physical organization rather than evolutionary descent\[13\]. While the physical and chemical cues coordinate morphogenesis and patterning\[14\], it is the topology of the fascial, circulatory and neurohumoral networks that build up biological robustness of the final contextual assembly of the body. Thereon, flexibly developing nervous directives and perfusion elements together adapt to new changes in the integral unit\[7\].

2.2 Nervous Directives and Perfusion Co-play to Live

Nerves and vasculature are patterned in similitude and closely alongside throughout development and up to adulthood. Since embryogenesis, growth cones for neurons and for vasculature, sharing receptor expression\[15\] and other features\[16\], both project onwards by extending filopodia, dissolving confronted obstructions by secreting proteases\[17,18\]. Following similar differential cues from the connective tissue, they go in parallel. In the embryo, pericytes as multi-functional mural cells generate early microvascular structures before recruiting endothelial cells to line vascular vessels\[19\]. Pericytes wrap around the endothelial cells in every vascularized tissue in the body. The embryonic neural tube provides positive blood vessel patterning signals\[20\]. It directly steers formation of both autonomic nerves, and smooth muscle cells and pericytes over the large thoracic arteries and forebrain vessels\[21,22\]. During development, certain arteries become innervated and form a pressurized circulation to allow proper control of the distribution of flow to vital organs\[23\]. In the periphery, nerve-artery congruence is established through nerve-derived signals that direct arterial differentiation and regulate patterns of angiogenic remodeling\[24\]. In the developing brain, pericytes appear early during vascular development\[25\], and neural tube-derived signals regulate sprouting capillaries to induce formation of blood-brain barrier characteristics in the neural tube vasculature.

In the adult, endothelial cells still show significant growth potential, even for regenerative or pathological processes\[26,27\]. Neurogenesis and angiogenesis are closely intertwined, with endothelial cells in vascular niches releasing cues for neural stem cells\[28,29\]. The close association between nerves and blood vessels as neurovascular bundles in the periphery and as neurovascular unit in the brain\[30\] as well as the correlation between neuronal and vascular cells\[31,32\] emphasize their symbiotic relationship. Information and perfusional resources go together to suit spatial and temporal body needs.

With body assets and with resources carried along nervous and perfusional elements, all kinds of activities spring forth. During activity, blood flow needs to reach the local tissues at the right time and place and in the right amount. The developmental, structural, and functional interdependence between neural and vascular elements is closely related in health and disease\[33\]. The fascial connective system where circulatory and neurohumoral elements are woven together (Figure 1), is organized as a network with paths for the transportation of energy signals including Meridians\[14\].

The three related dimensions of the fascial organization, the co-directed nervous and perfusional elements are adaptive assets for the body with operational organs and self-vitality subsystems. Their incorporation in the body both spatially and temporally prepare the core adapting to the variable demands of the surrounding while having controlled stability to ensure proper function.

3. Living Strong with Adaptive Body and Assets

Despite phenotypic variations, the human body core basically functions and sustains itself through cellular processes, operational organ systems, and self-vitality subsystems for survival in the environment\[39\].

3.1 The Hardcore

The body's rigid framework is provided by the skeletal system including cartilage, tendons, and ligaments. The whole musculoskeletal system is anchored together with the integumentary system to enable the body to
move about as these support and are supported by the body operational organ systems - cardiovascular lymphatic system, respiratory system, digestive system, nervous system, endocrine system, urinary system, and reproductive systems. The fascia sheets underneath would encase, separate and stabilize various parts.

As the body develops into the proper being, these organ systems are installed early. When early life biological robustness declines, the individual would sustain his body through well-patterned dynamics. Interestingly, an integral person may still have wholistic gaps yet not filled but the person remains positive as a whole [7]. To be snug and fit, the whole body builds up from organ complexes as self-vitality systems complementarily [35].

Well-patterned dynamics with self-regulatory core-vs-match and remodeling mechanisms to suit the environment would produce snug resource provisions to accommodate adaptation in the capacity range of the individual.

3.2 Being Snug to Fit

Successful survival in the real world counts on coping well with fluctuating levels of both internal and environmental perturbations. Rings after rings of development with well-patterned dynamics (Figure 2) would form patterned core responses to maintain the person as a whole for a stabilizing core, yet having flexible physiological and behavioral responses to context-appropriate behaviors. In fact, even since birth, the body is equipped with processes that the body core can continually match its environment without losing its formation and strength.

**Figure 1. Three body dimensions essential for adaptation**

**Figure 2. Well-patterned dynamics**

- Core and Match
  - Righting abilities, “rightness”
- Remodelling
  - Contingent poise
- Individual body congruence in body layers functional-anatomically
- Self-regulatory integrity maintenance mechanisms restituted for gaps reduced,

The more reserve for homeostatic stability, the smoother the facilitation over fundamentally ever-changing processes.

- Resources provision over fluctuating levels of variables for consistency (acting body state is in positive snug)
  - In the basal homeostatic range, variables and changes are accommodated in the ‘normal’ homeostatic range of the body
  - Over this range, the body capacity with resourceful adaptivity provides for certain resilience when change would not affect the core momentum.
  - Regulatory heterostasis, allostatic, hormesis and adaptive homeostasis are the reactive or anticipatory body-mind mechanisms in case. Positive accommodation allowed until adaptive mechanisms yield beyond capacity range.
3.2.1 Core and match Processes

With core-vs-match mechanisms, deviations tend to be corrected by self-regulating mechanisms [36].

A. Righting behaviors: setting the body in its own right position keeping least deviations from its optima.

Self-regulatory integrality maintenance mechanisms with evolutionary survival value [37-39] start with the innate motor setup, from righting reflexes, positive support reflex, symmetrical and symmetrical tonic neck reflex, Galant reflex, the Babkin reflex, the parachute, and the palmar grasp reflex, all preparatory responses born to match immediate environmental changes and demands. Following the first four rightly responses, an individual is subsequently consolidated in a poise as a stabilized stance whereby dynamics for all sorts of movements, contingent for common recurrent needs, can be tuned with ready autonomy on top of the poise to meet new situational needs. Following the latter four matching responses, the primitive rooting reflex and the visual grasp reflex facilitate development of the motor-visual coordinative setup, important for being optimized automatically to grasp the surrounding environment by reach and scenarios, avoiding off-matching and extraneous movements and mental strain that mean extra costs [40].

These many righty and matching responses automate the return of the individual to the stabilizable frame [36] while anticipatory and reactive mechanisms enhance effectiveness and efficiency. The body during activities adjusts itself to move in optimal ways, energy wise [41-43]. Oriented grasp of surrounding environment [40,44,45] stabilized within a mode of rightly processes, supports the individual conserving energy over his domains and terrains for forward thrust with stability (Figure 3).

B. Remodeling: continual recomposing the body state

Body adaptation essentially is to prepare the body core, elements and assets ready spatially and temporally to enable adjustment to the variable demands of the surrounding while retaining controlled core stability to ensure proper function. Physiologically, neuro-cardiac matching [46] can enhance this capacity. Integrally, non-verbal cues though face-to-face interactions demonstrate efficient interactional matching since infancy [47,48]. Infants’ gaze synchrony (matching social gaze between parent and child), and affect synchrony (matching affective expression) during interaction with parents contribute to internal development of self-regulatory capacities in the autonomic system [49] and cognitive growth [50].

In fact, all five self-vitality subsystems [35] evolve with repeated remodeling as they mature. The acquisition system during food handling would evolve memorized vegetative behavior during interaction between the nervous system and the enteric neural system, gut microbiota and immunologic system. Patterned dynamics for situations develop both in the patternable energy-process driver during acclimatization to environment and synchronization in lung patterns over activities. Same for the situational option-generation system for heart-mind congruence in determining mobility or motivation over

An integral unit
the Core

- One integral unit
- maintain integrality of the entirety of the individual
- core stabilized within a rightly mode
- stabilized core supports the individual over his domains and terrains in conserving energy for forward thrust and stability
- the more stable the core, the less it be disturbed

The Environment
Matching

- Expressing the world and body events
- to actuate in domains and terrains to live
- matching responses coordinated to suit the individual’s interactive operations
- facilitate adaptive input-output linkages
- the better the match, the more the person functions, with less difficulty to maintaining integrity

Figure 3. Core matching patterns well-coordinated for the individual develops and maintains his core to keep vitality against environmental perturbations

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motives or emotive moves according to their importance. These are some of the early core matching interactions wherewith repeated remodeling develop well-patterned dynamics, followed by other maturational changes, even for conformity norms\textsuperscript{[51]}.

### 3.2.2 Neural Vascular Effectively Coordinated

Closely associated neural and perfusional elements and their coordinated regulation during well-patterned dynamics provide for ready and matching mechanisms to act and react to surrounding variations and disturbances\textsuperscript{[57]}.

**A. Neuro-vascular coupling:** coordinated regulation in effective patterns

The body tends to maintain an adapting or positioned suspense with a forwarding stance. In assertional activities, neural and perfusional match to the surrounding environment will be a prime objective in securing the individual’s forward thrust supported. Going across variable grounds, capabilities and capacity vary in each instance and in each individual but strains would be especially significant in the brain which cannot stand interruption of cerebral blood supply for a few minutes. Extra effort is deemed necessary in activities when an individual needs to exert beyond basic levels of functioning or attempt alternative strategies even recruiting other resources to maintain performance.

A number of functionalities are in place to provide resourceful support governing appropriate microvascular density towards needs and for redistribution for snug coordinated neuro-vascular regulation in effective patterns for the whole body during and after assertion\textsuperscript{[7]}. These include neuro-vascular coupling with fascial support at tissue level, autoregulation at regional levels\textsuperscript{[52, 53]} to maintain stable blood flow to a region over a wide range of systemic conditions, recruitment of the hepatic splanchnic circulation during assertive activities at whole body level\textsuperscript{[54]}, and patternable metabolism and perfusion with body pacemakers according to daily activities\textsuperscript{[55]}. Execution is facilitated with assured dynamics and control by simply fine-tuning itself to match for discrepancies at task over a stabilized core that maintains the person as a whole.

**B. Brain-circulatory system match:** coordinated interplays

The whole body adaptivity depends on both the nervous system and the circulatory system in coordinated interplays temporally. The nervous system directly influences blood vessel patterning resulting in neuro-vascular congruence that is maintained throughout development and in the adult\textsuperscript{[56]}. The brain is conditioned with the heart which apart from the medullary autonomic nervous system regulating ordinary cardiac function, is directed by the limbic autonomic input which discretarily prepares circulation for motive or emotive endeavors\textsuperscript{[57]}. Common signaling pathways play a key role on cardiac and nervous system development\textsuperscript{[58]}. The brain-heart interplay is conditioned during maturation to anticipatorily and reactively provide constancy of perfusion support sufficiently during all modes of living. A series of vascular, neural and hormonal stabilization mode, consciously or subconsciously regulated, to balance himself in his setup with the environmental influences facilitate modulation of circulatory homeostasis for body and mental actualization during matching out\textsuperscript{[57]}. Pushing forward, information directives and perfusional resources go hand in hand. Local blood supply is matched to neuronal demand.

Full exposition of neuro-vascular coupling as well as snug and fit dynamics shows analogously how Chinese medicine views energy processes and blood perfusion prime for the body as a concept with Qi (Energy process) and Xue (Blood) together\textsuperscript{[7]}. There may be a depletion or a repletion\textsuperscript{[59]}. Emphasizing the interplay between Qi and perfusion, Huangdi Nei Jing described how the Zang organs are vitalized by meaningful ideas and intention up to the enduring will, well bred-in-the-bone and in essence so that, through body channels, the body’s five Zang systems become depot organs and are shaped\textsuperscript{[59]}. In other words, parts of mind and body interact with the environment through the energy processes, circulatory channels and blood as well as fascial system to consolidate related Zang organs to allow actuations with flexible physiological and behavioral responses to context-appropriate behaviors (Figure 4).

The better the matching responses, the stronger would be the individual to function. Herbal therapies can soothe matching processes in the body homeodynamics.

### 3.2.3 Snug Processes

Body snug provides further capacity for adaptiveness. The body would function better in its own right optima away from deviations. Snug is a body functioning poised at the energy-efficient body state that autonomously maintains activities without stresses. A paper summarized the salient evidence and features\textsuperscript{[7]}.

**A. Functioning in optima:** conditioned dynamics to produce unstrained effectiveness

To preserve its integral functionality for the years to come, living as an organized unit in congruity with least incompatibility would be effective and more cost efficient. The biochemical, hormonal or cephalic projective body responses are trying to establish homeostasis, and at times
of extra uncomely demands, to be regulated as heterostasis, allostasis, hormesis and adaptive homeostasis. While lives of any organism depend upon the entire ecosystems, the body developed pertinent energy efficient mechanisms. Human beings have core-vs-match processes and neuro-vascular setup for ensuring conservation of cost efficiency for effective living and assertion modes in different terrains. Sometimes continual demands may call for shifts or new body changes even in constitutions, psychotypes and adaptive structural types. Energy efficiency is an important principle for optimizing physiological functions within organisms. Conserving biological and physiological synchrony between the individual and environment in interactions produce snug. With more stable peaceful environment, there is more snug. In variable environment, this energy efficient state depends on the central systems well in disposition, the body toned up, and activities handled by functions wherein reserves are never over-drained. The core matching mechanisms extend inside the body to organ systems and outside the person to social systems. Maturation of body habits and brain circuits and associated physiological regulation and autonomic response would support self-actualization with social engagement. A close relationship between emotional phenomena and rational processes has certainly been established. To have conditioned dynamics maintaining unstrained effectiveness at activities as a snug state would contribute to cognitive, social, and emotional growth.

B. Restitution: restoration of proper order

Simply, individuals cannot go too much over just to fit. Overt demands above the basic provisions may happen in everyday activity, and the body core and internal processes may become deflected. In daily life, the deflected body and the internal processes need to be re-instated to their primary positions by restoration at sleep after all activities and demands finish. Notably, rest and sleep may not complete the re-setting of the body in snug when the person hangs on with impertinent activities and squandering thoughts. By fine-tuning over the readily prepared core, the body may form and put up normative responses promptly by sets of concretized external processes or tackling behaviors. In congruence and snug, one can work well and respond readily to diverse activities.

4. To Go Forth, Being and Striving

The above basic assets support the person for functioning through his developing years. There are certainly other body assets and skills attained in adulthood. However, it is most often these basic assets that the individual has to use to re-attain snug while reaching out for being fit. Survival quality depends both on fitness and snug of the individual.

4.1 Snug and Fit Dynamics for its Sophistication

Snug dynamics is one way to understand life (Figure 5). Recent advises tend to recommend multitasking in this world of many things happening together. As people want to add value, pushing for “fitness”, there will be assertive patterns built in the body and mind. On the other hand,
snug dynamics is another way to achieve being fit for the individual in the world. Then one sleep for to sleep, work for to work, play for play, and energized wisdom is built up with concentration. One would no longer be sleeping yet indulged in work thoughts, playing yet still concerned with work, nor working yet still hanging on play, as people nowadays are. Daily function can be metaphorically likened to the performance of the Olympic karate winner who strikes sharply and rigorously yet in between strikes is in calm breathing and composed.

4.2 Re-patterning with Snug and/or Fit at New Encounters

Stabilized patterns and remodeling allow other full functional changes. Matching fascial, circulatory and neurohumoral elements and mind-body-environment interactions throughout the body start early and contribute to shaping interactions between external and internal domains. The surrounding dragging forces on the whole conformable alignment would be acting to match or dissociate it. Each chanced event encountered would add multi-dynamic changes inside according to pertinence and weightiness of issues and forces. Core functions constantly righting, myofascial organization more flexible and properly aligned, and autonomous adaptive behaviors well-patterned, the responses suited to the individual’s center of balance would support better carrying out and coping further demands. More reserves with strength and capacity would meet situations and even stresses with less traumatic confrontations. Fitter are those with more snug.

Some may develop with a setup for being snug internally and fit externally, while others with dysfunctional patterns may develop into strained and stressful states even with depression in repetitive cycles. Sleep loss could affect the capacity for performance and access to energetic resources and associated with global cognitive decline. Real-time lags in the mutual support between neural directives, vascular perfusion and metabolic needs are obvious to the patient subjectively. Remarkably, viewing these domains as separate entities would not see real-time lags as problems. Yet, as nervous-perfusion poorly matched, electrocardiographic changes is associated with the severity of acute cerebral ischemic stroke. In Chinese medicine, Qi deficiency syndromes, described as having fatigue, weak voice, weak pulse and pale tongue with teeth marks on the side, are believed to be at the root of many common Western disorders. When his nervous-perfusional resources are not providing necessary timely matching with environmental needs, his complexion should show it up.

When the snug capacity reaches critical inadequacy to allow being adaptive, the body yielding to new changes may decompensate. When inadequate to overcome, stress evokes a variably wide range of physiological responses as general adaptation. These responses can be undue, inappropriate or exaggerated response to the situation. In the aging, a decline in functionality is often kept up by compensation mechanisms.

5. Clinical Directions

In general, for the person to function well, the core
must be integrally firm and stable as it continually keeps timely matching to the surrounding, dealing with fluctuating levels of variables for consistency. The core bio-physiological self-regulatory capacities during self-actualizations are recurrently remodeled in patterns of body processes and brain circuits. Snug living changes body. More snug, more reserves and capacity to face new situations without necessity for stressful changes. Neurovascular coupling mechanisms at all levels and the core-vs-matching capacity enhance each other. Contingency surpluses from physique built up and resources acquired set an individual at ease to go across rough terrains and variable grounds to complete various tasks. Well-patterned dynamics tend to further provide more capacity for adaptiveness.

Only understanding these can clinicians realize the body’s strength and direct it into real health with therapies. A new overviewed body map[35], and re-understanding of the three related dimensions of the fascial organization, the co-directed nervous and perfusional elements could be the restarting basis for re-defining our therapies, whether eastern or western medicine. The operational organs may be likened to administrative compounds in a city. Underneath, likened to the subway network are the contextual neuro-vascular coupled channels with information, resources and meaningful entities co-routed. The fascial system provides, like related pipes and groundwork, the textual discoursive infrastructure. When mature, the mantle, operational organs and cells constitute the body form installed in topological position. The five self-vitality subsystems depict the body state adapting to the body-environment changes. The way the individual actuates and asserts is his body disposition. The body form, the body state, and the body disposition could comprehensively describe the whole person for how he lives in health and disease.

The body form, the body state, the body disposition are areas to assess for good therapy. Any clinician who cannot make out a full picture as well as life dynamics would be at best a technical expert. Thereby, for therapy to achieve making body snug, reconstituting the body state as well as re-instating a good body form (such as a smooth lumbar curvature and postural dynamics without nodular bumps) would be important. More details could be elaborated for molding the body disposition better in disease and in health.

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ARTICLE

Fine-tuning Mast Cells is Essential for the Maintenance and Regulation of the Systemic and Immune Homeostasis

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ABSTRACT

During the past decades, populous expansion in mast cell scientific literature came forth with more, than forty-four thousand PubMed publications available to date. Such surge is due to the appreciation of the momentous role of mast cells in the evolution of species, in the development and maintenance of vital physiological functions, such as reproduction, homeostasis, and fluids, diverse immunological roles, and the potential of far-reaching effects despite minute numbers. While the emerging knowledge of the importance of mast cells in equilibrium comes of age when looking at the matter from an evolutionary perspective, the recognition of mast cells beyond detrimental performance in allergies and asthma, during protection against parasites, falters. Beyond well-known classical functions, mast cells can process and present antigens, can serve as a viral reservoir, can respond to hormones and xenobiotics, initiate antiviral and antibacterial responses, phagocytosis, apoptosis, and participate in important developmental cornerstones. During evolution, upon the development of a sophisticated niche of innate and adaptive cell populations, certain mast cell functions became partially transmutable, yet the potency of mast cells remained considerable. Reviewing mast cells enables us to reflect on the certitude, that our sophisticated, complex physiology is rooted deeply in evolution, which we carry ancient remnants of, ones that may have decisive roles in our functioning. This communication sets out the goal of characterizing mast cells, particularly the aspects less in limelight yet of immense significance, without the aspiration exhaust it all.

1. Introduction

Reflection on mast cells allows us to understand that the human and natural environment is subject to constant adaptation and there is a fine-tuned balance established with careful selection of the fittest throughout evolution. The message from looking at mast cells must trigger a warning signal in judging our choices, because harefooted, rough disturbances to the delicate balance of homeostatic organization may have far-reaching, irreversible consequences. Due to the genuine qualities of mast cells, therapeutic interventions may not always be feasible, it appears to be prudent to concentrate our efforts at preventing harsh perturbations into our balance, because during quick-fired changes, time for adaptation is not allowed, and selection of the fittest may be outweighed by shifting the phenotype towards disease and degeneration.

This review is a recollection to emphasize how

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pathophysiological happenings are interconnected, how external influences are widespread, and how obsolete it is to analyze and conclude from a narrow-angle. The second major objective is to direct the attention of healthcare professionals towards unsettling matters of contention, the process of understanding from a research perspective. There is an increasing price for the comforts of modern lifestyle, which is often driven by a material growth-oriented attitude, inconsiderate demand, producing excess waste, spreading all over the world.

Overall features

Phylogenetically at the beginning of the emergence of vertebrates, mast cells (MCs) were the primordial immune cells, carrying out the majority of self-defensive functions\(^1\). Their appearance reaches back to invertebrates chordae, two hundred million years before the emergence of immunoglobulins, when mast cells served wide housekeeping functions, beyond immunological ones. MCs are pleiotropic and plastic, even today in highly sophisticated and specialized organisms. MCs are well fed as their name implies, filled with preformed forcible mediators with the proficiency of very early release upon stimulation, powerful, multifarious, and long-reaching effects. The diverse nature of granule content reflects their primordial origin. It is heparin, histamine, proteases, (such as tryptase, carbboxypeptidase, and chymase), TNFα, prostaglandins, leukotrienes, and many more. MCs are customarily categorized based on their granule content tryptase and chymase, their strategical location on interfaces between host and environment, tissue and vessels to mucosal and connective tissue mast cells\(^2\). Despite their rarity, the effector potency can be immense, best demonstrated by the abrupt, profound, life-threatening anaphylactic reactions, elicited by disproportionately small stimuli in susceptible individuals. Intensivists, who appreciate the crucial notion of early effective interventions in facing forceful noxious stimuli severely compromising organ functions, may turn their eyes towards the early and persuasively reacting mast cells.

2. Origin, Ligands, Signaling Pathways

The fundamental cradle of immature mast cells is the bone marrow. They are derived from pluripotent hematopoietic progenitors. The main driver of mast development is SCF (stem cell factor), eliciting its effect upon binding to the ckit receptor (CD117)\(^3\). SCF is a growth factor produced by endothelial cells and fibroblasts, present in membrane-bound and soluble forms, leading to proliferation and differentiation of hematopoietic cells, melanocytes, and germ cells, inhibiting their apoptosis. Mast cells are derived from the bone marrow CD13+, CD34+, CD117+ progenitors, upon egress circulate in the blood as immature progenitors. When homed, complete their development in a cytokine environment of the target tissue to achieve mature phenotype. Mast cells are abundant in tissues with proximity to the external environment. SCF is upregulated by hypoxia-inducible factor1-alpha (HIF-1α), epidermal growth factor, ultraviolet B light, for example.

SCF binds to ckit (CD117), a type III tyrosine kinase receptor, which is present on early hematopoietic cells, later ckit expression is lost, except mainly on mast cells, which express high levels and depend on ckit for their survival, proliferation, homing, and function, such as degranulation and cytokine production. Upon ckit dimerization or oligomerization, the downstream PI3K-Akt prosurvival pathway is activated. CD117 is also present on melanocytes, germs cells, interstitial cells of Cajal, eosinophils, NK cells. Dendritic cell (DC) ckit expression is upregulated by Th2/Th17 inducing stimuli and ckit induced PI3K-Akt pathway mounts IL-6 production\(^6\). Mice devoid of SCF or CD117 are deficient in mast cells (W, S1) and have further abnormalities. If completely devoid of ckit receptor, mice die within 10 days after birth for severe macrocytic anemia, ckit hence is indispensable for survival and development, it is a prerequisite for the development of myeloid cells. In utero transplantation of wild type, fetal liver rescues mice and enables research application. To overcome the lack of exclusivity, diphtheria toxin-, and cre- inducible, more mast cell-specific ablation techniques were developed\(^5\).

A multitude of mutations exists within the ckit gene, often leading to enhanced or aberrant activation and behavior of mast cells. These mutations are clinically difficult to pinpoint, there is only one mutation clinically screenable, the remaining fifty or so already described mutations are only possible to identify in research settings. Mast cell activation syndrome is a recognized entity, due to the usually concomitant presence of several ckit gene mutations in the same individual. The diagnosis of the syndrome is cumbersome for the transient nature and vast variability in the clinical and laboratory presentations\(^6\).

Mutations in stem cell factor may lead to leukemia, small cell lung cancer, gastrointestinal- and germline tumors.

As mice and humans age, mast cells increase in numbers, beyond that strain specificity is present, BALB/c mice are more abundant in mast cells than C57BL6/J mice, females have more mast cells than males, and chronic allergic conditions are associated with increased...
mast cell numbers.

Recently, white adipose tissue (WAT) is becoming appreciated as an important source of mast cells and precursors, that are committed to the mast cell lineage, home to peripheral tissues. Adipose tissue-derived colony-forming cells when cultured with IL-3 and SCF, gradually acquire mast cell phenotype, and more than 40% express tryptase, can differentiate into both mast cell types and very efficiently colonize the periphery, but they do not home to hematopoietic tissues [7]. In the WAT of lean subjects without inflammatory activity, mast cells represent a minute proportion, but their numbers and tryptase activity [8] increase in metabolic syndrome and obesity, characterized by low-level inflammation [9]. Mast cells regulate adipocyte adaptation to cold in the white adipose tissue [10].

There is an abundance of signaling receptors present on mast cells [11]: FcεRI, FcεRII/low affinity (CD23), Fcγ receptors, pattern recognition receptors (PRRs, TLRs), complement receptors C3aR, C5aR [12], purinergic P2X4, prostanoïd PR3 [13], etc, hormone receptors. MC activation upon IgE cross-linking is the prototypic MC response to allergens, but MCs are multifaced cells with a multitude of immune functions. The variety of recognition receptors enables mast cells to react to environmental, exogenous, and endogenous triggers, as primary effectors or adjuvants, aggravating immune response, deriving towards a Th1, Th17, or Th2 phenotype, presenting processed antigens in an inflammatory environment, unfolding cryptic epitopes, enhancing inflammatory cell death, serving as viral reservoirs, modulating cytokines, and impelling the extent of the immune response.

While a significant redundancy may be present in receptor utilization and immune functions in innate microbial defense, MCs may become prevalent in an aberrant immune environment, such as irradiation for example into which mast cells in comparison to other immune cell types are relatively more resistant [14]. Mast cells respond to various types of stimuli with differential degranulations, or chemokine, cytokine production. Anti-IgE or IgG cross-linking produces a slower, progressive, and sustained degranulatory response, while activation of MCs via substance P (SP), C3a, C5a, produces a fast, expulsive response. Differences exist also in intracellular degranulatory signaling pathways in the context of stimulus [15]. The direction and extent of the immune response are based on the complexion of the exogenous or endogenous danger signals, the engaged recognition receptors with their signaling pathways, and the underlying cytokine milieu. TLRs, particularly TLR4 function in this manner proximally, including mast cells, setting the stage for an overall immune response [16].

3. MC Secretory Products

The variety of mast cell secretory products is overwhelming, a few examples are highlighted here. As opposed to pancreatic proteases, MC proteases are released from the cell in active form. Proteases are constitutive and distinct components of mast cells, with low levels present in basophils and macrophages, too. MC subpopulation signature chymase, a peptidolytic serine protease has a wide assortment of targets: fibronectin, procollagenase, high mobility group box 1 (HMGB1), tight junction proteins, thrombin, angiotensin I, substance P, IL-1β, IL-6, IL-13, TNFα, etc. [18]. Human MC chymase can degrade alarmins (HMGB1), degrade the virulence factor of Trichinella spiralis, cleave influenza hemagglutinin to increase virulence [19,20]. A male predominance is known in acute coronary events and dilated cardiomyopathy, and interestingly estrogen can inhibit MC chymase release and prevent pressure overload-induced adverse cardiac remodeling [21]. The chymase effect can be thereupon detrimental or beneficial, depending on the context.

MC tryptase, another serine protease has the potential to induce extracellular matrix proliferation, by stimulating fibroblast migration factor release [22]. MC tryptase contributes to the pervasive macrophage population of chronic obstructive pulmonary disease (COPD) lungs and dynamizes their pro-inflammatory cytokine expression (IL-1β, TNFα) [23]. During chronically ongoing inflammation, in COPD for example, mast cells contribute to the development of fibrosis via Tgfβ induction. An important excretory product of mast cells is granzyme D (GD), a serine protease produced by NK and CD8 cells, likewise. GD released upon stimuli from gram-positive or gram-negative bacteria, or IgE cross-linking enters target cells via perforins and induces apoptosis per caspase-dependent and independent pathways, activation of reactive oxygen species (ROS) production. GD expression is decreased in TLR2 KO mice, pointing towards TLR signaling pathway involvement at GD production [24].
Histamine generated and released from mast cell granules serves physiological roles by regulating the sleep and wakefulness cycle, inducing vasodilation, vasopermeability, smooth muscle contraction, and mucus production. Histamine has a profound effect on monocyte and T lymphocyte phenotypes. Myeloid-derived suppressor cells (MDSCs), harboring the histamine (HR1-3) receptors are conditioned by mast cells, in their presence both granulocytic and monocytic monocytes increase in numbers and histamine promotes their survival and proliferation in culture, and coculture of monocytes and histamine enhances IL-10 and decreases Th1 related IL-12 production. Allergic patients have higher MDSCs in comparison to healthy control [25]. MDSCs, mast cells, and regulatory T cells assemble in an immune-suppressive network, that promotes tumor growth [26]. Mast cells histamine can upregulate TLR2 and TLR4 expression.

4. Mast Cells and Endocrine Disruptors

Behavioral neuroscience is deeply engaged in understanding the regulation of social and sexual behavior. The important function of mast cells, sexual/gonadal development during intrauterine and postnatal life comes to scrutiny when realizing how deeply they can be affected via minute dysbalances in hormonal levels, and how decisive an impact the environment may have on human health and reproduction.

In many aspects of immunity, sexual hormones have important functions and the reversed question, how immunity affects sexuality, has been asked less frequently. Brain mast cells are strategic mediators of brain sexual differentiation. Male mice have more mast cells and dendritic cell synapses in the preoptic area (POA), the main center of sexual behavior. When newborn female mice were exposed to either testosterone or its active metabolite estradiol, phenotypic switching took place by mast cell proliferation and engagement. Dendritic cell spine proliferation in POA was increased via the estradiol-mast cell-histamine-microglial axis [27].

Epigenetic modification has been suggested to have the capability to modify sexual orientation. According to Lenz et al, for example, prenatal exposure to allergens may contribute to adulthood social and sexual behavioral changes via disrupting sexual differentiation. Adult female mice, exposed to an allergen during prenatal development demonstrated male patterns of mounting behavior, while male mice had decreased copulatory behavior and decreased olfactory preference towards females. Prenatal indirect exposure of mice to microbial TLR ligands via maternal infection, has led to behavioral "difficulties" and it appears that mast cells may be responsible for the effect of sex hormones on the developing brain [28].

Mast cells harbor on their surface the estrogen receptors (ER), progesterone receptors (PR), and testosterone receptors. Beyond the influence on sexual differentiation, EDCs -for example, phthalates and parabens- can interfere with prostaglandin (PG) synthesis in Sertoli cells and mast cells. PG is inhibited via EDC binding to the active site of the cyclooxygenase (COX) enzyme [29]. Sertoli cells fulfill important functions during spermatogenesis. The PG pathway is important in the masculinization of the male reproductive tract during prenatal development.

Endocrine disruptors (EDCs) are environmental chemicals that interrupt or modify various natural hormonal pathways, they modulate biotransforming enzyme activities, ion channels, occupy plasma protein transporters, induce mast cell degranulation, etc [30]. EDCs are a diverse entity of chemicals used in food processing, cosmetics, toys, drinking containers, plastic containers, water pipes, laundry detergents, herbicides, pesticides, etc. Among important disruptors are phthalates, benzophenones, parabens, estradiol, bisphenols, dioxins, etc. EDCs interface with many hormonal receptors, such as estrogen receptors, progesterone receptors, aryl hydrocarbon receptors (AhR), adrenal, and thyroid receptors. EDCs influence signaling pathways through the engagement of the above receptors variably, in a competitive manner. Their dose-effect may be proliferative, suppressive, mutagenic, they can inhibit, activate, modulate enzymatic activities, they can have binary dose effect, delayed effect, epigenetic effect through DNA, and histone methylation, importantly their effect is often cumulative. EDCs may be soluble in water or often in lipids, may have metabolically active and toxic degradations products, and due to often long half-lives their amount may sore up in the adipose tissue or organs, and with a depo effect, they may continuously assault the human body to the point beyond repair. The brain is a lipid-rich tissue and prenatal to toddler exposure may be relatively higher than in adults [31]. Around 62000 different chemicals are in use currently, in which the Environmental Protection Agency could not produce evidence in their separate, singular toxicity to the environment or humans. There are many attempts to regulate the use of EDCs. To be considered EDC, the producing company or scientific literature must present data regarding adverse health effects. Certain chemicals have proven harmful to humans and animals. But while many chemicals introduced into the environment, particularly with the speedy technical, biochemical, and agricultural development of the past 20th century comply individually with the "safety dose limits", the potential cumulative effect on the limited number of...
human and animal hormonal receptors represents a real, underrated danger. The disputes over the potential toxicity of already preaced chemicals are lengthy, the causative relationship between the environmental chemical and disease development providing long term low dose exposure is hard to prove, and during the period of legal debate and often beyond, contamination is proceeding.

Among the important endocrine disruptors (EDCs) is atrazine, the herbicide used in eliminating broadleaf weeds in crops, particularly corn. The triazine type chemical, soluble in water, has a long half-life, including metabolites. When 14C isotope of atrazine was administered to the soil for three consecutive years, follow-up studies demonstrated the presence of the radioactive substance in 83% of original levels 9 years later, and 25% still present 22 years later [32]. Due to the slow biodegradation, atrazine currently represents one of the major water pollutants despite the fact, that in 2004 it has already been officially banned in the European Union. Atrazine introduction to the environment from the 1950s has had faster dynamics than its biodegradation, creating a cumulative effect on its own. In several animal species, atrazine has been shown to interfere with gonadal development and thyroid function, slow porcine oocyte maturation, disrupt DNA integrity, and cause the death of oocytes [33]. Atrazine’s influence is complex, it decreases sperm count, delays puberty and has obsogenic effect [34]. Atrazine in vitro induces degranulation of rat thyroid mast cells and in the RBL2H3 basophil cell line [35].

Alkylphenols, used as surfactants in washing detergents, are poorly water-soluble, may cumulate in adipose tissue, bind to ER receptors, and stimulate mast cell degranulation in a FcεR dependent and independent way, they also promote Th2 polarization [36].

Aryl hydrocarbon receptor (AhR) is one of the most important environmental gatekeepers in the human body. AhR is an intracellular transcription factor, that in communication with multiple signaling pathways influences proliferation, apoptosis, energy metabolism, and cholesterol synthesis, immune cell development, and function, serves constitutively in an antiinflammatory position, cell migration, hormonal pathways, and stimulates the xenobiotic detoxifying CYP genes [37,38]. The receptor is ancient in its origin, dates more than five hundred million years back, and is essential for existence, as AhR deficient mice die a few weeks after birth. AhR has several exogenous and endogenous ligands, such as dioxin, polycyclic aromatic hydrocarbons (PAH), and from endogenous ligands tryptophan metabolites, like kynurenine acid and tryptophan metabolic products of microflora activate AhR. The exogenous ligands come from tobacco smoke, diesel exhaust, indoor heating, electronics, construction material, plastics, inner coating of water pipes, and food containers. AhR is present in many cell types in the body including mast cells, epithelial cells, etc, highly expressed in the liver, lungs, etc. Because AhR possesses important biological homeostatic functions, the inappropriate and overt engagement, disabling the physiological function of the receptor, overwhelming biotransformation capacity, may lead to organ and immune dysfunction. There is variability in the character of AhR response to different ligands depending on the dose or timing, they may promote Th17 or Treg phenotype [39]. Dioxin undergoes slow degradation of seven to ten years, impersonating a particular danger skewing the immune response towards overt proinflammatory or immunosuppressed phenotype. Accidentally, the effect of xenobiotics may become protective, in the case of estrogen-dependent breast cancer AhR decreases estrogen receptor activity, or coal tar in skin psoriasis improves skin barrier function, occupationally may however cause skin cancer [40]. Polycyclic aromatic hydrocarbons (PAH) represent environmentally frequent AhR ligands, some with cancerogenic potential, upon binding to AhR and via alternative routes. They are present in the ambient air, arising upon combustion, unburned hydrocarbons in automobile exhaust, resuspended road dust, coal burning, metal manufacturing, tobacco smoke, etc. AhR enhanced CYP1B1 activity is correlated with poor outcomes in glioblastoma patients. The adverse effects may be related to overt reactive oxygen species (ROS) production and DNA damage, proliferation, loss of differentiation [41]. AhR is abundantly expressed in mast cells, colocalizes with tryptase, and is upregulated in endometriosis patients on lesion-specific mast cells [42]. Mouse bone marrow-derived MCs (BMMCs) constitutively express the AhR receptor and upon stimulation with FICZ (endogenous tryptophan ligand) show CYP1A1 and CYP1B1 activation and enhanced ROS production [43]. Bisphenol A and estradiol stimulated BMMCs in vitro showed enhanced histamine release. Perinatal Bisphenol A exposure induced decreased DNA methylation in adult mice [44]. Mast cells and AhR are antiquated motifs, essential bricks in living organisms from very early and primitive forms, to highly complex mammals. Toxic exogenous insults therefore may have far-reaching consequences for many lifefoms on Earth.

5. MC Hormonal Interactions

Mast cells contribute in alliance with stress hormones to acute and chronic stress responses. Genetic mapping demonstrated over eight thousand genes differentially expressed among
female and male mouse mast cells in response to immunological and psychological stress. IgE-mediated allergic response induced a more aggravated and speedy response in female mast cells, in terms of tryptase, histamine serum levels and BMMCs from female mice had increased TNFα, histamine, β-hexosaminidase, and tryptase release. Among the upregulated genes, differential expressions were observed in TNFα, mast cell protease 1, 2, 4, 8, tryptase, cathepsins, and several genes involved in granule biogenesis and maturation underscoring the relevance of sex differences in mast cell-mediated immune responses [45].

Acute stressors, by the means of corticotropin-releasing hormone (CRH), excreted from the paraventricular nucleus of the hypothalamus, activate the hypothalamic-pituitary-adrenal axis of the flight or fight reaction. CRH receptors are present on the surface of mast cells, CRH itself is secreted by mast cells, inducing an inflammatory response, degranulation exocytosis with a massive 5-hydroxytryptamine release [46]. Perceived stress is a major trigger for CRH release in the periphery, for example, skin sensory neurons and mast cells, nasal mucosa, react with CRH-R1 mediated mast cell degranulation [47]. Stress-induced increase in serum CRH levels, for example, if ignited by acute restraint, leads to brain, skin, lung MC degranulation and worsens blood-brain barrier tightness, neuroinflammation, and acute asthma attacks [48]. Patients with psoriasis and atopic dermatitis too, have increased serum CRH levels and their symptoms can be aggravated by psychological stress. CRH is implicated in gut hypersensitivity in irritable bowel syndrome (IBS) and inflammatory bowel disease [49]. Ex vivo exposure of porcine intestinal cells to CRF showed increased mast cell degranulation, tryptase, and TNFα release, and gut permeability due to disruption of tight junctions, these effects were prevented with cromolyn pretreatment [50]. Mice exposed to 60 or 120 minutes of restraint stress produced high levels of serum IL-6, increased vascular permeability measured by 99Tc extravasation, and skin CRH content. These changes were remarkably diminished in MC deficient mice [51].

The interaction of mast cells with the hormonal milieu shapes the immune response. Mast cells have been shown to interact with virtually all hormonal systems, the sex, stress hormones, the thyroid, adrenal gland. The predominant hydroxyreductase in mast cells is 11 beta-hydroxyreductase (11βHSD1). The function of this enzyme is to convert cortisone in target cells to cortisol, produced mostly, but not exclusively in the liver, contributing to obesity, diabetes. 11βHSD1 is upregulated in the sites of inflammation, in mast cells, in macrophages, etc. In mast cells, they lead to decreased degranulation [52].

The interaction of mast cells and aldosterone was demonstrated using mast cell knockout mice. Aldosterone, one of the main regulators of fluid, sodium, and potassium homeostasis is produced by the cells of zona glomerulosa of the adrenal cortex in response to low potassium levels, in order to stimulate by the renin-angiotensin system. Subcapsularly located adrenal mast cells via serotonin production stimulate aldosterone synthesis and release from the adrenal gland. Low sodium levels triggered mast cell tryptase synthesis and aldosterone production in aged female BALB/c mice [53]. While deficiency of mast cells is rare, the question remains how inadvertent adrenal mast cell activation, for example during sepsis, influences electrolyte and fluid balance.

Thyroid hormones and metabolites are present in mast cells, and the thyroid receptors are present on their surface, consequently the MC – thyroid interaction is bidirectional. Antithyroid peroxidase (TPO) antibodies present in autoimmune thyroiditis can directly activate and degranulate mast cells, while during Graves ophthalmopathy mast cells in elevated numbers contribute to orbital fibroproliferation. The number and the distribution of mast cells are influenced by thyroid hormones in many organs. Mast cells interact with chondrocytes and osteoblasts and contribute to the T3 actions on bone remodeling and chondrocyte growth. Non-thyroidal illness (NTI) or euthyroid sick syndrome is characterized by low T3 and or T4 without increased feedback thyroid-stimulating hormone (TSH) levels. Among clinical intensivists, the role of peripheral thyroid silence is debated, whether it is a maladaptive change rather than recuperation of resources during bacterial infection and sepsis. NTI is a rather frequent finding during prolonged sepsis and low T3 levels represent an independent negative predictor for sepsis mortality. The notion, that bacterial lipopolysaccharide (LPS) causes peripheral suppression of thyroid hormones via MyD88 signaling and mast cells skews the dialogue towards the maladaptive hypothesis. Mice deficient in MyD88 did not develop a hypothyroid response. The thyroid dysfunction in inflammation is also due to LPS induced hypothalamic dysfunction and can be reversed with thyrotropin-releasing hormone (TRH) administration [55]. There is no consensus as per thyroid substitution in sepsis, and there are experimental indications that intravenous T3 administration may have beneficial relevance [56].

6. MC in Viral and Bacterial Infections

MCs contribute to immune response fronting primarily helminth, but also viral and bacterial infections. Mast cells are a reservoir for HIV. FcεR crosslinking on the surface of mast cells leads to enhancement of CXCR4 homing
receptor expression and increased HIV viral tropism. During hepatitis B and C infection, the Fv sialoprotein is produced in large amounts from the liver, binds to the heavy chain of the main immunoglobulin subfamilies, including IgE and leads to mast cell and basophil degranulation, histamine, and tryptase release from lung and skin mast cells.

Mast cells have been shown to participate and degranulate upon bovine respiratory syncytial virus (RSV) infection. Involvement of virus-specific IgE and RSV infection predisposes to the future development of asthma and allergy. RSV upon nasal inoculation to guinea pigs persisted for up to 60 days.

MC enhances the vireolytic ability of CD8 virus-specific cells in lymphocytic choriomeningitis virus (LCMV) infection, thus controlling the infection, but their function in asthmatic and COPD patients may become ambivalent due to a concomitant increase in airway reactivity. Postmortem analysis of alveolar mast cells of COVID-19 patients shows increased density, enhanced IL-4 production by alveolar macrophages and type II pneumocytes in comparison to control or H1N1 infected lungs. Significantly elevated serum levels of chymase, tryptase, CPA3 (mast cell carboxypeptidase A) were found in COVID-19 patients when compared to healthy volunteers. Another study showed significantly elevated CPA3 serum levels in severe COVID disease. Mast cells may have a protective role in microbial clearance, and detrimental function in aggravating airway hyperresponsiveness and fibrosis.

7. Mast Cells, Allergies, and Asthma

Mast cells and basophils are essential components of type I immediate hypersensitivity reactions. The prototypic anaphylactic response is triggered by crosslinked IgE in previously sensitized individuals and mediated by histamine, tryptase, platelet-activating factor (PAF), etc. IgE crosslinking leads to abrupt activation of signaling pathways: IgE binds to high-affinity FcεRI on the surface of mast cells. There are approximately 3x10^5 FcεRs on the surface of mast cells and a 0.3% or antigen-specific IgE receptor occupancy is needed for mast cell activation, and importantly certain mono-antigenic IgEs render polymreactivity. Several viral, bacterial, helminth superallergen proteins possess the capacity to crosslink IgE and contribute to an aggravated mast cell sensitivity. Fine particular matter of the ambient air has been shown to crosslink FcεR and worsen asthma symptoms in susceptible patients. Upon IgE engagement, prestored mediators are rapidly released via degranulation (histamine, heparin, proteases, TNFα), later de novo synthesized prostaglandins, leukotrienes, cytokines follow. A predominantly Th2 immune environment enhances the allergic phenotype. Th2 associated cytokines IL-4 and IL-13 promote IgG and IgM antibody switch to IgE subtype, and pattern recognition receptor (PRR) activation may serve a synergistic function. Human DCs harbor histamine receptors and histamine modulates the cytokine profile of DCs by downregulating IL-12 and stimulating IL-10 production to skew T cells toward an IL-4 producing phenotype. As to why certain individuals are susceptible to allergens, remains to be fully understood, the number of allergic and asthmatic individuals in recent decades however is growing. House dust mite (HDM) induced allergies, female sex, airway hyperresponsiveness in childhood are all independent predictors of adulthood asthma persistence. Importantly, anaphylactoid or pseudo-allergic reactions can occur without previous sensitization independent of IgE crosslinking, via activation of Mas-Related G-protein coupled receptor-X2 (MRGPRX2) on the surface of connective tissue mast cells, inducing a more transient and speedy degranulation, with dominant tryptase contribution. Drug-induced anaphylactoid reactions represent a sizeable group of potentially severe adverse medication effects, particularly in intravenously applied cationic small molecules. Among the most widely used medications are nondepolarizing nonsteroidal muscle relaxants and opioids used in general anesthesia and intensive care, fluoroquinolone antibiotics, vancomycin, antidepressants. Endogenous mediators like substance P(SP) can lead to MC degranulation via MRGPRX2 receptor, mediating pain and itching. Further, somatostatin, defensins can act via binding to MRGPRX2. There are 30 single nucleotide polymorphisms (SNP) described in the receptor structure, rendering some individuals more, others less reactive to receptor agonists.

Protease allergens (PA) represent a particularly dangerous group of allergens because they possess multiple immunomodulatory properties and trypsin, cysteine, chymotrypsin-like protease activities. Protease allergens are particularly abundant in house dust mites, moreover, cockroaches, certain fungal allergens, and staphylococcal proteins possess protease activity. HDM PAs are dispersed upon fecal contamination of fine particular matter (FPM). In susceptible individuals, PAs activate MCs employing crosslinking IgE, via SP release and MRGPRX2 engagement, and a dominant PA, Depr2 mimics the MD-2 molecule of the TLR signaling complex, promoting inflammation via adjuvant effect. According to studies, HDM is present in 70% of the central European households, and 50% of allergic patients are sensitized to dust mites. The effect of inhalational PA on the immune...
system of the host is surprisingly manyfold. Derp1, with cysteine-like protease activity, can disrupt the tight junctions between airway epithelial cells, can destroy IL-2 receptors, required for Th1 and Treg proliferation, further skewing T cells towards Th2 phenotype. The lung-protective surfactant A can bind allergens, Derp1 however overcomes this obstacle by cleaving the surfactant protein. CD23, the low-affinity IgE receptor on the surface of B cells, negatively regulates IgE levels but is cleaved too by Derp1. A similar immunomodulatory effect has been shown experimentally for Per a 10, a major cockroach allergen [69]. Fine particular matter, defined by size below 2.5mm associated with air pollution in urbanized areas, is of preponderant interest for its small size and ability to reach small airways, alveoli. Inhalation of the fine particular matter is associated with acute agitation of asthma symptoms, particularly in children with allergic asthma. In vitro experiments confirm reactivity of bone marrow-derived mast cells, particularly to higher doses of FPM in terms of apoptosis, enhanced IgE mediated degranulation [70], increased IL-6, TNFα, and MCP-1 secretion.

8. Mast Cells and the Heart

The human heart accommodates mast cells in vascular intima, perivascularly, and interstitially. The involvement of mast cells and immune-mediated processes is compelling in acute and permanent atrial fibrillation, heart failure, myocardial ischemia, atherosclerotic plaque development, and rupture.

Cardiomyocytes are not capable of proliferation and nonphysiological stress leads to their hypertrophy and/or degeneration. The renin-angiotensin-aldosterone system (RAS) is a driving force behind hypertensive cardiomyopathy and human mast cells are involved due to renin production and angiotensin-II generation from angiotensin-I by chymase, behaving in a convertase manner [71]. The profibrotic potential of RAS has been demonstrated, and angiotensin-converting enzyme inhibitors carry the potential of reducing cardiac remodeling. The signature profibrotic cytokine is TGFβ, and mast cell degranulation leads to the activation of profibrotic signature, myofibroblast generation, and collagen deposition [72]. Pressure overload due to increased peripheral vascular resistance leads to infiltration and proliferation of cardiac mast cells, with interstitial fibrosis and increased sensibility towards ectopic atrial pacing, irregular ectopic atrial activity- atrial fibrillation by creating aberrant pathways for cardiac conduction. Both pathognomonic features are abrogated upon mast cell stabilization with cromolyn and in MC knockout (KO) mice [73]. Repeated paroxysms of atrial fibrillation may conclude in permanent atrial fibrillation, signatures of acute versus chronic inflammation and degeneration, mast cell degranulation versus fibrosis. Histamine release from cardiac mast cells may trigger coronary spasm in predisposed patients. Experimentally, injection of histamine caused spasm in cadaveric coronaries [74]. Due to the strategical location of MCs in and around coronary vessels, their contribution to atherosclerotic changes is of interest. Beyond obesity, diabetes, and dyslipidemia, acute and chronic stress represents contributors to the development of the acute coronary syndrome. When apolipoprotein E (apoE) KO mice on Western diet were subjected to 120 minutes of restraint stress, significant activation of mast cells in the heart was observed, altogether with increased corticosterone and IL-6 levels, a shift towards neutrophils, with large size intraplaque hemorrhages, these changes were abrogated in MC KO mice. Increased numbers of mast cells were observed in the areas of atherosclerotic lesions, with the highest numbers and greatest level of degranulation in the areas of ruptures, and the intimal thickness showed a correlation with chymase activity. MC chymase can promote apoptosis of smooth muscle cells by degrading fibronectin, by disrupting focal adhesion complexes and Akt dephosphorylation, events that are needed for cell survival and adhesion. Chymase blocks NF-κB mediated survival of smooth muscle cells, NF-κB’s translocation to the nucleus is abolished and Bcl2 mRNA levels are decreased, leading to mitochondrial swelling and cytochrome c release [75]. Mast cells have been shown to promote lipid accumulation and foam formation. Instances of acute myocardial ischemia associated with severe anaphylactic reactions, the Kounis syndrome, are documented in the literature [76]. These reports indicate mast cell-mediated coronary plaque rupture upon allergic reactions to known pharmacological and environmental allergens, such as myorelaxants, aspirin, acetaminophen, antibiotics, gadolinium, food. Three types of Kounis syndrome have been described, type I in non-stenotic coronaries, when inflammatory mediator release leads to coronary vasospasm and myocardial ischemia, type II in silent atheromatous coronaries when inflammatory mediator release leads to coronary vasospasm and myocardial ischemia, type II in silent atheromatous coronaries when spasm may lead to ischemia or/and ultimately to coronary plaque rupture, finally type III representing stented patients with thrombosis or stent restenosis due to xenobiotic induced anaphylactic or anaphylactoid reaction. As mentioned earlier, mast cell degranulation may be prompted via complement component C5a, a potent anaphylatoxin arising upon C5 cleavage by alternative, mannose-binding lectin or classical complement pathway serine

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protease - C5 convertase activation, and binding upon CD88 receptor on the surface of mast cells, macrophages. C5a is a potent chemoattractant, causes smooth muscle contraction and increases vascular permeability, and can trigger mast cell degranulation. ApoE deficient mice, prone to severe atherosclerosis have been treated with C5a. Plaque disruptions increased from 13% to 56%, and the length of ruptured plaque was increased likewise, but cromolyn treatment could not reverse C5a effects. C5a induced apoptosis via the caspase apoptotic pathway, and while caspase-1 and TNFα expressions were unchanged, caspase-3 levels were increased and TUNEL assay revealed doubling of apoptotic cells from 2.4% to 5.9% of total cell number upon C5a treatment [77]. Mast cells are implicated in Coxsackie B3 virus-induced experimental myocarditis, where signature proximal positive and negative regulators of heart passaged virus-induced inflammation on mast cells and macrophages-TLR4 and TIM3- set the inflammatory events to a degree and direction that is influenced by sex [78].

9. Mast Cells and the Brain

The pathophysiology of neurodegeneration is complex. Neuroinflammation, autoimmunity, and inflammatory cell death are common features of Alzheimer's disease, Parkinson's disease, and dementia. As per Parkinson's disease, a form of chronic neurodegeneration, the alpha-synuclein misfolded protein accumulates in dopaminergic neurons of the substantia nigra, Lewy bodies are formed and neurodegeneration materializes [83]. Among important environmental triggers paraquat(herbicide), and rotenone (pesticide) are emphasized. These substances interfere with the mitochondrion electron transport system, ultimately by producing reactive oxygen species leading to the death of dopaminergic neurons in substantia nigra. There is a locally disrupted blood-brain barrier and soon autoreactive activated, mostly Th17 producing T cells populate the region aggravating neuroinflammation. The herbicide paraquat shows a clear association between inhalational, dermal, or oral exposure and the development of Parkinson's disease (PD) [84]. Paraquat is an enhancer of reactive oxygen species (ROS) formation and in its pure form leads to severe acute respiratory distress syndrome (ARDS). MCs treated with MPP+(experimental neurotoxin) showed degranulation and MMCP6,7 (corresponding to human tryptase) release, moreover, co-culture of BMMCs with neurons aggravated this response. Mast cells are located adjacent to microglia, and upon stimulation, a persistent glial activation emanates. MMCP6 and 7 lead to CCL2 release from astrocytes and glial cells activation, neurodegeneration measured by neurite outgrowth [85]. In the brain lesions of Alzheimer's and, Parkinson's patients autoimmune T cells and Foxp+ regulatory T cells are present likewise. The emergence of autoimmune conditions is manyfold, and under preexisting inflammatory conditions presentation of previously unseen cryptic epitopes may occur, leading potentially to autoimmunity. Likewise, the presence of ROS further aggravates the apoptotic process, creating a multimodal self-perpetuating pathological circuit of death and autoinflammation. The involvement of antigen processing and presentation appears to be necessary for PD to develop, this has been demonstrated on major histocompatibility complex(MHC)II KO mice, in whom MPTP (a prodrug to MPP+) intoxication did not lead to cell death, nor release of pro-inflammatory cytokines. Even when α-synuclein is overexpressed, but MHCII is not present, mice are protected from microglial activation, and neuronal cell death [86].

Concerning acute traumatic and inflammatory conditions, intracerebral bleeding, and ischemia, beyond augmenting inflammation in the central nervous system, mast cells also contribute to the increased permeability of the blood-brain barrier (BBB), enabling toxins previously precluded from entering the brain to centralize [87]. MCs are located in the pia mater, brain parenchyma, and vessels, facing the brain. Experimental stabilization with cromoglycate, similar to mast cell deficiency, led to a 50-60% decrease in brain swelling and BBB leakage. Considering our limited resources in the management of brain swelling using traditional methods, these data must be taken seriously. Histamine by its virtue increases edema formation in general, together with circulating platelet aggregating factor (PAF) from neutrophils and monocytes, upon enhancing nitric oxide (NO) production, and by VE-cadherin rearrangement in endothelia [88]. In the mouse model of focal cerebral ischemia, lack of MCs in C57BL6/J mice, similarly to intraventricular MC stabilization using cromoglycate, significantly decreased edema formation upon transient - 45 minute- middle cerebral artery occlusion [89]. The study demonstrates the contribution of mast cells to increased BBB permeability, vasogenic edema, and ischemia-reperfusion injury to disease pathology. The effect of mast cells on leukocyte infiltration, BBB permeability was present within four hours after injury, and sustained for up to 70 hours of injury, demonstrating how mast cells are immediate mediators, and later followed up by macrophages and neutrophils. The delicate response of the brain to trauma is demonstrated in experimentally induced midshaft tibial fracture under general anesthesia with sevofoxarine, in C57BL6/J male mice. A significant increase in mast cell numbers was present in the hippocampus one day after surgery. While the baseline level of proinflammatory
TNFα and IL-1β was not different among experimental groups, the increased TNFα and IL-1β production were decapitated on day one upon intraventricular cromoglycate treatment in wild type mice [90]. The role of TLR4 signaling in the ischemia-reperfusion injury has been previously emphasized, the abrogation of this pathway experimentally leads to improved tissue viability and organ functionality [91].

10. MC Stabilization

Physiologically vitamin D stabilizes mast cells. Thanks to the presence of 25-hydroxyvitamin D1-alpha-hydroxylase, mast cells can convert vitamin D to its active form, which conversely has a negative regulatory role on mast cell maturation in the bone marrow. Both vitamin D and mast cell deficiencies are linked to allergic disorders, eczema, multiple sclerosis, and tumor development. Active vitamin D can induce IL-10 production in mast cells and dampen IgE mediated mast cell activation, in the presence of the functional CYP27B1 hydroxylase, that is constitutively expressed in the mast cells. In the presence of active vitamin D, percutaneous anaphylaxis is significantly blunted [79]. The clinically available mast cell stabilizers are cromolyn (disodium cromoglycate) and ketotifen [80]. Cromolyn is a preventive mast cell stabilizer, approved for mastocytosis, allergic rhinitis, and asthma. It alleviates the extracellular calcium influx, preventing the degranulation of mast cells. Cromolyn has a good safety profile, but very low oral bioavailability and a short half-life [81]. Omalizumab, a humanized monoclonal antibody, binding IgE is in the introductory stages of application in severe steroid-resistant asthma and urticaria, indications carefully selected. Antihistamines, by blocking the action of histamine by binding to H1 or H2 receptors have proven to be instrumental in the management of allergy and asthma symptoms. Inhaled dexamethasone inhibits mast cell numbers within a few days of the administration, smooth muscle reactivity, and restrains IgE and IL-33 mediated mast cell reactivity [82]. Ultimately adrenaline with potent alpha- and beta-adrenergic activity counteracts the profound degranulation-induced disturbances in anaphylactic reactions.

11. Conclusions

The delicate balance in homeostasis is disrupted during illness. Understanding the pathognomic features of the illness, particularly in critical care with multiple organ dysfunctions, and treating adequately is a true challenge. This review was set to analyze and understand the less traditional mast cell aspects, it is a glimpse at mast cells in a broader context. Mast cells are inevitable or instrumental parts of the immune response and homeostasis, but when inappropriately engaged or directed, may become detrimental. Current clinical options at influencing them therapeutically further, beyond allergies and asthma, beyond well-known established ways with cromolyn, antihistamines, corticosteroids are limited, various shared receptor and pathway modulators are clinically tested. Mast cell „stabilization” from an evolutionary and inflammatory perspective may come by the virtue of indirect measures, by the considerate choice of environmental chemicals, particularly during periods of heightened developmental sensitivity. An important psychosomatic motif can be discovered in the article, that the pathological incentives are deeply embedded on a molecular level, can be ignited by stress and restraint. The epidemics of hypertension can partially be derived back to nonphysiological life choices. Mast cells as part of overall immune response optimally should clinically be monitored. In the complex niché of the immune response, that is determined by the etiology of the disease and the overall state of the patient due to comorbidities, sex, age, race, location. The disease pathology that is time sensitive, creates an immune cell and cytokine environment, that is to some degree exclusive, individual. Basic cell, mediator, survival, apoptosis, and cytokine panels, receptor levels would enable us to appreciate the governing forces driving and limiting the immediate immune response in time. Strategies to influence and direct such responses could be developed in an educated manner. Reverberations of research incentives in a complex and dynamic system, mapping, analyzing, and reacting, are extremely desirable attitudes of the future approach to immunological challenges.

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