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Gas-Therapy in Rheumatoid Arthritis Treatment: When West Meets East – Actual Medical Concepts with Ancient World Ideas

Michal Gajewski, Slawomir Maslinski,
Przemyslaw Rzdokiewicz and
Elzbieta Wojtecka-Lukasik

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1. Introduction

The significant number of rheumatoid arthritis (RA) patients do not respond to the typical treatment. To develop new anti-inflammatory therapies, studies on identification of new pathways involved in modulation of inflammation are still conducted. Inflammation is a local, protective response to injury and pathogen invasion. Following tissue damage manifests clinically by swelling, pain, redness and heat. Relations between the processes which enhance or suppress the inflammatory response are subject of precise regulation. Disorders of this delicate balance between proinflammatory and anti-inflammatory agents may even lead to necrosis or tissue damage. Too weak response to the agent that causes inflammation and may effect in immunodeficiency and on the other hand, too intense inflammatory response, causes the tissue damage such as occurs for example in RA. Regulation of inflammation by anti-inflammatory processes is an important condition for maintaining health and homeostasis [1,2].

The acute inflammatory process lasts from minutes to days. Its development depends on hemodynamic alterations, mechanisms of specific leukocyte-endothelial adhesive interactions, chemotaxis, and leukocyte activation and phagocytosis. These steps are regulated on humoral way by variety of soluble inflammatory mediators (eg. cytokines, histamine, NO, prostaglandins etc.) produced both by stationary cells (eg. fibroblasts, mast cells etc.) and circulating cells (lymphocytes, neutrophils etc.). Inflammatory reaction can be also regulated by neural signaling. In contrast to humoral regulation neural control is short-lived: after a

brief refractory period, responding cells can resume function as required in the absence of further neural input. Recovery of immune function after transient inhibition enables necessary local inflammatory responses to be mobilized during persisting threat or infection [1,2]. Neural regulation of discrete, distributed, localized inflammatory sites provides a mechanism for integrating responses in real time. In RA patients imbalance between an overly active adrenergic nervous system (ANS) and reduced cholinergic neural system (CHNS) activity is observed [3]. ANS stimulation results in bringing the body to a state of raised activity and attention, usually called the *fight or flight* response. In contrast, stimulation of the cholinergic nervous system (CHNS) can be summarized as the *rest and digest* response, as this returns the body functions back to normal. Idea of manipulation of the autonomic nervous system (adrenergic and cholinergic) could be interesting approach in RA treatment [4].

2. Cholinergic neural system

The CHNS consists mainly (75%) of the vagus nerve, which is the largest nerve and owes its name because of the wandering course along the body [5]. CHNS, through the vagus nerve, plays an important role in regulation of inflammation. Main neurotransmitter of CHNS is acetylcholine (ACh). ACh acts through two, widely expressed types of receptors - the muscarinic (mAChR) and the nicotinic (nAChR) receptors. The ACh receptors are found not only on neurons, but also are widely expressed on other non-neural cells such as endothelial cells (EC), monocytes, macrophages, T and B lymphocytes, dendritic cells and neutrophils, which can act in an autocrine/paracrine way, modifying immune responses [1,2]. There is no neuroanatomical evidence for parasympathetic or vagal nerve innervations of any immune organs but ACh can be also locally expressed and released from splenic immune cells [6]. ACh is detectable in blood of several animal species [7]. In humans, the mean concentration of ACh in plasma is approximately 3 nmol L⁻¹ (or 456 pg mL⁻¹, range 151–1312 pg mL⁻¹). Sixty per cent of the total ACh in human blood is contained in mononuclear leukocytes and the rest is found in plasma [8]. The nonneuronal cells that can produce ACh are for example: epithelial, endothelial, mesothelial, muscle cells and immune cells. Release of ACh from these sources is regarded as nerve-independent, which raises the possibility of immune cell-derived cholinergic activity that can modulate inflammation. These cells possess also all functional components of the cholinergic system and the cholinergic signaling in these cells is comparable to regular neurotransmission, this could be relevant in inflammatory conditions occurring with decreased activity of the vagus nerve [1,2,4].

3. Cholinergic anti-inflammatory pathway

Identification of 'cholinergic anti-inflammatory pathway' which is neural mechanism that deactivates macrophages through parasympathetic outflow changed our understanding of the mechanisms that regulate inflammation. It was shown that experimental activation of the cholinergic anti-inflammatory pathway by direct electrical stimulation of the efferent va-

gus nerve inhibits the synthesis of TNF in liver, spleen and heart, and attenuates serum concentrations of TNF during endotoxaemia [9,10]. Vagotomy significantly exacerbates TNF responses to inflammatory stimuli and sensitizes animals to the lethal effects of endotoxin. This connection between the nervous and immune systems functions as an anti-inflammatory mechanism and is observed also in other models of inflammation [1].

Human macrophages exposed to ACh inhibit release of TNF- α , IL-1 β , IL-6, primarily through nAChRs; contrary to that, production of the anti-inflammatory cytokine IL-10 was unaffected [4]. It was observed that exposure of human macrophages, but not peripheral blood monocytes, to nicotine or ACh inhibits the release of TNF, IL-1 and IL-18 but not anti-inflammatory IL-10, in response to endotoxin. Because tissue macrophages are responsible for production of most of the TNF that appears during an excessive inflammatory response, it may be concluded that a nicotinic, α -bungarotoxin sensitive macrophage ACh receptor is responsible for connection between the cholinergic nervous system and the innate immune system [11]. Because ACh is produced also by nonneural cells such as epithelial cells, T lymphocytes and EC, inflammatory response can be modulated by these cells [1,2,4].

4. Reflex inhibition of inflammation

The presence of pathogens in the body activates inflammatory response. Innate immune cells release number of cytokines regulating development of the process. Inflammatory process activates sensory fibres that ascend in the vagus nerve to synapse in the nucleus tractus solitaries [1]. Increased efferent signals in the vagus nerve suppress peripheral cytokine release through macrophage nicotinic receptors and the cholinergic anti-inflammatory pathway[1,2]. The inflammation-sensing and inflammation-suppressing functions provide the principal components of the inflammatory reflex. The 'inflammatory reflex' is described as localized, rapid and discrete response. It can also induce systemic humoral anti-inflammatory responses. This occurs because vagus nerve activity can be relayed to the medullary reticular formation, to the locus ceruleus and to the hypothalamus, leading to increased release of corticotrophin from the anterior pituitary gland [2,4].

Increased cytokine production in tissues causes pain, providing another mechanism for transferring information from the immune system[1]. Neural regulation of discrete, distributed, localized inflammatory sites provides a mechanism for integrating responses in real time, moreover, Tracey suggest that the cholinergic anti-inflammatory pathway might also modulate processing events that promote neural memory of the peri-inflammatory events (that is, the 'hissing snake' or 'charging lion' that caused the wound and/or infection) [1].

5. Dysregulation of autonomic nervous system in rheumatoid arthritis

Both ANS and CHNS are important actors in maintaining immune homeostasis. Inflammatory mediators signal to the brain via the circulation or via afferent fibers of the vagus nerve.

Result of this action is activation of the ANS and/or CHNS. Tracey suggested that the tonic activity of the vagus nerve is crucial to maintain immune homeostasis [2]. Efferent ANS and vagal nerve fibers induce local catecholamine and ACh production by neurons or nonneuronal cells. Impairment of this activity could lead to unrestrained cytokine responses and damage to the host [4].

The way in which autonomic nervous synthesis is dysregulated is still unknown. It seems likely that it may be result of increase in ANS activity (for example, stress). Pain and stress can activate the flight-or-fight responses. Because of resultant increase of adrenaline and noradrenaline, macrophages activation is inhibited and synthesis of TNF and other proinflammatory cytokines decreases [12]. Result of this response is also increased release of IL-10, which is an important anti-inflammatory cytokine from monocytes [13]. Flight-or-fight activation of sympathetic responses also stimulates increased vagus nerve output. The combined action of these neural systems is significantly anti-inflammatory through both local (neural) and systemic (humoral) anti-inflammatory mechanisms [1].

Usually actions of the sympathetic and parasympathetic nervous systems are in opposition. But in some situations the two systems function synergistically. For example, simultaneous stimulation of both sympathetic and vagus nerves produces a higher increase in cardiac output than does isolated stimulation of either nerve alone [14]. Similar situation is observed in RA - autonomic dysfunction in RA patients is characterized by an increased overall sympathetic tone and decreased activity of the vagus nerve [4]. It seems probable that the autonomic imbalance observed in experimental arthritis and RA patients is at least partially responsible for sustaining the inflammatory status [4]. Imbalance between ANS and CHNS observed in RA may effect in induction and/or persistence of the inflammation [3,15,16]. Low tone of the vagus nerve means low activity of the cholinergic anti-inflammatory pathway, which results in higher cytokine levels, thereby contributing to this proinflammatory status [4].

6. Cytokine disease theory

For nearly 1,500 years, the dominant medical doctrine of the occurrence of disease was the humoral theory of disease, whose founder was Galen. The theory assumed that disease was due to an imbalance of bodily fluids (called "humors"). Produced by internal organs, humors maintain health, until their level are properly regulated and balanced [17].

Contemporary cytokine theory of disease, similarly to humoral theory of disease assumes that cytokines produced by the immune system are somehow the equivalent of "humors," and may themselves cause not only symptoms of disease, but also the damage characteristic to diseases. For example, the presence of a single cytokine, TNF which is completely sufficient to cause lethal septic shock analogous to a serious infection caused by Gram-negative bacteria. Administration of TNF to healthy mammals makes exactly the same changes in metabolism, such as immune response or pathological manifestations of the disease, as in the case of bacterial infection [17]. It has been suggested that some diseases can be devel-

oped as a simple result of the presence of abnormal amounts of cytokines. This discovery opened a new field of research on the physiological control mechanisms that maintain health by restraining or counterregulating cytokine release. As was shown by Kokkonen [18] blood samples obtained from individuals before the onset of symptoms of RA have elevated concentrations of proinflammatory cytokines, cytokine-related factors, and chemokines, indicating activation of the immune system. Observed in this study activation, occurred before any symptoms of joint involvement. These findings present an opportunity for better predicting the risk of developing RA and, therefore, possibly preventing disease progression [18]. In the case of RA, the typical symptoms may be caused merely by exposure to TNF or IL-1. The success of drug therapy specifically blocking TNF in patients with RA clearly show the fundamental role of this cytokine in the course of inflammation, validating fundamental premise of the cytokine theory of disease. Anti-TNF and anti-IL-1-based therapeutics are currently widely used in doses that reduce cytokine activity to levels compatible with health without causing significant immunosuppression. Today, it seems that ancient Greek concepts were not far off, since our modern theories about disease causation implicate an imbalanced production of body substances [17].

7. Ancient medical concepts and modern clinical problem

The balance between the activity of the vagus nerve, and the cholinergic anti-inflammatory pathway in modulating the activity of the inflammatory response seems to be the guarantor of the proper course of inflammation. It is possible that a permanent dysfunction of the vagus nerve, and thus distortion of the inflammatory reflex may contribute to the emergence of diseases characterized by chronic inflammation such as RA. The therapeutic approach based on direct stimulation of the vagus nerve has already been used to treat epilepsy. Also, hypnosis or meditation can significantly affect the transmission of stimuli through the vagus nerve and inhibit immediate and delayed type hypersensitivity [19]. It should now be possible to determine clinically whether these or other approaches activate the cholinergic anti-inflammatory pathway [1,2,4].

Early clinical observations suggested that cigarette smoking might be beneficial to patients with ulcerative colitis. Randomized clinical trials of nicotine administration demonstrated significant benefit in a subset of patients [20]. Nicotine inhibits cytokine production via an nAChR-dependent mechanism makes it plausible to consider whether this pathway is activated in the subset of patients that derive benefit from nicotine. Cigarette smoking confers some increased risk of the development of RA but is protective against osteoarthritis [21]. Cholinergic deficiency and decreased vagus nerve activity characterize Alzheimer disease and other brain degenerative disorders. Peripheral immune responses can be modulated by cholinergic agonists used in treatment of Alzheimer disease through activation of the cholinergic anti-inflammatory pathway [1,17].

Physical exercises reduces synthesis of TNF and other cytokines. It is widely known that physical activity reduces risk of cardiovascular disease, type 2 diabetes and atherosclerosis

[22]. This observation is at least partially connected with increased vagus nerve activity and increased in cholinergic anti-inflammatory pathway activity. Obesity, on the other hand, is characterized by diminished vagus nerve output and elevated cytokine levels, which have been implicated in mediating insulin resistance and atherosclerosis [22]. Since weight loss and exercise are each associated with increasing vagus nerve activity. One can consider whether enhanced activity in the cholinergic anti-inflammatory pathway might decrease cytokine production and reduce the damage and metabolic derangements mediated by chronic, low-grade systemic inflammation that is characteristic of the metabolic syndrome [1].

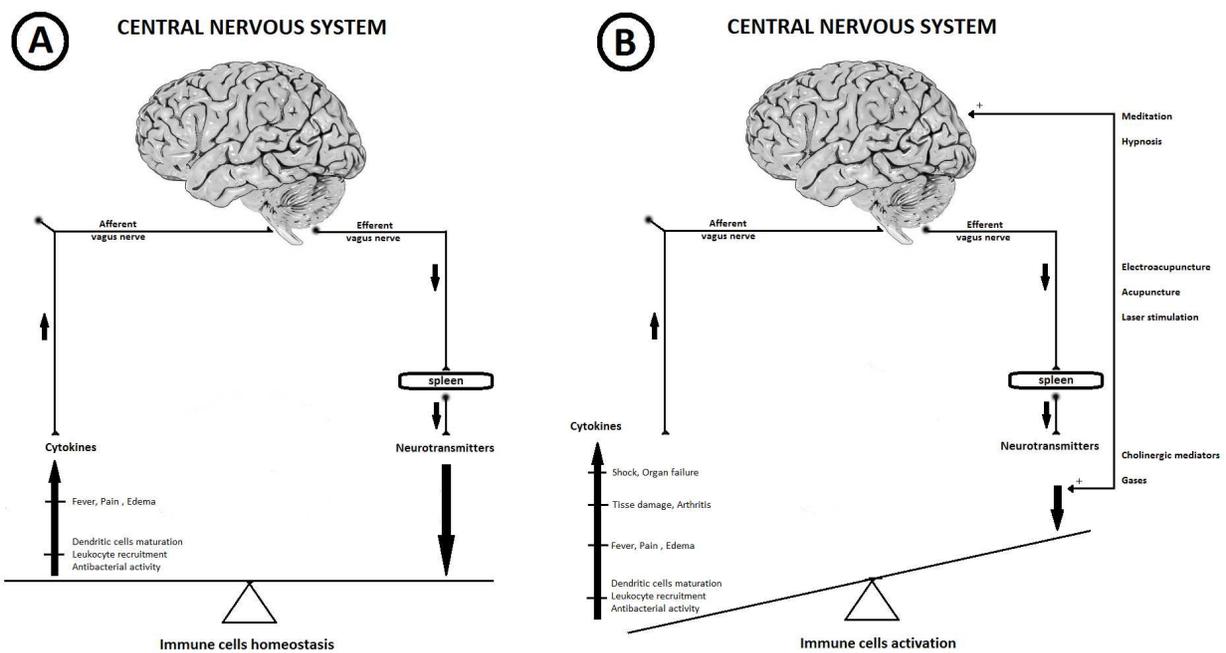


Figure 1. The therapeutic approach based on direct stimulation of the vagus nerve. A) At health cytokine production is balanced: low levels are required to maintain homeostasis. B) Overproduction of some cytokines causes diseases for example arthritis. Humoral and neural regulatory pathways regulate the magnitude of the inflammatory response. Cytokines released at the inflammatory site activate afferent fibres of the vagus nerve and reach the nucleus tractus solitarius in the brain stem. Compensatory signals are conveyed by the efferent vagus nerve and reach the site of inflammation where neurotransmitters act upon macrophages and other cells of the immune system to attenuate the inflammatory response. Hypnosis, meditation, acupuncture, electroacupuncture or laser stimulation can affect the transmission of stimuli through the vagus nerve and inhibit immediate and delayed type hypersensitivity. Complex interactions between vagus nerve modulation, acupuncture, cholinergic neurotransmitters, “biological gases” and redox status of inflammatory cells, involved in joint tissue damage, might be useful for the development of novel therapeutic strategies for RA.

One of the fundamental premises of the ancient Greeks was that dietary manipulation controlled humoral balances. This concept is now, at least in principle, supported by new evidence of a direct link between dietary composition and the regulation of cytokines by the cholinergic anti-inflammatory pathway [1]. In the folk medicine we see examples of diet, modulating the immune system functioning. For example, a diet rich in fats derived from fish may increase parasympathetic activity affecting the inhibition of synthesis of proinflammatory cytokines

such as TNF- α , IL-1 β , IL-2, IL-6 and IL-18. At the same time increased production of antiinflammatory IL-10 is observed. It has long been suspected that the presence of large amounts of fatty acids derived from fish may explain the reduced incidence of heart disease in Eskimos and Japanese [23]. Now, we understand that this is due to the increased activity of the cholinergic system. Suggested mechanisms for this cardio-protective effect focused on the effects of n-3 fatty acids on eicosanoid metabolism or inflammation, beta oxidation; but, none of these mechanisms could adequately explain the beneficial actions of n-3 fatty acids [23].

Contemporary clinical studies recognize the validity of dietary supplementation with fish fats, oil or soy oil. These particular oils cause an increase in vagal activity and reduce severity of inflammation in chronic inflammatory bowel disease or RA [21,22]. These clinical antiinflammatory responses may be linked to the fat-induced stimulation of the cholinergic anti-inflammatory pathway, as observed in rats [24]. Main source of TNF which is a major factor determining the course of inflammatory pathway is the spleen, the source of Galen's black bile. As Tracey said, jokingly: "One can not help but wonder: How did the ancient Greeks know?" [17].

The physiology of the cholinergic anti-inflammatory pathway can also be used to consider the design of clinical experiments for anti-inflammatory therapies that were previously difficult to reconcile with classical mechanisms. Hypnosis and meditation can significantly affect the transmission of stimuli through the vagus nerve and inhibit immediate and delayed type hypersensitivity [1]. Acupuncture is used to modulate the activity of the vagus nerve to change the course of inflammatory bowel function and heart rate [1,17]. Modulation of autonomic nervous system, especially the vagus nerve, by various means (including acupuncture) may be a breakthrough in the treatment of inflammatory diseases [1]. Because measurable increases in vagus nerve activity after acupuncture treatment was observed, it is theoretically possible that acupuncture can modulate the cytokine response via the cholinergic anti-inflammatory pathway [1]. High concentrations of neurotransmitters and hormones, including ACh and Norepinephrine were found within the boundary of most acupuncture points and meridian lines [25].

8. Acupuncture – Integrative functions of connective tissue

The ancient Chinese believed that there are two forces the Yang and the Yin. They are not two independent forces, as it is sometimes called, but they represent two extreme and opposite varieties of Chi energy, and thus - two opposing properties of the body, nature and the universe. Yang and Yin are defined opposites in nature, the cosmos and in man. As two opposite poles, Yin and Yang are represented in any substance at the same time, however, cannot exist alone, cannot be separated, exist in mutual relationship, in a constant balance and harmony. Imbalance between them leads to a disequilibrium in the world of phenomena, processes of change in charge of the life and phenomena of nature (homeostasis). Balance between Yin and Yang is basis of traditional Chinese medicine, which was created over 2500 years ago [26,27]. This concept is very similar to the pneuma of ancient Greek medicine. The

great historian and biochemist Joseph Needham suggested that both Qi and pneuma are rarefied form of energy [28].

Acupuncture is an unconventional technique for treatment that came from the East but it seems that similar techniques were used in ancient Europe and America - ancient mummies (stone aged) that have tattoo lines partly overlapping meridians were discovered in Europe ("Ice Man") [29].

Acupuncture is used to reduce pain and maintain or restore the body's Qi energy balance, which is the condition for its effective functioning. The technique involves piercing the body with silver or gold needles in appropriate points, corresponding to the highest activity of internal organs. These points are located along the so-called meridians [30].

According to traditional Chinese medicine, the human body has designated pathways (meridians), which circulates in the Qi. The harmonious, stable and smooth flow of Qi through the meridians is health. While the symptoms appear when the energy flow stops (which does not mean that less and less Qi, only that it does not participate in the circulation) or is impaired. Each meridian is connected with region of cerebral cortex of specific internal organ [25]. It is believed that needling acupuncture point is the way to influence on flow of Qi and regulate homeostasis of the body. Significant correlation between the distribution of connective tissue and the meridians has been shown. Distribution of acupuncture points is consistent with the distribution of connective tissue, what's more acupuncture points are places with the largest grouping of connective tissue [30,31]. It was found an 80% correspondence between the sites of acupuncture points and the location of intermuscular or intramuscular connective tissue planes in postmortem tissue sections. Langevin et al. [30] hypothesized that the network of acupuncture points and meridians can be viewed as a representation of the network formed by interstitial connective tissue. Transmission of stimuli (mechanical, bioelectrical and biochemical) through connective tissue may have a powerful potential for integration across physiological systems (connective tissue surrounds all the organs, nerves, blood vessels and lymphatic) and between different parts of the body [31].

It was suggested by Fung [27] that the anatomical structure of meridian channels and acupoints are related not only to the connective tissues but also the connective tissue interstitial fluid (CTIF) system. This hypothesis, called CFMDD (Connective Fluid, Mechanotransduction, and Degranulation Durotaxis cells), postulated to be valid not only the connective tissue (hard part), but also liquids (soft part) is an integrated network to maintain the integrity of body shape against gravity [27]. Connective tissue consists of cells and extracellular material secreted by some of those cells. Ground substance consists largely of proteoglycans, hyaluronic acid, collagen fibers, proteins such as elastin, fibronectin, laminin. Various cell types were found in connective tissue interstitial fluid: macrophages, lymphocytes, T- and NK cells, eosinophils, adipocytes, plasma cells, fibroblasts, chondroblasts, osteoblasts, stem cells and mast cells [27]. Moreover, the CTIF system embeds a large numbers of nerve endings which contain receptors that are near mast cell migration tracks would have much better chances of interaction with the mast cells, leading to the degranulation of these cells. There is evidence that acupuncture could also cause degranulation of mast cells directly through mechanical stress [32].

Mast cells have a circular or oval shape (size ~10–20 μm) are produced in bone marrow BM arising from myeloid precursors (probably the same as basophils), to the final settlement they reach the bloodstream. Mast cells are important actors in inflammatory reaction. Their granules are rich in histamine and heparin. In addition, activated secrete prostaglandins and cytokines (eg IL-4 and TNF- α). They also contain proteases (eg tryptase and chymase). On the surface there is a receptor Fc ϵ RI IgE antibody binding. Mast cells are believed to interact with connective tissue matrix components through integrins. The interaction between mast cells and nerve cells would cause degranulation of the former leading to the release of said biomolecules as physiological or pathophysiological responses. In fact, the mast cell densities were found to be high around acupoints [33] and these cells could be mediators of the effector functions of acupuncture action. Fung [27] suggested that the solid substrate tissues can serve as the tracks of migration for the cells for physiological functions. He hypothesized that there are special migration tracks for fibroblasts and mast cells. The anatomical findings suggest that the densities of the collagen fibers plus some proteins are higher along certain tracks correlating with the Chinese medicine meridian channels. Mechanical properties of extracellular matrix not only affect the cell structure, but also cell locomotion. It was demonstrated that when cells are cultured on substrates of different rigidities (but with the same chemical properties), the morphology and motility rates of cells are different [27].

Manipulation of acupuncture needle may result in modification of extracellular matrix surrounding needle. Mechanical stimulus of the meridian can be transduced into bioelectrical and/or biochemical signals and can lead to downstream effects, including cellular actin polymerization, signaling pathway activation, changes in gene expression, protein synthesis, and extracellular matrix modification [30]. Actin polymerization in connective tissue fibroblasts may cause further pulling of collagen fibers and a “wave” of connective tissue contraction and cell activation spreading through connective tissue during acupuncture treatment [31].

Acupuncture meridians for more than 2500 years were believed to form a network throughout the body, connecting “via” connective tissue all internal tissues and organs. A form of signaling (biochemical, bioelectrical, or mechanical) transmitted through tissues or organs, therefore may have integrative functions. It is commonly known that connective tissue permeates all organs and surrounds all nerves, blood vessels, and lymphatics. Langevin et al. [30] propose that connective tissue plays a key role in the integration of several physiological functions with ambient levels of mechanical stress. Finally, as was concluded by E.S. Yang: “the ancient model appears to have withstood the test of time surprisingly well confirming the popular axiom that the old wine is better than the new” [26].

9. Intercellular communication or energetic modulation – Gasotransmitters as Qi equivalent?

As was suggested by Ralt [34] the transmission of Qi along the meridians is based on small molecules that travel *via* connective tissue. Acupuncture at specific points enhances the flow

of these small signaling molecules. In this hypothesis the nitric oxide (NO) is a prime candidate to be a signaling molecule in the meridian system. The tight relations between NO and acupoint manipulation was observed [35]. NO has been shown to play a role in mediating the cardiovascular responses to electroacupuncture at point 36ST [35], moreover it was demonstrated that acupuncture increased the level of iNOS mRNA in macrophages [36]. It was also shown that NO contents and nNOS expression were consistently higher in the skin acupoints/ meridians associated with low electric resistance [36]. These data clearly show that NO is associated with the functions of acupoint/meridian including their low electric resistance. It is described that NO contents of peripheral blood increases significantly after acupuncture/moxibustion at point 36ST. Manipulating a distant point on the meridian and measure NO in another part of the body, demonstrate the meridian net via the NO biochemistry and the essentiality of NO in the control mechanisms of Chinese medicine. The similar results were obtained by blocking NO in one part of the body and measure inhibition of a distant meridian manipulation [36].

The three gases; CO, NO, and H₂S, are generated endogenously [37-39]. Cytochrome oxidase (COX), the terminal enzyme of mitochondrial complex is a mediator of mitochondrial respiration not only through its natural ligand, O₂, but also through the binding of CO, NO, and H₂S [37]. At physiological concentrations, all three gases act as anti-inflammatory and cytoprotective agents [37-39]. The inhibition of COX by CO, NO, and H₂S suppresses oxidative phosphorylation reduces energy production and decrease intensity of energy metabolism. Simultaneously, this down regulation of this phosphorylation changes the redox state of the electron transport chain and produces reactive oxygen species (ROS). In many cases, ROS function as signaling molecules, thereby controlling cell and tissue functions. This process is known as "mitochondrial redox signaling (MRS)". It is noting that near 1% of the O₂ consumed is used for this reaction. The production of NO, H₂S, and CO is determined by stimulated NO synthase (eNOS), cystathionine γ -lyase, and heme oxygenase, respectively. All these enzymes are activated by Ca²⁺-calmodulin, via stimulation of ACh receptors on EC. NO and CO diffuse into the adjacent smooth muscle cells and activate soluble guanylyl cyclase to produce cyclic GMP, being a functional part of cholinergic system. H₂S also diffuses into smooth muscle cells, where it likely activates K⁺ channels, part of cholinergic system. These three gasotransmitters are known to inhibit O₂ consumption by inhibiting cytochrome oxidase, the terminal and crucial enzyme of the electron transport chain in mitochondria. In result, all these enzymes finally reduce energy production.

NO, similarly to CO and H₂S is known to inhibit O₂ consumption by inhibiting COX, the terminal electron acceptor of the electron transport chain. NO binds COX both reversibly and irreversibly. The tissue levels of NO is in a range between 10 and 450 nM, and NO can bind to COX in vivo. It was suggested that the effect of NO inhibition of COX may be the induction of MRS, which secondarily regulates many cellular responses, including, O₂ redistribution, and regulation of energy metabolism [37]. NO also has a vasodilatory effect through the GMP pathway, which can increase the blood flow to the tissue. Thus, both the classic cGMP pathway and the MRS pathway seem to cooperate [37].

The tissue CO concentrations are in the micromolar range, and it may be assumed that CO inhibits COX in cells and tissues. CO is known to produce antiinflammatory and antiapoptotic effects, what seems to be regulated at the level of COX blocking and mediated by MRS. Furthermore, CO upregulates superoxide dismutase 2 (SOD) expression and because SOD converts O₂ to the signaling molecule H₂O₂, this effect may also enhance the MRS [37,38].

H₂S is an additional competitive inhibitor of COX [37], in a range of H₂S tissue concentration between 1 to 10mM. H₂S can also induce this phenomenon of “suspended animation” which is a physiologic response to anoxia in which all life processes reversibly arrest the most of physiological functions. This phenomenon completely returns to normal without any damage when animals are released from stress. In experiments of Blackstone et al. [40] inhalation of a nontoxic level (80 ppm) of H₂S in awake self-breathing mice could reversibly reduce the metabolic rate to as low as 10% of the normal state. Similar results were obtained in experiments with CO and NO. It seems, although these gases may have different pathways to induce the suspended phenomenon, COX inhibition is the common characteristic [40].

O₂ stimulation metabolism factor, may be recognized as yang (hot, light), contrary to CO, NO, and H₂S, inhibiting metabolism factors, which may be recognized as yin (cold, dark). It was also proposed that yin-yang balance may be an antioxidation-oxidation balance with yin representing antioxidation and yang as oxidation. These hypothesis opens an avenue to systematicall study the yin-yang balance and its health implications with the use of modern biochemical tools [41].

10. Qi as a modulator of redox imbalance?

In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining health. Very similar concept of balance called yin-yang has existed in Traditional Chinese medicine for more than 2000 years. As was suggested by Ou [41] the yin-yang balance may be compared to the antioxidation-oxidation balance with yin representing antioxidation and yang as oxidation. This hypothesis is supported by the fact that the “yin-tonic” traditional Chinese herbs have high efficiency in detoxification of ROS. They show about six times more antioxidant activity and polyphenolic contents than the “yang-tonic” herbs [41].

ROS are produced during normal aerobic cell metabolism, mainly during oxidative phosphorylation and by activated phagocytic cells during oxidative burst. Many studies have demonstrated a role of ROS and oxidative stress in the pathogenesis of RA. Oxidative stress is defined as an imbalance between oxidants and anti-oxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage [42]. The living cells possess antioxidant molecules, including thiols- mainly glutathione (GSH) for defense. The major barrier against oxidative stress is the redox equilibrium of sulfhydryl/disulfides [43]. It is commonly accepted that ROS and the resulting redox imbalance play an important role in chronic inflammatory states like RA [43].

As was shown by Crapo [44] this stress may be a potent initiator of cytokine release. The redox conditions may be important factor in determining the reactivity of the innate immune cells. In the tissues an extremely high level of extracellular antioxidants activity to maintain extracellular spaces in a highly reduced state to keep the tissue and extracellular fluids in a highly reduced state is necessary to normal physiological functions was observed [44]. Immune system activation is regulated by oxidation and reduction balance and thus the responsiveness of the immune system is influenced by the general tissue redox state, moreover, it was suggested that the imbalance in redox equilibrium may be associated with hyperresponsiveness of innate immune system [44].

11. Redox regulation of endothelial progenitor cells

The pathogenesis of RA is critically dependent on neovascularization of the synovium. It is important to note, that this microvascular expansion is occurring before clinical symptoms appear. Synovial neovascularization creates a direct conduit for the entry into the joint of circulating leukocytes that exacerbate inflammation, and provides nutrients to the hyperproliferative synovium [45].

The discovery of endothelial progenitor cells (EPC) indicated the contribution of circulating bone marrow-derived (BM) progenitor cells to new blood vessel formation, rather than migration and replication of local adult EC. Recently it was shown that EPC are not only restricted to the BM, but could also be detected in the peripheral circulation of adults. It was indicated the inverse correlation of circulating EPC numbers with cardiovascular risk factor. Moreover that conditions, which reduce the cardiovascular risk increase the levels of EPC [46].

It was observed by Paleolog [47] that the EPC cells were generated at a higher rate from BM samples taken from RA patients compared with normal subjects. The capacity of BM-derived EPC from RA patients to progress into mature EC positively correlates with the synovial microvessel density [47]. It was also shown that, in patients with active RA, EPC levels in peripheral blood, contrary to BM, were significantly lower than in healthy control. The presence of EPC in RA synovium could result from their enhanced recruitment from peripheral blood. This might then lead to increased RA synovial blood vessel formation in pannus, perpetuating chronic inflammatory disease [47]. In collagen induced arthritis Silverman et al. [45] observed EPC cells in the inflammatory infiltrate of arthritis joints, whereas they did not detect these cells in the joint lining from control animals. This correlates well with observations that the number of EPC in human RA synovial tissue samples was elevated 25-fold over the number of EPCs localized in normal synovial tissue [45]. Regulation of EPC function may have to important clinical beneficial effects. In RA the inhibition of neovascularization and in consequence inhibition of expansion of invasive tissue of pannus may be very positive clinically effect [45].

Actually it is commonly accepted that blood vessels in adult can arise not only during angiogenesis (formation of new capillaries from preexisting vessels), but also be result of

vasculogenesis (de novo new vessel formation from BM-derived progenitor cells after recruitment of a progenitor subpopulation into blood vessels) [45]. Dernbach et al [48] examined whether EPCs are equipped with an antioxidative defence to provide resistance against oxidative stress occurring in chronic inflamed joints. It was shown that the expression of the intracellular antioxidative enzymes catalase, and superoxidase dismutase was significantly higher in EPCs than EC. They are excellently equipped to be protected against redox imbalance, consistent with their progenitor cell character in chronic inflamed environment [48].

It is known that many progenitor cells functions are under redox regulation [49]. Factors promoting self-renewal cause the reduction of the redox state, whereas molecules promoting differentiation lead to excess oxidation in neural progenitor cells [50]. ROS, in non-toxic amounts, seem to be involved in the balance between self-renewal and differentiation of progenitor cells. ROS are involved in normal progenitor cells functions, such as proliferation, differentiation, apoptosis and survival. Although excess amounts of ROS are toxic, they are balanced by ROS-generating and antioxidant enzymes [50]. The stem cells are embedded in a local BM environment (so-called stem cells “niche”) and are maintained to be quiescent with low oxygen and ROS level. The increased ROS in BM facilitates stem cells to exit from the quiescent state, thereby stimulating proliferation and differentiation [50].

ROS at low levels function as signaling molecules to mediate cell proliferation, migration, differentiation, and survival. ROS regulated both angiogenic gene expression in ECs and vasculogenic gene expression in EPC [51]. Understanding these mechanisms may provide insight into NADPH oxidase and its mediators as potential therapeutic targets for chronic inflammatory disease.

It was suggest that essential attribute of the “stemness” is high resistance to stress. It was observed that the ROS levels in EPC are lower than those in mature ECs. Moreover, the higher expression of antioxidant enzymes (SOD, CAT, GPx) in EPC than in EC was shown. It seems that this is required for preserving stemness, such as undifferentiated, self-renewal state [49]. It seems that oxidative stress increases circulating cytokine levels, thereby stimulating NADPH oxidase dependent ROS production in BM, which may promote reparative mobilization of EPC from BM and the resulting revascularization of synovial tissue [50,51].

In summary, the ROS are involved in BM stem cells proliferation, differentiation, and migration, moreover, the NADPH oxidase-dependent ROS play an important role in redox signaling involved in the mobilization of BM progenitor cells [49]. Moreover there is strong evidence that EPC express eNOS- enzyme generating NO. The activity of this enzyme is regulated under inflamed conditions [51]. Compounds or molecules that increase eNOS expression improve EPC function, contrary to that the eNOS inhibitory substances have deleterious effects on EPC activity. Besides overexpression of eNOS, carbon monoxide (CO) also enhanced EPC proliferation [52]. Since NO serves as an important factor for mobilization of EPC use of organic nitrates, which are powerful NO donators, should enhance the number and function of circulating EPC. In general, low levels of ROS may activate EPC, whereas higher ROS levels significantly impair EPC function. In diseases,

which result in increased ROS levels such as RA, EPC numbers and function may be modified by redox potential in environment [52].

12. Regulation of endothelial progenitor cells by low density granulocytes

The presence of low density granulocytes (LDGs) in mononuclear cell fractions from patients with lupus or RA was observed by Denny [53]. It was shown that mature neutrophils are capable of producing many factors in response to certain stimuli, including the proinflammatory cytokines TNF- α and IL-1 β or IFN- γ . Inhibition of neutrophil-derived cytokines is viewed as a potentially useful strategy for therapeutic in chronic diseases as was suggested by Denny [53]. LDGs induce significant EC cytotoxicity and synthesize sufficient levels of type I IFNs to disrupt the capacity of EPC to differentiate into mature EC. Moreover LDG depletion restores the functional capacity of EPC. As was concluded by Denny [53] LDGs may play an important dual role by simultaneously mediating enhanced vascular damage while inhibiting vascular repair. Patients with systemic lupus erythematosus SLE and RA have a strikingly higher risk of developing cardiovascular complications when compared to age- and gender-matched controls. It was proposed that this is due to a strong imbalance between vascular damage (EC apoptosis) and repair (by EPCs,) [53].

Further, IFN- α is cytotoxic to EPCs. This suggests that this neutrophil subset may play an important role in the induction of premature vascular damage in SLE and RA. Therefore, future strategies aimed at characterizing the origin of these cells and therapeutic mechanisms to deplete them or replenish are warranted [53].

13. Redox regulation of circulating inflammatory cells

The functioning of cells infiltrating inflamed joints is markedly influenced by alterations in the intracellular redox balance. The study of Biagioni et al. [54] clearly demonstrated a markedly decreased GSH/GSSG ratio index of an increased redox imbalance in neutrophils from patients with chronic inflammation.

Neutrophils from synovial fluid (SF) of patients with RA undergo transdifferentiation to cells with dendritic-like characteristics [55] and start to express MHC class II molecules [56]. Because MHC class II positive PMN activate T cells, the activation of neutrophils and T cells might contribute to the perpetuation of the local inflammatory process, and consequently to the enhanced of destructive process in RA [55]. Transdifferentiated into dendritic like cells neutrophils might be responsible for controlling migration and activation of lymphocytes

In T lymphocytes, intracellular GSH/GSSG levels seem to be a critical for their functions. However T lymphocytes require exogenous thiols for activation and function, therefore to sustain lymphocytes activation exogenous thiols can be generated in the microenvironment

of an immune response. Angelini et al. [57] have shown that human dendritic cells (DCs) release cysteine in the extracellular space thus providing a reducing microenvironment that facilitates immune response. On the other hand, the migration of monocytes in inflamed joint is depended upon neutrophils activity. The two subpopulations of monocytes “resident” monocytes and “inflammatory” monocytes have been identified [58]. Extravasation of inflammatory monocytes, but not resident monocytes, may be enhanced by local treatment with secretion of activated neutrophils. Lysates of neutrophils were shown to exert chemotactic activity on monocytes suggesting an important role for the neutrophils-monocyte axis in the physiology of the onset, intensity and resolution of inflammatory process. Components of the neutrophils secretion were found to directly activate inflammatory monocytes and further enhanced of redox imbalance [58]. The hyporesponsiveness state observed in SF T lymphocytes from patients with RA strictly correlates with markers of oxidative stress and redox imbalance. Decreased of intracellular levels of antioxidant GSH and increased of GSSG in circulating cells was observed [59]. The redox balance alterations play a critical role in the abrogation of the cellular activation of the SF T lymphocytes from patients with RA [59]. Similar conclusion was performed by Cemersky et al. [60]. Oxidative stress and redox potential imbalance plays an important role in the induction of T lymphocyte hyporesponsiveness observed in RA.

The results obtained by Remans [61] show that chronic oxidative stress observed in synovial T lymphocytes is not secondary to exposure to extracellular ROS, but originates from intracellularly produced ROS. Chronic redox imbalance in SF T lymphocytes inhibits T cell receptor-dependent activation of signaling molecules required for efficient T cell proliferation, thus contributing to severe hyporesponsiveness of these cells to antigenic stimulation [61].

It seems that modification of the inflammatory environment by neutrophils and their granule proteins creates a milieu favoring further extravasation of inflammatory subtypes of monocytes. The targeting neutrophils without causing serious side effects seems futile, it may be more very promising to aim at interfering with subsequent neutrophils-driven proinflammatory events [58]. On the other hand, accumulating evidence suggests that intracellular redox status regulates many cellular function in macrophages [66].

Two classes of macrophages, ie. reductive macrophages (RMF), with a high intracellular content of glutathione, and oxidative macrophages (OMF) with a reductent content of glutathione were described by Murata et al.[66]. Moreover, it was observed that the Th1/Th2 balance is regulated by the balance between RMF and OMF. Type 1 helper T cells (Th1) are characterized by the production of pro-inflammatory cytokines like IFN- γ , IL-2, and TNF- β . These cells are involved in cell-mediated immunity. RA have been described as Th1 dominant disease. Type 2 helper T cells (Th2) are characterized by the production of IL-4, IL-5, IL-9, IL-10, and IL-13, and these cells are thought to play a role in humoral-mediated immunity. The balance Th1/Th2 was due to the disparate production of IL-12 vs IL-6 and 10. The OMF showed an elevated IL-6 and IL-10 production, and reduced NO and IL-12 production. Contrary to that RMF elicited a elevated IL-12 and NO production and reduced IL-6 and IL-10 production [66].

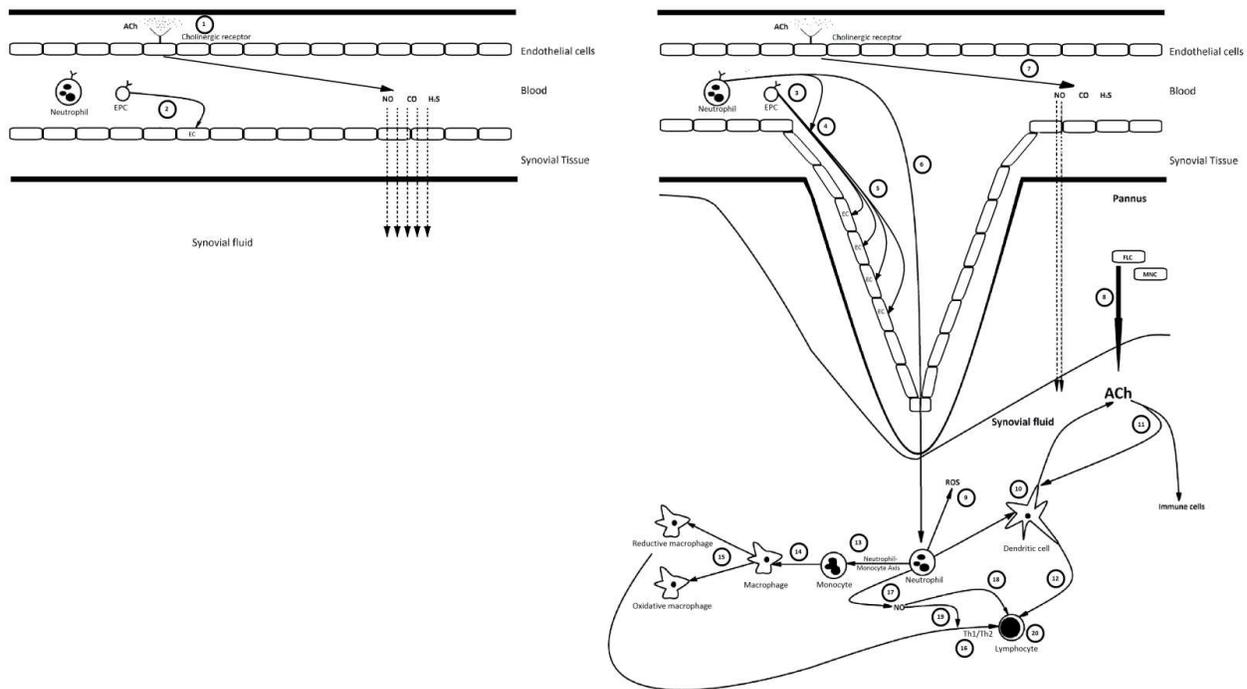


Figure 2. The role of neutrophils and importance of cholinergic anti-inflammatory pathway in interactions neutrophils-cells in RA. 1. Stimulation of ACh receptors on EC cells results in release of gases which may regulate function of neutrophils and EPC. 2. EPC have ability to differentiate into EC. 3. Acetylcholine by its receptor on EPC improves the functional activity of EPC [62]. 4. The low density granulocytes (LDGs) partially disrupt the capacity of EPC to differentiate into mature EC. 5. Increased vasculature of ST in RA is a result of intensified processes of angiogenesis and neovascularisation. 6. Raised neutrophil migration into the articular cavity in RA patients is observed. 7. Decreased activity of the vagus nerve results in inhibited production of biologic gases. 8. In RA tissue in which cholinergic innervation is not proven to exist is supplied with ACh via production in non-neural cells within the tissue. Fibroblast-like cells and mononuclear-like cells may produce ACh [63]. 9. Reactive Oxygen Species released by neutrophils cause toxic effects. 10. The neutrophils are capable to transdifferentiate into dendritic like cells (DC). 11. DC express the muscarinic receptors and enzymes for production of ACh. ACh modulates the function of DC through autocrine/paracrine loop [64]. 12. DC are responsible for controlling migration and activation of lymphocytes. 13. The neutrophils activate inflammatory monocytes. 14. Monocytes transform to macrophages. 15. There are two classes of macrophages- reductive macrophages (RMF) and oxidative macrophages (OMF) with a reductant content of glutathione. 16. The Th1/Th2 balance is regulated by the balance between RMF and OMF. 17. The neutrophils are capable of generating NO in the inflamed synovium. 18. The NO regulate T cell functions under physiological conditions, but overproduction of NO may contribute to T lymphocyte dysfunction [65]. 19. The NO profoundly alters T cell activation and Th1/Th2 balance. 20. Chronic redox imbalance in SF contribute T lymphocytes hyporesponsiveness to antigenic stimulation.

The cytokines propensities of OMF and RMF were interconverted to each other. Taken together RMF induction may generate the amplification loop of a RMF/Th1 circuit and OMF that of OMF/Th2. The findings implicate that the alteration in MF functions because altered intracellular glutathione may play a relevant role in the pathological progression of inflammation, as was suggested [66]. IL-12 is secreted physiologically by MF and plays a pivotal role in regulating cell-mediated immunity. Moreover, IL-12 also plays an important role in maintaining the in vivo balance between TH1 and Th2 responses. The development of Th1 cells from TH0 cells requires IL-12 and is prevent by prostaglandin E2 and IL-10. The differentiation TH0 into TH2 cells requires IL-4 and IL-6. It was proven that the Th1/Th2 balance is certainly regulated by the balance between RMF and OMF [66]. Moreover, the lympho-

cytes functions may be regulated by biological gases, it is commonly accepted that NO regulate T cell functions under physiological conditions, but overproduction of NO may contribute to T lymphocyte dysfunction [65].

Several different cell types are capable of generating NO in the inflamed synovium, including neutrophils and other circulating and stationary cells [67]. NO-dependent tissue injury has been implicated in RA. It was shown that NO serves as a potent immune-regulatory factor by influencing the cytoplasmic redox balance and may inhibit cytochrome c oxidase, leading to cell death through ATP depletion. It seems that T cell activation is associated with NO production [13]. Several studies have documented evidence for increased endogenous NO synthesis, suggesting that overproduction of NO may be important in the pathogenesis of RA [67].

It seems that the NO profoundly alters T cell activation and Th1/Th2 balance. Because NO may play an important role in the pathogenesis of RA, it seems reasonable that “biologic gases” may represent a novel therapeutic approach in the treatment of chronic autoimmune diseases like RA.

14. Conclusions – Proposed therapies

The parasympathetic nervous system, through the vagus nerve down-regulate inflammation. The vagus nerve exerts anti-inflammatory effect via ACh, primarily on the nicotinic receptor on macrophages. As was shown by Maanen et al. [68], experimental arthritis may be ameliorated by cholinergic agonist, nicotine. Bruchfeld et al. [69] showed that addition of nicotine to the blood cultures, from patients with RA, significantly suppressed cytokine production. These experiments showed that it is possible to therapeutically target the ACh receptors dependent control of cytokine release in RA patients with suppressed vagus nerve activity.

Strong expression of nicotinic receptor in the synovium of RA patients was detected by Das [70]. Both peripheral macrophages and synovial fibroblasts respond to specific cholinergic stimulation with potent inhibition of proinflammatory cytokines. It was proposed by Das [70] that vagal nerve stimulation, or nicotinic agonists may augment the formation of anti-inflammatory lipid molecules: lipoxins, resolvins, protectins and maresins. This implies that new therapies focused on directed regulation of the cholinergic mediated mechanisms and enhancing the formation of lipoxins, resolvins, protectins and maresins may halt and/or ameliorate RA [70]. It is very important to note, that electrical stimulation of the vagus nerve, ie. activating anti-inflammatory pathway, attenuates inflammatory injury without unwanted secondary effects on organ and tissue functions, such as heart rate or gut motility [11].

It is commonly accepted that, similarly to vagus nerve stimulation, acupuncture may activate the cholinergic anti-inflammatory pathway in treatment of inflammatory diseases [12]. Moreover, stimulation of auricular afferents excites vagal efferents [14], and may modulate

the cholinergic anti-inflammatory pathway activity [71]. Auricular acupuncture (AA) has been used for a wide varieties of pain conditions but unlike body acupuncture, the mechanism of AA remains largely uninvestigated. Since the auricle is innervated by a mix of V, VII, IX and X cranial sensory nerves and has central connections distinct from those of body acupoints, the physiological responses produced by AA may be substantially different from those produced by body acupuncture.

AA is a method based on normalizing the body's dysfunction through stimulation of some definite points on the ears [72]. AA, a distinct form of acupuncture. It is based on a somatotopic relation of the external ear to other body regions. It as was shown that ear acupressure is effective in increasing oxygen uptake and lowering lactic acid following exercise. Lin et al. [72] shown that AA enhance the physiological abilities by lowering athletes' heart rates at rest, decreasing oxygen intake, and expediting excretion of post-exercise blood lactic acids. Electroacupuncture, another form of acupuncture increases the cannabinoid receptor expression not only on "stationary" keratinocytes but also on "circulating" infiltrating inflammatory cells, like macrophages or T-lymphocytes [73].

Acupressure or physical exercise has been also proposed to be a physiological way to modulate immunity and inflammation. These processes are controlled mainly by alterations in ROS level and redox balanced [74]. Moreover it was observed that acute severe exercise (ASE) usually impedes immunity, contrary to chronic moderate exercise (CME) which improves it [74]. It was shown that ASE stimulates the secretion of many pro-inflammatory cytokines, such as TNF- α , IL-6, and IL-1 β . Nevertheless, CME potently elevates the an anti-inflammatory cytokine like IL-10. Since ASE and CME have opposite effects on inflammatory cytokines, they differentially regulate ROS level as well as redox balance [74].

A number of drugs or drug candidates (in clinical trials) exert their effects via donation of "biological gases", on redox balance, such as NO, CO and H₂S [39,75]. Szabo [39] suggested that the endogenous generated molecules may have a distinct advantage when applied as therapeutics, because the body 'already knows' how to 'deal with them'. Moreover, the cellular responses are predictable, and specific elimination pathways are present. It seems that gaseotransmitter therapy may be viewed as 'hormone replacement therapy' [38]. Many therapeutic approaches (in clinical or preclinical stages) are based on various aspects of gaseotransmitter pharmacology, for example the therapeutic administration of these gases in inhaled gaseous form [39].

The main anti-inflammatory effects of NO, CO and H₂S involve multiple more complex pathways, but at high local concentrations, the cytotoxic effects of NO, CO and H₂S cause a direct inhibition of mitochondrial respiration [76]. It was demonstrated that the inhibition of cytochrome c oxidase (followed by inhibition of mitochondrial respiration, and generation of ROS) is responsible for both the toxic effects of this gases and for the therapeutic modulation of anti-inflammatory phenomenon [76]. It may be assumed that the direction of the net biological effect (protection or toxicity) is a function of the degree of cytochrome c inhibition by "biological gases", and in consequence modulation of redox homeostasis. Since NO, CO and H₂S are produced in human body, there are basal levels of these gases in many cells and tissues. Szabo postulated that endogenous levels of all three gaseotransmitters may increase

or decrease in various disease conditions, moreover, it may be interesting for diagnostic purposes [77]. The beneficial anti-inflammatory effects of these gases typically occur at low (near-physiological) concentrations, while at higher concentrations the effects diminish, and toxicity may ensue, according to Paracelsus (1493-1541) definition that “all drugs are poisons; the benefit depends on the dosage”.

Taken together, understanding of the complex interactions between vagus nerve modulation, acupuncture, cholinergic neurotransmitters, “biological gases” and redox status of inflammatory cells, involved in joint tissue damage, might be useful for the development of novel therapeutic strategies for RA. The significant number of RA patients do not respond to the typical treatment. To develop new anti-inflammatory treatments, studies on identification of new pathways involved in modulation of inflammation are still conducted. One of interesting approaches could be idea of manipulation of the autonomic nervous system. In conclusion, it may be posited that the combined medical knowledge of the East and the West, the ancient and the actual concepts may offer new possibilities to the modern medicine.

Author details

Michal Gajewski¹, Slawomir Maslinski², Przemyslaw Rzdokiewicz^{1,2} and Elzbieta Wojtecka-Lukasik¹

1 Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland

2 Department of General and Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

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