v. 4, n. 20, 2024

International Journal of Health Science

HYPOTHESIS OF THE MECHANISM OF ACTION OF ACUPUNCTURE THROUGH GHK-CU

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Abstract: One of the great challenges currently facing acupuncture is the biological explanation of its actions at the molecular level. Deciphering how the stimulus based on the insertion of a needle can modulate physiological functions as complex as the release of neurotrophic factors is complex. For example, we know that the expression of BDNF is stimulated by the action of acupuncture, also that acupuncture is capable of increasing the amounts of neurotransmitters and receptors in the brain, etc… Understanding how this happens is a challenge for basic science in acupuncture, in this hypothesis I propose a molecular pathway that may be behind many of the phenomena that observe in the clinic. The emerging paradigm of Psychoneuroimmunoendocrinology helps us to understand the implications of acupuncture at the systemic level, however, how we explain its molecular actions.
In this case, that acupuncture could stimulate the synthesis of the biopeptide GHK-Cu, and in this sense, many of the molecular actions that trigger acupuncture and that we still do not understand could be justified. These phenomena could be explained through this biopeptide of great gene expression, since we know that it stimulates the action of many genes, which in turn can explain the clinical observations of acupuncture. This biopeptide acts on many physiological levels. In the present work I focus on the Spinal Cord Injury model and how acupuncture can help in its treatment by taking advantage of the actions of GHK-Cu.
Palabras Clave:
Keywords: GHK-CU, acupunture, electroacupuncture, spinal cord injury, stem cells, neurotrophic factors.

Currently we have evidence of the action of acupuncture at the systemic level in relation to the three levels of integration of the human body, namely: neurological, immunological and endocrine. The paradigm of Psychoneuroimmunoendocrinology helps us to understand the action of acupuncture at the systemic level (Moltó., 2018[1]). This discipline is very interesting, because it explains how the entire system is interconnected, being very similar to the systemic postulates of acupuncture. In 1973 Robert Ader showed that mental activity could somehow affect the immune system, Adler coined the term: psychoneuroimmunoendocrinology. (PNIE). With his work published in Psychocomatic Medicine in 1975 under the title: Behaviorally conditioned immunosuppression. In his conclusions they wrote:

> << These results demonstrate that an intimate and virtually unexplored relationship may exist between the central nervous system and immunological processes.>>
>
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One of the central ideas of the PNIE is that the molecules, be they neurotransmitters, cytokines, hormones or biopeptides, are information molecules. Somehow life is information, and our immune system, as well as the endocrine and nervous systems, communicate through chemical substances. Acupuncture intervenes in biological information patterns, through stimuli at acupuncture points, generating modulations of cellular responses, according to the PNIE. The PNIE is based on the homeostatic principles of biological modulation. Several experimental studies show that acupuncture can act by regulating these homeostatic principles and consequently restore health (Escobar-Corona C, et al., 2017., [2] ; Moltó Ripoll., 2015[3]). We know that acupuncture generates observable changes in the puncture sites (Xia Y, Zhou F., 2010[4]). The puncture will generate the famous microtrauma, which will injure the local epithelial cells, as well as

those of the surrounding tissues, causing the intracellular substances to come out by rupture of the membrane, this will cause an immune activation, since these substances will activate the innate response. Remember, for example, that mitochondria are supposed to have been bacteria at some point in evolution (Philipp Dettmer., 2022[5]).

The microtrauma (Martha P., el tal., 2011[6]) generated by the needles initiates the secretion of histamine, bradykinin, substance P, serotonin, among others, which lead to local inflammation. The Hageman Factor (factor XII), plasminogen, kinins and the activation of the complement system are also part of it, as well as adrenocorticotropic hormone (ACTH). Its secretion causes vasodilation and increased local permeability, and as these acupuncture points have a greater number of nerve endings, the effects are amplified by generating local leukocyte migration with secretion of cytokines (tumor necrosis factor alpha (TNF-α), IL-6, IL-1 that stimulate the hypothalamus in the secretion of CRH, activating the hypothalamus-pituitary-adrenal axis that induces the secretion of glucocorticoids (cortisol) to regulate inflammation and repair processes (Cabioglu MT., 2008[7]). , it has been confirmed by neurophysiological analysis that acupuncture points have a lower electrical resistance than adjacent non-specific areas (Ahn AC., et al., 2008,[8] ;2010[9]; Kagitani F., et al., 2010[10]). in animals and humans they have found that these areas have all the families of neurological receptors: free nerve endings, Merkel, Meissner, Ruffini and Pacini, muscle spindles and Golgi tendon organs. Somatic efferent fibers have also been found to innervate neuromuscular components. Another fact of special importance is that plexuses of autonomic fibers were found, especially noradrenergic fibers (Zhang Z-J., et al., 2012[11]). The pattern of neural acupuncture points varies depending on the path and stimulation mode, as well as the direction of the needles and their depth (Kong J., et al., 2012)[12].

Acupuncture has also been shown to participate in regenerative processes as it acts on immune cells and fibroblasts that release growth factors that participate in the remodeling of damaged tissue, epidermal growth factor (EGH) as well as gene growth factor (AGF). All these factors participate in tissue regeneration (Wang G, et al., 2016[13]).

As we can see, the action of acupuncture is complex and very interactive, many systems come into action, which explains many of the actions that we observe in clinical practice, however, it remains to be understood how acupuncture can promote the synthesis of neurotrophic factors, which biological mechanisms trigger many of the molecular actions we observe.

GHK-CU

GHK-Cu copper peptide is a natural copper complex of the tripeptide glycyl-L-histidyl-L-lysine. The tripeptide has a strong affinity for copper(II) and was first isolated from human plasma, it can also be found in saliva and urine (Pickart, L. 2018[14]). GHK was discovered by researchers who wanted to compare the blood of young people with that of people over 50 and see if there were or were any measurable differences in determining why we age. They realized that the blood of younger people had a tendency to inhibit the synthesis of fibrinogen, a protein that is involved in numerous pathological processes. Going deeper, they managed to identify the active factor causing this phenomenon, which is none other than GHK.

Following this discovery, dozens of studies have shown that this simple molecule could improve wound healing, promote tissue regeneration (especially skin, scalp,

bone, and liver tissue), increase collagen, glycosaminoglycans, improve angiogenesis and neurogenesis and have antioxidant and anti-inflammatory effects (L. Pickart., et al., 2008[15]; S. Jose, M. et al., 2014[16]). As we can see actions very present in the acupuncture clinic.

Acupuncture could also act on the action of the GHK-CU, a phenomenon that would explain many of its actions. This hypothesis concludes that many of the general effects of acupuncture could be behind the stimulation of GHK-CU produced by acupuncture, and this biopeptide is responsible for many molecular actions observable in acupuncture. As many authors point out, we must explain how a stimulus from a needle can modulate the release of neurotrophic factors. GHK-Cu is released by tissue injury, in this case microtrauma would be behind this release, and once this peptide is released it can stimulate a large number of genes.

ACUPUNCTURE, GHK-CU AND NERVE REGENERATION

Current research indicates that GHK-Cu is activated after tissue injury (Pickart, L. 2018)[14]. And as we have mentioned, acupuncture generates a microtrauma and with it, a tissue injury. This injury could stimulate GHK-Cu, which acts as a powerful tissue protector and an anti-inflammatory agent that controls the oxidative damage that occurs after tissue injury. In this sense, we know that it can help in the repair of nervous tissue.

Spinal cord injury (SCI) or myelopathy is a disorder of the spinal cord that can cause loss of sensation and/or mobility. It is important to note that the spinal cord does not have to be completely severed to experience loss of function. It is a medical emergency whose poor evolution can determine a state of permanent paralysis that will depend on the level at which the compression occurs, with greater affectation the greater the proximity to the brain (Gorelick, P. 2016[17]), we have verified evidence in the action of acupuncture in this injury (Jiang K, Sun Y, Chen X. 2022[18])

Spinal Cord Injury promotes structural and functional damage by direct and indirect factors, altering the neurological functions of the affected subjects. (McDonald and Sadowsky, 2002[19]). There is evidence that acupuncture has a neuroprotective effect in SCI (Paola and Arnold, 2003[20]; Shin et al., 2009[21]; Ma et al., 2015[22]; Fan et al., 2018[23]; Lu et al., 2020[24]). In this hypothesis, we maintain that part of this healing phenomenon is thanks to the tissue stimulation of GHK-Cu, which in turn generates the molecular reactions observed in the clinic. This peptide plays an essential role in tissue remodeling, breaking down damaged tissue and generating new tissue. GHK-Cu has also been shown to have the ability to increase axon differentiation and proliferation within neurons, indicating that it has an effect on the nervous system to ensure proper functionality.

In the present work going to use spinal cord injury to present the proposed hypothesis.

The pathophysiological changes that occur after an SCI are included in primary ones that are unpredictable and secondary, produced by the primary responses, on these are the ones that we can act on. (Belegu et al., 2007[25]; Jeong et al., 2021[26]).

OXIDATIVE STRESS

After a spinal cord injury have the presence of a multitude of free radicals, this generates an imbalance between the oxidation and antioxidant systems, generating an oxidative chain reaction (Bringans et al., 2022[27]) this may be behind the cell damage and the worsening of SCI symptoms. Due to its large amount of fatty acids, the spinal cord is very sensitive to oxidative stress with little capacity to eliminate these, which makes it very vulnerable. (Genovese and Cuzzocrea, 2008[28];

Figueroa et al., 2013[29]; Lim et al., 2013[30]; Wojdasiewicz et al., 2020[31]).

Superoxide dismutase (SOD) is an active protease that scavenges free radicals and protects cells from oxidative damage. It has the ability to eliminate free radicals and plays a vital role in the balance between oxidation and anti-oxidation. (Woźniak et al., 2016[32]; Wu et al., 2017[33]). There is evidence that acupuncture can reduce oxidative stress (Wu et al., 2002[34]; On-Ong-Arj et al., 2018[35]; Alvarado-Sánchez et al., 2019[36]). For example, GV26 reduces hydroxyl radical concentration and increases lipid peroxidation. GV4 decreases oxidative stress and improves recovery of motor function in the hind limbs of paralyzed rats (Juárez Becerril et al., 2015[37]).

Understanding how acupuncture can generate this antioxidant effect is challenging, however, we know that needle microtrauma causes tissue damage, and this damage can stimulate GHK-Cu synthesis. GHK is supposed to stimulate 14 genes related to antioxidant systems, while slowing the expression of 2 genes related to oxidation. As animal experiments show, treatment of wounds with GHK-Cu leads to increased levels of antioxidant enzymes. GHK-Cu also possesses strong antioxidant and anti-inflammatory actions. GHK-Cu inactivated harmful free radical byproducts of lipid peroxidation, such as 4-hydroxynoneal, acrolein, malondialdehyde, and glyoxal, by protecting cultured skin keratinocytes from ultraviolet (UV) radiation (Cebrián J., 2005[38]). GHK-Cu was shown to completely block Cu(2+)-dependent oxidation of low-density lipoprotein (LDL). Another well-known antioxidant, also widely used in skin care, superoxide dismutase (SOD1), gave only 20% protection (Thomas C.E., 2016[39]). GHK also prevents the damaging effects of lipid peroxidation by binding its byproducts such as acrolein and 4-hydroxynonenal (T. Arodz, et al, 2013[40]; Pickart L. 2006[41]).

GHK-Cu also increased superoxide dismutase (SOD) activity while terminating TNF-1 and IL-6 production through p65 and p38 MAPK (mitogen-activated protein kinase) blockade activation of NFκB . Mitogen-activated protein kinases are kinase enzymes that play a crucial role in cell signaling. P38 MAPK pathways enable cells to respond to a wide range of external stressors and impair skin differentiation, apoptosis, motility, and gene expression. Activation of NFκB p65 has been found to correlate with many diseases of aging and the development of cancer (Park J.R., 2016[42]). It can reduce the level of reactive oxygen species (ROS) with its ability to quench hydroxyl radicals much stronger than glutathione (GSH), making it a strong endogenous antioxidant (Sakuma S. 2018[43]).

Therefore, we can understand that acupuncture stimulation can trigger microtrauma, and this stimulates GHK-Cu, which in turn can improve the function of the antioxidant system in the body by increasing the activity and content of various antioxidant enzymes. as SOD and GSH peroxidase (GSH-Px). The stress-induced synthesis of some of these enzymes is primarily triggered by Nrf2, which plays a central role in protecting cells against oxidative and xenobiotic damage (Kensler TW, Wakabayashi N (2010)[44]; Sykiotis GP, Bohmann D (2010)[45].

It can also inhibit lipid peroxidation. Lipid peroxidation is a common way of tissue damage by oxygen free radicals through the following ways: oxygen free radicals + lipid peroxidation reaction → cell membrane → lipid peroxidation → MDA + cell components → lipofuscin Xu AX . et al (2006)[46].

INFLAMMATORY REACTION

After a spinal cord injury we are going to

have a great innate immune response, this will go against the health of the tissue because we know that an excessive inflammatory response damages the tissue of the spinal cord. Proinflammatory cytokines and chemokines will be secreted, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF-α), which aggravate local inflammation and damage axons and neurons (Zhou et al. al., 2014a[47]; Tang et al., 2020a[48]; Brockie et al., 2021[49]; Hellenbrand et al., 2021[50]). It will be essential to inhibit inflammation as soon as possible. There is evidence supporting that the peptide GHK-Cu has anti-inflammatory properties (Dou Y., 2020[51]), Both GHK-Cu and its copper(II) complex GHK-Cu have been studied for antioxidant and anti-inflammatory activities. Without being bound to copper, GHK has been shown to quench the cytotoxic end products of lipid peroxidation such as α,β-4-hydroxy-trans-2-nonenal and acrolein, which can be associated with serious pathologies such as diabetes

and neurodegenerative diseases (Beretta G. et al, 2007[52]; 2008[53]).

Regarding the action of acupuncture and the control of inflammation, there is evidence in this regard as we know that it acts in the reduction of proinflammatory cytokines such as IL-6, TNF-α, nitric oxide synthase and cyclooxygenase-2 (Choi et al. , 2010[54]).

PROMOTION OF NEURAL STEM CELL PROLIFERATION AND DIFFERENTIATION

LME induces damage to segmental neurons, axons, and glial cells at the site of injury, forming a hole in the center of the spinal cord (Jiang K, 2022)[55]. Loss of neurons within the injured section and disruption of ascending sensory and descending motor tracts of axon conduction caused loss of neurological function.

Several experimental studies have shown that acupuncture can induce the proliferation and differentiation of Neural Stem Cells (NSC), thus promoting the repair of injured nerves; however, the mechanism remains unclear

The mechanism that would explain how acupuncture and EA promote the proliferation and differentiation of endogenous stem cells is not completely well defined, several studies maintain that it could be through the regulation of numerous endogenous signals. As increased exosomal miR-146b, NeuroD1, Notch pathway activation, and reduced PTEN expression are associated with acupuncture-induced improvement of neurological injury after ischemic stroke (Tao et al., 2014[56]; Zhao et al., 2015[57]; Sha et al., 2019[58]; Zhang et al., 2020[59]). Similarly, two other studies have shown that AD facilitates integration of the mesenchymal stem cell (MSC)-derived neural network in the spinal cord by elevating neurotrophin-3 (NT-3) (Ding et al., 2013[60]; Yang et al., 2021[61]). On the other hand, the team of Xu et al (2021)[62] showed that AD promotes the growth of spinal neurons after SCI by activating the kinase/NT-3 pathway related to the calcitonin gene/slope-pending protein kinase. α calcium/calmodulin. EA treatment can promote differentiation and remyelination of MSCs and oligodendrocyte precursor cells, protect spinal motor neurons, and alleviate muscle atrophy after SCI, along with upregulation of NT-3 expression (Huang et al, 2011[63]; Yan et al., 2011[64]; Ding et al., 2015[65]; Liu et al., 2015[66]).

The action mechanisms of acupuncture in relation to the mentioned effects are yet to be elucidated, however, following the hypothesis we can suspect that GHK-Cu is behind this phenomenon, example, we know that Ahmed[67] and his colleagues in the laboratory of Neurochemists in Chennai, India, wrote that when severed nerves inside a rat were placed in a GHK-impregnated collagen tube,

there was increased nerve growth. GHK-Cu increased the production of nerve growth factor and the neurotrophins NT-3 and NT-4, accelerated the regeneration of nerve fibers from nerve pieces placed in a collagen tube, and increased axon count and cell proliferation.

GHK also appears to act in regeneration through its influence on the growth factors of the TGF-β superfamily (Growth Transforming Factor-β). Its action seems to be especially important in the last stage of healing, remodeling. The treatment of various animals with GHK has shown an effective activation of the healing system (P. V. Peplow, 2012[68]; T, Arodz, 2013[69]).

As we can see, GHK-Cu may be behind many of the observed effects, some of which are related to the skin. The skin is an organ that repairs itself very efficiently in children and young people. However, with age, the skin's ability to repair damage decreases. GHK content is highest in the plasma of young, healthy individuals. At age 20 years, the plasma level of GHK is approximately 200 ng/mL (10−7 M), and at age 60 years, it decreases to 80 ng/mL. In the 1980s, Maquart et al[70]. proposed that GHK may be an early signal for skin repair. The GHK amino acid sequence is present in the alpha 2 (I) chain of type I collagen, and when damage activates proteolytic enzymes, GHK is released at the site of injury.

In the 1973s pioneering Pickart L, et al[71], made the discovery that led to the discovery that adding GHK from plasma of young individuals to liver tissue obtained from older individuals causes the old liver tissue to produce proteins more characteristic of younger individuals.

BIOLOGY OF GHK-Cu

GHK-Cu has a fairly short life in the body, about 1 hour. However, this time interval is sufficient to act, since the small size of GHK allows it to circulate rapidly in the extracellular space and easily access cellular receptors. This time is sufficient and easily stimulated by the microtrauma generated by the needle.

In contrast, the classic administration in the form of capsules would have very few effects on the body due to the extreme sensitivity of GHK to the action of carboxypeptidase (Schlesinger D.H., et al., 1977[72]), an enzyme found in the intestine, This has led to the development of several technologies to stimulate GHK-Cu. For example, the stimulation of GHK-Cu was recently developed through patches based on nanotechnology and on the physicochemical properties of the patches (Melinda H, et al 2021[73]), these are very interesting proposals that may give us surprises in the future. Well, it is known that most of the GHK-Cu molecules would not cross the intestinal barrier intact (P. Li., et al., 2012[74]; J. Swaminathan and C. Ehrhardt, 2012[75]). For this reason, researchers essentially work with injections, liposomal or sublingual tablets, etc.

HYPOTHESIS OF THE MECHANISM OF ACTION OF ACUPUNCTURE THROUGH GHK-Cu

As we can see, there are initiatives that try to use GHK-Cu to improve many biological phenomena, in this work I have focused on the LME, but the field of action goes much further, we know that it acts on (Pickart, L, 2008[76]; Ahmed, MR; Basha, et al 2005[77]; Choi, HR 2012[78]):

- The Proteasome
- Activation of DNA repair genes
- Activation of Antioxidation genes
- Control of Genes involved in Cancer
- Tissue Repair

- Facial enhancements
- Hair growth

As we can see, the action of GHK-Cu is very broad, promoting many actions that remain to be explained in acupuncture. Although we know that acupuncture stimulates growth factors, for example, we do not know very well how this happens. Our proposal is that thanks to microtrauma, the resulting tissue injury stimulates the synthesis of GHK-Cu, this being responsible for the activation of the molecular reactions necessary for the activation of the different metabolic pathways observed in the action of acupuncture, as can be the synthesis of growth factors among other phenomena, these in turn can stimulate the expression of stem cells. As a working hypothesis, believe that it should give rise to the necessary stimulus for future Chinese Medicine laboratories to corroborate these ideas.

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