A review of the potential effect of electroacupuncture and moxibustion on cell repair and survival: the role of heat shock proteins

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In recent years, a considerable amount of research has been focused on the underlying mechanisms of electroacupuncture and moxibustion assisted tissue repair. Intracellular protein denaturation is a significant pathological step of acute conditions such as stroke, myocardial infarction and acute pancreatitis. Protein aggregation can be observed after the protein denaturation step in chronic diseases of the central nervous system like Alzheimer’s and Parkinson’s disease, and also in other chronic systemic diseases including cataract formation. Heat shock proteins (HSPs) are fundamental for intracellular protein repair and work by preventing protein aggregation and assisting denatured proteins to refold. Further, HSPs can also function for extracellular signalling. The focus of this review is to analyse the data studying electroacupuncture and moxibustion induced HSPs, and how acupuncture can survive cells from apoptosis under stress.

Heat shock proteins (HSPs), also called stress proteins, are a group of proteins present in all cells in all life forms. Although HSPs are named after an investigation studying heat exposure in drosophil (fruitfly), they are produced in any kind of stress when a cell undergoes various types of environmental stresses such as heat, heavy metal, UV-B, ethanol, oxygen deprivation. Experiments with bacteria, yeast, fruitflies, plants, fruits, mice and humans have shown that increased production of HSPs can protect an organism against stress-induced damage. It is worth noting that even noise can be a stress factor for cochlear cells and triggers HSP production, which inhibits the cochlear hair cell death. The aim of this review is to clarify the relationship of HSPs and mechanisms underlying the electroacupuncture (EA) and moxibustion assisted regeneration.

Protein folding

When harmful substances denature proteins and cause them to unfold, the proteins lose their original configuration and are no longer able to function properly. In such cases, these denatured proteins can undergo the three main pathways in the cell: repair, degradation and aggregation (cell death, apoptosis). See figure 1.

The underlying mechanism of HSP dependent cell protection belongs to their repair action on misfolded and unfolded proteins. Linear unfolded proteins become active when they are folded into functional three-dimensional shapes with the help of HSPs. These active folded proteins can be misfolded when the cell experience stressful conditions. In such a case, the most significant action of HSPs occurs. HSPs stabilise and repair misfolded proteins and thereby help to protect the cell against irreversible damage, apoptosis and death. HSPs (especially HSP70 and HSP90) repair misfolded proteins by refolding them back to their original size. If these processes are ineffective, proteins are targeted for degradation in the ubiquitin-proteasome pathway. Further, these misfolded or unfolded proteins tend to accumulate. If they accumulate they trigger apoptosis and cell death. Even if an accumulation occurs, HSPs (HSP100-HSP70) can still help cell survival by stimulating disaggregation. HSPs are mandatory molecules for the cells to shift the apoptosis pathway to the survival pathway. See figure 1.

There are many different subgroups of heat shock proteins—each one of them performs a variety of functions that help the cell in both stressful and non-stressful conditions. HSP40 presents unfolded (nonfunctional) or newly formed aminoacid chain to HSP70. HSP70 folds newly formed aminoacid chains into their functional folded forms, but HSP70 also has another significant function for cell survival. HSP70 promotes the renaturation of the misfolded proteins by refolding them. Another HSP, HSP60, works by refolding the unfolded proteins that have become inactive after cell stress exposure. In the mammalian system, HSP90 and HSP70 cooperate as a team in folding and the maturation of large proteins such as cellular receptors in the cell (see figure 2). Further, it is worth noting that the HSP70 and HSP100 bichaperone system is able to disentangle polypeptides from aggregates which, in turn, results in the disaggregation process. See figure 1.

Acupuncture and heat shock proteins

The first study on HSP and acupuncture related techniques was published in 1995. It has been shown that levels of HSP70, HSP85 and HSP100 are increased by moxibustion, in which heat is applied to acupuncture points directly or through needles via burning of artemisia vulgaris (mugwort). It is not surprising that heat assisted acupuncture techniques triggers an increase of HSP levels. However, it is worth noting that HSPs act on moxibustion rather than the smoke of mugwort.

The first evidence of the relationship between HSPs and EA was demonstrated by Zamotinsky et al in 1997. Zamotinsky et al applied 3Hz auricular EA daily for 10 days to patients with coronary artery disease. After the seventh and eighth EA no ECG signs of myocardial ischaemia are observed. The patients could tolerate a bicycle exercise test and they also could climb 5-7 flights of stairs without requiring sublingual glyceryl trinitrate or developing angina. Three days after the final EA session, HSP70 amounts, whose presence is specific for ischaemic heart pathology, were not detectable in samples of the EA group. This was the first, indirect evidence of HSP levels increasing with EA and EA assisted tissue repair via HSPs.

In 2001, Lin YH et al applied local somatothermal stimulation by using heated rods on the intersection point of the midclavicular line and right seventh intercostal space at the liver ischaemia-reperfusion model of rats. Although the point at which the heated rod was applied fits with acupuncture point of GB24, according to World Health Organization description, it has not been indicated in the study. The results of the study demonstrated that preconditioning somatothermal stimulation on right seventh intercostal nerve territory increases HSP70 and protects the liver from ischaemia-reperfusion injury in rats.

In 2003, Sun N et al performed a study to explore anti-apoptotic role of EA on an animal model of middle cerebral artery occlusion. HSP70 increased after EA in the ischaemic cortex and in the hippocampus the decrease of HSP90 is modulated after
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**Figure 1** Electroacupuncture and moxibustion assisted intracellular protein repair pathways.

EA. In the same year, Wang XR et al reported myocardial protective effects of electroacupuncture that were attributed to HSPs in a porcine heart after ischaemia/reperfusion. The results of the study revealed that HSP70 expression increased in EA in addition to lowered creatinine kinase MB levels.

In 2007, Yan XY demonstrated that HSP70 markedly increased after 15 min of EA (4/60 Hz) at GV26, PC9 and GV16 acupoints in the brain tissue of rats with cerebral ischaemia-reperfusion injury, and helped brain tissue to repair. In addition to brain and myocardial repair, it has also demonstrated that moxibustion at ST36 (Zusanli) and Liangmen decreased the gastric injury and apoptosis of gastric mucosal cells by up-regulating HSP70 expression in studies of Yi et al and Chang et al in 2007. Moreover, An et al performed a study to investigate the role of EA on acute pancreatitis in rats. After the EA application on ST36, pancreatic levels of HSP60 and HSP72 increased, in addition to a decrease of β-amylase and lipase levels, which are associated with acute pancreatitis.

**The central nervous system**

Shifting the denaturated misfolded intracellular proteins from the aggregation pathway to the repair pathway is the most critical mechanism for neurodegenerative diseases. In Alzheimer’s disease, hyperphosphorylated tau aggregates to form intracellular neurofibrillary tangles. It has been demonstrated that there are beneficial effects of EA on a rat model of Alzheimer’s disease by Zhu et al. In Parkinson’s disease, α-synuclein also tends to aggregate to form intracellular Lewy bodies. It has been shown that EA is effective in halting the degeneration of dopaminergic neurons in substantia nigra with individual studies, however the role of HSP had not been studied.

Further, in familial amyotrophic lateral sclerosis (FALS) mutant superoxide dismutase 1 (SOD1) aggregates to form intracellular inclusions. Remarkably, primary spinal cord cultures of FALS fails to upregulate HSP70 in response to heat shock, whereas cerebellar, cortical and pyramidal neurons, as well as astrocytes, efficiently upregulate HSP70 in response to heat shock. Moreover, it has been shown that intranuclear co-microinjection of expression vectors for HSP70 and mutant SOD1 into primary motor neurons reduces the toxicity of mutant SOD1, decreases SOD1 aggregation and enhances survival.

Mutant Huntington, mutant ataxin and mutant androgen receptor are the main aggregate components in Huntington’s disease, spinocerebellar ataxias, and spinal and bulbar muscular atrophies. It has been found that simply increasing the amounts of HSP70 and HSP40 is enough to reduce the Huntington aggregates. Earlier studies of Machado-Joseph disease in animals have also demonstrated that the overproduction of HSP70 and HSP40 suppresses protein aggregation and subsequent nerve cell death.

Although EA or moxibustion may have beneficial effects on FALS, Huntington’s disease and Machado-Joseph Disease by preventing protein aggregation and saving neurons from apoptosis, there are no reports in the literature for those diseases in which EA or moxibustion is used.
Clinical application of EA for ophthalmological diseases is worth giving attention to. It has been demonstrated that the lens expresses a series of HSPs that have critical roles in preventing lens opacification (cataract). The opacification of the crystalline occurs by aggregation of cytoplasmic lens proteins. In 2006, Cariello et al. revealed that EA prevents cataract formation in Wistar rats but attributed nothing to the HSP role for the underlying mechanisms of the EA effects on preventing cataract formation.

Mechanisms
In addition to the intracellular response, stress also triggers the release of HSPs into the extracellular space. The release and the ability to bind HSP70 is common to multiple cell types including the haemopoietic lineage, neuronal cells, vascular and other epithelial cells. The circulating HSPs induced by EA and moxibustion may be the reason for distance acupoint needing benefits.

One of most significant cell survival processes is the disaggregation process which needs the HSP70 and HSP100 bichaperone system. It has been shown that moxibustion can improve the disaggregation process of both components (HSP70 and HSP100). On the other hand, although improving the HSP70 levels with EA is clearly demonstrated. the relationship of HSP100 and EA has not been revealed yet. Further studies are needed to investigate whether EA assist both HSP100 and HSP70 or only HSP70 in the disaggregation process.

The ubiquitin-proteasome degradation pathway is another survival pathway for cells to escape from apoptosis. Under certain conditions, when HSPs cannot repair misfolded proteins, HSP-mediated targeting of the ubiquitin-proteasome system or lysosomes results in selective degradation. Carboxy terminus of HSP70 mediates crosstalk between molecular chaperones and the ubiquitin–proteasome system by associating with it BCL2-associated athanogene I, a protein that binds to the proteasome and assists in the degradation of specific chaperone substrates. HSP70 also contributes to the delivery of protein substrates to lysosomes, a process known as chaperone-mediated autophagy. The collective activities of HSPs, the ubiquitin–proteasome system and lysosome-mediated autophagy are also helpful in preventing the accumulation of misfolded proteins. To explore the cellular effects of mild electrical stimulation (ES), Morino et al. treated cells with low-intensity direct current at 5 V applied for 10 min. Mild ES did not induce cell cytotoxicity or the unfolded protein response. Interestingly, the expression of ubiquitinated proteins and HSP72 was increased after mild ES application and regulates the fate of many proteins. The effects of EA and moxibustion on the ubiquitin-proteasome degradation pathway still needs to be investigated.

On the other hand, although the repair and regeneration effects of the underlying mechanism of EA are usually attributed to blood flow improvement and the anti-inflammatory cholinergic pathway, the role of HSPs on the EA assisted tissue repair has been well documented in the literature. Further research is needed to clarify the acupoint, heat degree and frequency specific role of HSPs, and whether or not the extracellular HSP levels can be influenced by EA (or moxibustion).

CONCLUSION
Taken as a whole, the literature summarised in this review has clarified the mechanisms underlying the cell survival effects of EA and moxibustion, and provides a sound basis for the investigation of EA and related techniques as treatments for appropriate clinical conditions.

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