ABSTRACT

The Potential for Acupuncture to Mediate Hippocampal Apoptosis: A Systematic Review

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Apoptosis is programmed cell death that can be maladaptive when not properly regulated, especially when it occurs in the hippocampus. The exact mechanisms for apoptotic malfunctioning are yet to be fully understood. Acupuncture has seen a recent rise in western medicine and is used to treat various blood flow related conditions such as strokes or ischemia. The exact mechanisms for acupuncture are also still unknown. This systematic review evaluated the potential for acupuncture to help mediate hippocampal apoptosis. A search was conducted through PubMed, Scopus, and WebofScience using the keywords “hippocampus,” “apoptosis,” and “acupuncture” and found 37 qualified articles from January 2009 to March 2019. All of the articles supported acupuncture decreasing the incidence of hippocampal apoptosis. The most frequently suggested mechanism was decreased BAX expression and increased BCL-2 expression, often in the CA1 region of the hippocampus; although, it was only seen in about 1/3 of the articles. While no exact mechanism is understood within current literature, acupuncture does indeed have the potential to mediate hippocampal apoptosis.

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The Potential for Acupuncture to Mediate Hippocampal Apoptosis: A Systematic Review

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CHAPTER ONE

Introduction

Apoptosis is the natural process of programmed cell death that the body utilizes to maintain homeostasis and employ in processes such as cell turnover, immune system regulation, embryonic development, hormone-dependent atrophy, and chemically induced cell death. However, untimely or excessive apoptosis can instead inflict damage on the body and the ability to manipulate the life and death of a cell has the potential to produce significant therapeutic benefits (Elmore, 2007).

Necrosis is yet another form of cell death; however, it is typically understood to be unprogrammed. It occurs as the result of noxious stimuli such as infectious agents, hypoxia, or severe environmental factors including heat, radiation, or ultraviolet irradiation. While apoptosis is part of the body's regulative functioning, necrosis is almost always premature problematic cell death. When cells die by necrosis, they exhibit either liquefactive necrosis or coagulative necrosis (Golstein & Kroemer, 2007). It can result in loss of membrane integrity, cellular swelling, damage to organelles, disruption of lysosomes, random degradation of DNA, and inflammation from cell lysis (McKernan, Dinan, & Cryan, 2009).

In recent years, there has been a rise in acupuncture clinical trials within western medicine. According to the previous studies, acupuncture may improve conditions such as depression, migraines, Bell's palsy, herpes zoster, and post-stroke dysphagia (Zhuang, Xing, Li, Zeng, & Liang, 2013). A variety of mechanisms associated with the positive effects of acupuncture on aforementioned conditions

have been previously proposed. For instance, the musculoskeletal conditions are thought to be improved by increased local blood flow, microinjury, facilitated healing, and analgesia. For other conditions, it is suggested that acupuncture may activate the somatic nervous system or manipulate neurotransmitter levels. Acupuncture functions with the hypothalamic pituitary adrenal (HPA) axis and reduces some hormone levels such as Luteinizing Hormone (Cheng, 2014). Acupuncture has been researched as a treatment for various conditions that involve hippocampal apoptosis such as Alzheimer's, cerebral infraction, and post-stroke pain (Huang, Gong, Ni, Jia, & Zhao, 2019; G.-H. Tian et al., 2016; R. Tian & Wang, 2019)

The exact processes employed for acupuncture are still under investigation. The same is true for the mechanisms behind hippocampal apoptosis. Moreover, only the limited amount of studies pertaining to this topic exist in the current literature. Nevertheless, a multitude of studies have demonstrated the occurrence and connection of these processes paired together. Therefore, the current study investigated the relationship between hippocampal apoptosis and acupuncture to see if acupuncture can work as a mediator to decrease apoptotic activity. While the specific mechanisms for the mediation are still not fully known and understood, the current systematic review examined the current data available to suggest any potential pathways for which further studies can investigate.

CHAPTER TWO

Methods

This systematic review included the keywords “hippocampus,” “apoptosis,” and “acupuncture” using the inclusive qualifier “AND” through the databases including PubMed, Scopus, and Web of Science. It was limited to only clinical trials available in English that were published between January 2009 and March 2019. Relevance was determined by the article being a clinical trial that involved application of a form of acupuncture (acupuncture, electroacupuncture, or Mongolian warm acupuncture) with an analysis of apoptotic levels in the hippocampus as part of the results.

CHAPTER THREE

Results

A total of 107 articles were found among PubMed, Scopus, and Web of Science under the aforementioned search criteria. PubMed was utilized first to produce 51 results, but 20 articles were removed after being evaluated for relevance (total =31). Scopus yielded 31 results and had 24 articles removed for being identical articles found in other databases. Additionally, 3 more articles were excluded after being screened for relevance (total = 4). Web of Science produced 24 results and had 18 removed for duplication and 4 additional articles were excluded due to their lack of relevance (total = 2). As presented in Figure 1, a total of 37 articles were qualified and included in the current systemic review. The results were synthesized into Table 1.

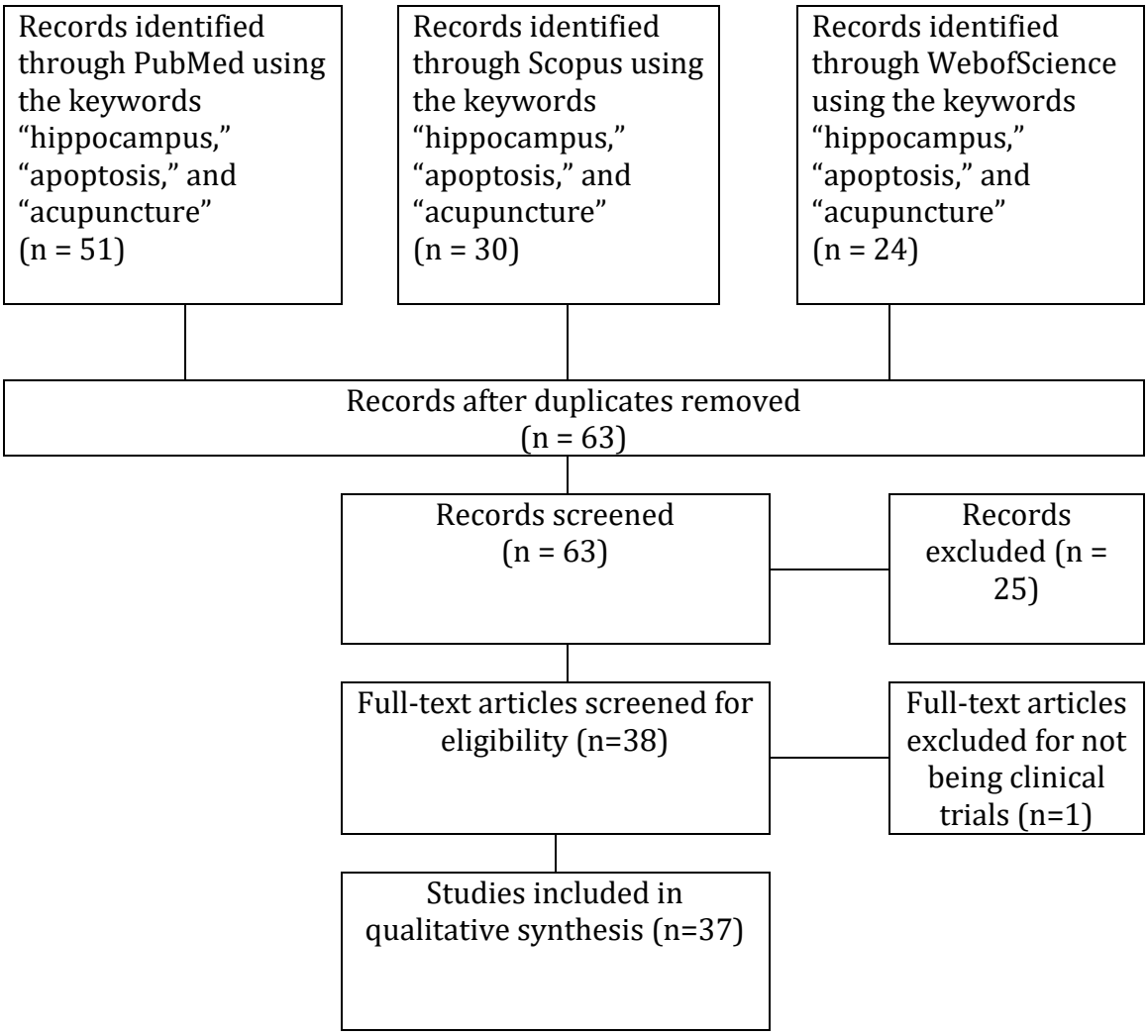

Figure 1. PRISMA chart for systematic review.

Interestingly, all of the studies were animal research studies and showed a statistically significant decrease in hippocampal apoptosis. As summarized in Table 1, 14 of the studies looked at the occurrence of an ischemia or infarction, 4 looked at Alzheimer's, 4 looked at heroin related conditions, and 4 looked at Dementia. 11 out of the 37 studies reported an increase in B-cell lymphoma 2 (BCL-2) expression as

the anti-apoptotic mechanism or a decrease in BCL-2-associated X protein (BAX) expression. As for potential mechanism, 2 studies proposed the p38 mitogen-activated protein kinases (MAPK) pathway, 12 studies did not report any specific mechanism, and the remaining 13 studies suggested a variety of mechanisms.

Author(s), year	# of subjects	Results	Mechanism
1. Huang R, et al., 2019	60 adult Sprague-Dawley rats	EA[1] + gastrodin has synergistic effect to inhibiting hippocampal apoptosis in AD[2] rats. P<0.05	Increased BCL-22[3] expression in CA1[4] region and decreased BAX[5] expression in the CA1[i] region
2. Tian R, et al, 2019	30 Male C57mice weighing 16–18 g	EA inhibited hippocampal apoptosis in mice with cerebral infarction by stimulating the Notch3 signaling pathway and triggering corresponding protein expression. P<0.01	Stimulated the Notch 3 pathway and triggered the expression of the protein TNA-α[6] to ultimately reduce area of infarction, level of inflammatory factors, and expression of caspase-3
3. Han L, et al., 2018	84 rats	The infarction volume and the hippocampal neuron's total apoptosis rate of the EA group decreased. P<0.05	The protein expression of BCL-2 and BCL-2/BAX of the EA group increased; and the protein expression of BAX of the EA group decreased
4. Juan L, et al., 2017	60 12-week-old, male Wistar-Kyoto rats	All tested acupuncture methods prevent target organ damage by inhibiting cell apoptosis in the hippocampus in spontaneously hypertensive rats. P<0.05	Increasing the hippocampal BCL-2/BAX ratio and inhibiting cell apoptosis in the hippocampus
5. Zhu W, et al., 2018	80 Male Wistar rats averaging 200–220 g	Acupuncture treatment improved VD[7] though anti-oxidative and anti-apoptotic mechanisms	Up-regulations of Trx-1/TrxR-1 and inhibitions of ASK1-JNK/p38 pathway.

[1] Electroacupuncture
[2] Alzheimer's Disease
[3] B-cell Lymphoma 2
[4] Cornu Ammonis 1
[5] Bcl-2-associated X protein
[6] Tumor necrosis factor alpha
[7] Vascular Dementia

		which involved the up-regulations of Trx-1/TrxR-1 [8]and inhibitions of ASK1-JNK/p38 pathway[9]. P<0.05	
6. Qing P, et al., 2016	46 Sprague Dawley Rats	EA plus RT[10] lessened the neuronal apoptosis and enlargement of intercellular space. P<0.05	Increasing the expression of GAP-43[11] and SYP[12] in hippocampal CA 3[13] region.
7. Gao YL, et al., 2017	48 male Sprague-Dawley rats	Lower levels of neuronal apoptosis in the hippocampus and VTA[14] were observed in the Heroin +acupuncture group. P=0.013	Inhibition of CHOP[15] and JNK[16] upregulation
8. Li W, et al., 2017	120 Sprague Dawley Rats	EA or Prozac treatment significantly decreased the apoptosis rate in hippocampal cells compared to chronic unpredictable mild stress group. P<0.05	Activated ERK[17] signaling and RSK[18]
9. Lan X, et al., 2017	250 male Sprague Dawley rats	Electroacupuncture reduced hippocampal apoptosis in the CA1 region in rats with cerebral ischemia/reperfusion injury.	Inhibits p38 MAPK[19] signaling pathway

[8] Thioredoxin/Thioredoxin reductase 1
[9] Apoptosis Signal-regulating Kinase 1- c-Jun N-terminal kinase/p38 pathway
[10] Rehabilitation Training
[11] Neuronal growth associated protein 43
[12] synaptophysin
[13]Cornu Ammonis 3
[14] ventral tegmental area
[15] CCAAT-enhance rbinding protein homologous protein
[16] c-Jun N-terminal kinase
[17] Extracellular receptor kinase
[18] ribosomal s6 kinase
[19] p38 mitogen-activated protein kinase

		P<0.05	
10. He X, et al., 2016	Sprague Dawley rats	EA pretreatment plays a crucial role in neuroprotection by decreasing levels apoptotic levels in the hippocampus. P<0.05	Wnt/β-catenin agonist to upregulate the BCL-2/BAX ratio
11. Lin Y, et al., 2016	90 Sprague Dawley rats	After treatment on the middle cerebral artery ischemia model with acupuncture and hypothermia, the apoptotic levels significantly decreased. P<0.01	Down-regulation of BAX level, and up-regulation of BCL-2 level, which is related to reducing the levels of p-MEK2[20] and p-ERK1/2[21]
12. He XL, et al., 2018	5-month-old male SAMP8[22] and age-matched homologous normal aging mice)	EA preventive treatment might improve cognitive deficits and neuropathological changes in SAMP8 mice by reducing neuronal apoptosis in the CA1[23] region and other processes. P<0.05	No mechanism given
13. Tian GH, et al., 2016	75 Adult male Sprague Dawley rats	After EA, the apoptotic levels in the neocortex and hippocampus sections of the central poststroke pain model rats decreased sharply with low-frequency having the best efficacy. P<0.05	No mechanism given
14. Chen Y, et al., 2016	Sprague Dawley Rats	EA pretreatment with different waveforms alleviates sepsis-induced brain injury by improving a variety of	No mechanism given

[20] Phosphor mitogen-activated protein kinase
[21] phospho extracellular receptor kinase
[22] senescence-accelerated mouse prone 8
[23] Cornu Ammonis 1

		factors, including decreasing apoptotic levels. P<0.01	
15. Wu C, et al., 2015	200 male Sprague Dawley Rats	EA alleviates neurological deficit, reduces apoptosis index, and simultaneously upregulates the expression of p-ERK signal pathway in rats subjected to ischemia reperfusion injury P<0.01	No mechanism given
16. Guo HD, et al., 2016	42 rats	EA reduced this Aβ[24]-induced neuronal apoptosis in the hippocampal CA1 region. P<0.001	Upregulation of the autophagy pathway in the Hippocampus
17. Zhang Y, et al., 2016	60 Sprague Dawley rats	The degree of neuronal apoptosis in the hippocampus of rats in the Heroin+ acupuncture and Heroin+ methadone groups was significantly reduced compared with the untreated Heroin group. P<0.001	No mechanism given
18. Zhang Y, et al., 2015	80 Sprague Dawley rats	Acupuncture may exert neuroprotective effects via inhibiting cellular apoptosis, increased GDNF[25] and BDNF[26] expression levels in rat hippocampus experiencing hypoxia-ischemia in the CA 1 region.	No mechanism given

[24] Amyloid beta
[25] Glial cell-derived neurotrophic factor
[26] Brain-derived neurotrophic factor

		P<0.05	
19. Tian WJ, et al., 2015	40 male Sprague Dawley rats	Scalp-acupuncture can regulate the expression of apoptosis related proteins BCL-2 of astrocytes in the CA 1 region of hippocampus in vascular dementia model rats. P<0.05	Up-regulation of decreased BCL-2 protein expression
20. Lin R, et al.,2015	48 male Sprague Dawley rats	EA activated the CREB[27] signaling pathway to inhibit apoptosis in the ischemic penumbra. P<0.05	Activated the CREB signaling pathway
21. Guo HD, et al., 2015	Sprague Dawley Rats	EA alleviated the cellular apoptosis caused by Aβ[28] infusion in hippocampus CA1 regions. P<0.01	Upregulation of the expression of BCL-2 and downregulating the expression of BAX
22. Chen HL, et al., 2014	144 male Sprague Dawley Rats	EA pretreatment can effectively suppress the number of hippocampal apoptotic neurons and increase survival rate of neurons in rats with global cerebral ischemia/reperfusion injury. P<0.05	up-regulation of the expression of GRP 78[29] protein and down-regulating the expression of GADD 153[30] protein in the hippocampus
23. Liu Z, et al., 2015	C57BL/6 mice	EA pretreatment improved neurological outcome, promoted cell survival by inhibiting neuronal apoptosis, and decreasing the BAX/BCL-2 ratio after reperfusion. P=0.013	Decreasing the BAX/BCL-2 ratio

[27] cyclic adenosine monophosphate response element-binding protein
[28] insoluble β-amyloid (Aβ) plaque
[29] glucose regulated protein 78
[30] growth arrest and DNA damage-inducible gene 153

24. Hou X, et al., 2014	40 rats	Acupuncture can prevent brain cell apoptosis in heroin readdicted rats. P<0.05	altering cell ultrastructure thorough regulating the expression of the apoptosis-related genes BCL-2 and Bax and changing BCL-2/BAX ratio
25. Yuan S, et al., 2014	40 rats	Differences in the percentage of TUNEL[31]-positive cells within the hippocampal CA1 region on the 1st day, 3rd day, and 7th day after the AMIR[32] event were significant among all the groups. P<0.05	No mechanism given
26. Zhou HP, et al., 2011	120 senile male Wistar rats	The numbers of apoptotic neurons and positive neurons of caspase-3 significantly decreased in the acupuncture pre-conditioning group versus the cerebral ischemic group. P<0.01	Lowered expression of caspase-3 protein
27. Feng S, et al., 2010	54 male Sprague Dawley rats	EA pretreatment inhibited hippocampal cell apoptosis and decreased hippocampal CA1 caspase-3 activation by +Gz[33] exposure. P<0.05	No mechanism given
28. Ma HF, Ren XJ, Tu Y, Zhou L, 2010	40 male Sprague Dawley rays	Compared with model group, the percentages of apoptotic cells of CA 3 area in acupuncture I and acupuncture II groups lowered remarkably. P < 0.01	Upregulated the expression of Cannabinoid 1 receptor

[31] Terminal deoxynucleotidyl transferase dUTP nick end labeling
[32] Acute myocardial ischemia-reperfusion
[33] High-sustained positive acceleration exposures

29. Zhu Y, Zeng Y, 2011	40 male Sprague Dawley rats	EA improves learning and memory ability and protects pyramidal cells from hippocampal apoptosis in vascular dementia rats. P<0.01	Inhibits expression of p53 and Noxa in the hippocampal CA1 region
30. Dai W et al., 2010	65 Sprague Dawley rats	EA can reduce apoptosis and down-regulate p-JNK level in the hippocampus of depression rats P<0.05	No mechanism given
31. Liu ZB, et al., 2011	40 Sprague Dawley rats	The expression of hippocampal BCL-2 was up-regulated significantly and that of hippocampal BAX protein downregulated considerably in the EA group P<0.01	Up-regulated BCL-2 and down-regulated BAX
32. Li-Da Z, et al., 2018	30 Sprague Dawley rats	Compared with the model group, rat's hippocampus and VTA in the acupuncture group showed significantly fewer apoptotic cells P<0.01	No mechanism given
33. Bao L, et al., 2017	72 Wistar rats	Mongolian medical warm acupuncture was able to protect the hippocampal neurons by changing the content of the apoptosis factors (P=0.024)	up-regulate the expression of the BCL-2 protein in the hippocampus, down-regulate the expression of the BAX protein, and increase the BCL-2/BAX ratio
34. Kim S-T, et al., 2012	64 Male C57BL/6 mice	Acupuncture stimulation at HT8, but not in the tail area, significantly reduced the neuron death, microglial and other	No mechanism given

		factors in the hippocampus. P<0.01	
35. Wang T, et al., 2009	70 male Wistar rats	In Hippocampal CA 1 region, acupuncture decreased the number of apoptotic cells P<0.05	Increase BCL-2 and decrease BAX
36. Yang, J-W, et al., 2018	84 Eight-week-old male Wistar rats	Acupuncture resulted in a total of 31 proteins were considered DEP[34]s, 13 of which were related to reduced apoptosis. P<0.05	Alteration of 13 different proteins
37. Lin R, et al., 2016	30 APP/PS1 double-transgenic mice	EA at the Baihui (DU20) acupoint, but not at a non-acupoint, reverses the aberrant cell death observed P<0.01	altering the expression and processing of BDNF

Table 1. Table synthesizing the important information from the 37 studies being examined from the systematic review.

[34] Differentially expressed proteins

CHAPTER FOUR

Discussion

This systematic review did find a connection between acupuncture and hippocampal apoptosis in the animal model, with all the 37 studies showing a statistically significant decrease in apoptotic levels. However, no clear mechanism was identified as the mechanisms listed varied. Furthermore, many of the studies did not examine a pathway specific to the anti-apoptotic effects as it was not the primary focus of the research but was instead a justification for a condition being mediated by acupuncture as there was either a preestablished connection or apoptosis itself was the mechanism. However, 2 of the studies suggested that the positive effects of acupuncture may be related to the p38 pathway (Lan et al., 2017; Zhu et al., 2018). The studies gave proposed mechanisms seen only once including stimulation of the Notch 3 pathway, upregulation of the hippocampal autophagy pathway, and activation of the cyclic adenosine monophosphate response element-binding protein signaling pathway(Guo et al., 2016; Lin et al., 2015; R. Tian & Wang, 2019).

As shown in Figure 2, the most frequently mentioned mechanism was decreased BAX expression and/or increased BCL-2 expression, often in the CA1 region of the hippocampus. This mechanism was utilized in 11 of the studies. This is a logical pathway as BAX is an apoptosis regulating protein. Under stress conditions, BAX undergoes a conformation change that moves it from the cytosol to the membrane of the mitochondria to release cytochrome c to ultimately promote CASP

3 which is the key player in the execution phase of apoptosis (Q. Wang et al., 2016). Downregulating BAX would reduce this process to in turn reduce apoptosis. BCL-2 inhibits BAX's movement by stabilizing the mitochondrial membrane's barrier function so that BAX can no longer pass through (Q. Wang et al., 2016). It has been suggested that as it pertains to the middle cerebral artery, electroacupuncture promotes BCL-2 expression in the mitochondria by reducing the expression of Death Receptor 5 (Kim et al., 2013).

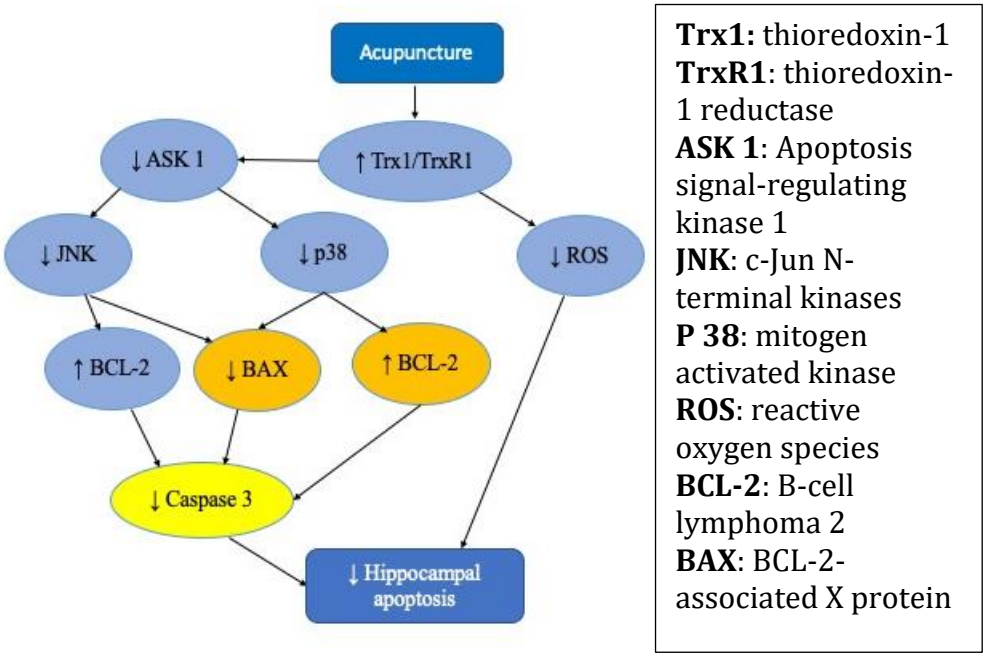

Figure 2. A potential pathway for the downregulation of hippocampal apoptosis by acupuncture

Nevertheless, the results overwhelmingly suggested that acupuncture does have the potential to treat hippocampal apoptosis in the animal model. Further studies are needed to establish a potential mechanism to confirm this connection. The specific acupoints repeated in multiple studies were also recorded on a separate list (Table 2). Some of them were used across multiple studies, including Baihui (GV 20) used in 19 studies, Zusanli (ST 36) used in 9 studies, Dazhui (GV 14) used in 5 studies, and Sanyinjiao (SP 6) used in 4 studies.

Acupoint Name	Acupoint Code	Studies Using Acupoint
“Baihui”	GV 20	1, 2, 5, 8, 12, 13, 14, 18, 20, 21, 22, 23, 24, 26, 27, 29, 32, 36, 37
“Dazhui”	GV 14	1, 22, 24, 29, 32
“Zusanli”	ST 36	1, 5, 6, 9, 13, 14, 15, 35, 36
“Taichong”	LR 3	4
"Quchi"	LI 11	6
“Yintang”	GV29	8, 12, 31
“Chize”	LU 5	9, 15
“Hegu”	LI 4	9, 15
“Sanyinjiao”	SP 6	3, 9, 15, 28
“Si shencong”	HN 1	18
“Shenting”	DU 24	20
“Shenshu”	BL 23	21, 29
"Yingxiang"	LI 20	31
“Shao Fu”	HT 8	35
“Neiguan”	PC 6	3
"Shuigou"	GV 26	3
“Fenglong “	ST 40	28
“Tanzhong”	CV 17	35
“Zhongwan”	CV 12	35
“Qihai”	CV 6	35
“Xuehai”	SP 10	35

Table 2. List of the acupoints used throughout the systematic review and the corresponding studies that used them

Apoptosis, when in excess, can result in degenerative diseases. When insufficiently applied, it can result in cancer or autoimmune diseases. Cell responses that increase or limit apoptotic activity are triggered by various forms of stress such as “hypoxia, energy deprivation, DNA damage or inflammation” (Milisav, Poljšak, & Ribarič, 2017).This literature review focuses specifically on apoptosis in the hippocampal region of the brain. Some conditions involving detrimental damage as the result of hippocampal apoptosis are arsenic exposure (Y. Wang et al., 2015), Cognitive deficits with aging (Yu et al., 2017), acute hypoxia (Coimbra-Costa, Alva, Duran, Carbonell, & Rama, 2017), radiation exposure (El-Missiry, Othman, El-Sawy, & Lebede, 2018), and Lead exposure (Ebrahimzadeh-Bideskan et al., 2016). It can also be the result of certain medications such as the result of Sevoflurane exposure, as used in general anesthesia (Zhou et al., 2016) or chronic use of methylphenidate, also known as Ritalin (Motaghinejad et al., 2017). Furthermore, it can play a role in diseases such as diabetes-induced Ca^{2+} entry and oxidative stress (Kahya, Nazıroğlu, & Övey, 2017) or Alzheimer’s Disease (Hashemi-Firouzi, Komaki, Soleimani Asl, & Shahidi, 2017).

As seen in the various studies utilized by this systematic review, downregulating hippocampal apoptosis is either a mechanism for or indicator of reduction in health conditions including: Alzheimer's, cerebral infarction, cerebral ischemia reperfusion, spontaneous hypertension, vascular dementia, brain injury by heroin addiction, depression like symptoms, accelerated senescence, central poststroke pain, myocardial ischemia, hyper gravity induced impairment, insomnia, and kainic acid induced neuronal death.

The World Health Organization lists acupuncture as an alternative and complementary strategy for treatment post-stroke. In an ischemic stroke, the main mechanisms are suggested to be promotion of central nervous system neurogenesis and cell proliferation, cerebral blood flow regulation in the ischemic area, decreased apoptosis in the ischemic area, and neurochemical regulation (Chavez et al., 2017). Various forms of infractions and ischemia were being studied in 15 out of the 37 articles in this study, a condition also signifying blood flow blockage. It is possible that acupuncture achieves its anti-apoptotic effects either through or with increasing blood flow. Chavez's 2017 systematic review found the most used acupoints to be Baihui (GV20), Zusanli (ST36), Quchi (LI11), Shuigou (GV26), Dazhui (GV14), and Hegu (LI4), showing similarity to this systematic review that had the highest incidence of Baihui (GV 20), Zusanli (ST 36), Dazhui (GV 14) and Sanyinjiao (SP 6) (Chavez et al., 2017).

Another treatment that has been suggested to aid in preventing hippocampal apoptosis is antidepressant drugs, like selective serotonin reuptake inhibitors (SSRIs). While the exact mechanisms are not clearly defined besides the acute upregulation of monoamine neurotransmission, they hold therapeutic potential. It is generally understood that SSRIs inhibit reuptake of serotonin at the synapse, leaving more to bind to the serotonin receptors and go into the bloodstream. There is conflicting data as to whether they increase neurogenesis in the hippocampus or lead to an increase in neuronal turnover (McKernan et al., 2009). Antidepressants were found to help prevent cell death by increasing neurotrophin release and the expression of neurotrophin receptors, activating survival kinases. With repeated

stress, Fluoxetine has been shown to reverse denate gyrus cell death that succeeded chronic stress (Lee et al., 2001).

CHAPTER FIVE

Conclusion

While the specific mechanisms for acupuncture mediating hippocampal apoptosis are still not yet known, the current systematic review concludes that acupuncture does have the ability to do so. The ability to downregulate excessive hippocampal apoptosis has the potential to improve multiple conditions such as Alzheimer's, stroke, and other ischemia related conditions. The mechanisms proposed by many studies involved the intermediary steps of increasing the BCL-2/BAX ratio though both upregulation of BCL-2 and downregulation of BAX, often in the CA1 or CA3 region. Further research is still needed to conclude on a potential pathway. Apoptosis can be induced directly through a variety of mechanisms including irradiation of ultraviolet B, small molecule drug treatments, ligation of death receptors like the mouse monoclonal anti-Fas antibody, and exposure to granule components of cytotoxic lymphocytes (Roberts, Rosen, & Casciola-Rosen, 2004). One of the primary weaknesses in this area of research is the lack of human trials. All of the 37 articles examined in the current study were animal research studies utilizing rats. Of course, it is extremely difficult to examine hippocampal apoptosis in humans due to a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, which is the most widely used methodology for detecting and quantifying hippocampal apoptosis. It requires the cranium to be opened and hippocampal brain tissue to be extracted, which creates significant ethical restrictions from utilizing human subjects (Gavrieli, Sherman, & Ben-Sasson, 1992).

Moreover, it is not certain that the research regarding animal models will directly reflect a human response. However, if a specific mechanism was discovered within the animal model, biomarker testing could be utilized to check for that same pathway as the result of acupuncture in the human model without having to perform a TUNEL assay.

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