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AN ANALYTIC STUDY ON GAP-43 AND S100 EXPRESSION ON INJURED SCIATIC NERVE TREATED WITH CURCUMIN

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ABSTRACT

Sciatic nerve injury causes damage to the muscles and a lack of movement. Effective treatment for this disease is still being studied. This study used the basis of treatment of sciatic nerve injuries with Curcumin and aerobic training and involved the analysis of the neuronal regeneration of brain nerve cells upon damage or injury. Therefore, this research aimed to analyze and determine the effect of the curcumin medicine administration on the proteins in the nerve cells that trigger the regeneration of the sciatic nerve cell that is GAP-43 and S100. The posterior vertebral column's sciatic nerves from mice (young 7-10 days and a middle-aged 4-6 weeks old) were used in this study. Immunocytochemistry and luxol fast blue staining were performed to analyze tissue and protein. Real-time Polymerase Chain Reaction and Bioinformatics analysis study were used in this study. The analysis results show a close correlation between curcumin's medication and the regeneration of the nerve cell. The hypothesis of the study was somewhat confirmed of a correlation of Curcumin and the proteins that is GAP-43 and S100. The study's test results comparison confirmed the relationships between the drug and the proteins.

Keywords
Curcumin, GAP-43, S100, sciatic nerve

Introduction

A sciatic injury is an injury that is caused by a deep and slow stretch that occurs in a muscle due to a singular motion in a few seconds. Sciatic injuries cause significant muscle damage. The sciatic injury that results in tissue damage results in decreased muscle damage results due to an increase in creatinine kinase levels increased in the blood circulation [1,2]. Several studies over the years have investigated the various epidemiological causes of static injury [3, 4, 5]. This paper will discuss the different genetic and biological markers that can be used to identify a sciatic treatment and the basis of treatment of the sciatic nerve injuries with Curcumin and aerobic training

The difficulty of treatment of any neuronal injury has proven difficult in the past years. In some affected individuals with neuronal damage over the years, the remedies that have been consistently used are pharmacological interventions. Speculation that the neuronal injuries have intrinsic healing procedures that are there are molecules in the body that are directly involved in the healing process of the damages. According to research, several pathways are related to the intrinsic pathway of healing. However, comparing the superiority of one pathway to another in the healing process is somewhat difficult. For instance, PI3K Phosphatidylinositol 3-Kinase and cyclic adenine monophosphate contain molecules involved in the healing pathway of neuronal injuries [6]. The susceptibility of human's brain to trauma is increasingly becoming a significant challenge in the relative cases of neuronal injuries. The molecules involved in the healing process can be promoters, inhibitors, or activators of the healing process.

Moreover, other than the molecules involved, some markers are very significant in identifying the availability of neuronal damage after an injury [7]. GAP-43 and S100 are significant biologic

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markers in the neuronal damage and are thus relevant in determining the neuronal nerves' function [8,9,10]. The various therapeutic process of the neuronal injury treatment and pharmacological treatments currently used in the therapeutic procedures of treating neuronal injuries are used to aid the process in which the neurons are regenerated [5]. GAP-43 growth-associated protein is a structural protein that is encoded by the GAP43 gene [11,12]. It is referred to as growth associated since the protein expression is found in masses in neuronal growth and axon development. Thus, the proteins help in the elongation of the axon tips [13,14]. The GAP-43 protein is activated by active phosphorylation through the protein kinase, contributing to the polymerization of actin in the protein [15].

The other type of genetic marker that is directly involved in neuronal damage is the S100 marker. S100 is a group of proteins that are encoded by the S100 genes. The S100 protein comprises two active sites where calcium is bound and has several amino acid sequences. The S100 designates the growth of Schwann cells [16]. During a neuronal injury, cells of the neuronal system tend to adapt to a procedure that leads to the regeneration of new cells, thus the recovering process beginning by the generation of mRNA [1,17,18]. In this research using a laboratory mouse, the study aims at investigating which the most appropriate marker in the neurodegeneration process and the treatment remedies that can be applied in the condition. The aim of this research was to analyze and determine the effect of the curcumin medicine administration on the proteins in the nerve cells that trigger the regeneration of the sciatic nerve cell that is GAP-43 and S100.

MATERIALS AND METHODS

The study is conducted to establish the various biomarkers that reinforce the growth of the neurons.GAP-43 is one biomarker protein involved in the study, directly associated with elongation and regeneration of axons. S100 which is also a protein biomarker of a distressing brain injury. This research will also look into Curcumin drug administration's insights through the intraneural and intravascular, and each's effectiveness. The method of sampling the posterior vertebral column's sciatic nerves from the mice (young 7-10 days and a middle-aged 4-6 weeks old) involved in the study should be done through the harvesting of the sciatic nerve at a maximal length.

The sciatic nerve sampling through the harvesting at maximal length is essential since it will help in the further segmentation of the samples, which will be used in the more profound analysis. A laboratory mouse in which euthanasia was done by deep anesthesia than the mouse vertebral column that is the spine is rapidly detached and frozen. Cryostat sections are then made, stained with immunohistochemical staining, and luxol blue fast staining. A statistical analysis of the results is done—cryostat, luxol blue fast stain, immunohistochemical staining, freezer, healthy laboratory mice, microscope.

Luxol fast blue staining

After the administration of Curcumin to sciatic nerves, the cells were fixed in formaldehyde for 72 hours. The longitudinal sections of the tissues incubated for a minimum of 5 minutes in 95% ethanol and an average of 15 seconds in Lithium carbonate, then washed in 70% ethanol and DDH_2O. The slides were then completely dehydrated and then were ready for observation under a microscope.

Tissue preparation procedure

In the process of tissue preparation of the mice (aged 7-10 and 4-6 weeks respectively), after euthanasia is performed and the spinal cord tissue is rapidly detached and the tissue dissected by

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cryostat in several longitudinal segments, the sciatic nerves are harvested aseptically which are then digested in an enzymatic solution to destroy the sciatic nerves. The longitudinal components of the tissue obtained through cryostat are then mounted into slides and frozen at a temperature of about -20 degrees Celsius.

Immunocytochemistry

The spinal cord tissue slides after days of administration of Curcumin are cultured for 48 hours, after which the tissues become well fixed and can be used for real-time polymerase chain reaction RT-PCR after staining with luxol blue fast stain. The cells are then observed under a tissue microscope.

Real-time Polymerase Chain Reaction

The total quantity of RNA extracted from the spinal cord tissue after weeks of curcumin administration through the TRIzol reagent method. This method was used to quantify the total mRNA that was in the tissues that were extracted. In the real-time PCR, some primers of multiplying the biomarkers of GAP-43, S100, and Beta-actin used were;
GAP43 5'AAGGCAGGGGAAGATACCAC 3'forward primer
GAP43 5'TTGTTCAATCTTTTTGGTCCTCAT3'reverse primer
S100 5`GCTGAGCAAGAAAGAACTGAA3` reverse primer
S100 5`AGCCACCAGCACAACATAC 3`forward primer
B-actin 5`CCTCTATGCCAACACAGT 3` forward primer
B-actin 5'AGCCACCAATCCACACAG 3` reverse primer
The probe sequence for S100 was 5'GTCATGGAGACGCTGGACGAAGATGG 3`, probe sequence for GAP-43 was 5'CAAGGCCGAGAATGGGAAGCTTGTC3`

Bioinformatics analysis study

Studies have shown that the overexpression of GAP-43 impacts greatly on memory enhancement.GAP-43 protein interacts with heterotrimeric G proteins thus activates the G-coupled receptor. In the brain GAP-43 is one of the relevant protein kinase receptors. The location of chromosome 16 in mice and chromosome 3 in humans. The common transcription sites for the GAP-43 protein are located between 52-56 nucleotides, the most relevant codons being CCAAT and TATA. An RNA binding protein is strictly required for the GAP-43 protein to be stable. The molecular weight of GAP-43 protein is about 25kDNA, moreover the protein contains many hydrophobic sites including phenylalanine,2leucine, Isoleucine, Valine and Tyrosine. The S100 protein is known to contain two calcium active sites which are the largest sites has several amino acid sequences. The S100 designates the growth of Schwann cells since it contains genes that code for the growth of the Schwann cells. S100 gene has 2 introns and 3 exons. The first exon which has 54 base pairs codes for the untranslated region of the protein, the other two exons codes carboxyl terminal end. Around four copies of S100 gene in the human genome is likely to be associated with S100A15.S100A7 is a protein containing a calcium binding terminal.

RESULTS

The analysis of the expression of the various biomarkers that indicate neuronal tissue injury is the GAP-43, S100, and the Beta-actin, a significant indication of the three biomarkers using multiple analysis methods were found. In the real-time PCR results, it was evident that the levels of mRNA of S100 in the middle-aged mice were relatively higher than that of the newborn mice.

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However, the levels of GAP-43 in the middle-aged mice were less than in the newborn mice of 7-10 days old (Figure 1).

Table 1: The role of Curcumin in up-regulating S100 mRNA

Dose levels of Curcumin	Expression of S100 mRNA in percentage
High levels	80%
Moderate levels	60%
Low level	30%
control	45%

Thus, it is evident that the high and moderate curcumin drug levels increase the expression of S100 and mRNA. This is because of the high percentages of S100 on the administration of high and moderate doses of Curcumin. However, in the low and control procedure, the levels of S100 are relatively low (Table 1).

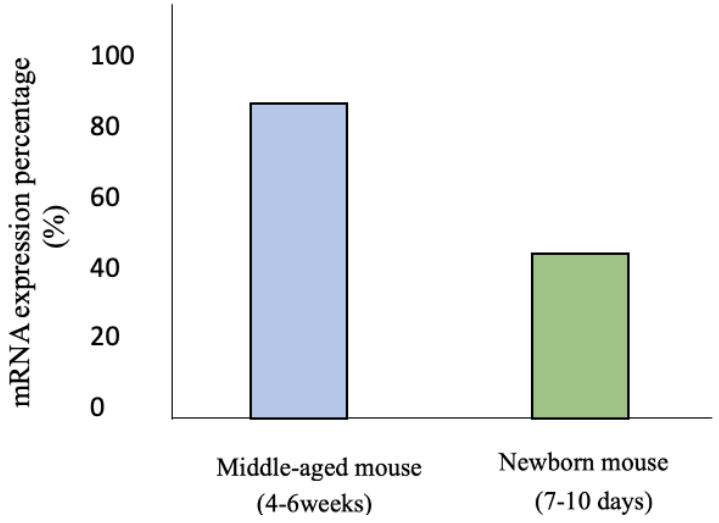

Fig1: The expression of mRNA (Comparison between the middle-aged mouse and the newborn)

Table 2: The expression of various biomarkers in the middle-aged mouse and the newborn mouse

Subjects	Test	Biomarkers	
		GAP-43	S100
Middle-aged mouse (4-6weeks)	+	**45%**	**60%**
Newborn (7-10 days)	+	**40%**	**70%**

To obtain the positive rates in percentage in the cells, the marker -the positive rate in respect to the total number of cells expressed in percentage as follows;

$$\text{Marker positive rate} = \frac{\text{marker positive cell number}}{\text{Total cell number}} * 100\%$$

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In the results, it was evident that S100 is not a reliable marker due to its low expression levels, and it is expressed at the final stages of division. S100 is not detectable in the sciatic nerves. In the cells' numerous culture stages, the expression of S100 protein was at the very last stages with very minimal quantity. Thus, in the experiment, S100 levels were down-regulated, resulting in a very insignificant amount of m-RNA levels. However, it is speculated that the low expression of S100 levels is due to the protein expression in other cells like the adipocytes, chondrocytes, skeletal muscles, and cardiomyocytes, among other cells. Therefore, the S100 biomarker may not necessarily give accurate results as a biomarker of neuronal tissue damage (Table 2).

DISCUSSION

Curcumin's protective effects in the nervous system have been reported in many studies; however, the aspect of nerve regeneration by the Curcumin is not vividly established. The experiment's analysis in determining the presence and the positive impacts of identifying the biomarkers in the neuronal system was successful. GAP-43 growth-associated protein is a structural protein that is encoded by the GAP43 gene [19]. It is referred to as growth associated since the protein expression is found in masses in neuronal growth and axon development. Thus, the proteins help in the elongation of the axon tips [13,20].

The GAP-43 protein is activated by active phosphorylation through the protein kinase, contributing to the polymerization of actin in the protein.GAP-43 and S100 were identified in the tissues of the brain of the two mice used. The young mouse aged 7-10 days was the control of the experiment. The two mice on the experiment generated varied results but within the expected range.GAP-43 protein is a cytoplasmic protein that is found attached to the membrane [6, 21]. Apart from the axonal role of GAP-43, it is also a component of the centrosome. GAP-43 protein plays a critical role in the development of the axon fibers, also it helps in the growth of the central nervous system and helps in the correction of a rat synaptic conditions [14, 20,22].

Neuroregeneration is the process by which nerves and tissues redevelop, thus generating new axons and myelin—the destruction of axon results in the nerve losing a myelin sheath. Therefore, the protein is involved in supporting the growth of nerves in the axon elongation process. In the results, the expression of GAP-43 in the middle-aged mouse was higher than the newborn mouse [23]. This is because the newborn mice's levels are not fully developed, and therefore their expression in invitro could be minimal. However, in the almost fully developed cells of the middle-aged mouse, the GAP-43 were fully developed the higher value of expression of 45% in the test results. The GAP-43 protein leads to the instant development of initial synapses.

Nonetheless, the protein results in boosting the ability of the injured sciatic nerves to sprout and redevelop. Therefore, any mutation in the GAP-43 gene that results in the coding of GAP-43 protein may interrupt the synapses creation system and the sciatic nerve development upon an injury. The GAP-43 protein also plays a crucial role in signal transduction. In the process of Kinase C protein, which is relatively involved in the growth and regeneration of a nerve terminal. Nevertheless, GAP-43 protein is not suitable in the immunohistochemistry application since the method portrays less quantity of proteins available in body tissue. Therefore, if the immunohistochemistry method is used, it may prove difficult to identify the sciatic injury's specific point on a neuron.

Therefore GAP-43 is a vital assay that could be used to establish neuronal injury and administer medication in the injured areas of the neuronal system [24]. The implication that the curcumin drug likely influences the upregulation of S100 proteins protein has proven correct in the experiments. Thus, it is evident that the high and moderate curcumin drug levels increase the expression of S100 and mRNA. S100 is a group of proteins that are encoded by the S100 genes.

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The S100 protein comprises two active sites where calcium is bound and has several amino acid sequences. The S100 designates the growth of Schwann cells [13,25,26]. During a neuronal injury, sections of the neuronal system tend to adapt to a procedure that leads to the regeneration of new cells, thus the recovering process beginning by the generation of mRNA. This is because of the high percentages of S100 on the administration of high and moderate doses of Curcumin. However, in the low and control procedure, the levels of S100 are relatively low. S100 is not detectable in the sciatic nerves. In the cells' numerous culture stages, the expression of S100 protein was at the very last stages with very minimal quantity. Thus, in the experiment, S100 levels were down-regulated, resulting in a very insignificant amount of m-RNA levels. However, it is speculated that the low expression of S100 levels is due to the protein expression in other cells like the adipocytes, chondrocytes, skeletal muscles, and cardiomyocytes, among other cells. Therefore, the S100 biomarker may not necessarily give accurate results as a biomarker of neuronal tissue damage.

The luxol blue fast staining is done mainly to distinguish the myelin sheath. It is accurate to state that in the experiment’s myelin sheath was very detectable in samples in which the cell had high administrations of Curcumin than those with lesser dosages of curcumin drug. The immunohistochemical staining method also established the same basis where the staining in the high and moderate groups was more pronounced than in the low doses of and in the model, which had lesser quantification. However, the accuracy of the S100 biomarker, the biomarker, can never be used solely to determine neuronal damage. The use of curcumin drugs has proved useful in various diseases such as Alzheimer's disease.

The administration of curcumin doses in high quantities is significantly essential. The mRNA degrades quickly at the same time that proteins accumulate due to the growth of sciatic nerves. Thus, the development and regeneration of sciatic nerves consequently indicate the recovery process of the nerve. Since there is high growth and proliferation when Curcumin is administered in the cells, it is appropriate to say that Curcumin's effect in cell proliferation and development is very relevant. Therefore, the sciatic nerve's regeneration is relatively associated with the increase in functionality of S100 protein and thus increasing the immune reactivity.

CONCLUSION

In summary, the administration of curcumin doses in high quantities is significantly essential. During a neuronal injury, cells of the neuronal system tend to adapt to a procedure that leads to the regeneration of new cells, thus the recovering process beginning by the generation of mRNA. This is because of the high percentages of S100 on the administration of high and moderate doses of Curcumin. However, in the low and control procedure, the levels of S100 are relatively low. S100 is not detectable in the sciatic nerves. In the cells' numerous culture stages, the expression of S100 protein was at the very last stages with very minimal quantity. Thus, in the experiment, S100 levels were down-regulated, resulting in a very insignificant amount of m-RNA levels.

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