Effect of electroacupuncture to prevent selenite-induced cataract in Wistar rats

Efeito da eletro-acupuntura na prevenção da catarata induzida por selenito de sódio em ratos Wistar

INTRODUCTION

Cataract is the main cause of blindness worldwide. Currently the only form of treatment is surgical removal of the opacified lens(1-2). It is estimated that a delay of 10 years in the development of the cataract would reduce the necessity for surgery in 45%, saving millions. Also, even after cataract surgery, some postoperative-related complications might occur such as posterior capsular opacity, uveitis, glaucoma, cystoid macular edema, error in intraocular lens power calculation, retinal detachment, corneal edema or endophthalmitis(3-4).

The opacification of the crystalline occurs by aggregation of cytoplasmic lens proteins due to modifications in the intermolecular interactions that are the result of chemical actions that include oxidative stress, glycation, proteolysis, transamidation, phosphorylation and increase of the calcium levels(5). Theoretically, conditions that influence these chemical ac-
Acupuncture has been performed by inserting fine metallic needles at specific loci of the human body, which may be manipulated manually or by electrical stimulation (electroacupuncture)\(^{(1)}\). Its effects are based on secretion of neurotransmitters (e.g., endorphins, dynorphins, serotonin, kinins) within neural synapses in the spinal cord and central nervous system, which cause reduction in pain threshold, vasodilation and muscular relaxation\(^{(9,10)}\). According to some Chinese authors, many ocular disorders could be treated with acupuncture (e.g., dry eye, allergic conjunctivitis, glaucoma, and cataract) and many textbooks covering acupuncture specialization courses recommend some acupoints for the treatment and prevention of cataract\(^{(11-13)}\). However there is only anecdotal basis to support this treatment modality and its real benefits are not known. The aim of this study is to evaluate the effect of the electroacupuncture preventing sodium-selenite-induced cataract in an experimental model.

### METHODS

Five pregnant Wistar-albino rats were obtained from the Center of Development of Experimental Models for Medicine and Biology (CEDEME) at the Federal University of São Paulo, Paulista School of Medicine (UNIFESP - EPM) in São Paulo, Brazil. All experiments were performed in accordance with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research and "Guiding Principles in the Care and Use of Animals"\(^{(16-17)}\). The experimental protocol was approved by the Research Ethics Committee of the university.

Each Wistar albino female rat and its litter of 10 pups were housed in plastic cages and remained in a separate animal room with adequate air turnover, constant control of temperature (22°C), lighting was set for 12-hour on and 12-hour off, receiving standard rat chow (Purina-Nuvilab, São Paulo, Brazil) and water ad libitum. At 10-12 days of age, two pups from each female rat were randomized into five groups of 10 animals each, as follows:

In Group 1, Control group, no specific procedure was performed. In Group 2, Selenite group, cataracts were induced by a single subcutaneous injection of sodium selenite (Ophthalmos Ltda., São Paulo, Brazil) (30 μmol/kg body weight) to 10-12-day-old rat pups as previously described\(^{(15)}\). In Group 3, Anesthesia group, the same amount of sodium selenite was injected and rats underwent inhalation of ethyl ether (Quimesp, São Paulo, Brazil) anesthesia for 10 minutes daily for one week. In Group 4, Electroacupuncture group, pups underwent the same procedure as in Group 3, but also receiving electroacupuncture by an electrical stimulator Plexus AP 585 WMV 2 Hz, 50 mA (Biotherapy, São Carlos, Brazil) once a day applied to acupuncture needles (0.25 mm x 15 mm / Lautz-Brazil) which were inserted in the equivalent of the human

### RESULTS

Both male and female 10-12-day-old Wistar albino rats were used. There was no statistically significant difference between genders (p=0.934). Table 1 shows cataract grade distribution by group. All control rats lenses (Group 1) were clear - stage zero (Figure 1A). Eight of ten rats (16 eyes, 80%) in Group 2 (Selenite group) developed bilateral stage 5 cataract (Figure 1B), whereas one rat developed a bilateral stage 4, and another a bilateral stage 6. In Group 3 (Anesthesia group) 70% of eyes (14 eyes) had a stage 5 cataract (Figure 1C), and 30% (6 eyes) presented as stage 4.

In rats treated with electroacupuncture (Group 4), no cataract was observed in 9 eyes (45% of eyes) (Figure 1D), while two eyes developed a stage 1 cataract (10%), one eye stage 2 (5%), one eye stage 3 (5%), two eyes stage 4 (10%), and the remaining five eyes had stage 5 cataract (25%). On the other hand, seventy per cent of pups’ eyes (14 eyes) in Group 5 (Sham group) developed stage 5 cataract (Figure 1E), ten per cent (2 eyes) had stage 6, and twenty per cent (4 eyes), stage
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Although the rate of opacification in the selenite model is much more rapid than in human cataract, it has many general similarities to human cataract like increased calcium, protein aggregation, decreased water soluble proteins and level of reduced glutathione\(^5,24\). A study using confocal microscopy and a lipid membrane probe suggested that morpho-

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Stage zero</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>0.00</td>
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<tr>
<td>Selenite</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>5.00 ± 0.46</td>
<td>5.00</td>
</tr>
<tr>
<td>Anesthesia</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>14</td>
<td>0</td>
<td>4.70 ± 0.47</td>
<td>5.00</td>
</tr>
<tr>
<td>Electroacupuncture</td>
<td>20</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1.85 ± 2.01*</td>
<td>1.00</td>
</tr>
<tr>
<td>Sham</td>
<td>20</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>4.90 ± 0.55</td>
<td>5.00</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>29</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>46</td>
<td>4</td>
<td>3.29 ± 2.24</td>
<td>4.50</td>
</tr>
</tbody>
</table>

* = Statistically significant (p<0.001)

**DISCUSSION**

In accordance with the previous studies\(^5\), a single injection of selenite (Group 2) resulted in the formation of 100% nuclear cataracts by day 7 after selenite treatment in our study. Groups 1 and 2 acted as normality and disease controls, respectively, allowing exclusion of environmental interaction or absence of the effect of the drug. Group 3 was important to exclude the possible interference of anesthetic drug effect. In Group 5, non-acupoints were chosen near real points on the upper and lower members to confirm the effectiveness of acupoints according to the traditional Chinese theory. Our results demonstrated that daily electroacupuncture treatment prevented or inhibited opacification in a rat model for cataract formation in most of the cases. The variability in the response in Group 4 may indicate that acupuncture has an individual susceptibility factor in the treatment.

Acupuncture theory suggests many other acupoints to treat cataract, including systemic and local points. In this study we used only two systemic acupoints bilaterally, and no local points, because it would not be possible to insert many needles in these little rat pups due to anatomical conditions.

With regard to cataract, the selenite model was selected because of the rapid, effective and reproducible cataract formation. Although the rate of opacification in the selenite model is much more rapid than in human cataract, it has many general similarities to human cataract like increased calcium, protein aggregation, decreased water soluble proteins and level of reduced glutathione\(^5,24\). A study using confocal microscopy and a lipid membrane probe suggested that morpho-

**Table 1. Experimental cataract classification, mean ± standard deviation of cataract grade according to groups (N=100 eyes)**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
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<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
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<th>Stage 5</th>
<th>Stage 6</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Selenite</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>5.00 ± 0.46</td>
<td>5.00</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>14</td>
<td>0</td>
<td>4.70 ± 0.47</td>
<td>5.00</td>
</tr>
<tr>
<td>Electroacupuncture</td>
<td>20</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1.85 ± 2.01*</td>
<td>1.00</td>
</tr>
<tr>
<td>Sham</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>4.90 ± 0.55</td>
<td>5.00</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>29</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>46</td>
<td>4</td>
<td>3.29 ± 2.24</td>
<td>4.50</td>
</tr>
</tbody>
</table>

* = Statistically significant (p<0.001)

**Table 2. Percentage of weight gain in all groups one week later compared to baseline**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean (%)</th>
<th>Median (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>34.14</td>
<td>31.41</td>
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<tr>
<td>Selenite</td>
<td>20</td>
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<tr>
<td>Anesthesia</td>
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<td>Electroacupuncture</td>
<td>20</td>
<td>30.25</td>
<td>31.25</td>
</tr>
<tr>
<td>Sham</td>
<td>20</td>
<td>26.36</td>
<td>28.17</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>30.38</td>
<td>31.25</td>
</tr>
</tbody>
</table>
logical changes in the nucleus of the human cataractous lens were best approximated by those seen in the selenite model. Free radicals secondary to cell metabolism and photochemical reaction have the potential to damage lipids, proteins and nucleic acids. The lens presents antioxidant mechanisms like ascorbate, catalase and glutathione systems to protect against oxidative stress. A reduction in the activity or level of these antioxidants occurs in old age and causes cumulative damage that leads to formation of senile cataract. Some studies suggest that selenite probably acts as an oxidative agent.

Wang et al. demonstrated that electroacupuncture could regulate the production and clearance of free radicals decreasing the contents of nitric oxide and malondialdehyde and increasing the activities of superoxide dismutase and glutathione peroxidase. Pogosyan et al. found improvement in the antioxidant status of erythrocytes after acupuncture treatment measuring contents or activities of several antioxidants including the glutathione system. Electroacupuncture probably would act modulating the concentration or activities of antioxidants in lens cell.

Several free radical scavenger and antioxidant substances, such as pantethine, caffeic acid phenethyl ester, WR-77913, flavonoids and disulfiram have been tested in experimental research for the prevention of cataract in animal models with satisfactory results but for reasons of effectiveness, research for the prevention of cataract in animal models with satisfactory results but for reasons of effectiveness, these drugs have not been studied yet for human senile cataracts. Conversely, acupuncture is a practical and cheap therapeutic modality and, when applied by a trained acupuncturist with standard disinfection method, has a very low adverse effect rate.

Längle et al., found that systemic intake of SDZ ICT 322, a selective serotonin receptor antagonist, induced cataract in rats, suggesting possible interference of neurotransmitters in cataract development. Some animal data and clinical observations suggest that acupuncture modulates activities in the central nervous system and influences treatment areas via release of neurotransmitters or hormones, or even by direct modulation of neural pathway. Secretion of many neurotransmitters has been associated with acupuncture mechanisms like endorphins, dynorphins, serotonin, catecholamines. It is possible that acupuncture plays a neurohumoral role in preventing cataract.

Although the prevalence of acupuncture use has increased in the western world, scientific data about its efficacy and mechanisms is scarce; mainly in the ophthalmologic area. This experimental study is the first step in the scientific research to evaluate the effect of acupuncture on prevention of cataract. It was performed according to scientific criteria and represents a new subject for future research. Cataract is a primary cause of human blindness and prevention should be the most important therapeutic approach for this disease. The results of the present study cannot be interpreted as evidence that acupuncture can protect humans from cataract; therefore it is necessary to carry out further experimental studies to elucidate the mechanisms involved in this kind of therapy and clinical trials involving humans to support this hypothesis.

REFERENCES


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