FUNCTIONAL EVALUATION OF THE VESTIBULAR SYSTEM OF GUINEA PIGS POISONED BY AN ORGANOPHOSPHATE THROUGH CALORIC STIMULATION

Avaliação funcional do sistema vestibular de cobaias intoxicadas agudamente por organofosforado por meio da prova calórica

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ABSTRACT

Purpose: this study aimed to assess the functioning of the vestibular system of guinea pigs exposed to the organophosphate chlorpyrifos, acutely, through caloric stimulation electronystagmography. **Methods:** the research conducted an experimental electronystagmography of guinea pigs exposed to organophosphate for 10 consecutive days, at doses 0.5 mg / kg / day and 1.0 mg / kg / day intraperitoneally and compared with a control group that received distilled water administration. We performed caloric ice (10 º C) and compared the variables frequency of appearance of nystagmus in 10 seconds (u / s) and angular velocity of the slow component (º / s). **Results:** the results showed no statistically significant difference in the comparison of variables between groups. **Conclusion:** we conclude that the tested doses of the pesticide organophosphate chlorpyrifos caused no detectable functional damage in the caloric test.

KEYWORDS: Postural Balance; Vestibule, Labyrinth; Insecticides, Organophosphate; Pesticides; Drug Toxicity

INTRODUCTION

Body balance is maintained by the joint operation of three structures: the vestibular system, the visual system and the proprioceptive system. The vestibular system detects the sensations of balance, assisting in the coordination of head and eye movements, and also in adjustments of body posture. Dysfunctions in this system may result in unpleasant sensations such as dizziness, nausea and a feeling of imbalance accompanied by uncontrollable eye movements, i.e., nystagmus[1,2].In humans, the vestibular system consists of three key components: a peripheral sensory system, a central processor, and a motor response mechanism. The peripheral system consists of a set of motion sensors which send information to the central nervous system (CNS)[3].

The central vestibular system produces a response to head movement which is transmitted to the extraocular muscles and the spinal cord, triggering the vestibulo-ocular reflex (VOR) and vestibulo-spinal reflex (VSR).The VOR generates eye movements, which maintain vision stable during head movement. Electronystagmography (ENG), in its different forms, is the most appropriate procedure for assessing ocular and/or vestibular information. It records eye movement, which represents the major element in the investigation and assessment of vestibular disorders [4,5]. The standard response for

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the exam is nystagmus, which appears every time the vestibular system is stimulated. This is due to an imbalance between antagonistic vestibular forces which helps keep the orientation of the eyeballs. Likewise, a pathology in the peripheral system or its central connections may determine the onset of nystagmus. Nystagmus is the fundamental element for analysis of VOR in ENG. Abnormalities of VOR can be found in the research on caloric stimulation[4].

Some chemicals cause harm to humans and the environment, e.g., pesticides, some of which are known for their neurotoxic properties. Pesticides are often considered responsible for poisoning agricultural workers that are exposed to them, especially in developing countries[6,7]. Pesticides are an important risk factor for human health. These products are used in large-scale by various productive sectors and more intensely by the agricultural sector[8,9].

As a type of pesticides, organophosphate (OP) insecticides are cholinesterase inhibitors that cause varying degrees of toxicity to humans. Because of the benefits that this pesticide brings to plantations, agricultural workers usually underestimate its effects on their own health when they are exposed to the product[10].This exposure often causes clinical changes that silently affect the lives of workers[11]. It is known that short-term exposure causes the so-called acute effects. This clinical picture varies in intensity, and may be characterized by nausea, vomiting, headache, dizziness and other symptoms. Dizziness is a major symptom, considered one of the clinical manifestations of exposure to pesticides[7,8].

It is important to note that not only agricultural workers are exposed to pesticides, but also their families, which live next to plantations. This fact highlights the need for research that deeply analyzes methodologies for assessment of exposure to toxic agents and the risks associated with these products, both in cases of acute poisoning and long-term exposure to pesticides[11].

The objective of this research was to evaluate, by means of electronystagmography, the functioning of the vestibular system of guinea pigs acutely exposed to organophosphate chlorpyrifos.

METHODS

This research was a prospective cohort study, based on Severino (2008)[12]. It was conducted in accordance with the Ethical Principles in Animal Research adopted by the Brazilian Society of Laboratory Animal Science (SBCAL) and with Law No. 11.794, of October 8, 2008,enforced by the National Council for Control of Animal Experimentation (CONCEA), which establishes procedures for the scientific use of animals. It was approved by the Ethics Committee on Animal Research of the School of Medicine of Ribeirão Preto, University of São Paulo, under no. 135/2011, on November 8, 2011.

This study used 18 male albino guinea pigs, species *Caviaporcellus*, English breed, weighing between 300 and 500g, with Preyer's reflex but no signs of otitis externa and/or media, and absence of tympanic perforation after otoscopic assessment. Preyer's reflex is assessed by observation of small twitching movements in the pinna of guinea pigs, when stimulated with sounds of small and medium intensity. Such reflex is used to assess auditory function in rodents[12].

The animals were housed in the Animal Laboratory of Experimental Surgery of the Department of Surgery of the School of Medicine of the university. They were kept in collective cages with shavings, sorted by group, under light/dark for 12 hours, controlled temperature and humidity, with unlimited access to feed and water.

For poisoning purposes, the animals received intraperitoneal application of the pesticide OF Pyrinex 480 CE®, marketed by Milenia Agrociências S/A, whose active ingredient is chlorpyrifos. Pyrinex ® 480 EC is an OP insecticide whose mode of action is by contact and ingestion, It is recommended for pest control in cotton, potatoes, coffee, citrus, beans, apples, corn, soybean, what and processing tomato for industrial use. The product is registered with the Ministry of Agriculture, Livestock and Food Supply (MAPA) under No. 09 298.

The intraperitoneal route was chosen to ensure the absorption of the rate; otherwise the exact rate might not have been absorbed.

The guinea pigs were divided into three groups: group I (control) received intraperitoneal administration of distilled water once daily, in the same volume as the rate of pesticide for the weight of the animal; in group II, the pesticide was intraperitoneally administered in a single daily rate of 0.5 mg / kg / day; in group III,pesticide was administered intraperitoneally in a single daily rate of 1.0 mg / kg / day – all of them for ten consecutive days. The weight of the guinea pigs was monitored daily for calculation of the appropriate rate of pesticide to be used. The control had five guinea pigs, and the treated groups II and III had six and seven guinea pigs, respectively. The number of guinea pigs per group was defined according to the standards of the National Health Surveillance Agencyfor toxicological studies, with a minimum of five animals for each rate tested in the experiment. Thus, the minimum number of animals required was chosen for the control group, and one animal was added as the rate increased because of the risk of losing animals.

For animal handling and administration of the pesticide, personal protective equipment was used as recommended in the product information. The rate of the pesticide was chosen based on oral L_D 50 for rats of Pyrinex 480 CE ®, found in the product information, which is 300 mg / kg, and it considered the standards of ANVISA whereby the rate must not exceed 80% of L_D50.

The functional assessment selected to be performed in this study was the ice water caloric test(water at 10 º C), according to the protocol of Marseillan, Grellet and Colafêmina (1969) within the spectrum of the ENG test. The variables selected were: frequency of nystagmus onset (Fn) in 10-second recording, measured in units per second (u / s) and angular slow-component velocity (SCV), measured in degrees per second (° / s).

ENG was performed 24 hours before starting the experiment and 24 hours after the last administration of pesticides and distilled water in the control group. The first test was carried out so that it could be used as a comparison of the situation of the vestibular system of guinea pigs, before and after poisoning. The second procedure was performed to assess the effect of the pesticide in the vestibular system of these guinea pigs by means of a functional test by analyzing their vestibulo-ocular reflex.

The technique used for the study of vestibular responses to thermal stimulation was developed by Marseillan, Grellet and Colafêmina in 1969. This technique is based on the electronystagmographic recording of ocular reactions to thermal stimulation in guinea pigs prepared for chronic experiments with permanent electrodes[13]. All animals selected were submitted to an aseptic surgical procedure for implanting one frontal subcutaneous electrode and two subcutaneous electrodes in the periorbiculares regions (left and right) under intramuscular anesthesia with 2% xylazine hydrochloride (0.5 ml / kg - Dopaser ®; Laboratórios Calier do Brasil Ltda) and ketamine hydrochloride 10% (0.9 ml / kg - Ketamin ® 50mg/ml; Laboratório Cristália).

The implanted electrodes were made of metal wire as the model developed by the creators of the technique (Figure 1), and the head of the animal had an external electrical connector to capture the signal (Figure 2).

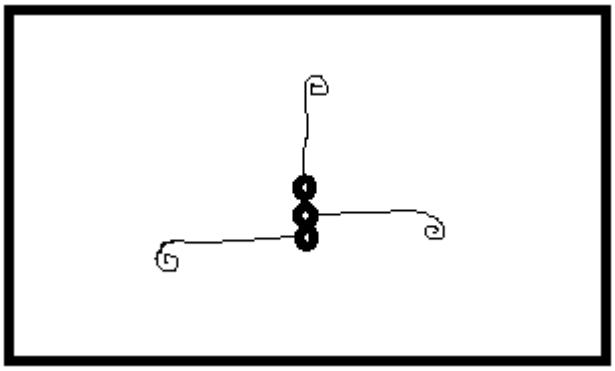

Figure 1 – Image of permanent electrodes implanted

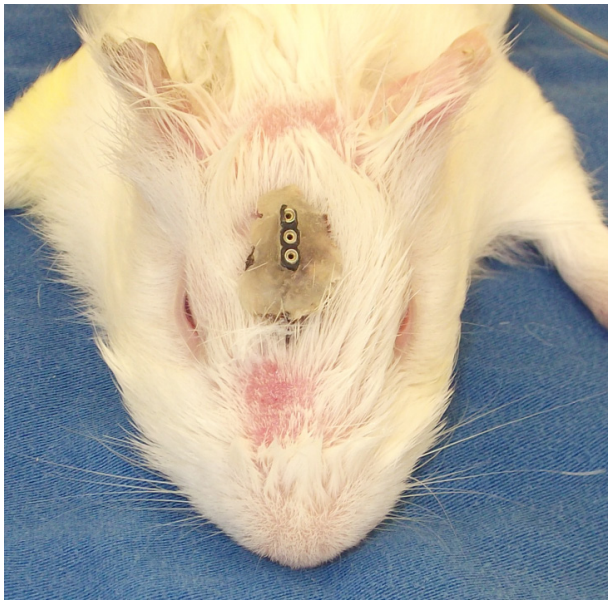

Figure 2 – Photograph of guinea pig's head with permanently implanted electrodes and external electrical connector

After healing of the surgical wound, the animals were kept in boxes suitable for recording of nystagmus, caused by irrigation with 20 ml of ice water at 10 º C in the external auditory canal.

Ten days after administration of the pesticide, the guinea pigs were subjected to a new recording of nystagmus (Figure 3), using the permanent electrode previously implanted, again by irrigation of the external auditory canal with 20 ml of water at 10 º C.

For test recording, single-channel Nistagmocil ® equipment was used to record nystagmus induced by the RALPAC method (air or water caloric stimulation). The equipment is portable and simple to use, consisting of a registration system with special pre-amplifiers coupled by capacitors which record changes in corneo-retinal potential but do not record the absolute values of these potentials.

The preamplifiers were adjusted for the ink pen movement within 2cm on the recording paper. The electrodes are polarized so that right-sided eye movement moves the ink pen upwards and left-sided eye movement moves the ink pen downwards.

Movements were calibrated by stabilizing the ink pen and keeping the guinea pigs' heads in a stable position.

Figure 3 – Photograph of electronystagmography recording system

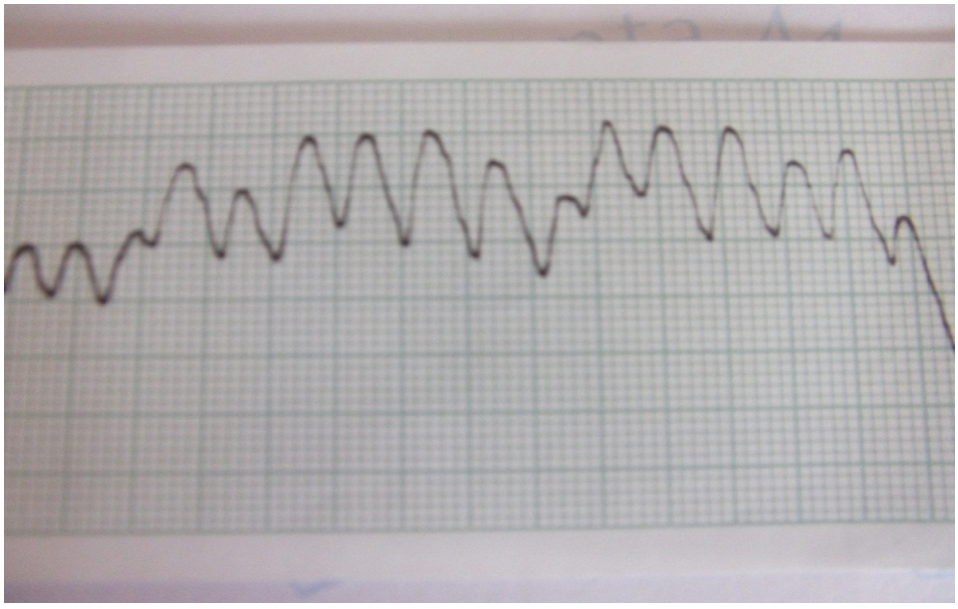

Figure 4 – Photograph of recording of right-sided nystagmus of a guinea pig in the Control Group, with stimulation of left ear

The values of frequency of nystagmus onset (Fn) were obtained by counting the number of nystagmus present in 10-secondrecording, based on paper speed of 25 mm / s in the machine.

The values for angular slow-component velocity (SCV) were obtained with three measurements of nystagmus with equal bases at three different locations: at the beginning, middle and end of the record. The value considered for SCV for each guinea pig ear was the average of the nine values measured.

The data were treated statistically using the program Statistica, version 9.0. The collected data were tested for normality with Lilliefors test. For normal data within groups, Student's t-test was used for dependent variables, while ANOVA was used for data among groups, considering the level of significance of 5%.

RESULTS

ENG results, when compared among groups, showed no significant difference across ears in any of the groups, which shows similarity among ears both for the variable Fn and the variable SCV. The comparison test in the pre-experiment among groups I, II and III showed no significant difference between them; thus, the groups were homogeneous.

These tests were performed to ensure homogeneity among the ears and among the groups. This fact allows the comparison of the results in the post-experiment, considering they were statistically similar beforehand.

The mean Fn and SCV for the ears of the animals in each group are shown in Tables 1 and 2.

Table 1 - Comparison of mean values of frequencies of nystagmus onset (u/s) between the pre-and post-experiment of Groups I, II and III (#)

Group	Pre-Experiment	Post-Experiment	*p-value*
G I (n= 5)			
RE	5.18±1.12	4.08±1.29	0.28
LE	4.78±0.70	4.28±1.39	0.37
G II (n= 6)			
RE	4.95±2.04	3.96±1.18	0.16
LE	4.63±0.98	4.08±1.30	0.20
G III (n= 7)			
RE	4.42±1.52	3.94±0.77	0.47
LE	4.45±1.27	5.04±0.79	0.37

(#) Data were shown as mean ± standard deviation
RE–right ear; LE –left ear; n - number of guinea pigs; u/s - unit/second
Intra-group test –*Student's t-test;* Intergroup test - *ANOVA*

Table 2 - Comparison of mean values of angular slow-component speeds(°/s) between pre- and post-experiment of Groups I, II and III(#)

Group	Pre-Experiment	Post-Experiment	*p-value*
G I (n= 5)			
RE	59.75±19.19	63.21±21.39	0.79
LE	55.59±23.25	73.39±22.70	0.09
G II (n= 6)			
RE	59.05±17.42	57.34±15.74	0.76
LE	44.31±14.37	52.86±11.19	0.36
G III (n= 7)			
RE	55.85±23.17	69.45±24.18	0.17
LE	62.66±16.71	68.31±25.69	0.54

(#) Data were shown as mean ± standard deviation
RE–right ear LE –left ear; n –number of guinea pigs; ° /s – degree per second
Intra-group test –*Student's t-test;* Intergroup test - *ANOVA*

The comparison of the groups in the post-experiment showed no statistically significant difference in the variables Fn and SCV.

DISCUSSION

There was no statistically significant difference in the post-experiment tests, showing that the OP pesticide chlorpyrifos at the rates of 0.5 mg / kg / day and 1mg/kg for 10 days did not cause detectable damage in the functional test of the vestibular system of guinea pigs.

Researchers warn that the vast majority of serious and fatal accidents caused by pesticides, including accidental work-related exposure and suicidal intake, is due to highly toxic OP insecticides and carbamates[14,15]. A study on the quality of life and hearing health of workers exposed to pesticides noted that these compounds cause worse life quality scores and hearing health effects in comparison with population that had not been exposed, showing the influence of these substances on human health[16].

Several studies were conducted to evaluate the impact of using OP pesticides on human health. As a method, these studies used interviews with rural workers exposed to these compounds[17-24].The results showed dizziness as a recurrent symptom reported by these workers. This suggests functional impairment, but the present study did not find significant differences between intoxicated and non-intoxicated guinea pigs by the caloric test.

It should be noted that interviews with workers are subjective and, therefore, are not as reliable as the functional test performed in the present study. This examination gives a measurable result of the effect caused by exposure to the substance in question. In contrast to this research, those studies assess chronic symptoms on humans, while the sample of this research was exposed for a brief period and assessed the acute effect on animals.

The study that evaluated morphological changes of the vestibulocochlear apparatus of guinea pigs exposed to OPs found evidence of changes by electron microscopy scanning in cochleae, saccules and utricles. This result shows that a morphological change may suggest a functional disorder[25]. However, in the present study, there has been no manifestation of functional symptoms, and this may have been due to difference of the active ingredient tested, although it was an organophosphorus compound.

The results of this study showed no statistically significant difference between the variables studied. This is at odds with other researchers, who found functional changes in sensorimotor tests in humans as a change in the measure of postural sway with eyes closed and on a smooth surface, suggesting subclinical effect of the pesticide on the vestibular and proprioceptive systems[7]. It also disagrees on the research which examined the vectoelectronystagmography results of workers chronically exposed to OPs, where changes were found in 50% of cases, with these changes being translated into caloric hyperreflexia in VENG; however, the present study evaluated the acute effects of this organophosphate compound[26].

The study on acutely exposed rural workers is difficult to conduct, since most of these subjects have been previously exposed to these substances for long periods. Another limitation of the study with humans is that they are rarely exposed to only one agent, which hinders the evaluation of the specific effects of a single active. For this reason, experimental studies are an alternative to search the isolated effect of an active ingredient.

The findings of this research are similar to those found in a study conducted in China, a country with strong agricultural production, which sampled 301 children aged 23-25 months exposed to OPs. This study revealed the presence of high levels of OP metabolites in urine tests, but found no relationship between these data and the level of child development[27].This reinforces the notion that even in the presence of this compound in the body, a functional relationship with this result is often not found.

Acute exposure to organophosphates may not be able to represent changes in functional tests, as shown in the study that evaluated the effects of these compounds in the auditory system of guinea pigs. The research found changes in outer hair cells of the cochlea after acute intoxication by OPs, but this morphological lesion did not translate into functional changes in the peripheral auditory system of guinea pigs assessed by tests for distortion product otoacoustic emissions and brainstem auditory evoked potential[11].

The acute period of exposure and assessment may have been the determining factor for the non-significant results in this study, pointing to the need for further research with different rates and for a longer period of time so that the use of this substance can be determined more reliably.

Although the results remain controversial with respect to morphological effects of pesticide exposure, studies show the observation of signs and symptoms in the population exposed to these compounds, and they also show that such reports are due to the incorrect use of the substances, the lack of proper product handling by workers and underestimation of the effects of these compounds on human health[28,29].

CONCLUSION

The organophosphate pesticide chlorpyrifos, at the rates tested, showed no functional damage to the vestibular system of acutely poisoned guinea pigs.

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RESUMO

Objetivo: avaliar o funcionamento do sistema vestibular de cobaias expostas ao organofosforado clorpirifós, de forma aguda, por meio da prova calórica da eletronistagmografia. **Métodos:** a pesquisa do tipo experimental realizou a eletronistagmografia de cobaias expostas a organofosforado durante 10 dias consecutivos, nas doses 0,5mg/kg/dia e 1,0mg/kg/dia por via intraperitoneal e comparadas com grupo controle que recebeu administração de água destilada. Foi realizada prova calórica gelada (10ºC) e comparadas as variáveis frequência de aparecimento de nistagmos em 10 segundos (u/s) e velocidade angular da componente lenta (º/s). **Resultados:** os resultados não demonstraram diferença estatisticamente significante na comparação das variáveis entre os grupos. **Conclusão:** conclui-se que nas doses testadas o agrotóxico organofosforado clorpirifós não causou danos funcionais detectáveis na prova calórica.

DESCRITORES: Equilíbrio Postural; Vestíbulo do Labirinto; Inseticidas Organofosforados; Praguicidas; Toxicidade de Drogas

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