SCIENTIFIC ARTICLE

Effects of malathion nebulization on the histopathology of the respiratory tract of Wistar rats, treated with sublingual atropine sulfate eye drops

Efeitos da nebulização de malathion na histopatologia do aparelho respiratório de ratas Wistar, tratadas com colírio de sulfato de atropina pela via sublingual

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Abstract

This study sought to evaluate the histological damage to the respiratory tract caused by inhaling an aqueous solution of 50% malathion, as well as the protective effects of sublingually-applied 1% atropine sulfate eye drops in rats. We searched the literature for the signs and symptoms associated with malathion inhalation for rats, humans, and livestock. We divide 24 female Wistar rats into three groups exposed to distilled water, malathion, and plastic boxes coupled to nebulizers kept at 22- 24°C. At the end of the experiment, the mice were sacrificed and their lungs and trachea were harvested. Histopathological examination revealed that the trachea in the treatment group is similar to that of the control group.

Keywords: sublingual atropine, intoxication, malathion, histopathology.

Resumo

Este experimento buscou estudar e avaliar os danos histológicos ao aparelho respiratório, pela inalação de solução aquosa de Malation a 50% em ratas e o tratamento pela via sublingual com colírio de sulfato de atropina a 1%. Buscamos na literatura os sinais e danos não somente nesta espécie como também em animais domésticos e de produção além de estendermos para seres humanos que eventualmente são intoxicados pela substância. Foram utilizadas 24 ratas, fêmeas, da espécie Wistar em três grupos expostos a água destilada ou malation e outro tratado por 21 dias, utilizando caixas plásticas acopladas a nebulizadores, com controle da temperatura da sala entre 22° e 24°C. Ao final do experimento e eutanásia seguindo as normas de bem-estar dos animais, foram coletados os pulmões e traquéias de todos os grupos, e a partir do exame histopatológico constatou-se recuperação do parênquima de traquéia no grupo tratado, semelhante ao grupo controle.

Palavras-chave: atropina sublingual, intoxicação, malathion, histopatologia.

Introduction

Malathion is an organophosphate that has been used in Brazil since the 1960s. Recently, pesticides have been in use due to the Green Revolution aimed to increase agricultural production through new technologies and end world hunger. This pesticide is relatively stable in neutral aqueous media, but is hydrolyzed at pH <5 or >7. It produces thiomalic acid and dimethylthiophosphate, which are generally stable to photolysis (IARC, 2017).

Studies have shown that malathion is effective against various pests including the *Aedes aegypti* mosquito (Andrighetti et al., 2013), which causes dengue, chikungunya, and zika in humans. This product is used to eliminate this pest in urban residences when there is an outbreak or epidemic. Malathion does this by inhibiting the propagation of nerve impulses, thus killing mosquitoes (Alout et al., 2007; Colovic et al., 2013).

In addition, there are occurrences of occupational and indiscriminate use of malathion in rural areas, mainly in small lots with monocultures and livestock, according to the Pesticide Registration System of MAPA (Brasil, 2021). This is due to its low cost. The lack of guidance and information on

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the correct use of malathion and its consequences for the handler and the environment coupled with deficient monitoring lead to abuse, including criminal use as in intentional poisoning.

The median lethal dose of this product in mammals is 1,500 mg/kg, and it takes approximately a week for 75% of the product to disappear from the soil, according to data from the São Paulo State Environmental Company. According to the latest data from the National Toxic-Pharmacological Information System of 2017, there have been 748 cases of accidental and intentional poisoning of animals in the states of Santa Catarina and Rio Grande do Sul; among these, 64 cases involved pesticides for agricultural use. Through a literature search, it is possible to observe high toxicity in humans and animals.

Grecco et al. (2009) reported that malathion works by inhibiting the acetylcholinesterase, an enzyme which promotes the accumulation of acetylcholine in the synaptic clefts. Acetylcholine is, a fast-acting neurotransmitter that, has excitatory and inhibitory effects on the parasympathetic peripheral nerve endings, located throughout the body. The intensity of the signals varies depending on the route, dose, and duration of exposure to the product.

Thus, the present study sought to investigate the effects of malathionon the respiratory system, which have been rarely described in literature. The study also sought to evaluate its response to sublingual atropine, in the search for new therapeutic modalities available to the clinician, such as allopathic medicine, phytotherapy, and homeopathy.

Material and methods

For this experiment, 24 female Wistar rats, approximately 6 weeks old, were used. They were divided into three groups with eight animals each: group 1 (Control),2 (Malathion), and 3 (Treated), following the scheme below: G1, Control: exposed to distilled water nebulization; G2, Malathion: exposed to nebulization with malathion at 50% based on the Technical Report of the State Department of Health (Goiás, 2014); G3, Treated, malathion and *Atropa belladonna*: exposed to nebulization with malathion at 50% and treated with sublingually-administered Allergan® eye drops (atropine sulfate 1%).

The concentration of the eye drops was determined through a pilot experiment in which we compared one drop of 0.5% and 1% eye drop (Figure 1) in two animals that would receive the treatment. Here, we observed mild mydriasis at higher concentrations. According to Neves et al. (2013), the dose of atropine recommended for laboratory animals is 0.05mg/kg. However, in this study, this dose was computed according to the mean weight of the animals in the treated group, which was 207g, and the concentration of the eye drops. We used three drops, which resulted in a volume of 0.15mL and a new dose of 0.007mg/g.

Figure 1. Atropine sulfate eye drops and chemical concentrations used in the pilot study.

A model of a human inhaler was used with some adaptations. This included a hose in the plastic boxes (Figure 2.A and 2.B) where the animals would be accommodated. Then, these were closed as tightly as possible. The temperature was controlled with an air conditioner (Figure 2C) because it is a volatile product (Escada, 2020). For 21 days, the animals received malathion as previously explained. During the experimental period, the animals were weighed to control their food and water consumption. This study was approved by the Ethics Committee on Animal Research and Experimentation under number 367/19, in accordance with the National Law 11.794 of October 8, 2008, Decree No. 6899 of July 15, 2009, and the National Council of Animal Experimentation.

After the experimental period, the animals were anesthetized with ketamine (100–200 mg/kg, intramuscular, IM) and xylazine (5–10 mg/kg, intraperitoneal, IP). Then, sodium thiopenthal at a dose of 120 mg/kg IP, which is three times the recommended dose to euthanize the rats (Neves et al., 2013). Afterwards, exploratory laparotomy and thoracotomy were performed to collect the lungs and trachea, for histopathological analysis. According to Paschoal and Moreira (2016), different parameters are defined for each organ because of the peculiarity of each organ. In the lungs, leukocyte infiltration, vascular congestion, and thickening of the interalveolar septa were assessed. A scoring system based on the presence and severity of the afore mentioned features was used to grade the lesions in the lungs. Five random regions of each tissue were observed under various magnifications (objectives ranging from 4x to 400x). Gradations were assigned for each type of lesion: 0, absent; 1, mild; 2, mild to moderate; 3, moderate; and 4, intense, according to their relevance. Calculations were made for these scores, and the data obtained were catalogued in an Excel table for statistical analysis, using the Kruskal-Wallis test and Dunn's post-test.

Figure 2. Nebulizer system developed by the students of Centro Universitário Barão de Mauá for animal experimentation. A: Adaptation of plastic boxes with inhalers identified with the group of animals to be placed; B: Accomodation of the animals; C: Climatization.

Results

The histopathological examination of the trachea in the control group (Figure 3A) revealed a normal parenchymal epithelium and underlying connective tissue. In the malathion group (Figure 3D), the trachea had an altered structure with accentuated loss of cilia. The treated group (Figure 3G), had a tracheal histopathology similar to the control group. Paschoal and Moreira (2016) reported that respiratory epithelium degeneration occurs due to inflammation. Inflammation due to inhalation of toxic gases may result in mucociliary transport disorders manifesting as the loss of ciliary structures lining the trachea.

Lung photomicrographs in the treated group (Figure 3H and 3I) showed that the pulmonary parenchyma is very similar to that of the control group. This is marked by the interalveolar (arrow) and alveolar spaces (asterisk). In intoxicated animals (Figure 3E), intense thickening of the alveolar septum was observed (empty arrow) due to vascular congestion (full arrow). The types of lesions found and the scores obtained from the histopathological analyses, graded from 0 to 4 according to absence or presence and their intensity for each animal, were calculated and represented in three statistical charts.

Regarding the presence of inflammatory infiltrates (Figure 4), there was a significant difference between the control and malathion groups ($p < 0.0001$). These differences were as follows: 22.5% mild infiltrate, 32.5% mild to moderate, 35% moderate, and 5% intense. There is also a significant difference between the malathion group and the treated group ($p < 0.0001$). Here, there is a higher percentage of absence of infiltrates 40%. However, the lesion patterns were similar between the control and treated groups ($p > 0.9999$). This implies that the sublingual treatment may be effective, since it maintained the histological conformation of the organ.

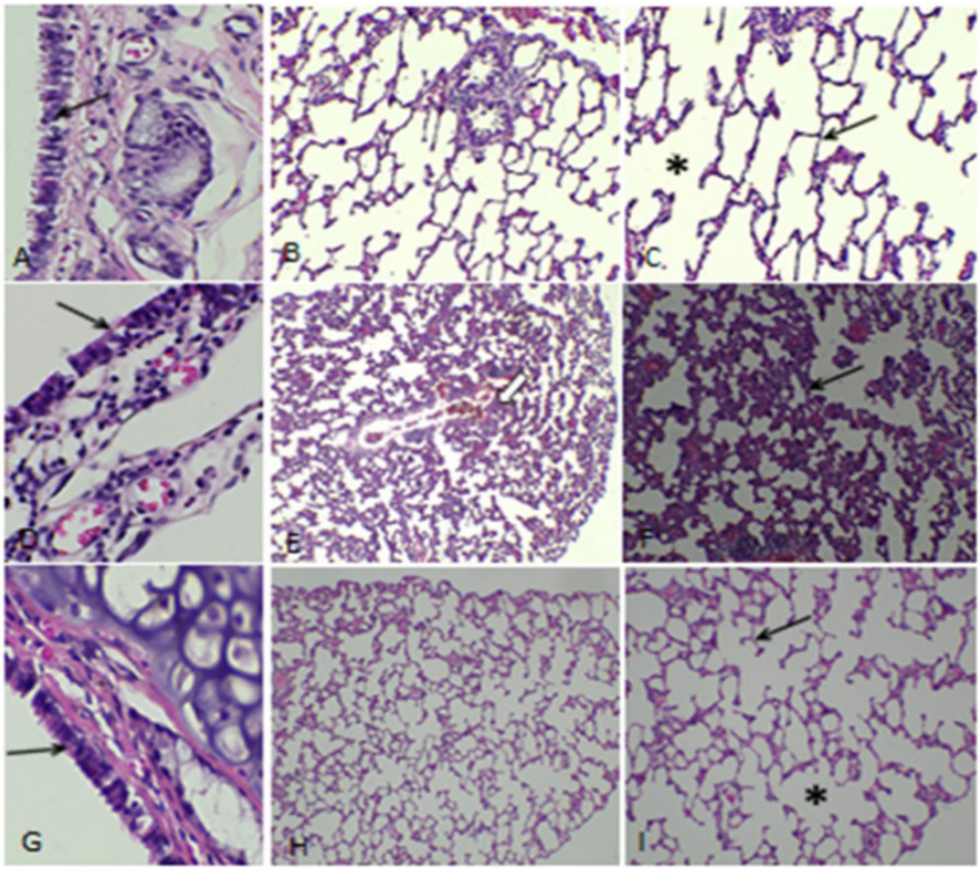

Figure 3. Photomicrographs of trachea and lungs sections. In A, B, and C Control group; In D, E, and F Malathion inhalation group; In G, H, and I Malathion inhalation and sublingual eye drops treatment group.

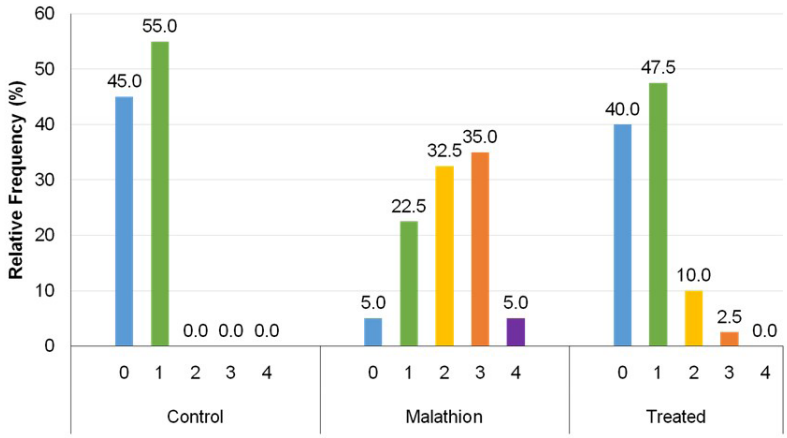

Figure 4. Leukocyte infiltrate. From left to right: Control, Malathion, and Treatment.

In Figure 5, which evaluates vascular congestion, there was a significant difference between the control, malathion, and treated groups ($p < 0.0001$). A moderate score of 32.5% was observed in the malathion group. On the other hand, 2.5% of animals that were treated had a moderate degree (score 3) of congestion. None of the animals had a severe degree of congestion, a finding comparable to the control group ($p > 0.9999$). For the interalveolar thickening (Figure 6), the intoxicated animals had the highest percentages in the different degrees of the histopathological findings: 25% mild, 27.5% mild to moderate, 32.5% moderate, and 5% severe. On the other hand, the treated rats did not present severe thickening compared to the malathion group ($p < 0.0001$), predominantly 40% absent and 47.5% mild.

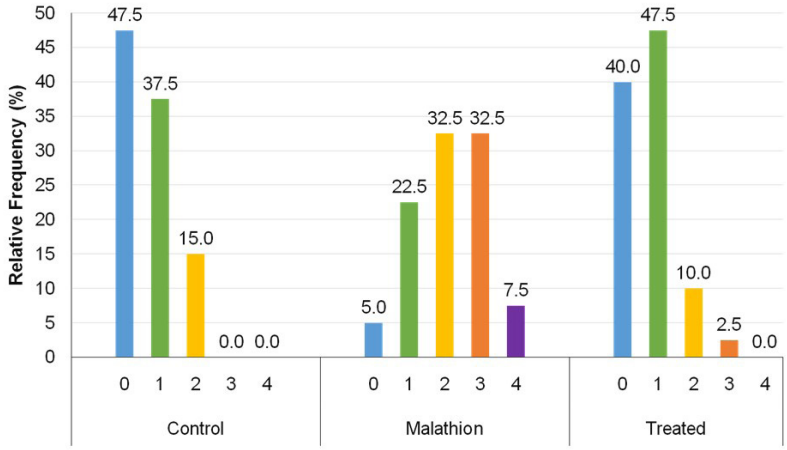

Figure 5. Vascular congestion

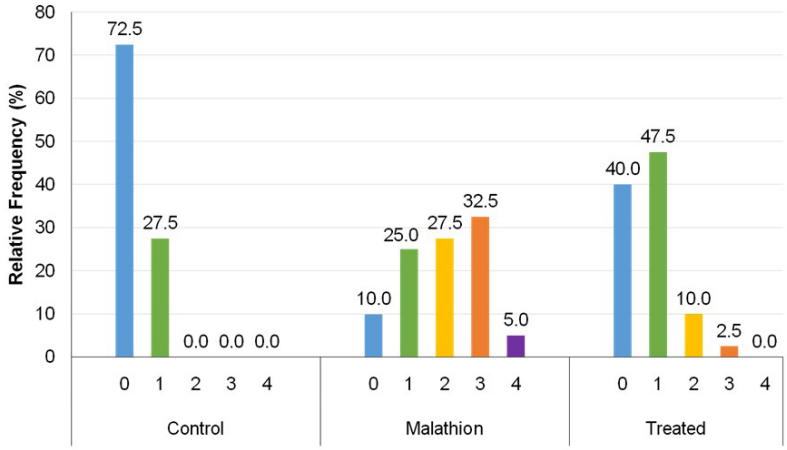

Figure 6. Interalveolar thickening.

In Figure 5, the malathion group presented a maximum congestion intensity of 7.5%, which did not occur in the other groups. However, the same group of animals that was intoxicated presented with 5% of interalveolar thickening, which was not observed in the treated group. This indicated that sublingual atropine eye drops were effective. There was no total suppression of milder lesions in the control group, due to the stress caused by the manipulation of the rats during the experiment, as well as the intrinsic behavior of their sex and age range.

Discussion

Atropine, a competitive antagonist of central and peripheral muscarinic receptors, is the main treatment instituted in organophosphate intoxications. It competes for the mentioned receptors and prevents the accumulation of acetylcholine in synapses by displacing it from the site of action. It is an alkaloid with fast gastrointestinal absorption and is applied topically to mucous membranes. It has beneficial effects on the respiratory system by relaxing the bronchiolar tone, reducing secretions, antagonizing bradycardia, and decreasing gastrointestinal motility (Vital & Acco, 2017).

In a study by Rajpal et al. (2010), the use of a formulation with 2% atropine sulfate, double-distilled water, and 0.9% sodium chloride by the sublingual route was tested in humans as a therapeutic strategy insituations of terrorist confrontations. The results obtained from the plasma peak of atropine with the chosen route were higher than those obtained with the intramuscular administration route while, the peak occurred at 30 min in the latter, it occurred at 15 min in the former.

Some studies have investigated possible reversal in guinea pigs, mice, rats, and rhesus monkeys through purified butyrylcholinesterase enzyme. This enzyme has an *in vivo* bioavailability of over 3 years and its administration in high doses does not appear to result in any pathological clinical signs (Saxena et al., 2006). The same enzyme was reported in a study by Mann et al. (2018) Who tested its efficacy in rodents, non-human primates, and minipigs poisoned percutaneously with toxic agents. Reversal of symptoms was more successful when the enzyme was administered before the onset of signs.

According to Raghavendran et al. (2008), acute lung injury and acute respiratory distress syndrome are caused by direct insults such as the inhalation of toxic substances, which promote lung inflammation with damage to the alveolar capillary membrane and directly influence systemic oxygenation. Pulmonary injury is divided into the acute exudative phase and the fibroproliferative phase, which occurs within 12 to 72 h after the initial pulmonary injury. In the initial phase, there was interstitial and alveolar edema with increased capillary permeability of the alveoli. After 3 to 7 days, alveolar damage extended with detachment of the basal lamina and formation of protein hyaline membrane, fibrin, and intra-alveolar cellular debris. At this stage, the coagulation cascade is activated, and cytokines, reactive oxygen species, and leukocytes are released.

Chronic insults to the lung parenchyma interrupt the production of surfactant substances, fibroblast migration, and a new collagen-rich architecture is deposited in the pulmonary interstitium, with consequent areas of fibrosis. The fibroblasts differentiate into myofibroblasts, and the alveoli are sealed as the tissue is reorganized, leading to pulmonary hypertension, which results in decreased blood flow and gas exchange (Raghavendran et al., 2008). The presence of vascular congestion and thickening of the interalveolar septum have also been described in dogs and cats in a study by Melo et al. (2002).

According to Vanova et al. (2018), the toxic effects of organophosphates, the cholinergic syndrome, varies in three ways: acute (within minutes to hours), intermediate or delayed (from 24 hours to 2 weeks), and chronic or late (after 2 weeks). The mechanisms responsible for the adverse effects are being studied. Although the present study is classified as a late cholinergic syndrome due to the duration of 21 days, the signs observed by some animals during intoxication were anxiety and restlessness, as reported by King and Aaron (2015). Other animals showed increased salivation and lacrimation of the eyes between 5 and 10 min after nebulization, hypotension, miosis, and muscle weakness. These data are related to the work of Melo et al. (2002), who describe other signs observed: vision disorders, bronchospasm, rhinorrhea, and dyspnea. In this study, observation of the abdominal breathing pattern revealed that some rats showed greater strength in the inspiratory phase of breathing, which characterized dyspnea.

Melo et al. (2002) reported that cats, in addition to being more sensitive to intoxication by organophosphates OF, developed delayed polyneuropathy syndrome in those who used flea collars impregnated with this substance. Delayed neuropathy progresses from 8 to 14 days after intoxication and occurs due to axonal destruction by the inhibition of esterases, which causes excessive calcium influx into the axons, preventing the transmission of nerve impulses (Caldas, 2000).

Conclusion

Since malathion was implemented in Brazil in 2012, it has been used to control *A. aegypti* in endemic cases of dengue along with three other pesticides (lambdacialothrin, deltamethrin and transcyphenothrin) according to the Ministry of Health (Brasil, 2018). The importance of changing the protocols should be further emphasized to combat the mosquito due to its resistance from adaptation and selection, as shown by Braga and Valle (2007), which man himself promotes through frequent spraying.

This organophosphate remains the subject of numerous investigations to elucidate the exact mechanisms through which it causes intoxication and irreversible injuries. This becomes even more worrying because of the lack of an antidote capable of reversing the remaining damage. Despite this, our study complements and reinforces the viability of treatment with sublingually-applied atropine sulfate using the dose found in the literature, both for humans and for domestic and production animals that may eventually be intoxicated.

In addition to the harm of intoxication in animals and humans, according to Soccol et al. (1995), there is a second problem in the form of residues in the soil. Infiltration into the soil can cause

leaching, with possible contamination of groundwater and food. Malathion may or may not be degraded by soil through volatilization or chemical reactions. In addition, spray particles may adhere to dust and cause air contamination, and wind and rain may extend the sprayed target zone.

Thus, malathion requires further studies to elucidate its possible consequences to other organ systems in humans and animal as well as its consequences to the environment. The search for easily accessible treatments and antidotes for cases of intoxication is necessary; other measures include therapeutic planning that aims to minimize the permanent damage at the muscle and neurological level, the development of new measures to combat the vector, the inspection of properties that still use this substance to safeguard inhabitants, and the adoption of educational measures to warn of the dangers and inform the population about the use of malathion.

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Ethics statement

The study has been approved at the Research Ethics Committee and Animal Experimentation (CEPan) of the Veterinary Institute of the Barão of Mauá University Center with protocol number 367/19.

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Conflict of interests

BAMA, JLRA e ARC – No conflict of interest.

Authors' contributions

AMA e ARC - Development of methodology; preparation and writing the initial draft. BAMA e JLRA - Application of statistical study data, Review and Editing manuscript. BAMA, JLRA e ARC - Writing, Review and Editing manuscript. BAMA e ARC- Acquisition of the financial support for the project leading to this publication.

Availability of complementary results

The authors must identify where readers can access any complementary information available, such as in an online repository or from the authors on request. We suggest consulting https://wp.scielo.org/wp-content/uploads/Lista-de-Repositorios-Recomendados_pt.pdf

The work was carried out at Biotério do Centro Universitário Barão de Mauá, Ribeirão Preto, SP, Brazil.

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