Malathion induces anxiety in the male adult mouse

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A b s t r a c t

The cholinergic nervous system and acetylcholine esterase are involved in chronic intoxication with organophosphorous insecticides. The present study aims to investigate the influence of the chronic toxicity of these chemicals on behaviors related to anxiety, using the elevated plus maze (EPM), in the male adult mouse.

Material and methods: Either water or 1% concentration of malathion was applied dermally to the male adult mice (10 s, once daily for 28 days) and, on day 29, the EPM test was done.

Results: Time spent in the open arms (TSAO) in intoxicated animals was decreased by over 50% compared to the controls (p = 0.047). In contrast, time spent in closed arms was significantly higher in the malathion-exposed mice (p = 0.025). Percentage of open arm entries (OAE) was slightly smaller in the malathion-treated group in comparison to the control animals. Percentage of closed arm entries (CAE) in the treated group was slightly higher than the value in the control animals.

Conclusions: The results showed that chronic toxicity of malathion may lead to an anxiety-like behavior in the animal model used in this study. It is difficult to extend these findings to clinical situations. However, more experimental work in different animal species as well as epidemiological studies in human subjects in this regard are highly recommended.

Key words: anxiety, elevated plus maze, malathion, mouse, organophosphates.

Introduction

Malathion is an organophosphate widely used as an insecticide in agriculture and in public health programs, causing risk to human health. Due to a wide range of usages in the agricultural sector, malathion is a high-scale chemical in use all over the world. The estimated average annual total domestic usage of malathion in the USA is approximately 15 million pounds of malathion as an active ingredient [1]. Acute toxicity in rodents following exposure to organophosphate compounds has long been understood [2]. Repeated exposure to organophosphates has been shown to cause a decrease in muscarinic cholinergic receptors in the central and peripheral nervous system [3]. The mechanism of acute toxicity of the organophosphorus insecticides has been known for many years to be inhibition of the critical enzyme acetylcholinesterase (EC 3.1.1.7), with the resulting excess acetylcholine accumulation leading to symptoms of cholinergic excess [4]. Acute organophosphate poisoning is known to result in substantial behavioral abnormalities. Psychiatric manifestations of exposure in workers less substantially exposed to organophosphate compounds

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and showing no obvious signs of toxicity were investigated. The commercial sprayers but not the exposed farmers showed elevation of anxiety and lower plasma cholinesterase than control subjects. These findings were viewed as tentative until confirmed by additional studies, but they point to the possibility that organophosphate compounds may produce subtle defects in workers who do not show obvious toxicity [5]. Previous studies support the value of detailed clinical observations to screen for the neurotoxic potential of organophosphates and a general standard of more than 20% inhibition of brain cholinesterase activity for cholinergic neurotoxicity [6].

The elevated plus maze (EPM) is a simple instrument to analyze some behaviors of mice and rats. It was introduced by Pellow et al. first in 1985 for anxiety studies in the rat [7]. It consists of two crossing arms which make a plus-like configuration. One arm, surrounded by walls, is called the closed arm and the other, not surrounded by walls, is called the open arm. Rodents generally prefer to stay longer in the closed arms and are afraid of moving and staying on the open arms. Their movement on the open arms, especially on the remote one third of this part, is limited to searching the environment and some sort of curiosity as the height of the instrument is a kind of stress for them. Two years after Pellow, the EPM was also designed in smaller dimensions to be used for the mouse [8]. A recent work published by our group demonstrated a valuable finding in the mouse exposed to lead acetate, using the EPM; as suggested by the study, the exposure caused a kind of increased risky behavior in this species [9].

It is regrettable that not much attention has been paid to the behavioral influences of environmental pollutants using more developed experimental models. The aim of the present study was to investigate whether a widely used insecticide, malathion, may affect the basic anxiety status in the male adult mouse as an animal model.

Material and methods

Animals and chemicals

Twenty male adult Balb/c mice were obtained from Razi Vaccine and Serum Research Institute (Marand, Iran). Their weight range was 20-25 g and they were 8 weeks old when transferred to the lab. They were kept for 1 week to adapt to the new conditions. The mice were kept at room temperature with free access to commercial chow and tap water. Light and dark was supplied in 12 h cycles. Malathion 57% EC was a product of Melli Agrochemical Company (Qazvin, Iran).

Experimental protocol

The protocol, including ethical points in case of animal care issues, was approved as an undergraduate research project by the Research Council of the Islamic Azad University of Tabriz. Animals were divided into two groups of 10 each. The controls were exposed topically to saline solution from the whole tail area for 10 s once daily for 28 successive days. The treated group was exposed in the same manner to a 1% concentration (100 ppm) of malathion. On day 29, the animals were placed individually in the elevated plus maze (EPM) for 5 min (300 s). The area was kept silent and dark while the EPM was under a slight light emitted from a red 60 W lamp placed at a 50 cm distance from the top of the maze. Movements of the animals were recorded by a video camera (Canon MV690 E) connected to a computer (Asus Eee PC) and viewed by Windows Media Player 11. Time spent in open arms (TSOA), time spent in closed arms (TSCA), open arm entries (OAЕ) and closed arm entries (CAE) were determined and recorded.

Statistical analysis

Data were averaged in every experimental group and expressed as means ± standard error of the means (SEM). Then, differences were evaluated by unpaired Student’s t test. A value of \( p < 0.05 \) was taken as statistically significant.

Results

Time spent in the open and closed arms

Time spent in open arms in intoxicated animals was decreased by over 50% compared to the controls \( (p = 0.047, \text{Figure 1 A}) \). In contrast, TSCA was significantly higher in the malathion-exposed mice \( (p = 0.025, \text{Figure 1 B}) \).

Open and closed arm entries

Open arm entries in 5 min was slightly smaller in the malathion-treated group in comparison to the control animals (not statistically significant, Figure 1 C). Closed arm entries in the treated group was slightly higher than the value in the control animals (not significant, Figure 1 D).

Discussion

The present study showed that chronic toxicity with malathion may lead to a state of anxiety-like behavior in the male adult mouse. The experiments were done in ordinary conditions with no stress imposed on the animal; therefore, the influence was exerted on basic anxiety levels in rodents. \( M_1 \) and \( M_2 \) receptor subtypes may be similarly regulated in response to subchronic exposure to organophosphates, including malathion [2]. \( \text{LD}_{50} \) values (median lethal dose for 50% of animals) values determined for many pesticides administered to adult and weanling male and female rats sug-

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gested that there were gender and age differences in the response of the animals to different agents [10]. Acute organophosphate intoxication in man is well known to result in substantial central nervous system dysfunction. However, relative resistance of higher nervous system functions to mild chronic organophosphate exposure has been suggested by existing results [11]. Malathion influences the reproductive function both by its cellular toxic action and by affecting the encephalic regulatory serotoninergic, besides acetylcholinergic, mechanism of the reproductive functions [12]. Malathion induces depressant-like behavior and oxidative damage to the brain of rodents [13]. Dichlorvos, another organophosphate agent, was shown to depress the open field behavior, ambulation, motor activity, and fine movements in the rat, with concordance of the open field behavioral changes with the levels of dopamine, norepinephrine, and serotonin in various regions of the brain [14]. This evidence is stressed only to note the relevance of behavioral abnormalities to organophosphate intoxication and it is not possible to make a connection between the existing findings and those of the current research work. However, peripheral effects cannot be ruled out in the present experimental conditions.

Considering some findings, which indicate the increase of brain serotonin by malathion, this mechanism may be the one which caused anxiety in our animal model. Serotoninergic neurotransmission increase could be acting within the amygdala, enhancing anxiety [15]. It is suggested in individuals suffering predisposition to anxiety disorders to minimize the exposure to malathion by means of sanitary measures. Summarized in one sentence, the message is that long-term exposure to insecticides is likely to cause not only somatological but also behavioral disorders in humans. Our findings suggest an area warranting more systematic and definitive investigation, and the role of cholinergic brain transmission in anxiety disorders could be a very interesting research line.

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