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Chlorpyrifos' influence on memory and movement processes in mice after transient oligemic brain hypoxia

Chlorpyrifos is a broad-spectrum insecticide, widely used in agriculture organophosphorus (OP). OP pesticides produce acute cholinergic effects *via* inhibition of acetylcholinesterase (AChE). Some OPs can cause organophosphorus compound-induced delayed neurotoxicity (OPIDN) through chemical modification of neurotoxic esterase (NTE).

Little is known about chlorpyrifos' influence on memory processes in mammals. Authors concentrate on its developmental effects, embriotoxicity and embriolethality if given to the dams on gestation. Few publications describe effects of massive incidental exposure of adult humans to OP pesticides. No articles can be found about neurotoxic effects of OPs on mammals exposed to transient brain ischemia similar to transient ischemic attacks (TIA) occurring in ageing humans.

The aim of the work was to evaluate the influence of subtoxic dose of chlorpyrifos on memory processes, movement co-ordination and spontaneous movement activity in mice exposed to transient oligemic brain hypoxia in BCCA model.

MATERIAL AND METHODS

Before the experiments the permission of The Local Ethical Committee was acquired. LD_{50} of chlorpyrifos was determined with a computer program based on Lichtfield and Wilcoxon's method (4). It was estimated at 275.3 mg/kg (173.3–337.3).

The experiment was conducted on randomly selected female Albino Swiss mice weighing 18–24 g. They were housed at least 4 days prior to the experiment in one group on a standard light-dark cycle (12 h light:12 h dark) at ambient temperature 21°C, humidity of 45–55%, given food and water *ad lib.* Each group examined consisted of 10 animals.

There were four groups of animals examined: I – sham–operated, II – after BCCA, III – sham- operated, treated with chlorpyrifos and IV – after BCCA, treated with chlorpyrifos.

Bilateral clamping of carotid arteries (BCCA) is an experimental model of transient ischemic attacks (TIAs). It was performed under Ketamine + Xylazinum anaestehesia. The anaesthetic solution was injected i.p. at the dose of 10 ml/kg of b.m. to the animals. Both carotid arteries were isolated and occluded with threads for 30 min. After that period the threads were removed and arteries inspected for reflow. During the procedure the mice were breathing spontaneously and kept at 37°C by a heating pad and a lamp. 2–3 hours after that they regained normal consciousness, ate, drank and showed no visible symptoms of CNS failure. Twenty-four hours after the surgery, the mice had a training in the passive avoidance task. The next day the animals from group III and IV were injected with 0,1 LD_{50} chlorpyrifos dissolved in bidistilled water intraperitoneally. Thirty minutes after administration the animals were examined in the passive avoidance task. Then, their movement co-ordination on a rotation-rod was examined. After that the mice were placed in Y-maze to examine their spontaneous movement alterations and later, their spontaneous movement activity during two 30-minute long periods was checked.

Results obtained were analysed with ANOVA and the *post hoc* tests. The results in passive avoidance task were analysed with Kruskal-Wallis' Test (nonparametric ANOVA) and *post hoc* Dunn's Test. The other results were analysed with parametric ANOVA and *post hoc* Student-Newman-Keuls' Multiple Comparisons Test. P value <0.05 was considered statistically significant. InStat version 3.0 for Windows-GraphPad Software, Inc.-USA and GraphPad Prism Version 3.0 GraphPad Software, Inc.-USA, were used.

RESULTS

The results obtained in the experiment show no statistically significant differences between the compared groups in passive avoidance task, Y-maze, nor spontaneous movement activity. Only in movement co-ordination significant differences were found (Fig. 1). In the test the animals after BCCA treated with chlorpyrifos performed significantly worse than the others.

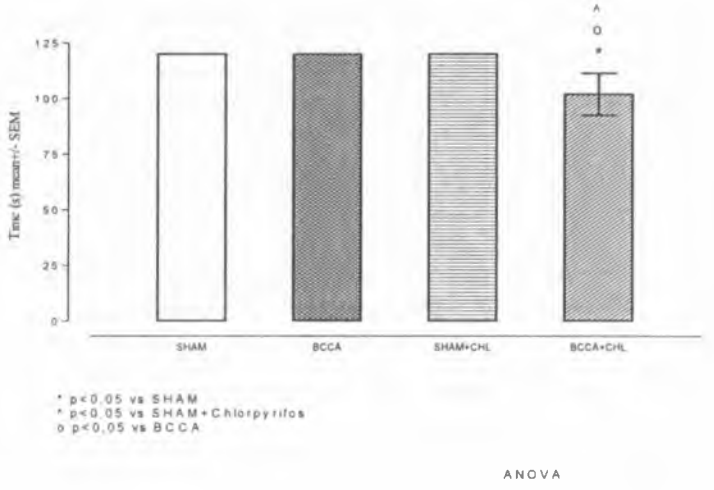

Fig. 1. Movement co-ordination in the experiment with chlorpyrifos (CHL). (n=10)

DISCUSSION

Numerous authors underline the significance of cholinergic system, brain cortex and hippocampus in memory processes. They blame age-related neurons' degeneration, brain ischemia, traumas and exposure to neurotoxic xenobiotics for cognitive deficits and memory impairment (1). It is known and understood that the OPs increase the brain ACh concentration *via* AChE inhibition. Therefore, some OP compounds are used in the treatment of neurodegenerative diseases like Alzheimer's disease. However, there is no evidence that they can significantly improve memory acquisition.

S i e k l u c k a et al. published a lot of data about biochemical changes occurring in brain tissues during BCCA procedure. Due to transient ischemia the temperature decreases locally and rises within 60 min. after reperfusion leaving no CNS deficits. They described no changes in EEG neither during nor after BCCA. They proved that BCCA leads to an increase in the brain GABA level and a decrease in the ACh level (2, 6, 7, 8, 9). J ó ź w i a k noted no memory impairment in passive avoidance task nor in Y-maze in mice after BCCA. She did not, either observe any significant movement co-ordination or spontaneous movement activity impairment after transient brain ischemia (3). The results obtained in our study are consistent with these findings.

Richardson published interesting results of experiments with high doses of chlorpyrifos (279 mg/kg s. c.) given to rats. It inhibited cortical and striatal cholinesterase in 58–60% but produced no significant changes in behavioural tests including spontaneous movement activity and co-ordination (5). The incongruent results regarding co-ordination may be due to additive BCCA's effect. On the other hand, BCCA may play a protective role against chlorpyrifos' action. Transient ischemia might be brain preconditioning for toxic agents.

CONCLUSIONS

Chlorpyrifos at 0.1 LD_{50} does not impair memory acquisition nor retention. It does not influence significantly spontaneous movement activity neither on its own nor in mice exposed to transient brain ischemia. However, movement co-ordination is significantly impaired by chlorpyrifos given to mice exposed to BCCA.

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SUMMARY

Chlorpyrifos is an organophosphorus insecticide. It produces acute cholinergic effects via inhibition of acetylcholinesterase. Little is known about its influence on mammalian brain exposed to transient ischemia. The aim of the work was to evaluate the influence of 0.1 LD_{50} of chlorpyrifos given i.p. on memory processes, movement activity and movement co-ordination in mice after brain ischemia in BCCA (bilateral clamping of carotid arteries) model. We observed no statistically important memory or movement impairment. Chlorpyrifos caused significant worsening of movement co-ordination in mice after BCCA compared to control groups.

Wpływ chlorpyrifosu na procesy pamięci oraz aktywność ruchową myszy po przejściowym niedokrwieniu mózgu

Chlorpyrifos to związek fosforoorganiczny stosowany jako insektycyd. Wywołuje on ostry efekt toksyczny w mechanizmie blokowania acetylocholinesteraz. Niewiele wciąż wiadomo na temat jego wpływu na mózgi ssaków po przebytym przejściowym niedokrwieniu. Celem pracy była ocena wpływu 0.1 LD_{50} chlorpyrifosu podanego dootrzewnowo na procesy pamięci, aktywność i koordynację ruchową u myszy po przejściowym niedokrwieniu mózgu. Niedokrwienie wywoływano poprzez okresowe obustronne zamknięcie tętnic szyjnych wspólnych zwierzętom doświadczalnym. Po przeprowadzeniu testów pamięci, aktywności i koordynacji ruchowej stwierdzono brak wpływu chlorpyrifosu na zapamiętywanie oraz przypominanie sobie informacji przez zwierzęta oraz brak wpływu na ich spontaniczną aktywność ruchową. Chlorpyrifos istotnie upośledza koordynację ruchową zwierząt po przejściowym niedokrwieniu mózgu.