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Effect of Methylmercury on Acetylcholinesterase and Serum Cholinesterase Activity in Monkeys, *Macaca fascicularis*

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The consumption of fish and fish-derived products is the main pathway of human exposure to methylmercury (MeHg). Methylmercury levels vary widely in fish, depending on age, size, the position of the species in the food chain, and most of all, on pollution levels (Imura et al. 1977).

The Mediterranean sea appears to be one of the most polluted seas, and high mercury levels have been found in fish as compared to the same species from the Atlantic Ocean (Auber 1975; Cunont et al.1975; Bernard and Renzoni 1977; Caleresu et al.1983).

Methylmercury accumulates in kidneys, liver, and the nervous system, causing damage and poisoning symptoms. The neurological symptoms observed are: tremor, hyposensitivity, double vision (Takeuki et al.1962; Takeuki et al.1970; Hunter and Russel 1954). MeHg also affects the Acetylcholinesterase activity (AChE) and the serum Cholinesterase activity (BChE). Dieter (1974) and Dieter and Ludke (1975) noticed that the AChE and BChE activities decreased in birds (Coturnix quail) treated with 4 ppm of methylmercury dycandiamide for 4 weeks. Miszta (1984) found that AChE activity of erythrocytes decreased in rats fed for 10 days with 0.8 mg/kg b.w./day of MeHg. However, MeHg did not inhibit AChE activity in vitro, when using mouse brain homogenate, or Torpedo ocellata membrane (Tunnicliff and Wood 1973; Rustam et al.1975; Kobayasi et al.1979; Eldefrawi et al.1977).

Histoenzymatic studies showed that 100mg Methoxy-ethylmercury chloride administered for 6 days to rats caused a reduction of AChE activity in the thalamus and an increase in different parts of the nervous central system (Wigowska-Sowisnska 1980).

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Acetylcholinesterase is present in the stroma of erythrocytes, in lung, spleen, grey substance, and synapses. Serum Cholinesterase is present in liver, pancreas, myocardium, the white substance of the brain and in serum.

The present study aims at verifying whether the dose permitted by F.A.O.(1976;0.0033 mg/kg b.w. weekly) and doses 10 and 100 fold higher affect the Cholinesterase activity in primates, and whether there is a correlation between AChE and BChE.

MATERIALS AND METHODS

Nineteen female adult monkeys, species Macaca fascicularis, mean weight 3080±220 gr were utilized. Prior to treatment, the monkeys were single caged (60x80x70 cm) for a 3-week acclimatization period. During this period, and for the duration of trials, the animals were kept at a constant temperature (23±2°C) and under relative humidity conditions, on a 12-hour light-dark cycle.
Monkeys were fed once a day on a diet of Purina Monkey Chow daily integrated with fresh fruit. Water was supplied ad libitum.

Methylmercury chloride, added to 3 mL apple juice, was dissolved in sterile isotonic saline and administred orally every day between 10 and 12 p.m..

The 19 monkeys were divided into a control group (4 subjects), and 3 experimental groups: one group (4 subjects) was treated with 0.4 µg/kg b.w./day; another one (5 subjects) was treated with 4.0 µg/kg b.w./day; the last one (6 subjects) was treated with 50 µg/kg b.w./day. MeHg was administered until the steady state was reached (150 days) (Burbacher et al.1984).

At the end of the treatment, on day 150, each monkey was given general anaesthesia with 0.2 mL/kg b.w. ketalar (as anaesthetic) associated with 0.1 mL/kg b.w. atropine, in order to take blood samples to assess the Acetylcholinesterase (E.C.3.1.1.7) and Butyrylcholine-sterase activities (E.C.3.1.1.8).

The material for analysing the Acetylcholinesterase activity in erythrocytes was prepared according to the recommendations specified by the International Committee for Standardization in Hematology (Beutler et al. 1977). The erythrocyte AChE activity was determined according to the spectrophotometric method developed by Elman et al. (1961) and modified by Sirchia et al. (1968). The activity of this enzyme was expressed in $mU/10^9$ erythrocytes.

BChE activity was determined according to the method developed by Szasz (1968). This activity was expressed in U/l.

Significant treatment effects were determined by one-way analysis of variance.

RESULTS AND DISCUSSION

During the 15 days of acclimatization and the 150 days of treatment neither tremors nor convulsions were observed; after a slight initial weight loss during the acclimatization period, in all groups receiving the toxicant and also in the control group, body weight returned to normal levels and kept so throughout the study.

In the control group AChE activity reached 312.8±82 mU $/10^9$ erythrocytes. In the groups treated with 0.4 and 50 µg/kg b.w./day the AChE activity was lower than in the control group, whereas in the group treated with 4.0 µg/kg b.w./day it was higher. The AChE activity of treated groups did not differ significantly from the control group. (Tab.1)

The value of BChE activity in the control group was 9882±2672 U/l; in the group treated with 0.4 µg/kg b.w./day the activity was lower than that of the control group, but it was higher in the groups treated with 4.0 and 50 µg/kg b.w./day. The analysis of variance did not show any difference between the treated groups and the control. (Tab.1)

AChE and BChE were not correlative.

The doses used in this study, considered to be the average MeHg ingested by people living along the coasts (Caleresu 1983), did not cause any significant decrease of AChE and BChE activities in the monkeys treated until the steady state was attained.

In other studies a reduction of Cholinesterase activity was found in birds treated with 4 ppm and rats fed with 0.8 mg/kg b.w./day of MeHg (Dieter 1974; Dieter and Ludke 1975; Miszta 1984). Other Authors found no reduction of cholinesterase in vitro (Tunnicliff and Wood 1973; Rustam et al.1975; Kobayasi et al.1979; Eldefrawi et al.1977).

Miszta (1984) noticed that rats treated with MeHg showed an improvement of AChE activity during the first period of treatment and, afterwards, a significant reduction (Miszta 1984).

It is difficult to compare these studies because the

doses, the length of administration, and the subjets used vary widely. Probably the effect of MeHg on both AChE and BChE depends on the species, daily ingested concentration, and route of administration.

MeHg might affect the enzyme inhibitors and activators, and change the substrate and product concentration, (Miszta 1984). It blocks the active site of acetylcholine receptors (Eldefrawi et al.1977). In any case, the positive or negative effects of MeHg on ChE activity is always dose-dependent (Miszta 1984)

Table 1. AChE activity expressed in $mU/10^9$ erythrocytes, and BChE activity expressed in U/l

	AChE	BChE
control	156±40.9	9882±2672
dose 0.4	141±32.6	8067±998
dose 4.0	162±47.5	12459±3137
dose 50	146±23.0	11406±1870

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