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THE EFFECT OF O,O-DIMETHYL-O-(2,4,5-TRICHLOROPHENYL) PHOSPHOROTHIOATE (FENCHLORPHOS) ON CHOLINESTERASES IN THE BLUE FOX (ALOPEX LAGOPUS)

By

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SØLI, N. E., R. A. ANDERSEN, J. UTNE SKAARE and A. MIKALSEN: *The effect of O,O-dimethyl-O-(2,4,5-trichlorophenyl) phosphorothioate (fenchlorphos) on cholinesterases in the blue fox (Alopex lagopus)*. Acta vet. scand. 1977, *18*, 408—415. — Four blue fox bitches were used in the experiments. Two foxes were given fenchlorphos in the feed, one 100 mg/kg body weight and the other 200 mg/kg daily for 30 days. The maximum inhibition of plasma cholinesterase was 65 and 69 %, respectively. The corresponding values of the erythrocyte acetylcholinesterase were 43 and 63 %.

For the third bitch given 0.4 mg/kg as a single dose i.v. the effect was only measurable as a small transient decrease of the plasma cholinesterase level.

Eighty % of the plasma cholinesterase of the fourth fox, given 500 mg/kg as a single oral dose, was inhibited on the third day. The erythrocyte acetylcholinesterase activity level only showed a slight decline. This fox vomited during feeding the day after administration.

Symptoms as salivation, tremors, diarrhea, pinpoint pupils and respiratory distress were never seen in any of the foxes.

It was concluded that fenchlorphos administration in the feed in doses recommended to dogs is well tolerated by healthy foxes as far as cholinesterase inhibition is concerned.

blue fox; fenchlorphos; cholinesterase inhibition

Ectoparasite attack in fur animals represent a practical problem with respect to treatment, because the animals are kept in outdoor cages which make dipping and spraying hazardous during winter time. It was therefore decided to examine the toxicity of systemically administered fenchlorphos measured by its cholinesterase inhibiting effect, with the aim to establish an alternative method of treatment.

MATERIALS AND METHODS

Chemicals

The following chemicals were used: DTNB (5,5-dithiobis-(2-nitrobenzoic acid)) and acetylthiocholineiodide (Sigma Chem. Comp. USA); physostigmine salicylate (C. H. Boehringer Sohn, Ingelheim am Rhein, Germany) and Ectoral® tablets with the active ingredient fenchlorphos (O,O-dimethyl-O-(2,4,5-trichlorophenyl)) phosphorothioate (Pitman-Moore, Inc., USA, Scandinavian dealer: Cilag-Chemie AB, Fack, S-19107 Sollentuna 7, Sweden).

The Merck index (eighth edition) claims the solubility of fenchlorphos in water at 25°C to be $1.25 \cdot 10^{-4}$M. A saturated solution was prepared by dissolving tablet powder in physiological saline, 0.1 M phosphate buffer and Tyrode solution. The anticholinesterase potencies of these solutions did not decrease for at least 10 hrs. at room temperature.

Enzyme preparations

Acetylcholinesterase from bovine erythrocytes and pseudocholinesterase from horse serum were obtained from Sigma. Erythrocytes and plasma were prepared by blood centrifugation $1000 \times$ g for 10 min. The erythrocytes were then washed and diluted to original blood volume by physiological saline. Acetylcholinesterase preparations from rat and chicken brains were prepared according to *Andersen et al.* (1972).

Isolated preparations

The isolated phrenic nerve diaphragm preparation from rat was used according to *Barstad* (1968).

Cholinesterase activity and inhibition studies

The measurements of cholinesterase activities were based on the method of *Ellman et al.* (1961). For erythrocytes, however, a modification of the original method was employed. In this case the substrate and the colour producing component DTNB were added to physiological saline containing a proper concentration of erythrocytes. After a convenient time interval the substrate hydrolysis was stopped by physostigmine (10^{-3}M). Before spectrophotometric reading the solution was cleared for erythrocytes by centrifugation.

Gas chromatography—mass spectrometry (GLC-MS)

GLC-MS were used to test the purity of Ectoral®. The data were obtained using a LKB Model 9000 mass spectrometer coupled to a gas chromatograph. The column was a 3 m × 3 mm i.d. glass tube packed with either 3 % OV-17 or 3 % OV-1 on a.w. DMCS-treated chromosorb W, 80/100 mesh. Operating temperature conditions were: column programmed from 135 to 270°C, 4°C/min.; flashheater at 250°C; molecular separator at 250°C and ion source at 270°C. Helium (35 ml/min.) was used as carrier gas. When recording gas chromatograms using only the total-ion-current detector, the ion source was operated at 20 eV. When scanning mass spectra the electron energy was changed to 70 eV automatically. Optimal amplification was used for the MS recordings. For testing the purity of Ectoral®, from 1 to 25 µl of a saturated (25°C) acetone solution of Ectoral®, assumed to contain approx. 700 g/100 ml, was injected into the gas chromatograph. Repetitive scanning of mass spectra was done all through the GC-run.

Clinical experiments with Ectoral®

Four blue fox bitches were used. The animals were housed individually in outdoor cages. They were fed a fresh food mixture prepared in own kitchen and given fresh water ad libitum.

Two of them were given fenchlorphos daily in the feed for 30 days, one 100 mg/kg body weight and the other 200 mg/kg. Ectoral® was mixed into the feed just prior to feeding. The third fox received a single dose, 40 ml i.v., of Ectoral® dissolved in physiological saline corresponding to 0.4 mg fenchlorphos/kg. The fourth one was given a single dose of 500 mg fenchlorphos/kg body weight through a stomach tube. The foxes receiving single doses only were fasted for 24 hrs. prior to administration and were fed 6 hrs. following administration. The foxes fed 100 and 200 mg fenchlorphos/kg per day were about two and four years old, respectively, while the others were one year old. Blood samples were drawn at suitable intervals from the cephalic vein.

RESULTS

The GC-runs of aliquots of the saturated Ectoral® acetone solution revealed, in addition to the fenchlorphos peak, eight small peaks having shorter retention times. However, the sum of the small peak areas constituted less than 1.5 % of the fen-

chlorphos peak area. The identities of the compounds corresponding to two of the small peaks were determined to be trichlorophenol and trichloromethoxybenzene. No effort was made to identify the other resulting small peaks, however, most of them seemed to contain at least one chlorine atom. Traces of the fenchlorphos oxoanalog in Ectoral® could not be detected in the GC-run when studying the mass spectra obtained from repetitive scanning done every fifth second. It was assumed that it should be possible at least to detect the oxoanalog when present in an excess of approx. 1/7000 of the concentration of fenchlorphos.

Plasma cholinesterase was inhibited significantly by a saturated solution of fenchlorphos in phosphate buffer, however, depending on the enzyme source, the rate of inhibition varied. The commercial pseudocholinesterase from horse serum was inhibited 60 % in 1 min., while cholinesterase in newly prepared blood plasma from human, rat and blue fox was inhibited about 10 times slower. Acetylcholinesterase, or true cholinesterase, however, from rat and chicken brains and also commercially obtained acetylcholinesterase from bovine erythrocytes were tested without being significantly inhibited by the fenchlorphos solution. When the isolated rat phrenic nerve diaphragm preparation was incubated with a saturated solution of fenchlorphos in Tyrode, neither increase in single contraction height nor high frequency inhibition were observed, thus indicating no acetylcholinesterase inhibition.

The cholinesterase activities of plasma and erythrocytes were followed during and after the 30 days' feeding experiments with fenchlorphos (Figs. 1 A, 1B). At the beginning of the treatment the plasma cholinesterase activity was seen to decrease rather sharply. For the animal given 100 mg/kg per day the maximum inhibition measured was 65 %, while it was 69 % for the one given 200 mg/kg per day. After the last administration of fenchlorphos the plasma cholinesterase activity increased rapidly. An overshoot was shown before the activity stabilized to normal. The acetylcholinesterase activity of the erythrocytes, however, did not show a comparable fast response to onset and termination of fenchlorphos administration. Maximum inhibition levels were 43 % for the animal given 100 mg/kg and 63 % for the one given 200 mg/kg per day. Both foxes seemed to attain normal cholinesterase levels in about three months after the end of fenchlorphos administration.

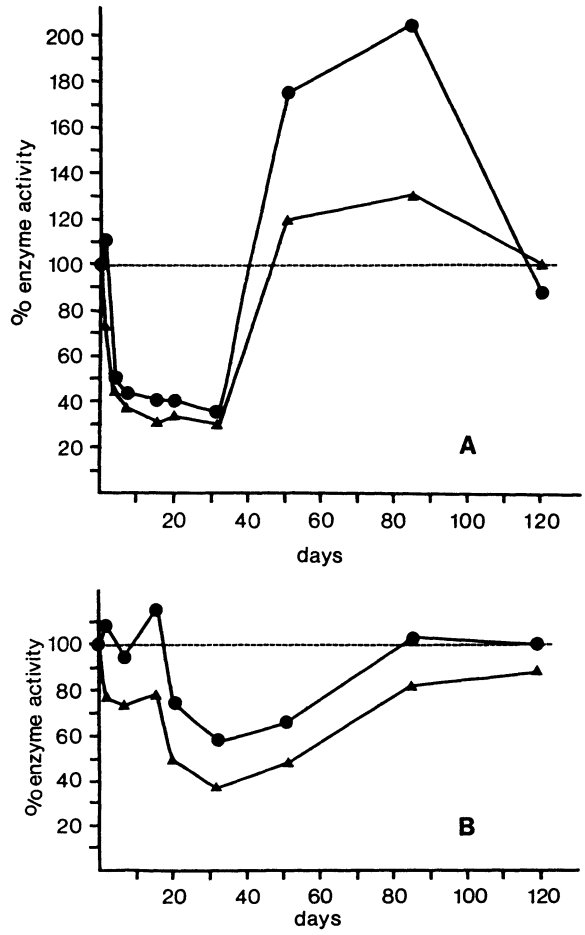

Figure 1. Time course of the cholinesterase activities during and after the 30 days' feeding experiment on two blue foxes. Fenchlorphos was dosed per kg body weight.

A. Pseudocholinesterase in plasma, ●——● 100 mg/kg daily, ▲——▲ 200 mg/kg daily.

B. Acetylcholinesterase in erythrocytes, ●——● 100 mg/kg daily, ▲——▲ 200 mg/kg daily.

The cholinesterase activities of plasma and erythrocytes were also followed after administration of a single oral dose of 500 mg fenchlorphos/kg and after i.v. injection of 40 ml saturated fenchlorphos solution. The effect of the dose given i.v. was only measurable as a small transient decrease (15 %) of the plasma

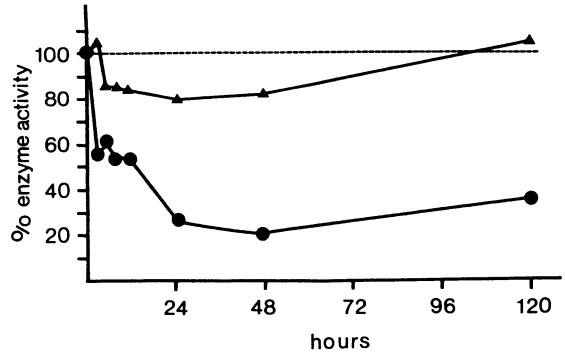

Figure 2. Time course of the cholinesterase activities after administration of 500 mg fenchlorphos/kg body weight as a single dose through a stomach tube to one blue fox, ●——● pseudocholinesterase in plasma, ▲——▲ acetylcholinesterase in erythrocytes.

cholinesterase level. The dose given orally, however, resulted in a sudden, large decline in plasma cholinesterase level, showing 80 % inhibition as maximum on the third day following administration (Fig. 2). After five days, when the last determination was performed, the activity still remained low, although a slight increase was observed. The acetylcholinesterase activity of the erythrocytes was not significantly affected.

Symptoms as salivation, tremors, diarrhea, pinpoint pupils or respiratory distress were never seen during the experiments performed, but the animal given the single oral dose of 500 mg fenchlorphos/kg vomited during feeding the day after administration.

DISCUSSION

The present investigation revealed that Ectoral® has an anticholinesterase potency of its own. This effect is probably caused by fenchlorphos isomers (*O'Brien* 1967). That fenchlorphos itself should act as an inhibitor is not likely, since the phosphorothionates of which fenchlorphos is an example have no significant anticholinesterase potency prior to its metabolism in the liver (*Spencer* 1972). Many workers have demonstrated that the pseudocholinesterases are often more susceptible to organophosphorous compounds than acetylcholinesterases (*Cohen & Oosterbaan* 1963). This is also the case for the unidentified inhibitor in

Ectoral®, because neither acetylcholinesterase from brain or erythrocytes were significantly inhibited by in vitro experiments. Nor could any inhibitory effect be demonstrated by using the diaphragm preparation. Because the acetylcholinesterase of the blue foxes was inhibited to a considerable extent in the present experiments, the inhibiting contaminants of Ectoral® could only play a modest role and only for the observed pseudocholinesterase inhibition. The cholinesterase inhibition caused by Ectoral® did not manifest itself by any clinical symptoms. This is in accordance with the observations of *Brimblecombe et al.* (1971) who showed that relatively large reductions in blood and brain cholinesterase levels are needed to establish significant clinical symptoms.

It is well established that fenchlorphos is easily absorbed and stored in the fat tissue of the body (*Hapke* 1975). The relatively low acute toxicity observed for fenchlorphos may be due to trapping of the compound in the fat. This may lead to only a slow release of the compound for activation to its oxoanalog by the microsomal enzyme system.

Successful control of ticks, fleas, lice and mites in dogs has been reported when given fenchlorphos orally. Depending on the kind of attack, the doses differed from 20 to 100 mg/kg body weight daily for a month. The same doses have been administered every other day or once weekly for longer periods (*Burch & Brinkman* 1962). Our results suggest that fenchlorphos administration in the feed in doses recommended for dogs is well tolerated by healthy foxes as far as cholinesterase inhibition is concerned.

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SAMMENDRAG

Effekten av O,O-dimethyl-O-(2,4,5-triklorfenyl) fosforothioate (fenklorfos) på kolinesteraser hos blårev (Alopex lagopus).

Fire blårevtisper ble brukt i eksperimentene. To fikk fenklorfos blandet i fóret, den ene 100 mg/kg kroppsvekt og den andre 200 mg/kg daglig i 30 dager. Maksimal hemming av plasmakolinesterase (pseudokolinesterase) var henholdsvis 65 og 69 %. Tilsvarende verdier for erythrocytkolinesterase (acetylkolinesterase) var 43 og 63 %. Begge revene hadde tilnærmet normale kolinesterasenivåer 3 mndr. etter avsluttet fenklorfosdosering.

Den tredje reven fikk engangsdose på 0,4 mg fenklorfos/kg i.v. I dette tilfellet ble effekten bare registrert som et lite fall (15 %) i plasmakolinesterasenivået.

Den fjerde reven fikk engangsdose på 500 mg/kg med magesonde. Maksimal hemming ble observert tredje dagen etter dosering. Da var 80 % av plasmakolinesterasen hemmet, mot bare 20 % av erythrocytkolinesterasen. Denne reven kastet opp under fóring dagen etter dosering.

Spyttsekresjon, skjelving, diaré, sammentrukne pupiller eller pustebesvær ble ikke observert på noen av revene under de utførte forsøk.

Det ble konkludert med at fenklorfos gitt i fóret, i doser anbefalt til hunder ved ektoparasittangrep, tolereres godt av friske rever.

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