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OBIDOXIME REACTIVATION OF ORGANOPHOSPHATE-INHIBITED CHOLINESTERASE ACTIVITY IN PIGS

By

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GYRD-HANSEN, N. and I. KRAUL: *Obidoxime reactivation of organophosphate-inhibited cholinesterase activity in pigs.* Acta vet. scand. 1984, *25*, 86—95. — The ability of obidoxime to reactivate organophosphate-inhibited cholinesterases was studied in pigs treated with either trichlorfon, dichlorvos or coumaphos. In 6 pigs cholinesterase activity was measured in the blood samples both before and after in vitro reactivation with obidoxime. Three pigs were treated with obidoxime 6 h after administration of the organophosphates in order to study the possibility of in vivo reactivation.

The results show a close correlation between the ability of obidoxime to reactivate the inhibited cholinesterases in vitro and in vivo. However, there was a marked difference in the possibility of reactivation between the 3 organophosphates. Thus no reactivation was possible after treatment with dichlorvos, while reactivation could be achieved for at least 6 h after administration of trichlorfon. After coumaphos treatment reactivation with obidoxime was possible for more than 24 h.

obidoxime; trichlorfon; dichlorvos; coumaphos; cholinesterase activity; pigs.

In 1951 *Wilson* showed that acetylcholinesterase inhibited by an organophosphate, tetraethylpyrophosphate (TEPP), could be reactivated by means of hydroxylamine or choline. This discovery started the use of cholinesterase-reactivators in the treatment of organophosphate poisonings. Among these reactivators pralidoxime[1] (*Wilson & Ginsburg* 1955) is probably the best known and has been proved efficient against poisoning with TEPP, di-isopropylfluorophosphate (DFP), paraoxon and parathion a.o. (vide *Hobbiger* 1957, *Karlog* 1960). Today pralidoxime has to a great extent been replaced by the more potent obidoxime[2]

[1] Pyridine-2-aldoxime methiodide.

[2] Bis(4-hydroxyiminomethyl-pyridinium-1-methyl) ether dichloride.

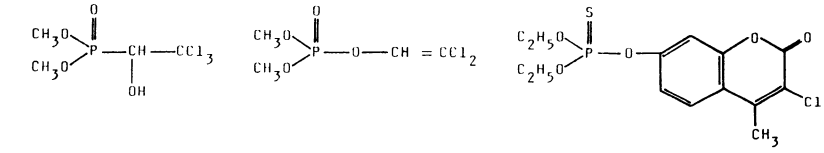
(*Heilbronn & Tolagen* 1965, *Natoff & Reiff* 1970). Treatment with reactivators was soon shown to be less efficient when the enzyme inhibition had persisted for some time than in acute cases (*Hobbiger* 1956, 1957, *Davies & Green* 1956). Furthermore the therapeutic value of the reactivators was found to vary considerably according to chemical structure and enzyme-phosphorylating properties of the organophosphates (*Sanderson & Edson* 1959).

In Denmark 3 organophosphates are being used in veterinary practice — trichlorfon for the treatment of ecto- and endoparasites, dichlorvos for endoparasites and coumaphos for ectoparasites. In high roses they all produce cholinesterase inhibition and it is the purpose of the present study to examine the reactivating capacity of obidoxime on the cholinesterase activity in blood from pigs treated with these 3 organophosphates.

MATERIALS AND METHODS

Nine pigs of the Danish Landrace breed with a body weight between 18 and 28 kg were used for this study. The pigs were kept separately with free access to water and were fed a commercial mixture twice daily according to weight.

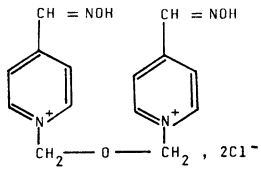
The following 3 organophosphates were administered orally to the pigs: Trichlorfon (Neguvon® vet.), dichlorvos (Atgard® vet.) and coumaphos[a] (Fig. 1). Trichlorfon and coumaphos were



TRICHLORFON
(METRIFONATUM NFN)

DICHLORVOS

COUMAPHOS



OBIDOXIME

Figure 1. Chemical structures of the drugs used.

[a] Kindly supplied by Bayer Kemi A/S.

given as the pure chemical while Atgard® vet. is a sustained release preparation where dichlorvos is incorporated into polyvinyl chloride pellets. The treatment of the individual pigs as well as the dosages used are given in Table 1. The organophosphates were administered in the feed and were mixed in one third of the normal ration, which was given before the remaining two thirds, in order to ensure complete consumption of the drugs.

In pigs nos. 3, 6 and 9 a cholinesterase reactivator obidoxime (Toxogonin®)[b] was administered intravenously 6 h after the pigs had been given the organophosphate-containing feed (Table 1).

Table 1. Dosages of organophosphates and obidoxime administered to 9 pigs.

Pig no.	Organophosphate	Dosage (mg/kg)	Obidoxime (mg/kg)
1 and 2	Trichlorfon	100*	0
3	Trichlorfon	100*	10
4 and 5	Dichlorvos	100*	0
6	Dichlorvos	100*	10
7 and 8	Coumaphos	25	0
9	Coumaphos	25	10

* 100 mg/kg is twice the recommended dose.

Blood samples were taken before administration of the organophosphates and 0.5, 1, 2, 4, 6, 24, 30, 48, 72, 96, 168 and 240 h after. In those pigs, which were treated with obidoxime additional blood samples were collected 0.5, 1, 2, 4 and 6 h after the injection of obidoxime. Heparin was used as anticoagulant.

Cholinesterase activity was measured in whole blood as well as in plasma by the manometric Warburg-technique described by *Augustinsson* (1948). Cholinesterase activities are given as percentages of the activity in the control samples. In the samples from pigs nos. 1, 2, 4, 5, 7 and 8 cholinesterase activity was measured in whole blood both before and after in vitro cholinesterase reactivation was tried by incubation with $2 \cdot 10^{-3}$ mol/l obidoxime (in Warburg buffer pH 7.6) for 2 h at 20—22°C.

[b] Kindly supplied by Nordisk Droge.

RESULTS

None of the pigs were seriously affected by the treatment they received although pigs nos. 1 and 2 showed some excitation and muscle tremor 1—4 h after the administration of trichlorfon.

The effects of trichlorfon, dichlorvos and coumaphos on the cholinesterase activity in whole blood and plasma are illustrated in Figs. 2A, 3A and 4A, respectively. In all pigs the organophosphates caused a marked decrease in the cholinesterase activity in blood and especially in plasma. However, within the observation period of 10 days a spontaneous reactivation took place with the activity in plasma as well as whole blood returning almost to control values.

In the Figs. mentioned the activities in whole blood before and after in vitro reactivation with obidoxime can be compared. For trichorfon (Fig. 2A) the activity in the reactivated blood samples is twice as high as in the untreated samples during the first 4 h, after which this difference decreases and has disappeared completely at 24 h. With dichlorvos there is no real difference between the cholinesterase activities in the reactivated and the untreated blood samples at any time. Coumaphos on the other hand causes a cholinesterase inhibition, which can be reactivated in vitro during the first 30 h. At 6 h the activity in the reactivated sample is nearly twice as high as in the untreated one and at 24 h the difference is still 50 %.

In order to examine the relationship between in vitro and in vivo reactivation of organophosphate-inhibited cholinesterases pigs nos. 3, 6 and 9 were treated with obidoxime 6 h after the organophosphate administration and the results are shown in Figs. 2B, 3B and 4B.

In the trichlorfon-treated pig (Fig. 2B) obidoxime is able to increase the cholinesterase activity from 25 to 40 % of the control value, which corresponds well with the in vitro effect of obidoxime (Fig. 2A).

In vitro reactivation had little effect on the enzyme activity in blood samples from dichlorvos-treated pigs (Fig. 3A) and the same was the case when obidoxime was administered to the pig itself (Fig. 3B).

However, when the coumaphos-treated pig received obidoxime a 100 % increase of the enzyme activity in the blood was observed within ½ h, i.e. a rise in activity from 45 to 90 % of the

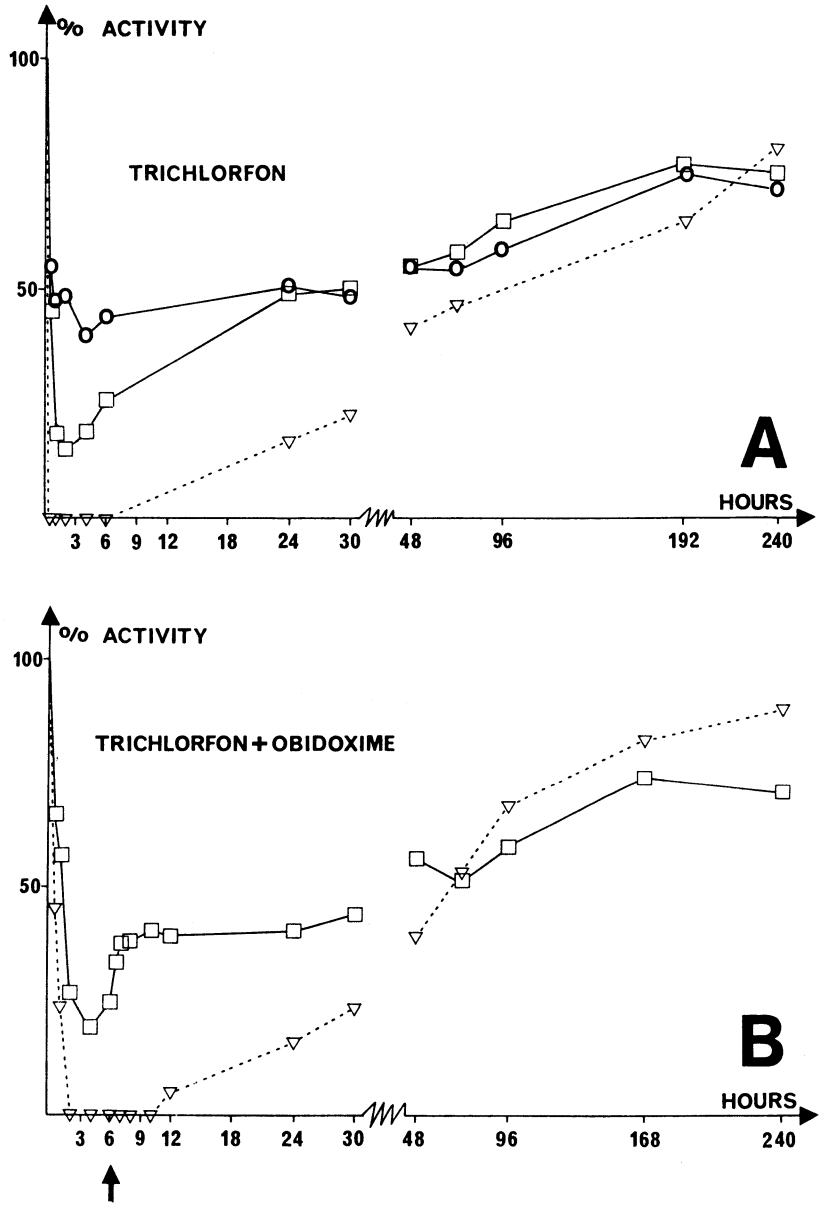


Figure 2A & B. Cholinesterase activity as per cent of control in plasma and whole blood from pigs nos. 1 (A) and 3 (B) both treated with trichlorfon. (▽) plasma; (□) whole blood; (O) whole blood after in vitro reactivation with obidoxime (A). The arrow indicates in vivo treatment with obidoxime (B).

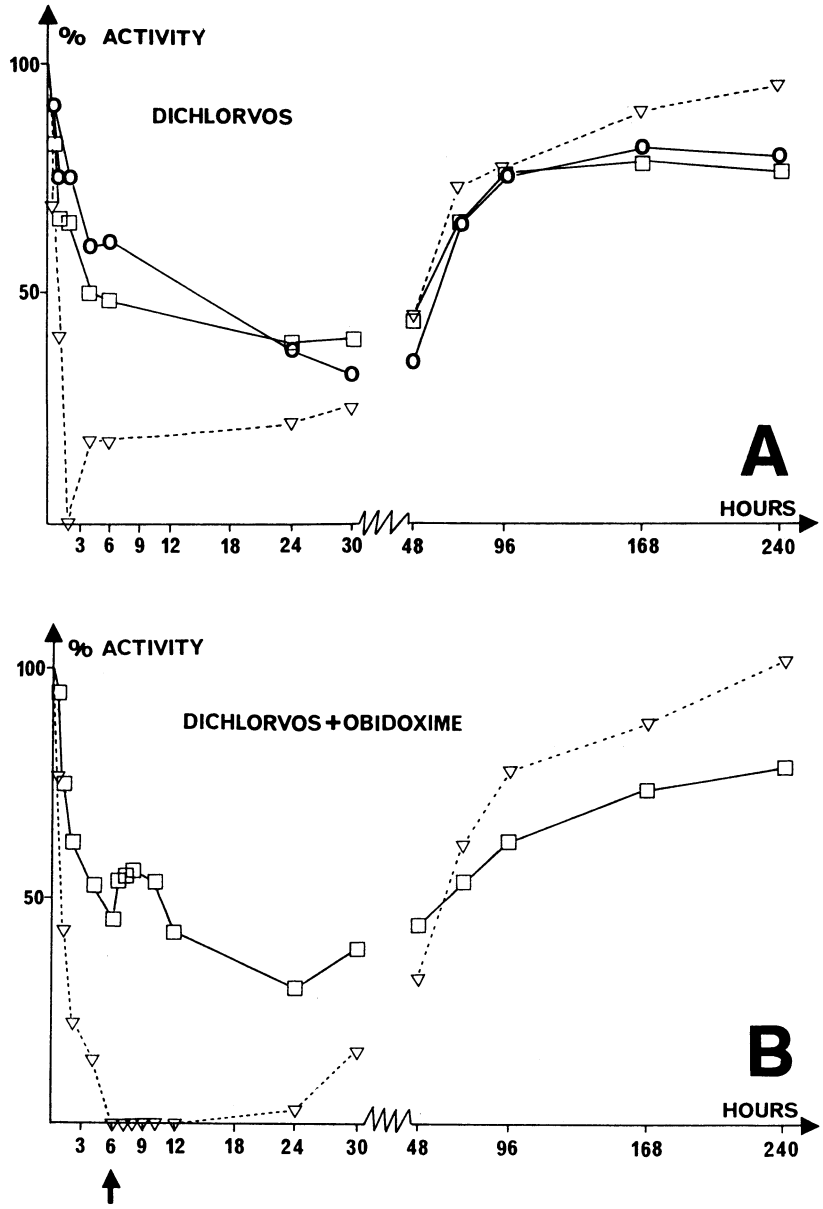


Figure 3A & B. Cholinesterase activity as per cent of control in plasma and whole blood from pigs nos. 4 (A) and 6 (B) both treated with dichlorvos. (▽) plasma; (□) whole blood; (O) whole blood after in vitro reactivation with obidoxime (A). The arrow indicates in vivo treatment with obidoxime (B).

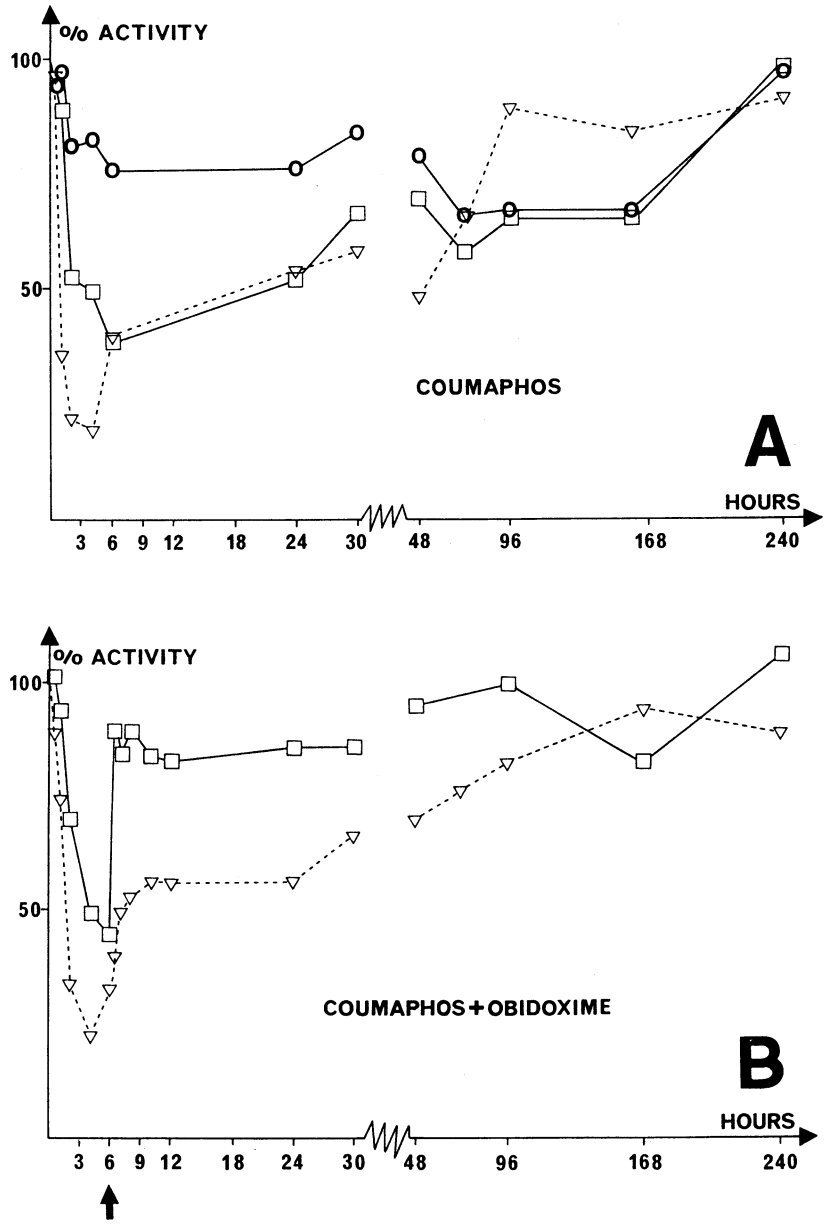


Figure 4A & B. Cholinesterase activity as per cent of control in plasma and whole blood from pigs nos. 7 (A) and 9 (B) both treated with coumaphos. (▽) plasma; (□) whole blood; (O) whole blood after in vitro reactivation with obidoxime (A). The arrow indicates in vivo treatment with obidoxime (B).

control value, which lasted for the rest of the observation period (Fig. 4B). This pronounced effect is very similar to the effect seen when obidoxime was added to the blood samples (Fig. 4A).

DISCUSSION

Trichlorfon has previously been shown quickly to inhibit the cholinesterase in whole blood and plasma of pigs treated with 50 or 100 mg per kg b.wt. orally (*Karlog et al.* 1971). As in the present study a spontaneous reactivation of the inhibited enzymes began few hours after administration with nearly normal enzyme activities being reached within 10 days.

Dichlorvos as Atgard® vet. has been given to pigs by *Bossen et al.* (1973) and *Gyrd-Hansen* (1982) who both found — as in the present study — that in this sustained release preparation dichlorvos caused a slowly occurring inhibition of the cholinesterase, the activity of which reached a minimum 30 h after administration. Spontaneous reactivation in whole blood took place from 48 h on and 80 % of normal activity was reached in 8—10 days both in the present study and in that of *Gyrd-Hansen* (1982) while *Bossen et al.* (1973) found it took somewhat longer to reach this level.

In vitro reactivation of inhibited cholinesterase by means of obidoxime was not possible after treatment with dichlorvos, while after treatment with the chemically closely related trichlorfon, obidoxime was effective for at least 6 h after administration of the organophosphate. The same observation that obidoxime is able to reactivate trichlorfon-inhibited cholinesterase was made by *Zech et al.* (1967) in experiments with horse serum. *Sanderson & Edson* (1959) observed that the ability of oximes to reactivate depends on the nature of the enzyme-phosphorylating portion of the organophosphates and that reactivation is not possible with certain dimethyl phosphates.

In the present study reactivation was only possible in less than 24 h after treatment with trichlorfon. This is in agreement with the observations made by *Hobbiger* (1956) and *Davies & Green* (1959) that when enzyme inhibition has lasted for some time reactivation can no longer take place. Other investigators (*Sanderson & Edson* 1959, *Coult et al.* 1966) have found that enzymes inhibited by di-isopropyl and dimethyl phosphates undergo this "ageing" much more readily than when inhibited

by diethyl phosphates. In accordance with this cholinesterase reactivation could be accomplished for more than 30 h after treatment with the diethyl phosphate coumaphos.

In the 3 pigs (nos. 3, 6 and 9) treated with obidoxime 6 h after administration of an organophosphates drug an increase in cholinesterase activity was obtained, which corresponds very well with the enzyme reactivation achieved by adding obidoxime to the blood samples taken from the other pigs 6 h after these were given the same organophosphate drugs.

If such good correlation between in vivo and in vitro reactivation always exists — and not only 6 h after exposure — it can be concluded that in case of overdosage or poisoning with one of the 3 organophosphates studied treatment with obidoxime would only be expedient in coumaphos poisonings — and maybe in severe cases of trichlorfon poisoning if the therapy could be administered within a few hours after the accident. Thus while atropin should always be used in the treatment of organophosphate poisoning obidoxime is only beneficial in special cases depending on the organophosphate involved.

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SAMMENDRAG

Reaktivering med obidoxim ved alkylfosfat-hæmmet kolinesteraseaktivitet hos grise.

Muligheden for at reaktivere alkylfosfat-hæmmet kolinesteraseaktivitet med obidoxim blev undersøgt på grise behandlet med enten trichlorfon, dichlorvos eller coumaphos. Kolinesteraseaktiviteten i blodet blev på 6 grise målt både før og efter in vitro reaktivering med obidoxim. Tre grise blev behandlet med obidoxim 6 timer efter indgift af alkylfosfat for at studere muligheden for in vivo reaktivering.

Undersøgelserne viste tydelig overensstemmelse mellem obidoxim's evne til at reaktivere hæmmet kolinesterase in vitro og in vivo. Samtidig observeredes en klar forskel i muligheden for reaktivering efter behandling med de 3 alkylfosfater. Reaktivering var således ikke mulig efter behandling med dichlorvos, mens hæmmet kolinesterase kunne reaktiveres i mindst 6 timer efter indgift af trichlorfon. Hos coumaphos-behandlede grise kunne reaktivering med obidoxim opnås i mere end 24 timer.

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