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INFLUENCE OF THE ROUTE OF EXPOSURE ON THE ACUTE TOXICITY OF CHOLINESTERASE INHIBITORS

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The acute toxicities of some cholinesterase inhibitor compounds have been determined by the oral, intraperitoneal, subcutaneous, intravenous and intracerebral routes of injection in female mice. The coupounds studied were monocrotophos, dicrotophos, mevinphos, crotoxyphos, dichlorvos, chlorfenvinphos, paraoxon, parathion, neostigmine and eserine.

Compounds which are rapidly degraded by the liver are less toxic following oral and intraperitoneal (hepatic routes) injection than they are by subcutaneous and intravenous (peripheral routes) injection. Parathion, which is activated by the liver, tends to be more toxic following administration by the intraperitoneal (hepatic) route.

Intracerebral injection by-passes the blood-brain barrier, and the compounds show, with one or two exceptions, a similar order of toxicity by this route of administration compared with the other routes of injection.

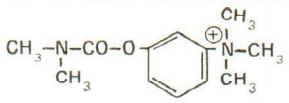
When measuring the median lethal doses of pesticidal cholinesterase inhibitors by different routes of injection, some striking differences and similarities were seen in the estimated values obtained (1).

Many of these compounds are known to penetrate the brain following their systemic injection (2), and exert an effect which may contribute to their toxicity. The variable of penetration of the blood-brain barrier was therefore obviated by measuring the acute toxicity of these compounds following their direct intracerebral injection in mice.

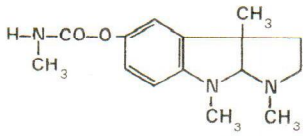
EXPERIMENTAL

The present observations were made using female mice of the CFE strain weighing between 18 and 26 g. They were divided into groups of 10, and received graded doses of the cholinesterase inhibitors. The compounds examined are shown in Figure 1. The doses of the compounds were prepared as logarithmically graded concentrations in physiological

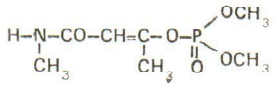
Neostigmine



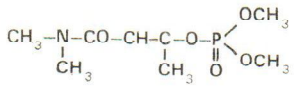
Eserine



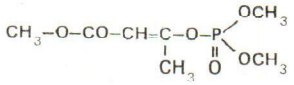
Monocrotophos



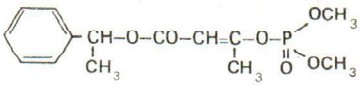
Dicrotophos



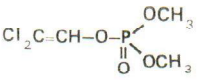
Mevinphos



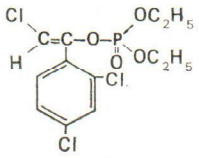
Crotoxyphos



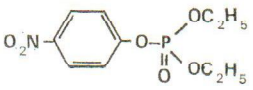
Dichlorvos



Chlorfenvinphos



Paraoxon



Parathion

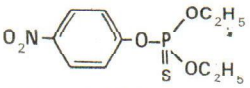


Fig. 1. *Cholinesterase inhibitors*

saline by solution, or by ultrasonic dispersion of insoluble materials. Chlorfenvinphos was dissolved in dimethylsulphoxide for the determination of its oral, intraperitoneal and subcutaneous toxicities.

A dose volume of 1 ml per 100 g body weight was used for the oral, intraperitoneal, intravenous and subcutaneous routes of injection.

For the study of intracerebral toxicity, injections were made in conscious mice by inserting a 26 gauge needle, 3 mm in length, vertically through the skull at the midpoint of a line joining the anterior bases of the ears. The dose volume was 0.02 ml/mouse. This form of injection delivers the material into the lateral, III and IV ventricles (3). In all studies of intracerebral toxicity, a control group of mice received an injection of physiological saline to ensure no effect due to injection trauma.

Deaths were recorded 24 hours after injection.

RESULTS AND DISCUSSION

Table 1 shows the estimated LD_{50} values of the different cholinesterase inhibitors following injection by the various routes. The routes of administration are grouped so that oral and intraperitoneal values appear under »hepatic routes« and subcutaneous and intravenous values appear under »peripheral routes«. For any one route of administration, a similar order of toxicity generally prevails.

The injection of a foreign compound into an animal by either of the »hepatic routes« infers that the total dose will pass mainly via the portal venous circulation during the first passage around the body, and so be subject to liver enzyme activity. If the order of toxicity is less by the oral than by the intraperitoneal routes this suggests the compound is either poorly absorbed from the gut or is broken down in its lumen.

Compounds administered by the subcutaneous or intravenous routes will enter the peripheral venous circulation directly, and only about 27.5% of the injected amount will pass through the liver during the first passage around the body (4). Should the compound be less toxic by subcutaneous injection than by intravenous injection, it would suggest poor, or slow, absorption from the subcutaneous site of injection. The major differences to observe, however, are those between the »hepatic« and »peripheral« groups, for these give some indication of the metabolic stability of the administered compound. Thus parathion is more toxic by intraperitoneal injection than by intravenous injection. Although this difference is not significant it nevertheless is in keeping with the known hepatic conversion of this compound to its active metabolite, paraoxon (5). However, paraoxon is inactivated in the liver by hydrolytic enzymes (6) and other enzyme systems (7), so that as parathion is activated it is simultaneously inactivated. This is substantiated by the higher toxicity of paraoxon itself by the peripheral routes than by the hepatic routes.

Table 1

Estimated median lethal doses (95% fiducial limits)

Cholinesterase inhibitor	Hepatic routes μ-mol/kg		Peripheral routes μ-mol/kg		Intracerebral n-mol/mouse
	i. p.	p. o.	i. v.	s. c.	
Neostigmine	1.9*	>15.0*	1.4 (0,8–2.4)	2.0 (1.7–2.4)	7.2 (6.1–8.5)
Eserine	2.4*	13.3 (9.7–18.9)	1.1 (0.9–1.4)	2.7 (2.1–4.0)	25.0 (20.8–28.4)
Monocrotophos	39.9 (19.3–82.4)	64.5 (43.9–95.4)	41.2*	39.0 (27.2–56.0)	571.3 (541.9–600.2)
Dicrotophos	43.2 (38.4–53.4)	73.2 (57.3–92.2)	36.2*	42.1 (35.1–50.1)	513.6 (466.8–565.2)
Mevinphos	11.2 (9.8–12.8)	54.9 (48.4–62.2)	3.0*	5.3 (3.4–8.0)	56.8 (47.3–64.6)
Crotoxyphos	193.7 (139.8–268.2)	509.5 (469.3–552.5)	12.3 (10.7–14.2)	41.3*	534.6 (484.1–584.6)
Dichlorvos	206.8*	832.6*	45.3*	108.6*	923.7 (816.5–1023.0)
Chlorfenvinphos	222.6** (181.7–286.5)	1018.3** (869.9–1192.2)	222.6 (163.7–301.9)	867.3** (655.0–1146.2)	>7675.0
Paraoxon	8.2*	46.1*	2.1 (1.9–2.4)	2.2*	49.9 (46.5–53.2)
Parathion	50.3 (42.6–58.6)	85.6*	58.0 (39.3–85.6)	71.3 (59.9–84.6)	>6866.5

* Data did not allow calculation of 95% fiducial limits

** Vehicle = dimethylsulphoxide

The two amides, monocrotophos and dicrotophos, have similar molar toxicities by all routes of administration. Monocrotophos is approximately equitoxic by the hepatic and peripheral routes, but dicrotophos is rather less toxic by the hepatic route. These compounds, in common with other dimethyl phosphate cholinesterase inhibitors, are *O*-demethylated by a soluble reduced glutathione-dependent enzyme, specific to methyl groups, in the liver (8). Thus the order dimethylphosphate compounds, crotoxyphos, mevinphos and dichlorvos, will also serve as substrates for this liver enzyme, and in fact are less toxic by the hepatic routes than by the peripheral routes.

An oxidative *O*-dealkylating enzyme associated with liver microsomes is responsible for the inactivation of both methyl and ethylphosphates (9). Both chlorfenvinphos and paraoxon will be substrates to this enzyme, but as there is little difference between the toxicities of chlorfenvinphos by either the hepatic or peripheral routes, this suggests a low rate of activity of this enzyme in the mouse (*Hutson* – personal communication).

The carbamates, eserine and neostigmine, are known to be inactivated by liver microsomal hydrolytic enzymes (10), and again this is borne out by the lower »hepatic« toxicity of these compounds.

Most of these catabolic enzymes may be inhibited by pre-treatment of the animals with the hepatic microsomal enzyme inhibitor SKF525-A (10), so increasing the toxicity of some of the cholinesterase inhibitors, while induction of these enzymes by pre-treatment of the animals with phenobarbitone (11) reduces their toxicity.

Although other means of inactivation of these compounds are present in the body (e. g. plasma protein binding, carboxylic ester hydrolysis, *p*-nitro reduction, oxidative *N*-dealkylation), this form of study of a new pesticide allows an insight into its possible break-down by hepatic enzymes, and the efficacy of its absorption following oral or dermal exposure.

When measuring the intracerebral LD_{50} values of these compounds it was found that at the highest doses administered, both parathion and chlorfenvinphos were without effect. Parathion has to be activated in the liver before it will inhibit cholinesterase (5), and so this observation is readily explained especially as its active metabolite, paraoxon, is one of the more toxic compounds by the intracerebral route. Chlorfenvinphos is a directly acting inhibitor of the enzyme, and its lack of toxicity at these high intracerebral doses is not possible to explain at the present time. Further studies to examine this phenomenon are in progress.

CONCLUSIONS

This study shows that there is an agreement between the route of exposure of cholinesterase inhibitors to mice and the known metabolism of these compounds. It also demonstrates that the order of toxicity of these compounds follows a similar trend irrespective of the route of exposure.

The use of various routes of administration in preliminary acute toxicity studies is therefore valuable in obtaining an indication of the fate of biologically active compounds in the body. Cholinesterase inhibitors have been used only as an example in this work. The principal illustrated applies to all biologically active materials.

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Sažetak

UTJECAJ NAČINA EKSPOZICIJE NA AKUTNU TOKSIČNOST INHIBITORA KOLINESTERAZE

Određena je akutna toksičnost nekih inhibitora kolinesteraze za ženke miševa nakon oralne, intraperitonealne, supkutane, intravenozne i intracerebralne aplikacije. Proučavani spojevi bili su: monokrotofos, dikrotofos, mevinfos, krotoksifos, diklorvos, klorfenvinfos, paraokson, paration, neostigmin i ezerin.

Spojevi što ih jetra brzo razgrađuje manje su toksični nakon oralne i intraperitonealne (hepatalni putevi) nego nakon supkutane i intravenozne aplikacije (periferni putevi). Paration, koji jetra aktivira, postaje toksičniji nakon intraperitonealne aplikacije (hepatalni put).

Intracerebralna aplikacija mimoilazi hematoencefalnu barijeru i, osim jedne ili dvije iznimke, ovi spojevi pokazuju sličnu toksičnost nakon ovog puta aplikacije u usporedbi s drugim putevima.

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