J. Pharm. Pharmac., 1967, **19**, 612–616 Received March 6, 1967

Influence of the route of administration on the toxicity of some cholinesterase inhibitors

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The acute median lethal doses of a series of cholinesterase inhibitors have been estimated in mice for different routes of administration Differences in the LD50 values obtained for intraperitoneal and oral routes ("hepatic" routes) and subcutaneous and intravenous routes ("peripheral" routes) suggest that the availability of the compound for metabolism by the liver is a major factor in their toxicity.

DIFFERENCES in the route of administration of biologically active compounds may influence their quantitative effects. Thus, tyramine has a more potent pressor effect in cats after intravenous injection than after intraportal injection (Natoff, 1965). Gaines, Hayes & Linder (1966) showed that solutions of some cholinesterase inhibitors produce muscular fasciculation more rapidly when infused into rats by the femoral vein than by the intestinal vein. Ramachandran (1966a,b) showed that di-isopropylphosphorofluoridate (DFP) is more toxic to mice when injected subcutaneously than when injected intraperitoneally.

These observations suggest that the availability of these compounds to the liver may account for the differences in their quantitative effects. To substantiate this, some cholinesterase inhibitors have been administered to mice by different routes and their median lethal doses (LD50) estimated.

Experimental

MATERIALS

Animals. Female albino mice, C.F.E. No. 1 strain, weighing 19 $\pm$5 g, were used at each dose level in groups of 10.

Drugs. The carbamates examined were physostigmine salicylate B.P. (B.D.H.) and neostigmine methylsulphate B.P. (Roche).

The organophosphates examined were Azodrin, Bidrin, chlorfenvinphos, Ciodrin, Phosdrin, parathion (Cheminova) and paraoxon (Baywood Chemicals Ltd.), the structural formulae of which are in Table 1.

TABLE 1. CHEMICAL FORMULAE OF THE ORGANOPHOSPHORUS CHOLINESTERASE INHIBITORS EXAMINED

$(RO)_2P(O)OC(R')=CH(R'')$

Compound	R	R′	R″
Azodrin	Me	Me	–CO·NH·Me
Bidrin	Me	Me	–CO·NMe$_2$
Chlorfenvinphos	Et	2,4-dichlorophenyl	Cl
Ciodrin	Me	Me	–CO·O·CH(Me)Ph
Phosdrin	Me	Me	–CO·OMe
Paraoxon	$(EtO)_2P(O)O(p\text{-}NO_2C_6H_4)$		
Parathion	$(EtO)_2P(S)O(p\text{-}NO_2C_6H_4)$		

From "Shell" Research Ltd., Tunstall Laboratory, Sittingbourne, Kent.

TABLE 2. 24 HOUR MEDIAN LETHAL DOSES OF CHOLINESTERASE INHIBITORS FOLLOWING ADMINISTRATION BY DIFFERENT ROUTES IN FEMALE MICE

Compound	Molecular weight	Active material %	LD50, mg/kg body weight (95% fiducial limits)				LD50, μmole/kg body weight			
			Hepatic routes		Peripheral routes		Hepatic routes		Peripheral routes	
			Intra-peritoneal	Oral	Sub-cutaneous	Intra-venous	Intra-peritoneal	Oral	Sub-cutaneous	Intra-venous
Physostigmine salicylate ..	413·45	100	*ca* 1·0 (—)	5·50 (4·02–7·83)	1·12 (0·85–1·65)	0·46 (0·38–0·56)	2·42	13·30	2·71	1·11
Neostigmine methylsulphate ..	334·39	100	0·62 (—)	>5·0† (—)	0·66 (0·56–0·80)	0·47 (0·28–0·80)	1·85	>15·00†	1·97	1·41
Azodrin	223·17	100	8·91 (4·31–18·4)	14·4 (9·80–21·3)	8·71 (6·06–12·5)	*ca* 9·2 (—)	39·92	64·52	39·03	41·22
Bidrin	237·20	86·8	11·8 (10·5–14·6)	20·0 (15·8–25·2)	11·5 (9·60–13·7)	*ca* 9·9 (—)	43·18	73·19	42·08	36·23
Chlorfenvinphos	359·59	92	87·0* (71·0–112·0)	398* (340–466)	339* (256–448)	87 (64–118)	222·59*	1018·27*	867·32*	222·59
Ciodrin	314·28	86	70·8 (51·1–98·0)	186·2 (171·7–201·9)	15·1 (—)	4·5 (3·9–5·2)	193·73	509·52	41·32	12·31
Phosdrin	224·14	100	2·51 (2·20–2·86)	12·30 (10·85–13·95)	1·18 (0·77–1·80)	0·68 (—)	11·20	54·88	5·26	3·03
Paraoxon	275·21	99	2·29 (—)	12·80 (—)	ca. 0·6 (—)	0·59 (0·53–0·66)	8·24	46·08	2·16	2·12
Parathion	291·27	97	15·1 (12·8–17·6)	25·7 (—)	21·4 (18·0–25·4)	17·4 (11·8–25·7)	50·29	85·59	71·27	57·95

*Vehicle = Dimethylsulphoxide. † Available material did not allow examination of higher concentrations.

These compounds were dissolved or suspended by ultrasonic agitation in physiological saline immediately before administration. Dimethylsulphoxide was used as the vehicle for estimating the LD50 of chlorfenvinphos by the oral, intraperitoneal and subcutaneous routes because of the poor stability of ultrasonic dispersions in saline, but the compound was suspended in physiological saline for intravenous administration and injected immediately.

METHODS

Median lethal doses were estimated in groups of mice by different routes of administration on the same day. The routes examined were classified as "hepatic" (oral and intraperitoneal) and "peripheral" (subcutaneous and intravenous). Preliminary experiments revealed the order of magnitude of the LD50 and the required logarithmic dose interval. The mice were observed for 24 hr after injection, and the LD50 values calculated with subsequent application of fiducial limits.

The volume of material injected was 10 ml/kg body weight for all routes of administration.

Results

Table 2 shows the estimated median lethal doses for each compound by different routes of administration. The values are expressed as mg of original compound per kg body weight, with 95% fiducial limits, and as μmol/kg body weight after correction for the proportion of active material. The term "active material" relates to the proportion of the original sample having biological activity.

In some instances, fiducial limits could not be calculated because of the high gradient of the log dose-mortality curve preventing a sufficient number of observations between 0 and 100% mortality.

Discussion

The toxicity and metabolism of organophosphorus and carbamate cholinesterase inhibitors have been examined by many workers. Although metabolic detoxification products have been identified, the site of their production within the body has not always been specified. Mazur (1946) demonstrated the occurrence of "DFPase" in the liver, and DFP has been shown to be more toxic to mice on intravenous or subcutaneous injection than on intraperitoneal injection. This suggested that DFP is detoxified by the liver (Ramachandran, 1966a).

Compounds administered intravenously or subcutaneously ("peripheral" routes) would enter the peripheral venous circulation directly and only about 27·5% would traverse the liver during the first passage through the body (Gaines & others, 1966). Administration of the compounds orally or intraperitoneally ("hepatic" routes) results in their access to the peripheral venous circulation being predominantly by way of the hepatic portal system. Differences in LD50 values between the "peripheral" and the "hepatic" routes therefore indicate the effect of the liver on the

biological activity of the compound. Moreover, differences between the intravenous and subcutaneous toxicities, or between the intraperitoneal and oral toxicities, indicate variations in the degree or rate of absorption from the appropriate sites. Any reduction in the rate of absorption would allow a greater net efficiency of the detoxifying process. Estimates of the LD50, which are similar between subcutaneous and intravenous routes but different between oral and intraperitoneal routes, suggest poor absorption of the compound from the alimentary tract, or its breakdown within the lumen.

Ciodrin, Phosdrin and paraoxon were more toxic by the "peripheral" routes than they were by the "hepatic" routes, suggesting detoxification during passage through the liver. Ciodrin and Phosdrin were also more toxic intravenously and intraperitoneally than they were subcutaneously and orally. This indicates poor absorption both from the intestine and from the subcutaneous site.

Paraoxon was equitoxic subcutaneously and intravenously. However, not only was it less toxic on "hepatic" administration, but the high LD50 value orally suggests poor absorption from the alimentary tract, or breakdown of the compound within the tract.

The carbamates physostigmine and neostigmine were more toxic intravenously than intraperitoneally, suggesting their detoxification by the liver. This agrees with the findings of Roberts, Thomas & Wilson (1965), who showed that within 10 min of the intramuscular injection of [^{14}C]neostigmine into rats, approximately 98% of the radioactivity in the liver was due to a metabolite, the production of which could be reduced by pretreatment with the liver microsome inhibitor SKF 525-A. Although the LD50 values of neostigmine and physostigmine were greatest after oral administration (Table 2), there is evidence of poor absorption of each compound because of differences in the subcutaneous and intravenous LD50 values.

The toxicity of Azodrin, Bidrin, and chlorfenvinphos did not appear to be reduced by "hepatic" administration. Azodrin and Bidrin are chemical congeners and Bidrin is *N*-demethylated *in vivo* to yield Azodrin (Bull & Lindquist, 1964; Menzer & Casida, 1965). The order of toxicity on a molar basis was similar by all four injection routes; oral administration caused the lowest toxicity.

Chlorfenvinphos was the only organophosphate studied which showed no evidence of a lower toxicity after administration by "hepatic" routes in mice. Hutson & Hathway (1967) found in the dog that the concentration of extractable chlorfenvinphos in the portal venous blood after oral dosing exceeded that in the peripheral venous blood, and that the compound had a lower toxicity in the dog than in either the mouse or the rat. Chlorfenvinphos therefore appears to be detoxified by the liver of the dog, but not by that of the mouse. The LD50 values obtained on "peripheral" and "hepatic" administration were similar, but differences for the values obtained for the individual routes within these groupings suggest that chlorfenvinphos is not readily absorbed from the alimentary tract and the subcutaneous site.

Parathion is a potent inhibitor of cholinesterase *in vivo* but not *in vitro*. It is oxidized by the liver to yield the active metabolite, paraoxon (Gage, 1953). Gaines & others (1966) found less parathion was required to produce muscular fasciculation in anaesthetized rats when it was infused into an intestinal vein than when it was infused into the femoral vein, suggesting that the liver is involved in the production of the biologically active product. Although parathion appeared to be more toxic to mice on intraperitoneal injection (Table 2) than on intravenous injection, this difference was not significant. As observations with paraoxon indicate that it is inactivated by the liver, the toxicity of parathion will depend on the resultant of the rate of hepatic conversion to paraoxon and the rate of inactivation of this compound.

Acknowledgements. The author wishes to thank Mr. G. R. Ellender for skilled technical assistance, and the Statistics Unit of Woodstock Agricultural Research Centre for the computer assessment of the median lethal doses, and of the fiducial limits.

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