

TOXIC EFFECTS OF TWO CARBAMATE INSECTICIDES IN DOGS

R. PLEŠTINA and B. SVETLIČIĆ

*Institute for Medical Research and Occupational Health,
Yugoslav Academy of Sciences and Arts and
Andrija Stampar School of Public Health, Medical Faculty,
University of Zagreb, Zagreb*

(Received for publication October 4, 1973)

In workers exposed to propoxur (2-isopropoxyphenyl methylcarbamate) and promecarb (3-methyl-5-isopropylphenyl methyl carbamate) the duration of plasma cholinesterase inhibition significantly differs. The aim of the present work was to find out whether this is due to differences related to enzyme-inhibitor interaction or to differences in insecticide absorption. Approximate equitoxic doses of the two carbamates were injected to dogs intravenously and intramuscularly. The intensity and quality of cholinergic symptoms were recorded and plasma cholinesterase activity measured at given time intervals. While the differences were found only after intramuscular application, the enzyme activity recovery was similar for both carbamates injected intravenously. The delayed plasma cholinesterase recovery in the case of promecarb could be explained by a much slower absorption after intramuscular injection of the compound.

Anticholinesterases are rather outstanding insecticides and their importance is growing rapidly as the use of more persistent organochlorine compounds is declining.

There are two groups of anticholinesterases: esters of phosphoric and carbamic acid. When these compounds react with cholinesterases a more stable phosphorylated and a less stable carbamylated enzyme is formed. The mechanism of this inhibitor-enzyme interaction is well understood (1, 2) and the overall process in both cases involves an essentially identical pathway (3). However, differences of great practical significance between the two groups of compounds have been established by *in vitro* studies (4, 5) and clearly demonstrated in experimental animals (6, 7) and exposed humans (8, 9).

This study has been supported in part by a research grant from the World Health Organization.

The experiments in which the $LD_{50} : ED_{50}$ ratios for the two groups of anticholinesterase insecticides were determined in rats and only preliminarily in dogs (6) yielded much higher values for all carbamates tested. Thus, in contrast to organophosphorus compounds, carbamates possess an important advantage from the point of view of occupational exposure since a very small fraction of the dangerous dose is capable of producing cholinergic symptoms which would warn exposed man and prevent the excessive absorption of a pesticide (10).

In the WHO programme for the evaluation and testing of new insecticides, which has been in operation for 13 years, nearly 1400 compounds were screened (11). Only three compounds, one carbamate and two organophosphates, proved to be both effective and safe insecticides for residual indoor application (12).

Among the abandoned compounds there was a very effective and promising insecticide OMS-716 (3-methyl-5-isopropylphenyl methyl carbamate). Although no clinical manifestations of poisoning were observed during its application, plasma cholinesterase activity in exposed persons remained inhibited for several days (13). Another similar carbamate insecticide — OMS-33 (o-isopropoxyphenyl methylcarbamate) — however, has been tested further and eventually released for wide use (14).

These two monomethylcarbamates (Fig. 1) differ in their acute toxicity to rats, i. v. LD_{50} for OMS-33 being 10.6 and for OMS-716 5.3 mg/kg (7), as well as in their inhibitory properties against erythrocyte and plasma cholinesterase.

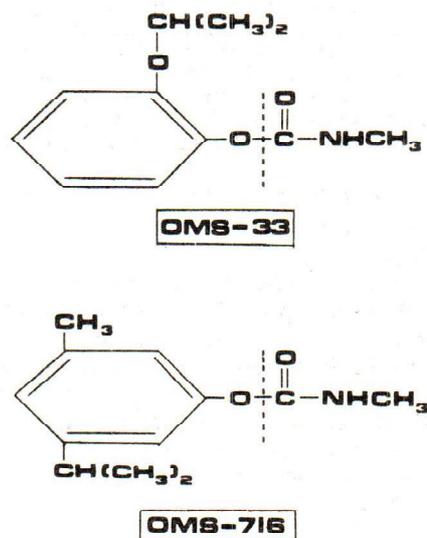


Fig. 1 Chemical structure of propoxur (OMS-33) and promecarb (OMS-716)

The aim of this work was therefore to try to explain a disagreement between the theoretical principles and actual findings in exposed subjects by studying further the effects of the two carbamates in dogs.

MATERIAL AND METHODS

The carbamate insecticides OMS-33 (propoxur) and OMS-716 (promecarb) were supplied by WHO and were purified before use. The compounds were dissolved in propyleneglycol in suitable concentrations on the day of application.

A total of 23 healthy cross-bred dogs of both sexes weighing 8–33 kg were injected a single intramuscular or intravenous dose of carbamate. The injected volume varied from 0.05–1.5 ml/kg body weight depending upon the dose. The doses were selected according to the experience obtained with rats and in preliminary tests on dogs, the lowest producing hardly noticeable symptoms and the highest causing severe symptoms or death. The dogs were carefully observed and the onset, duration and intensity of symptoms were recorded.

Blood samples were collected by venepuncture in dry heparinized tubes before and at given times after injection. After separating the plasma by centrifuging for 5 minutes at 2500 r.p.m., cholinesterase activity was measured instantly by Ellmans' spectrophotometric method (15, 16) using 50 μ l of plasma for the assay. Cholinesterase activity values are expressed as percentages of the normal (pretreatment) activity.

RESULTS

The onset, duration and severity of symptoms after intramuscular injection were as a rule dose dependent as shown in Table 1. The symptoms recorded after an injection of 60 mg/kg of OMS-716 were unexpectedly milder than those produced by half of this dose. It should be pointed out, however, that the concentration had to be increased from 50 to 80 mg/ml to enable the injection of the highest dose.

No qualitative difference in symptoms was observed between the two carbamates. Usually the first symptoms noticed were the tremor of the head, periodical trembling of auricles and palpebral muscles and occasional swallowing or dropping of saliva from the mouth. The intensity of symptoms rapidly increased so when reaching maximum usually a pronounced dyspnoea, tremor, fasciculations and at highest doses convulsions and staggering gait were recorded.

As shown in Fig. 2 a rapid fall of enzyme activity was found in dogs injected intramuscularly with either compound and the degree of inhibition was dose dependent. The recovery of enzyme activity, however, was much slower in case of OMS-716: at the highest dose it regained

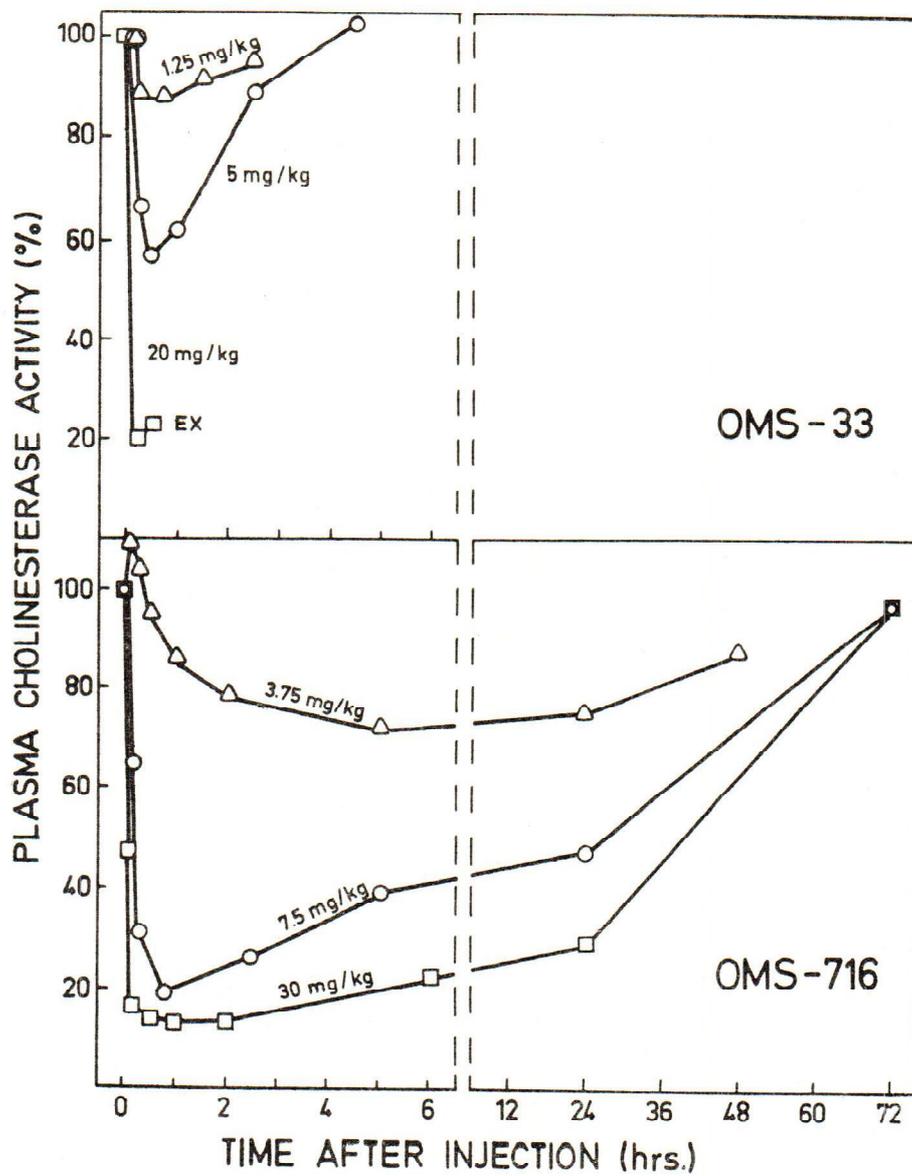


Fig. 2 Plasma cholinesterase activity in dogs after a single intramuscular injection of OMS-33 or OMS-716

Table 1.

Dose-response relationship in dogs treated with a single intramuscular injection of OMS-33 or OMS-716

Compound	Dose mg/kg	Symptoms		
		Onset (min.)	Duration (nrs)	Intensity
OMS-33	1.25	5	0.5	very slight
	1.50	13	1.5	slight
	2.00	5	1.0	very slight
	5.00	5	2.5	severe
	8.00	7	3.0	severe
	8.00	5	2.5	moderate
	10.0	8	5.0	very severe
	15.0	3	6.0	severe
20.0	5	Died at 42 min.		very severe
OMS-716	3.75	15	1	hardly noticeable
	7.5	15	2.5	slight
	15.0	8	1	moderate
	30.0	8	6	severe
	30.0	4	> 5 < 24	very severe
	60.0	0	5-7	severe

Table 2.

Dose-response relationship in dogs treated with a single intravenous injection of OMS-33 or OMS-716

Compound	Dose mg/kg	Symptoms		
		Onset (min.)	Duration (hrs)	Intensity
OMS-33	0.75	4	0.75	very slight
	0.75	3	0.75	slight
	3.0	5	2	very severe
	3.0	1	1.5	very severe
OMS-716	0.375	7	0.3	hardly noticeable
	0.357	2	1	slight
	1.5	2	2.5	moderate
	1.5	2	2	very severe

normal activity only by the fourth day after injection. This prolonged inhibition was most likely due to a much slower absorption of OMS-716 from the site of injection than of OMS-33.

In the second series of experiments animals were therefore injected with OMS-33 or OMS-716 by intravenous route. Again, as shown in Ta-

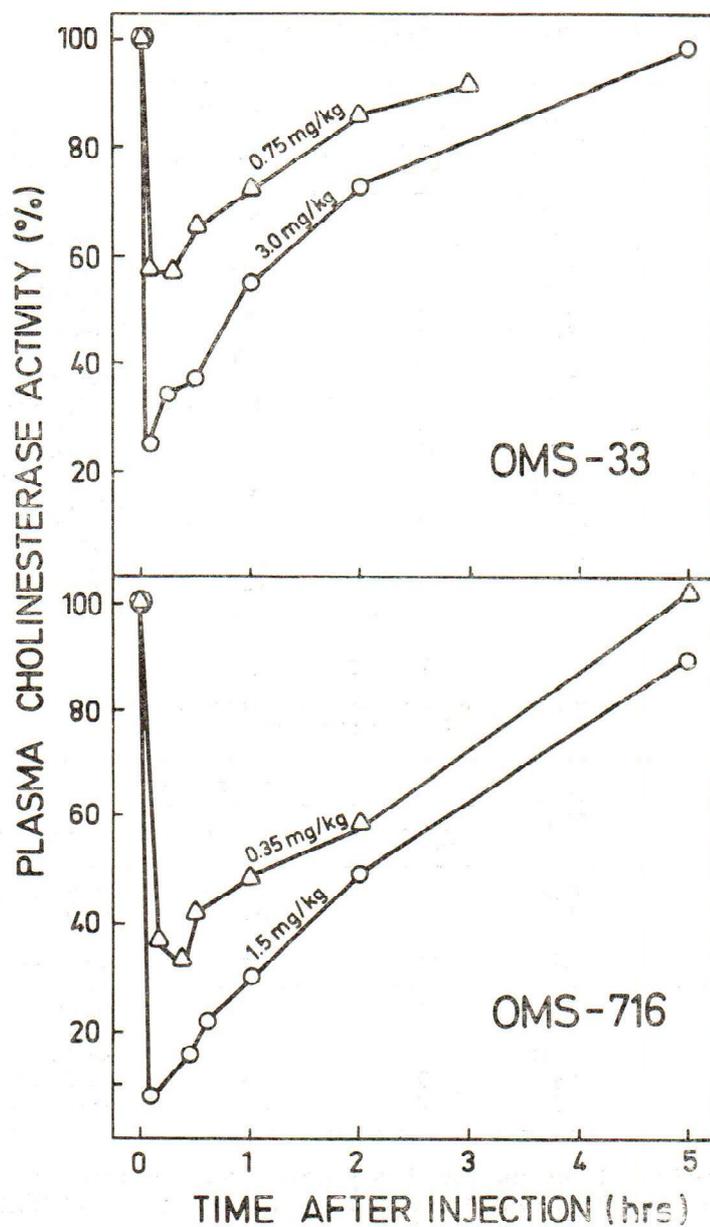


Fig. 3. Plasma cholinesterase activity in dogs after a single intravenous injection of OMS-33 or OMS-716

ble 2, the onset, duration and intensity of symptoms were dependent of the dosage applied, the quality of symptoms observed being similar for both compounds and very much alike to those described after intramuscular injection.

The values of plasma cholinesterase activity after intravenous injection of different dosages of the two carbamates are shown in Fig. 3. After this route of application no difference in the rate of recovery of enzyme activity was found between the two compounds. Again, the higher the dose the more pronounced plasma cholinesterase inhibition was found. At the highest doses applied OMS-33 and OMS-716 produced cholinesterase depression down to 25 and 8 percent respectively. The inhibition was relatively transient and the enzyme regained its pretreatment value within five hours.

DISCUSSION

The carbamates tested produced typical cholinergic symptoms in dogs, the intensity of which varied according to the dose applied. The onset of the symptoms was also dose dependent, and it was shorter after intravenous than after intramuscular injection of either compound. The symptoms lasted similarly for both compounds, 6—7 hours after maximal intramuscular dose and about 2 hours after the higher intravenous dose.

The intravenous toxicity of OMS-716 in rats is twice as high as that of OMS-33 (7). However, after intraperitoneal application the acute toxicities are similar and orally OMS-716 is even less toxic (7). These data fit with our observations on dogs. Namely, after intravenous application the relationship between the doses producing equally intensive symptoms was similar to that in rats. However, when using the intramuscular route we had to apply much higher doses of OMS-716 to produce equally intensive symptoms. In addition, the dose of 60 mg/kg of OMS-716, which was three times higher than the lethal dose of OMS-33 (20 mg/kg) was not only non-fatal, but produced milder symptoms than a dose of 30 mg/kg of OMS-716 which was administered less concentrated. All this points at differences in absorption between the two compounds tested.

It should be pointed out that no differences in quality were found if the symptoms observed in rats were compared with those described in rats (17).

Although plasma cholinesterase activity does not play any known role in cholinergic mechanisms, this enzyme was followed during the study because the dog's erythrocyte cholinesterase is too low to be measured. It was shown that plasma and brain cholinesterase of rats treated with OMS-33 are closely related to the symptoms observed (17). However, it is not known whether any similar correlation exists in dogs. In our experiments a reasonable correlation was found between the intensity of symptoms and plasma cholinesterase inhibition after treating dogs with

either compound. The rate of *in vitro* inhibition of dog's plasma cholinesterase differs significantly for the two compounds. At 37°C k_i for OMS-33 is $8.4 \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$ and for OMS-716 it is $6.1 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$ (18). These data are in agreement with our findings concerning the extent of inhibition after intravenous application of the inhibitors, since smaller doses of OMS-716 produced more pronounced enzyme inhibition. It was not so, however, after intramuscular application.

Long lasting plasma cholinesterase inhibition both in spraymen (13) and in dogs injected intramuscularly is not in agreement with the present knowledge of the kinetics of carbamylated enzyme. In such a short time, as in our experiments, it is not likely that any other mechanism except reactivation was involved in the enzyme recovery. There is no indication that the type of carbamylation for the two compounds is different and therefore, the rate of reactivation should not differ either. This was proved by measuring the rate of reactivation of carbamylated cholinesterases inhibited *in vitro* by the two compounds. The constants of reactivation were identical (18).

Prolonged inhibition after intramuscular application of OMS-716 could not be attributed to the longer persistence of this inhibitor in blood since its persistence in rats circulation was shown to be very short (19) and no such difference was observed after intravenous application.

The only possible explanation for the delayed enzyme recovery would be a delay in muscular resorption in our experiments and in the gastrointestinal and/or skin resorption in case of human occupational exposure.

Our results imply that the toxic effects and biochemical lesions found after application of chemically similar compounds may vary considerably although this variation may not necessarily be due to differences in their mode of action. This may largely depend on distinctive pathway in absorption, distribution and persistence both in experimental animals and in humans.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. Vandekar for stimulating discussions and critical comments and Mr. A. Fajdetić for skilful technical assistance.

References

1. Aldridge, W. N.: *Biochem. J.*, 53 (1953) 110.
2. Casida, J. E.: *Annual Rev. Entomol.*, 8 (1963) 39.
3. Wilson, I. B., Hatch, M. A., Ginsburg, S.: *J. Biol. Chem.*, 235 (1960) 2312.
4. Vandekar, M., Heath, D. F.: *Biochem. J.*, 67 (1957) 202.
5. Reiner, E., Simeon-Rudolf, V.: *Biochem. J.*, 98 (1966) 501.
6. Vandekar, M., Reiner, E., Svetličić, B., Fajdetić, T.: *Brit. J. Industr. Med.*, 22 (1965) 317.
7. Vandekar, M., Pleština, R., Wilhelm, K.: *Bull. Wld Hlth Org.*, 44 (1971) 241.
8. Vandekar, M.: *Bull. Wld Hlth Org.*, 33 (1965) 107.

9. Vandekar, M., Svetličić, B.: Arh. hig. rada, 17 (1966) 135.
10. Vandekar, M., Hedayat, S., Pleština, R., Ahmady, G.: Bull. Wld Hlth Org., 38 (1968) 609.
11. Wright, J. W.: Bull. Wld Hlth Org., 44 (1971) 11.
12. WHO Expert Committee on Insecticides: Wld Hlth Org. Techn. Rep. Ser. No 513 (1973) 16.
13. WHO Expert Committee on Insecticides: Wld Hlth Org. Techn. Rep. Ser. No. 356 (1967) 43.
14. Wright, J. W., Fritz, R. F., Hocking, K. S., Babione, R., Gratz, N. G., Pal, R., Stiles, A. R., Vandekar, M.: Bull. Wld Hlth Org., 40 (1969) 67.
15. Ellman, G. L., Courtney, K. D., Andres, V. Jr., Featherstone, R. M.: Biochem. Pharmacol., 7 (1961) 88.
16. Wilhelm, K.: Arh. hig. rada, 19 (1968) 199.
17. Pleština, R., Vandekar, M.: Proceedings 15th Internat. Congress of Occupat. Health, Vienna 2 (1966) 529.
18. Simeon, V.: Personal communication.
19. Wilhelm, K., Vandekar, M.: Proceedings 15th Internat. Congress of Occupat. Health, Vienna 2 (1966) 517.

Sažetak

TOKSIČNI UČINCI DVAJU KARBAMATNIH INSEKTICIDA NA PSIMA

U radnika eksponiranih propoksuru (2-izopropoksifenil N-metil karbamat) i promekarbu (3-metil-5-izopropoksifenil N-metil karbamat) nađene su razlike u trajanju inhibicije kolinesteraze plazme. Svrha ovog rada bila je utvrditi da li ova pojava počiva na razlikama u interakciji otrova s enzimom ili pak na različitim načinima ulaska otrova u organizam. Pokusi su rađeni na psima križancima kojima smo približno ekvitoksične doze istraživanih spojeva injicirali intravenski odnosno intramuskularno. Pratili smo i registrirali intenzitet i vrstu kolinergičnih simptoma i u zadanim vremenima mjerili aktivnost kolinesteraze plazme. S obzirom da smo razlike našli samo nakon intramuskularne aplikacije, a da je nakon intravenske aplikacije oporavak enzimske aktivnosti bio za oba spoja podjednak, dugotrajna inhibicija kolinesteraze plazme nakon promekarba može se tumačiti sporijom resorpcijom ovoga spoja pri intramuskularnoj aplikaciji.

*Institut za medicinska istraživanja
i medicinu rada Jugoslavenske akademije
znanosti i umjetnosti i Skola narodnog
zdravlja »Andrija Stampar« Medicinskog
fakulteta Sveučilišta u Zagrebu*

Primljeno 4. X 1973.