J. Pharm. Pharmac., 1969, **21**, 287–292 *Received October* 23, 1968

Comparison of bromisoval and carbromal in the rat

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Distribution experiments *in vitro* and *in vivo* with bromisoval and carbromal have shown carbromal to be more lipophilic and cumulative in brain and fat tissue. The half-life of bromisoval in the rat is approximately 2·5 h, that of carbromal is longer and variable. Stimulation and inhibition experiments revealed that both drugs are metabolized and follow different patterns. Carbromal toxicity is enhanced by bromide treatment, whereas bromisoval toxicity is not. The results show that carbromal is more toxic than bromisoval for the rat.

The hypnotic bromocarbamides (or bromoureides) carbromal and bromisoval, introduced in the beginning of this century, have largely been superseded by the evolution of the barbiturate hypnotics except as household remedies in some countries. Being effective and apparently innocuous when used occasionally they gave little cause for worry. When, however, in the years after the Second World War misuse and abuse of drugs became more widespread, the chronic and acute toxicity of both bromocarbamides became a source for concern (Magnussen, 1947; Anonymous comment, 1947; Kärber, 1952; Copas, Kay & Longman, 1959; Atkinson, 1960; Pihkanen & Harenko, 1962; van Heyst, 1966).

As pharmacological and toxicological knowledge of both drugs was defective, some of their properties which were expected to be related to their toxicity have been investigated. The tissue distribution and relative lipid solubilities were determined. Plasma half-lives were estimated and their dependence on sex and other factors were assessed. As acute intoxication with bromocarbamides often follows a period of prolonged misuse, the influence of bromide intoxication on the toxicity of both drugs was also determined.

EXPERIMENTAL

Materials. Bromisoval (Ph.Ned. Ed. VI) and carbromal (Ph.Ned. Ed.VI) were used and were shown to be pure by thin-layer chromatography. They were dissolved in propylene glycol B.P. for administration.

Animals. Wistar rats of either sex, approximately 150 g were used and were obtained from the TNO animal breeding farm, Zeist, the Netherlands, or from the breeding department of our own institute (RIV).

Partition coefficients. The bromocarbamide (5 mg) dissolved in deionized water (10 ml) was extracted for 4 h with an equal volume of organic solvent (nitrobenzene, chloroform, light petroleum b.p. 80–100°). Samples of both phases were taken, and the bromocarbamide content was determined as described by Rauws (1969), directly in the water phase and, after evaporation of the chloroform or light petroleum phase, in the residue taken up in water. After preceding alkaline hydrolysis of the bromocarbamide, the nitrobenzene phase was removed by steam distillation and bromide was determined in an aliquot of the aqueous residue.

Distribution in vivo. The drugs were administered to the rats by intraperitoneal injection as a solution in propylene glycol. Blood and tissue samples were taken when the animals awoke. It was hoped that this would minimize individual differences in distribution kinetics. Subcutaneous perineal fat was used in the analysis of fat tissue.

Determination of the half-life. Groups of six rats were bled (orbital puncture) to obtain blank values. At zero time 0·7–1·0 mmol/kg of the drug, was injected intraperitoneally. At least three times afterwards the rats were bled again, 0·4 ml of blood from each animal was pooled and the bulked blood of each group was used to determine the bromocarbamide concentration in duplicate. On the assumption that a linear relation exists between the logarithm of the blood level and time, the best fitting straight line was computed (de Jonge, 1962). The apparent half-life was calculated from the parameters of this line. No rate constants are derived from this empirical quantity.

Experimental bromide intoxication. Rats were given 0·1 M sodium chloride and 0·05 M sodium bromide in the drinking water. Control animals received 0·15 M sodium chloride. In three to four weeks blood bromide levels rose to approximately 25 m-equiv/litre. At these bromide levels, which in man are toxic, the rats showed no signs of depression as judged by their spontaneous motility, exploration and feeding habits. Grooming however was distinctly depressed and the otherwise clean fur of the rats became yellow and caked with dried urine.

Analytical procedures in biological material. These were carried out as described by Rauws (1969). As the plasma and blood concentrations in the rats used were essentially the same, determinations were made in whole blood.

Statistical analysis. Wilcoxon's two samples test was used to estimate the significance of some results.

RESULTS

Partition coefficients. The results are shown in Table 1. The two structurally related compounds exhibit large differences in physicochemical properties. The relative difference between the partition coefficients of the two compounds increases with the increase of the ratio for the dielectric constants of water and solvent.

Table 1. *Partition coefficients of bromisoval and carbromal between water and some organic solvents*

	Nitrobenzene: water	Chloroform: water	Light petroleum (80–100°): water
$K_{bromisoval}$	3·58	7·4	0·013
$K_{carbromal}$	26·3	163	0·53
K_{carbr}/K_{brIso}	7·35	22·0	40·7
$\epsilon_{water}/\epsilon_{solvent}$	2·27	16·9	42·6

Distribution in vivo. Table 2 shows the results of two distribution experiments. Carbromal accumulates in brain relative to blood and more so in fat tissue, whereas bromisoval concentration in fat tissue is strikingly low.

Comparison of half-lives. Results in Table 3 show a consistent and clear difference in half-life of carbromal and bromisoval in various lines of female rats of the Wistar strain. The same situation prevails in the male rat, as is illustrated in Tables 3 and 5. The unintentioned use of several lines of the Wistar strain in one investigation—a consequence of fluctuations in the supply—is instructive in so far as it shows the large

Table 2. *The distribution of bromisoval and carbromal in blood, brain tissue and fat tissue of the rat* (0·75 mol/kg, *intraperitoneally*). Samples were taken when the animals awoke. Concentration μmol/ml blood or μmol/g wet tissue (mean ± s.d.) Each group consisted of 5 or 6 animals.

		Experiment I	Experiment II
Bromisoval	Blood	0·200 ± 0·020	0·255 ± 0·055
	Brain	0·210 ± 0·020	0·250 ± 0·060
	Fat tissue	—	0·095 ± 0·025
Carbromal	Blood	0·180 ± 0·080	0·230 ± 0·035
	Brain	0·380 ± 0·050	0·370 ± 0·070
	Fat tissue	—	0·510 ± 0·225
Bromisoval	Brain*/Blood	1·05	1·00
	Fat tissue/Blood	—	0·37
Carbromal	Brain/Blood	2·11	1·60
	Fat tissue/Blood	—	2·23
Significance of difference between bromisoval and carbromal: *P* in %	Brain/Blood	<5	2
	Fat tissue/Blood	—	2

* Concentration ratio's are the mean values of the individual concentration ratio's.

differences that may be a result of slight differences in breeding and housing of animals.

The influence of sex on the half-life of carbromal and its lack of influence on that of bromisoval is shown in Table 3. Other differences between bromisoval and carbromal are the prolongation by SKF 525-A (β-diethylaminoethyldiphenylpropylacetate) of the half-life of bromisoval and the shortening by phenobarbitone pretreatment of the half-life of carbromal (Table 4). The increase in half-life of bromisoval by SKF 525-A

Table 3. *Plasma half-lives (h) of bromisoval and carbromal in the male and female rat*

Strain and line	Bromisoval		Carbromal	
	Female	Male	Female	Male
Wistar-RIV	3·0		12·5	6·9
Wistar-TNO	2·1		> 15	
	4·5		> 40	
Wistar-RIV inbred	1·2	1·2	6·5	2·8
	1·7		6·9	

Table 4. *Influence of pretreatment with phenobarbitone and SKF 525-A on the plasma half-lives (h) of bromisoval and carbromal in the rat*

		strain and line	Control	Phenobarbitone	SKF 525-A
Bromisoval	♀	Wistar-TNO	1·7	—	2·9
	♀♀	Wistar-TNO	4·5	4·9	8·6*
	♀	Wistar-RIV inbred	1·7	1·4	2·9†
	♂	Wistar-RIV inbred	1·2	1·2	3·8
Carbromal	♀	Wistar-TNO	> 15	—	> 15
	♀♀	Wistar-TNO	> 40	9·5	> 40
	♀	Wistar-RIV inbred	6·9	3·6	5·8
	♂	Wistar-RIV inbred	4·5	1·5	4·0

Pretreatment: phenobarbitone on both days preceding the experiment 50 mg/kg in saline intraperitoneally; SKF 525-A, 45 to 60 min before the experiment 80 mg/kg in saline intraperitoneally.

* 1 animal died (out of 6).

† 3 animals died (out of 6).

was accompanied by some mortality after the administration of doses which were non-lethal in controls. Benziodarone, an inhibitor of mercapturic acid conjugation (Boyland & Grover, 1967) had no effect on either half-life.

Table 5. *Influence of pretreatment with bromide on hypnotic activity and toxicity of bromisoval and carbromal in the rat (Wistar-TNO). Each subgroup consisted of 5 animals*

		Control group (chloride)			Bromide group (blood level 15 m-equiv/litre)		
	Sex	Mortality (24 h) %	Sleeping time (h) mean ± s.d.	Surviving animals arousable after 24 h (%)	Mortality (24 h) %	Sleeping time (h) mean ± s.d.	Surviving animals arousable after 24 h (%)
Bromisoval 1 mmol/kg i.p.	Female	0	4 ± 1	100	0	6 ± 1	100
	Male	0	3 ± 1	100	0	5½ ± ½	100
Carbromal 1 mmol/kg i.p.	Female	0	> 22½ (21, 22, 23, > 24, > 24)	60	60	> 24 (> 24, > 24)	0
	Male	0	9½ ± 1½	100	40	> 23 (21, 23, > 24)	67

Pretreatment (3 weeks): control group, 0·15 M sodium chloride in drinking water; bromide group, 0·10 M sodium chloride plus 0·05 M sodium bromide in drinking water.

In Table 5 the results of one of the experiments comparing the influence of preceding bromide administration on the toxicity of the bromocarbamides are shown. A large increase in the toxicity of carbromal is evident. The prolongation of the sleeping time in the male group is striking. Because of the restricted observation time an effect on the sleeping time in the female group is masked, but the incidence of mortality points to an increased toxicity. On the other hand, the influence of bromide on the sleeping time after administration of bromisoval is slight, but significant at the 5% level. The results of several other analogous experiments, although differing numerically, are consistent with this picture (Rauws, 1969).

DISCUSSION

The results of the partition experiments show a distinct difference between carbromal and bromisoval, although their molecules differ only in the location and length of an alkyl group (2-ethyl versus 3-methyl). Carbromal is more lipophilic. A comparison of the Stuart models of both molecules gives a hint of an explanation. The carbromal molecule is much more compact, the bromine atom being pinched between the two ethyl groups. These groups also limit the free rotation of the carbamide group. In contrast the bromisoval molecule is more flexible. The volatility and smell, and the lower melting point of carbromal are probably also related to this structural compactness. The results also show that the partition coefficient between light petroleum and water is a more sensitive indicator of slight differences in lipophilicity than that between more polar solvents and water. Analogous comparisons are given by Mayer, Maickel & Brodie (1959) and Bickel & Weder (1968).

The distribution pattern *in vivo* is consistent with the results *in vitro*. The samples were taken late in the experiment, to minimize the influence of vascularization on the distribution. When samples are taken shortly after the administration of the drugs, the differences in concentration are much less. Early brain levels are high, reflecting

early blood levels, whereas early levels in tissues with a low degree of vascularization are negligible. Only after redistribution in the tissues are steady state conditions, which reflect physicochemical relations between drug and tissues, approached. From these results, treatment of cases of acute carbromal overdosage would require consideration of the accumulation of the drug in fat depots and treatment with lipid haemodialysis might be well worth trying. In bromisoval poisoning, conventional haemodialysis would be more effective. Pilot experiments revealed no important binding of either bromocarbamide to proteins or cell membranes (Rauws, 1968).

The difference between the half-lives and their respective patterns of stimulation and inhibition suggest that the main biotransformation pathways of both drugs are not identical. A phenobarbitone stimulated dehalogenating, microsomal enzyme system is known (Van Dyke, 1966) and a SFK 525-A-inhibited microsomal amidase has been characterized by Hollunger (1960). These enzyme systems might well represent the main pathways, as 2-ethylbutyrylcarbamide is an important metabolite of carbromal (Butler, 1964) and 2-bromo-isovaleric acid and its mercapturic acid derivative are found as metabolites of bromisoval (Haruna, 1961, Rauws, 1968). Human and rat plasma also contains an enzyme which degrades bromisoval but not carbromal (Rauws, 1968). Assuming a degree of overlap in the biotransformation patterns of bromisoval and carbromal one might, by way of hypothesis, suggest the following scheme (Fig. 1). The difference in pattern could depend on degree of lipophilicity of the compounds, as is suggested in another context by Lien & Hansch (1968). This matter is the subject of further research.

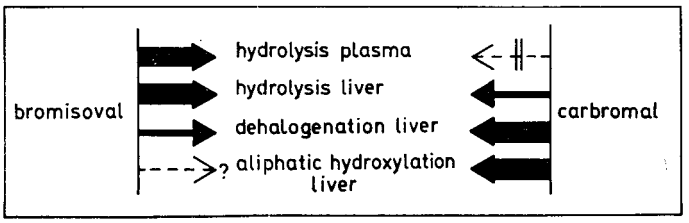

FIG. 1. Schematic representation of probable biotransformation patterns of bromisoval and carbromal.

The long half-life of carbromal in the Wistar-TNO rat is puzzling. It is associated with a greater apparent volume of distribution than in the other Wistar lines. A difference in the composition of the body fat might be the cause. It is tempting to speculate about a possible genetic cause of these differences. To obtain conclusions appreciably more data would be needed. As the TNO-animals grew up in another institute, environmental influences cannot be excluded as a possible cause.

It was to be expected that pretreatment with bromide to a level of approximately 25 m-equiv/litre would intensify the action of both bromocarbamides. Bromide itself has no influence on the biotransformation kinetics (Rauws, 1968) but the intensification of the action is probably not a purely pharmacological phenomenon, at least in the case of carbromal. Early experiments showed a raised brain concentration of carbromal in bromide-treated animals compared to chloride-treated controls. An explanation of this could be that the synergism of bromide with carbromal causes a circulatory depression, which in turn slows down the elimination of carbromal from the brain tissue. That bromisoval toxicity is not augmented by bromide to the same

extent as carbromal toxicity is interesting. Comparative data about the distribution of both compounds within the brain might contribute to an explanation of this contrast. In one respect, however, the two compounds are similar. Prolonged administration of both produces the same, fairly rapid accumulation of bromide in the body (Rauws, 1968).

The investigation, reported here in part, was begun with the expectation that the two compounds would present a closely analogous picture. Many unexpected but mutually consistent differences were found. The present conclusion is that, in the rat, carbromal is more toxic than bromisoval because of several interdependent reasons.

How far this conclusion may be transferred to man is a matter of conjecture. Clinical reports about acute intoxications generally do not contain the data required for an objective comparison.

The possibility of bromide accumulation as a consequence of prolonged administration of both bromocarbamides has already been examined in man by Wollheim (1958). Sufficient data would have now been gathered (Copas & others, 1959; Atkinson, 1960; Pihkanen & Harenko, 1962; Andrews, 1965) to conclude that both bromocarbamides are entirely unsuitable for prolonged use.

This work is part of a doctor's thesis at the University of Leiden. The full text is published in Dutch.

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