A NEW ANALEPTIC: 5,5-DIETHYL-1,3-OXAZIN-2,4-DIONE (DIOXONE)

II. Antagonism to some C.N.S. Depressants

By G. Maffii and M. G. Serralunga

From the Department of Pharmacology, Lepetit S.p.A. Research Laboratories Milan, Italy

Received December 5, 1960

Dioxone (5,5-diethyl-1,3-oxazine-2,4-dione), has been found to exhibit a strong antagonistic effect to barbiturates and chlorpromazine. Given subcutaneously, dioxone significantly reduces the mortality in mice from phenobarbitone and allows an increase in the LD50 of quinalbarbitone and hexobarbitone in mice, and pentobarbitone in rats and mice, thus showing its barbiturate antagonist action both at lethal and depressant dose levels. As with leptazol and bemegride, dioxone does not show any significant effect on the depressant activity of ethanol. It has no effect on lethal doses of chlorpromazine but it has an awakening effect on mice sedated with the drug. The pharmacological actions of dioxone, suggest that its mode of action is functional, rather than at a cellular competition level.

In a previous paper (Maffii, Dezulian and Silvestrini, 1961) the convulsant activity and toxicity of dioxone (5,5-diethyl-1,3-oxazin-2,4-dione) a new compound selected among a series of substituted oxazindiones (Maffii and Silvestrini, 1961) and synthetised by Testa, Fontanella, Cristiani and Gallo (1959) has been reported.

The convulsant activity of dioxone, studied in different species and under various experimental conditions resembles that of leptazol and bemegride. However dioxone appeared more active than leptazol and its margin of safety, as determined in mice, is greater than that of bemegride.

It was also shown that the convulsant activity of dioxone could be antagonised by pretreatment with other substituted oxazindiones which possess anticonvulsant activity, as well as by trimethadione and meprobamate.

It seemed of obvious interest to investigate whether or not dioxone could antagonise the depressant effect of barbiturates and other CNS depressants such as ethanol and chlorpromazine and to compare its effects with those of leptazol and bemegride.

Materials and Methods

CF 1 mice and CF-Wistar rats of both sexes were used. The average weight, respectively was 18–22 and 180–200 g. To ascertain the effect of the three compounds upon the toxicity of several depressant agents, two methods were used. In some experiments the LD50 of a given agent was determined in a control group and then the LD50 was retested in animals also receiving the supposed antagonist. In other experiments a dose of depressant of approximately 90 per cent of the lethal one was

taken as basis and the mortality checked in control animals and in animals also receiving the test agents. Finally in some experiments the changes in the most characteristic symptoms of depressant-poisoning, produced by the given stimulant were observed.

The tested drugs were injected either intraperitoneally (i.p.) or subcutaneously (s.c.) as solutions (sodium salt of phenobarbitone, pentobarbitone, hexobarbitone and quinalbarbitone and hydrochloride of chlorpromazine). For calculating the LD50 and ED50 the method of Litchfield and Wilcoxon (1949) was used. Dioxone, leptazol and bemegride were usually injected in doses that were a multiple of their respective LD50,

TABLE I

PROTECTION SHOWN BY DIOXONE, BEMEGRIDE AND LEPTAZOL IN MICE INJECTED WITH TOXIC DOSE OF PHENOBARBITONE (250 MG./KG.)

Compound	Dose mg./kg. s.c.	LD50 s.c.	Died/ treated	Survivors per cent	Death per cent within			
					0–12 hr.	12–36 hr.	36–84 hr.	84–108 hr.
Phenobarbitone alone		250 mg./kg. i.p.	59/70	25·8	54·2	28·8	17	0
Dioxone		60·5 (54·0–67·7)						
	15		7/10	30	42·8	57·2	0	0
	30		12/20	40	66·6	8·3	16·6	8·3
	60		12/20	40	66·6	16·6	8·3	16·3
	120		16/30	46·7	12·5	37·5	50·0	0
	240		4/20	80	25·0	25·0	50·0	0
Leptazol		101·0 (93·0–109·5)						
	60		11/20	45	81·8	18·2	0	0
	120		9/20	55	44·5	33·3	22·2	0
	240		14/30	53·4	7·1	28·5	64·3	0
	480		4/20	80	25·0	25·0	50·0	0
Bemegride		40·5 (36·1–45·3)						
	20		16/20	20	56·3	31·2	12·5	0
	40		14/20	30	50·0	42·8	7·2	0
	80		14/30	53·4	28·6	35·7	35·7	0
	160		3/20	85	33·3	66·6	0	0

as determined previously (Maffi, Desulian and Silvestrini, 1961). Determinations of the LD50 with the depressant agent alone and with an antagonist were always made on the same day.

To study the awakening action in chlorpromazine treated mice, the righting reflex was used. During the experiments the room temperature was kept between 23 and 24°.

RESULTS

Effects of Dioxone on the Action of Barbiturates

Phenobarbitone. In mice, this drug was given i.p. at 250 mg./kg., a dose found to correspond approximately to the LD90. The solution was 1·25 per cent in distilled water and the volume injected 0·4 ml./20 g. After 1 hr., animals received different doses of dioxone, bemegride and leptazol subcutaneously. The mortality was checked for 108 hr.

The results of this experiment are in Table I from which it appears that the potency of the three compounds, all effective in protecting mice

from death due to phenobarbitone, is inversely proportional to their LD50 determined by the same route.

Plotting the percentages of survivors against the doses expressed as multiples of the respective LD50, the plots obtained with bemegride, leptazol and dioxone may be considered similar (Fig. 1).

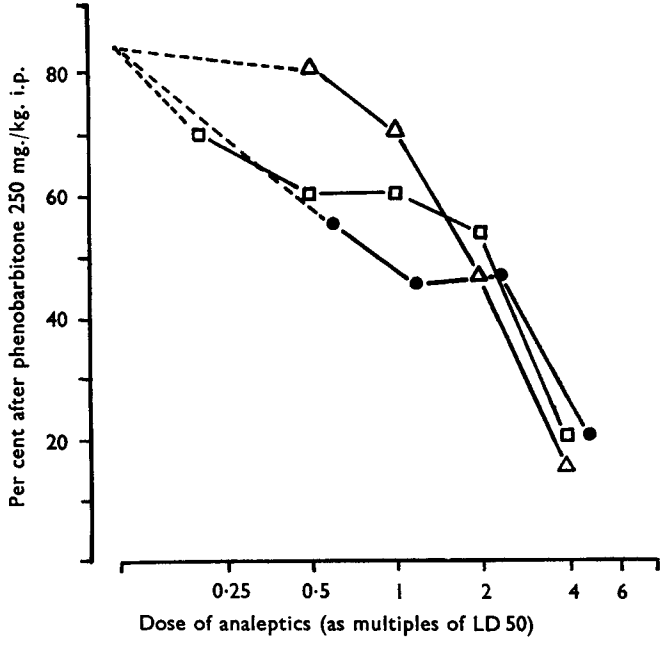

FIG. 1. Reduction in number of deaths due to phenobarbitone 250 mg./kg. i.p. in mice receiving □dioxone, △ bemegride and ● leptazol.

Since the three analeptics were always given in a single dose 1 hr. after the phenobarbitone, the effect of the compounds on mortality can be compared (see Table I). It appears that dioxone gives a significant protection even at half the LD50. The same does not seem to be true

TABLE II

EFFECTS OF DIOXONE, BEMEGRIDE AND LEPTAZOL ON THE TOXICITY OF PENTOBARBITONE IN MICE

Analeptic Agent	Doses mg./kg. s.c.	Number of doses of pentobarbitone	Animals/dose	LD50 mg./kg. and [Fiducial Limits (P = 0·05)]
	(Pentobarbitone alone i.p.)	4	17	124 [117–131]
Dioxone s.c.	15	4	20	138 [133–143]
	30	5	10	169 [155–184]
	120	4	10	167 [160–174]
	240	3	10	152 [144–160]
Bemegride	20	3	10	141 [135–147]
	80	3	10	179 [165–194]
	160	4	10	168 [158–178]
Leptazol	60	4	5	140 [134–146]

with bemegride and leptazol when the doses needed are at least twice the respective LD50.

Though the three analeptics were administered in much higher doses than the convulsant ones, no convulsions were observed.

Pentobarbitone. The antagonism of dioxone towards pentobarbitone toxicity was studied through the changes in the i.p. LD50 of the barbiturate in animals treated simultaneously with standard s.c. doses of dioxone. Similar experiments were made with bemegride and leptazol. In Tables II and III the results obtained in rats and mice are shown. They demonstrate that dioxone resembles bemegride quantitatively in the ability to lower the acute toxicity of pentobarbitone.

Both dioxone and bemegride appear much more active than leptazol on the basis of weight : weight or toxicity : activity ratios. Moreover the

TABLE III

EFFECTS OF DIOXONE, BEMEGRIDE AND LEPTAZOL ON THE TOXICITY OF PENTOBARBITONE IN THE RAT

Analeptic agent	Doses mg./kg. s.c.	Number of doses	Rats/dose	LD50 of pentobarbitone (mg./kg. i.p.) and Fiducial Limits (P = 0·05)
	Pentobarbitone (alone) i.p.	4	15	104 [100–108]
Dioxone	20	3	10	112 [107–118]
	40	5	14	127 [120–135]
	80	3	10	117 [112–122]
	160	4	12	145 [134–157]
Bemegride	20	3	10	113 [109–117]
	40	5	14	127 [118–137]
	80	3	10	132 [122–142]
	160	3	10	119 [103–137]
Leptazol	40	3	10	99 [95–103]
	160	3	10	124 [114–134]

relationship between doses and protective effect both with bemegride (the more active in the mouse) and with dioxone (the more active on the rat) shows the limitations of this antagonism. It is clear that for both dioxone and bemegride, there are dose levels over which no further amelioration of effect could be obtained and perhaps a deterioration could be expected. This end point is difficult to establish and, obviously, it largely depends upon experimental conditions. However, in the comparison of dioxone and bemegride, the evidence excluded any significant difference between their anti-pentobarbitone activity (see Fig. 2). Since subcutaneously dioxone has a toxicity significantly lower than bemegride in mice and in rats, it may be considered superior in reducing the probability of death in animals given toxic doses of pentobarbitone.

Antagonism of dioxone towards sub-lethal effects of pentobarbitone was also investigated in a preliminary experiment where the influence of s.c. dioxone was observed on the sleeping time of rats pretreated with 50 mg./kg. i.p. of pentobarbitone 10 min. before. The results, shown in Table IV, demonstrate that dioxone significantly reduces the sleeping time in the rat and in this respect it is at least twice as active as leptazol.

Quinalbarbitone and hexobarbitone. The protective effect of dioxone against quinalbarbitone and hexobarbitone was also compared with that of bemegride and leptazol. As in the previous experiment, the effects were evaluated on changes in the LD50. The barbiturates were administered i.p. immediately after the subcutaneous injection of the analeptic

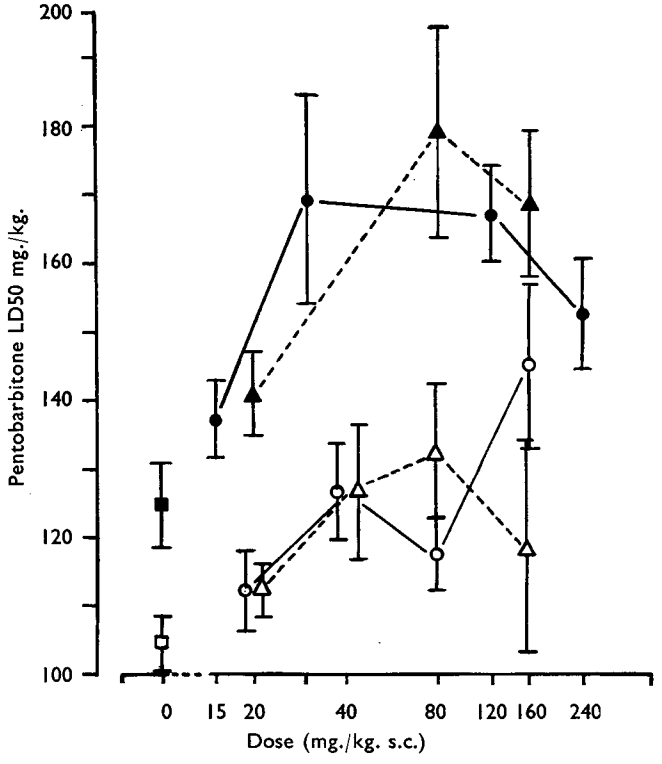

FIG. 2. LD50 of pentobarbitone i.p. alone and with the analeptic agents, in rats and mice.

■ Pentobarbitone alone in mice.
● Pentobarbitone + dioxone in mice.
▲ Pentobarbitone + bemegride in mice.
□ Pentobarbitone alone in rats.
○ Pentobarbitone + dioxone in rats.
△ Pentobarbitone + bemegride in rats.
⊢——⊣ = fiducial limits (P = 0·05).

agents. The results in Table V show the effectiveness of dioxone against these two short-acting barbiturates. Bemegride gave comparable results while leptazol had lower activity.

Interaction of dioxone with ethanol. The LD50 of ethanol was found to be in mice 7·10 ml./kg. In animals receiving dioxone in doses of 30 mg./kg. and bemegride 20 mg./kg. 15 min. after the ethanol, the frequency and rate of deaths was the same as in the controls (see Table VI). With 90 mg./kg. of dioxone a limited increase in the LD50 value was obtained

but this was not significant. As reported by May (1957) bemegride even at 100 mg./kg. has no effect on the mortality of mice poisoned with ethanol 6–7 mg./kg.

Interaction between dioxone and chlorpromazine. Dioxone in doses of 10–25 mg./kg. s.c. every hr. for 5 hr., and of 50 mg./kg. in a single dose,

TABLE IV

AVERAGE SLEEPING TIMES OF RATS TREATED WITH 50 MG./KG. I.P. OF PENTOBARBITONE, AND WITH VARIOUS DOSES OF DIOXONE AND LEPTAZOL

Treatment and dosage mg./kg.	Average awakening time (min.)
Pentobarbitone 50 i.p.	95 ± 12
,, ,, + leptazol 50 s.c.	70 ± 10
,, ,, + ,, 100 s.c.	45 ± 7
,, ,, + dioxone 25 s.c.	65 ± 6
,, ,, + ,, 50 s.c.	45 ± 5

TABLE V

EFFECTS OF DIOXONE, BEMEGRIDE AND LEPTAZOL ON THE TOXICITY OF QUINALBARBITONE AND HEXOBARBITONE IN MICE

Antagonist and dose mg./kg. s.c.	Barbiturate	Dose No.	Mice/dose	LD50 mg./kg.	[Fiducial Limits] (P = 0·05)
	Quinalbarbitone	4	10	100	[94–107]
Dioxone 15	,,	3	10	106	[91–123]
,, 30	,,	3	10	135	[117–155]
Leptazol 30	,,	3	10	108	[100–117]
,, 60	,,	3	10	121	[114–128]
,, 120	,,	3	10	128	[121–136]
Bemegride 20	,,	4	15	119	[112–126]
,, 40	,,	4	10	123	[115–132]
	Hexobarbitone	4	12	285	[271–299]
Dioxone 30	,,	3	13	318	[301–335]
Leptazol 60	,,	3	13	304	[290–318]

TABLE VI

INFLUENCE OF DIOXONE AND BEMEGRIDE UPON THE TOXICITY OF ETHANOL

Analeptic agent	Doses mg./kg. s.c.	Number of doses	Mice/dose	LD50 of ethanol ml./kg. and Fiducial Limits (P = 0·05)
	Ethanol i.p. (alone)	3	20	7·10 [5·96–8·44]
Dioxone . .	30	3	10	6·35 [5·20–7·74]
	90	5	12	7·50 [6·84–8·21]
Bemegride . .	20	3	10	6·80 [5·61–8·22]

failed to modify the mortality of mice due to chlorpromazine 40 mg./kg. i.p. Leptazol and bemegride tested in the same dose range also did not affect the frequency of death.

However, in mice given chlorpromazine, 20 mg./kg., the awakening activity of dioxone was easily demonstrated. In fact under our conditions no animal given chlorpromazine alone, was able to right itself during the 2 hr. after treatment. Of mice receiving dioxone s.c. 1 hr. after chlorpromazine, some righted themselves within 30 min. after the analeptic. Fig. 3 shows the relationships between doses of dioxone and this wakening action and compares it with the results after leptazol. The ratio of the ED50 leptazol : dioxone is 2·41, a value that corresponds

approximately to the activity ratios of the two compounds as shown in other experiments by Maffi, Dezulian and Silvestrini (1961).

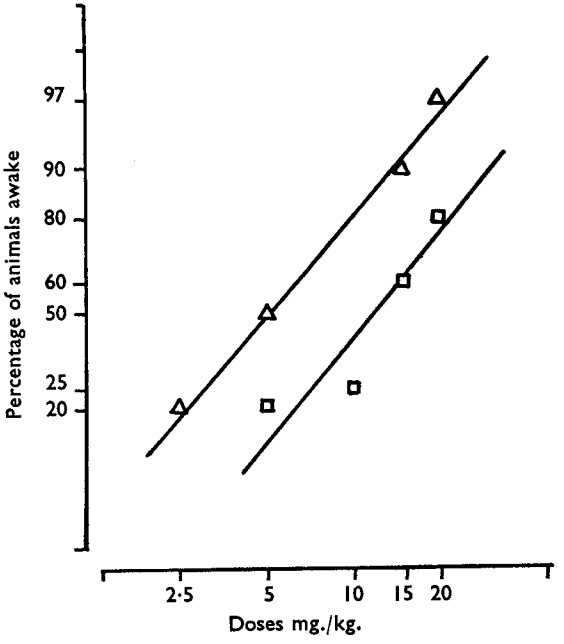

FIG. 3. Awakening effect of △ dioxone and □ leptazol in mice given chlorpromazine 20 mg./kg.

ED50 mg./kg. dioxone = 5·1 (8·0 — 3·2).
leptazol = 12·3 (15·9 — 9·4).

The convulsant doses of dioxone, leptazol and bemegride produce convulsions in the animals treated with chlorpromazine, however the seizures are only clonic and rarely followed by the tonic phase like the occurrence in animals receiving the analeptics alone.

DISCUSSION

In the study of the barbiturate-antagonist properties of dioxone we have shown its ability to reduce the mortality of a long acting barbiturate (phenobarbitone) a medium-acting one (pentobarbitone), a short acting derivative (quinalbarbitone) and a very short acting barbiturate (hexobarbitone). In most of our experiments the effect of dioxone was evaluated on the basis of the LD50 because of the obvious statistical weight of this kind of data and also for practical purposes.

By comparison with bemegride and leptazol, dioxone may be considered to be more active than leptazol and is comparable in potency with bemegride. However, some minor differences in the action of dioxone and bemegride are worth considering; they are quantitative and concern both the depressing agents and the species. Dioxone is more active against short and very short acting barbiturates than against phenobarbitone and

perhaps more effective in rats than in mice, and the opposite being true for bemegride. However, if the two analeptics are evaluated on the basis of their respective toxicity: activity ratios, the difference that has been found in the protective potency against phenobarbitone, tends to disappear and also leptazol may hardly be defined as less active (see Fig. 1). These results probably depend upon the experimental conditions used, that is a single administration of the analeptic 1 hr. after injection of phenobarbitone, but this hypothesis cannot affect the evidence and the value of the comparison. With hexobarbitone and quinalbarbitone, the toxicity: activity ratios allow a favourable comparison of dioxone with both bemegride and leptazol.

However, the pharmacological properties of dioxone strongly suggest that this drug counteracts the barbiturate action by its proper functional activity and not through a competition at the level of common cell receptors. This results from: (*a*) the nature of dioxone as a convulsant agent (which it shares with all other analeptics and bemegride); (*b*) the antagonism between dioxone and anticonvulsant and depressive agents that are also effective against other convulsant agents in a degree that is proportional to potency of the convulsant agent; (*c*) the primary pharmaco'ogical properties of dioxone that are specific to it such as the activation of cerebral electrical activity, the stimulation and the increase in excitability of bulbar centre (Maffii, Bianchi, Schiatti and Silvestrini, 1961); (*d*) the restoring action in other types of CNS depression. We thus consider the protection against mortality by barbiturates, as well as the wakening action, as expressions of a general exciting action of dioxone which extends over functions of the central nervous system other than the motor ones. This view is supported by the evidence that in laboratory animals, larger doses of dioxone—as well as of other analeptics—are often necessary to reduce barbiturate narcoses than are required for the production of convulsions. Then the structural relationship between dioxone and anticonvulsant oxazindiones (Maffii and Silvestrini, 1961) as well as that between dioxone and barbiturates, has probably no particular significance in view of the explanation of pharmacological antagonism, and we may only accept the fact that the exciting and depressing actions on the CNS are thus sometimes closely interrelated from the point of view of chemical structure.

Some practical implication may be drawn also from our results obtained in animals given chlorpromazine. It is true that no significant protection was produced by dioxone towards the lethal doses of this drug; but an obvious effect was found in animals which received paralyzing doses. The potency of dioxone in awakening the mice from paralysis by chlorpromazine is demonstrated by the low level of the ED50–6·1 mg./kg. s.c. Thus dioxone may be considered of some use in reducing the depression and hypnotic effect produced by excessive doses of chlorpromazine, and may also be of some help in severe intoxication, in addition to other drugs.

On the basis of experimental evidence it may be concluded that the new analeptic agent, dioxone, has a strong antagonistic action against lethal effects of barbiturates and depressive effects of other CNS depressants.

Acknowledgement. The technical assistance of Mr. Silvano Banfi is gratefully acknowledged.

REFERENCES

Litchfield, J. T. and Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.
Maffii, G., Dezulian, V. M. and Silvestrini, B. (1961). *J. Pharm. Pharmacol.*, **13**, 244–253.
Maffii, G. and Silvestrini, B. (1961). *Il Farmaco* (Ed. Scient.), **16** (in the press).
Maffii, G., Bianchi, G., Schiatti, P., and Silvestrini, B. (1961). *Brit. J. Pharmacol.*, **16**. (In the press.)
May, J. (1957). *Dissertationes Pharm.*, **9**, 131–142.
Testa, E., Fontanella, L., Cristiani, G., and Gallo, G. (1959). *J. org. Chem.*, **24**, 1928–1936.