J. Pharm. Pharmacol. 1981, 33: 672–673
Communicated February 3, 1981

0022–3573/81/100672–02 $02.50/0

Effect of combined treatment of phenytoin with diazepam on the susceptibility of mice to electroconvulsions

S. J. CZUCZWAR*, L. TURSKI, W. TURSKI, Z. KLEINROK, *Department of Pharmacology, Institute of Clinical Pathology, Medical School, Jaczewskiego 8, 20–090 Lublin, Poland.*

Both phenytoin (diphenylhydantoin) and diazepam can terminate status epilepticus (Wallis et al 1968; Nicol et al 1969) and they are also used in the chronic treatment of some types of seizures (Aird & Woodbury 1974). The enhancement of GABA-mediated inhibition has been proposed as a mechanism for these two antiepileptic drugs (Ayala et al 1977; Deisz & Lux 1977; Macdonald & Barker 1979; Simmonds 1980). According to Olsen & Leeb-Lundberg (1981), the picrotoxin-barbiturate receptor, which is a part of the GABA receptor-ionophore complex, may be the place where both phenytoin and diazepam exert similar effects. Gallager et al (1980) reported on the ability of phenytoin to increase the total number of benzodiazepine binding sites and found this effect independent of GABA-ergic transmission. Thus, phenytoin was shown to enhance the electrophysiological action of diazepam and this finding was distinctly correlated with phenytoin-induced binding of the benzodiazepine. The anti-leptazol (pentetrazol) activity of diazepam was considerably potentiated by phenytoin pretreatment while the protective effects of the benzodiazepine against bicuculline- and isoniazid-induced convulsions remained unchanged by prior administration of phenytoin (Czuczwar et al 1981). A question thus arises whether phenytoin potentiates the protective effect of diazepam against electroconvulsions since the possible efficacy of the combined treatment could be of value.

Experiments were on Albino Swiss male mice, 18 to 22 g, housed in standard conditions and having free access to food and water (except during the experiments). Phenytoin (Polfa, Warsaw, Poland) and diazepam (Relanium, Polfa, Poznań, Poland) were suspended in a 3% solution of Tween 81 (Loba Chemie, Wien, Austria) and injected intraperitoneally 75 and 60 min, respectively, before the test. Control animals received intraperitoneal injections of vehicle only. Electroconvulsions were produced according to Swinyard et al (1952) with the use of corneal electrodes and alternating current (50 Hz); the stimulus duration being 0·2 s. The convulsive threshold was evaluated as CS50, which is the current strength (in mA) necessary to produce tonic hind limb extension in 50% of the animals. Both CS50 values and statistical analysis of the results were calculated according to Litchfield & Wilcoxon (1949).

Phenytoin in doses of 4 and 8 mg kg^{-1} exerted a moderate anticonvulsant effect raising the CS50 value from 7·8 to 9·3 and 12·2 mA, respectively. Diazepam had also a relatively moderate effect on the convulsive threshold in doses of 4 and 6 mg kg^{-1} (CS50 values—11·3 and 14·0 mA, respectively). The combined treatment of phenytoin (8 mg kg^{-1}) with diazepam (2, 2·5, 3 and 4 mg kg^{-1}) resulted in a dramatic increase in the convulsive threshold. Also, phenytoin (4 mg kg^{-1}) combined with the benzodiazepine in doses of 3, 4 and 6 mg kg^{-1} was highly effective against electroconvulsions (Table 1). The intensified anticonvulsant effect was also shown to occur when different doses of phenytoin (4, 8, 12 and 16 mg kg^{-1}) were combined with diazepam (2 and 3 mg kg^{-1}; Table 2).

The results indicate that the combined treatment has considerable effect against electroconvulsions. In addition, it is evident that adding subthreshold doses of diazepam is far more effective than doubling the dose of phenytoin. The anticonvulsant action of phenytoin may be in some way related to GABA-mediated inhibition (Ayala et al 1977; Deisz & Lux 1977), and benzodiazepines potentiate GABA-mediated responses (Macdonald & Barker 1979; Simmonds 1980) while GABA itself facilitates benzodiazepine binding to the specific binding site (Martin & Candy 1978). However, the protective effect of phenytoin against electroconvulsions was poorly affected by GABA-ergic stimulation (Kleinrok et al 1980) so it is unlikely that diazepam enhances the action of phenytoin via GABA-ergic mechanisms. Nevertheless, the possibility of an interaction between the two drugs at the level of the receptor for picrotoxin-barbiturates (Olsen & Leeb-Lundberg 1981) should be considered. The demonstration of specific benzodiazepine binding sites in the central nervous system (Braestrup & Squires 1977; Mohler & Okada 1977) points to the possibility of mediation of at least some of benzodiazepine effects by these receptors. The present results suggest that phenytoin distinctly enhances the anticonvulsant action of diazepam probably as a result of the phenytoin-induced increase in the number of specific benzodiazepine receptors (Gallager et al 1980). If this is correct then the present data appear to indicate that the anticonvulsant potency of diazepam is not entirely due to GABA-ergic mechanisms as suggested before (Mao et al 1975; Löscher & Frey 1977; Macdonald & Barker 1979; Killam & Suria 1980) and may involve interactions with specific receptors. Paul et al (1979) showed a strict correlation between the occupation of benzodiazepine binding sites and the duration of anticonvulsant activity of diazepam in leptazol-induced seizures. Phenytoin-induced increase in the antileptazol action of the benzodiazepine and the simultaneous lack of such effect against bicuculline- and isoniazid-induced convulsions (Czuczwar et al 1981) seem to exclude a possibility that phenytoin potentiates the protective potency of diazepam by pharmacokinetic and

* Correspondence.

Table 1. Effect of combined treatment of phenytoin (4 and 8 mg kg^{-1}) with different doses of diazepam on the threshold for maximal electroconvulsions in mice.

Treatment	Diazepam (mg kg^{-1}) 0	2	2·5	3	4	6
None	7·8 (7·0–8·7)	9·0 (8·3–9·7)	9·0 (8·3–9·7)	9·2 (7·0–12·1)	11·3^{a} (9·5–13·4)	14·0^{b} (11·3–17·4)
Phenytoin (4 mg kg^{-1})	9·3* (8·6–10·0)	15·0**^{b} (13·3–17·0)	not tested	16·0*^{b} (13·2–19·4)	24·5**^{c} (16·9–35·5)	39·0***^{c} (33·3–45·6)
Phenytoin (8 mg kg^{-1})	12·2** (10·8–13·8)	18·5***^{b} (16·8–20·4)	45·0***^{c} (37·2–54·5)	76·0***^{c} (54·7–105·6)	>150***^{c}	not tested

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs untreated control or respective diazepam-treated group.
a, $P<0.05$, b, $P<0.01$, c, $P<0.001$ vs untreated control or respective phenytoin-treated group.
Phenytoin and diazepam were given intraperitoneally 75 and 60 min, respectively, before the test.
Table values are CS50 (in mA) with 95% confidence limits.
Control animals received intraperitoneal injections of vehicle.
Both CS50 values and statistical analysis of the results were calculated according to Litchfield & Wilcoxon (1949).
At least 30 animals were used to calculate one CS50 value.

Table 2. Effect of combined treatment of diazepam (2 and 3 mg kg^{-1}) with different doses of phenytoin on the threshold for maximal electroconvulsions in mice.

Treatment	Phenytoin (mg kg^{-1}) 0	4	8	12	16	20	24
None	7·8 (7·0–8·7)	9·3^{a} (8·6–10·0)	12·2^{c} (10·8–13·8)	14·0^{c} (11·2–17·5)	15·3^{c} (11·9–19·7)	20·0^{d} (14·3–28·0)	130·0^{d} (95·6–176·8)
Diazepam (2 mg kg^{-1})	9·0 (8·3–9·7)	15·0**^{c} (13·3–17·0)	18·5**^{d} (16·8–20·4)	32·0**^{d} (22·5–45·4)	>150***^{d}	not tested	not tested
Diazepam (3 mg kg^{-1})	9·2 (7·0–12·1)	16·0**^{b} (13·2–19·4)	76·0***^{d} (54·7–105·6)	>150***^{d}	not tested	not tested	not tested

**$P<0·01$, ***$P<0·001$ vs respective phenytoin treated group.
a, $P<0·05$, b, $P<0·02$, c, $P<0·01$, d, $P<0·001$ vs untreated control or respective diazepam-treated group.
Table values are CS50 (in mA) with 95% confidence limits in parentheses.
Phenytoin and diazepam were given intraperitoneally 75 and 60 min, respectively, before the test.
Control animals received intraperitoneal injections of vehicle.
At least 30 animals were used to calculate one CS50 value.
Both CS50 values and statistical analysis of the results were calculated according to Litchfield & Wilcoxon (1949).

GABA-ergic mechanisms. Phenytoin has no influence upon leptazol seizures which makes the interpretation of combined treatment more simple. On the contrary, both drugs are effective against electroconvulsions so the interaction may be more complex.

REFERENCES

Aird, R. B., Woodbury, D. M. (1974) in: Kugelmass, I. N. (ed.) Management of Epilepsy. Charles C. Thomas, Springfield, Illinois, pp 149–238

Ayala, G. F., Lin, S., Johnston, D. (1977) Brain Res. 121: 259–270

Braestrup, C., Squires, R. F. (1977) Proc. Natl. Acad. Sci. U.S.A. 74: 3805–3809

Czuczwar, S. J., Turski, L., Kleinrok, Z. (1981) Neuropharmacology 20: 675–679

Deisz, R. A., Lux, H. D. (1977) Neurosci. Lett. 5: 199–203

Gallager, D. W., Mallorga, P., Tallman, J. F. (1980) Brain Res. 189: 209–220

Killam, E. K., Suria, A. (1980) in: Glaser, G. H., Penry, J. K., Woodbury, D. M. (eds) Antiepileptic Drugs: Mechanisms of Action. Raven Press, New York, pp 597–615

Kleinrok, Z., Czuczwar, S. J., Kozicka, M. (1980) Epilepsia 21: 519–529

Litchfield, J. T., Wilcoxon, F. (1949) J. Pharmacol. Exp. Ther. 96: 99–113

Löscher, W., Frey, H.-H. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 296: 263–269

Macdonald, R. L., Barker, J. L. (1979) Brain Res. 167: 323–336

Mao, C. C., Guidotti, A., Costa, E. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 289: 369–378

Martin, I. L., Candy, J. M. (1978) Neuropharmacology 17: 993–998

Mohler, H., Okada, T. (1977) Science 198: 849–851

Olsen, R. W., Leeb-Lundberg, F. (1981) in: Costa, E., Di Chiara, G., Gaetano, G., Gessa, G. L. (eds) GABA and Benzodiazepine Receptors. Raven Press, New York, pp 93–102

Nicol, C. F., Tutton, J. C., Smith, B. H (1969) Neurology 19: 332–343

Paul, S. M., Syapin, P. J., Paugh, B. A., Moncada, V., Skolnick, P. (1979) Nature (London) 281: 688–689

Simmonds, M. A. (1980) Ibid. 284: 558–560

Swinyard, E. A., Brown, W. C., Goodman, L. S. (1952) J. Pharmacol. Exp. Ther. 106: 319–330

Wallis, W., Kutt, K., McDowell, F. (1968) Neurology 18: 513–528