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The Comparison of Anticonvulsant Properties of Commercial Diphenylhydantoin (DPH) and Its 3-Amino-Derivative (3-amino-DPH)

Porównanie przeciwdrgawkowych właściwości handlowej postaci dwufenylohydantoiny (DPH) i jej 3-amino pochodnej (3-amino-DPH)

The administration of anticonvulsant drugs has been and will continue to be the primary method of therapy for epilepsies. Phenytoin, since its introduction for treatment in humans by Merrit and Putnam in 1938 (8) occurred to be the agent of broad antiepileptic spectrum. Its effects can be observed in focal epilepsies of both symptomatic and idiopathic nature accompanied by simple motor or sensory (complex partial) grand mal seizures. It is also useful for treatment of grand mal and myoclonic fits within idiopathic generalized epilepsies (7, 9) and for the prevention in occasional epileptic symptoms of alcohol/drug dependency and febrile states (11).

However, during the past decades of DPH clinical use, numerous reports on the various side effects associated with its use have been published. Over 75 various clinical manifestations, as well as many alterations of the laboratory test values, can be counted as side effects (4, 10). Some of these effects appear to be clinically insignificant, whereas others may have fatal consequences. Since patients suffering from epilepsies are bound to continue using the drug for the rest of their lives, there arises the need for a broader spectrum of medicaments so that alternative treatments can be offered. Therefore, it is important to continue searching for new antiepileptic agents. We made a preliminary examination for anticonvulsant properties of quite a lot of various DPH derivatives in our department (2, 3). Among them, 3-amino-DPH appeared to be the most active compound in the MES test so, we decided to compare anticonvulsant and other properties of 3-amino-DPH with those of commercial DPH.

MATERIALS AND METHODS

Experiments were performed on Albino-Swiss mice. Groups consisted of 10 mice. The investigated compounds were given in doses corresponding to 1/10, 1/20 etc. their LD_{50} i.p. or p.o., as suspensions

in 3% Tween 81 (Lobachemie), in volumes 0.1 ml/10 g. Control LD_{50} and ED_{50} values were calculated on the log-probability paper.

Acute toxicity — The compounds were given i.p. or p.o. in increasing doses to groups consisting of 10 mice. The aproximate LD_{50} value was based on the mortality during 24 hrs. Values were calculated in mg/kg according to Litchfield and Wilcoxon (6).

Cumulative toxicity — According to Lim et al. (5) mice were divided into two subgroups A and B (5 animals in each) and treated i.p. for 4 consecutive days with tested drugs in a single dose corresponding to 9% of LD_{50}. Every four days the dose was increased to 13.5, 20 and 30% of LD_{50} etc. The observation was continued to the moment of death of all animals in the group. C-LD_{50} values were expressed as percentage of LD_{50} acc. to Thompson (13).

Motor coordination — Acc. to Gross and Tripod (1) the experiment was performed on trained mice, using rota-rod, turning at 3 rpm. The animals were tested 15, 30, 60, 90, 120, 150 and 180 min after i.p. administration of tested compounds (3-amino-DPH 78, 38, 19 mg/kg, DPH — 132, 66, 33, 16.5 mg/kg). The percentage of keeping balance during 2 min observation was estimated. The toxic neurologic dose ND_{50} (the dose causing a fall for 50% of the animals after 1 hr) and neurotoxic index NI — expressing relation ND_{50}/ED_{50} was calculated.

Anticonvulsant tests

A. Electroconvulsions were induced according to Swinyard et al. (12) with the use of corneal electrodes and alternating current (50 mA/0.2 s for mice). Animals pretreated with investigated compounds were challenged with maximal electroshock (MES) in order to evaluate the respective ED_{50} values (in mg/kg); at least 20 to 30 mice were used to calculate each ED_{50} value. Therapeutic indexes (TI) expressing relation LD_{50}/ED_{50} were calculated.

B. Chemically induced seizure tests: mice were injected i.p. with 2/5 LD_{50} of 3-amino-DPH or DPH one or 2 hrs before injection of pentylenetetrazol (120 mg/kg s.c.) or bicuculine (3.3 mg/kg i.p.) or strychnine (1.6 mg/kg s.c.). The animals were then placed into individual perspex Plexiglass cages (25 × 15 × 10 cm) and observed for occurrence of clonic pentylenetetrazol, bicuculine or clonic and tonic strichnine seizures. Percentages of protected animals were noted.

RESULTS

It was found that acute toxicity of 3-amino-DPH i.p. exceeded ca twice that of DPH. Both compounds when administered orally showed ca 4 times weaker acute toxicity than that following i.p. application. The tendency for cumulation was evidently weaker for 3-amino-DPH. The cumulation, as well as absorption and elimination, influences not only potency and time of convulsions, but also neurotoxic and other side phenomena (4, 10). We could see that 3-amino-DPH administered i.p. in the dose of ca 1/3 LD_{50}, influenced motor coordination in 50% of animals, whereas DPH evoked the same disturbances in the dose of ca 1/5 its LD_{50} (Table 1). It is worth stressing that 3-amino-DPH, in the dose of 1/10 LD_{50} (19 mg/kg i.p.), which was successful in MES, did not affect coordination at all. DPH in the corresponding dose (33 mg/kg i.p.) disturbed motor coordination in mice slightly and its effect was transient.

Neurotoxic indexes of both compounds are, to all intents and purposes, similar (Table 2). The strength and duration of anticonvulsant effect of evaluated compounds were similar in MES, when doses of 1/10, 1/20 LD_{50} were administered per os, and lasted over 24 hrs. 3-amino-DPH administered i.p.

showed weaker antiseizure potency. It should be stressed that although IT of DPH was superior to that of 3-amino-DPH, the latter is, nevertheless safe; it is only 12 times i.p. and 26 times p.o. overdosage which lead to lethal intoxication (Table 2). Both compounds showed negative influence on chemically evoked convulsions.

Table 1. Acute toxicity (LD_{50}), cumulative toxicity (C-LD_{50}) and disturbance of motor coordination (ND_{50}) of 3-amino-DPH and DPH in mice

Compound	LD_{50} (mg/kg)		C-LD_{50} as % of LD_{50}	ND_{50} (mg/kg)
	i.p	p.o.	i.p.	i.p.
3-amino-DPH	190 (176—205)	720 (600—864)	59.8±4%	56 (41.2—76.2)
DPH	330 (280—389)	1450 (1271—1653)	36.7±2.5%	68 (45.3—102)

Table 2. Maximal electroshock seizure test in mice, comparison of the influence of 3-amino-DPH and DPH on ED_{50} and their therapeutic indexes (TI) and neurotoxic indexes (NI)

Compounds	ED_{50} (mg/kg)			
	i.p.		p.o.	
	1 hr	3 hrs	1 hr	4 hrs
3-amino-DPH	16.5 (11.46—23.76)	15.5 (10.2—22.05)	28 (23.7—33)	18 (14.4—22.5)
DPH	15.5 (11.57—20.77)	12.5 (9.69—16.3)	32 (25—40.96)	16 (11.9—21.4)
	$TI = LD_{50} / ED_{50}$			
3-amino-DPH	11.5.	12.7	25.7	40.0
DPH	21.3	26.4	45.3	90.6
	$NI = LD_{50} / ND_{50}$			
3-amino-DPH	3.4	3.7		
DPH	4.4	5.4		

DISCUSSION

We have shown in the present paper that 3-amino-DPH has a marked anticonvulsant activity which, however, is a little weaker in comparison with DPH. The fact that 3-amino-DPH does not serve as protection against chemically evoked seizures, leads one to believe that these compounds exhibit similar range and mechanisms of anticonvulsant activity. Since 3-amino-DPH when administered in effective doses in MES did not affect motor coordination and exhibited positive therapeutic indexes and a smaller tendency for cumulation, it may be assumed that the compound will differ from maternal DPH in either intensity or its range of side effects. In view of the evidence adduced so far

it may be gathered that the 3-amino-DPH may comply with the requirements of a safe antiepileptic drug, especially in prolonged, small dosage or combined therapy.

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STRESZCZENIE

Z przeprowadzonych doświadczeń wynika, że efekt przeciwdrgawkowy 3-amino-DPH w teście maksymalnego szoku elektrycznego (MES) jest porównywalny do DPH, a przy podaniu p.o. zaznacza się nawet po upływie 24 h. Chociaż toksyczność ostra 3-amino-DPH jest 2-krotnie większa od toksyczności handlowej postaci DPH, to jednak 3-amino-DPH wykazuje w porównaniu z DPH ewidentnie słabszą tendencję do kumulacji.

Należy podkreślić, że indeks neurotoksyczny obu związków jest zbliżony, jednak 3-amino-DPH w dawce 19 mg/kg i.p., wykazującej działanie przeciwdrgawkowe w MES, nie wpływa na koordynację ruchową myszy, podczas gdy zastosowanie równoważnej dawki DPH, tj. 33 mg/kg i.p., przejściowo ją zaburza. Obydwa badane związki nie wykazują działania ochronnego w testach drgawek chemicznych.