

EFFECTS OF SULPHADIMIDINE ON THE TOXICITY OF PHENOBARBITAL, PENTETRAZOLE AND BEMEGRIDE IN MICE OF DIFFERENT AGES

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Received July 31, 1984

Abstract

Šimůnek J., Eva Hegerová, J. Jaroš and E. Tkadlec: *Effects of Sulphadimidine on the Toxicity of Phenobarbital, Pentetrazole and Bemegrade in Mice of Different Ages*. Acta vet. Brno, 54, 1985: 177–182.

Acute toxicity of phenobarbital sodium salt and of injection solutions of bemegrade and pentetrazole administered subcutaneously alone and after premedication with sulphadimidine sodium salt at 0.2 g/kg body mass was determined in conventionally reared white mice of 12–16 and 22–27 g respectively, in body mass. The toxicity of phenobarbital alone was higher for the younger mice, whereas that of the two analeptics of the central nervous system (CNS) was lower for the younger than for the older animals. Premedication with sulphadimidine increased the toxicity of all the drugs under study, having a particularly marked effect in the younger mice.

Acute toxicity, phenobarbital, bemegrade, pentetrazole, sulphadimidine premedication, mice, age-dependence.

A previous study from our laboratory (Šimůnek et al. 1985) was concerned with the effects of sulphadimidine premedication on the acute toxicity of phenobarbital in cockerels of different ages. The impetus to the study was possible simultaneous action of the two pharmaceuticals in flocks where Sedophen^B, a drug containing phenobarbital, is used to tranquilize chickens and sulphadimidine is used therapeutically, e. g. to combat coccidiosis. Considering the differences in the response to drugs between birds and mammals, the present study was designed to investigate this possible interaction with regard to acute toxicity in white mice. This time the experiments were extended to cover possible effects of sulphadimidine premedication on the acute toxicity of two central analeptics, pentetrazole and bemegrade.

No published data were available to us for direct comparison, particularly as regards the ontogenetic point of view. Nevertheless, it is well established that phenobarbital, like other barbiturates, plays a major role in changing the effects of concurrently administered drugs by affecting the enzyme systems of the body. Thus a number of clinically important interactions have been described. Květina and Fendrich (1978), e. g., mentioned the potentiation of the action of the CNS inhibitors; reduced effects of anticoagulants, corticosteroids (glucocorticoids) and riphampicine as a result of enzyme induction; reduced effects of griseofulvine as a result of both enzyme induction and reduced gastrointestinal absorption; and the observation that antacids reduce gastrointestinal absorption of orally administered phenobarbital. Krishna and Bonanomi (1974) described enhanced binding of chloramphenicol to macromolecules of various tissues of phenobarbital-premedicated rats. Dunajev et al., in a long-term experiment with rats given a phenobarbital solution (1g per l) instead of drinking water, demonstrated the activation of hepatocyte enzymes.

According to Skovsted et al. (1974) sulphonamides can prolong the action of other drugs presumably by retarding their metabolism in the liver. The competitive mechanism of the interaction of sulphonamides with a number of other drugs has been universally recognized (Květina and Fendrich 1978, a. o.) and is in fact the underlying principle of their antimicrobial action (Šimůnek as cited by Bentz 1982, a. o.). Lee and Foley (1944) pointed out as early as 1944 that where sulphonamides act concurrently with other drugs, the possibility of both antagonism and potentiation should be considered. As to barbiturates, Csögör et al. (1971) and Csögör

and Papp (1969) found in their experiments on rats that sulphathiazol potentiated the depressive effect on the CSN of both thiopental and hexobarbital, apparently by its binding to plasma proteins. In this connexion it is of interest to note that Anton (1968, as cited by Mitchell 1970) drew attention to the possibility of unexpected reactions occurring in the binding of sulphonamides to plasma proteins not only upon interaction with other drugs but also as a result of disease. Acetazolamide, chemically ranking among sulphonamides, prolonged sleeping time in mice injected intraperitoneally with phenobarbital by depressing its blood level and enhancing its brain level (Sato et al. 1983).

With respect to central analeptics, pentetrazole and bemegride, few published data were available to us for comparison. According to Horáková et al. (1963) sulphamethoxyypyridazine potentiates excitatory effects of pentetrazole; experiments conducted by the latter authors demonstrated a similar effect of sulphadimethoxin but not of sulphamethoxydiazine. In our previous experiments, sulphadimidine blood levels of white mice of two different ages were not much affected by either pentetrazole or bemegride or phenobarbital present in their bodies (Šimůnek 1974). Exploratory investigations into possible effects on toxicity yielded positive results for the two analeptics, whereas the toxicity of phenobarbital was unaffected. Stone and Javid (1979), in their experiments on mice, studied among other things the antagonism of phenobarbital and pentetrazole and reported its relatively weak manifestations. In the light of their further results they concluded that convulsions observed after administration of pentetrazole or bemegride could not be ascribed primarily to the blocking of GABA-mediated inhibitions. From our previous experiments (Šimůnek 1983) it appears that sulphadimidine administered to chickens affects the diazepam-altered LD₅₀ of pentetrazole by partly cancelling the anti-pentetrazole effect of diazepam.

Materials and Methods

For experiments with phenobarbital, a total of 576 conventionally-reared white mice were used. Half of them were young animals with an average body mass of 16 g, the other half consisted of adult mice averaging 27 g in body mass. Each of the two groups was divided into three subgroups. Two of them were premedicated with sulphadimidine 15 or 120 minutes before phenobarbital administration and the remaining subgroup received no premedication. For determination of the LD₅₀, phenobarbital was given in doses of 150, 180, 210, 240, 270, 300, 330 and 360 mg/kg body mass using 12 mice (6 males and 6 females) with each dose in each subgroup. Aqueous solutions of phenobarbital (phenobarbitalum natricum pulv.) were prepared immediately before subcutaneous injection. Na-sulphadimidine, dissolved in water, was given at 0.2 g per kg body mass. Toxicity was expressed in terms of LD₅₀ values with confidence limits according to Litchfield and Wilcoxon (1949) on the basis of deaths occurring within 48 hours.

For experiments with bemegride, a total of 360 conventionally-reared white mice of both sexes were used. Half of them were young animals with an average body mass of 12 g, the other half consisted of adult mice averaging 22 g in body mass. Half of the animals within each of the two groups were allotted for experiments with bemegride and the other half for experiments with pentetrazole. The bemegride- and pentetrazole-treatment groups were each divided further into three subgroups. One of them received no premedication and the remaining two were premedicated with sulphadimidine three hours previously at 0.1 g or 0.2 g/kg body mass, using Na-sulphadimidine in aqueous solution. Pentetrazole and bemegride were given in doses of 100 mg and 45 mg per kg body mass, respectively, using solutions prepared from commercial products. All drugs were injected subcutaneously and the animals were observed for convulsions and mortality up to 24 hours after administration. The differences of the death rates were evaluated using the law of probability distribution of chi-square values according to a procedure recommended by Myslivec (1957).

All experimental mice were kept in a constant environment. They were fed Larsen's diet ad libitum and had free access to drinking water.

Results

In all experiments with phenobarbital, deaths occurred within 24 hours of its administration. In the group of young mice no differences in the LD₅₀ values between sulphadimidine-premedicated and non-premedicated animals were recorded for males, whereas in female mice the toxicity of phenobarbital was increased by premedication, the differences between premedicated and non-premedicated animals being

significant. In adult mice no effects of premedication were observed. In general, the toxicity of phenobarbital proved age-dependent, being higher in young than in adult mice including those of sulphonamide-premedicated subgroups where the relative toxicity for females was even significantly higher.

In experiments with pentetrazole and bemegride the toxicity of the two central analeptics was higher after premedication with sulphadimidine, particularly in the younger mice, irrespective of the sulphadimidine dose level employed. Used without sulphadimidine premedication, the two central analeptics were less toxic for the younger animals. However, the differences between the older and younger animals in the occurrence of convulsions and deaths were not significant.

The results of the experiments are summarized in Tables 1 and 2.

Table 1

LD₅₀ values (in mg per kg body mass) of phenobarbital administered subcutaneously alone and after Na-sulphadimidine premedication (0.2 g per kg body mass s. c.), together with percentages of relative toxicity, in white mice of different ages

Premedication	Mice of 27 g in body mass *			Mice of 16 g in body mass		
	♂	♀	♂×♀	♂×♀	♀	♂
None	250±25	256±12	255±12	223±17 115±7 %	230±17 114±8 %	217±23 116±16 %
15 min previously	235±21	250±14	249±18	211±19 120±12 %	* 202±20 125±13 %	213±17 110±13 %
120 min previously	241±23	248±26	248±16	* 211±7 118±10 %	* 208±19 121±16 %	212±26 114±15 %

* - Values significantly different ($P < 0.05$) from those obtained in non-premedicated animals.

Table 2.

Occurrence of convulsions and deaths among white mice injected subcutaneously with pentetrazole or bemegride alone and after premedication with Na-sulphadimidine

Group	Mice averaging 22 g in body mass				Mice averaging 12 g in body mass			
	No. animals	No. with convulsions	No. died	No. survived	No. animals	No. with convulsions	No. died	No. survived
Pentetrazole (100 mg/kg)								
Without premedication	15	15	11	4	15	14	7	8
Na-SD 0,1/kg	15	14	11	4	15	14	13	2
Na-SD 0,2 g/kg	15	14	14	1	15	15	11	4
Bemegride (45 mg/kg)								
Without premedication	15	14	9	6	15	14	4	11
Na-SD 0,1 g/kg	15	14	6	9	15	15	10*	5
Na-SD 0,2/kg	15	14	9	6	15	14	10*	5

* - Values significantly different ($P < 0.05$) from those obtained in non-premedicated animals.

Discussion

The results of the experiments reported here demonstrated age-dependent differences in the toxicity of the two drugs acting on the CNS. In our previous experiments with pentetrazole and bemegride alone (Šimůnek et al. 1971a) the acute toxicity of the former was lower for young than for adult mice, whereas the toxicity of the latter was higher for young mice, amounting to 117.5 % as compared to its toxicity for adult animals. This result for bemegride alone was not confirmed in our present experiments. After premedication, however, the toxicity of bemegride was significantly higher for young mice, this difference being the only one that reached statistical significance in the present study.

The doses of the two central analeptics were chosen according to the results of our previous experiments (Šimůnek et al. 1968) where the LD₅₀ values for bemegride and pentetrazole upon subcutaneous administration to mice were 41.5 and 102.0 mg/kg body mass, respectively. The LD₅₀ of phenobarbital after subcutaneous administration was found by us in another study (Šimůnek et al. 1971b) to be 218 mg/kg body mass for young mice and 257 mg/kg body mass for adult animals.

Considering that our previous experiments with drugs acting on the CNS and with sulphadimidine (Šimůnek 1974) revealed no changes in the toxicity of phenobarbital, our present tests with phenobarbital included determination of its LD₅₀ after sulphadimidine premedication, whereas those with the analeptics were confined to evaluation of the changes in the occurrence of convulsions and subsequent mortality. The effect of sulphadimidine premedication on acute toxicity of phenobarbital was manifested by an increase in its toxicity, particularly in the younger group where even phenobarbital alone was more toxic than in adult mice. No direct explanation of the mechanism underlying the interaction emerges from our experiments. One possibility to be considered is competition for the binding sites of proteins as suggested in studies on similar drugs by Csögör et al. (1971) and Csögör and Papp (1969). Determination of sulphonamide levels in our previous experiments (Šimůnek 1974) showed no major changes upon interaction with phenobarbital (the same being true also for bemegride and pentetrazole) but it should be pointed out that the technique used in the study, a modification of the Bratton-Marshall method by Wagner (1950), makes no distinction between the quantities of free (directly reacting) and protein-bound sulphonamide so that changes in the binding to proteins, if present, cannot be determined in this way. The age-dependent difference in sulphonamide blood levels reported by us previously (Šimůnek 1974) made itself felt even upon interaction. Berecký and Lopuchovský (1979), in a study on adult white mice, found a decrease in sulphonamide concentration of some tissues only after as many as three phenobarbital treatments given during 24 hours, an observation ascribed by them - in keeping with relevant literature - to enhanced biotransformation of sulphonamide after enzyme induction by phenobarbital.

The manifestations of interactions of the two central analeptics with sulphadimidine in the present study were particularly marked in the younger mice, without being much affected by sulphadimidine dose. An increase in the toxicity of the two analeptics after sulphonamide premedication was also found in our previous exploratory experiments (Šimůnek 1974). As regards pentetrazole, our present results are in reasonable agreement with the data reported by Horáková et al. (1963) for sulphadimethoxin. No direct data on the mechanism of such action have been published. However, a certain role in the interaction of sulphadimidine with central analeptics can presumably be ascribed to their sulphonamide-retarded metabolism in the liver as was pointed out, for example, by Skovsted et al. (1974).

Ovlivnění toxicity fenobarbitalu, pentetrazolu a bemegridu sulfadimidinem u rozdílně starých myši

U bílých myši konvenčního chovu o hmotnosti 12–16, resp. 22–27 g byla stanovena akutní subkutánní toxicita sodné soli fenobarbitalu, injekčního roztoku bemegridu a pentetrazolu, a to jak těchto farmak samotných, tak po premedikaci sodnou solí sulfadimidinu v dávce 0,2 g/kg ž. h. Toxicita fenobarbitalu samotného byla vyšší pro myši mladší; toxicita obou analeptik CNS pro mladší myši byla nižší než pro starší. Po premedikaci sulfadimidinem se zvýšila toxicita všech zkoušených farmak, a to zvláště u mladší věkové skupiny myši.

Влияние на токсичность фенobarбитала, пентетразола и бемегида сульфадимидином у мышей разного возраста

У белых мышей классического разведения массой 12—16 или 22—27 г проводились определения острой подкожной токсичности натриевой соли фенobarбитала, инъекционного раствора бемегида и пентетразола, собственных фармакологических препаратов и после премедикации натриевой солью сульфадимидина дозой 0,2 г/кг живого веса. Токсичность собственного фенobarбитала была выше у мышей младшего возраста, токсичность обоих аналептиков CNS для молодых мышей была ниже чем у старших. После премедикации сульфадимидином токсичность у всех проверяемых фармакологических препаратов увеличилась, в особенности у младшей возрастной группы мышей.

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