PATHOGENETIC COMBINATION THERAPY OF EXPERIMENTAL BOTULISM

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Botulinum toxin (BT) specifically blocks release of the neurotransmitter in various central and peripheral cholinergic synapses [15]. As a result of this block to synaptic transmission generalized paralyses of the skeletal and respiratory muscles develop and are complicated by anoxia and a syndrome of asphyxia. The possibility cannot be ruled out that anoxia aggravates the pathogenic effects of BT, for the use of artificial respiration in botulism, with consequent abolition of respiratory anoxia, reduces the severity of the functional and biochemical changes [9, 10].

Taking into account the fact that until now there have been no effective methods of pathogenetic therapy of botulism, it was decided to study the effect of antianoxic agents and also of combined treatment with drugs, some of which facilitate neurotransmitter release in cholinergic synapses, whereas the others have an antianoxic action. Such a combination of drugs may constitute the basis for pathogenetic combination therapy of botulism, on the principal of goal-directed measures directed toward the principal pathogenetically interconnected components of the pathological process [7, 8].

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-22 g and albino rats weighing 200-220 g. Type C BT was injected intramuscularly into the hind limb: in doses of 0.2, 0.16, and 0.14 μg per mouse and 10 μg/kg per rat. The effectiveness of experimental therapy of animals receiving the toxin was studied by determining the rate and duration of survival of the animals. The effectiveness of therapy was calculated by means of a fourfold table and Fisher's exact test [5]. Each group consisted of 20 mice or 10 rats. LD_{50} of BT was determined in mice by the probit-analysis method of Miller and Tainter [2].

As the drug activating acetylcholine release in the synapses we chose 4-aminopyridine (4-AP; from Reanal, Hungary), which was injected in doses of 1 and 2 mg/kg intraperitoneally, twice daily. As the antianoxic agent gutimin† was used and was injected twice daily, intraperitoneally, in doses of 50, 100, and 200 mg/kg. The first injection of the drugs was given immediately after the injection of BT.

EXPERIMENTAL RESULTS

After injection of BT into the mice, the appearance of symptoms of poisoning and the time of death depended on the dose of toxin injected. BT in a dose of 0.2 μg (the "strict" model of botulism) caused death of the animals 3.5-4 days after injection of the toxin. When smaller doses were injected, death of 100% of the mice took place after 6-7 days (Fig. 1). Gutimin in doses of 50 and 100 mg/kg had virtually no effect on the percentage of surviving animals at different stages of poisoning in the "strict" model of botulism. Only injection of high doses of the drug (200 mg/kg) gave some degree of protective effect. However, the mean duration of survival of the animals also was increased after administration of gutimin in doses of 50 and 100 mg/kg (Table 1). In a less "strict" model of botulism (injection of BT in a dose of 0.16 μg) the protective effect of gutimin was much more marked: a signifi-

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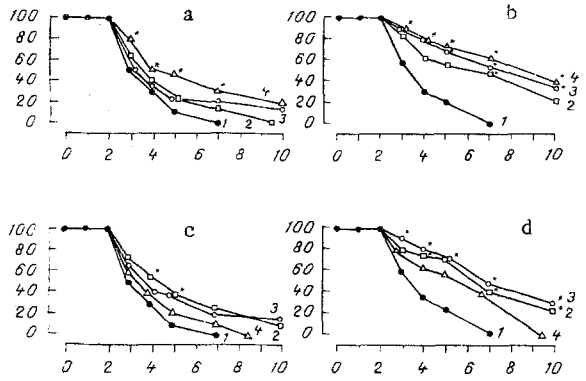

Fig. 1

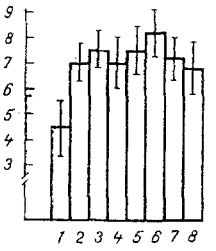

Fig. 2

Fig. 1. Effect of gutimin and 4-AP on survival rate of mice poisoned with BT. Abscissa, time of poisoning (in days); ordinate, survival rate, in %: 1) without injection of preparation; 2) gutimin; 3) gutimin + 4-AP (1 μg/kg); 4) gutimin + 4-AP (2 mg/kg). a, b) Gutimin in a dose of 50 mg/kg; c, d) gutimin in a dose of 100 mg/kg. a, c) Dose of BT was 0.16 μg; b, d) dose of BT was 0.14 μg. *$p < 0.05$.

Fig. 2. Effect of combination therapy of LD_{50} of BT. 1) No drug injection; 2, 3, 4) gutimin in doses of 50, 100, and 200 mg/kg, respectively; 5) gutimin (50 mg/kg) + 4-AP (1 mg/kg); 6) gutimin (50 mg/kg) + 4-AP (2 mg/kg); 7) gutimin (100 mg/kg) + 4-AP (1 mg/kg); 8) gutimin (100 mg/kg) + 4-AP (2 mg/kg).

cant increase in the percentage of surviving animals was found at different stages of botulism after injection of gutimin in doses of 100 and 200 mg/kg, and a significant increase in the mean duration of survival when gutimin was used not only in these doses, but even in a dose of 50 mg/kg. The therapeutic effect of gutimin was more marked still when BT was injected in a dose of 0.14 μg (Fig. 1; Table 1). It must be pointed out that whereas the greatest protective action of gutimin on the "strict" model of botulism was exerted in a dose of 200 mg/kg, on the "mild" model (i.e., after injection of smaller doses of BT) smaller doses of the antianoxic agent also were more effective.

Injection of gutimin also caused an increase in LD_{50} of BT (Fig. 2).

Similar results were obtained in experiments on albino rats poisoned with a lethal dose of BT. Two injections of gutimin daily (100 mg/kg, intraperitoneally) increased the percentage of surviving animals at different periods after injection of the toxin and increased the duration of survival from 5.0 ± 0.1 to 6.2 ± 0.4 days ($p < 0.01$).

In the next series of experiments the effect of combination therapy including the antianoxic agent gutimin and 4-AP was studied. The latter compound can intensify neurotransmitter release from nerve endings, temporarily removes the block to neuromuscular transmission, and exerts a protective action on the development of experimental botulism [12, 14]. With the combined use of these preparations, 10-15 min after their injection the animals became noticeably excited, and their motor activity was increased. Injection of a combination of these preparations had a stronger protective action than that of these two substances separately. The maximal protective effect was observed after injection of relatively small doses of the preparations in this combination (Fig. 1; Table 1). Their combined administration also caused an appreciable increase in the mean lethal doses of BT (Fig. 2). It is very important to note that because of potentiation of the action of the substances in combination, the doses of each can be considerably reduced, thus reducing the likelihood or the severity of any side effects.

The suggested use of a combination of the antianoxic agent gutimin and of 4-AP is thus a highly effective method of protecting animals in models of experimental botulism with varied degree of "strictness".

When the results are analyzed it must be pointed out that whereas 4-AP acts mainly on synapses, sharply intensifying neurotransmitter secretion in various cholinergic and adrenergic synapses [16], gutimin is characterized by a rather wide spectrum of action. In

TABLE 1. Effect of Combination Therapy on Duration of Survival of Mice (in days) with Experimental Botulism (M ± m)

Preparation	Dose of preparation, mg/kg	Dose of BT, μg		
		0,2	0,16	0,14
None given (BT alone)	—	2,85±0,12	3,62±0,21	4,00±0,21
Gutimin	50	3,03±0,21	4,60±0,42*	8,05±0,90***
	100	3,35±0,15**	5,65±0,48***	8,02±0,87***
	200	3,48±0,21***	5,10±0,42**	7,15±0,69***
Gutimin + 4-AP	50+1	3,34±0,16**	4,08±0,51	7,92±0,87***
	50+2	3,20±0,15	5,80±0,75**	8,34±0,71***
	50+	3,30±0,2	4,28±0,3	6,10±0,45***
	100+1	3,43±0,23*	5,05±0,68*	7,15±0,78***
	100+2	3,18±0,15	4,08±0,36	5,95±0,42***

Legend. *p < 0.05, **p < 0.01, ***p < 0.001.

botulism just as, evidently, in other anoxic states, gutimin has an activating effect on inactivated enzymes of iron pumps [1, 13] and promotes normalization of the acid-base balance [3, 11]. Moreover in anoxia gutimin increases the resistance of the nerve cells, which are able to function, after administration of this substance, at critical values of pO_2 for the brain under ordinary conditions [4]. Gutimin significantly lowers the tissue oxygen consumption and this plays an important role under the conditions of anoxia arising in botulism. Another fact of definite importance is that gutimin can exert a direct stabilizing and antioxidative action in anoxia on membranes of cells, mitochondria, and lysosomes, and this is accompanied by preservation of functions linked with the formation and utilization of high-energy compounds [4, 6]. The use of combination therapy including the antianoxic agent gutimin and 4-AP, which facilitates neurotransmitter release, thus has a marked protective action on the development of experimental botulism. It can accordingly be postulated that the binary approach to correction of disturbances used in this investigation may provide an addition to the list of existing methods for the treatment of botulism.

LITERATURE CITED

1. A. E. Aleksandrova and L. V. Govorova, Farmakol. Toksikol., No. 1, 53 (1977).
2. M. L. Belen'kii, Elements of Quantitative Evaluation of a Pharmacologic Effect [in Russian], Leningrad (1963).
3. G. A. Boyarinov, N. A. Shvets, S. P. Peretyagin, et al., Vopr. Med. Khim., No. 1, 54 (1985).
4. V. M. Vinogradov, L. V. Pastushenkov, and É. N. Sumina, Patol. Fiziol., No. 4, 81 (1981).
5. V. S. Genes, Tables of Significant Differences between Groups of Observations with Respect to Qualitative Parameters [in Russian], Moscow (1964).
6. O. R. Grek, Farmakol. Toksikol., No. 1, 101 (1978).
7. G. N. Kryzhanovskii, Determinant Structures in the Pathology of the Nervous System [in Russian], Moscow (1980).
8. G. N. Kryzhanovskii, Principles and Mechanisms of Activity of the Human Brain [in Russian], Moscow (1985), pp. 46-49.
9. V. V. Morrison, Respiratory Failure in Clinical and Experimental Medicine [in Russian], Kuibyshev (1977), pp. 81-82.
10. V. V. Morrison, Vopr. Med. Khimii, No. 6, 749 (1979).
11. V. V. Morrison, Abstracts Lodged in the All-Union Institute of Scientific and Technical Information, No. 8469-V, December 11, 1985.
12. V. V. Morrison, and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 10, 445 (1985).
13. N. P. Chesnokova and T. A. Nevvazhai, Patol. Fiziol., No. 2, 43 (1983).
14. H. Lundh, Brain Res., 153, 307 (1978).
15. L. L. Simpson, Pharmacol. Rev., 33, 155 (1981).
16. S. Thesleff, Neuroscience, 5, 1413 (1980).