of the natural cytokine complex. Combined application of NCC with ointments potentiates their effects.

Thus, our results point to the prospectiveness of NCC use in the treatment and prevention of purulent-inflammatory pathologies.

REFERENCES

1. L. V Gankovskaya, L. V. Koval'chuk, and B. Bayart, *Byull. Eksp. Biol. Med.*, **108**, No. 9, 213-217 (1989).
2. L. V. Gankovskaya, L. V. Koval'chuk, R. E. Titovets, *et al.*, *Dokl. Akad. Nauk SSSR*, **323**, 354-358 (1992).
3. G. N. Dudikova, *Voen.-Med. Zh.*, No. 2, 26-29 (1982).
4. V. A. Ivanov, *Ibid.*, No. 5, 62 (1983).
5. L. V. Koval'chuk and L. V. Gankovskaya, *Allergol. Klin. Immunol.*, No. 1, 19-26 (1993).
6. L. V. Koval'chuk, L. V. Gankovskaya, T. A. Krainova, and T. I. Khoroshilova-Maslova, *Byull. Eksp. Biol. Med.*, **115**, No. 3, 284-287 (1993).
7. M. I. Kuzin, *Khirurgiya*, No. 11, 5-7 (1980).
8. V. B. Skopintsev, "Treatment of purulent wounds with adsorbing hydrophilic ointments with silver ions," Author's Synopsis of Dissertation [in Russian], Moscow (1992).
9. K. M Fenchin, in: *Wound Healing* [in Russian], Kiev (1979), pp. 66-69.
10. J. M. Dayer, B. Beutler, and A. Ctrami, *J. Exp. Med.*, **162**, 2163-2168 (1985).
11. D. N. Sauder, P. L. Kikian, J. A. McLane, *et al.*, *Lymphokine Res.*, **9**, No. 4, 465-473 (1990).

Benzamide and Its Derivatives Are Active Against Botulinal Intoxication

G. A. Ugryumova, I. I. Krasil'nikov, O. F. Alferova, I. D. Vinogradova, and Yu. V. Vertiev

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A high antibotulinal activity of benzamide injected for prevention and of 3-N-butyrylaminobenzamide injected for treatment was observed in mice with intoxication caused by type A botulin toxin. 3-aminobenzamide and 3-N-butyrylaminobenzamide did not inhibit botulinal toxin *in vitro*.

Key Words: *experimental botulinal intoxication; botulinal neurotoxin; benzamide; benzamide derivatives*

Specific antibotulinal sera at late stages of botulism are often ineffective and can induce allergic reactions. The search for pharmacological antagonists of botulinal toxin (BT) is difficult because little is known about the mechanism of BT action. Damaging effect of BT is explained by its capacity to block mediator release in the cholinergic myoneural synapses, which results in paralysis and, in severe cases, in death [10].

Guanidine [9], 4-aminopyridine and its derivatives [5,6], tusendanine [8], and other agents are capable of blocking botulinal neurotoxin. However, therapeutic effectiveness of these agents is low, therefore, new agents for treating botulism are required.

Recently, the inhibitors of endogenous ADP-ribosylation in body tissues and isolated cells have been studied [7]: primarily benzamide and its 3-substituted derivatives, i.e., structural analogs of the nicotinamide moiety of NAD whose ADP-ribosyl fragment is transferred onto the acceptor proteins by mono-ADP-ribosyltransferases [11]. Some of these substances can decrease the intensity of endogenous ADP-ribosylation in experimental animals by 90-95% [2], which permits us to regard them as potential pharmacological antagonists of some bacterial toxins.

In this study we assessed antitoxic effectiveness of benzamide, 3-aminobenzamide (3-ABA), and 3-

Department of Clostridiosis, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow; Institute of Military Medicine, Ministry of Defense of Russia, St. Petersburg

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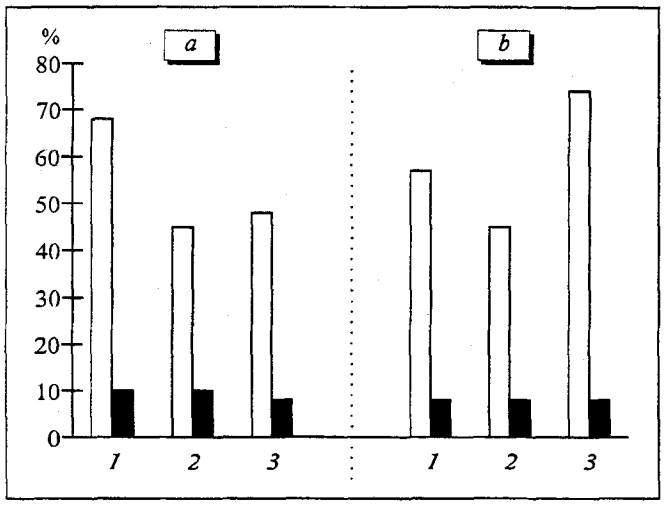

Fig. 1. Effects of benzamide and its derivatives on survival of mice. Preventive (*a*) and therapeutic (*b*) effects. *1*) benzamide; *2*) 3-aminobenzamide; *3*) 3-N-butyrylaminobenzamide. Light bars: experiment; dark bars: control. All results are statistically significant ($p<0.01$).

N-butyrylaminobenzamide (3-N-BABA) in intoxication induced in mice by type A botulinal toxin. These agents are specific inhibitors of ADP-ribosylation.

MATERIALS AND METHODS

Experiments were carried out on 600 outbred male albino mice weighing 18-20 g. Acute toxicity was assessed in mice by determining LD_{50} as described elsewhere [1]. 3-ABA was dissolved in distilled water and injected intraperitoneally. Benzamide and 3-N-BABA was dissolved in 0.05% Twin-80 and administered orally.

Botulinal toxin was isolated from culture filtrates of type A *Clostridium botulinum* strain 501. It was concentrated by salt precipitation and partially purified by gel filtration and ion exchange chromatography. The specific activity of BT was 3.0×10^6 LDM (minimal lethal dose) per mg, protein concentration in solution 2.5 mg/ml. The preparation was stabilized with glycerol (1:1).

TABLE 1. Drug Effects *In Vitro* on Biological Activity of 1 LDM of Type A BT

Survival rate*	Dose, mg/kg	Drug	
		abs.	%
Benzamide	25.0	4/20	20
3-ABA	5.0	0/20	0
3-N-BABA	0.5	0/20	0
Control		0/20	0

Note. Values are statistically significant ($p<0.05$) in comparison with the control. *Numerator: number of surviving mice, denominator: number of mice in experiment.

The activity of the antibotulinal agents was assessed in the survival test on mice infected with BT in 1 LDM (0.2 ml).

Experiments were carried out during the preclinical period of botulin intoxication. The prophylactic effect of the agents was assessed after their injection 1 h before the toxin, the therapeutic effect after injecting the drugs 1 h after the toxin. Each experimental and control group consisted of 6-10 mice. The controls were injected only BT (1 LDM). Control mice died in 3-4 days. Two groups of mice (infected and noninfected with BT) were administered 0.2 ml aqueous suspension of Twin-80. To experimental animals the agents were injected once in the same volume in the minimal active dose: 25.0 for benzamide, 5.0 for 3-ABA, and 0.5 mg/kg for 3-N-BABA. The animals were observed for 10 days.

The capacity of the substances to neutralize BT was assessed *in vitro*: a mixture of equal volumes of the agent in the effective dose and 1 LDM of BT was allowed to stay at ambient temperature for 40 min and then injected intraperitoneally (0.4 ml).

The results were statistically processed using the χ^2 method, the differences were considered significant at $p<0.5$ [1].

RESULTS

First we determined the toxicity of the agents. Injection of the agents in toxic doses led to adynamia, dyspnea, and death within 3-3.5 h. Acute toxicity of substances expressed in LD_{50} was 1005 for benzamide, 502 for 3-ABA, and 1080 mg/kg for 3-N-BABA. These values indicate that these agents have moderate toxicity [3].

Then we studied the antibotulinic activity of benzamides. A single injection of these compounds after lethal dose of BT saved the animals from death. The proportion of survived mice significantly increased. Clinical manifestations of botulism were observed in the majority of mice 2-3 days after injection of BT. In comparison with the controls injected only BT (1 LDM), the life span of experimental mice was much longer: from 4 to 10 days. The 10-day survival of mice was 45-68% after preventive and 45-74% after therapeutic injection of benzamides, whereas in the controls this value was only 8-10%. Injection of the solvent did not influence the survival of animals infected with BT. No animals died in the group injected with the solvent (Twin-80). Figure 1 shows the effectiveness of benzamide and its derivatives. Benzamide displayed the highest antibotulinic activity (68% survival) when injected 1 h before BT. 3-N-BABA was the most effective when injected 1 h after BT (74% survival).

The therapeutic index reflecting the LD_{50} ratio to the minimal effective dose is the highest for 3-N-BABA: 2000, whereas the index of benzamide is 40 and of 3-ABA 100 ($p<0.01$). 3-ABA and 3-N-BABA did not inhibit BT *in vitro* (Table 1).

Thus, benzamide and its derivatives produce preventive and therapeutic effects when used during the preclinical period of botulinal intoxication. Disorders of cell metabolism, ion transport, and functional activity of proteins develop during this period [4]. The observed protection of mice from death (68-74% survival) may be due to depression by the studied substances of unknown ADP-ribosylating activity of BT, which plays an essential role in the toxic effect of type A BT, or to depression of the ADP-ribosylating activity, intrinsic for all eukaryotic cells. Besides the known effects of these substances as mono-ADP-ribosylation antagonists, they are capable of regulating through some other mechanism cell metabolism or pathogenetic processes during the preclinical period of botulinal intoxication.

3-ABA and 3-N-BABA are apparently functional antagonists, because *in vitro* they do not inhibit biological activity of BT.

High therapeutic and prophylactic effectiveness, broad spectrum, and a relatively low toxicity of 3-N-BABA open new prospects in the development of new drugs for prevention and therapy of botulism.

REFERENCES

1. M. L. Belen'kii, in: *Elements of Quantitative Assessment of Pharmacologic Effect* [in Russian], Riga (1959), p. 40.
2. V. G. Vladimirov, I. I. Krasil'nikov, Yu. E. Belyaev, *et al.*, *Radiobiologiya*, **32**, No. 2, 261-265 (1992).
3. S. D. Zaugol'nikov, A. O. Loit, and A. M. Ivanitskii, in: *General Problems of Industrial Toxicology* [in Russian], Moscow (1967), p. 46.
4. N. P. Chesnokova and G. Yu. Kulyash, *Vopr. Med. Khimii*, No. 1, 65-68 (1985).
5. G. E. Lewis, in: *Biomedical Aspects of Botulism*, New York - London - Paris (1981), pp. 261-270.
6. J. Molgo, H. Lundh, and S. Thesleff, *Eur. J. Pharmacol.*, **61**, 25-34 (1980).
7. Y. Ohashi, T. Kamiya, and M. Fujiwara, *Biochem. Biophys. Res. Commun.*, **142**, No. 3, 1032-1038 (1987).
8. L. Peizhoneg, L. Jing, M. Wuyang, *et al.*, *Zhongcaoyao*, **13**, No. 6, 28-30 (1982).
9. M. Puggliari and M. Cherington, *JAMA*, **240**, 2276 (1978).
10. L. L. Simpson, *Pharmacol. Rev.*, **33**, 155-188 (1981).
11. J. L. Sims, G. W. Sikorski, D. M. Catino, *et al.*, *Biochemistry*, **21**, No. 8, 1813-1821 (1982).