EFFECT OF VERAPAMIL, CROMOGLYCATE, AND DIPHENHYDRAMINE ON SURVIVAL OF MICE AFTER ENDOTOXIC SHOCK

B. Z. Shenkman

UDC 616-001.36-02:616.94/-085.218.2-092.9

KEY WORDS: endotoxin; verapamil; cromoglycate; diphenhydramine; LD_{50}.

Endotoxic shock develops in 40% of cases of Gram-negative bacteriemia, and terminates in death in 40-80% of cases [2, 8, 10]. Since interaction of endotoxins with platelets and macrophages, followed by the release of various mediators from them, plays a trigger role in the pathogenesis of shock, when methods of pathogenetic treatment are developed attention must be concentrated on substances capable of blocking these reactions. The writer showed previously that verapamil, cromoglycate, and diphenhydramine inhibited endotoxin-induced platelet aggregation [4, 5]. Verapamil is known to block intracellular calcium channels, cromoglycate prevents the release of allergic mediators from mast cells, while diphenhydramine reversibly binds histamine H_1 receptors [3].

This paper describes an attempt to depotentiate the lethal action of endotoxin by means of verapamil, cromoglycate, and diphenhydramine.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 18-22 g, divided into groups with 15 animals in each group. The endotoxin of *Shigella sonnei*, obtained at the I. I. Mechnikov Central Research Institute of Vaccines and Sera (series 5063, February 14, 1984) was used. The preparations were dissolved in physiological saline and injected intraperitoneally in a volume of 0.2 ml. Mortality parameters of the mice were used as the test of effectiveness of the preparations: the percentage mortality, the mean survival time, and the value of LD_{50}. The last parameter was calculated by probit analysis by the method of Muller and Tainter [1]. The significance of differences in mortality was estimated by the chi-square test.

EXPERIMENTAL RESULTS

In the experiments of series I endotoxin was injected into animals of the 4 groups in doses of 10, 20, 40, and 60 mg/kg The first signs of poisoning appeared in the mice after 45-50 min: apathy, hypodynamia, dyspnea, followed by diarrhea. After 2-3 h the body surface temperature fell and muscular tremor appeared, leading to the development of convulsions. The first deaths were recorded 3 h after injection of the endotoxin. Their frequency reached a peak between 8 and 21 h, and fell thereafter until 32 h. After 48 h there were no clinical features of poisoning in the surviving animals. During subsequent observation for 10 days no deaths were observed among the mice.

Comparison of the length of survival of animals of the different groups showed that this criterion depends to some degree on the severity of poisoning. For instance, the mean duration of survival of the mice after receiving endotoxin in a dose of 10 mg/kg was 16.4 h, with 20 mg/kg it was 13.7 h, with 40 mg/kg — 11.9 h, and with 60 mg/kg — 5.8 h. The mortality levels were 53.3, 68.9, 86.7, and 100%, respectively. The calculated value of LD_{50} was 9.4 ± 3.4 mg/kg.

A study of the effectiveness of the pharmacologic agents when injected 1 h before and 4 and 9 h after the endotoxin showed that LD_{50} was 21.3 ± 3.8 mg/kg in the group of mice treated with verapamil ($p < 0.05$), 23.2 ± 3.7 mg/kg ($p < 0.01$) in those treated with cromoglycate and 21.8 ± 3.9 mg/kg ($p < 0.05$) in those treated with diphenhydramine. The effectiveness of the

Department of Pathological Physiology, Saratov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 428-430, October, 1987. Original article submitted January 5, 1987.

TABLE 1. Effect of Intraperitoneal Injection of Verapamil, Cromoglycate, and Diphenhydramine on Mortality (in percent) of Mice from Endotoxic Shock

Preparation	Time of first injection of preparation relative to time of injection of endotoxin		
	simultaneously	after 1 h	after 2 h
Verapamil (2.5 mg/kg)	46,7	60,0	53,3
Chromoglycate (25.0 mg/kg)	46,7	60,0	53,3
Diphenhydramine (5.0 mg/kg)	46,7	67,7	60,0

Legend. Mortality in the control group (30 mice) after injection of endotoxin in a dose of 20 mg/kg intraperitoneally, was 68.9%. The preparations were injected 3 times with an interval of 4-5 h. Changes in all parameters are not significant.

compounds with respect to length of survival of the mice was estimated at the total mortality level, i.e., with endotoxin in a dose of 60 mg/kg. Under the influence of verapamil, cromoglycate, and diphenhydramine this value rose from 5.80 ± 0.66 h in the control to 9.80 ± 1.21, 12.53 ± 1.98, and 14.20 ± 1.36 h ($p < 0.01$), respectively.

In the experiments described above, the first injection of the drug was given 1 h before the endotoxin. If, however, the first injection of the drugs was given simultaneously with the endotoxin or 1 or 2 h thereafter, they were virtually ineffective (Table 1). These results are not unexpected. In experiments on mice, rats, and rabbits, other workers also observed a preventive, but not a therapeutic action of glucocorticoids, indomethacin, and other preparations in endotoxic shock or Gram-negative septicemia [6, 7, 9]. This is evidently because in our present and previous investigations shock was produced by a single, "exciting" dose of the endotoxin or bacterial suspension.

Experiments were accordingly carried out on a model of poisoning in which three injections of toxin were given at intervals of 4 h. The preparations were injected 2 and 8 h after the first injection of the toxin. In this modification of the experiments, when verapamil (total dose 7.5 mg/kg), cromoglycate (75 mg/kg), diphenhydramine (15 mg/kg), and combinations of them were given, some decrease of the lethal action of the endotoxin, or a tendency for it to decrease, when injected in total doses of 10, 20, and 40 mg/kg, was observed. The calculated value of LD_{50} for the control group was 12.2 ± 4.0 mg/kg, 23.4 ± 4.4 mg/kg for the verapamil group ($p > 0.05$), 18.4 ± 3.6 mg/kg ($p > 0.2$) for the cromoglycate, and 22.5 ± 3.6 mg/kg ($p > 0.05$) for the diphenhydramine group. The value of LD_{50} for a combination of verapamil and cromoglycate was 24.4 ± 3.8 mg/kg ($p < 0.05$), for a combination of verapamil and diphenhydramine it was 28.0 ± 4.2 mg/kg ($p < 0.05$), and for cromoglycate and dimidrol it was 22.5 ± 4.2 mg/kg ($p < 0.01$).

A combination of drugs possessing antiaggregating and antimediator properties may thus have not only a prophylactic, but also a therapeutic action against endotoxic shock.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Evaluation of a Pharmacologic Effect [in Russian], Second Edition, Leningrad (1963).
2. M. I. Lytkin, E. D. Kostin, A. L. Kostyuchenko, and I. M. Tereshin, Septicemic Shock [in Russian], Leningrad (1980).
3. M. D. Mashkovskii, Therapeutic Substances: A Textbook of Pharmacotherapy for Physicians [in Russian], 10th Edition, Moscow (1985), Part 1, pp. 309, 319, and 427.
4. B. Z. Shenkman, Mechanisms of the Cytopathogenic Action of Bacterial Endotoxins; Teaching Aid in Methodology [in Russian], Saratov (1985).
5. B. Z. Shenkman, "The importance of platelets and vasoactive mediators in the pathogenesis of bacterial endotoxicosis and the principles of its pathogenetic therapy," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Tomsk (1986).

6. T. Ebata, Sapporo Med. J., 47, 186 (1978).
7. J. Ferluga, A. Kaplun, and A. S. Allison, Agents and Actions, 9, 566 (1979).
8. F. R. Hierro, A. Palomeque, M. Calvo, and A. Torralba, Paediatrician, 8, 93 (1979).
9. J. Ottosson et al., Circ. Shock, 9, 571 (1982).
10. M. M. Parker and J. E. Parillo, J. Am. Med. Assoc., 250, 3324 (1983).