STIMULATION OF NATURAL RESISTANCE TO EXPERIMENTAL INFECTION BY NEW BENZIMINAZOLE DERIVATIVES

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Systematic studies of the possiblity of increasing resistance to infection by means of benziminazole derivatives showed that, of the 43 compounds studied, two (1-methyl-6, 7-dimethoxybenziminazole and 1-methyl-2-dimethylamino-6, 7-dimithoxobenziminazole hydrochloride) possessed biological activity in this respect. The preliminary subcutaneous injection of these heterocyclic compounds into albino mice in a dose of 10 mg/kg for 3 days increased the survival rate among animals infected with Bacterium pyocyaneum [3, 8].

The object of the present investigation was to study the protective action of these two preparations on the model of staphylococcal septicemia in albino mice, and to examine their effect on the phagocytic activity of the micro- and macrophages.

EXPERIMENTAL METHOD

The effect of 1-methyl-6, 7-dimethoxybenziminazole (1M67D) and of 1-methyl-2-dimethylamino-6, 7-dimethoxybenziminazole (1M2D67D), and also of dibazol (2-benzyl-benziminazole hydrochloride), for comparison, on the process of infection was studied in 100 pure-line albino mice weighing 17 g. The animals were divided into four equal groups. The preparations were injected into the experimental mice daily for 3 days in a dose of 10 mg/kg, while controls received 0.2 ml of physiological saline. Twenty four hours after the last injection of the preparations the animals were inoculated intraperitoneally with 1 LD_{50} of Staphylococcus aureus (strain No. 75) in a volume of 0.3 ml (600 million bacterial cells). Observations on the survival of the mice continued for 10 days.

The effect of 1M67D and 1M2D67D on phagocytosis was studied in 220 albino mice receiving the preparations in the same doses as in the experiments with staphylococcal infection. The phagocytic activity of the microphages (polymorphonuclear neutrophils) was investigated in vivo by a method described previously [2]. The indices of phagocytosis were: the phagocytic index (the mean number of microoganisms phagocytosed by one neutrophil), the intensity of phagocytosis (the percentage of the total number of microphages engaged in active phagocytosis), the attraction (the ratio between the number of cells surrounded by uningested microorganisms and the total number of neutrophils counted), and the degree of completion of phagocytosis (the percentage of the total number of neutrophils containing digested bacteria). The ingestive activity of the macrophages from the peritoneal cavity of the albino mice was investigated 1 h after incubation of the macrophages with bacteria (S. aureus or Escherichia coli) in the ratio of 1:40 [1]. The digestive power of the macrophages was studied by a method modified slightly by the authors [9]. In this cases the ratio between bacteria and macrophages was 1:1, and the incubation time for ingestion of the microorganisms by the macrophages was 40 min at 37°. At the end of this time, and again after cultivation of the macrophages for 1 h, the number of surviving, viable microorganisms in the cultures was determined (D_1 and D_2 respectively). The index of the digestive power (IDP) of the macrophages was determined from the formula:

$$IDP = \frac{D_1 - D_2}{D_1} \times 100.$$

TABLE 1. Effect of Benziminazole Derivatives on Indices of Phagocytosis (IP) of Microphages)

Preparations	Ratio of IP_e to IP_c (in %)			
	attraction	phagocytic index	intensity	completion
1M2D67D	74	165	222	140
Dibazol	92	108	240	101
1M67D	80	124	214	143

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TABLE 2. Effect of Benziminazole Derivatives on Ingestive Activity (IA) and Digestive Activity (IPD) of the Macrophages

Preparation	Object of phagocytosis	Ratio of IA_e to IA_c	P	Ratio of IDP_e to IDP_c	P
1M2D67D	St. aureus	179,3	0,01	150	0,01
1M67D	Ditto	192,1	0,01	144	0,01
Dibazol	Ditto	162,0	0,01	108	0,05
1M2D67D	E. coli	203,0	0,01	161	0,01
1M67D	Ditto	191,7	0,01	164	0,01
Dibazol	Ditto	171,1	0,01	103	0,05

The change in the digestive power of the macrophages under the influence of the studied preparations was estimated from the ratio between the values of the IDP in the experimental (IDP_e) and control (IDP_c) series.

EXPERIMENTAL RESULTS AND DISCUSSION

After injection of 1M67D and 1M2D67D the number of survivors from a group of 25 mice infected with S. aureus was 20 and 18 respectively (in the control group 11 mice survived; the difference between the experimental and control series is statistically significant, $P < 0.05$). So far as the prophylactic action of dibazol is concerned, in these experiments no statistically significant difference was found between experimental and control series (15 mice survived in the experimental series; $P = 0.25$). Dibazol was used for comparison with the benziminazoles on the basis of reports in the literature [5] that it improved the prognosis of staphylococcal inflammatory conditions in rats.

The results of the study of the phagocytic activity of the microphages are given in Table 1. The differences between the indices of phagocytosis in the experimental (IP_e) and control (IP_c) series are statistically significant, $P = 0.05$.

The preliminary injection of each of the two preparations increased the phagocytic index and the intensity and completion of phagocytosis. It is concluded that a direct relationship exists between the fall in the attraction index under the influence of the benziminazoles and the increased intensity of the phagocytic reaction. The results concerning the effect of dibazol on phagocytosis obtained in these experiments were in agreement with those reported in the literature [6].

Similar results were obtained from the study of the ingestive activity of the macrophages (Table 2). It is clear from these results that administration of the benziminazoles essentially increased the ability of the macrophages to ingest microorganisms. The same is true of the results of determination of the digestive power of the macrophages (see Table 2).

Table 2 shows that the two benziminazole derivatives under investigation, in contrast to dibazol, led to an increase in the digestive power of the macrophages (the difference between the experimental and control series is significant).

The conclusion may be drawn that 1-methyl-6, 7-dimethoxybenziminazole and 1-methyl-2-dimethylamino-6, 7-dimethoxybenziminazole, although stimulating phagocytosis, as the results of the earlier investigation showed have no direct antibacterial action [3, 4, 8]. It follows that the efficacy of the two preparations in experimental infection is due to the increase which they bring about in the resistance of the experimental animals to infection—to what N. V. Lazarev [7] calls the state of nonspecifically increased resistance. The activity of the two preparations in this respect is identical.

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