ENHANCEMENT OF ANTIBACTERIAL AND ANTIVIRAL RESISTANCE AND IMMUNE RESPONSE BY A PHARMACOPOEIAL PREPARATION OF RNA

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Sodium nucleate (SN) substantially increased the resistance of mice to pathogenic strain Escherichia coli 026, Proteus vulgaris, Pseudomonas aeruginosa, and Serratia marcescens and had a general stimulating action on nonspecific antibacterial resistance; homologous low-polymer RNA from the liver had a similar stimulating activity. SN enhanced the resistance of animals to viruses of tick-borne encephalitis and encephalomyelitis and increased the number of antibody-forming cells (AFC). Manifestation of the side effect of heat-inactivated vaccine prepared from pathogenic E. coli cells was weakened in animals previously treated with SN.

KEY WORDS: sodium nucleate; RNA; resistance to infection.

The adjuvant properties of natural DNAs and synthetic polynucleotides has now been studied [2-4, 7, 11], but there is little information on the corresponding properties of natural RNAs.

The object of the present investigation, in which a pharmacopoeial preparation of RNA, namely sodium nucleate (SN), such as is used for the treatment of agranulocytosis, was chosen, its effect on nonspecific resistance to conditionally pathogenic bacteria and viruses and on immunogenesis was investigated.

EXPERIMENTAL METHOD

Different batches of SN, sterilized at 100°C, were tested. No contamination with polysaccharides was found on chromatography of an acid digest of SN in a butanol–pyridine–water (4 : 3 : 3) system on Filtrak FN-11 (GDR) paper, with development by aniline phthalate and using mannose, glucose, xylose, and ribose as reference substances. The ratio between the absorption of SN in UV light was $E_{260}/E_{280} = 2.1$ and $E_{260}/E_{230} = 2.5$. SN was found to be a sufficiently homogeneous preparation with molecular weight equivalent to ~ 3-4S, as shown by gel filtration through a column with Sephadex G-100 and by electrophoresis in polyacrylamide gel (PAG). Total RNA was extracted [12] from mouse liver by the phenol method, the low-molecular-weight 4S fraction was separated from it, and it was then purified on Sephadex G-25 and by electrophoresis in 10% PAG [6]. Marker RNAs (5S, 16S, and 23S) were obtained from E. coli ribosomes on a Hitachi ultracentrifuge at 105,000g [5].

SN was injected into mice in a dose of 0.4-32 mg singly or repeatedly, intraperitoneally, subcutaneously, or intramuscularly. Resistance to infection by pathogenic strain E. coli 026, Proteus vulgaris, Pseudomonas aeruginosa, and Serratia marcescens was tested. In some experiments the animals were immunized with titrated doses of E. coli killed at 60°C for 1 h. SN was injected by different methods in a dose of 16-36 mg daily for 3 days into mice weighing 6-8 g, and 24 h later the animals were infected intraperitoneally with a series of doses of Western American equine encephalomyelitis and tick-borne encephalitis viruses. Control mice received 0.85% NaCl solution.

CBA mice were immunized with $0.25 \cdot 10^7$ sheep's red cells (SRBC) or a mixture of SRBC with 32 mg SN, incubated for 30-40 min at 37°C. The number of AFC was determined in the spleen [10]. The results were subjected to statistical analysis with determination of LD_{50} for bacteria and viruses and confidence intervals of other indices [1].

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TABLE 1. Enhancement of Resistance of Mice to Pathogenic Strain of E. coli by Sodium Nucleate (SN)

Time of injection of SN before infection	Sessional dose of SN, mg	Method of injection of SN	Method of infection	$LD_{50} \cdot 10^9$	P
3,5 h	3,5	Intraperitoneally	Intraperitoneally	1,109	<0,01
Control	—	—	»	0,67	—
3,2, and 1 days	32	Intraperitoneally	»	>3,076	<0,01
Control	—	—	»	1,33	—
4, 3, 2, and 1 days	4, 8, 16, 32	Subcutaneously	»	2,0	<0,01
4, 3, 2, and 1 days	4, 8, 16, 32	Intramuscularly	»	2,0	<0,01
Control	—	—	»	1,21	—
4, 2, and 1 days	32	Subcutaneously	Subcutaneously	8,5	<0,01
4, 2, and 1 days	32	Intraperitoneally	»	7,82	<0,01
Control	—	—	»	3,37	—
48, 24, and 8 h	32	Subcutaneously[1]	Subcutaneously[1]	8,5	<0,01
48, 24, and 8 h	32	"	Subcutaneously[2]	8,5	<0,01
24 h	32	Subcutaneously[1]	Subcutaneously[1]	6,75	<0,01
Control	—	—	Subcutaneously	3,98	—
3, 2, and 1 days	0,4	Subcutaneously	»	6,76	<0,01
Control	—	—	»	4,44	—
1 day	32	Intramuscularly	Intramuscularly	2,95	<0,01
Control	—	—	—	1,95	—

[1]Injected into dorsal surface.
[2]Injected into ventral surface.
Legend. Here and in Table 2, each group consisted of 16 mice.

TABLE 2. Enhancement of Resistance of Mice to Conditionally Pathogenic Microorganisms by SN

Time of administration of SN before infection, days	Sessional dose of SN, mg	Infection with	$LD_{50} \cdot 10^9$	P
1	4	Pr. vulgaris	5,433	<0,01
1	32		>7,0,	<0,01
Control	—		3,273	—
1	4	Ps. aeruginosa	21,13	<0,01
Control	—		12,77	—
3, 2, and 1	32		40,7	<0,01
Control	—		12,4	—
1	4	Ser. marcescens	3,11	<0,05
Control	—		2,23	—
3, 2, and 1	32		4,0	<0,01
Control	—		2,63	—

EXPERIMENTAL RESULTS

Injection of the stimulator by different methods on infection of mice with pathogenic E. coli cells by the same or different methods proved to be highly effective in all cases (Table 1).

SN considerably enhanced the resistance of the animals to E. coli whether injected once or repeatedly and in small or large doses (0.4, 3.5, or 32 mg); repeated injection enables the stimulating dose to be reduced at least 80-fold. Different schemes of administration of SN and of infection were used and they all demonstrated the general stimulating action of SN on the nonspecific resistance of the mice; the compound increased the resistance of the animals to Pr. vulgaris, Ps. aeruginosa, and Ser. marcescens, which are frequently responsible for bacterial complications that respond with difficulty to antibiotic treatment (Table 2).

Low-polymer homologous RNA, injected in a dose of 8 mg daily for 3 consecutive days, also significantly enhanced the resistance of the mice to E. coli: LD_{50} in the experimental group was $1.523 \cdot 10^9$ and in the control group $0.777 \cdot 10^9$ cells ($P < 0.01$).

It has recently been shown that one of the mechanisms of the stimulating action of SN is stimulation of the detoxication of bacterial endotoxins [4]. An attempt was made to use this phenomenon to reduce the side effects of killed E. coli vaccine ($3 \cdot 10^8$ cells) with respect to the following indices: the leukocyte count after 90 min, changes in the individual body weight and water consumption in the course of 18-24 h [8, 9]. In the stimulated mice the values were: before and after injection of vaccine $10,575 \pm 1208$ and $10,912 \pm 1735$ leukocytes/μl blood respectively ($P > 0.05$), gain in weight 0.65 ± 0.22 g, water consumption 3.33 ± 0.33 ml before vaccination; in the vaccinated animals $12,967 \pm 1596$ and 8433 ± 831 leukocytes/μl blood ($P < 0.001$), a decrease

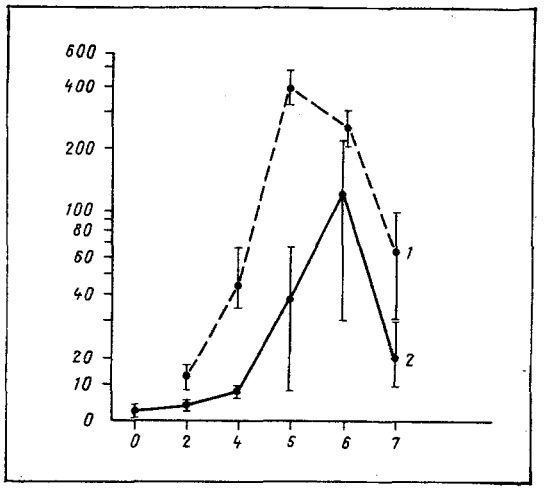

Fig. 1. Number of AFC in spleen of CBA mice immunized with mixture of SRBC and SN (1) or with SRBC only (2). Curves plotted for mean numbers of AFC with standard error at $P < 0.05$ ($n = 6$). Semi-logarithmic scale. Abscissa, time after immunization (in days); ordinate, number of AFC per 10^6 nucleated cells.

in body weight by 1.21 ± 0.51 g, and water consumption 1.11 ± 0.17 ml; in the control group $12{,}041 \pm 1537$ and $11{,}208 \pm 1903$ leukocytes/μl blood ($P > 0.05$), and gain in weight 0.63 ± 0.3 g respectively. These figures are evidence that the toxic properties of the killed vaccine were not manifested in mice stimulated by SN. The immunogenicity of the vaccine was not reduced under the circumstances in these animals: When mice stimulated with SN and vaccinated were infected 2 weeks later, LD_{50} was 932.4 million, in animals vaccinated only it was 932.2 million, in those receiving SN only it was 563 million, and in the control 437.4 million cells. Preliminary injection of SN could thus be a way of reducing the side effects of bacterial vaccines.

It is important to note that in the case of all the effects described above administration of SN in analogous doses parenterally, nasally, and perorally increased the resistance of the mice to infection by encephalomyelitis and tick-borne encephalitis viruses more effectively by the peroral route: In the last two cases LD_{50} was 5.88 ($P < 0.01$) and 7.94 ($P < 0.01$) times greater than in the corresponding control groups.

The preparation also possessed marked adjuvant properties: on the 2nd, 4th, 5th, 6th, and 7th days the number of AFC in the experimental series was 2.04-10.55 times greater than in the control (Fig. 1), and it thus surpassed the adjuvant effect of DNA [7].

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