Rapid Paper

**Effect of Intraperitoneally Administered Nucleoside-Nucleotide
on the Recovery from Methicillin-resistant *Staphylococcus
aureus* Strain 8985N Infection in Mice**

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Summary The effect of intraperitoneally administered nucleoside-nucleotide on the recovery from methicillin-resistant *Staphylococcus aureus* (MRSA) strain 8985N infection was studied in mice. Mice fed nucleic acid-free 20% casein diet were administered intraperitoneally with a nucleoside-nucleotide mixture or with saline (control group) daily for 30 days. On the tenth day on this treatment, mice were challenged with the bacteria. The survival rates were 25% and 72% for the control and nucleoside-nucleotide groups, respectively. The recovery of the survived mice from the infection was confirmed by the increment of body weight and the reduction of the bacteria in the organs. The results show the effectiveness of the intraperitoneal administration of the nucleoside-nucleotide mixture for the recovery from the MRSA strain 8985N infection in mice.

Key Words nucleoside, nucleotide, MRSA, mouse

The microbial causes of nosocomial infections in the immunocompromised host is a problem and outbreaks of infection caused by MRSA, which is multi-resistant against many antibiotics, are the most important among the infections and have become increasingly prevalent world wide (1-3). Therefore some attempts ought to be made in order to alleviate the problem. The enhancement of the immunocompetence by nutritional management may be one of the attempts; however, no such efforts have been reported.

The revelation by Rudolph and his colleagues that dietary RNA enhances the immune response (4) prompted us to investigate the effect of nucleic acid components (nucleoside-nucleotide) on the recovery from the MRSA strain 8985N

infection in mice. Although they administered RNA orally, we administered the components intraperitoneally because of the poor utilization of orally administered nucleic acid components as compared to the parenteral route of administration (5–7), and the non-immunogenic effect of nucleosides and nucleotides when administered parenterally (8).

Materials and methods. BALB/c female mice, 4 weeks old, were maintained on a nucleic acid-free 20% casein diet for 30 days. This dietary composition is the same as shown elsewhere (9). From 10 to 11 a.m. every morning, the animals were weighed and the food and water were renewed. Twenty-five mice were administered 0.35 ml of nucleoside-nucleotide mixture interaperitoneally daily throughout the experiment. This mixture was formulated by Ogoshi *et al.* (8) for the promotion of protein synthesis after surgical operations. It consisted of (w/v%): inosine 0.80, GMP·2Na 1.22, cytidine 0.73, uridine 0.55, and thymidine 0.18. Twenty-four mice were administered 0.35 ml saline (control group). *Staphylococcus aureus* strain 8985N used in this study was isolated from a clinical specimen, identified by standard taxonomic criteria, checked for purity by standard bacteriologic methods and confirmed to be methicillin-resistant. The bacteria was maintained in nutrient semi-solid agar until used for the experiment. It was subcultured in brain heart infusion broth for about 18–24 h at 37°C. The cells were harvested by centrifugation (10,000 rpm, 15 min) and then suspended in saline to give an optical density reading of 1.0 at 660 nm in an Erma spectrophotometer. This suspension gave the number of 6.7×10^8 colony-forming units (CFU)/ml which was determined at 24 h on nutrient agar plates using the dilution plating method (10).

On the tenth day mice were administered 0.3 ml of the bacterial suspension through the tail vein and monitored for mortality. Twenty days after the bacterial challenge, all the surviving mice were killed. The number of CFU of the bacteria were determined for heart, spleen and kidney on mannitol salt agar by the plate dilution method (10). The mice that had died during the course of the experiment were also assayed for the presence of the bacteria in the organs. The data was analysed by χ^2 test and Student's *t*-test.

Results and discussion. Figure 1 shows the percent survival in both groups. The survival rate of the control group was 25% (6 out of 24), which was very different from the group injected the nucleoside-nucleotide mixture, which showed 72% (18 out of 25).

The changes in body weight after the bacterial challenge are shown in Fig. 2. The mice administered the nucleoside-nucleotide mixture or saline were further categorized into two groups, the surviving mice and those mice which died. After the bacterial challenge a loss of weight was noted in both the experimental and the control groups. About 10 days after the challenge with the bacteria, the surviving mice in both groups started to gain weight at a similar rate, while the mice which died continuously lost weight.

Recovery of the mice which survived at the 20th day after the challenge with

Fig. 1. Survival rates of BALB/c female mice administered nucleoside-nucleotide (●) or saline (■) intraperitoneally following inoculation with viable *Staphylococcus aureus*, 2.0×10^8 CFU. Number of mice were 25 and 24 for both the experimental and control groups, respectively. Asterisk indicates significant difference from the control group by χ^2 test (* $p < 0.01$).

Fig. 2. Body weight changes of surviving mice (●; $n=18$) and mice which died (○; $n=7$) administered nucleoside-nucleotide mixture and in the mice which survived (■; $n=6$) and those which died (□; $n=18$) in the group administered saline. Body weight was noted for 20 days and is expressed as the mean of the group; bars are SD.

Table 1. Recovery of viable *Staphylococcus aureus* in organs harvested from BALB/c mice inoculated with 2.0×10^8 *Staphylococcus aureus* and organ weights.

	Control group		Nucleoside-nucleotide group	
	Dead mice (n = 18)	Survived mice (n = 6)	Dead mice (n = 8)	Survived mice (n = 17)
CFU in kidney ($\times 10^5$)	3,690 $\pm$ 1,620	19 $\pm$ 27*	2,200 $\pm$ 1,250	18 $\pm$ 31*
CFU in spleen ($\times 10^4$)	17 $\pm$ 18	6 $\pm$ 10	24 $\pm$ 13	3 $\pm$ 1*
CFU in heart ($\times 10^5$)	14 $\pm$ 31	2 $\pm$ 1	16 $\pm$ 22	4 $\pm$ 14
Kidney weight (g)	0.24 $\pm$ 0.03	0.21 $\pm$ 0.03	0.21 $\pm$ 0.01	0.23 $\pm$ 0.04
Spleen weight (g)	0.06 $\pm$ 0.01	0.21 $\pm$ 0.06*	0.06 $\pm$ 0.01	0.13 $\pm$ 0.05*
Heart weight (g)	0.09 $\pm$ 0.03	0.07 $\pm$ 0.01	0.08 $\pm$ 0.01	0.09 $\pm$ 0.02

*Significantly different from the group which died, by Student's *t*-test ($p < 0.01$).

the bacteria was also confirmed by the bacterial number in the kidney, heart and spleen. Table 1 shows for both groups the number of CFU of the bacteria in the organs and organ weights of mice which survived and the mice which died in both groups. Although the average number of the CFU in the kidney of the mice which died was more than 2.0×10^8 , the number in those which survived was less than 2.0×10^6 in both groups. The bacterial number in heart and spleen was low in both surviving and dead mice. Furthermore, in both groups, the spleen weight noted in the surviving mice was more than twice that of the mice which died. Since the survival rate of the nucleoside-nucleotide group was much higher than that of the control, the result may indicate that nucleosides and nucleotides affect the activation and function of spleen cells, especially when we consider the findings of Sonoda and Tatibana (6). They observed greater incorporation of orally administered ^{14}C -thymine into spleen than in any other tissues.

The problem of MRSA infection often becomes evident after surgical stress. For the nutritional management after the stress, enteral and parenteral formulas are often used; however, considering the fact that, with a few exceptions (8, 11), most formulas contain little or no nucleic acid components the importance of our studies need be further assessed. Further studies are necessary to ascertain the effects of nucleosides and nucleotides on the recovery from MRSA infections by using other bacteria and with other animal models.

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