Protection of BALB/c Mice against Methicillin-Resistant *Staphylococcus aureus* Infection by Intraperitoneal Administration of Nucleoside-Nucleotide Mixture

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Adjei, A.A., Matsumoto, Y., Kina, T., Takamine, F., Yonabaru, M., Asato, L., Yokoyama, H., Imamura, T. and Yamamoto, S. Protection of BALB/c Mice against Methicillin-Resistant *Staphylococcus aureus* Infection by Intraperitoneal Administration of Nucleoside-Nucleotide Mixture. Tohoku J. Exp. Med., 1993, 169 (3), 179-186 — The effect of intraperitoneally administered nucleic acid components (nucleoside-nucleotide mixture) on the recovery from methicillin-resistant *Staphylococcus aureus* (MRSA) strain 8885N infection was studied in mice. Two experiments were conducted in which BALB/c mice were fed a nucleotide-free 20% casein diet for 30 days. On the 10th day, the mice were inoculated intravenously with viable MRSA organisms. The mice were intraperitoneally administered nucleoside-nucleotide mixture or saline (control) daily from the onset of the experiment (experiment 1) or from the day of inoculation (experiment 2). The survival rate in the nucleoside-nucleotide group in experiment 1 (70%) was significantly higher than that in the saline group (20%) (p <0.05). In experiment 2, the survival rate in the nucleoside-nucleotide group (55%) tended to be higher than that in the saline group (36%) without statistically significant difference; furthermore, in the surviving mice, the viable MRSA organisms recovered from the spleen and the kidney were fewer in the former group than in the latter group (p <0.05). The studies showed that the intraperitoneal administration either before or after the MRSA challenge of the nucleoside-nucleotide mixture was effective for the recovery of the mice from the infection. ——— MRSA; nucleoside; nucleotide; mouse

Recent reports have pointed to the existence world wide of a particular epidemic strain of *Staphylococcus aureus*, often called methicillin-resistant *Sta-
*Staphylococcus aureus* (MRSA), which appears to have spread and caused infection in susceptible patients (Townsend et al. 1987; Cookson and Phillips 1988). This problem has persisted and many reports have shown that most strains are virulent and are associated with considerable mortality (Boyce et al. 1983; Bradley et al. 1985; Cookson et al. 1985). The management of MRSA infection is difficult (Crossley et al. 1979; Boyce et al. 1981), and many investigators have attempted to develop efficient control measures against the bacteria (Standiford 1987; Walsh et al. 1987). We focused attention on the development of efficient parenteral and oral feeding formulas that can be used to manipulate the immune response against MRSA infection, as the incidence of infection is most common after stress such as surgery.

The revelation (Van Buren et al. 1983, 1985; Kulkarni et al. 1986, 1987, 1989; Barankiewicz and Cohen 1987; Fanslow et al. 1988) that dietary nucleotides enhance the immune system prompted us to investigate a possible effect of the nucleoside-nucleotide mixture on the recovery from MRSA infection. Although they administered the nucleotides orally, we administered the nucleic acid components parenterally because of the poor utilization of orally administered nucleic acid components as compared to the parenteral route of administration (Bennett 1953; Sonoda and Tatibana 1978; Savaiano et al. 1980).

**MATERIALS AND METHODS**

*Animals and diets*

BALB/c female mice 4 weeks old were obtained from Kyudo (Kumamoto) and were kept in a specific pathogen-free constant temperature room at 25±2°C with a 12 hr light period from 08:00 to 20:00 hr. The mice were maintained on a nucleotide-free 20% casein diet (NF) for 30 days. The composition of the NF was the same as shown elsewhere (Yamamoto et al. 1988). From 10 a.m. to 11 a.m. every morning, the animals were weighed and food and water were renewed. The mice were intraperitoneally administered 0.35 ml nucleoside-nucleotide mixture or saline (control) daily from the first day (experiment 1), or from the 10th day (experiment 2). This mixture was formulated by Ogoshi et al. (1985) for the promotion of protein synthesis after surgical operation. It consisted of inosine 0.80 w/v%, GMP-2Na 1.22 w/v%, cytidine 0.73 w/v%, uridine 0.55 w/v%, and thymidine 0.18 w/v%. On the 10th day the mice were challenged with MRSA 8985N intravenously.

*Cultivation and inoculation of Staphylococcus aureus 8985N*

*Staphylococcus aureus* 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 were maintained in nutrient semi-solid agar until use for the experiment. They were subcultured in brain heart infusion broth for about 12-24 hr at 37°C. The cells were harvested by centrifugation (10,000 rpm, 15 min) and then suspended in saline to give an optical reading of 1.0 at 660 nm in an Erma spectrophotometer. This suspension gave the number of 6.7×10⁸ colony forming units (CFU)/ml which was determined at 24 hr on nutrient agar plates using the dilution plating method (Eklund and Lankford 1970). Prior to the experiment, a dose response curve was examined to determine LD₅₀. We used 2.0×10⁸ *Staphylococcus aureus* since that dose resulted in a 40% control survival at twenty days, which was considered to be an appropriate duration for the experimental studies. On the 10th day, mice in each
group were administered 0.3 ml of the bacterial suspension \((2.0 \times 10^6 \text{ CFU/ml})\) through the tail vein and monitored for mortality.

**Organ culture for MRSA**

The spleen and kidney of the surviving mice in each group were assayed for the presence of MRSA organisms on mannitol salt agar by the plate dilution method (Eklund and Lankford 1970). Briefly, the organs were removed aseptically, weighed and homogenized using a Dounce homogenizer in 3 ml of 0.85% sterile saline. After preparation of 10-fold serial dilutions, 100 μl was inoculated onto mannitol salt agar plates and the number of CFU was determined after overnight incubation at 37°C.

**Statistical analysis**

Survival rates and percent distribution of CFU of the kidney and spleen were analyzed by the chi-square test. Differences in the CFU and the weights of body, spleen, and kidney of the surviving mice between saline and nucleoside-nucleotide mixture groups were determined by Student's t-test.

**RESULTS**

**Survival rates after MRSA challenge**

Fig. 1 shows the percent survival in each group. The sharp decline in the survival rates were observed between days 9 and 15 and may appear to be due to the presence of load of viable bacteria in the system. But during the course of time, the mice seem to respond differently among the groups. The survival rate in the control group in experiment 1 (20%; 2 out of 10) was different from that of the group administered the nucleoside-nucleotide mixture (70%; 7 out of 10).

![Fig. 1. Survival rates of BALB/c female mice fed nucleotide-free 20% casein diet and intraperitoneally administered nucleoside-nucleotide mixture or saline from the onset of the experiment (experiment 1) or after the bacterial challenge (experiment 2). On the 10th day, mice were inoculated intravenously with MRSA strain 8985N \((2.0 \times 10^6 \text{ CFU/ml})\) and mortality was noted for 20 days. •, nucleoside-nucleotide mixture group \((n = 10)\) and ▲, saline group \((n = 10)\) of experiment 1. ○, nucleoside-nucleotide mixture group \((n = 11)\) and △, saline group \((n = 11)\) of experiment 2. Survival rates on day 20 were analyzed and values with the same letter are not significantly different \((p < 0.05)\).](image-url)
(p < 0.05). In experiment 2, the rates in the mixture and the control groups were 55% (6 out of 11) and 36% (4 out of 11), respectively. There was no statistical difference, but the rate in the mixture group tended to be higher as compared to the saline group.

**Body, spleen, kidney weights and CFU in the organs**

Table 1 shows the body, spleen, and kidney weights and the CFU values of the spleen and kidney of the surviving mice at the end of the experiment in each group. Following the MRSA challenge, a persistent weight loss was noted in all the mice for about 10 days; thereafter the mice that survived consistently gained weight, though the rest continuously lost weight and died during the experiment. There was a significant increase in the spleen weights of the surviving mice in each group when compared with the weights of the dead mice in a corresponding group (data not show). The numbers of viable MRSA organisms recovered from the spleen and kidney on day 20 of the surviving mice were much smaller in all the groups than the corresponding numbers of the mice that died during the observational period.

**Percent distribution of CFU in spleen and kidney**

Fig. 2 shows the percentage of the surviving mice of which CFU of MRSA

| Table 1. Body, spleen, and kidney weights and the recovery of viable MRSA organisms in these organs of surviving mice administered saline or nucleoside-nucleotide mixture |
|---------------------------------|-----------------|-----------------|
| **Experiment 1**                |                 |                 |
| Number of mice                  | 2               | 7               |
| Body weight (g)                 | 16.90, 11.10^a  | 15.52±1.27^b    |
| Spleen weight (mg)              | 160, 100        | 170±10          |
| Spleen CFU (×10^6)              | 2.40, 0.20      | 0.30±0.80       |
| Kidney weight (mg)              | 250, 200        | 240±40          |
| Kidney CFU (×10^7)              | 0.12, 0         | 0.16±0.30       |
| **Experiment 2**                |                 |                 |
| Number of mice                  | 4               | 6               |
| Body weight (g)                 | 15.30±1.68      | 16.51±1.56      |
| Spleen weight (mg)              | 120±23          | 165±28          |
| Spleen CFU (×10^6)              | 0.20±0.30       | 0.10±0.20       |
| Kidney weight (mg)              | 240±20          | 265±29          |
| Kidney CFU (×10^7)              | 1.24±2.38       | 0               |

^aIndividual values are shown because of the small number of the surviving mice which may skew the mean±S.D.

^bThe values are mean±S.D.
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organisms was less than $10^2$ in the spleen or kidney in each group. In experiment 1 where nucleoside-nucleotide mixture had been administered prior to the bacterial challenge, higher percentages were observed in the splenic (85%) and kidney (85%). These were significantly different from the percentages (0% and 25%) in the saline group. A similar tendency was noted in experiment 2. Of the surviving mice, 75% and 100% had the splenic and kidney CFU values less than $10^2$ in the nucleoside-nucleotide mixture administered group, whereas 0% and 50% in the saline administered group.

**DISCUSSION**

In this experiment, we studied the effect of the intraperitoneal administration of the nucleoside-nucleotide mixture on the response of BALB/c mice challenged with MRSA 8985N. The administration of the mixture prior to the bacterial challenge (experiment 1) was clearly effective to protect the mice as measured by survival and CFU recovered from spleen and kidney. In experiment 2, in which the mice were administered the mixture after the challenge, the survival rates of the saline group (36%) and the nucleoside-nucleotide group (55%) were not different statistically; however, the effectiveness of the mixture in increasing the host resistance was evident when the splenic and kidney CFU values were taken into account. Of the surviving mice administered the mixture in experiment 2, 100% and 83% had kidney and splenic CFU values of less than $10^2$ as compared to 50% and 25% in the saline group, respectively. These results demonstrate that of the mice administered saline, half (2 out of 4) of the surviving mice failed to clear the bacteria by the end of the experimental period; whereas all the 6 surviving mice in the group administered the nucleic acid components almost
eliminated the organisms. Hence, the results show that of the total mice in experiment 2, 18% (2 out of 11) in the saline group and 55% (6 out of 11) in the nucleoside-nucleotide group cleared the organism. The values differed significantly \( p < 0.05 \).

Similar results were reported by Fanslow et al. (1988). They noted decreased splenic and kidney CFU recoveries and higher percent survival in mice fed nucleotide prior to the intravenous administration with \textit{Candida albicans} as compared to mice fed NF diet.

Enhanced blastogenic responses have been proposed as a possible mechanism for the beneficial effects of nucleic acids or their components. Van Buren et al. (1985) reported that splenic lymphocytes from NF diet-fed mice had diminished blastogenic responses to phytohemagglutinin (PHA). Increased proliferation of T-lymphocyte after stimulation with PHA was also reported by Barankiewicz and Cohen 1987. Szondy and Newsholme (1991) noted that addition of adenosine and uridine to the culture medium increased the proliferation of rat cervical lymph-node T-lymphocytes after stimulation by concanavalin A. We (Yokoyama et al. 1991) also showed that splenic lymphocytes from mice administered nucleoside-nucleotide mixture increased the blastogenic responses to concanavalin A.

Several other mechanisms have also been proposed. Van Buren et al. (1983) observed in mice that dietary nucleotides enhanced the immune responses of delayed-type hypersensitivity reactions to various antigens (purified protein derivative, dinitrofluorobenzene, and sheep red blood cells). Kulkarni et al. (1986, 1987, 1989) showed in mice that by the addition of RNA and uracil to NF diet, the production of interleukin-2 (IL-2) and macrophage increased significantly and that the increased production of IL-2 effected the activation and function of T helper and monocyte cells. From these studies, we can speculate that the supply of nucleic acid by the de novo synthesis is not sufficient to maintain optimal immune function. This requirement may be evident in certain circumstances such as during periods of infection as more nucleic acid is necessary to effect the proliferation, activation and function of the immune cells.

The need for exogenous supply of nucleic acids other than for the maximum function of the immune system has also been reported. Uauy et al. (1990) observe that addition of dietary nucleosides to a nucleic acid-free diet enhances the gut growth, maturation of the intestine and the production of disaccharide in the gut mucosa of rapidly growing rat.

In summary, the studies showed that intraperitoneal administration of the nucleic acid components either before or after the MRSA challenge appeared effective in offering protection to the mice. The problem of MRSA infection often becomes evident in conjunction with stress such as surgery. But as most formulas do not contain nucleic acid components, addition of these components to parenteral and oral formulas must be evaluated. Further studies are being initiated in our laboratory to verify the beneficial effects and define the role of nucleosides in
parenteral and oral nutrition.

References


