# Synergistic effect of rabbit specific antiserum and amikacin on the treatment of mice with lethal infection due to *Klebsiella pneumoniae*

K. Yoshida, M. Takahashi and S. Narikawa[1]

*Department of Microbiology, St. Marianna University School of Medicine, Sugao, Takatu-ku, Kawasaki 213 (Japan), 2 December 1980*

*Summary.* A combination of rabbit specific antiserum and amikacin showed a synergistic effect on the treatment of mice with lethal infections due to a strain of *Klebsiella pneumoniae.*

Specific serum antibody has been postulated as an important factor in the resistance of hosts against opportunistic infections. Protective antibodies in normal human sera against strains of *Staphylococcus aureus* and *Klebsiella ozaenea* were found in a high ratio, according to the descriptions by Yoshida et al.[2] and Takahashi et al.[3], respectively. In a patient with pneumonia due to *K. ozaenea,* Takahashi et al.[3] noted an antibody response to these organisms during the course of the disease. Also, Davis[4] obtained passive protective antibody to *Pseudomonas aeruginosa* from human serum. These facts make it necessary to reevaluate the effects of antibiotics in vivo in the presence of specific serum antibodies. Attempts were therefore made to observe the effect of rabbit specific protective antibody and amikacin in mice with lethal infections due to *K. pneumoniae.* This paper is concerned with our experimental results.

*Material and methods.* Strain K-9 of *K. pneumoniae* was used throughout the experiments. The biological properties of the organisms have been described elsewhere[5]. Normal female mice of the DD strain (Nihon Clea Farm Co., Ltd, Tokyo) weighing approximately 20 g, and rabbits (Japanese white rabbit, Nihon Clea Farm, Co., Ltd, Tokyo) weighing 2 kg were used. The method for the challenge infection of mice with the strain K-9 was that described previously[5]. The organisms were cultured in tryptosoycase broth (Eiken Co., Ltd, Tokyo) at 37 °C overnight, then washed once with sterile saline, and a cell suspension giving 1.0 OD nephelometrically at 430 nm was prepared. The viable cell number of this cell suspension was $4.56 \times 10^8$ per ml enumerated by the usual plate count method. 0.5 ml of $1:10^{-5}$ dilution of this cell suspension in saline was injected i.p. into a group of 5 mice. With this procedure virtually all the mice were already killed by the 4th day (from 72 to 96 h) after the challenge infection; exceptions were very rare. For the preparation of rabbit hyperimmune serum, strain K-9 was cultured and a cell suspension was prepared as mentioned above, this was then formalinized by the usual procedure. 0.1 ml of this formalinized vaccine was injected i.v. into rabbits on 3 successive days during the 1st week. The dose was increased to 0.15 and 0.2 ml for the 2nd and 3rd weeks, respectively, and the final dose was administered for 3 weeks. 7 days after the final injection, rabbits were exsanguinated and the sera were separated. To determine the antiserum activity, primarily, 1:100 diluted rabbit antiserum was prepared with saline, the 2-fold serial dilutions were made with saline down to 1:6400. 0.5 ml of each dilution was injected i.p. into a group of 5 mice which had been challenged with strain K-9 3 days before as mentioned above. The numbers of dead animals were recorded for 3 weeks after the challenge infection. With this procedure 0.5 ml of maximal antiserum dilution exhibiting 100% protection was designated as 1 unit. Amikacin, provided by Bristol Myers Co., Ltd, (New York, N.Y.), was dissolved in M/15 phosphate buffer, pH 6.6, and an appropriate concentration of the antibiotic was prepared. The minimal inhibitory concentration (MIC) of amikacin against strain K-9 of *K. pneumoniae* in vitro was determined by the method described by the Japanese Society for Chemotherapy[6]. For the quantitative determination of the antibiotics in body fluids and tissues, the procedure followed was the method of Nasu et al.[7], using spores of *Bacillus subtilis* ATCC 663; the amount of amikacin per g of tissue was calculated. To observe the therapeutic effect of rabbit specific antiserum and/or amikacin, 0.5 ml of rabbit specific antiserum, containing 1.0–0.125 unit of the activity was injected once i.v. into the animals. As to the antibiotics, varying amounts of amikacin (0.31–320 mg/kg) were injected i.m. 4 times at 3-h intervals into a group of 5 mice who had been challenged with strain K-9 of *K. pneumoniae* 3 days before. The number of dead animals was recorded for 3 weeks after the challenge infection; however, experimental results obtained by the first 10 days after the infection were noted in the table since no change in survival was shown after 10–30 days. These experiments were carried out simultaneously twice and the total number of dead animals was recorded.

*Results.* When 0.31–1.25 mg/kg of amikacin was repeatedly given to the mice challenged 3 days before with strain K-9 of *K. pneumoniae* no effect was observed when treated mice were compared with controls. When the amount of antibiotic was further increased, up to 320 mg/kg, a slight prolongation of survival was shown with the administration of more than 40 mg/kg of amikacin, although every animal was eventually killed. Regarding the rabbit specific antiserum, 1 unit of the activity enabled the animals to recover from the lethal infection; with 0.5 units of the activity a decrease in the mortality rate and prolongation of survival were shown. However, when a rather small amount of amikacin was combined with a low activity of antiserum (0.25 units), which was unable to protect from death, prolongation of survival and recovery from the infection were observed, depending upon the amount of antibiotics. A dose response was exhibited, as shown in the table. These results indicate a significant synergistic effect in the combination of rabbit specific antiserum and amikacin. When the effect of amikacin in vitro and in vivo was observed in order to analyze this phenomenon, the reported MIC of this antibiotic in vitro was found to be 3.12 µg against the strain K-9, as in our previous paper[8]. When 40 mg/kg of amikacin was injected i.m. into mice the concentrations of antibiotic in the blood after 15, 30, 60 and 90 min were 75, 62, 25 and 13 µg per ml respectively, and no detectable antibiotic was found 120 min after the injection. In the kidney, 15, 30, 60, 90, 120, 150, and 180 min after the injection the concentrations were 98, 130, 110, 95, 60, 57 and 57 µg per g respectively. In the lung and bile, small amounts of antibiotic were found after the first 60 and 90 min; however, no amikacin was shown thereafter. In the spleen and liver, no antibiotic was detected throughout the experiments.

*Discussion.* Although the effects of antibiotics in vivo are known to be much less than those found in vitro, published descriptions are usually of positive effects of antibiotics in the treatment of experimental infections with bacteria, when the organisms are sensitive in vitro against these chemotherapeutic reagents. With the administration of antibiotics in vivo significant low concentrations of the

Effect of treatment with amikacin and/or rabbit specific antiserum on mice with a lethal infection due to *Klebsiella pneumoniae* K-9

| Treated with | | Day and number of mouse deaths after the challenge infection | | | | | | | | | | Final results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amikacin (mg/kg) | Antiserum (unit) | 1 | 2 | 3* | 4 | 5 | 6 | 7 | 8 | 9 | 10 | (No. dead/No. used) |
| 0.31 | 0.25 | 0 | 0 | 0 | 10 | | | | | | | 10/10 |
| 0.62 | 0.25 | 0 | 0 | 0 | 4 | 0 | 2 | 1 | 1 | 0 | 0 | 8/10 |
| 1.25 | 0.25 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 0 | 5/10 |
| 2.5 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 |
| 5.0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 |
| 0.31 | None | 0 | 0 | 0 | 10 | | | | | | | 10/10 |
| 0.62 | None | 0 | 0 | 0 | 10 | | | | | | | 10/10 |
| 1.25 | None | 0 | 0 | 0 | 9 | 1 | | | | | | 10/10 |
| 2.5 | None | 0 | 0 | 0 | 6 | 4 | | | | | | 10/10 |
| 5.0 | None | 0 | 0 | 0 | 0 | 10 | | | | | | 10/10 |
| 10 | None | 0 | 0 | 0 | 0 | 10 | | | | | | 10/10 |
| 20 | None | 0 | 0 | 0 | 0 | 6 | 4 | | | | | 10/10 |
| 40 | None | 0 | 0 | 0 | 0 | 0 | 10 | | | | | 10/10 |
| 80 | None | 0 | 0 | 0 | 0 | 0 | 10 | | | | | 10/10 |
| 160 | None | 0 | 0 | 0 | 0 | 0 | 10 | | | | | 10/10 |
| 320 | None | 0 | 0 | 0 | 0 | 0 | 10 | | | | | 10/10 |
| None | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 |
| None | 0.5 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 3/10 |
| None | 0.25 | 0 | 0 | 0 | 1 | 9 | | | | | | 10/10 |
| None | 0.125 | 0 | 0 | 0 | 10 | | | | | | | 10/10 |
| Normal rabbit serum | | 0 | 0 | 0 | 10 | | | | | | | 10/10 |
| None | | 0 | 0 | 0 | 9 | 1 | | | | | | |

* Treatment was begun on day 3.

antibiotics have been detected in tissues, especially in inflammatory lesions[9,10]. Also, a decrease in the effectiveness of antibiotics in the presence of inflammatory exudate has been observed[10]. Matsumoto et al.[11] postulated 3 phases of bacterial multiplication in infections, in order to explain the lower activity of antibiotics in vivo than in vitro. In these experiments, it is presumed that lethal infection of the animal by the organisms, involving endotoxin effect and severe tissue damage was already established, when treatment with the specific antiserum and/or antibiotics was given. However, prolongation of survival was observed only when an extremely high dose of amikacin was administered. In this case an antibiotic concentration higher than the MIC was maintained for a period of time only in blood and kidney; low concentrations of antibiotic were detected for a short time in bile and lung. Further, no amikacin was found in liver or spleen throughout the examination. These results suggest that even with extra high doses of this antibiotic no effect was shown on the treatment of a lethal infection of mice with *K. pneumoniae;* this agrees with similar results reported by many investigators[2,12,13]. On the contrary, with the administration of rabbit specific antiserum, animals could recover from lethal infection. This finding suggests that the organisms disappeared rapidly from the lesions in these cases, as in the case of a majority of pathogenic organisms associated with opportunistic infections, such as *S. aureus*[2], *S. epidermidis*[14], *K. ozaenea*[3], *Pseudomonas aeruginosa*[4], *Serratia marcescens*[15], *K. pneumoniae*[16], *Streptococcus pneumoniae*[17], and group B streptococcus[18]. As to the synergistic effect of the combination of specific antiserum and amikacin represented in these experiments a similar phenomenon was noted with the combination of specific antibody to *P. aeruginosa* and dibekacin[19]. This might mean that the therapeutic effect of an antibiotic is unexpectedly higher than it is in vitro, in some cases, since normal human sera possess high ratios of specific antibodies to the organisms related to opportunistic infections, as noted by Yoshida et al.[2] and Takahashi et al.[3]. Inhibition of the adherence of microorganisms by antibiotics as reported by Sugarman and Donta[20], is regarded as an important factor for the synergistic effect of specific antiserum and antibiotics. These investigations are currently in progress in our laboratory.


1 Present address: Department of Clinical Sciences, St. Marianna University School of Medicine, Sugao, Takatu-ku, Kawasaki (Japan).
2 K. Yoshida, Y. Ichiman, M. Takahashi and C. L. San Clemente, J. med. Microbiol. *12*, 277 (1979).
3 M. Takahashi, Y. Ichiman, S. Narikawa, K. Yoshida and S. Iwami, J. Bact. *33*, 16 (1978).
4 S. D. Davis, J. infect. Dis. *134*, 717 (1975).
5 M. Takahashi, K. Yoshida and C. L. San Clemente, Can. J. Microbiol. *23*, 448 (1977).
6 S. Ishiyama, Y. Ueda, S. Kuwabara, N. Kozakai, G. Fruya, M. Konno and R. Fujii, Chemotherapy *16*, 89 (1968).
7 M. Nasu, A. Saito, N. Mori, K. Izumikawa, T. Tsutsumi, M. Hirota, M. Iwanaga, M. Nakatomi, N. Horiuchi and K. Hara, Chemotherapy *23*, 2117 (1975).
8 M. Takahashi, Y. Ichiman, Y. Usui, S. Narikawa and K. Yoshida, Jap. J. Antibiotics *32*, 830 (1979).
9 K. Matsumoto, T. Nagatake, H. Shishida, H. Suzuki, Y. Noguchi, K. Tamaki, M. Ide and K. Watanabe, Chemotherapy *27*, 109 (1979).
10 M. R. Smith and W. B. Wood, Jr, J. exp. Med. *103*, 509 (1956).
11 K. Matsumoto, Y. Uzaka, T. Nagatake, H. Shishida and K. Watanabe, Chemotherapy *27*, 80 (1979).
12 S. Ishiyama, I. Nakayama, H. Iwamoto, S. Iwai, M. Takatori, T. Kawabe, I. Sakata, H. Kawamura, H. Mizuashi and K. Shibata, Chemotherapy *23*, 2144 (1975).
13 K. Mashimo, K. Fukaya, O. Kunii and M. Suzuki, Chemotherapy *23*, 2096 (1975).
14 K. Yoshida and Y. Ichiman, J. med. Microbiol. *11*, 371 (1978).
15 M. S. Simberkoff, N. H. Moldover and J. J. Rahal, Jr, J. infect. Dis. *134*, 348 (1976).
16 T. Fukutome, M. Mitsuyama, K. Takeya and K. Nomoto, J. gen. Microbiol. *119*, 225 (1980).
17 L. C. Russel and F. G. Blake, J. exp. Med. *31*, 657 (1980).
18 R. C. Lancefield, M. Mac Carty and N. W. Everly, J. exp. Med. *142*, 165 (1975).
19 K. Kawaharajo and J. Y. Homma, Jap. J. exp. Med. *46*, 155 (1976).
20 B. Sugarman and S. T. Donta, J. infect. Dis. *140*, 622 (1979).