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Investigation of the Therapeutic Effect of Tobramycin in Mice with Experimental Pneumonia in One-shot Versus Divided-dose Schedules

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ABSTRACT. We measured the plasma levels of tobramycin administered by different methods and evaluated the therapeutic effects of tobramycin in mice with experimental pneumonia in various methods and dose schedules.

(1) Mice were dosed with tobramycin intramuscular, intravenous one-shot injection and drip infusion, and intramuscular injection in divided doses at intervals of 15 minutes. The plasma concentration of the drug was determined at timed intervals after administration in each instance. Intramuscular and one-shot intravenous injections resulted in substantially parallel time courses of plasma concentration and plasma concentration with drip infusion was similar to that after divided intramuscular injection.

(2) A therapeutic experiment was conducted in mice with *Klebsiella pneumoniae* pneumonia using one-shot and divided intramuscular injection methods of tobramycin. The one-shot injection group showed a satisfactory result at the dose level of 2 mg/kg, but the injection in four divided doses yielded better results at 1 mg/kg. Therefore, in cases where high doses are not feasible or are undesirable, the time of contact of the bacteria and the drug should be prolonged, rather than trying to increase the peak plasma concentration of the drug.

In an experimental study of the metabolic fate and acute toxicity of dibekacin (DKB) in mice, Komiya et al.[1] reported that compared with rapid intravenous injections, intravenous drip infusions result in a significantly increased LD_{50} value approaching that attainable by an intramuscular injection, and commented that if an appropriate drip infusion time is selected, intravenous drip infusions could be clinically as useful as intramuscular injections. The authors[2] also conducted an experimental study on DKB in mice infected with

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klebsiella pneumoniae and reported that even when the dosage is constant, administration of the drug in divided doses, which would mean a longer time of contact between the bacteria and the drug, lead to a slightly faster decrease of bacteria in the lung and tend to inhibit their regrowth more effectively.

In recent years, even for aminoglycoside antibiotics, intravenous administration has been considered desirable in not a few cases.[3]

However, since it is not easy to compare the therapeutic effects of different administration methods in clinical situations, we have used mice as models, and measured the plasma levels of tobramycin (TOB) administered by different methods and evaluated the relative clinical efficacies.

METHODS

1. Determination of Plasma Concentration

Mice of the ICR strain were dosed with TOB and the plasma levels of the drug were determined, using blood samples taken at intervals over 120 min after administration. These plasma level determinations were carried out by the agar well technique using *B. subtilis* ATCC 6633 as the test organism, and the standard curve was prepared by dilution of mouse plasma.

The doses and methods of administration of TOB were as follows.

(1) 5 mg/kg/mouse : one-shot i.m., one-shot i.v., 1-hour i.v. drip, and four divided doses i.v. (1.25 mg/kg/dose × 4) at intervals of 15 min.

(2) 1 mg/kg/mouse : one-shot i.m., and four divided doses i.m. (0.25 mg/kg/dose × 4) at intervals of 15 min.

(3) 2 mg/kg/mouse : one-shot i.m., and four divided doses i.m. (0.5 mg/kg/dose × 4) at intervals of 15 min.

2. Therapeutic Efficacy in Mice with *K. pneumoniae* Pneumonia

(1) Determination of viable bacterial count in mice lugs :

Mice of the ICR strain with body weights of 15 to 25 g were infected with *K. pneumoniae B-54* using a nebulizer. After 18 hours, the animals were intramuscularly treated once with 1 or 2 mg/kg of TOB or four times with 0.25 or 0.5 mg/kg/dose of TOB at intervals of 15 min. The viable count in the lung was serially determined at intervals up to 24 hours after administration.

(2) Survival rate :

Groups of mice were given 1 or 2 mg/kg/dose of TOB intramuscularly twice daily or 0.25 or 0.5 mg/kg/dose of TOB intramuscularly four times at intervals of 15 min twice daily for 3 to 5 consecutive days, and the survival rates for the groups were compared. In addition, the efficacies of further intramuscular injections of 0.5 mg/kg in a single dose and of 0.5 mg/kg in four divided doses were also investigated.

(3) Therapeutic efficacy in mice with formalin-induced airway injury[4,5]

Mice with the airway injury induced by 1% formalin were infected with *K. pneumoniae* was in inoculated in the same manner as above. The animals were then dosed intramuscularly with 1 or 2 mg/kg/dose of TOB twice daily

or 0.25 or 0.5 mg/kg/dose of TOB in four divided doses at intervals of 15 min twice daily for 5 consecutive days, and a group-to-group comparison was made of the survival rates.

RESULTS

1. Plasma Concentration

(1) Plasma concentration after administration of 5 mg/kg of TOB : Fig. 1 shows the courses of plasma concentration after a single intramuscular injection, a one-shot intravenous injection and a 1-hr intravenous drip infusion of 5 mg/kg each, and four intravenous injections of 1.25 mg/dose at 15-min intervals.

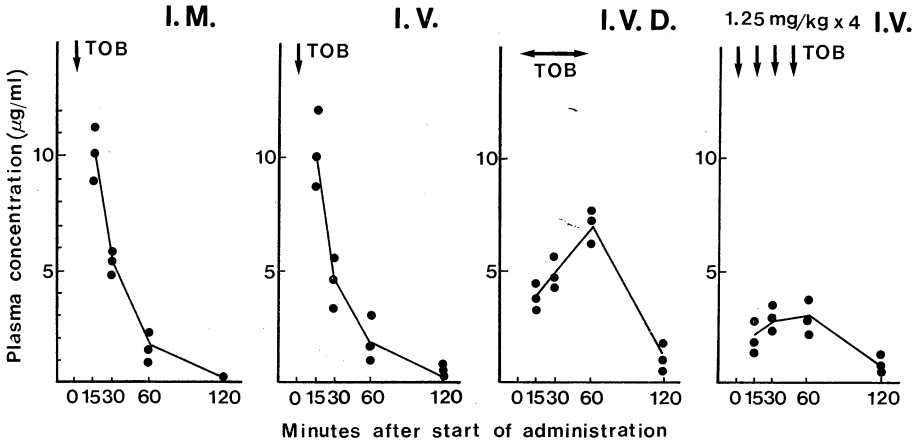

Fig. 1. Plasma concentration of tobramycin after 5 mg/kg/mouse administration

In the group treated once with 5 mg/kg, the peak plasma level of 10.05 μg/ml on the average was attained 15 min after intramuscular administration. The plasma concentration at 30 min was 5.33 μg/ml, but subsequently underwent a rapid decrease to less than 0.2 μg/ml at 120 min. A more or less similar pattern was found in the one-shot intravenous administration group, where the plasma concentration of TOB was 10.2 μg/ml at 15 min, 4.54 μg/ml at 30 min and 0.4 μg/ml at 120 min after administration. In the 1-hr i.v.d. group, the concentrations at 15, 30, 60 and 120 min were 3.81, 4.94 7.14 and 1.16 μg/ml, respectively. Thus, while the peak level was lower, the time before disappearance was prolonged. In the group dosed i. v. every 15 min, plasma concentrations were invariably low but this was found due to the fact that the determinations were made at 15 min after administration, and the actual course of plasma concentration is likely to be that indicated by the broken line in the right hand side of Fig. 2.

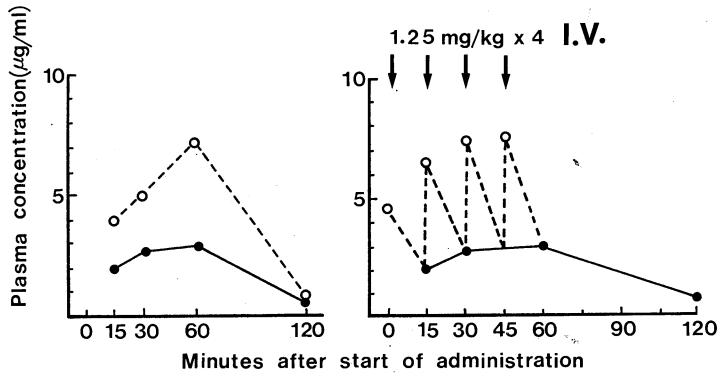

Fig. 2. Plasma concentration of tobramycin after divided injection of 5 mg/kg/mouse
·····, I.V.D. 5 mg/kg/hr ; ——, Divided I.V. 1.25 mg/kg×4

(2) Plasma concentrations after administration of 1 and 2 mg/kg of TOB

As shown in Fig. 3, the plasma concentrations of TOB, after a single intramuscular injection of 1 mg/kg of TOB were 1.47, 0.49, <0.32 and <0.32 μg/ml at 15, 30, 60 and 120 min, respectively. Thus, by the end of 60 min after administration, the plasma concentration was already less than 0.39 μm/ml, which was the MIC of TOB against the infective agent *K. pneumoniae B-54*. The plasma concentrations after a single i.m. injection of 2mg/kg were 2.47, 0.77, <0.32 and <0.32 μg/ml at the corresponding time points, and the course after 60 min was similar to that noted above. In contrast, when 0.25 mg/kg/dose was given 4 times at intervals of 15 min, the concentrations were <0.32, 0.57, 0.57 and <0.32μg/ml and when 0.5 mg/kg/dose was similarly administered, the values were 0.63, 0.71, 0.60 and <0.32 μg/ml and still above 0.39 μg/ml even after 60 min.

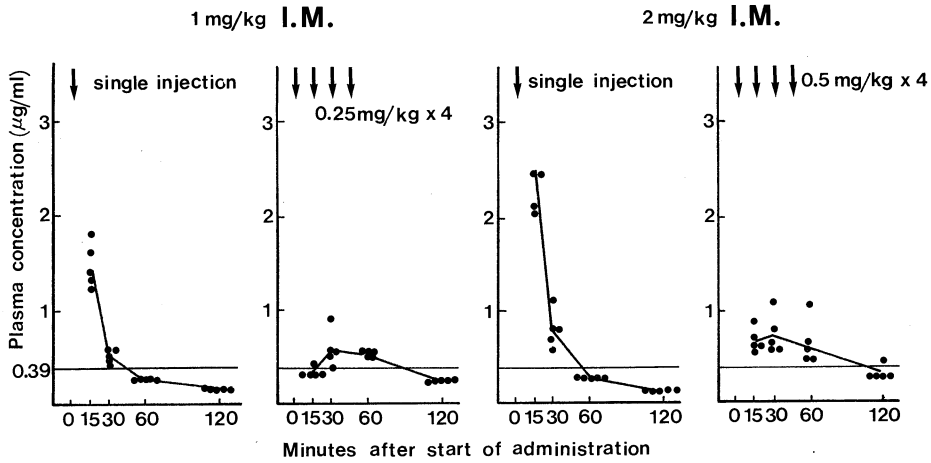

Fig. 3. Plasma concentration of tobramycin after 1 and 2 mg/kg/mouse administration

2. Therapeutic Efficacy in Mice with *K. pneumoniae* Pneumonia

(1) Course of the viable bacterial count in the lung:

As shown in Fig. 4, the viable cell count in the lung after a single intramuscular injection of 1 mg/kg of TOB, decreased consistently up to 6 hours after administration and then increased again. In the 2 mg/kg i.m. group, the decrease within 6 hours was very rapid but regrowth started at 9 hours. In contrast, in the animals given four divided doses at 15 min' intervals, both for the 0.25 and 0.5 mg/kg/dose groups, the time that regrowth started tended to be delayed compared with the one-shot injection schedule.

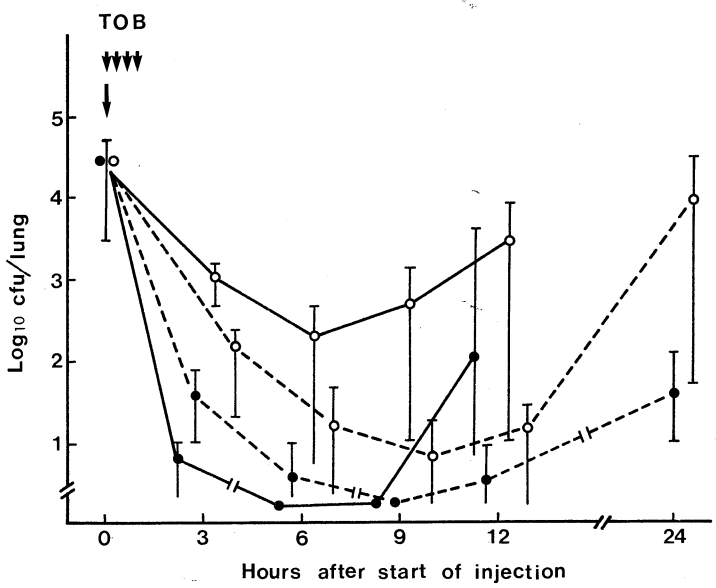

Fig. 4. Viable counts and decreasing rate of bacteria in murine lung treated with tobramycin ——control mice——.
○——○, 1 mg/kg × 1, I.M. ; ●——●, 2 mg/kg × 1, I.M. ;
○·····○, 0.25 mg/kg × 4, I.M. ; ●·····●, 0.5 mg/kg × 4, I.M.

(2) Survival rate :

Figs. 5, 6 and 7 show the survival rates found in the models constructed by infecting healthy mice with *K. pneumoniae B-54*. Fig. 5 shows the results obtained by 3-day treatment and Fig. 6 represents the results of 5-day treatment. Fig. 7 shows the results obtained by additional administrations of 0.5 mg/kg in one-shot and in four divided doses for 5 days. In both instances, the administration schedule of 0.25 mg/kg×4 at 15 min intervals×2/day yielded more satisfactory results than did the one-shot administration of 1 mg/kg twice daily. However, in the group treated with 2 mg/kg for 3 days, the one-shot administration gave a more satisfactory result. In the group given 5-day

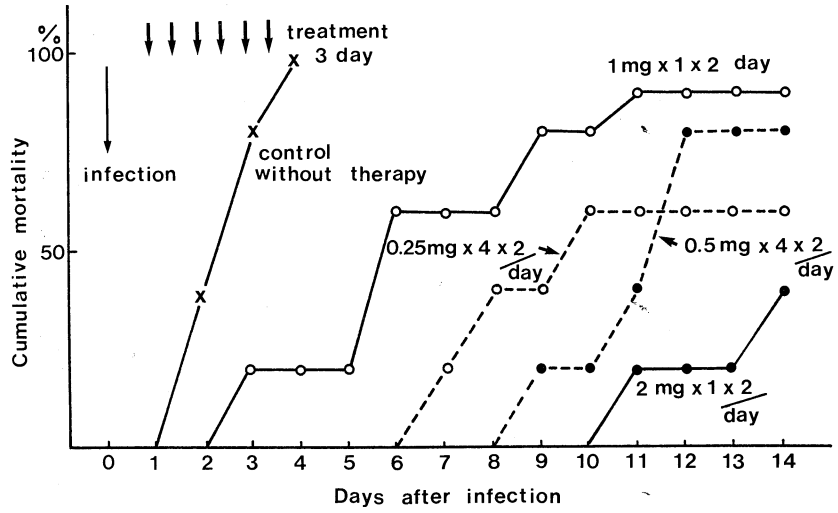

Fig. 5. Cumulative mortality of mice (I) challenged with *Klebsiella pneumoniae* and treated with tobramycin ——control mice (n=10)——
×——×, control without therapy ; ○——○, 1 mg×1×2/day ; ○·····○, 0.25 mg×4×2/day ; ●·····●, 0.5 mg×4×2/day ; ●——●, 2 mg×1×2/day

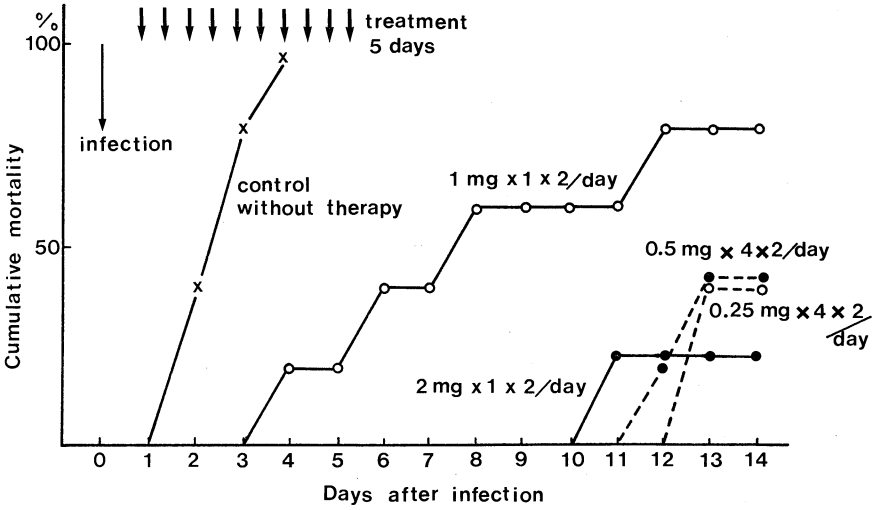

Fig. 6. Cumulative mortality of mice (II) challenged with *Klebsiella pneumoniae* and treated with tobramycin ——control mice (n=10)——.
×——×, control without therapy ; ○——○, 1 mg×1×2/day ; ●——●, 2 mg×1×2/day ; ●·····●, 0.5 mg ×4×2/day ; ○·····○, 0.25 mg ×4×2/day

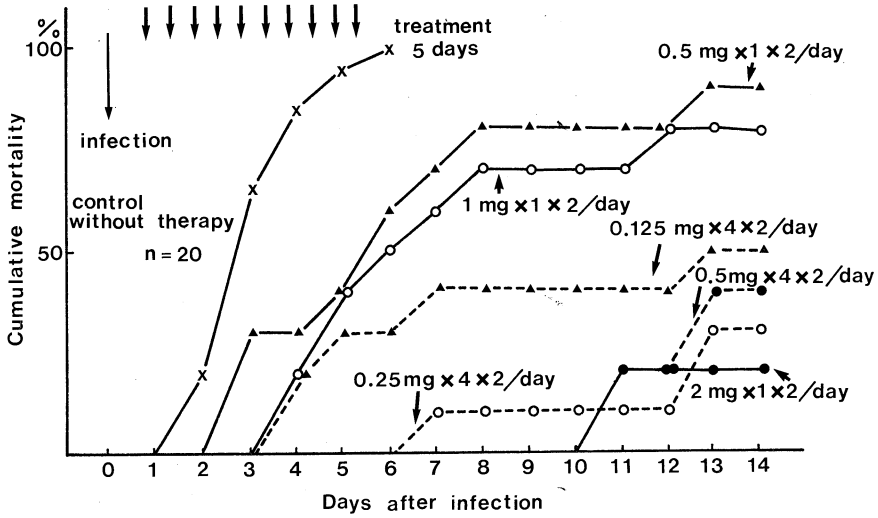

Fig. 7. Cumulative mortality of mice (III) challenged with *Klebsiella pneumoniae* and treated with tobramycin ——control mice (n=10)——.
×——×, control without therapy (n=20) ; ▲——▲, 0.5 mg 1×2/day ; ○——○, 1 mg×1×2/day ; ▲·····▲, 0.125 mg 4×2/day ; ●·····●, 0.5 mg×4×2/day ; ○·····○, 0.25 mg 4×2/day ; ●——●, 2 mg×1×2/day

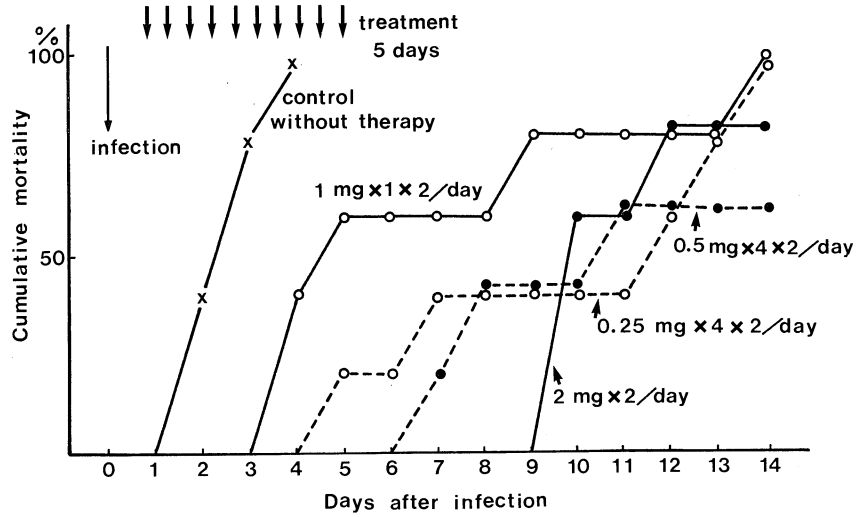

Fig. 8. Cumulative mortality of mice (IV) challenged with *Klebsiella pneumoniae* and treated with tobramycin ——formaldehyde-inhaled mice——.
×——×, control without therapy ; ○——○, 1 mg×1×2/day ; ●——●, 2 mg ×1×2/day ; ●·····●, 0.5 mg×4×2/day ; ○·····○, 0.25 mg×4×2/day

treatment, there was no obvious difference between the one-shot and divided-dose schedules. In the case of 0.5 mg/kg in one-shot versus four divided doses, too, the results obtained by administration of four divided doses tended to be more satisfactory.

(3) Therapeutic efficacy in mice with formalin-induced airway injury :

As shown in Fig. 8, the results were relatively poor as a whole, but the survival time tended to be prolonged in the group treated in four divided doses compared with the one-shot administration of 1 mg/kg. Thus, the divided-dose administration seemed to give more satisfactory therapeutic results.

DISCUSSION

In recent years, intravenous administration of aminoglycoside antibiotics has been reported in many cases[2,6,7] and considered to be desirable. In our opinion, the rate of ingress of the drug into the bloodstream and, hence, the plasma concentration of the drug can be easily controlled by selection of the proper drip-time, and such an administration method would contribute not only to the prevention of side effects but also to a prolonged effective concentration of the drug in the blood, thus leading to more satisfactory therapeutic results.

However, since it is not easy to compare the therapeutic effects of different administration methods in clinical situations, we have used mice as models. Mice were dosed with TOB in various ways, i.e., intramuscularly, by intravenous one-shot injection and drip infusion, and intramuscularly in divided doses at short intervals, and the plasma concentration of the drug was determined at timed intervals after administration in each instance. We found that while the intramuscular and one-shot intravenous injections resulted in substantially parallel time-courses of plasma concentration, intravenous drip infusion over a period of an hour resulted in a lower peak concentration but a sustained effective plasma level. Since intravenous drip infusions are not feasible in the case of small animals such as mice, the plasma levels after divided intramuscular doses given at intervals of 15 min were determined. As it turned out, while peak levels were low, effective levels, i.e., concentrations over 0.39 μg/ml, the MIC value against *klebsiella B-54* used for infection, could be sustained for a long time.

Therefore, a therapeutic experiment was conducted in mice with pneumonia, induced by airborne-infection with *K. pneumoniae B-54* using one-shot and divided injection methods. The one-shot injection group showed the most satisfactory result at the dose level of 2 mg/kg, but the injection in four divided doses yielded better results at the dose level of 1mg/kg. While therapeutic results may be improved by increasing the dose, this will of course lead to a higher frequency of side effects. Therefore, in cases where high doses are not feasible or are undesirable, it would be advisable to prolong the time of contact of the bacteria and the drug, rather than trying to increase the peak plasma concentration of the drug.

While therapeutic results in laboratory animals can not be directly extrapolated to humans, this study indicated that the therapeutic effect of TOB can be improved with suppression of adverse reactions by selecting an appropriate drip time.

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