INFLUENCE OF DEHYDRATION AND D-GLUCARATE ON DISTRIBUTION AND EXCRETION OF KANAMYCIN IN RATS

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Abstract—Effects of dehydration and sodium D-glucaro-1,4-lactone on kanamycin distribution in the kidneys, liver, lungs, spleen and cochleae were determined after intramuscular injection of the antibiotic into male rats. Coincident changes in plasma concentration and urinary excretion of the antibiotic were also measured. After administration of 30 mg/kg of kanamycin, dehydrated rats showed slower plasma elimination and urinary excretion of the drug as compared with normal animals. When the dehydrated rats were pretreated with sodium D-glucaro-1,4-lactone, however, both the plasma half-life of drug elimination and the rate of urinary drug excretion were in good parallel to those of normal animals. In the organ distribution study, dehydrated rats showed delayed removal of kanamycin from all organs as compared with normal animals. The extremely high level of the antibiotic in the kidney was about two orders of magnitude higher than found in other organs. In addition, the kidney showed different time course patterns for tissue levels in dehydrated rats. In dehydrated rats given sodium D-glucaro-1,4-lactone, the antibiotic levels in all organs followed the same pattern as in normal rats. When a lower dose of kanamycin was given to the dehydrated rats, the drug was cleared from the plasma with a decreased half-life. The tissue distribution percentages also decreased in all organs as the dose was lowered in dehydrated rats and with 1 mg/kg of kanamycin, only the kidney content was higher than that in sodium D-glucaro-1,4-lactone-treated animals.

The aminoglycoside antibiotics are known to have two serious side effects such as ototoxicity and nephrotoxicity (1, 2), and these toxic effects may be closely correlated (3). In our previous paper (4) we reported that dehydrated rats regularly developed acute renal failure following injection of aminoglycoside antibiotics, and that such was prevented by administration of some monosaccharides. In the present work, organ distribution and urinary excretion of \(^3\)H-kanamycin were compared in normal and dehydrated rats given saline or sodium D-glucaro-1,4-lactone (D-glucarate).

MATERIALS AND METHODS

\(^3\)H-kanamycin sulfate preparation

Kanamycin disulfate (Meiji Seika Co., Ltd.) was tritiated by the Wilzbach method. The labile tritium was removed by treating the substance with water, followed by lyophilization of the product. The labeled antibiotic was purified by passing through a column of Amberlite CG 50 (NH\(_4\) type). Chemical identity and determination of radiochemical
purity of the kanamycin were carried out by thin layer chromatography (Silica Gel-Merck) with n-propanol: pyridine: 30% NH$_4$OH: water (1:2:2:1) as a developing solvent, followed by ninhydrin coloration and radio-chromatographica scanning. Radiochemical purity was 98% and specific activity was 16.6 $\mu$ci/mg.

**Animal treatment**

Male Wistar-Imamichi rats weighing 350-400 g were deprived of drinking water for 48 hr to induce dehydration. Both normal and dehydrated rats were given kanamycin i.m. dissolved in physiological saline solution at a volume of 0.5 ml per animal. Throughout the present study, the amount of kanamycin sulfate which was employed was estimated as the base form. D-glucarate was given i.p. 5 min before antibiotic injection. The rats were then maintained under standard conditions with free access to water. At definite time intervals, the rats were sacrificed and organs were excised. In some experiments, blood samples were collected from the cervical vein with the rats under ether anesthesia. Urine was collected after placing the rats in individual metabolic cages.

**Radioactivity measurement**

Specimens of about 500 mg were obtained from each organ and 0.1-0.8 ml of plasma or urine were placed on paper disks. They were then burned by a sample oxidizer (Packard-305 type) and $^3$H$_2$O produced was dissolved in 15 ml of dioxane base scintillator. The samples thus prepared were counted in a liquid scintillation system (Nuclear-Chicago Mark I).

**RESULTS**

**Pattern of plasma elimination and urinary excretion of kanamycin**

Plasma levels and urinary cumulative excretion rate after injection of 30 mg/kg of kanamycin in normal and dehydrated rats on saline or 100 mg/kg of D-glucarate are shown in Figs. 1 and 2, respectively. When kanamycin was injected into normal rats, a peak level in plasma was seen after 15 min, the earliest time estimated, after which it rapidly disappeared with a constant half-life of 0.63 hr. The urinary excretion of the drug was almost completed during the first 6 hr, reflecting the quick elimination of the drug from the plasma, and 92% of the initially given dose was recovered in the urine within 48 hr.

In the dehydrated rats, there was an evident delayed excretion of kanamycin from the body. The plasma level was equal to that of normal rats at the first measured interval, decreased only slightly at 3 hr and then with a prolonged half-life. The urinary excretion of the drug reached a maximum for 12-24 hr and was not completed even after 48 hr. Recovery in the urine over 48 hr was 71% of the total dose.

In the dehydrated rats on D-glucarate, kanamycin was rapidly cleared from the body with almost the same plasma half-life and urinary excretion rate as observed in normal rats.

**Time course of tissue levels of kanamycin**

Fig. 3 represents the time course of level of antibiotic in the kidneys, liver, lungs, spleen and cochleae after injection of 30 mg/kg of kanamycin in normal and dehydrated rats on
Fic. 1. Plasma levels of kanamycin in normal and dehydrated rats on saline or D-glucarate, when given 30 mg/kg of the drug i.m. Normal and dehydrated rats deprived of water for 48 hr were given kanamycin-sulfate i.m. in a dose of 30 mg/kg. Saline or 100 mg/kg of sodium D-glucaro-1,4-lactone (D-glucarate) was given i.p. 5 min before kanamycin injection. Each point represents the mean (±S.D.) from three normal (– — – – –) and five dehydrated animals on saline (● – –●) or D-glucarate (● – –●).

Fic. 2. Urinary excretion of kanamycin in normal and dehydrated rats on saline or D-glucarate, when given 30 mg/kg of the drug i.m. Normal and dehydrated rats on saline or sodium D-glucaro-1,4-lactone (D-glucarate) (100 mg/kg) were given kanamycin (30 mg/kg). Experimental conditions were the same as in Fig. 1. Each point represents the mean (±S.D.) from three normal (– — – – –) and five dehydrated animals on saline (● – –●) or D-glucarate (● – –●).

saline or 100 mg/kg of D-glucarate. When kanamycin was injected into normal rats, it peaked in all organs after 15 min, decreased for 3 hr exponentially and, thereafter, slowly, except in the kidneys, where there was no sharp diminution of the drug from the start. Tissue levels were similar among organs such as the liver, lungs and spleen, while markedly high tissue levels were found in the kidneys, that is, the drug level was about one hundred times higher in the kidney 48 hr after administration. The level of the drug in the cochlea was expressed as mg per cochlea specimen which weighed approx. 200 mg.

Following injection in dehydrated rats, kanamycin was removed from all organs with a striking slow diminishing pattern. The tissue levels of the drug in all organs except the kidney were a little higher than those seen in normal rats after 15 min, remained high for 3 hr and then decreased gradually with time. The tissue levels of the drug in dehydrated rats...
Tissue levels of kanamycin in normal and dehydrated rats on saline or D-glucarate, when 30 mg/kg of kanamycin was given i.m. Organs were excised after administration of kanamycin in normal and dehydrated rats on saline or sodium D-glucaro-1,4-lactone (D-glucarate) (100 mg/kg). Animals were the same as those used for data in Fig. 1. The concentration of the drug was expressed as \( \mu \text{g per g wet weight} \) for organs such as the kidney, liver, lung, and spleen, and as \( \mu \text{g per specimen} \) for the cochlea. Each point represents the mean (± S.D.) from three normal (×—×) and five dehydrated animals on saline (●—●) or D-glucarate (○—○).

The level of drug in the kidneys of dehydrated rats was apparently higher than in normal rats after 15 min, almost doubled after 3 hr and then hardly decreased for 48 hr. The kidney content of the drug in dehydrated rats accounted for about 20% of the dose administered (Table 1.)

When kanamycin was injected into dehydrated rats on D-glucarate, however, all tissues showed the same pattern of drug decrease as seen in normal rats, although there was a positive rise of the antibiotic level as compared with that in normal rats. The drug level was apparently lower in the kidney of dehydrated rats pretreated with D-glucarate than in non-pretreated dehydrated rats soon after administration (p<.001), whereas there were no
Distribution percentages of kanamycin in organs:

| Tissues    | Treatment   | 30 mg/kg | 8 mg/kg | 1 mg/kg (Dosage) |
|------------|-------------|----------|---------|------------------|
| Kidneys    | Control     | 18.7     | 14.8    | 10.4 ± 0.7       |
|            | D-glucarate | 4.95     | 5.11    | 5.66 ± 0.41      |
| Liver      | Control     | 1.14     | 0.481   | 0.268 ± 0.041    |
|            | D-glucarate | 0.210    | 0.246   | 0.235 ± 0.059    |
| Lungs      | Control     | 0.317    | 0.072   | 0.0294 ± 0.0050  |
|            | D-glucarate | 0.0265   | 0.0013  | 0.0256 ± 0.0058  |
| Spleen     | Control     | 0.0944   | 0.0441  | 0.0203 ± 0.0022  |
|            | D-glucarate | 0.0187   | 0.0032  | 0.0183 ± 0.0033  |
| Cochlea    | Control     | 0.0349   | 0.00891 | 0.00392 ± 0.00092 |
|            | D-glucarate | 0.00344  | 0.00118 | 0.00312 ± 0.00071 |

a. Kanamycin was injected into dehydrated rats on saline or sodium D-glucarate-1,4-lactone in a dose five times that of kanamycin. Animals were sacrificed 6 hr after the kanamycin injection. b. Values represent the mean ± S.D. from five animals. c. Significantly different from control (p < .001). d. Not significantly different from control (p > .10).

significant differences (p < .10) between other organs of both groups at that time.

Plasma elimination pattern of lower doses of kanamycin

Plasma levels of kanamycin in dehydrated rats on saline or D-glucarate after administration in a dose of 8 or 1 mg/kg were examined (Fig. 4). Levels for D-glucarate-treated dehydrated rats decreased sharply with an almost constant half-life at both doses. The half-life of drug disappearance was about 0.6–0.7 hr, the same as that in normal rats.

Dehydrated rats showed changes in the pattern of drug disappearance depending to a great extent on the dose administered. In a dose of 8 mg/kg, kanamycin disappeared from plasma faster than in the case of a dose of 30 mg/kg, although plasma elimination was still greatly delayed in comparison with that observed in D-glucarate-treated dehydrated rats. When the dose was decreased to 1 mg/kg, the plasma levels of the drug in dehydrated rats became identical to those observed in D-glucarate-treated dehydrated rats at all times after administration.

Distribution rates in organs as a function of dose

Distribution percentages of kanamycin in the kidneys, liver, lungs, spleen and cochlea of dehydrated rats on saline or D-glucarate 6 hr after administration in doses of 30, 8 and 1 mg/kg were studied. As shown in Table 1, kanamycin was found in a large amount in the kidneys, followed by large amounts in the liver, lungs, spleen and cochlea, in that order. When D-glucarate-treated dehydrated rats were injected with kanamycin in various doses, an almost identical distribution percentage was obtained in each organ despite the large differences in doses.

In dehydrated rats, however, all organs showed lower distribution percentages with lower doses. Rates of decrease were large in the lungs, cochlea, liver and spleen but less only in the kidneys. When a dose of 1 mg/kg was used, statistically significant differ-
FIG. 4. Plasma levels of kanamycin in dehydrated rats on saline or D-glucarate, when given 8 or 1 mg/kg of the drug i.m. Dehydrated rats were injected with kanamycin following administration of saline or sodium D-glucaro-1,4-lactone (D-glucarate) in a dose five times that of kanamycin. Blood samples were collected from the cervical vein at defined times. Other experimental conditions were as in Fig. 1. Each point represents the mean (± S.D.) from five animals on saline (●—●) or D-glucarate (○—○).

ences were not found in distribution percentages between dehydrated rats on saline and D-glucarate in all organs with the sole exception of the kidney in which the value in the former was about twice that in the latter.

DISCUSSION

Specific high localization of kanamycin in the kidney in dehydrated rats clearly demonstrated the high degree of nephrotoxicity of the antibiotic. Dehydrated rats showed slow drug elimination from plasma in association with high drug levels in the kidney after a high dose of kanamycin was given i.m.. However, with lowering of the dose, the drug was cleared faster from the plasma in dehydrated rats. The content of the drug in the kidney rose more markedly in dehydrated rats than in normal animals soon after administration when there was no difference in plasma levels of the drug. Increase in drug content in the kidney of dehydrated rats is attributed to an enhanced incorporation of the drug into kidney tissue, and the high drug plasma levels are probably due to a lack of drug excretion into the urine as the result of renal dysfunction. The enhanced drug accumulation in the kidney in de-
hydrated rats was suppressed by D-glucarate administration. Suppression of antibiotic accumulation in the kidney by D-glucarate was obtained at the lowest dose in which the plasma and other tissue levels of the drug were identical in dehydrated rats on saline or D-glucarate. Thus D-glucarate may directly suppress the enhanced incorporation of kanamycin into kidney tissue in dehydrated rats.

Dehydrated rats also showed higher drug levels in organs such as the liver, lungs and spleen. However, antibiotic distribution percentages in these organs in dehydrated rats, decreased as the dose was lowered and became equal to levels obtained in D-glucarate-treated dehydrated rats given the lowest dose, whereas rise of the drug level could still be detected in the kidney of non-treated dehydrated rats. It is likely that in these organs the antibiotic is distributed in a volume corresponding to the extracellular space and when rapidly eliminated from the plasma, is removed from the space. When the antibiotic remains for a long time at high levels in the plasma, it may be distributed in the intracellular compartment, possibly due to slow penetration.

The antibiotic level in the cochleae was also investigated since aminoglycosides cause lesions in the cochleovestibular system. The cochlea, unlike the kidney, showed a comparatively fast drop in drug level with time after administration. Since the cochlea is rich in lymphatic fluid, the following is suggested; kanamycin quickly penetrates the lymphatic fluid and the antibiotic content obtained in the cochleae soon after administration is present in the lymphatic fluid.

Aminoglycosides have a high affinity for kidney tissue even in normal rats. Normal rats, however, rarely develop renal failure even with large doses and consecutive administration of antibiotics (5). We did not observe renal dysfunction in normal rats despite high drug levels in the kidney which caused serious renal failure in the dehydrated rats (Fig. 3). Such data indicate that there is a different antibiotic distribution location which is involved in renal impairment in the kidney of dehydrated rats. Aminoglycoside-induced renal failure is apparently responsible for necrosis of epithelial cells of convoluted proximal tubules (2, 6), however, the underlying mechanism has not been elucidated. If indeed there is a different antibiotic distribution location in the kidney of dehydrated rats, then increase in antibiotic content in the kidney would be due to incorporation of the drug into the epithelial cells. The brush border is evidently exposed to antibiotics since the renal excretion of these compounds appears to be primarily through glomerular filtration (7). If the brush border is the initial area of intoxication, then antibiotics would produce renal damage in normal rats when given in large doses and consecutive administration of the drug. Roman et al. (8) observed ultrastructural alterations of subcellular organelles with the apical microvilli intact. According to Hirata et al. (6) the antibiotic was localized in phagolysosomes which become more fagils. It may be that in dehydrated rats, aminoglycosides penetrate the epithelial cells and are bound to subcellular organelles, thereby inducing sublethal cellular functional disorders.

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REFERENCES

1) Finegold, S.M.: *Ann. N.Y. Acad. Sci.* 132, 942 (1966)
2) Kosek, J.C., Mazze, R.I. and Cousins, M.J.: *Lab. Invest.* 30, 48 (1974)
3) Akiyoshi, M., Sato, K. and Tamura, H.: *Audiology* 12, 435 (1969)
4) Furuno, K., Ando, K., Suzuki, S. and Hirata, K.: *J. Antibiotics* 29, 187 (1976)
5) Flandri, O. and Damant, M.: *Gentamycin. First International Symposium*, p. 47. Basel, Schwabe and Co. (1967)
6) Hirata, K., Ueda, T., Morita, K., Takamatsu, C., Goto, K., Aramaki, R., Kai, M. and Ishikawa, T.: *Japan. J. Nephrol.* 15, 371 (1973)
7) Tisch, D.E., Huttaln, J.B. and Dickison, H.L.: *Ann. N.Y. Acad. Sci.* 76, 44 (1958)
8) Roman, J.V., Krishnakanth, T.P. and Cuppage, F.E.: *Lab. Invest.* 33, 412 (1975)