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Kinetics of gentamicin accumulation in subcellular structures of the mouse kidney

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Gentamicin adsorption to brush-border and basement membranes of the proximal tubule cells, followed by endocytosis and lysosomal sequestration, are considered to be the steps of the process of gentamicin accumulation in the kidney cortex (Just & Habermann 1977: Watanabe 1978; Kuhar et al 1979; Silverblatt & Kuehn 1979). Although electron microscope autoradiographic puctures indicate a very rapid transfer of gentamicin from the tubular lumen into the endocytic vesicules and lysosomes following administration of gentamicin (Just & Habermann 1977), the time dependence of gentamicin uptake into subcellular particles has not yet been described so far.

Materials and methods

[^{3}H]Gentamicin, specific radioactivity 0·44 μCi ^{3}H g^{-1} (Radiochemical Centre Amersham) was injected i.v. into male Swiss mice, 18–24 g, in a dose of 4 mg kg^{-1} (the dose was adjusted with gentamicin sulphate, Schering). Blood samples (≈ 100 μl) were taken from the retro-orbital venous plexus for determination of gentamicin in plasma 1, 2·5, 5, 10, 20, and 40 min; 1, 3, and 6 h; 1, 3, 7, and 10 days post injection. Heparinized plasma 40 μl was dissolved in 25% KOH in 20% ethanol, and mixed with 15 ml of Bray's solution. For determination of gentamicin in kidneys the animals were killed at the same time intervals. Both kidneys were removed, decapsulated and homogenized by five complete strokes in a Potter-Elvehjem homogenizer in a solution of 0·25 M saccharose and 0·05 M Tris, pH 7·4, 9 ml g^{-1} tissue. For ^{3}H determination an aliquot of 0·45 ml from the homogenate was combusted using a Sample Oxidizer 306 (Packard). The homogenate was centrifuged for 30 min at 105 000 *g* and 10 ml Insta-gel (Packard) was added to the total supernatant. The pellet was combusted as indicated previously. Liquid scintillation counter TriCarb (Packard) was used for determination of ^{3}H radioactivity in samples. To check the integrity of lysosomes, the activity of β-*N*-acetylglucosaminidase and cathepsin D were determined (Ruth et al 1978) in the supernatants and pellets after disruption of lysosomes with Triton X-100 (Koch-Light). The percentages of gentamicin in the supernatant, which was supposed to represent unbound gentamicin, and in the sediment composed of subcellular particles, were calculated. The mean ± s.e.m. recovery of ^{3}H, expressed in percentages obtained from the ratio of the sum of ^{3}H in the supernatant and in the sediment to ^{3}H in homogenate before centrifugation, was 93·8 ± 1·4%. The time dependences of the logarithm of gentamicin concentration in the plasma and kidneys were approximated by functions composed from 3 or 2 exponential terms, respectively using the Monte Carlo and least-square methods.

Results and discussion

A 3-compartment pharmacokinetic model, consisting of one central and two peripheral compartments, with elimination from the central compartment, was used to approximate gentamicin behaviour in mice. The concentration-time curve of plasma gentamicin (Fig. 1) could adequately be described by a function composed from three exponential terms $C_{pl} = 57{\cdot}4e^{-1{\cdot}435t} + 10{\cdot}2e^{-0{\cdot}062t} + 1{\cdot}22e^{-0{\cdot}000065t}$, t being the time in min. The time dependences of gentamicin concentration in the kidneys, in the sediment of the kidney homogenate and in the computed second and third compartments of the 3-compartment pharmacokinetic model are shown in Fig. 2. The concentration-time dependence of gentamicin in the kidney could be approximated by a biexponential function $C_{kid} = 39{\cdot}9e^{-0{\cdot}00123t} + 10e^{-0{\cdot}000098t}$, t being the time in min, beginning from the peak at 60 min post administration. The early time intervals reflecting gentamicin distribution were not included in the kinetic analysis. The shape of the concentration-time curve of gentamicin in the kidney sediment parallels the concentration-time curve of gentamicin in the whole kidney, the difference between the curves being greater in the early time intervals and gradually diminishing later. This indicates that the sedimentable structures represent

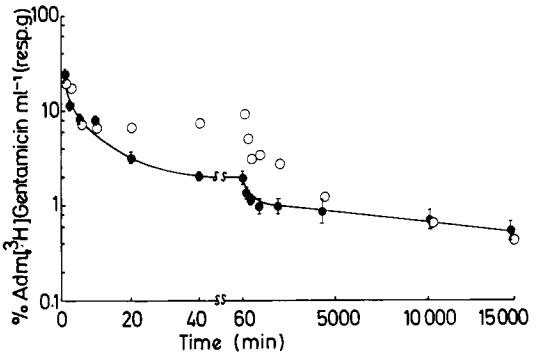

FIG. 1. The concentration-time curve of gentamicin in plasma of mice (●) (% administered activity ml^{-1}) and of gentamicin in the kidney supernatant (○) (% administered activity g^{-1} kidney).

* Correspondence.

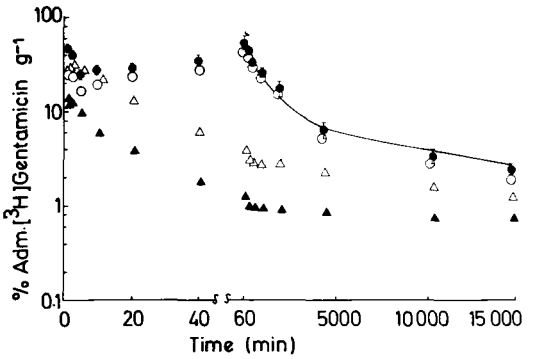

FIG. 2. The concentration-time dependence of gentamicin in kidney (●) (% administered activity g^{-1}), of gentamicin in the kidney sediment (○) (% administered activity g^{-1} kidney), in the 2nd (△) and 3rd (▲) compartments (% administered activity g^{-1}).

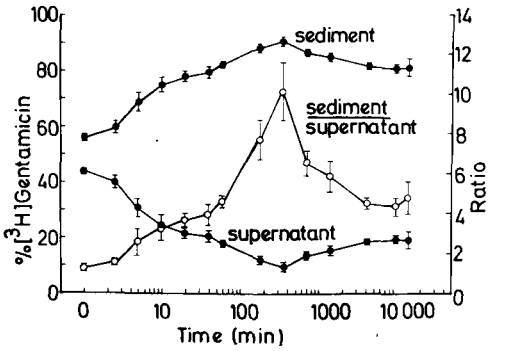

FIG. 3. The distribution of ^3H into 105 000 *g* sediment and supernatant after centrifugation of mouse kidney homogenate at various times after i.v. administration of [^3H] Gentamicin (left-hand axis). Ratio of ^3H in sediment/^3H in supernatant (right-hand axis). Each value is the mean ± s.e.m. from 6 experiments.

the fraction accounting for kidney gentamicin. On the other hand gentamicin in the supernatant does not closely parallel gentamicin plasma concentration (Fig. 1) rather it follows gentamicin in the sediment mainly during the first 24 h. As can be seen, the kidney gentamicin cannot be directly related to either tissue compartment computed from the gentamicin plasma curve (Fig. 2). The terminal half-life of gentamicin in plasma is 173 h and in the kidney, 117 h, which is close to 109 h (Luft & Kleit 1974) and 98 h (Fabre et al 1976) found in rats. Gentamicin serum terminal half-life in man reaches a value of 112 h corresponding to the slow release of the drug from the tissue compartment represented mainly by the kidneys (Schentag & Jusko 1977). The time course of gentamicin distribution into subcellular particles and the supernatant is shown in Fig. 3. As early as 1 min after i.v. administration, 55·9% of gentamicin present in the homogenate was found to be in the sediment. The percentage sedimenting increased up to 6 h, at which time it attained the peak value of 90·7%, the sediment/supernatant ratio of gentamicin being 10. A continuous and slow decrease of the fraction of gentamicin accumulated in subcellular particles, along with the steady increase of the fraction of gentamicin in the supernatant, was observed until the end of the 10th day of the observation period.

Table 1. The activity of β-*N*-acetyl-glucosaminidase and cathepsin D in supernatant, and sediment after addition of Triton X-100, obtained by mouse kidney homogenate centrifugation at 105 000 *g* for 60 min.

	Activity of β-*N*-acetyl-glucosaminidase (units* mg^{-1} protein)	Activity of cathepsin D (units* mg^{-1} protein)
Sediment	1·01 ± 0·015	21·6 ± 1·39
Supernatant	0·28 ± 0·036	7·1 ± 0·44

* One unit of β-*N*-acetyl-glucosaminidase activity is equivalent to formation of 1 nM 2-nitrophenyl-*N*-acetyl glucosamide/min and that of cathepsin D to 1 nM tyrosine/min.

The results of this experiment in which a single and rather small dose of gentamicin was administered have been considered to represent a kinetic picture which occurred independently of the effects of the antibiotic on the kidney function.

The method used in this experiment could not differentiate between the uptake of gentamicin into subcellular particles and the adsorption of gentamicin to sedimentable structures. The whole sediment, not particular density fractions of the homogenate, was examined for gentamicin, as other studies (Morin et al 1977) have unequivocally shown that gentamicin is stored exclusively in lysosomes. The mean ± s.e.m. activity of β-*N*-acetylglucosaminidase and cathepsin D in the sediments after addition of Triton X-100, and in the supernatants is shown in Table 1. The ratio of the activities of both enzymes in the supernatant to that in the pellet was approximately 1:3, indicating a careful homogenization procedure and preservation of most lysosomes. A similar value for acid phosphatase was found by Just & Habermann (1977) and for β-*N*-acetylglucosaminidase by Tulkens & Trouet (1978).

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