Tissue Gentamicin Concentrations in the Newborn and Adult rat

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Summary

Rats 4 or 5 days old and 8 to 10 weeks old were injected ip with 16 mg [14 C]gentamicin sulfate/kg body weight and between 4 and 15 animals were killed at 0.5, 1, 2, 4, and 8 hr. Blood, liver, heart, kidneys, skeletal muscle, lung, and brain were collected and analyzed for radioactivity. Tissue gentamicin measured by 14 C correlated well with the results of radioimmunoassay. The extracellular fluid space of neonatal and adult heart, muscle, kidney, lung, and liver was measured *in vitro* with [14 C]carboxyl inulin. Gentamicin disappeared from the adult circulation exponentially with a rate constant of $1.54\% \text{ min}^{-1}$ but more slowly from neonatal plasma. The semilogarithmic plot of neonatal plasma gentamicin against time was curvilinear, suggesting that the drug was distributed in more than one body compartment. The renal concentration of gentamicin in the adult rat was four times greater than the peak plasma level at 0.5 hr and continued to rise thereafter to a maximum of 240 ng/mg at 4 hr, which was sustained at 8 hr. In the neonate a qualitatively similar result was obtained but the concentrations were lower. In neonatal muscle the mean gentamicin concentration was three times greater than that in the adult at 0.5 hr and the difference between the two ages became greater as time passed, rising to 10-fold at 8 hr. The mean lung concentrations in the adult and neonate were similar at 0.5, 1, and 2 hr but at 4 and 8 hr neonatal lung gentamicin levels were greater than those in the adult. Gentamicin was detectable in adult brain at 0.5 and 1 hr only, values thereafter being insignificantly different from zero. In contrast, the mean brain gentamicin concentration of the neonates rose progressively to a peak of 1.26 ng/mg at 4 hr and had not fallen significantly at 8 hr. In all tissues the extracellular space was significantly greater in the neonate but the degree of difference varied markedly, it was greatest in the liver and least in the lung. The renal concentration of gentamicin in both the adult and neonate was always in excess of the plasma concentration. In the adult there was no evidence of intracellular gentamicin in heart or muscle, but the mean lung concentration exceeded that in the lung extracellular space by 0.9 to 3.0 ng/mg at all times studied, and the mean "intracellular" gentamicin concentration in liver was 0.5, 1.5, and 1.4 ng/mg at 2, 4, and 8 hr, respectively. In neonatal lung and muscle, intracellular gentamicin was detectable from 2 hr on and the concentration remained stable at 1.21 to 2.32 ng/mg. In liver intracellular gentamicin was first detected at 4 hr when the mean concentration was 1.6 ng/mg, but at 8 hr this had risen to 3.71 ng/mg. Cardiac intracellular gentamicin was present at 8 hr in a low but significant concentration of 0.59 ng/mg.

Speculation

In the neonatal rat gentamicin penetrates the intracellular compartment and traverses the blood-brain barrier. The pharmacology of aminoglycoside antibiotics may be influenced by immaturity in other species also.

Gentamicin is commonly believed to be distributed in the extracellular space and to be excreted unaltered via the kidneys

(12). Both facts influence neonatal dosage because the newborn infant has poorer renal function, but a larger extracellular space than the adult. The former point led to infants receiving inappropriately low doses of the antibiotic until clinical trials (8-10) established the need for a higher dose, expressed as milligrams per kilogram body weight, in the baby than in the adult. In one of the above studies (8), measurements were made of tissue gentamicin concentrations in four infants who died. Not only the kidney, but also the lung, liver, and heart were found to retain gentamicin after the drug had been cleared from the plasma. This observation led to speculation that, in the newborn at least, gentamicin might penetrate the intracellular compartment. The concept has been explored in the work reported here using [14 C] gentamicin and the rat as an experimental model.

MATERIALS AND METHODS

Sprague-Dawley rats of both sexes and two age groups were studied. The neonatal rats were 4 or 5 days old and weighed 6 to 9 g while the adults weighed 200 to 250 g and were 8 to 10 weeks old. Each animal was injected ip with a solution of [14 C]gentamicin sulfate [specific activity, $0.83 \mu\text{Ci}/\text{mg}$ (21)] dissolved in phosphate-buffered saline (Dulbecco A; Oxoid Ltd.) at a concentration of 1-3 mg/ml. The nominal dose was 16 mg gentamicin sulfate/kg body weight and the exact dose given was calculated by weighing the syringe before and after injection. All calculations of plasma and tissue concentration were performed after correcting the results as if the animal had received 16 mg/kg.

Blood samples were obtained from adult rats at intervals until the time they were killed via the tail vein using a heparinized micropipette (Bilbate Ltd.). Blood was obtained in the same manner from neonatal rats by section of the axillary artery immediately before they were killed. After decapitation the following tissues were dissected and blotted free from blood: liver, heart, kidneys, anterior axillary skeletal muscle, lung, and brain. Between 4 and 15 animals were killed at 0.5, 1, 2, 4, and 8 hr. Blood samples were also collected at 10, 20, 40, and 50 min from adults and at 40 and 50 min from neonates. Plasma and tissues were stored at -20° until the time of analysis.

LIQUID SCINTILLATION COUNTING

Approximately 100 mg tissue was chopped with scissors into pieces of less than 1 mm in diameter, transferred to a scintillation vial, and weighed to the nearest 0.1 mg. Plasma was pipetted into a scintillation vial and weighed similarly. After bleaching with 0.2 ml hydrogen peroxide (30% w/v) and 0.2 ml isopropyl alcohol, 1 ml Soluene 350 (Packard Ltd.) was added and the tissue was solubilized overnight at 56° . After the addition of 1.2 ml water and 10 ml scintillation fluid (4 g, 2,4-diphenyloxazole in 333 ml Triton X-100 (Hopkin and Williams Ltd.) and 667 ml toluene scintillation grade (Inter technique Ltd.), each sample was counted for 10 min on a S.L. 30 counter (Inter technique Ltd.). Quench curves were constructed by the addition of an internal [14 C]hexadecane standard (Radiochemical Centre, Amersham) to control

samples of plasma and the other six tissues. The radioactivity of the sample of the gentamicin solution of known concentration used in each experiment was measured, thus enabling the dose of gentamicin per rat to be calculated accurately. This also enabled the results received as dpm to be expressed as nanograms gentamicin sulfate per milligram plasma or tissue.

PROCESSING OF TISSUE

Gentamicin was extracted from tissues by homogenization and sonication. Weighed amounts of tissue were homogenized for 15 min on a minihomogenizer (MSE Ltd.) in phosphate-buffered saline to produce a breis containing approximately 500 mg tissue/ml. The mixture was sonicated for 10 min at maximum energy using a Rapidis 50 sonicator (Ultrasonics Ltd.). After centrifugation for 45 min at 2400 rpm on a Mistral 2L centrifuge, aliquots of the supernatant were stored frozen at -20° until assay. Preliminary experiments using tissues labeled with [^{14}C]gentamicin *in vivo* showed that this extraction recovers 85–95% of the total radioactivity.

The extracellular fluid space of the neonatal and adult tissues was determined *in vitro* (11) with [^{14}C]carboxyl inulin (Radiochemical Centre, Amersham).

RADIOIMMUNOASSAY OF GENTAMICIN

The gentamicin concentration of different tissues was measured by a modification of a double antibody radioimmunoassay (5) using gentamicin fluorescein thiocyanate iodinated with ^{125}I by the chloramine T method as tracer (2), a first antibody raised in rabbits to bovine serum albumin-gentamicin conjugate, and donkey anti-rabbit globulin (Wellcome Ltd.) as the second antibody. The standard curve prepared from a 100 $\mu\text{g}/\text{ml}$ standard (Nicholas Laboratories Ltd.) was 50 μl gentamicin sulfate solution at a concentration range of 6.4–0.05 $\mu\text{g}/\text{ml}$. Each tissue homogenate was assayed against a standard curve which contained an equal volume of homogenate of the same tissue but from similar animals that had not received gentamicin.

RED CELL AND PLASMA PROTEIN BINDING OF [^{14}C]GENTAMICIN

Heparinized arterial blood was obtained from five adult and six neonatal rats and the hematocrit measured. After the addition of [^{14}C]gentamicin at a concentration of approximately 25 $\mu\text{g}/\text{ml}$, the blood was incubated for 1 hr at 37° with shaking. Samples of blood and plasma were then processed for scintillation counting. From knowledge of the radioactivity of whole blood, plasma and the hematocrit, the proportion of gentamicin bound to red cells was calculated.

Plasma was incubated with [^{14}C]gentamicin at a final concentration of 30 $\mu\text{g}/\text{ml}$ for 20 min at 37° . Radioactivity was then measured before and after precipitation of the proteins with 10% trichloroacetic acid. Estimations were made on five individual adult rats and on three pools each from two neonatal rats.

RESULTS

CIRCULATING GENTAMICIN LEVELS

The mean plasma gentamicin concentrations in adult and newborn rats at different times after ip injection are presented in Table 1. In the adults a maximum level occurred at 30 min but the levels between 30 and 60 min did not differ significantly. In the neonates the maximum concentration was similar to that in the adults and occurred at 60 min. After the first hr, gentamicin disappeared from the adult circulation exponentially with a half-life of 45 min and a disappearance constant (K_d) of 1.54%. When neonatal plasma gentamicin concentration was plotted semilogarithmically as a function of time the disappearance was slower and curvilinear, suggesting that the drug was distributed in more than one body compartment (Fig. 1).

When gentamicin was incubated with adult blood *in vitro* the

mean ($\pm\text{SE}$) percentage binding by red cells was 3.1 ± 1.0 (range, 0–5.3%, $n = 5$). In the neonates the percentage bound was 2.9 ± 0.9 (range, 0.8–6.6%, $n = 6$). The mean fraction ($\pm\text{SE}$) of gentamicin precipitated by trichloroacetic acid after addition to adult plasma was 0.427 ± 0.005 ($n = 7$) and to neonatal plasma 0.468 ± 0.016 ($n = 3$).

TISSUE GENTAMICIN LEVELS

The change of gentamicin concentration with time not only varied between adult and neonate but also between different tissues (Table 2), and the results are therefore considered organ by organ.

Kidney. The renal concentration of gentamicin in the adult rat at 0.5 hr was four times greater than the peak plasma level and continued to rise thereafter to a maximum of 240 ng/mg at 4 hr, which was sustained at 8 hr. In the neonate a qualitatively similar result was obtained but the concentrations were lower. At 0.5 hr the renal concentration was similar to that in plasma and thereafter a gradual rise occurred to a peak concentration of 73 ng/mg at 8 hr. In contrast to the kidney, the mean concentration of gentamicin in other neonatal tissues at 0.5 hr was equal to or greater than that in the adult.

Table 1. Mean ($\pm\text{SE}$) plasma gentamicin sulfate concentration (ng/mg) in neonatal and adult rats after the ip injection of 16 mg [^{14}C]gentamicin sulfate/kg body weight

Time	Neonate	Adult
10 min		16.1 ± 3.0 (8)
20 min		23.7 ± 3.5 (8)
30 min	20.4 ± 1.0 (9) ¹	26.2 ± 3.3 (8)
40 min	15.0 ± 1.2 (8)	24.4 ± 2.3 (7)
50 min	15.8 ± 2.0 (9)	23.3 ± 2.5 (7)
1 hr	23.3 ± 1.2 (8)	20.9 ± 2.0 (8)
2 hr	12.1 ± 0.4 (7)	8.4 ± 1.5 (7)
4 hr	6.2 ± 0.4 (15)	0.9 ± 0.3 (5)
8 hr	2.6 ± 0.1 (11)	0.1 ± 0.1 (5)

¹ Number of observations shown in parentheses.

Fig. 1. Semilogarithmic plot of mean ($\pm\text{SE}$) plasma gentamicin sulfate concentration in neonatal (○) and adult rats (●) as a function of time after the ip injection of 16 mg [^{14}C]gentamicin sulfate/kg body weight.