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Distribution of Gentamicin and Amikacin in Rabbit Tissues

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Rabbits were injected intramuscularly with gentamicin and amikacin (15 mg of base per kg), and the antibiotic levels in tissues were determined 20 h after either a single or multiple injections. Disk diffusion assays, with *Bacillus subtilis* ATCC 6633, were carried out both on homogenates and on supernatants deproteinized with trichloroacetic acid. Both assays indicated that the kidney is the major site of antibiotic deposition. Antibiotic levels increased after multiple doses. Gentamicin levels in other tissues were higher than those of amikacin. The assay of trichloroacetic acid-treated material was more sensitive than the assay of the total homogenate.

Aminoglycoside antibiotics are used extensively for the treatment of severe bacterial infections. Several studies have indicated that these antibiotics reach peak levels in serum within several hours of administration and subsequently decline, whereas high levels in the kidney persist much longer (1-4, 9-11).

Usually, tissue aminoglycoside levels are assayed by disk diffusion techniques. The amount of drug in the tissue is extrapolated from the standard curve of the drug in buffer or in control tissue homogenate. Since aminoglycosides become partially bound to tissue macromolecules and thus inactivated (7), the assay with the standard curve in buffer measures only the free drug. The assay with the standard in the homogenate presumably corrects for the bound portion of the drug in tissue and thus gives a better estimate of the total drug. An alternative method is to dissociate the bound antibiotic by a chemical method and subsequently assay the total drug.

The present study reports on the assay of two aminoglycosides, gentamicin and amikacin, in rabbit tissues after a single and multiple injections. The antibiotics were assayed in tissue homogenates directly and in the homogenate supernatants after trichloroacetic acid precipitation of protein. A comparison of the results from these two methods is presented as well as data regarding the binding of these antibiotics by the tissue homogenates in vitro.

MATERIALS AND METHODS

Materials. Gentamicin sulfate (Garamycin, injectable; 40 mg/ml) was obtained from Schering Corp., Kenilworth, N.J. Amikacin (BB-K8) was obtained from Bristol Laboratories, Syracuse, N.Y. (lot no. 76F 1598 and 76F 439). It was dissolved in sterile saline to give 40 mg of amikacin base per ml.

Administration of antibiotics in vivo. Male and female white rabbits (Klubertanz, Edgerton, Wis.), varying in weight from 1.6 to 2.4 kg, were divided in groups of three rabbits each. They had free access to water and were fed Purina Rabbit Chow Checkers W. The rabbits in each group received either a single injection or seven daily injections of gentamicin or amikacin, 15 mg/kg, in the hind leg muscle. The control group received an equivalent volume of saline. Sterile techniques were used throughout these manipulations. In experiments in which antibiotic levels in tissues other than kidney were measured, one experimental group and the controls were injected. In kidney experiments, both gentamicin and amikacin groups, as well as the controls, were injected simultaneously.

Preparation of tissue homogenates. Twenty hours after the last injection, blood was removed from each rabbit by cardiac puncture, and the animal was sacrificed by cervical dislocation. The following tissues were removed and placed on ice in sterile cups: kidney, liver, lung, heart, brain, and hind leg skeletal muscle from the side opposite the injection. The tissues were minced and homogenized in 3 volumes of 0.25 M sucrose-0.01 M tris(hydroxymethyl)aminomethane buffer, pH 7.4 (25%, wt/vol, homogenate), with a Potter-Elvehjem homogenizer. The homogenates were filtered through cheesecloth and then used in the microbiological assay. Three additional dilutions of the experimental kidney homogenates were made with control kidney homogenates. One additional dilution of experimental urine with control urine was also made. Blood samples were allowed to clot at room temperature for 2 h. Sera were obtained after centrifugation at 1,000 × *g* for 20 min. Urine was withdrawn from rabbit bladders after sacrifice. Most of the urine samples were alkaline and required the addition of 1 N HCl to lower the pH to 7.4. Protein in tissue homogenates was determined by the Lowry procedure (8).

Trichloroacetic acid precipitation. To 2-ml portions of tissue homogenates, 0.2 ml of concentrated trichloroacetic acid (Sigma, St. Louis, Mo.) was added. The tubes were mixed vigorously on a Vortex mixer and then centrifuged at 1,000 × *g* for 15 min. Portions, 1 ml each, of the supernatants were transferred to a new set of tubes, and each sample was adjusted to pH 7 to 8 (Beckman Zeromatic pH meter, model SS-3, equipped with a combination electrode) by the addition of NaOH. Assay of control samples at pH 7 and 8 indicated that there was no significant difference in antimicrobial activity at these two pH values.

Tissue homogenates from control rabbits were incubated with four different concentrations of either gentamicin or amikacin (2.5 to 20 μg/ml; twofold dilutions) at 25°C for 30 min and then treated with trichloroacetic acid as described above. The microbiological assay of these samples provided the standard curves from which the antibiotic levels of the experimental trichloroacetic acid-treated samples were extrapolated. No correction was made for the small increase in volume caused by the addition of trichloroacetic acid and base, because all the samples, experimental as well as standards, were treated identically.

Microbiological assay. Disk diffusion assays were carried out as described by Grove and Randall (6). *B. subtilis* ATCC 6633 was the assay organism, and antibiotic medium no. 5 (BBL, Div. of Becton, Dickinson & Co., Cockeysville, Md.) was used to prepare the bilayered plates. The top layer was prepared from 5 ml of the seeded inoculum. Disks were saturated with the test solution, placed on the agar, and incubated overnight at 37°C. The zones of inhibition were recorded the following morning. All assays for each experiment were carried out simultaneously, and the agar plates were prepared on the day of the experiment.

Each experiment included the following test solutions: (i) tissue homogenates from each of the injected rabbits; (ii) four concentrations (2.5, 5, 10, and 20 μg/ml) of gentamicin or amikacin or both in tissue homogenates from control rabbits for the construction of standard curves; (iii) trichloroacetic acid-treated homogenate supernatants from each of the injected rabbits; (iv) trichloroacetic acid-treated homogenates containing gentamicin and amikacin for standard curves (see Trichloroacetic acid precipitation); (v) control samples of homogenates, trichloroacetic acid supernatants, urine, and sera from uninjected rabbits; (vi) dilutions of gentamicin and amikacin in homogenization buffer and in trichloroacetic acid-treated buffer for standard curves.

The antibiotic level of an experimental homogenate or trichloroacetic acid supernatant was extrapolated from the standard curve (zone size versus the log of antibiotic concentration) corresponding to that particular tissue and treatment. All dilutions of kidney homogenates and of urine were assayed, and the mean values were calculated. Standard curves in homogenization buffer and in trichloroacetic acid-treated buffer were included in each experiment to provide reference points for the other standard curves in which homogenates and trichloroacetic acid supernatants were used as diluents. The percent activity was calculated from these data.

RESULTS

Tissue levels of antibiotics after in vivo administration. Gentamicin and amikacin levels in tissues from injected rabbits were assayed both in the homogenates and in trichloroacetic acid-deproteinized supernatants. The data, derived by either method, indicated that kidney was the major site of antibiotic deposition and that the drug levels increased after multiple injections (Table 1). After a single injection, amikacin was found only in kidney and in urine; gentamicin concentrations in kidney and

TABLE 1. *Antibiotic levels in rabbit tissues after a single and repeated injections*

Tissue	Concn in tissues (μg/g of tissue)[a]							
	Single injection				Seven daily injections			
	Gentamicin		Amikacin		Gentamicin		Amikacin	
	Homogenate	Trichloroacetic acid supernatant	Homogenate	Trichloroacetic acid supernatant	Homogenate	Trichloroacetic acid supernatant	Homogenate	Trichloroacetic acid supernatant
Kidney	70 ± 11	110 ± 54	56 ± 41	50 ± 19	135 ± 65	214 ± 70	84 ± 36	169 ± 74
Liver	0	10 ± 5	0	0	18 ± 5.5	24.5 ± 14	1.7 ± 3	9 ± 3
Heart	0	3 ± 3	0	0	5.8 ± 0.3	22 ± 14	1 ± 2	4.3 ± 2
Lung	5 ± 1.4	6 ± 5	0	0	7.2 ± 1	23 ± 10	0.4 ± 0.7	3 ± 2
Brain	0	0	0	0	0	15 ± 10	0	0
Muscle	0	0	0	0	0	6 ± 4	0	2 ± 4
Serum	0.8 ± 0.9		0		0.5 ± 0.7		0.2 ± 0.3	
Urine	26 ± 14		8.0 ± 8.5		36 ± 7		10.3 ± 2	

[a] Numbers represent the mean antibiotic concentration ± standard deviation. In kidney experiments nine rabbits were used, except for gentamicin, single injection, in which there were eight rabbits. For all other tissues the data are presented for three rabbits.

urine were higher than those of amikacin, and in addition it was detected in lung, heart, and liver by the trichloroacetic acid procedure. After seven daily injections, the kidney levels of both antibiotics were not significantly different; however, again higher amounts of gentamicin were present in the other tissues.

The trichloroacetic acid supernatant procedure was more sensitive than the homogenate procedure in detecting small amounts of antibiotics in tissues other than kidney; in addition, it indicated significantly higher levels of both antibiotics in kidney after multiple injections (gentamicin, $P < 0.05$; amikacin, $P < 0.001$). The difference in the two methods with respect to the kidney was not observed after a single injection.

Effect of tissue homogenates on antimicrobial activity. Homogenates of the rabbit tissues inhibited the activity of amikacin and gentamicin as compared with the homogenization buffer alone (Table 2). This is consistent with the aminoglycoside-binding capacity of homogenates previously reported from this laboratory (7). With liver and kidney homogenates, the lowest percentage of remaining antimicrobial activity was observed. Considering the inhibitory effect in relation to the protein content, it was apparent that no one tissue was more effective than another in binding these drugs in vitro. Serum, on a unit protein basis, was less effective as a binder than the other tissues.

Urine as a diluent consistently augmented the activity of gentamicin and amikacin. Control urine samples did not produce any zones of inhibition, thus eliminating the possibility that endogenous antibacterial compounds were responsible for this enhancement. The careful pH adjustment of all samples before assay eliminated this factor as a variable.

Addition of trichloroacetic acid to the homogenates previously incubated with the antibiotics resulted in the precipitation of protein and in the recovery of the antimicrobials in the supernatant solution (Table 2). Full recovery was obtained with kidney and most other homogenates. Reduced recovery of amikacin was observed with heart and skeletal muscle tissue, which may be related to difficulties of homogenizing these fibrous tissues.

DISCUSSION

The present study indicates that kidney is the site of amikacin as well as gentamicin deposition in rabbits. This is similar to data previously reported for gentamicin in dogs, rats, and man (1, 3, 9) and more recently for amikacin in man (5).

The mode of deposition of aminoglycoside antibiotics is of particular interest because of their nephrotoxic potential. They are excreted mainly by glomerular filtration, but some reabsorption takes place in the proximal tubule, the major portion of which is located in the cortex (3). Thus, a certain cell population is exposed to high levels of the antibiotics. As this process continues, more subcellular organelles and more binding sites may be affected, finally leading to alteration of morphology and function. The identification of the subcellular organelles and the specific macromolecules responsible for binding of the aminoglycosides would be helpful in understanding their mode of accumulation and their potential toxicity.

Amikacin at equal doses tended to accumu-

TABLE 2. *Effect of rabbit tissue homogenates on antimicrobial activity of gentamicin and amikacin*

Diluent	Remaining antimicrobial activity (%)[a]				Protein in 25% homogenates (mg/ml)
	Homogenates		Trichloroacetic acid supernatants		
	Gentamicin	Amikacin	Gentamicin	Amikacin	
Buffer	100	100	100	100	0
Kidney	59.0 ± 19.1	66.5 ± 13.9	100.9 ± 25.4	100.6 ± 16.8	42
Liver	41.0 ± 4.7	45.0 ± 17.3	95.5 ± 5.2	87.2 ± 19.0	48
Heart	82.0 ± 7.9	68.3 ± 0.6	87.0 ± 9.6	72.7 ± 17.0	21
Lung	75.0 ± 1.0	68.7 ± 31.5	101.0 ± 9.0	91.0 ± 15.7	28
Brain	68.0 ± 10.7	72.5 ± 20.7	94.8 ± 18.6	86.5 ± 31.7	31
Muscle	77.7 ± 4.5	68.7 ± 22.5	94.7 ± 6.4	69.3 ± 20.5	25
Serum	77.0 ± 20.5	74.6 ± 20.1	109.2 ± 23.4	85.6 ± 11.8	57
Urine	288.0 ± 102	138.4 ± 40.3			

[a] Results are expressed as percentage of antimicrobial activity in each diluent ± standard deviation; for kidney, $n = 8$; serum, $n = 5$; liver, brain, and urine, $n = 4$; heart, lung, brain, and muscle, $n = 3$. Antimicrobial activity in buffer, 0.25 M sucrose–0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, or in trichloroacetic acid-treated, neutralized buffer was set at 100% in each experiment.

late less than gentamicin in kidney after a single injection and in other tissues both after single and multiple injections. It is difficult, however, to extrapolate these findings to toxicity in man because of species differences, lack of direct information that tissue binding is related to toxicity, and the fact that higher doses of amikacin are recommended for treatment of infection.

This report also compares the utility of two assay procedures. Antibiotic deposition in tissues is most frequently determined with the aid of standard curves in the corresponding diluent, i.e., tissue homogenates. The assumption underlying this method is that even if part of the drug is tissue bound, this effect will be corrected by the standard curve, and a quantitative assessment of the total antibiotic will be obtained. In the present study we have used this method for the determination of gentamicin and amikacin levels in tissues, but in addition we have used trichloroacetic acid to obtain protein-free supernatants. Since most of drug-protein interactions are noncovalent in character and reversible, denaturation and precipitation of protein lead to release of the bound drug into the supernatant. This method is particularly applicable to aminoglycosides because of their stability.

Theoretically, both methods should give identical results, assuming that the addition of the drug in vitro to the homogenates (used for standard curves) is equivalent to drug-tissue interactions in vivo (experimental homogenates). In the present study, similar drug levels in the kidney were found by either method after a single injection. After multiple injections, the trichloroacetic acid method gave significantly higher results, suggesting the possibility that, after prolonged administration, both gentamicin and amikacin become sequestered in a manner such that the in vitro standard curves did not correct for the in vivo binding.

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