Altered Tissue Distribution and Elimination of Amikacin Encapsulated in Unilamellar, Low-Clearance Liposomes (MiKasome®)

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Purpose. Amikacin in small unilamellar liposomes (MiKasome®) has prolonged plasma residence (half-life > 24hr) and sustained efficacy in Gram-negative infection models. Since low-clearance liposomes may be subject to a lower rate of phagocytic uptake, we hypothesized this formulation may enhance amikacin distribution to tissues outside the mononuclear phagocyte system.

Methods. Rats received one intravenous dose (50 mg/kg) of conventional or liposomal amikacin. Amikacin was measured for ten days in plasma, twelve tissues, urine and bile.

Results. Liposomal amikacin increased and prolonged drug exposure in all tissues. Tissue half-lives (63–465 hr) exceeded the plasma half-life (24.5 hr). Peak levels occurred within 4 hours in some tissues, but were delayed 1–3 days in spleen, liver, lungs and duodenum, demonstrating the importance of characterizing the entire tissue concentration vs. time profile for liposomal drugs. Predicted steady-state tissue concentrations for twice weekly dosing were >100 μg/g. Less than half the liposomal amikacin was recovered in tissues and excreta, suggesting metabolism occurred. Amikacin was not detected in plasma ultrafiltrates. Tissue-plasma partition coefficients (0.2–0.8 in most tissues) estimated from tissue-plasma ratios at T_{max} were similar to those estimated from tissue AUCs.

Conclusions. Low-clearance liposomal amikacin increased and prolonged drug residence in all tissues compared to conventional amikacin. The long tissue half-lives suggest liposomal amikacin is sequestered within tissues, and that an extended dosing interval is appropriate for chronic or prophylactic therapy with this formulation.

KEY WORDS: liposomes; aminoglycosides; amikacin; MiKasome; tissue distribution; elimination/excretion.

INTRODUCTION

Amikacin, an aminoglycoside antibiotic with a broad spectrum of Gram-negative bactericidal activity, is not widely used for first-line therapy due to its narrow therapeutic index and the requirements for frequent intravenous dosing and plasma level monitoring. Encapsulation of amikacin in small unilamellar liposomes resulted in a markedly altered pharmacokinetic profile, which differed from both conventional amikacin and other liposomal aminoglycosides (1). These liposomes, composed of high transition temperature phospholipids and cholesterol, remain in the gel state under physiological conditions, resulting in a lower clearance than conventional liposomes, and a plasma half-life many-fold longer than conventional amikacin. Previous efforts to develop liposomal aminoglycosides largely focused on the treatment of intracellular infections, such as *Mycobacterium avium* (2). Studies with low-clearance liposomal amikacin show it is effective against extracellular infections caused by Gram-negative aerobes, including *Klebsiella* pneumonia and *Pseudomonas* endocarditis, at doses less than or equal to therapeutic doses of conventional amikacin (1). Low-clearance liposomal amikacin (70 mg/kg) also had prophylactic efficacy when administered 1, 2, and 3 days prior to a lethal *Klebsiella pneumoniae* challenge in immunosupressed mice. Under these conditions, neither conventional amikacin (70 mg/kg) or ampicillin/sulbactam (1050 mg/kg) treatment was effective (3).

The long plasma half-life and prophylactic activity of low-clearance liposomal amikacin suggest it could be administered with a prolonged dosing interval, perhaps once weekly. Although the mechanism of bactericidal activity of liposomal aminoglycosides is not well understood, conventional amikacin rapidly penetrates the extracellular fluid space, and exhibits concentration-dependent killing of susceptible organisms. Thus, the hypothesis that low-clearance amikacin liposomes can provide prolonged efficacy is based on the expectation of prolonged antibiotic exposures in tissues and sites of infection, as well as prolonged plasma residence times.

In conventional liposomes, aminoglycosides are rapidly cleared, and appear to accumulate in tissues associated with the mononuclear phagocyte system (MPS), principally the liver and spleen (4,5). Understanding the potential of low-clearance liposomal amikacin to provide prolonged and effective antibiotic exposures to tissues and sites of infection outside the MPS may be critical to understanding its potential spectrum of activity, and in developing appropriate dosing schedules. In this study, amikacin was measured in plasma, urine, bile and twelve tissues for up to ten days following single intravenous injections of low-clearance liposomal amikacin and conventional amikacin in rats. These data were used to compare the extent and timecourse of antibiotic distribution in each tissue, to predict antibiotic levels that might be achieved in these tissues after repeated dosing with liposomal amikacin, and to compare the recovery and elimination of amikacin between the two formulations.

MATERIALS AND METHODS

Test Articles and Reagents

Liposomal amikacin (MiKasome®, NeXstar Pharmaceuticals, Inc., San Dimas, CA) was supplied as a sterile intravenous formulation of amikacin (10 mg/ml) in small unilamellar liposomes. Hydrogenated soy phosphatidylcholine, cholesterol and distearoylphosphatidylglycerol (2:1:0.1 mole ratio, respectively) were dissolved in chloroform and spray dried. Liposomes were formed by rehydration of the dried lipids with amikacin HCl in 9% sucrose/succinate (pH 6.5) buffer. The liposomes were then homogenized to form small unilamellar liposomes with a median diameter < 100 nm by laser light scattering, then dialyzed against formulation buffer to remove unencapsulated drug. More than 85% of the amikacin was encapsulated within the liposomes, while the remainder of the amikacin was exter-

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nally associated with the liposomes. The lipid-to-drug ratio was approximately 5:1 by weight. Conventional amikacin (Chem Werth, Woodbridge, CT) was formulated as amikacin HCl in the same vehicle as liposomal amikacin. Taurocholic acid, sodium salt, trichloroacetic acid and Triton X-100 were obtained from Sigma (St. Louis, MO).

Animals

For the plasma, tissue and urine studies, male Sprague-Dawley rats (250–300 g body weight) were obtained from Harlan Bioproducts (Indianapolis, IN). For bile recovery studies, closed-loop bile duct-cannulated male Sprague-Dawley rats (275–300 g body weight) were purchased from Hilltop Laboratories (Scottsdale, PA). Animal care and use adhered to the principles in "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996). Animals had *ad libitum* access to food and water throughout the study and were not fasted prior to dosing.

Treatment with Conventional and Liposomal Amikacin

Each animal received a single intravenous injection of liposomal amikacin (50 mg/kg) or conventional amikacin (50 mg/kg) via the caudal vein while under isoflurane anesthesia. Blood samples (approx. 0.5 ml) were obtained by venipuncture (caudal vein) from each animal at 4 to 6 time points during the study, using EDTA as an anticoagulant. Groups of 4 to 6 animals were sacrificed by exsanguination under isoflurane anesthesia at 4, 24, 48, 72, 120, 168 and 240 hours after dosing (the last three timepoints were omitted for conventional amikacin). During the exsanguination procedure, an additional blood sample (4–5 ml) was obtained from the posterior aorta into an evacuated EDTA-containing blood collection tube. Plasma was harvested by centrifugation of blood samples. A portion (0.5 ml) of the plasma obtained from the aortic blood sample was placed into a centrifugal ultrafiltration device (Microcon-100, Amicon, Inc. Beverly, MA) and centrifuged (15 min @ 3000 rcf) to obtain 100–200 μl of plasma ultrafiltrate. All plasma and plasma ultrafiltrate samples were frozen (−80°C) prior to analysis. After the animal was exsanguinated, the following organs and tissues were excised, blotted to remove any remaining blood, weighed and frozen (−80°C): liver, kidneys, heart, lungs, spleen, brain, duodenum, testes, mesenteric lymph nodes, skeletal muscle (portion of the m. gracilis), skin (approx. 1 cm^2 from the abdominal midline) and bone marrow (red marrow from femur). For urine collection, rats were placed into individual metabolic cages (Nalge Co., Rochester, NY) immediately after dosing. Urine was collected into tubes placed on dry ice. Urine was collected at 4 and 24 hours (conventional amikacin, n = 4) or over 24 hour intervals for 10 days (liposomal amikacin, n = 4). At the end of each urine collection period, the collection tube contents were thawed, mixed, the total volume recorded, and a portion (4–5 ml) frozen (−80°C) for analysis. For bile collection, the closed-loop bile duct cannulae were cut just prior to dosing. The distal end was infused with sterile-filtered bile replacement salts (taurocholic acid, 27.8 g/l in sterile 0.9% saline solution) at a rate of 0.9 ml/hr. Bile secreted by the animal was collected from the proximal end into a tube placed in wet ice. Bile was collected over 24 hour intervals for up to 9 days (liposomal amikacin, n = 6) or 2 days (conventional amikacin, n = 7). At the end of each collection period, the collection tube contents were thawed, mixed, the total volume recorded, and a portion (4–5 ml) frozen (−80°C) for analysis.

Measurement of Total Amikacin in Biological Samples

Total amikacin was measured in all plasma, plasma ultrafiltrate, tissue, bile and urine samples using a commercial fluorescence polarization immunoassay (TDx/FLx, Abbott Diagnostics, Abbott Park, IL). Tissues were thawed, minced and then homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in 10% trichloroacetic acid (10 ml/g tissue, 5 ml/g for lung and testes). Samples of bone marrow and lymph nodes were similarly homogenized using a motorized pestle (VWR Scientific, West Chester, PA) in a 1.8 ml microcentrifuge tube. Tissue homogenates were centrifuged (60 min @ 9000 rcf) at 4°C, and portions of the supernatants were frozen (−80°C) prior to amikacin analysis. Bile samples were diluted 1:5 in phosphate buffer (50 mM, pH 7.0) prior to analysis. The assay was performed using reagents supplied by the manufacturer, except that Triton X-100 was added to the assay dilution buffer (final concentration: 0.05%) to release liposome-associated amikacin. The assay lower limits of quantitation were 0.3 μg/ml for plasma and plasma ultrafiltrates, 0.6 μg/ml for urine, 1.5 μg/ml for bile, 3.3 μg/g for testes and lungs, and 6.6 μg/g for other tissues. Unknown samples were serially diluted (1:10) in phosphate buffer until they fell within the validated range of the assay. Standardization solutions were prepared from USP amikacin. Liposomal amikacin quality control standards (2000, 555 and 0.925 μg/ml) were prepared in each matrix, and run in triplicate with each assay. Runs were accepted if the means of quality control samples at each concentration fell within ±20% of the expected values.

Pharmacokinetic Analysis

For timed bile and urine collections, total amikacin recovery was calculated by multiplying the sample concentration by its volume. Tissue recoveries were calculated by multiplying tissue concentration by the measured total tissue weight (liver, kidneys, spleen, lungs, heart, testes, brain) or by the total tissue weight estimated from the animals' body weight (muscle, skin and bone marrow) (6). Group mean tissue concentration vs. time curves were fit using a multiexponential weighted least-squares fitting program (RSTRIP, Micromath, Salt Lake City, UT) to estimate terminal half-lives of amikacin. Group mean plasma concentration vs. time curves were similarly fit, and the resulting exponential slopes and intercepts used to calculate standard pharmacokinetic parameters (7). For tissues, the area under the concentration vs. time curve (AUC) to the last time point was determined by the linear trapezoidal method. The tissue-plasma partition coefficient (K_p) was estimated as the ratio of tissue AUC to the last timepoint to plasma AUC over the same time period (8). For tissues that exhibited a clear maximum concentration at times greater than 4 hours, K_p was also estimated as the ratio of tissue concentration to plasma concentration at the time at which the maximum tissue concentration was observed. Plasma steady state concentrations (C_{ss}) were calculated as the dosing rate times the plasma clearance.

For tissues, C_{ss} was estimated as tissue K_p times plasma C_{ss}. Urinary clearance (Cl_u) was calculated as the amount of unchanged drug recovered in the urine divided by the plasma AUC during the urine collection period.

RESULTS

Plasma Pharmacokinetics

The plasma concentration profiles of low-clearance liposomal amikacin and conventional amikacin (Table I, Figs. 1 and 2) differed substantially, as previously reported (9). Conventional amikacin was rapidly cleared (plasma clearance approx. 90% of glomerular filtration rate in the rat), and distributed into a volume close to that of the extracellular fluid compartment (10). In contrast, liposomal amikacin had a lower clearance and lower volumes of distribution, resulting in higher and greatly prolonged plasma levels of total amikacin. In physiologic terms, the clearance of liposomal amikacin represented only a small fraction of the plasma flow to any of the major tissues, and its volume of distribution was close to that of the plasma compartment (10). The disposition of both formulations was adequately described using bi-exponential equations, but for conventional amikacin a majority of the plasma AUC was under the initial rapid phase, while for liposomal amikacin the majority of the plasma AUC was under the slow terminal phase. As a result, the total plasma amikacin AUC and mean residence time were increased by 130-fold and 61-fold, respectively, for the liposomal formulation. Plasma levels fell to below 1 μg/ml within 5 hours after administration of conventional amikacin, but remained above 100 μg/ml for 2–3 days after administration of liposomal amikacin.

Amikacin levels measured in plasma ultrafiltrates were below detection (<0.3 μg/ml) at all timepoints after the administration of liposomal amikacin. It was previously shown that free amikacin added to rat plasma which contained liposomal amikacin was quantitatively recovered in the ultrafiltrate under these conditions (data not shown).

Table I. Plasma and Urine Pharmacokinetic Parameters of Conventional and Liposomal Amikacin After Intravenous Injection (50 mg/kg) in Rats

Parameter	Liposomal amikacin	Conventional amikacin
AUC (μg*hr/ml)	23360	178
CL (ml/min/kg)	0.036	4.7
C(0) (μg/ml)	1301	555
$t_{1/2}$ (α) (hr)	0.28	0.21
$t_{1/2}$ (β) (hr)	24.5	3.0
MRT (hr)	34.9	0.57
V_c (ml/kg)	38.4	90.0
V_{ss} (ml/kg)	74.8	161
AUC_α (%)	1.1	93.2
AUC_β (%)	98.9	6.8
CL_u (ml/min/kg)	0.0098	5.38

Note: AUC, Area under plasma concentration vs. time curve from zero to infinity; CL, plasma clearance; C(0), plasma concentration at time zero; α, β, refer to the initial and terminal phases, respectively; MRT, mean residence time; V_c, volume of the central compartment; V_{ss}, steady-state volume of distribution; AUC_α, AUC_β, partial AUCs of the respective phases; CL_u, urinary clearance.

Distribution in Tissue

The tissue concentration profiles of low-clearance liposomal amikacin and conventional amikacin also differed substantially (Table II, Figs. 1 and 2). After administration of conventional amikacin, tissue levels of amikacin were below quantitation (<6.6 μg/g) at all timepoints in all tissues except the kidneys. Kidney levels of amikacin peaked rapidly, then fell with a half-life longer than the plasma half-life, so that kidney amikacin levels exceeded plasma levels at most timepoints. Kidney amikacin concentrations remained above 50 μg/g for at least 72 hours after conventional amikacin administration.

In contrast, amikacin was detectable in all twelve tissues sampled after the administration of liposomal amikacin. Considerable variation in the concentration vs. time profiles was observed between tissues and individuals (Fig. 1). Maximum tissue concentrations were highest (180–700 μg/g) in spleen, kidney and bone marrow, intermediate (50–100 μg/g) in lymph node, liver, lungs, duodenum, skin and heart, and lowest (<15 μg/g) in muscle, brain and testes. Some tissues (kidney, heart, muscle, brain, skin and testes) reached peak levels at or before the first time point (4 hr), while in other tissues (spleen, liver, lungs and duodenum) maximum tissue concentrations were reached 1–3 days after dosing. Concentrations in the lymph nodes and bone marrow appeared to remain constant or increase over the entire course of the study. Mean tissue levels fell below the limit of quantitation after 24 hr and 120 hr in the brain and muscle, respectively. In the remaining tissues, amikacin levels were still measurable (range: 5 to 318 μg/g) ten days after liposomal amikacin administration. In all tissues where it could be estimated, the tissue elimination half-life was longer than the plasma elimination half-life. Tissue AUC exceeded plasma AUC (K_p was >1) only for the spleen and kidneys. For most other tissues, estimated K_p values ranged between 0.2 and 0.8. In the tissues where K_p was also estimated from tissue C_{max}, these values were similar to those calculated from tissue-plasma AUC ratios. In the kidneys, tissue C_{max} and T_{max} were similar for the two formulations, but the tissue half-life and AUC were greater for liposomal amikacin than for conventional amikacin.

Excretion and Mass Balance

Amikacin recoveries in plasma, tissues, urine and bile are shown in Table III. As previously reported for other aminoglycosides (4), conventional amikacin was rapidly excreted unchanged in the urine during the first 24 hr after dosing, with a small percentage of the dose being sequestered in the kidneys (11), and minimal biliary excretion (amikacin was not detected in bile at most timepoints). In contrast, a large fraction of the liposomal amikacin remained in the plasma, from which it was slowly cleared. Urinary recovery of amikacin was slower and less extensive, with about one third of the administered dose excreted unchanged over the 10 day study period. During the first 2 days, a small amount of amikacin was also excreted in the bile. Tissues contained over 40% of the dose after 4 hours,

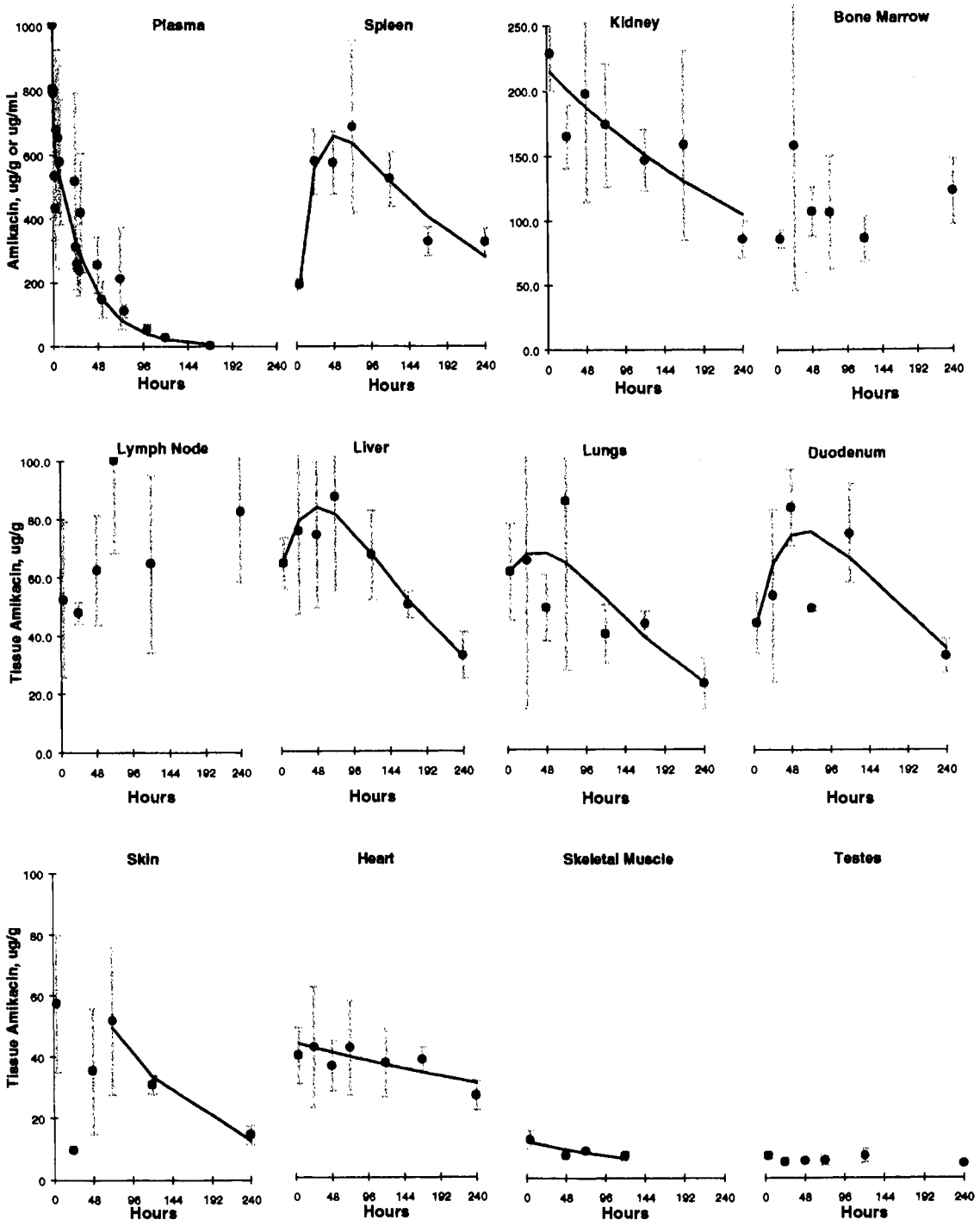

Fig. 1. Plasma and tissue amikacin concentrations (mean ± S.D., n = 4–6) after a single intravenous injection of liposomal amikacin (50 mg/kg) in rats. Lines show curve fits used to estimate terminal half-lives. Amikacin concentrations in brain were 12.1 ± 0.9 μg/g (mean, n = 4) at 4 hr, 8.5 μg/g (median, n = 3) at 24 hrs, and <6.6 μg/g at all subsequent timepoints.

and more than 10% of the dose after ten days. The total recovery of amikacin in plasma, tissues and excreta fell over the course of the study, so that only about half of the administered amikacin was accounted for ten days after dosing with liposomal amikacin. The urinary clearance of liposomal amikacin was less than 30% of its total clearance, and less than 0.2% of the urinary clearance of conventional amikacin.

DISCUSSION

These results support the hypothesis that the prolonged plasma residence time of low-clearance liposomal amikacin is associated with increased distribution to tissues. After administration of a single dose (50 mg/kg) to rats, liposomal amikacin increased both the magnitude and duration of amikacin distribu-

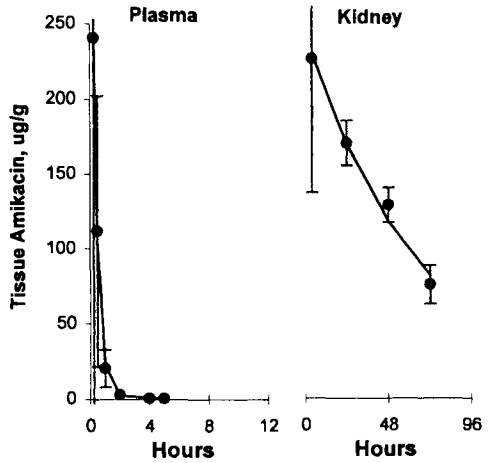

Fig. 2. Plasma and tissue amikacin concentrations (mean ± S.D., n = 4–6) after a single intravenous injection of conventional amikacin (50 mg/kg) in rats. Lines show curve fits used to estimate terminal half-lives.

tion to all tissues studied. Administration of conventional amikacin provided only brief (<4 hr) drug exposures in plasma and all tissues except the kidneys. In contrast, low-clearance liposomal amikacin produced much higher exposures in most tissues over the entire 10 day study. Liposomal amikacin, which disappeared from plasma with a half-life of 24.5 hr, remained in tissues even longer (half-lives ranged from 63–465 hr). These data indicate that once weekly administration could maintain relatively constant drug levels in most tissues, and that a single dose of low-clearance liposomal amikacin could provide sustained levels of antibiotic for several days.

The plasma clearance of this liposomal amikacin formulation was lower than that of conventional amikacin (9) and other liposomal aminoglycosides (4,12) and represented approximately 0.1% and 2.5% of plasma flow to the liver or spleen, respectively (10). This is in contrast to other liposomes, which have hepatic extraction ratios of up to 60% in rodents (13,14). Free (ultrafilterable) amikacin was undetectable (<0.3 μg/ml) in the plasma at any time point after liposomal amikacin administration. This is also in contrast to another liposomal aminoglycoside for which 33% of plasma drug was ultrafilterable (15).

Although liposomal amikacin displayed bi-exponential plasma disposition, the physiologic significance of the initial rapid phase is not clear. While small unilamellar liposomes may freely diffuse into some portion of the extravascular space (16), it is uncertain to what extent the accumulation of liposomes at extravascular and intracellular sites is reversible (17), and whether these processes are governed by the law of mass action. The plasma pharmacokinetic profile we observed for liposomal amikacin is consistent with the hypothesis that amikacin is largely sequestered within circulating liposomes, which are slowly cleared into sequestered sites in many tissues. After tissue uptake, amikacin was cleared at an even slower rate by mechanisms that may include metabolism, or the release of free or liposomal amikacin from extravascular or intracellular sites. The low apparent volume of distribution of liposomal amikacin, the failure to detect amikacin in plasma ultrafiltrates and the fact that tissue half-lives exceed the plasma half-life argue that tissue and plasma amikacin are not in rapid equilibrium, and that amikacin is sequestered within one or

Table II. Tissue Pharmacokinetic Parameters of Conventional and Liposomal Amikacin After Intravenous Injection (50 mg/kg) in Rats

Treatment	Tissue	C_{max} (μg/g)[a]	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{0-240hr}$ (μg*hr/g)	K_p[b]	K_p[c]	Css (μg/g)
Liposomal amikacin	Plasma	1003 ± 599	0.25	24.5	30444	[d]	[d]	278
	Spleen	670 ± 260	72	134	107428	3.53	3.13	981
	Kidney	229 ± 29	4	228	37587	1.23	[e]	343
	Bone Marrow	181 ± 100	24	[e]	24648	0.81	[e]	225
	Lymph Node	100 ± 32	72	[e]	17187	0.56	[e]	157
	Liver	86.5 ± 33	72	95	14837	0.49	0.40	135
	Lungs	85.9 ± 59	72	62.9	11940	0.39	0.40	109
	Duodenum	82.6 ± 13	48	110	13696	0.45	0.32	125
	Skin	57.3 ± 23	4	85.1	7085	0.23	[e]	64.7
	Heart	47 ± 18	24	465	9012	0.30	[e]	82.3
	Muscle	12.6 ± 3.0	4	136	[e]	[e]	[e]	[e]
	Brain	12 ± 7.6	4	[e]	[e]	[e]	[e]	[e]
	Testes	7.3 ± 0.9	4	[e]	1453	0.05	[e]	13.3
Conventional amikacin	Plasma	241 ± 359	0.25	0.21	178	[d]	[d]	1.1
	Kidney	220 ± 87	4	45.3	10608	59.6	[e]	63.2

Note: C_{max}, maximum observed concentration in tissue; T_{max}, time at which C_{max} occurred; $t_{1/2}$, elimination half-life; $AUC_{0-240hr}$, Area under concentration vs. time curve from zero to 240 hours; K_p, tissue-plasma partition coefficient; C_{ss}, steady-state concentration assuming twice weekly dosing at 50 mg/kg.

[a] Mean ± S.D. (n = 4–6).

[b] Calculated from tissue-plasma $AUC_{0-240hr}$ ratio.

[c] Calculated from tissue/plasma concentration ratio at T_{max}.

[d] Calculation not applicable.

[e] Could not be determined from the data.

Table III. Total Amikacin Recovery (% Dose) in Plasma, Tissues and Excreta After Intravenous Injection (50 mg/kg) of Conventional and Liposomal Amikacin in Rats

Treatment	Time (hr)	Plasma	Liver	Kidneys	Muscle	Skin	All Tissues[a]	Urine[b]	Bile[b]	Total
Liposomal amikacin	4	72.6	5.2	3.5	11.4	20.6	43.2	[c]	[c]	115.8
	24	49.2	5.1	2.3	[d]	3.5	16.1	9.5	0.6	75.3
	48	20.3	5.9	3.1	8.6	12.7	34.3	12.1	1.0	67.7
	72	17.1	5.9	2.0	8.1	18.7	39.9	15.4	1.0	73.3
	120	2.1	5.8	2.3	7.2	11.1	30.3	21.7	1.0	55.1
	168	0.1	4.0	2.6	[d]	[c]	8.9	31.2	1.0	41.2
	240	[d]	2.6	1.0	[d]	5.3	11.6	35.9	1.0	48.5
Conventional amikacin	4	2.0	[d]	3.1	[d]	[d]	3.1	62.6	[d]	67.7
	24	0.7	[d]	2.3	[d]	[d]	2.3	115.0	0.1	118.4

[a] Liver, kidneys, muscle, skin, heart, lungs, spleen, brain, bone marrow and testes.
[b] Cumulative recovery to the indicated time point.
[c] Not collected at this time point.
[d] Below limit of quantitation (6.6 μg/g).

more extravascular compartments after liposomal amikacin administration.

The timecourse of amikacin disposition appeared to differ among the tissues studied. In the kidneys, heart, skeletal muscle, brain, skin and testes, maximum tissue concentrations were observed soon (≤4 hrs) after administration, and then fell slowly. In the spleen, liver, lungs and duodenum, maximum tissue concentrations were observed 1–3 days after dosing, by which time the plasma level had fallen to 10–20% of its initial value. In the lymph nodes and bone marrow, maximum tissue levels did not appear to have been reached by the end of the ten day study. Thus, tissue elimination half-lives could not be estimated, and calculated K_p values probably underestimate the true values for these tissues. The ability of this formulation to be slowly taken up into many different tissues, rather than rapidly cleared by MPS-associated organs, may prove a significant clinical benefit for treatment of extracellular Gram-negative infections. Prolonged antibiotic residence in both the plasma and tissue compartments after a single dose of low-clearance liposomal amikacin is in direct contrast to conventional amikacin, which is rapidly cleared from both compartments.

These data also demonstrate the importance of characterizing the entire tissue concentration vs. time profile when comparing the performance of drug delivery systems. Reports of improved tissue or tumor targeting with drug delivery systems based on the ratio of concentrations measured at a single time-point should be viewed with critical skepticism.

Tissue-plasma partition coefficients (K_p) were used to estimate steady-state concentrations (C_{ss}) in tissues. For twice weekly administration of liposomal amikacin (50 mg/kg), a plasma C_{ss} of nearly 300 μg/ml and tissue $C_{ss} > 100$ μg/g in most tissues were predicted (Table II). These levels are much higher than those achieved by conventional amikacin and may help explain the observed efficacy of liposomal amikacin, at doses less than those required for conventional amikacin, against *Klebsiella* and *Pseudomonas* in vivo (1,3).

Tissue-plasma partition coefficients were also estimated by an alternative method for those tissues which exhibited a clear maximum concentration (C_{max}) at some time (T_{max}) after dosing. This method is based on the observation that at T_{max}, the rate of change of tissue concentration with time is zero. At this instant, there is no net drug transport between the tissue and the vascular compartment, if the tissue is non-eliminating. This is a reasonable assumption given the low clearance of liposomal amikacin. Thus, at T_{max}, K_p can be estimated as C_{tissue}/C_{plasma}, where C_{plasma}, the mixed venous plasma concentration, is used to estimate the efferent venous concentration in the tissue. This approximation is also reasonable given the low clearance of liposomal amikacin. Values of K_p calculated by the T_{max} method were found to be in close agreement with those calculated by the AUC ratio method (Table II).

Because of the high amikacin concentrations in plasma after liposomal amikacin, some of the drug measured in tissues may represent amikacin in plasma contained within the tissue. To minimize the blood content of tissues, animals were exsanguinated prior to harvesting tissues. The fact that tissue levels did not decrease in concert with plasma levels, and in many cases increased while plasma levels were decreasing, suggests that the contribution of plasma drug to the measured concentrations in tissue was minimal. Also, because a detergent was used to disrupt liposomes during the assay, measured amikacin levels reflect total amikacin concentrations in tissue. The analytical methodology used did not differentiate between free and encapsulated, or between intracellular and extracellular amikacin, although these pools my differ in their antimicrobial activity.

Liposomal amikacin produced higher and more prolonged drug levels in the kidneys, a target organ for amikacin toxicity, than did conventional amikacin. The implications of this observation are not yet clear, but previous studies suggest liposomal formulations can increase tissue levels substantially without toxic effects by altering drug distribution within tissues (18,19). The sequestration of amikacin in circulating liposomes and the low urinary clearance we observed for liposomal amikacin suggest this formulation may reduce the exposure of the renal tubular epithelium to amikacin. In a one-month, repeated-dose safety study, the ratio of renal cortical to renal medullary amikacin was significantly lower in dogs treated with liposomal amikacin than in those treated with conventional amikacin (1). Although amikacin concentrations in the kidney exceeded 1000

μg/g after liposomal amikacin treatment, no evidence of altered renal function was observed. These data support the hypothesis that the amikacin present in the kidneys after liposomal amikacin is not localized in the tubular epithelium as it is with conventional amikacin (11).

As a result of the longer plasma and tissue residence times observed for liposomal amikacin, the rate of urinary amikacin excretion was reduced. The extent of biliary excretion was not increased, so that by the end of the 10 day study, less than 40% of the administered dose had been excreted unchanged. This mass balance deficit could be explained by drug metabolism, or distribution to unmeasured tissues. Although conventional aminoglycosides are not metabolized after administration, there is evidence that gentamicin is metabolized in vitro by a hepatocyte fraction (20). Thus, if some fraction of liposomal amikacin enters cells as our data suggest, it may undergo metabolism. Hepatic metabolism could explain why hepatic amikacin concentrations were lower than those in other MPS tissues (spleen and bone marrow) in this study.

CONCLUSIONS

A low-clearance liposomal formulation of amikacin was shown to provide prolonged drug residence in tissues as well as plasma, while reducing urinary clearance and excretion. Tissue uptake and elimination rates were slow, suggesting that amikacin was sequestered within the tissue compartment. These data demonstrate that tissue amikacin levels could be maintained with infrequent dosing, and that amikacin may remain in tissues for many days after treatment with low-clearance liposomal amikacin. These unique properties may result in clinical benefits, including reduced dosing frequency and prolonged tissue antibiotic residence for chronic or prophylactic therapy of extracellular Gram-negative infections.

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