Pharmacokinetics of Liposomal Gentamicin

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We present data on pharmacokinetics of free and liposomal forms of gentamicin. Comparative study of two drug forms showed that immobilization of gentamicin in liposomes significantly increased most important pharmacokinetic parameters of the antibiotic (AUC, Cmax, MRTpo, $T^{1}/_{2}$, Kel, Vzpo) and reduced Clpo and Kel.

Key Words: *antibiotics; liposomes; pharmacokinetics*

Effectiveness of antibacterial drugs is determined by their stability, selective effect on target cells, ability to pass through cell membranes, and the lack of side effects. Difficulties in the therapy of various bacterial infections, especially with intracellular localization of the pathogens, necessitates the search for drugs penetrating into cells and affecting intracellular microorganisms without loss of specific activity [1,4]. Development of liposome techniques made possible directed transport of drugs including gentamicin [2,5,10,11]. However, pharmacodynamics and pharmacokinetics of liposomal drugs are not well studies [6,8].

Here we compared the pharmacological properties of free and liposomal forms of gentamicin.

MATERIALS AND METHODS

Liposomes were prepared by reverse-phase evaporation method [9] using chromatographically pure lecithin and cholesterol (Serva) in a weight ratio of 7:3. Gentamicin sulfate (Sintez, Kurgan) was used for incorporation into liposomes. Extrusion of liposomal gentamicin was performed through polycarbonate membranes with a pore size of 100 nm. The average liposome size and its uniformity were assessed by electron microscopy at an instrumental magnification of ×120,000. The amount of the antimicrobial drug in liposomes was estimated microbiologically [3]. The degree of lipid peroxidation in liposomal membrane (Klein test) was determined spectrophotometrically [7].

The study was conducted on 156 laboratory white mice weighing 18-20 g. The animals were divided into 4 groups (42 mice to study the distribution of liposomal antibiotics and 36 mice, of free antibiotics). ^{125}I-gentamicin was used as a marker. The drugs were administered intraperitoneally in a dose of 200 μl (120 μg) with gentamicin activities of 0.01 mBq. Six mice from each group were sacrificed under halothane anesthesia 1, 2, 4, 6, 8, 24, 48 h after introduction of free and liposomal antibiotic. Blood, lungs, liver, spleen, kidneys were taken for examinations. Radiometric measurements of the samples were performed using LKB RIA Gamma Counter. The content of radiolabel was determined in 1 ml whole blood and 1 g tissue as a percentage of the administered dose.

The data were processed statistically using M-IND software developed at the Laboratory for Pharmacokinetic Studies of State Research Center for Preventive Medicine.

RESULTS

Liposomes contained 71.0±2.1% gentamicin; their size ranged up to 100 nm. Klein oxidation index was 1.0 arb. unit, which was a normal value for non-oxidized lipids.

After a single intraperitoneal injection of liposomal and free gentamicin in a dose of 0.12 mg, a linear relationship was also observed between the blood levels of tested drugs and time of sampling. The dynamics of mean concentrations of gentamicin sulfate in

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the blood of healthy animals gives an indication of fairly rapid entry and distribution of the drug in the bloodstream, as well as its rapid elimination from the circulating blood. After reaching a maximum concentration within 2 h (1.67 μg/ml) and a certain decrease in the next 4 hours, the drug concentrations decreased by 2-5 times by the 6th and 8th hours, respectively. In 24 h after administration, free gentamicin was not determined in the blood. Incorporation of gentamicin sulfate into phospholipid vesicles made it possible to increase the level of mean concentrations and circulation in the bloodstream by not less than 48 h. Recorded levels of liposomal antibiotic (3.71-15.64 μg/ml) exceeded those of the free drug by 8-10 times for the same time points (Fig. 1).

Comparison revealed differences in the main pharmacokinetics parameters of liposomal and free gentamicin (Table 1).

Thus, maximum concentrations (Tmax) of examined preparations were determined in the blood 2 h after administration, which apparently reflects the same rate of absorption from the peritoneal cavity and the possible timing of the effect of the studied formulations. At the same time, all other calculated parameters differed. Area under the curve (AUC) for liposomal gentamicin proved to be 38 times higher ($p<0.05$) than that for free antibiotic, which probably indicates unequal absorption of the test drugs into circulation. The differences in kinetic volume of distribution (Vzpo) between liposomal antibiotic (0.0084 liter) and free gentamicin sulfate (0.053 liter) suggest that antibiotic immobilized in liposomes had better penetrated into tissues. It was found that liposomal antibiotic had longer elimination half-life ($T^1/_2$=22.64) and lower elimination rate constant (Kel=0.0306), which may indicate slow excretion of liposomal drug from the blood compared with free drug. Mean retention time of liposomal gentamicin after extravascular administration (MRTpo=31.92) increased by 5.5 times ($p<0.05$). In addition, maximum concentrations of gentamicin in liposomes (Cmax=15.64 μr/ml) were 9 times higher ($p<0.05$) than those of the free antibiotic (Cmax=1.67 μg/ml).

Thus, our study revealed significant differences in pharmacokinetic parameters of liposomal gentamicin which manifest itself in an increase in most pharmacokinetic parameters (AUC, Cmax, MRTpo, $T^1/_2$, Kel, Vzpo). Clpo of liposomal gentamicin was reduced by 32 times ($p<0.05$) and Kel by 6 times in comparison with gentamicin sulfate. These findings attest to its prolonged circulation in the blood and slow excretion from the body, which agrees well with obtained values of AUC and $T^1/_2$.

The distribution of antibiotics in the organs (Table 2) showed different patterns of their penetration into tissues.

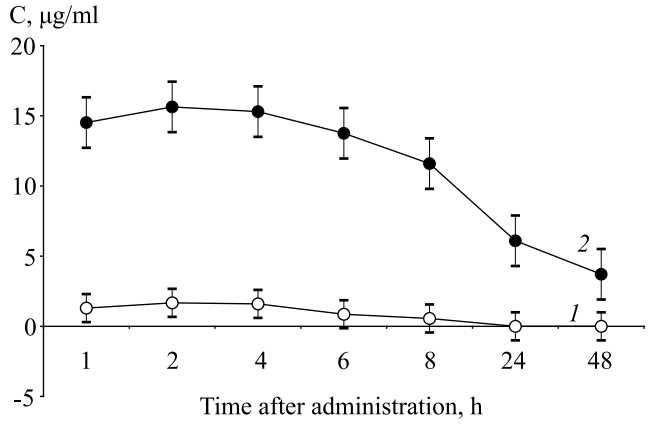

Fig. 1. Dynamics of mean concentrations of free (*1*) and liposomal (*2*) gentamicin sulfate in the blood of mice. Ordinate: mean drug concentration.

Gentamicin immobilized in liposomes is primarily accumulated in the mononuclear phagocytic system organs, namely liver, spleen and lungs at high concentrations than its free form. Mean levels of liposomal antibiotic in the examined organs were significantly superior ($p<0.05$) to those (3-10 times) of the free drug. Relatively low levels of accumulation in the kidney suggest decreased nephrotoxicity of antibiotic in liposomes compared with free form.

The observed differences are probably due to special properties of liposomes as drug delivery systems. Our studies showed that incorporation of gentamicin in liposomes significantly ($p<0.05$) increased drug retention in the blood (10 times) and lengthened their

TABLE 1. Main Parameters of Pharmacokinetics of [^{125}I]-Liposomal and [^{125}I]-Free Gentamicin Sulfate after a Single (120 μg) Intraperitoneal Injection to White Mice (*M*±*m*)

Parameter	Liposomal gentamicin sulfate	Gentamicin sulfate
AUC, ng×h×ml^{-1}	467.6569±17.4*	12.2646±1.28
Tmax, ng×h×ml^{-1}	2.00±0.32	2.00±0.14
Cmax, μg /ml	15.64±1.32*	1.67±0.23
Clpo, liter/h	0.00030±0.00008*	0.0098±0.0021
Kel, liter/h	0.0306±0.0010*	0.1845±0.07
MRTpo, h	46.06±3.20*	5.124±0.320
$T^1/_2$, h	22.64±0.65*	3.750±0.053
Vzpo, liter	0.00840±0.00034*	0.0530±0.009

Note. AUC: area under the curve; Tmax: time of attaining maximum concentration; C max: maximum concentration; Clpo: clearance for extravascular administration; Kel: elimination rate constant; MRTpo: mean residence time after extravascular administration; $T^1/_2$: elimination half-life; Vzpo: kinetic volume of distribution. *$p<0.05$ in comparison with administration of free antibiotic.

TABLE 2. Dynamics of Mean Concentrations (μg/g) of [^{125}I]-Liposomal and [^{125}I]-Free Gentamicin Sulfate in Mice after Single (120 μg) Intraperitoneal Administration (*M*±*m*)

Drug	Organ	Time after injection, h					
		1	2	4	8	24	48
Gentamicin sulfate	lungs	0.73±0.06	0.84±0.07	0.9±0.1	0.76±0.04	0.73±0.05	0.71±0.03
	liver	14.54±0.90	15.82±1.40	19.58±1.70	14.54±1.10	4.45±0.70	2.10±0.18
	spleen	1.57±0.34	2.18±0.46	1.34±0.17	0.97±0.08	0.90±0.06	0.30±0.04
	kidneys	10.30±1.62	10.60±1.53	11.10±1.97	12.10±2.38	1.36±0.12	1.40±0.10
Liposomal gentamicin sulfate	lungs	3.37±0.08*	3.31±0.10*	2.97±0.07*	2.50±0.09*	2.04±0.12*	1.44±0.14*
	liver	33.2±4.3*	33.8±4.5*	33.6±4.2*	30.0±4.2*	14.60±3.63*	12.1±3.2*
	spleen	3.36±0.60*	3.46±0.70*	8.07±1.50*	7.34±1.30*	5.22±0.80*	3.2±0.7*
	kidneys	4.15±1.02*	7.45±1.30*	5.5±1.1*	8.71±1.60	6.9±1.3*	5.12±0.90*

Note. Values are significant (p<0.05) compared with those after administration of free gentamicin.

circulation time. The long circulation half-life time of the antibiotic in the blood compared with its free form is probably due to good tissue penetration. High levels in the blood and reduction in clearance rate of antibiotic immobilized in liposomes probably attests to its higher therapeutic activity.

These results suggest that further study of liposomes as delivery system for various drugs and biologically active compounds may be promising approach for development of fundamentally new therapeutic and preventive drugs.

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