International Journal of Pharmaceutics, 42 (1988) 193–198
Elsevier

IJP 01425

Decreased distribution of gentamicin in rat kidney by complexation with dextran sulfate

Shinji Kikuchi, Asaichi Inaba, Masayuki Takahashi, Yukihiko Aramaki and Seishi Tsuchiya

Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo (Japan)

(Received 6 December 1986)
(Modified version received 10 July 1987)
(Accepted 15 September 1987)

Key words: Aminoglycoside; Dextran sulfate; Binding; Nephrotoxicity

Summary

The effect of dextran sulfate on aminoglycoside concentration in rat renal cortex was studied. Dextran sulfate has one class involving many binding sites for aminoglycosides. Gentamicin has a higher affinity for dextran sulfate than either amikacin or dibekacin. A complex of dextran sulfate and gentamicin had no influence on the elimination of gentamicin from the blood, but gentamicin concentration in the renal cortex showed a significant decrease. The binding of gentamicin to brush-border membrane vesicles was inhibited non-competitively by dextran sulfate. Substantial amounts of administered dextran sulfate were found in the urine, possibly indicating that dextran sulfate may inhibit the interaction between aminoglycoside and brush-border membranes at the lumen of the renal proximal tubule. There is thus the possibility that dextran sulfate may effectively inhibit the distribution of aminoglycosides on the reabsorption process and decrease the accumulation within the renal cortex.

Introduction

Aminoglycoside antibiotics (AGs) are widely used to treat Gram-negative bacterial infectious diseases. However, nephrotoxicity is one of serious side-effects incurred by patients, and is strongly related to the concentration of AGs within the renal proximal tubular cells (Kaloyanides and Pastoriza-Munoz, 1980). Many investigators consider that AGs penetrate renal proximal tubular cells through brush-border membranes (Silverblatt and Kuehn, 1979; Wedeen et al., 1983; Aramaki et al., 1986). Consequently, reduction in this distribution may be an essential means for avoiding the nephrotoxicity. Josepovitz et al. (1982) reported that a decrease in the uptake of gentamicin by rat renal cortex results from the coadministration of polycations, spermine and tetralysine. But polyamines themselves are nephrotoxic.

AGs, polycationic drugs, have been reported to bind easily with substances such as phosphatidylinositol (Feldman et al., 1982; Sastrasinh et al., 1982), ATP (Inaba et al., 1986), and acidic mucopolysaccharides (Deguchi et al., 1978; Aramaki et al., 1987a) through ionic interactions between the amino group(s) of AGs and the phosphate, carboxyl or sulfate group(s) of binding substances.

In the present study, an investigation was made of the effects of dextran sulfate (DS) on the distribution of AGs in rat renal cortex in vivo.

Correspondence: S. Tsuchiya, Tokyo College of Pharmacy, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan.

Materials and Methods

Materials

Dibekacin (DKB; Meiji Seika Co., Tokyo), [^{3}H]DKB (66 μCi/mg, Meiji Seika Co., Tokyo), gentamicin sulfate (GM; Shionogi Pharmaceutical Co., Osaka) and Amikacin sulfate (AMK, Banyu Pharmaceutical Co., Tokyo) were used. Dextran sulfate (DS; mean mol. wt. = 5000, S-content was 15%) was obtained from Sigma Chemical Co. (St. Louis, MO). The GM–DS complex (molar ratio of GM/DS = 1 : 2) was prepared by incubation at 37 °C for 15 min. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) was purchased from Sigma Chemical Co. and other chemicals were from Wako Pure Chemical Industries (Osaka). Male Wistar rats (250–280 g) were purchased from Shizuoka Agricultural Co. (Shizuoka).

Binding studies

The binding of AGs to DS was studied by the equilibrium dialysis method. DS was dissolved in 10 mM Tris-HCl buffer (pH 7.0) at a concentration of 200 μg/ml. AGs were also dissolved in the buffer at various concentrations. Equal volumes of each of these solutions were dialyzed against the buffer at 25 °C for 24 h. The non-specific binding of AGs to the dialysing equipment was negligible. Analysis was performed using the program MULTI (Yamaoka et al., 1981).

In vivo studies

Male Wistar rats were used for all the experiments. Under light ether anesthesia, a polyethylene catheter (PE-50) filled with heparin was secured to the femoral vein and artery followed by placing the rat in a Bollmann cage. Following complete recovery of the rat from anesthesia, i.v. administration of GM (20 mg/kg) or GM-DS (450 mg/kg, equivalent to 20 mg/kg of GM) was conducted. Blood samples (0.2 ml) were withdrawn from the femoral artery at the indicated times, and urine samples were collected in the Bollmann cage over 3 h. The rats were sacrificed by decapitation at 3 h following the administrations and their kidneys were removed. Pharmacokinetic parameters were obtained using the program MULTI (Yamaoka et al., 1981).

Binding of [^{3}H]DKB to brush-border membrane vesicles

Brush-border membrane (BBM) vesicles were prepared from the renal cortex by the calcium precipitation method (Malathi et al., 1979). Their purity was determined by alkaline phosphatase, a marker enzyme. Activity was enriched by about 9 times that of the renal cortical homogenate. Contamination of the basolateral membranes was negligible, since Na^+,K^+-ATPase activity was very low. Cytochrome c oxidase, *N*-acetyl-β-D-glucosaminidase and glucose-6-phosphatase activity in the BBM vesicles was also lower than that of the homogenate. The membrane preparation was suspended in 20 mM Tris-HEPES buffer (pH 7.0) containing 250 mM mannitol.

The effects of DS on the [^{3}H]DKB binding to BBM vesicles were determined by the rapid filtration technique using a Millipore filter (type HA, 0.45 μm). One-hundred μl of the BBM suspension (20–30 μg of protein) was mixed with 100 μl of 20 mM Tris-HEPES buffer (pH 7.0) containing 250 mM mannitol, 5 μM of [^{3}H]DKB, and various concentrations of DS. Following incubation at 37 °C for 30 min, 2.0 ml of ice-cold buffer was added and filtered through a Millipore filter under light suction. The filter was washed twice with 2.0 ml of the ice-cold buffer and then dried. The BBM vesicles remaining on the filter were dissolved in 1.0 ml of ethylene glycol monoethyl ether and measured for radioactivity using a liquid scintillation counter (Aloka 903).

Analytical method

In the equilibrium dialysis experiment, AGs concentrations were determined by HPLC (Anhalt and Brown, 1978) using Licrosorb RP-18 or RP-8 column (5 μm, 250 × 4 mm, Merck). In the plasma, urine and renal cortex, AGs concentrations were determined by bioassay using the *Bacillus subtilis* ATCC 6633 as the test organism. Extraction of GM from rat kidney cortex was carried out by the method of Ruben et al. (1984). Protein concentration was determined by the method of Lowry et al. (1951). Alkaline phosphatase (Bessey et al., 1946), Na^+,K^+-ATPase (Jorgensen, 1974), *N*-acetyl-β-D-glucosaminidase (Niebes and Ponard, 1975), cytochrome c oxidase (Wharton and

Tzagoloff, 1967), and glucose-6-phosphatase (Aronson and Touster, 1974) activity was measured by the reported methods.

Results

Binding of aminoglycosides to DS

The Scatchard plots in Fig. 1 of 3 kinds of AGs are linear, indicating DS to have one class of binding site for each AG. The AGs and DS binding parameters are given in Table 1. The highest binding constant (K) was obtained for GM, but the AGs had essentially the same number of binding sites. Since it had the highest affinity ($n \times K = 604$) to DS, GM was used in in vivo experiments.

Concentration in plasma GM

Fig. 2 shows plasma concentrations of GM following intravenous administrations of GM or the GM–DS complex. In both cases, the elimination of GM is about the same, and GM was eliminated from the plasma circulation bi-exponentially. Pharmacokinetic parameters were listed in Table 2. These results suggest that GM in GM–DS complex may be subjected renal elimination as well as free GM, i.e. DS has no effect on the elimination of GM from the blood.

TABLE 1

Binding parameters of aminoglycosides binding to dextran sulfate

Aminoglycosides	$K(\times 10^4\ M^{-1})$	n
Gentamicin	58.30	10.4
Amikacin	21.63	12.9
Dibekacin	4.47	10.7

Concentration in renal cortex and urinary excretion

Three hours after the administration of GM–DS complex, the GM concentration in the renal cortex (146.9 ± 25.6 μg/g cortex) was significantly lower than that of free GM administration (300.9 ± 89.3, Fig. 3A). The urinary excretion of GM during the 3 h after the administration of the GM–DS complex was 6102.0 ± 895.0 μg, and that of free GM

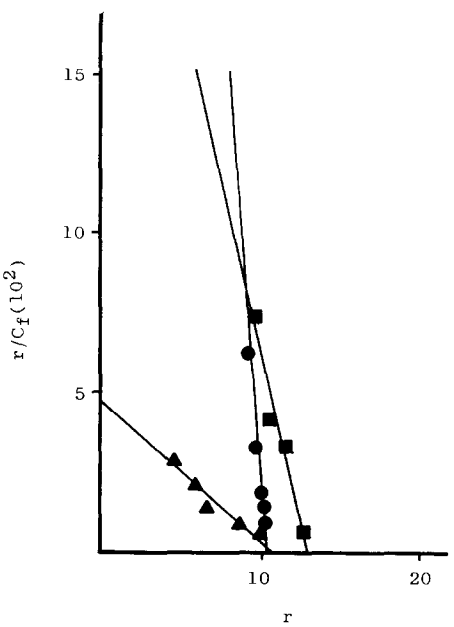

Fig. 1. Scatchard plots of aminoglycosides binding to dextran sulfate. Cf, the concentration of free GM; r, the ratio of GM binding to DS. ●, gentamicin; ■, amikacin; ▲, Dibekacin.

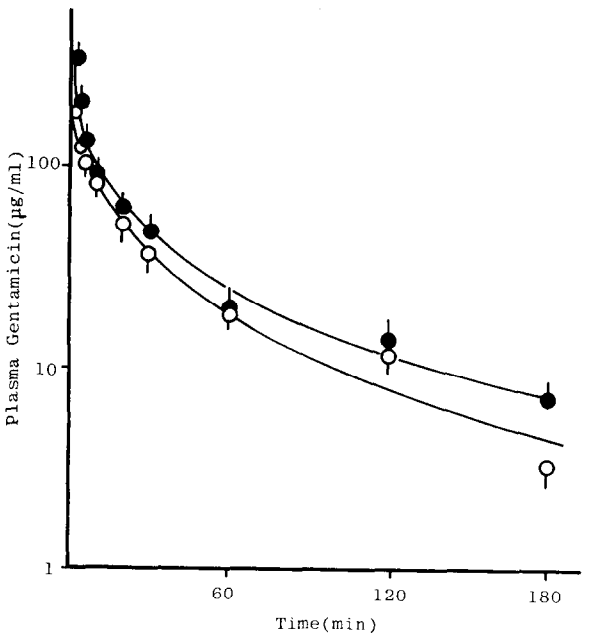

Fig. 2. Plasma gentamicin level. Rats were injected i.v. with gentamicin (20 mg/kg, ○) or gentamicin–dextran sulfate complex (●). Values shown represent mean ±S.D. for 3 experiments.

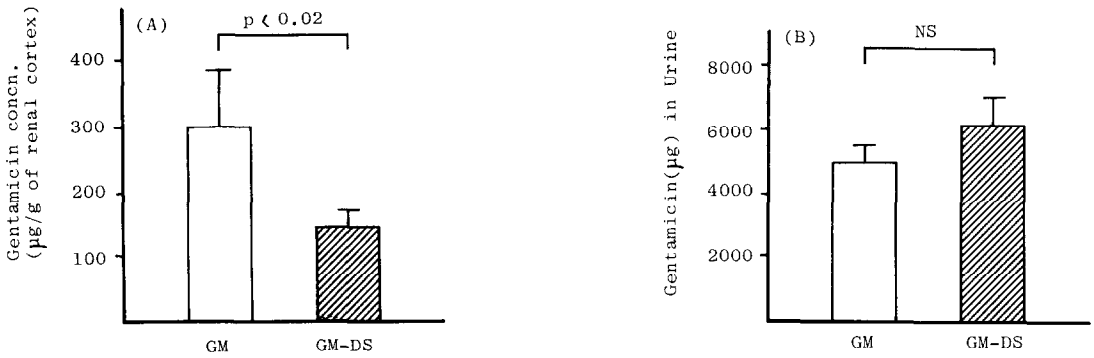

Fig. 3. Renal cortical distribution (A) and urinal excretion (B) of gentamicin. Rats were injected i.v. with gentamicin (□) or gentamicin–dextran sulfate complex (▨). Values represent mean ± S.D. for 3 experiments.

TABLE 2

Pharmacokinetic parameters of gentamicin and gentamicin–dextran sulfate complex

Values shown represent the mean of 3 experiments ± S.D.

Parameter	GM	GM-DS
A	201.0 ± 29.3	359.5 ± 14.6 **
α	15.6 ± 1.2	24.1 ± 0.7 **
B	56.7 ± 5.4	77.1 ± 5.2 *
β	0.8 ± 0.1	1.0 ± 0.2
AUC(μg·h/ml)	78.2 ± 5.6	91.2 ± 8.2
Cl_{total} (ml/h)	64.2 ± 4.8	55.1 ± 5.2
$t_{1/2}$ (h^{-1})	0.8 ± 0.1	0.7 ± 0.1

Statistical analysis was carried out by t-test. *; $P < 0.05$, **; $P < 0.01$

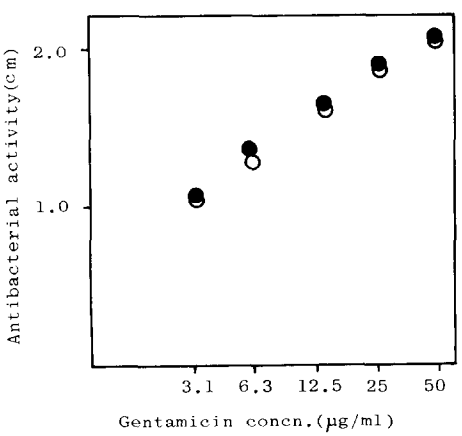

Fig. 4. Comparison of antibacterial activity of gentamicin and gentamicin–dextran sulfate complex. The concentration of gentamicin in gentamicin–dextran sulfate complex is equivalent to that of gentamicin only. ●, gentamicin only; ○, gentamicin–dextran sulfate.

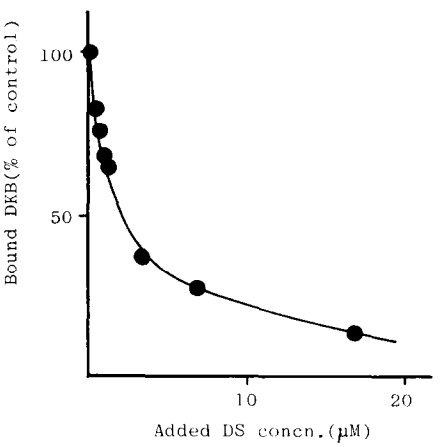

Fig. 5. Effect of dextran sulfate on [^{3}H]dibekacin binding to BBM vesicles.

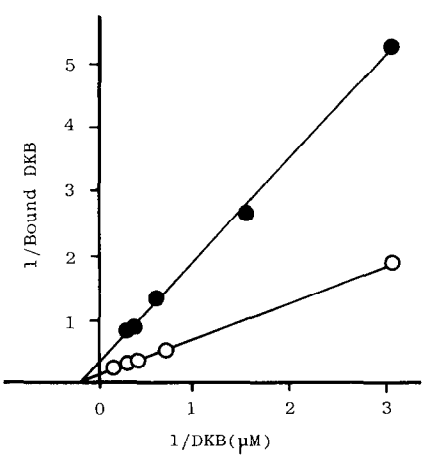

Fig. 6. Double reciprocal plot of dibekacin binding to BBM vesicles in the presence (●) and absence (○) of dextran sulfate.

was 4986.9 ± 512.2. There is a difference in mean values, but statistical significance could not be obtained (Fig. 3(B)).

Antibacterial activity of GM-DS complex

The effect of DS on antibacterial activity of GM was studied by the bioassay method using *Bacillus subtilis* ATCC 6633 as the test organism. As shown in Fig. 4, GM and GM–DS complex have equal antibacterial activity.

[^{3}H]DKB binding to BBM vesicles

To clarify the effect of DS on the renal distribution of AGs in vitro, the effect of DS on [^{3}H]DKB binding to BBM vesicles was examined. As shown in Fig. 5, the binding of [^{3}H]DKB to BBM vesicles decreased with increasing DS concentration, and showed about 80% inhibition at 17 μM DS. A double reciplocal plot of this inhibitory effect indicated DS to inhibit non-competitively the binding of DKB to BBM vesicles (Fig. 6).

Discussion

The selective accumulation of aminoglycosides into the renal cortex is closely related to their nephrotoxicity. It is considered that their accumulation within renal proximal tubular cells occurs during reabsorption of AGs (Bennett, 1983; Francke and Neu, 1983; Whelton, 1985). Reabsorption through BBM may be the primary route for proximal tubular accumulation (Collier et al., 1979; Aramaki et al., 1986). Thus, it should be possible theoretically to reduce aminoglycoside nephrotoxicity by blocking this route. To find some means for this, the interactions of DS with AGs and their disposition in the rat were investigated in the present research. DS was chosen as inhibitor for the following reasons: (1) the stability of DS is sufficient and its toxicity is low (William et al., 1969); (2) the urinary excretion of DS is very high (Wada et al., 1976); and (3) DS of various molecular weights is available.

In the binding study, 3 kinds of aminoglycosides were made to interact with DS and GM was found to have the highest affinity for DS. The binding site of DS for AGs is considered to be its sulfate groups, since AGs in a previous study were found to have no affinity for dextran (Aramaki et al., 1987b). These results are consistent with the finding that AGs have an affinity for substances with a negative charge (Aramaki et al., 1987a; Deguchi et al., 1978; Sastrashinh et al., 1982).

The antibacterial activity of GM and GM–DS mixture was examined by bioassay, and both cases were found to have essentially the same activity (Fig. 4). Thus, the addition of DS to a GM solution will not interfere with the antibacterial activity of GM. In vivo experiments showed that there was no significant difference in the elimination of GM from the blood between the administration of GM and the GM–DS complex. Thus, glomerular filtration is the primary route for AG elimination and the rate of filtration of GM and GM in the GM–DS complex may be nearly the same. The renal distribution of GM significantly decreased by administration of the GM–DS complex (Fig. 3). Because the recovery of GM from renal cortex and urine was almost complete in GM administration (100.9 ± 10.3%) and GM–DS administration (113.8 ± 16.7%), the distribution of GM within the renal cortex may be disturbed by DS.

AGs are considered by some investigators to accumulate within the proximal tubular cells during the reabsorption process (Collier et al., 1979; Aramaki et al., 1986). Consequently, a study of the interactions between AGs and BBM is very important for understanding their nephrotoxicity. Sastrasinh et al. (1982) reported that AGs bind to phosphatidylinositol on BBM vesicles of rat renal proximal tubular cells. We found that DKB binds to BBM vesicles with the same affinity as that of GM (Takahashi et al., 1987). Thus, the effect of DS on AG binding to BBM vesicles was studied using [^{3}H]DKB. The binding of [^{3}H]DKB was clearly inhibited by the addition of DS (Fig. 5). As discussed above, GM has affinity toward the sulfate group(s) of DS and thus, [^{3}H]DKB binding to BBM vesicles is inhibited non-competitively by the addition of DS.

The results of an in vivo experiment showed about 30% of the administered DS to be recovered in the urine within 3 h (data not shown). Thus, DS in GM–DS complex reduces the renal accumula-

tion of GM by interfering with the binding of GM to BBM.

The data of the present research demonstrate DS to be an effective substance for reducing the accumulation of AGs within the renal cortex.

References

Anhalt, J.P. and Brown, S.D., High-performance liquid-chromatographic assay of aminoglycoside antibiotics in serum. *Clin. Chem.*, 24 (1978) 1940–1947.

Aramaki, Y., Takahashi, M., Inaba, A., Ishii, Y. and Tsuchiya, S. Uptake of aminoglycoside antibiotics into brush-border membrane vesicles and inhibition of Na^+, K^+-ATPase activity of basolateral membrane. *Biochim. Biophys. Acta*, 862 (1986) 111–118.

Aramaki, Y., Inaba, A., Hosoda, J. and Tsuchiya, S., Interaction of aminoglycosides with heparan sulfate from rat kidney. *Chem. Pharm. Bull.*, 35 (1987a) 756–760.

Aramaki, Y., Niibuchi, J., Tsuchiya, S. and Hosoda, J., Interaction of 3′, 4′-dideoxykanamycin B and submaxillary mucin *Chem. Pharm. Bull.*, 35 (1987b) 320–325.

Aronson, N.N. and Touster, O., Isolation of rat liver plasma membrane fragments in isotonic sucrose. *Meth. Enzymol.*, 31 (1974) 90–102.

Bennett, W.A., Aminoglycoside nephrotoxicity. *Nephron*, 35 (1983) 73–77.

Bessey, O.A., Lowry, O.H. and Brock, M.J., A method for the rapid determination of alkaline phosphatase with fine cubic millimeters of serum. *J. Biol. Chem.*, 164 (1946) 321–326.

Collier, V.U., Lietman, P.S. and Mitch, W.E., Evidence for luminal uptake of gentamicin in the perfused rat kidney. *J. Pharmacol. Exp. Ther.*, 220 (1979) 247–251.

Deguchi, T., Ishii, A. and Tanaka, M., Binding of aminoglycoside antibiotics to acidic mucopolysaccharides. *J. Antibiot.*, 31 (1978) 150–155.

Feldman, S., Wang, M. and Kaloyanides, G.J., Aminoglycosides induce a phospholipidosis in the renal cortex of the rat: an early manifestation of nephrotoxicity. *J. Pharmacol. Exp. Ther.*, 220 (1982) 514–520.

Francke, E. and Neu, H.C., Nephrotoxicity of aminoglycosides. *Infect. Surg.*, March (1983) 205–214.

Inaba, A., Aramaki, Y., Takahashi, M. and Tsuchiya, S., Binding of 3′, 4′-dideoxykanamycin B to ATP and its related compounds. *Life Sci.*, 39 (1986) 1345–1351.

Jorgensen, P.L., Purification and characterization of Na^+, K^+-ATPase III. Purification from the outer medulla of mammalian kidney after selective removal of membrane components by sodium dodecylsulfate. *Biochim. Biophys. Acta*, 356 (1974) 36–52.

Josepovitz, C., Pastoriza-Munoz, E., Timmerman, D., Scott, M., Feldman, S. and Kaloyanides, G.J., Inhibition of gentamicin uptake in rat renal cortex in vitro by aminoglycosides and organic polycations. *J. Pharmacol. Exp. Ther.*, 223 (1982) 314–321.

Kaloyanides, G.J. and Pastoriza-Munoz, E., Aminoglycosides nephrotoxicity, *Kidney Int.*, 18 (1980) 571–582.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193 (1951) 265–275.

Malathi, P., Preiser, H., Fairclough, P., Mallett, P. and Crane, R.K., A rapid method for the isolation of kidney brush-border membranes. *Biochim. Biophys. Acta*, 554 (1979) 259–263.

Niebes, P. and Ponard, G., Stabilization of rat liver lysosomes by (+)-cyanidanol-3 in vivo. *Biochem. Pharmacol.*, 24 (1975) 905–909.

Ruben, A.G., Gert, A.V., Dirk, E.P., Ludo, V., Simon, L.S. and Marc, E. D-B., Improved procedure for extracting aminoglycosides from renal cortical tissue. *J. Antimicrob. Chemother.*, 25 (1984) 783–784.

Sastrashinh, M., Knauss, T.C., Weinberg, J.M. and Humes, H.D., Identification of aminoglycoside binding site in rat renal brush-border membranes. *J. Pharmacol. Exp. Ther.*, 222 (1982) 350–358.

Silverblatt, F.J. and Kuehn, C., Autoradiography of gentamicin uptake by the rat proximal tubule cell. *Kidney Int.*, 15 (1979) 335–345.

Takahashi, M., Aramaki, Y., Inaba, A. and Tsuchiya, S., Inhibition of alkaline phosphatase activity and D-glucose uptake in rat renal brush-border membrane vesicles by aminoglycosides, submitted.

Wada, S., Nagashima, R., Ogura, T., Sakamoto, T. and Yamaguchi, T. Studies on absorption, distribution, metabolism and excretion of orally administered ^{35}S-dextran sulfate in rats and dog(1) Time course of ^{35}S-radioactivities. *Pharmacometrics*, 12 (1976) 537–542.

Wedeen, R.P., Batuman, V., Cheeks, C., Marquet, E. and Sobel, H. Transport of gentamicin in rat proximal tubule. *Lab. Invest.*, 48 (1983) 213–223.

Wharton, D.C. and Tzagolff, A., Cytochrome oxidase from beef heart mitochondria. *Meth. Enzymol.*, 48 (1983) 245–250.

Whelton, A., Therapeutic initiatives for the avoidance of aminoglycoside toxicity. *J. Clin. Pharmacol.*, 25 (1985) 879–885.

William, A.B., Ho, H.K. and Saverio, F.R., *Dextran sulfate treatment of peptic ulcers*, United States Patent Office, (1969) 3, 487, 150.

Yamaoka, K., Taniguchi, Y., Nakagawa, T. and Uno, T., A Pharmacokinetic Analysis Program (MULTI) for Microcomputer. *J. Pharm. Dyn.*, 4 (1981) 875–885.