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Effect of age on gastrointestinal absorption of tobramycin in rats

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Abstract

Using newborn, suckling, weanling and adult rats, we studied the effect of age on the intestinal absorption of tobramycin, which is poorly absorbed in adult humans. The mean (±S.D.) bioavailability (F) values after administering tobramycin (30 mg/kg) orally to newborn, suckling and weanling rats were 39.4 ± 5.8, 19.9 ± 4.6, and 2.8 ± 0.9%, respectively (significant differences). A marked difference was observed between suckling and weanling rats. After duodenal administration of tobramycin (30 mg/kg) to suckling rats, absorption occurred more readily than in adult rats (F = 104.0 ± 33.6 vs 27.2 ± 5.7%), and the mean F values for both ages were significantly higher than those in the respective oral sham experiments. Therefore, a high concentration of tobramycin in the intestine promotes intestinal absorption. When the cumulative amounts of tobramycin transferred from the mucosal to serosal side were determined in suckling, weanling and adult rats by an everted sac method, the amount was highest for suckling rats, with an abrupt change being found between suckling and weanling rats. The permeation of tobramycin from the mucosal to serosal side was not a saturable process in rats of all ages. An aminoglycoside (AMG), neomycin (10 mM), and a polyamine, spermine (10 mM), significantly increased the permeation of tobramycin in the jejunum and ileum of suckling rats and the jejunum of adult rats. Choline chloride (10 mM), tetraethylammonium (10 mM) and 2,4-dinitrophenol (0.5 mM) did not affect the permeation of tobramycin in any small intestinal regions (jejunum and ileum) of either suckling or adult rats. The following conclusions were drawn: (1) tobramycin absorption occurs readily from the small intestine of suckling rats, but markedly decreases during the weaning period; (2) tobramycin is transferred predominantly via passive diffusion in the immature small intestine of suckling rats; and (3) the intestinal absorption of tobramycin can be enhanced by direct delivery of a high drug concentration exposed to the small intestine, and this absorption is further facilitated with other AMGs or polyamines in the small intestine, particularly in suckling rats.

Key words: Tobramycin; Aminoglycoside antibiotic; Suckling rat; Weaning period; Age; Bioavailability; Intestinal absorption; Developmental intestinal function

1. Introduction

Tobramycin is an aminoglycoside (AMG) widely used in the treatment of pediatric gram-negative infections. Its excretion in premature and newborn infants with immature kidneys is delayed relative to adults (McCracken and Nelson, 1977; Nahata et al., 1984). Thus, the pharmacokinetics of tobramycin in neonates differ from those in adults. Therefore, it is clinically impor-

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tant to study the effect of age on the pharmacokinetics in order to avoid serious side-effects such as nephrotoxicity and ototoxicity and to maintain an effective serum concentration.

Because AMGs are poorly absorbed from the fully mature gastrointestinal tract in adults, they are given mainly as intravenous and intramuscular injections, with oral administration used only for the treatment of intestinal infection or for decontamination before colorectal surgery. However, at the newborn and suckling stages, the mammalian gut remains immature. During the weaning period, when the nutrient input changes from milk to an adult diet which is more variable as well as quantitatively and qualitatively different, the need for functional changes arises in the gut. Accordingly, the gut undergoes developmental changes in many of its physiological and morphological characteristics (Lev, 1981; Koldvsky, 1985; Henning et al., 1987; Buddington et al., 1990). The gastrointestinal absorption of many nutrients and macromolecules in the suckling period differs from that in the adult (Younozai et al., 1981; Henning, 1987; Buddington et al., 1990). However, little work has been carried out on the effect of developmental changes on gastrointestinal absorption of drugs (Smith et al., 1980). In our previous studies, we showed that sulfaguanidine (Mizuno et al., 1986), several β-lactam antibiotics (cefazolin, cefamandole and cefmethazole) (Morita et al., 1990, 1992), and acyclovir (Fujioka et al., 1990), all of which are poorly absorbed from the adult rat intestine, could be readily absorbed from the intestine of suckling rats. Thus, changes in drug absorption are considered to occur during the weaning period. In the present study, we examined the effect of age on the gastrointestinal absorption of tobramycin using newborn, suckling, weanling and adult rats.

2. Materials and methods

2.1. Materials

Tobramycin sulfate was donated by Shionogi & Co., Ltd (Osaka, Japan). Neomycin sulfate, spermine tetrahydrochloride, choline chloride, tetraethylammonium chloride and 2,4-dinitrophenol were obtained from Nakarai Tesque, Inc. (Kyoto, Japan). All other chemicals used were of analytical reagent grade.

Animals: Wistar rats, aged 1 week (males and females, 8–11 g), 2.5 weeks (males and females, 19–26 g), 3 weeks (males and females, 28–37 g) and 8–10 weeks (males, 170–235 g) were used. All rats were fasted overnight before and during the study. Rats were kept at 37°C using thermostatically controlled plates during the experiments.

2.2. Animal experiments

In vivo experiments

(a) Intravenous (i.v.) administration: Rats were anesthetized with ether. Part of the cervicalis of each rat was opened and tobramycin sulfate solution (30 mg/ml per kg) was injected into the jugular vein using a microsyringe.

(b) Oral (p.o.) administration: Each rat was given 30 mg/10 ml per kg of tobramycin solution, via a stomach tube for adults and via a polyethylene tube (i.d., 0.28 mm; o.d., 0.61 mm) for newborn, suckling and weanling rats.

(c) Duodenal administration: The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p. for adult rats, 120 mg/kg s.c. for suckling rats), and the pylorus was exposed by an abdominal incision. Tobramycin solution (30 mg/10 ml per kg) was injected into the duodenum through a metal syringe from a small opening made just distal to the stomach. The pylorus and the small opening were ligated immediately, and the incision was discontinuously sutured. In the sham experiments, the pylorus was exposed for a few minutes. The incision was then sutured, and tobramycin was administered orally.

Blood samples (0.15 ml) were collected from the jugular vein at regular intervals after drug administration while the rats were under a light ether anesthesia (except in the case of intraduodenal administration). The blood was centrifuged immediately after collection, and plasma samples were stored at −40°C until HPLC analysis, as described later.

In vitro experiments (everted sac method)

The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p. for adult rats, 120 mg/kg s.c. for suckling rats) and a midline abdominal incision was made. The rats were killed by cardiac puncture. The small intestine from the ligament of Treitz to the ileocecal junction was used. Jejunal and ileal intestines were rapidly removed and everted with a glass rod. The length of the intestine and the volume at the serosal and mucosal sides at different weeks of age were as follows:

Age	Length (cm)	Number of sacs used	Volume at serosal side (ml)	Volume at mucosal side (ml)
Adult	7	1	0.7	40
Weanling	5	2	0.3	40
Suckling	5	2	0.3	40

The everted sac was filled with Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 0.4% glucose and treated with 95% O_2/5% CO_2 gas prior to the experiment. The drug solution containing the same buffer was incubated at 37°C in a polypropylene vessel and gassed with 95% O_2/5% CO_2. After 5 min, the sac was added to the polypropylene vessel and further incubated. The concentrations of tobramycin in the serosal and mucosal sides were determined by HPLC, except for inhibitor experiments which were examined using the TDX system (Dainabot Co., Ltd, Tokyo, Japan).

2.3. Determination of tobramycin concentration in plasma

Plasma extraction procedure

AMGs (e.g., tobramycin) were separated from interfering compounds in the plasma by ion-exchange gel chromatography. A short column (0.8 cm) with a bed volume of 0.5 ml was prepared from CM-Sephadex (C-25) with a 0.2 M solution of Na_2SO_4 used as the initial buffer. A disposable polypropylene column (Terumo 2.5 ml) was used with a bed support cut from Toyo filter paper (Toyo Filter Paper No. 51). A filter paper was placed on the top of the gel. A 50 μl plasma sample was then applied to the column, and 500 μl of buffer (0.05 M KH_2PO_4-NaOH buffer, pH 6.5) was added. The column was then eluted with 4 ml of the 0.2 M Na_2SO_4 solution. After the column had drained completely, 1 ml of alkaline buffer (0.2 M Na_2SO_4, 0.01 M NaOH) was added, and the eluate was collected. To this eluate (100 μl), 0.01 N H_2SO_4 (200 μl) was added, and 100 μl of the resulting solution was subjected to analysis (Anhalt et al., 1978).

Tobramycin assay

The tobramycin concentration was determined by modifying the post-column HPLC method of Anhalt et al. (1978). After tobramycin had been separated, using a stainless-steel column (25 cm × 4.6 mm, i.d.) packed with RP-18 (5 μm particles) (E. Merck, Darmstadt, Germany) at 24°C, the fluorescent derivative with *o*-phthalaldehyde was prepared at 16°C. Fluorescence was detected with a fluorescence detector (RF 535 model, Shimadzu Co., Tokyo, Japan) at 340 nm for excitation and at 430 nm for emission.

Mobile phase: The mobile phase for these assays contained, per l, 0.045 mol of Na_2SO_4, 5 mmol of sodium pentanesulfonate, and 1 ml of acetic acid, in water. The solution was filtered (0.45 μm membrane filter) before use. A flow rate of 1.2 ml/min was used.

o-Phthalaldehyde reagent: Potassium borate buffer was prepared from 0.2 M boric acid and 0.14 M potassium borate. The solution was filtered through a 0.45 μm membrane filter (Toyo Roshi, Tokyo, Japan). A flow rate of 0.6 ml/min was used.

2.4. Data analysis

Plasma concentration-time curves were analyzed using the least-squares regression analysis program, MULTI (Yamaoka, 1981). The areas under the plasma concentration-time curves (AUCs) and the mean residence time (MRT) were calculated by standard linear trapezoidal integration with extrapolation to infinite time. The steady-state distribution volume per body weight (Vd_{ss}) and the total body or plasma clear-

ance per body weight (CL_t) were estimated as described by Yamaoka et al. (1983). The absolute bioavailability (F) was expressed as:

$$F = AUC_{0-\infty}(p.o.)/AUC_{0-\infty}(i.v.)$$

The mean absorption time (MAT) was calculated as follows:

$$MAT = MRT(p.o.) - MRT(i.v.)$$

The data are given as the mean ± standard deviation (S.D.) throughout the text and were assessed by means of Student's t-test.

3. Results

3.1. Developmental changes in tobramycin elimination

Fig. 1 represents the mean (±S.D.) plasma concentration-time data of tobramycin after an i.v. administration (30 mg/kg) to newborn, suckling, weanling and adult rats. The mean pharmacokinetic parameters are listed in Table 1. The data were fitted to the two-compartment model, expressed by the following formula, for rats of all ages:

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$$

where C_p is the drug concentration in the plasma at time t. The constants C_1 and C_2 are the intercepts on the y-axis, and λ_1 and λ_2 are their slopes for the respective fast and slow exponential segments of the curve generated by the equation.

In newborn rats, the elimination of tobramycin from plasma was the slowest (λ_2, K_{10}, CL_t) among the rats studied, and the values of V_c, Vd_{ss} and $AUC_{0-\infty}$ were the greatest. Elimination of the drug in suckling and weanling rats was delayed relative to adult rats (λ_2, K_{10}, CL_t), but there was no significant difference in their pharmacokinetic parameters. The distribution rate of the adult rats was the highest (λ_1, K_{12}, K_{21}).

3.2. Developmental changes in intestinal absorption in vivo

Fig. 2 shows the mean plasma concentration-time data of tobramycin after a p.o. administration (30 mg/kg) to rats of different ages (newborn, suckling and weanling). The mean pharmacokinetic parameters calculated for these groups are listed in Table 2. The absolute bioavailabilities (i.e., F values) for newborn and suckling rats were about 14- and 7-fold greater, respectively, than that of weanling rats. In addition, to-

Table 1
Pharmacokinetic parameters after an i.v. injection of tobramycin (30 mg/kg) to newborn, suckling, weanling and adult rats

Parameter	Newborn	Suckling	Weanling	Adult
C_1 ($\mu g\ ml^{-1}$)	78.9 ± 6.5 [b]	127.3 ± 32.9	158.8 ± 40.3	119.8 ± 25.6
C_2 ($\mu g\ ml^{-1}$)	45.4 ± 3.0 [a,c]	70.2 ± 3.8	47.2 ± 6.6	78.2 ± 8.1
λ_1 (h^{-1})	6.2 ± 4.1 [a]	6.7 ± 4.6 [a]	11.4 ± 2.9 [a]	34.6 ± 5.9
λ_2 (h^{-1})	0.2 ± 0.0 [a,c]	0.6 ± 0.1 [b]	0.5 ± 0.1 [b]	1.3 ± 0.3
K_{10} (h^{-1})	0.5 ± 0.1 [b,d]	1.4 ± 0.4	2.0 ± 0.9	3.1 ± 1.2
K_{12} (h^{-1})	3.5 ± 2.5 [a]	3.2 ± 2.9 [a]	7.0 ± 2.2 [a]	18.2 ± 4.3
K_{21} (h^{-1})	2.4 ± 1.5 [a]	2.6 ± 1.3 [a]	2.9 ± 0.3 [b]	14.7 ± 3.5
V_c ($l\ kg^{-1}$)	0.2 ± 0.0 [a,d]	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
AUC ($\mu g\ h\ ml^{-1}$)	271.4 ± 36.0 [a,c]	147.4 ± 18.7 [a]	115.4 ± 16.8 [b]	69.3 ± 18.6
MRT (h)	5.3 ± 0.7 [a,c]	1.8 ± 0.1 [a]	1.6 ± 0.2 [a]	0.8 ± 0.2
Vd_{ss} ($l\ kg^{-1}$)	0.6 ± 0.0 [a,c]	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
CL_t ($l\ h^{-1}\ kg^{-1}$)	0.1 ± 0.0 [b,c]	0.2 ± 0.0 [b]	0.3 ± 0.0	0.5 ± 0.2

Each value represents the mean ± S.D. of 3–4 determinations.
[a] $p < 0.01$.
[b] $p < 0.05$, compared with adult rats.
[c] $p < 0.01$.
[d] $p < 0.05$, compared with suckling rats.

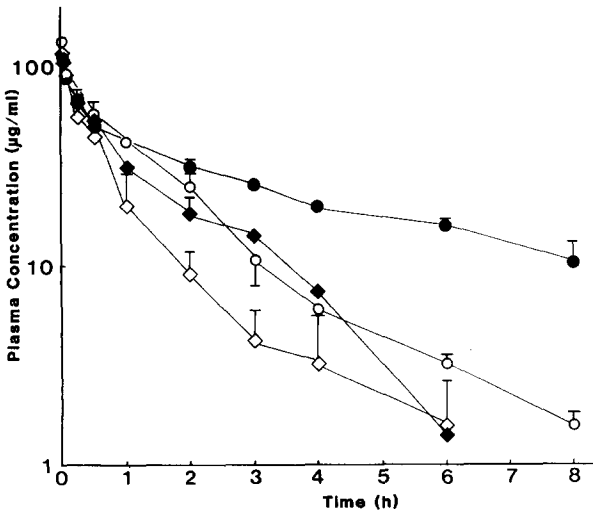

Fig. 1. Mean plasma concentration-time data of tobramycin (30 mg/kg) after i.v. administration to newborn (●), suckling (○) weanling (◆) and adult (◇) rats. Each point represents the mean ± S.D. of 3–4 determinations.

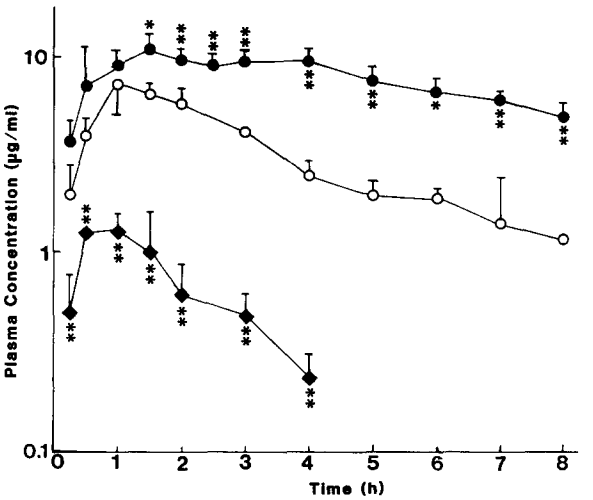

Fig. 2. Mean plasma concentration-time data of tobramycin (30 mg/kg) after p.o. administration to newborn (●), suckling (○) and weanling (◆) rats. Each point represents the mean ± S.D. of 3–4 determinations. * $p < 0.05$, ** $p < 0.01$ compared with the mean values from suckling rats.

bramycin was poorly absorbed from the small intestine in adult rats just as in weanling rats (data not shown).

Next, we studied the influence of a duodenal administration of tobramycin (30 mg/kg) on the drug's absorption in adult and suckling rats (Table 3). The mean F values were greater after the duodenal administration of tobramycin than those in sham p.o. experiments in both adult and suckling rats, and the mean F in sucklings was greater than that in adults.

3.3. Developmental changes in intestinal permeation of tobramycin in vitro

We determined the cumulative amounts of tobramycin (100 μM) transferred from the mucosal to serosal side in the jejunum and ileum of suckling, weanling and adult rats by using the everted sac method. Since the amounts for all of the examined ages increased linearly vs time up to 30 min (data not shown), the transferred amounts were determined at 30 min in this study. Fig. 3 shows the effect of the initial mucosal concentrations of tobramycin, ranging from 50 to 500 μM, on the cumulative transferred amounts. The mean values determined in both the jejunum and ileum of suckling rats were significantly greater than those of weanling and adult rats, implying that an abrupt change occurred in the intestinal absorption of tobramycin during the weaning period. These in vitro results showed the same trend as those determined in vivo. As the relationship between the initial mucosal concentrations of tobramycin and the cumulative transferred amounts represented a direct proportion over the range of 50–500 μM, no saturation phenomenon was de-

Table 2
Pharmacokinetic parameters after a p.o. administration of tobramycin (30 mg/kg) to newborn, suckling, weanling and adult rats

Parameter	Newborn	Suckling	Weanling
C_{max} (μg ml^{-1})	10.7 ± 1.8 [a]	7.5 ± 1.7 [a]	1.4 ± 0.2
T_{max} (h)	1.5 ± 0.0 [a]	1.2 ± 0.3	0.7 ± 0.2
MAT (h)	3.9 ± 2.0 [b]	2.2 ± 0.1 [a]	0.1 ± 0.2
$AUC_{0-\infty}$ (μg h ml^{-1})	106.8 ± 15.7 [a,c]	29.3 ± 6.8 [a]	3.3 ± 1.0
Bioavailability (%)	39.4 ± 5.8 [a,d]	19.9 ± 4.6 [a]	2.8 ± 0.9

Each value represents the mean ± S.D. of 3–4 determinations.
[a] $p < 0.01$.
[b] $p < 0.05$, compared with weanling rats.
[c] $p < 0.01$.
[d] $p < 0.05$, compared with suckling rats.

Table 3
Pharmacokinetic parameters after duodenal administration of tobramycin (30 mg/kg) to suckling and adult rats

Parameter	Suckling rats		Adult rats	
	Duodenal administration ($n = 4$)	Oral sham experiment ($n = 3$)	Duodenal administration ($n = 6$)	Oral sham experiment ($n = 4$)
$AUC_{0-\infty}$ ($\mu g\ h\ ml^{-1}$)	153.3 ± 49.6 [a,b]	40.8 ± 15.9	21.8 ± 4.6 [c]	4.4 ± 0.8 [a]
MRT (h)	5.6 ± 0.2	6.4 ± 1.2	2.0 ± 0.1	1.8 ± 0.9 [a]
Bioavailability (%)	104.0 ± 33.6 [a,b]	27.7 ± 10.8	27.2 ± 5.7 [c]	5.5 ± 1.1 [a]

Each value represents the mean ± S.D. of n experiments.
[a] $p < 0.05$, compared with oral sham experiment of suckling rats.
[b] $p < 0.001$, compared with duodenal administration of adult rats.
[c] $p < 0.01$, compared with oral sham experiment of adult rats.

tected for the ranges of initial concentrations studied.

3.4. Effect of metabolic inhibitors and analogues on tobramycin permeation

Fig. 4 shows the effect of neomycin, spermine, tetraethylammonium, choline chloride (10 mM) and 2,4-dinitrophenol (0.5 mM) on the mean cumulative amounts of tobramycin (100 μM) transferred from the mucosal to serosal side in the jejunum and ileum of suckling and adult rats. Neomycin and spermine significantly increased the transferred amounts of tobramycin in the jejunum and ileum of suckling rats and in the jejunum of adult rats. Thus, the coexistence of analogues facilitated the intestinal permeation of tobramycin. However, in the ileum of the adult rats, no such influence was found. Choline chloride and tetraethylammonium, which were transferred by the choline transport system (Sheard et al., 1986; Tsubaki et al., 1986), and the metabolic inhibitor, 2,4-dinitrophenol, did not affect the amounts in any of the regions at either age. This

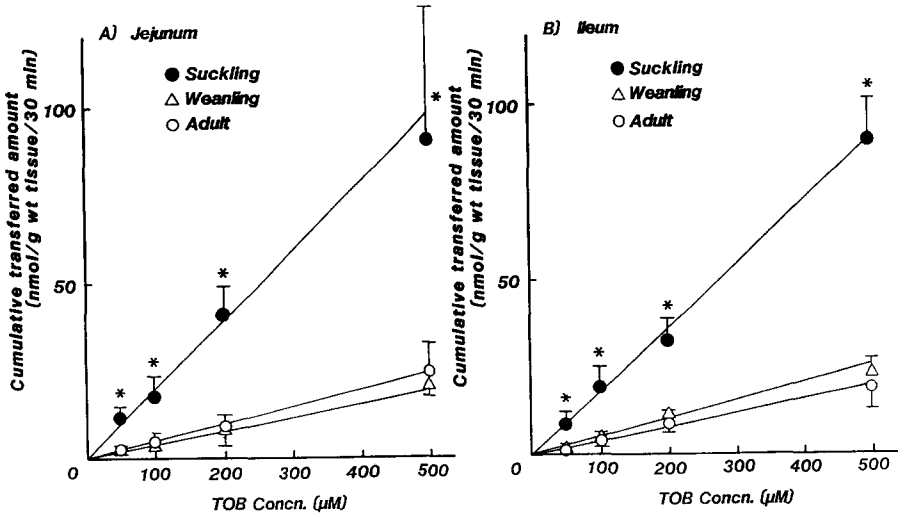

Fig. 3. Effect of the initial mucosal concentrations of tobramycin (TOB) (50–500 μM) on the cumulative amounts transferred from the mucosal to serosal side in 30 min at 37°C as observed by the everted sac method with (A) jejunum and (B) ileum of suckling (●), weanling (△) and adult (○) rats. Each point represents the mean ± S.D. of 3–8 determinations. * $p < 0.01$ compared with the mean values from adult rat.

suggests that the permeation of tobramycin in the jejunum and ileum of both suckling and adult rats may occur via passive diffusion without energy expenditure.

4. Discussion

Tobramycin, an AMG, is primarily eliminated from human and rat sera to the kidneys via glomerular filtration without being metabolized (Yamada et al., 1975; Haughey et al., 1980) and remains in the renal tissues for a long time (Regamey et al., 1972; Winslade et al., 1987). However, in premature and newborn infants (particularly 0–3 days after birth) with immature renal function, the elimination of tobramycin from the serum is delayed (McCracken and Nelson, 1977; Nahata et al., 1984). In the present study, we found that the elimination kinetic variables (CL_t, K_{10} and λ_2) after the i.v. injection of tobramycin to newborn rats significantly declined and the MRT value increased. These results are coincident with those for humans, as cited above.

The values of distribution at steady state per body weight (Vd_{ss}/BW) for many drugs are influenced by some age-dependent factors including protein binding, body compartment size and renal and/or hepatic disposition. Since tobramycin shows little binding to protein (Gordon et al., 1972) and rapidly diffuses into extracellular water fluid, the distribution value of tobramycin corresponds to the extracellular water volume per body weight (V_{ecw}/BW) (Koshida et al., 1987). The V_{ecw}/BW in the newborn is larger than that in the adult (Milsap et al., 1986). Therefore, our observations that the initial plasma concentration of tobramycin after i.v. administration to newborn rats was significantly lower than those of other ages and that the Vd_{ss}/BW of the newborn was greater than those of others support the findings described above.

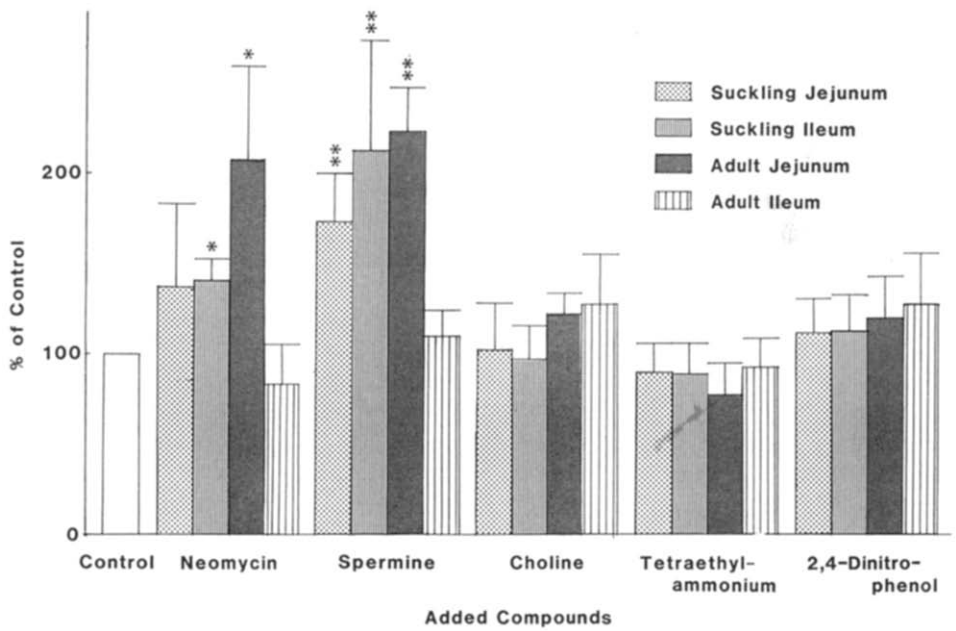

Fig. 4. Effect of neomycin, spermine, choline chloride, tetraethylammonium (10 mM), and 2,4-dinitrophenol (0.5 mM) on the cumulative amounts of tobramycin (100 μM) transferred from the mucosal to the serosal side in 30 min at 37°C as observed by the everted sac method with jejunum and ileum of suckling rats and jejunum and ileum of adult rats. Each point represents the mean ± S.D. of 3–8 experiments. * $p < 0.05$, ** $p < 0.01$ compared with the control group.

It is well known that AMGs are poorly absorbed from the fully mature adult intestine, since they are ionized and only slightly liposoluble at physiological pH. In addition, bile acids (Thompson et al., 1970) and/or intestinal mucin (Niibuchi et al., 1986) produce insoluble complexes with AMGs in the intestinal duct. However, this study revealed that tobramycin was readily absorbed from the intestine of newborn and suckling rats, but poorly absorbed from those of weanling and adult rats (Table 2). The highest bioavailability was observed in newborn rats. It has been reported that a raised plasma concentration rarely occurred when neomycin, an AMG, was orally administered to patients with impaired renal functions (Kunin et al., 1960). In our study, the C_{max} after oral administration in newborn rats did not significantly differ from that in suckling rats. Also, the mean elimination kinetic variables (λ_2, K_{12}, CL_t) after i.v. administration to newborns were lower than those in the suckling rats. Therefore, the difference in bioavailability after the p.o. administration between newborn and suckling rats can be attributed, at least in part, to their elimination process. However, in spite of the absence of distinction in the elimination between suckling and weanling rats, the bioavailability after p.o. administration to weanling rats markedly decreased compared with suckling rats. The cumulative transferred amounts of tobramycin in the jejunum and ileum of suckling rats by the in vitro everted sac method were significantly greater than those of weanling and adult rats (Fig. 3). Such an abrupt change was observed only at the weaning period.

The findings as mentioned above can be explained in several ways from the views of the functional development of the gastrointestinal tract. One explanation is that the developing gastrointestinal system during the weaning period undergoes functional transformations due to dietary changes (from milk for sucklings to solid diet for adults) and thereby morphological alterations, e.g., lengthening of the villi and proliferation of epithelial cells (Lev, 1981) as well as changes in membrane composition (Chu et al., 1988). Another explanation is that several biophysical alterations, e.g., pH, motility (Murray et al., 1989), enzyme activity (Raul et al., 1977; Biol et al., 1987), hormone activity (Henning, 1978) and membrane fluidity (Schwarz et al., 1985; Hiibner et al., 1988), would occur during the developing stages (Henning, 1987). It has been reported that the intestinal absorption of nutrient metals [iron (Gallagher et al., 1973) and copper (Mills et al., 1979)], toxic elements (Henning, 1987), amino acids (Rubino et al., 1975; Buddington et al., 1990), glucose (Ghishan et al., 1985), water, electrolytes (Younozai, 1981), lipids (Meddings et al., 1989) and macromolecules (Daniels et al., 1973) including proteins will change markedly during the weaning period (Henning, 1987). Thus, a similar phenomenon may, in theory, also occur for drugs. Consequently, the higher bioavailability of tobramycin in suckling rats than in weanling rats is attributable to the difference in membrane structure related to intestinal membrane permeability and/or developmental environments surrounding the intestine rather than that in the excretory function between suckling and weanling rats.

In suckling humans and rats, there has been evidence suggesting that both the secretion and reabsorption of bile acids are lower than in adult humans (Henning, 1987) and rats (Yousef et al., 1982). Therefore, it seems likely that a decline in the concentration of bile acids, which form insoluble complexes with tobramycin in the intestinal tract of suckling rats, may lead partly to an improvement of the intestinal absorption of tobramycin in vivo. More detailed studies are required to clarify this point.

Tobramycin permeation in vitro showed no saturation phenomenon with respect to the initial tobramycin concentrations (Fig. 3) and was not reduced by the coexistence of another AMG, polyamine, quaternary compounds and metabolic inhibitor (Fig. 4). Therefore, the permeation of tobramycin in the jejunum and ileum of both suckling and adult rats was mediated predominantly via a passive diffusion mechanism. Further studies are needed to determine the permeation route (paracellular or transcellular pathway) and the factor responsible for the intestinal permeation of tobramycin in suckling rats.

Although the cumulative transferred amounts

of tobramycin were linear over the concentration range of 50–500 μM tobramycin (Fig. 3), the other aminoglycoside and polyamine enhanced permeation in the jejunum of adult rats and in the jejunum and ileum of suckling rats (Fig. 4). Moreover, the bioavailability after duodenal administration of tobramycin in vivo was significantly greater than that in sham oral experiments (Table 3). Tobramycin was scarcely degraded in the gastrointestinal tract, and showed little binding to gastrointestinal mucin obtained from pigs (our unpublished observation, data not shown). Therefore, a high concentration of AMGs or polyamines seemed to enhance the intestinal permeability of tobramycin.

The surface of the small intestinal membrane is known to be rich in anionic charge regions (e.g., phospholipids, mucopolysaccharides). Ionic interaction between the liposomes containing anionic phospholipids and AMGs having several amino groups (cationic groups) has been found to increase the liposome membrane permeability of glucose (Aramaki et al., 1989) and fluorescent probes (Au et al., 1987). Thus, in the present study, the ionic interaction between several amino groups in AMGs and polyamines and the surface of the small intestinal membrane might have changed the intestinal membrane permeability of tobramycin. Further investigation is needed along these lines.

In closing, the present results suggest two important implications for future studies on gastrointestinal drug absorption: (1) if the mechanism(s) by which a less lipophilic drug like tobramycin is enhanced in the suckling rats compared with the mature (weanling and adult) rats can be clarified, it should be possible to improve the absorption capacity of drugs with a low lipophilicity by pharmaceutical manipulation; and (2) a hydrophilic drug like tobramycin is better absorbed in the suckling than in the adult rats, and therefore the drug transferred to suckling babies from the mothers via breast milk may be better absorbed from the suckling gastrointestinal tract, thereby leading to a possibly greater incidence of ototoxicity and nephrotoxicity of suckling babies born to mothers receiving AMG (e.g., tobramycin) therapy.

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