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# Fasting Increases Tobramycin Oral Absorption in Mice▽

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**The pharmacokinetics of the aminoglycoside tobramycin was evaluated after oral administration to fed or fasting (15 h) mice. As expected, under normal feeding conditions, oral absorption was negligible; however, fasting induced a dramatic increase in tobramycin bioavailability. The dual-sugar test with lactulose and L-rhamnose confirmed increased small bowel permeability via the paracellular route in fasting animals. When experiments aimed at increasing the oral bioavailability of hydrophilic compounds are performed, timing of fasting should be extremely accurate.**

The aminoglycoside antibiotic tobramycin is often employed in the treatment of serious infections caused by *Pseudomonas aeruginosa*, in particular in patients with cystic fibrosis (4). Aminoglycosides are polyaminated compounds and, at physiological pHs, exist as polycations that are too hydrophilic to diffuse freely across cell membranes. As a consequence, they are distributed into the extracellular space, are not metabolized, and are excreted from the body primarily by renal glomerular filtration (7); in addition, they are not absorbed from the gastrointestinal tract and have to be administered parenterally. Oral delivery presents a series of advantages, such as the avoidance of pain and discomfort associated with injections and the convenience of home treatment, that are particularly relevant for pediatric patients with chronic diseases; considerable attention has therefore been directed at finding ways to increase the oral bioavailability of these compounds.

In the course of experiments aimed at increasing tobramycin bioavailability in mice, we observed that its oral absorption was increased in fasting animals. The pharmacokinetics of the aminoglycoside in fed and fasting animals was therefore studied; an innovative dual-sugar test based on the drop whole-blood assay in mice, commonly used to evaluate the permeability of the paracellular route (14, 16), was also applied.

Tobramycin sulfate was diluted in physiologic solution; lactulose (L) and L-rhamnose (R) were dissolved separately in Milli-Q water, and the sugar solutions were mixed before administration. A volume of 0.1 ml per 10 g of animal weight was administered. All chemicals were purchased from Sigma-Aldrich (Milano, Italy).

BALB/c male mice (Harlan, Udine, Italy) aged 6 to 8 weeks (25 to 30 g) were used. All animals were acclimatized under standard conditions to the laboratory environment for 1 week before the experiment, with free access to tap water and pelleted food. In some experiments, mice were deprived of food for 15 h before treatment but had free access to tap water. All experiments were carried out in accordance with the current European and international regulations, and approval for research was obtained from the Ethical Committee for Animal Experimentation of the University of Trieste.

Tobramycin was administered intravenously in the tail vein or intramuscularly in the hind leg muscle in normally fed mice (10 mg/kg of body weight) and orally (50 mg/kg) by gavage in fed and fasted animals. Mice were randomly divided into groups of 3 animals each, corresponding to the time points of blood collection (0, 5, 15, 30, 60, 120, and 180 min). In addition, the drug was orally administered at doses of 25 and 10 mg/kg in 2 groups of 3 fasted animals each, and blood was obtained 15 min after drug administration.

For the dual-sugar test, animals were randomly selected and divided into two groups of 6 animals each; the L and R solutions (90 mg/kg of each sugar) were orally administered in fed (group 1) or fasting (group 2) mice. After 1 week, the same solution was orally administered to mice of group 2 that, this time, had free access to food. Blood was obtained 60 min after the administration of the sugar solution.

Blood was collected from the submandibular vein directly into heparinized test tubes, and plasma was recovered by centrifugation at 2,000 × *g* at 4°C and stored at −20°C until used. Tobramycin concentration was determined by a homogeneous enzyme immunoassay (Emit 2000 Tobramicina; Siemens Healthcare Diagnostic SRL, Milano, Italy) according to the manufacturer's instructions. The concentrations of L and R were determined by a high-performance liquid chromatography (HPLC) assay as previously described (14), and the L/R ratio was calculated.

A noncompartmental method using WinNonLin software version 5.2.1 (Pharsight Co., Mountain View, CA) was employed to calculate values for pharmacokinetic parameters.

Statistical significance was calculated using the nonparametric Mann-Whitney U test and the Wilcoxon matched-pairs test.

The plasma concentration time profiles observed after oral administration of tobramycin (50 mg/kg) in fasting and fed mice are reported in Fig. 1, and the values for the pharmacokinetic parameters are shown in Table 1. Under normal feeding conditions, tobramycin plasma levels were extremely low,

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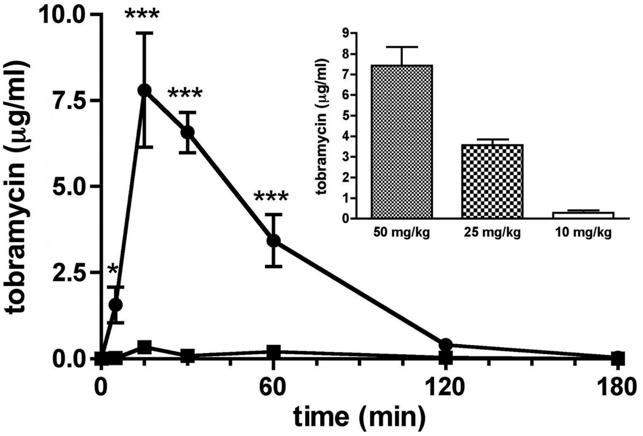

FIG. 1. Mean plasma concentration-time profiles observed after oral administration of tobramycin (50 mg/kg) to fasted (●) or fed (■) mice. Bars represent the standard errors (SE) (3 animals per point). Significant differences from the levels observed for normally fed animals at $P$ values of $<$0.05 (*) and $<$0.001 (***) (two-way analysis of variance [ANOVA] with the Bonferroni posttest) are indicated. (Insert) Tobramycin plasma concentrations 15 min after oral administration of tobramycin at 50 mg/kg, 25 mg/kg, and 10 mg/kg to 3 fasted mice (means ± SE).

confirming its extremely poor gastrointestinal absorption (7); a 15-h fasting induced a dramatic increase in the area under the curve (AUC) as well as a level of bioavailability of tobramycin that was 25-fold greater than that observed in normally fed mice (Table 1). A concentration-dependent absorption of the antibiotic was also evident (Fig. 1).

The paracellular route is the dominant pathway for transepithelial flow of hydrophobic drugs in the small intestine (15), and permeability depends on the presence and regulation of intercellular tight junctions (1). These structures are subjected to physiological regulation and are modulated in response to a variety of stimuli, including dietary state, humoral or neuronal signals, inflammatory mediators, and a variety of cellular pathways that can be activated by microbial or viral pathogens (2). Fasting and malnutrition increase movements of ions and large molecules through the paracellular route (5, 23, 26) and decrease the number of tight junction strands in jejunal epithelia in animals (20); in addition, dietary state also modifies intestinal permeability in humans (25).

To assess small intestine permeability in normally fed and fasting mice, we used the dual-sugar test with L and R, a noninvasive method useful for monitoring changes in the permeability of the paracellular pathway in the small intestine (2, 14, 17). In fasting mice, the plasma L/R ratio 60 min after oral administration of the solution was 4 times higher than in fed mice (Fig. 2), further suggesting that the increased permeability was mainly via the paracellular route. The phenomenon appears to be a reversible process: when the same fasted mice were maintained at a normal diet for 1 week, the L/R ratio returned to values obtained with normally fed mice.

The observation that fasting significantly increases the gastrointestinal absorption of tobramycin is of particular interest; indeed, a number of studies have recently been performed with the aim of improving the oral absorption of low-bioavailability drugs, increasing their paracellular transport (3, 11, 13, 18, 24). Most reported absorption enhancers produced increases in *in vivo* absorption up to 15- to 50-fold; however, in the present study, we show that fasting increases tobramycin bioavailability up to 25-fold. This suggests that, when this type of study is performed, timing of fasting should be extremely accurate and clearly indicated.

Mice and rats are hindgut fermenters and have developed characteristic strategies of digestion (22); however, a number of studies have shown that gastrointestinal drug absorption rates are comparable in rodents and humans (8, 9, 27). These species also show similar transporter expression pathways in the small intestine but distinct expression levels and patterns for metabolizing enzymes (6); since aminoglycosides are not metabolized (7), the rodent should be considered a good predictor of oral tobramycin absorption. Anecdotic reports of increased serum concentrations after oral administration of aminoglycosides in patients with gastrointestinal diseases, characterized by a high level of intestinal permeability, have been published (12, 19, 21), and this condition also leads to drug-induced toxicity (21). Studies are therefore needed to clarify if the increase in oral permeability induced by fasting, and described in this paper, could result in enhanced blood concentrations also in humans.

In conclusion, we have demonstrated for the first time that tobramycin bioavailability depends on a physiological condition; indeed, the oral absorption of the drug is significantly improved when animals are fasted for 15 h. As all aminogly-

TABLE 1. Values obtained for pharmacokinetic parameters after oral, intramuscular, and intravenous administration of tobramycin to fasted and fed mice (3 animals per point)[a]

| Property | Mean ± SE for indicated route and condition | | | |
|---|---|---|---|---|
| | Oral | | i.m. | i.v. |
| | Fed | 15-h fasted | | |
| Dose (mg/kg) | 50 | 50 | 10 | 10 |
| $C_{max}$ (µg/ml) | 0.33 ± 0.09 | 7.8 ± 0.95 | 16.46 ± 0.89 | 35.26 ± 0.79 |
| $T_{max}$ (min) | 15 | 15 | 15 | 5 |
| $t_{1/2}$ (min) | 41.61 | 21.97 | 17.70 | 20.33 |
| $AUC_{last}$ (µg · min/ml) | 16.43 ± 1.26 | 423.68 ± 24.23 | 658.69 ± 37.00 | 682.93 ± 29.10 |
| $AUC_{inf}$ (µg · min/ml) | 18.94 | 437.80 | 667.02 | 687.47 |
| $F$ (%) | 0.48 | 12.40 | 96.45 | 100 |

[a] $C_{max}$, maximum concentration of drug in serum; $T_{max}$, time to maximum concentration of drug in serum; $F$, bioavailability; $t_{1/2}$, half-life; $AUC_{last}$, area under the curve from time zero to last determined concentration-time point; $AUC_{inf}$, area under the curve from time zero to infinity; i.m., intramuscular; i.v., intravenous.

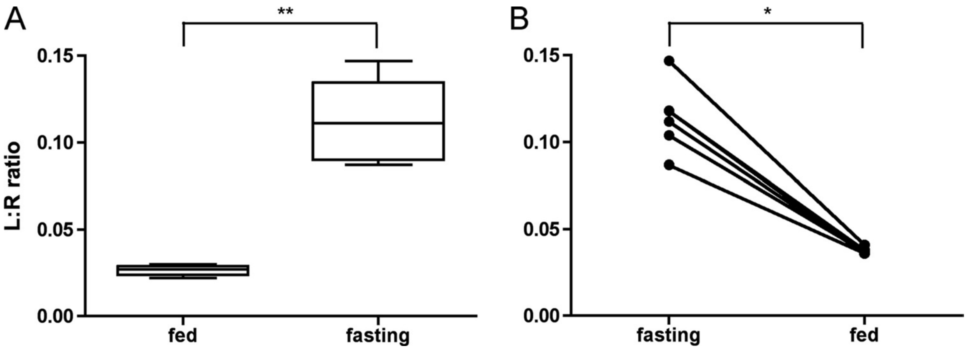

FIG. 2. (A) L/R ratios in fasting ($n = 6$) and fed ($n = 6$) mice 60 min after oral administration of dual-sugar solution (means ± SE). $**$, $P < 0.01$ (Mann-Whitney U test). (B) L/R ratio in the same mice ($n = 6$) under fasting and fed conditions. $*$, $P < 0.05$ (Wilcoxon matched-pairs test).

cosides are polar and exist as polycations, it is possible that all compounds of this family are absorbed in this situation. Animal studies of oral delivery of aminoglycosides, and probably other hydrophobic compounds, should define timing of fasting with particular precision; in addition, caution should be used when patients (for example, children with short bowel syndrome [10], who are often treated with rotating courses of enteral antibiotics, including aminoglycosides, for prevention or treatment of bacterial overgrowth in the small intestine) are orally treated with these compounds.

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