Notes

Modulation of the Intestinal Flora of Mice by Treatment with Aztreonam and Tigemonam

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Oral and parenteral administration of aztreonam and oral administration of tigemonam to conventional mice caused a decrease in the number of aerobic gram-negative rods in the feces. Oral treatment with high doses of aztreonam (≥25 mg/kg/day) and tigemonam (100 mg/kg/day) adversely influenced colonization resistance, whereas oral treatment with lower doses of the monobactams or parenteral treatment with aztreonam did not.

Monobactams are a relatively new group of beta-lactam antibiotics with antibacterial activity against most aerobic gram-negative rods but limited activity against gram-positive and anaerobic bacteria (2). Aztreonam has been used in regimens for empirical antimicrobial treatment of suspected infections in granulocytopenic patients (8) because of its potent in vitro activity against gram-negative rods. Furthermore, it has been suggested that orally administered aztreonam may be suitable for selective elimination of gram-negative bacilli from the gastrointestinal tract (4). Tigemonam is a monobactam which, unlike aztreonam, is well absorbed after oral administration. Its in vitro antibacterial spectrum resembles that of aztreonam.

In another article (15), we described the antibacterial effect of aztreonam and tigemonam in an experimental infection with Escherichia coli and Klebsiella pneumoniae in irradiated mice. The present study was undertaken to evaluate the potential of aztreonam and tigemonam to eliminate aerobic gram-negative rods from the gastrointestinal tract and to evaluate the effect of these drugs on colonization resistance in mice, in order to obtain information about the potential usefulness of these two drugs.

Female specific-pathogen-free Swiss mice were given aztreonam orally or subcutaneously or tigemonam orally. Several parameters of intestinal ecology were measured as described previously (14) during treatment with single daily doses of these antimicrobial agents up to 100 mg/kg/day.

The administration of the monobactams resulted in a dose-dependent decrease in the number of gram-negative rods in the feces; subcutaneous administration of aztreonam was the most effective in this respect, oral administration of tigemonam was less effective, and oral administration of aztreonam was the least effective (Fig. 1). The gram-negative species isolated from the feces before treatment were mainly E. coli, Proteus mirabilis, and Morganella morganii, among which E. coli accounted for more than 90%. These bacteria were all susceptible to the monobactams in vitro (MIC, <0.25 μg/ml). None of the treatment schedules with a monobactam led to complete eradication of the aerobic gram-negative rods from the feces. No change in the susceptibility of the isolated bacteria to either aztreonam or tigemonam was found during the study. Spontaneous colonization with low numbers of a strain of Pseudomonas aeruginosa that was moderately susceptible to aztreonam in vitro (MIC, 1 to 4 μg/ml) was observed during treatment with parenterally administered aztreonam but not during oral treatment with the monobactams. These results showed that in mice treatment with monobactams leads to a decrease of the number of aerobic gram-negative rods in the feces. The finding that the decontaminating effect of oral administration of aztreonam is less than that of parenteral administration is somewhat puzzling, because the concentration of aztreonam recovered from cecal contents is lower after subcutaneous administration than after oral administration. A possible explanation for the superior decontaminating efficacy of parenterally administered aztreonam could be that aztreonam reaches the deep crypts of the intestinal epithelium only after parenteral administration and there exerts its antibacterial effect against aerobic gram-negative rods attached to the epithelium.

Oral treatment with aztreonam and tigemonam led to higher numbers of Candida albicans in the feces in a murine model of gastrointestinal candidiasis (12), but the difference with the controls was only statistically significant at a dose of 100 mg/kg (Fig. 2). In animals treated subcutaneously with aztreonam, the numbers of Candida albicans in the feces were not significantly different from those in the controls. These findings indicate that oral treatment with monobactams reduces colonization resistance of the gastrointestinal tract for Candida albicans, although to a small extent since the observed difference in the numbers of Candida albicans did not amount to more than 10-fold.

After the mice had been contaminated with an aztreonam-resistant strain of Citrobacter freundii, oral or parenteral treatment with aztreonam had no demonstrable effect on the numbers of this strain in the feces (data not shown but summarized in Table 1). Likewise, oral treatment with tigemonam had no demonstrable effect on the number of...
tigemonam-resistant \textit{Pseudomonas aeruginosa} in the feces after oral contamination with this strain (data not shown but summarized in Table 1).

Since an increase of the relative cecal weight is generally observed in mice with a disturbed microbial ecology of the intestinal tract (10), we determined this parameter after 10 days of treatment with the monobactams. Oral treatment with 100 mg of aztreonam per kg per day led to a significant increase of the relative cecal weight from 16.6 mg/g of body weight in the controls to 32.4 mg/g of body weight (standard deviation, 8.2), and oral treatment with 25 mg of aztreonam per kg per day led to an increase to 22.9 mg/g of body weight (standard deviation, 4.1). Oral treatment with lower dosages of aztreonam, parenteral treatment with aztreonam, or oral treatment with tigemonam did not lead to a significant increase in the relative cecal weight (Table 1).

Since the anaerobic bacteria outnumber the aerobic bacteria in the cecum by 1,000 to 1, the total number of anaerobic bacteria can be determined by direct microscopical counts of the homogenized cecum, as described by Holdeman and Moore (6). Treatment with the monobactams did not have a significant effect on the total number of microscopically detectable bacteria in the cecum 10 days after the start of treatment (Table 1).

After administration of a single dose of 100 mg/kg, concentrations of the monobactams in cecum contents were determined by high-performance liquid chromatography as described previously (3, 16). The peak concentration and the area under the concentration-time curve from 0 to 5 h were 506 \( \mu \text{g/g} \) and 300 \( \mu \text{g} \cdot \text{h/g} \) for subcutaneously administered aztreonam, 6,130 \( \mu \text{g/g} \) and 22,000 \( \mu \text{g} \cdot \text{h/g} \) for orally administered aztreonam, and 902 \( \mu \text{g/g} \) and 1,400 \( \mu \text{g} \cdot \text{h/g} \) for orally administered tigemonam, respectively.

The results show that oral treatment with high doses of the monobactams caused a decrease of colonization resistance but that parenteral administration of aztreonam did not, although the spontaneous colonization with low numbers of \textit{Pseudomonas aeruginosa} could indicate that the intestinal microbial ecology was slightly disturbed. Several studies have shown that oral or parenteral treatment with aztreonam in humans causes a pronounced decrease in the number of aerobic gram-negative rods without altering the composition.

\textbf{FIG. 1.} Effect of treatment with the monobactams for 9 or 10 days on the indigenous aerobic gram-negative flora in the feces of mice. Each symbol represents the geometric mean of the number of aerobic gram-negative bacteria per gram of feces of five mice. The numbers at the end of each curve refer to the daily dose administered. AGNR, Aerobic gram-negative rods.

\textbf{FIG. 2.} Effect of treatment with the monobactams for 10 days on the recovery of \textit{Candida albicans} in the feces of mice. Each symbol represents the geometric mean of the number of \textit{Candida albicans} per gram of feces of eight mice. The numbers at the end of each curve refer to the daily dose administered.
TABLE 1. Comparison of various effects of the monobactams on intestinal ecology in mice

| Drug           | Dose (mg/kg) | Relative cecal wt | Total bacterial count[a] | No. of Candida albicans in feces | No. of resistant AGNR[b] in feces |
|----------------|--------------|-------------------|--------------------------|----------------------------------|----------------------------------|
| Aztreonam (sub- | 100          | =                 | =                        | =                                | =                                |
| cutaneously)    | 25           | =                 | =                        | =                                | =                                |
|                | 6.25         | =                 | =                        | =                                | =                                |
| Aztreonam      | 100          | =                 | =                        | =                                | =                                |
| (orally)       | 25           | =                 | =                        | =                                | =                                |
|                | 6.25         | =                 | =                        | =                                | =                                |
| Tigemonam      | 100          | =                 | =                        | =                                | =                                |
| (orally)       | 25           | =                 | =                        | =                                | =                                |
|                | 6.25         | =                 | =                        | =                                | =                                |

[a] = not significantly different from the value in the control mice; ↑, significantly higher than in control mice at day 10 after start of treatment.
[b] Total bacterial count, Number of microscopically detectable bacteria in the cecum.
[c] AGNR, Aerobic gram-negative rods.

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