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Influence of Administration Routes of Sodium Ampicillin on the Cecal Flora in Rats: Role of Biliary Excretion[1)]

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The role of biliary excretion of sodium ampicillin (ABPC Na) was studied in relation to the influence of drug administration routes on the cecal flora in rats. When ABPC Na was administered intravenously, orally or rectally to normal rats, populations of bacteria such as *Enterobacteriaceae*, *Streptococci*, *Staphylococci*, and *Bacteroidaceae* in the cecal contents decreased by a factor of 10 to 100 at 9 h after administration. However, the changes of the cecal flora by ABPC Na were not influenced by the administration routes. To investigate the role of biliary excretion of ABPC Na intravenous administration to bile-fistula rats was performed. However, in the absence of bile in the intestinal lumen, the bacterial populations in the cecal contents were markedly modified even in rats without drug treatment. Therefore, the influence of biliary excretion of ABPC Na on the cecal flora was investigated using bile-supplied bile-fistula rats.

When ABPC Na was given orally to these bile-supplied bile-fistula rats, *Enterobacteriaceae*, *Streptococci*, and *Staphylococci* decreased markedly. On the other hand, when ABPC Na was given intravenously, changes in the cecal flora were not observed. These observations suggest that antibiotics which show low biliary excretion will have little effect on the intestinal flora if administered intravenously or rectally.

Keywords——ampicillin; oral and rectal ampicillin administration; biliary excretion of ampicillin; cecal flora; influence of ampicillin on cecal flora; bile fistula rats

In the previous report,[2)] the influence of administration routes of sodium ampicillin (ABPC Na) on the cecal flora was investigated in rats. It was found that although the concentration of ABPC Na in the cecal contents after single or multiple oral administration was higher than that after intravenous or rectal administrations, rather high concentrations of ABPC Na were observed in cecal contents even after intraveneous and rectal administrations. ABPC Na in the cecal contents after administration by each of the three different routes caused changes of cecal bacterial populations, and these changes seemed to be independent of the route of administration. These results were attributed to the fact that the concentration of ABPC Na in the cecal contents after administration by all three routes was larger than the minimum inhibitory concentration of ABPC Na for all the cecal flora studied. The presence of ABPC Na in the cecal contents after oral administration was considered to be mainly due to low absorbability of ABPC Na from the gastrointestinal tract,[3)] while that after intravenous or rectal administration was considered to be mainly due to the biliary excretion of ABPC Na.

In the present study, th role of biliary excretion of ABPC Na was studied in relation to the influence of drug administration routes on the cecal flora in rats.

Experimental

Materials——ABPC Na (912 μg/mg) was obtained from a commercial source and used without further

purification. All other reagents and culture media (described in the previous report[2]) were of reagent grade and were used without further purification.

Animal Study——Male Wistar rats weighing 180—200 g were used. All rats were maintained in laboratory cages for at least one week after purchase. All rats received commercial chow pellets (MF, Orientalkobo Industries, Ltd.) and water *ad libitum*. Intravenous, oral and rectal administrations of ABPC Na were performed as described previously.[2]

Intravenous Administration: ABPC Na dissolved in saline at the concentration of 50 mg/ml was administered into the tail vein at a dose of 50 mg/kg.

Oral administration: ABPC Na dissolved in deionized water at the concentration of 50 mg/ml was administered through a stomach tube at a dose of 50 mg/kg.

Rectal Administration: Fifty mg of ABPC Na was well dispersed in a melted mixture of 20 mg of BL-9EX as an absorption promoter and 0.91 g of Witepsol H-15 (Dynamic Nobel Chemicals, West Germany) as a suppository base by sonication in an ultrasonic cleaner for 30 seconds at 40 °C. The melted suppository was introduced into a glass tube (inner diameter of 4 mm) and kept horizontally at room temperature. After solidifying, the cyclindrical suppository was pushed out of the glass tube. One gram of the suppository/kg body weight (equivalent to 50 mg ABPC Na/kg) was applied to the rectum. After application of the suppository, the anus was closed with a drop of surgical cement (Aronalpha, Sankyo Co., Ltd., Tokyo) to prevent leakage of the melted suppository.

Biliary Excretion of ABPC Na——Male Wistar rats, 180—200 g, were anesthetized by ether inhalation. After laparotomy, the proximal bile duct was cannulated with 15 cm of polyethylene tubing (PE 10, Clay Adams). The bile duct-cannulated rats were housed in Bollman cages to collect bile after oral or intravenous administration of ABPC Na. The amount of ABPC Na excreted *via* the bile duct was determined by microbioassay. During bile collection, rats were given food and water freely. In the case of administration of bile to the bile-fistula rats, another piece of polyethylene tubing was cannulated into the duodenal lumen *via* the distal bile duct, and bile freshly collected from untreated control rats was injected through this tubing at the rate of 1 ml/h with a micro tube pump.

Cecal Flora Culture——Cecal flora of rats was cultured as described previously.[2] Aerobic and anaerobic microorganisms in the cecal contents were cultured on each selective medium after appropriate dilution.

Analytical Methods——Blood, bile and the cecal contents sample were diluted appropriately with deionized water. The concentration of ABPC Na in samples was determined by microbioassay with *Bacillus subtilis* ATCC 6633 as a test organism by the usual paper disc method.

Results and Discussion

Cecal bacterial populations after oral, intravenous or rectal administration of ABPC Na at the dose of 50 mg/kg were determined 9 and 24 h after the drug administration. As shown in Fig. 1, populations of bacteria such as *Enterobacteriaceae, Streptococci, Staphylococci,* and *Bacteroidaceae* in the cecal contents decreased by a factor of 10 to 100 at 9 h after administration. However, they had recovered again to the normal levels at 24 h. These decreases and recoveries corresponded to the amount of ABPC Na observed in the cecal contents after administration of ABPC Na.[2] The changes of the cecal flora by ABPC Na were not influenced by the administration routes.

Generally, the drug observed in the intestinal lumen after intravenous administration is

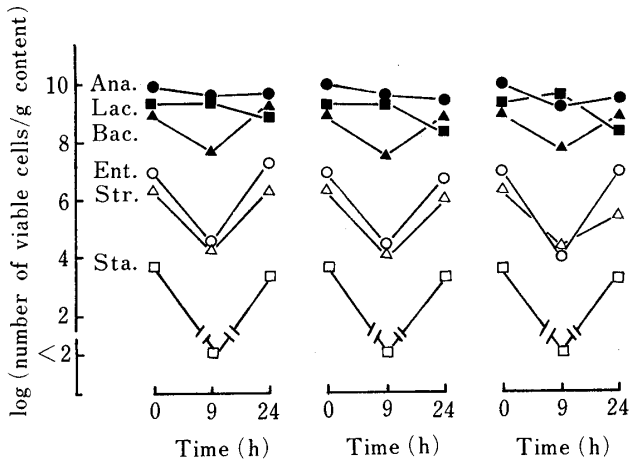

Fig. 1. Changes of Cecal Flora after Oral (Left), Intravenous (Center), or Rectal (Right) Administration of ABPC Na at the Dose of 50 mg/kg in Rats

Ent. (—○—), *Enterobacteriaceae*; Str. (—△—), *Streptococci*; Sta. (—□—), *Staphylococci*; Ana. (—●—), anaerobic bacteria; Bac. (—▲—), *Bacteroidaceae*; Lac. (—■—), *Lactobacilli*.

Rectal preparation: ABPC Na (5%) and BL-9EX (2%) were suspended in Witepsol H-15.

Dose: 50 mg ABPC Na/g suppository/kg.

Each point represents the mean of the logarithm of the number of viable cells/g content for 5—26 rats. The coefficients of variation of points are less than 13%.

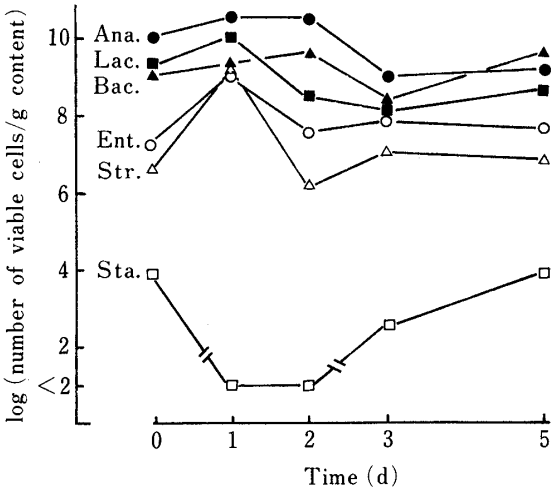

Fig. 2. Changes of Cecal Flora in Bile Duct-Ligated Rats

Abbreviations are the same as in Fig. 1.
Each point represents the mean of the logarithm of the number of viable cells/g content for 5—6 rats. The coefficients of variation of points are less than 13%.

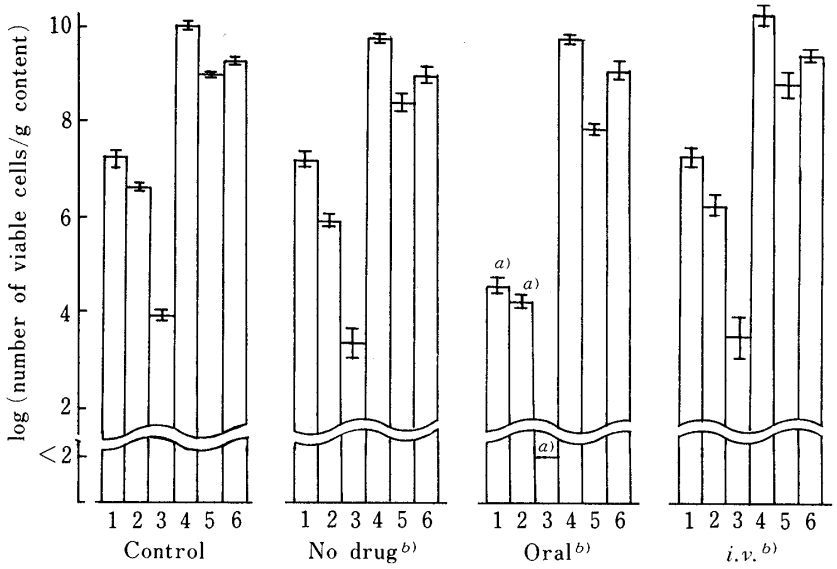

Fig. 3. Changes of Cecal Flora after Oral or Intravenous Administration of ABPC Na at the Dose of 50 mg/kg in Bile-Supplied Bile-Fistula Rats

1, Ent.; 2, Str.; 3, Sta.; 4, Ana.; 5, Bac.; 6, Lac. Abbreviations are the same as in Fig. 1.
a) Data were analyzed by means of Student's *t*-test. $p<0.01$: *versus* no drug.[1)]
b) Bile freshly collected from untreated control rats was supplied to the intestinal lumen of bile-fistula rats at the rate of 1 ml/h. Cecal flora was cultured at 9 h after drug administration. Each point represents the mean ± S.E. of the logarithm of the number of viable cells/g content for 3—26 rats.

considered to be derived from biliary excretion and secretion *via* the intestinal membrane. In rectal administration, spreading of the drug in the suppository base through the colon must also be taken into consideration.[4)] However, when ABPC Na was administered intravenously or rectally to bile-fistula rats, ABPC Na was not detected in the cecal contents by microbioassay. The biliary excretion of ABPC Na within 6 h after intravenous administration at the dose of 50 mg/kg was 18.8 ± 3.4%. This amount corresponds to the amount observed in the cecal concents 6 h after intravenous administration of ABPC Na. These results suggest that biliary excretion *via* the bile duct rather than secretion and/or spreading is mainly responsible for the appearance of ABPC Na in the intestinal lumen and thus for changes of bacterial population in the cecal contents after intravenous or rectal administration. To confirm this suggestion, intravenous administration was performed in bile-fistula rats. Since the bile fistula itself may influence the bacterial population in the cecal contents, the effect of

bile on the cecal flora was investigated using bile duct-ligated rats.

As shown in Fig. 2, the absence of bile in the intestinal lumen in bile-fistula rats resulted in a marked modification of the bacterial population in the cecal contents. This result indicates that bile secreted from the bile duct has a role in maintaining the normal flora in the rats. Therefore, the influence of biliary excretion of ABPC Na on the cecal flora should be investigated using bile-fistula rats which are supplied with adequate bile to the intestinal lumen by another route. In the present study, bile freshly collected from untreated control rats was supplied to the intestinal lumen of bile-fistula rats at the rate of 1 ml/h *via* polyethylene tubing cannulated into the duodenum through the distal bile duct.

As shown in Fig. 3, cecal bacterial populations in bile-supplied bile-fistula rats were similar to those of the untreated control rats. When ABPC Na was given orally at the dose of 50 mg/kg to the bile-supplied bile-fistula rats, *Enterobacteriaceae*, *Streptococci*, and *Staphylococci* decreased markedly, as found in normal rats (Fig. 1). On the other hand, similar results to the untreated control rats were observed after intravenous administration of ABPC Na at the same dose. As reported previously,[2] plasma levels of ABPC Na and concentrations of ABPC Na in the cecal contents after rectal administration were similar to those after intravenous administration. Thus, in bile-supplied bile-fistula rats, rectal administration is expected not to influence the bacterial populations, as observed in the case of intravenous administration. These observations confirm that the biliary excretion of ABPC Na in normal rats contributes to the modification of cecal flora by the antibiotic after intravenous or rectal administration. It is likely that antibiotics which show low biliary excretion will have little effect on the intestinal flora if administered intravenously or rectally.

It has been reported that the biliary excretion of most antibiotics except rifamycin SV and rifamide is quantitatively low in man, although that in rats is rather high.[5,6] Acocella *et al.* reported that the biliary excretion of ABPC was 0.03% of administered dose/12 h in man.[7]

It is generally known that orally administered ABPC often causes enteritis and/or diarrhea as side effects, especially in children.[8] The present results suggest that rectal administration of ABPC Na will have some advantages over oral administration in regard to the effect on the cecal flora.

References and Notes

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