Effect of Pretreatment with Antibiotics on the Hydrolysis of Salicyluric Acid in Rabbit Intestinal Microorganisms

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The effect of pretreatment with antibiotics on the hydrolysis of salicyluric acid in rabbit intestinal microorganisms was investigated. Latamoxef sodium (LMOX, 25 mg/kg/d, intravenously) and cephalixin (CEX, 16.7 mg/kg/d, orally) were administered for 1 or 3 d. The blood concentration of salicyluric acid and salicylic acid following oral, intracecal and rectal administration of salicyluric acid was determined. By the pretreatment with LMOX for 1 or 3 d, the blood concentration of salicylic acid following oral administration of salicyluric acid was slightly decreased. In rabbits pretreated with CEX for 3 d, the blood concentration of salicylic acid was detected at low concentration. By the pretreatment with LMOX and CEX, however, the decrease in the blood concentration of salicylic acid following rectal administration of salicyluric acid was not observed. Although the examination of population of intestinal microorganisms induced by the pretreatment with antibiotics was not performed, the metabolic activity of intestinal microorganisms may be changed.

Keywords — salicyluric acid; salicylic acid; gut flora; intestinal microorganism; prodrug; rabbit; antibiotic; cephalixin; latamoxef; glycine conjugate

Introduction

The significance of the intestinal microorganisms to pharmacokinetics and biopharmacy has been emphasized with respect to their ability to metabolize drugs and foreign compounds.1–6 In the previous reports, we examined the blood concentration of salicyluric acid and salicylic acid following the intravenous, oral, intracecal and rectal administration of salicyluric acid in rabbits,7–10 rats11 and dogs.12 In these species, salicyluric acid is metabolized to salicylic acid by intestinal microorganisms. Following rectal administration of salicyluric acid, prolonged blood concentration of salicylic acid was observed. However, species difference in the metabolic fate of salicyluric acid following oral and intravenous administration was recognized.

The administration of antibiotics often results in an alteration of the bacterial population in the gastrointestinal tract. This may be the therapeutic goal when overgrowth of indigenous or nonindigenous microorganisms produce disease. The bacterial population of the small intestine has been shown to influence the absorption of a variety of dietary substances.13–17 Also, antibiotics have been shown to change the disposition of drugs susceptible to metabolism by intestinal microorganisms.18–25

To provide more information on the physiological factors in the hydrolysis of salicyluric acid by intestinal microorganisms, we examined the oral, intracecal and rectal administration of salicyluric acid in rabbits pretreated with antibiotics.

Materials and Methods

Materials — Salicyluric acid was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Acetonitrile, acetic acid, methanol and o-methoxybenzoic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Cephalixin (CEX) and latamoxef sodium (LMOX) were kindly supplied from Shionogi & Co., Ltd. (Osnaka, Japan). All other chemicals used in these experiments were of reagent grade.

Animal Experiments — Male albino rabbits weighing approximately 2 kg were used throughout the study. The animals were individually

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housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd., Tokyo, Japan). Pretreatment of rabbits with antibiotics were carried out as follows. CEX dissolved in distilled water was administered by gastric intubation at a dose of 16.7 mg/kg/d. LMOX dissolved in 0.9% NaCl was administered intravenously via an ear vein at a dose of 25 mg/kg/d. Antibiotics were administered for 1 or 3 d. The rabbits were starved for 24 h prior to use for absorption experiments but had free access to water. Salicyluric acid was dissolved in NaOH (equivalent to salicylic acid). Appropriate amounts of drug solution were administered orally, intracecellarly and rectally.

Oral Administration of Drug: The drug solution (4 ml/kg) was administered by gastric intubation.

Intraveccular Administration of Drug: Animals were anesthetized with sodium pentobarbital, given intravenously, via ear vein. After complete anesthesia, a midline incision (2—3 cm) was made, and the drug solution (6 ml/kg) was administered by direct injection into the cecum by syringe. Leakage of drug solution at the injection site was not observed. The abdomen was closed with operative stitching.

Rectal Administration of Drug: The drug solution (2 ml/kg) was administered rectally, and the anus was closed with a plastic clip to prevent leakage of the rectal contents during the experiments. Following oral, intracecellar and rectal administration of drug, blood was collected with a heparinized syringe at appropriate time intervals from an ear vein.

Analytical Method — Salicyluric acid and salicylic acid in blood were analyzed by high-performance liquid chromatography after modifying the method described by Cham et al.26 We used fluorescence intensity for detection instead of absorption measurement at 313 nm, which was employed by Cham et al. Blood samples (0.4 ml) were added to an equal volume of acetonitrile containing 30 μg of the internal standard, o-methoxybenzoic acid, in 1 ml. The samples were mixed on a vortex-type mixer and centrifuged at 10000 rpm for 10 min. Then 20 μl of the supernatant fluid was withdrawn using a Hamilton syringe and loaded onto the column. Calibration curves were constructed from data on the peak-area ratios of salicyluric acid and salicylic acid to the internal standard. We used a LC-6A pump, a RF-530 fluorescence detector, a Chromatopac C-R3A recorder (all from Shimadzu Co., Ltd., Kyoto, Japan) and a model 7125 sample injection valve (Rheodyne Inc., CA, U.S.A.). The prepared column was a bonded octadecylsilane-silica gel type (Fine SIL C18, Japan Spectroscopic Co., Ltd., Tokyo, Japan), with an average particle size of 10 μm and 250 × 4.6 mm i.d. This column was used at room temperature. The peak area of fluorescence intensity was recorded at excitation and emission wavelengths of 300 and 410 nm, respectively. The chromatographic mobile phase consisted of a mixture of acetic acid—methanol—water (4:40:60, v/v/v) and was filtered by passing through a 0.5 μm pore size membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan) before use. The flow rate was 1.5 ml/min. The retention times of salicyluric acid, salicylic acid and the internal standard were 4.9, 9.5 and 7.5 min, respectively.

**Results and Discussion**

In the previous report,10 we examined the oral, intracecellar and rectal administration of salicyluric acid in fasted rabbits to provide in-

![Fig. 1. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Oral Administration of Salicyluric Acid in Control Rabbits](image)

○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (60 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of four experiments.
formation on the physiological factors in the hydrolysis of salicylic acid by intestinal microorganisms. In the present study, the effect of pretreatment with antibiotics on the hydrolysis of salicylic acid in rabbit intestinal microorganisms was investigated. LMOX and CEX were chosen as intravenous and oral antibiotics, respectively. In humans, the dose of LMOX and CEX are 1–2 g/d and 1 g/d, respectively. In rabbits, therefore, LMOX was administered intravenously at a dose of 25 mg/kg/d. CEX was administered orally at a dose of 16.7 mg/kg/d.

The blood concentration of salicylic acid and salicylic acid following oral administration of salicylic acid was determined. In control rabbits (Fig. 1), salicylic acid reached a peak blood concentration (15.9 μg/ml) at 30 min after the dose and then decreased. The blood concentration of salicylic acid was maintained at 10.8–12.4 μg/ml from 4 to 12 h. In Fig. 2, the blood concentration of salicylic acid and salicylic acid in rabbits pretreated with LMOX is shown. By the pretreatment with LMOX for 1 d (Fig. 2a) or 3 d (Fig. 2b), the blood concentration of salicylic acid was slightly decreased (Fig. 2a: 6.5–7.4 μg/ml, Fig. 2b: 4.6–8.5 μg/ml from 4 to 12 h), probably due to LMOX excreted in bile. It was reported that 2.1% of LMOX administered intravenously (20 mg/kg) was recovered in bile after 6 h in rabbits. In Fig. 3, the blood concentration of salicylic acid and salicylic acid in rabbits pretreated with CEX is shown. By the pretreatment with CEX for 3 d (Fig. 3b), the blood concentration of salicylic acid was detected at low concentration (<5.5 μg/ml).

In order to examine the mechanism of change of salicylic acid concentration in the blood following the oral administration of salicylic acid in rabbits pretreated with CEX for 3 d, salicylic acid was administered intracelally. In control rabbits (Fig. 4), salicylic acid reached a peak
blood concentration (13.9 μg/ml) in 2 h, after which it slowly declined. Salicyluric acid was detected at low concentration (<0.7 μg/ml). In the previous report, the blood concentration of salicylic acid following intracecal administration of salicylic acid (5 mg/kg) was determined. Salicylic acid reached a peak blood concentration (14.5 μg/ml) in about 15 min. These results indicate immediate and very extensive salicylic acid formation from salicyluric acid in the cecum. By the pretreatment with CEX for 3 d, the blood concentration of salicylic acid was decreased at 1—4 h (Fig. 5). From these results, the decrease of metabolic activity in intestinal microorganisms may occur by the pretreatment with CEX for 3 d. It was reported that 52% of CEX administered orally (10 mg/kg) was recovered in urine after 6 h in dogs. CEX unabsorbed may affect the intestinal microorganisms. Murakami et al. demonstrated that the concentration of ampicillin in the cecal contents after a single oral administration or multiple oral administration was higher than that after intravenous administration in rats.

Fig. 4. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Intracecal Administration of Salicyluric Acid in Control Rabbits
○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of five experiments.

Fig. 5. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Intracecal Administration of Salicyluric Acid in Rabbits Pretreated with CEX Orally
The rabbits are pretreated with CEX for 3 d. ○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of six experiments.

Fig. 6. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Rectal Administration of Salicyluric Acid in Control Rabbits
○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of five experiments.

Fig. 7. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Rectal Administration of Salicyluric Acid in Rabbits Pretreated with LMOX Intravenously
(a), pretreatment with LMOX for 1 d (5); (b), pretreatment with LMOX for 3 d (5). ○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. Numbers in parentheses represent number of experiments.
Figure 6 shows the blood concentration of salicylic acid and salicylic acid following rectal administration of salicylic acid in control rabbits. A small amount of salicylic acid was absorbed in intact form. A part of the rest was hydrolyzed to salicylic acid, which was subsequently absorbed. The blood concentration of salicylic acid was maintained at 1.0—2.2 μg/ml from 3 to 12 h. In the previous report, the blood concentration of salicylic acid following rectal administration of salicylic acid (5 mg/kg) was determined. Salicylic acid reached a peak blood concentration (9.0 μg/ml) in 30 min, suggesting rapid absorption from the rectum. These results indicate that microbial metabolism of salicylic acid may be responsible for the prolonged retention of salicylic acid in the blood. In rabbits pretreated with LMOX (Fig. 7) and CEX (Fig. 8) for 3 d, the blood concentration of salicylic acid was increased. The metabolic activity of intestinal microorganisms in the rectum may be changed by the pretreatment with LMOX and CEX. Further studies will be required to clarify the mechanism of increase in the blood concentration of salicylic acid.

Walsh and Levine reported the effect of alterations of the bacterial population produced by antibiotics on the intestinal absorption of drug in rats. A mean 10 d consumption of 100 mg/kg/d of neomycin sulfate and 24 mg/kg/d of potassium penicillin V produced a marked reduction in the counts of *coli* forms and *proteus*, *enterococci*, *lactobacilli* and anaerobic *lactobacilli* and *streptococci*. No significant differences between control and pretreated animals were found, however, in the absorption of tetracycline, diphenylhydantoin or benzethamine. These results suggest that the changes observed in the indigenous flora of the rat do not significantly affect the factors that are critical to the normal absorptive process of drugs. Also, in the present study, significant change of the blood concentration of salicylic acid following oral, intracecal and rectal administration of salicylic acid was not found by the pretreatment with LMOX and CEX. Judging from these results, probably, the permeation of salicylic acid and salicylic acid in the intestine is not affected by the pretreatment with LMOX and CEX. In the present study, the examination of population of intestinal microorganisms induced by the pretreatment with antibiotics was not performed.

In the previous report, we examined the effect of fasting on the hydrolysis of salicylic acid in rabbit intestinal microorganisms. In fasted rabbits (24 or 48 h), the blood concentration of salicylic acid after oral administration of salicylic acid was changed compared to the control. However, a significant effect of fasting was not observed in the blood concentration of salicylic acid after rectal administration of salicylic acid. In the present study, the blood concentration of salicylic acid following rectal administration of salicylic acid was not decreased by the pretreatment with LMOX and CEX compared to the control. From these results, it appears that with rectal administration of salicylic acid there is substantial conversion to salicylic acid by intestinal microorganisms. Oral administration of antibiotics may affect the hydrolysis of prodrug in the intestinal microorganisms, a process which is avoided by intravenous administration of antibiotics.
Additional studies are needed to clarify the effect of other antibiotics on the formation of parent drug from prodrug by intestinal microorganisms. Certainly, additional studies are needed in other animal species.

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