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Prolonged Blood Concentration of Salicylic Acid Following the Simultaneous Oral Administration of Salicylic Acid and Salicyluric Acid in Rabbits

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Salicylic acid was detected in the blood at 2 h after oral administration of salicyluric acid (60 mg/kg; salicylic acid equivalent) to rabbits and reached the maximum level at 5 h (about 9.7 $\mu\text{g/ml}$). The levels of salicylic acid remained above 7 $\mu\text{g/ml}$ for several hours, after which they slowly declined. Following the simultaneous oral administration of salicylic acid (7.5 mg/kg) and salicyluric acid (60 mg/kg; salicylic acid equivalent), the blood concentration of salicylic acid was maintained at 8.5—15.3 $\mu\text{g/ml}$ from 6 min to 12 h. Different blood level patterns of salicylic acid could be produced by simultaneous oral administration of salicyluric acid and various doses of salicylic acid.

Keywords—salicyluric acid; salicylic acid; coadministration; prolonged blood concentration; rabbit; gut flora; microorganism; presystemic deconjugation; glycine conjugate; prodrug

The objective of most drug therapy is to produce and maintain a therapeutic response. In recent years, a considerable body of evidence has been accumulated from animal experiments and from studies in man, indicating that response is better correlated with plasma concentration or with the amount of drug in the body than with the dose administered. Therefore, the maintenance of plasma concentration is considered to be a desirable objective.

Drugs will often come into contact with the microorganisms which comprise the normal gastrointestinal flora. It is becoming widely recognized that the gastrointestinal flora may be of great significance in determining the metabolic fate of drugs.^{1,2)} Despite the well-documented importance of the ability of the gut microflora to metabolize drugs, little work has been done on the prolongation of blood concentration of a drug by utilizing the gut flora to metabolize a prodrug.

In the previous report, we demonstrated that salicyluric acid is metabolized to salicylic acid by gut flora in rabbits.³⁾ Salicylic acid and unchanged salicyluric acid were detected in the blood after oral administration of salicyluric acid. The present investigation was undertaken in an attempt to prolong the blood concentration of salicylic acid by means of simultaneous oral administration of salicylic acid and salicyluric acid in rabbits.

Experimental

Materials—Salicyluric acid was prepared according to the method reported by Frömring and Vollenberg.⁴⁾ Sodium salicylate, *o*-methoxybenzoic acid, and acetonitrile were of reagent grade. All other chemicals used were of the finest grade available.

Animal Experiments—Male albino rabbits weighing 1.8—2.3 kg were used. The animals were fasted for about 20 h prior to use for experiments but had free access to water. Sodium salicylate was dissolved in 25 ml of distilled water and salicyluric acid (530 mg) in 25 ml of 0.12 N NaOH (equivalent to 15.0 mg/ml salicylic acid solution).

Appropriate amounts of drug solution were administered orally *via* a stomach tube. The blood was collected at appropriate time intervals from an ear vein.

Analytical Method—Salicylic acid in blood was analyzed by high-performance liquid chromatography (HPLC) after modifying the method described by Cham *et al.*⁵⁾ We used fluorescence intensity 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 100 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 directly injected into the HPLC column. The calibration curve was constructed from data on the peak-height ratio of salicylic acid to the internal standard. We used a Trirotar-II pump, an FP-110 fluorescence detector, and an RC-125 recorder (all from Japan Spectroscopic Co., Ltd., Japan). The prepared column was a bonded octadecylsilane-silica gel type (Fine SIL C₁₈, Japan Spectroscopic Co., Ltd. Japan), average particle size 10 μm , 4.6 \times 250 mm internal dimensions. This column was used at room temperature. The peak height of fluorescence intensity was recorded at excitation and emission wavelengths of 300 and 410 nm, respectively. The mobile phase consisted of a mixture of acetic acid-methanol-water (4:40:60) and was filtered by passing it through a 0.45 μm pore size membrane filter (Toyo Roshi Co., Ltd. Japan). The flow rate was 1.5 ml/min. The retention times of salicylic acid and the internal standard were 9.5 and 7.5 min, respectively.

Results and Discussion

The metabolism of glycine conjugates in the intestine has been studied mainly with regard to the bile acids (glycocholic acid, *etc.*). Norman and Grubb reported that enterococci are able to hydrolyze hippurate to glycine and benzoic acid.⁶⁾ *p*-Aminohippuric acid and *p*-acetylaminohippuric acid undergo extensive hydrolysis in the alimentary tract following their oral administration to man.⁷⁾

In the previous report, we examined the fate of salicylic acid after oral administration in rabbits.³⁾ Salicylic acid was detected in the blood, as shown in Fig. 1. After intravenous administration of salicylic acid, only unchanged salicylic acid was detected in the blood, suggesting that presystemic deconjugation of glycine was involved. After treatment of rabbits

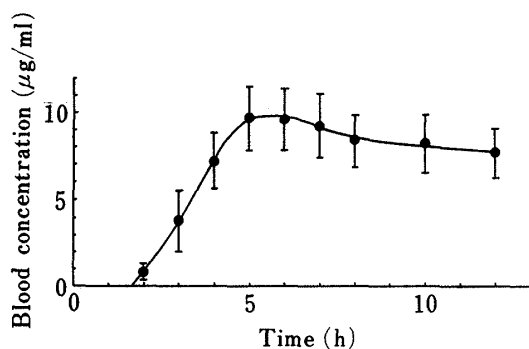


Fig. 1. Blood Concentration of Salicylic Acid Following the Oral Administration of Salicylic Acid

Dose: 60 mg/kg (salicylic acid equivalent). Each point represents the mean \pm S.E. of four experiments.

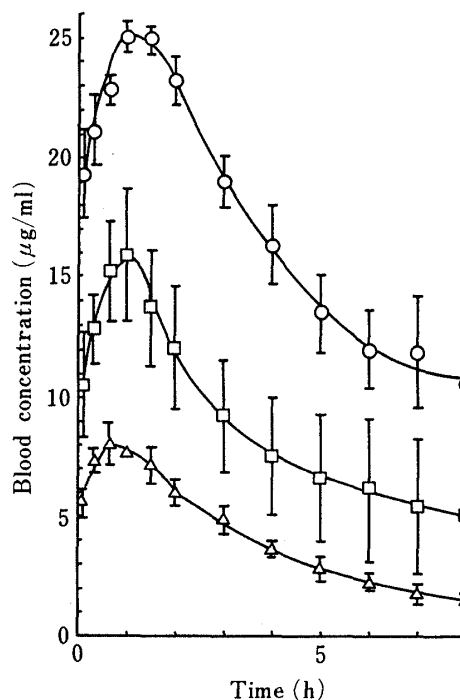


Fig. 2. Blood Concentration of Salicylic Acid Following the Oral Administration of Salicylic Acid

Dose: \circ , 15 mg/kg; \square , 7.5 mg/kg; \triangle , 3.75 mg/kg. Each point represents the mean \pm S.E. of at least three experiments.

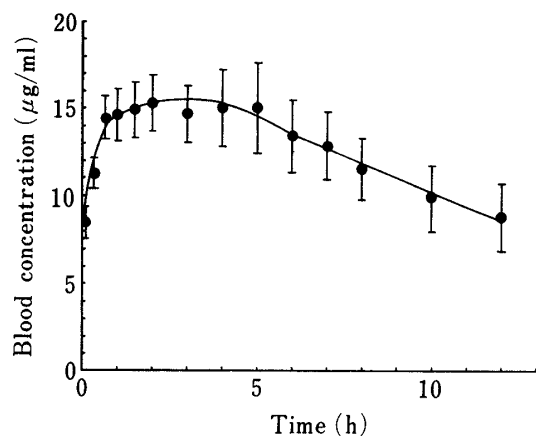


Fig. 3. Blood Concentration of Salicylic Acid Following the Oral Administration of Salicylic Acid (7.5 mg/kg) and Salicylic Acid Simultaneously

Dose: 60 mg/kg of salicylic acid (salicylic acid equivalent). Each point represents the mean \pm S.E. of seven experiments.

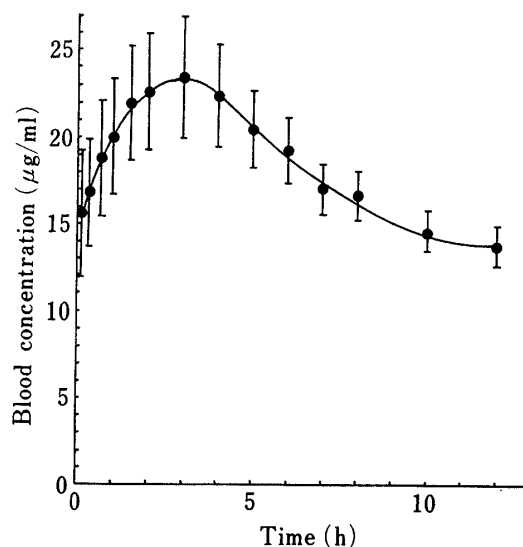


Fig. 4. Blood Concentration of Salicylic Acid Following the Oral Administration of Salicylic Acid (15 mg/kg) and Salicylic Acid Simultaneously

Dose: 60 mg/kg of salicylic acid (salicylic acid equivalent). Each point represents the mean \pm S.E. of five experiments.

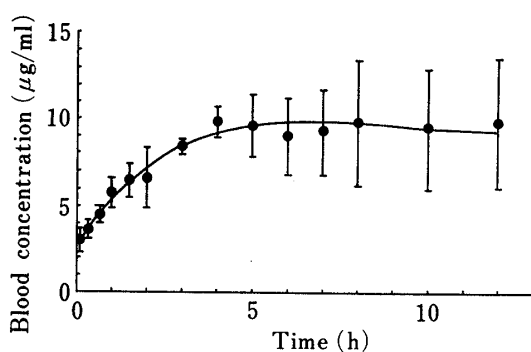


Fig. 5. Blood Concentration of Salicylic Acid Following the Oral Administration of Salicylic Acid (3.75 mg/kg) and Salicylic Acid Simultaneously

Dose: 60 mg/kg of salicylic acid (salicylic acid equivalent). Each point represents the mean \pm S.E. of five experiments.

with kanamycin sulfate, complete inhibition of the formation of salicylic acid after oral administration of salicylic acid was demonstrated, indicating that the intestinal microflora were responsible for the biotransformation. Furthermore, *in vitro* incubation of salicylic acid with gut contents showed that the major location of the hydrolysis was the hind gut. As shown in Fig. 1, salicylic acid was detected at 2 h after dosing and reached the maximum level at 5 h (about 9.7 $\mu\text{g/ml}$). The levels of salicylic acid were maintained above 7 $\mu\text{g/ml}$ for several hours, after which they slowly declined. In the present study, we investigated the blood concentration of salicylic acid following the simultaneous oral administration of salicylic acid and salicylic acid. First, three doses of salicylic acid were administered orally; the results are presented in Fig. 2. The peak level increased with dose. The simultaneous administration of salicylic acid (7.5 mg/kg) and salicylic acid was next examined. As shown in Fig. 3, the blood concentration of salicylic acid was maintained at 8.5–15.3 $\mu\text{g/ml}$ from 6 min to 12 h. The results with a higher dose (15 mg/kg) or lower dose (3.75 mg/kg) of salicylic acid are presented in Figs. 4 and 5. The blood concentration of salicylic acid following the simultaneous administration of salicylic acid (15 mg/kg) and salicylic acid increased rapidly, with a slow decrease from 3 h after dosing. In the case of coadministration of salicylic acid (3.75 mg/kg) and salicylic acid, the blood level of salicylic acid increased gradually and

tended to maintain a plateau after 4 h. From these observations, it should be possible to design a dosage regimen to give a desired pattern of blood level of salicylic acid based on simultaneous administration of salicylic acid and salicyluric acid.

These results suggest the importance of oral coadministration of a parent drug and its prodrug, which is converted to the parent drug by gut flora, to obtain therapeutic blood concentrations of the parent drug. Differences in the metabolic fate of salicyluric acid may occur among different animal species. In addition, interindividual differences in the nature of the gut flora, the extent of absorption, and the intestinal transit time may result in large differences in blood concentration of salicylic acid. Therefore, additional studies are needed before this technique can be applied in humans.

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