SALICYLAMIDE GLUCURONIDE FORMATION IN LIVER DISEASE AND ITS CHANGE BY DRUGS

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Summary

Salicylamide glucuronide (SAMG) in 0-6 and 6-12 hours-urine specimens was determined after oral administration of salicylamide in 7 normal volunteers (NV), in 51 cases of various liver diseases and hyperbilirubinemias, and in 19 cases after drug administration, to predict the in vivo drug metabolism in man and its change by drugs. Maximal glucuronide formation was obtained by 1.0 g of salicylamide administered to NV; thus, this dosage was used in the present study. SAMG as percent of total salicylamide, the percent of SAMG, from 0-6 hours-urine specimens was high and constant in NV (71.3 ± 8.3 (mean ± S.D.)). 0-0.08% of the total salicylamide was confirmed as free salicylamide in 0-12 hours-urine specimens of NV. The percent of SAMG of 0-6 hours-urine specimens was 57.2 ± 8.6 in acute hepatitis, 66.6 ± 10.9 in chronic hepatitis, and 48.6 ± 10.7 in liver cirrhosis (mean ± S.D.). Free salicylamide increased slightly in liver diseases. Serum bilirubin levels tended to be inversely correlated with the percent of SAMG. In most cases of Gilbert's syndrome, the percent of SAMG remained at a normal level. The percent of SAMG in cases with unconjugated hyperbilirubinemias of other geneses were almost within normal limits. Bucolome and phenobarbital increased the percent of SAMG in patients with various liver diseases. After rifampicin or phenytoin administration, the percent of SAMG of the patients with lung tuberculosis or epilepsy did not surpass that of NV.

Key Words: Salicylamide, Glucuronate, Glucuronyltransferase, Bilirubin, Hyperbilirubinemia, Liver disease, Gilbert's syndrome, Phenobarbital, Bucolome, Rifampicin, Phenytoin, Drug metabolism, Enzyme induction

Introduction

After a long term administration, the steady-state plasma concentrations and therapeutic effects of drugs metabolized by the liver vary widely according to individuals15. This variability is thought to be mainly caused by individual differences in the hepatic drug-metabolizing activity. In general, lipid-soluble drugs are taken up into the liver, and metabolized by the mixed-function oxidase, glucuronyl-transferase in microsomal fraction, sulfo-transferase and/or reductase in supernatant fraction. Becoming water-soluble, the metabolized drugs are then excreted into the bile or urine.

Since the rate of drug metabolism in patients with liver diseases is an important determinant of the therapeutic response and/or drug...
interactions, convenient and simple methods to assess hepatic drug-metabolizing activity are highly desirable. The purpose of the present study is to assess, by determining the glucuronide of salicylamide, the in vivo hepatic drug metabolism in various liver diseases and its change by administration of drugs which are thought to influence hepatic drug metabolism.

Methods

Subjects: The subjects consisted of the following: 7 healthy volunteers, 5 cases of acute hepatitis, 12 cases of chronic hepatitis, 15 cases of liver cirrhosis, 3 cases of hepatic tumors, 5 cases of Gilbert's syndrome, 3 cases of hemolytic anemia, 8 cases of jaundice of other geneses, 8 cases of lung tuberculosis who had been administered 450 mg of rifampicin daily for more than 2 months, and one epileptic case who had been treated daily with 300 mg of phenytoin for 4 years. All were fully conversant with the nature of the study and freely agreed to take part in it. Patients other than tuberculosis and epilepsy were not taking any of the enzyme-inducing drugs at the time of investigation.

Procedure: After overnight fasting, each subject received salicylamide orally. Urinary specimens of 0 to 6 and 6 to 12 hours after the drug administration were collected separately. The specimens were examined either immediately or after stockling below −20°C. Metabolites of salicylamide were determined by the method of Levy and Matsuzawa with a slight modification. Total salicylamide (SAMT) in the urine was determined by adding the same volume of 6 N HCl to the urine specimen to hydrolyze all metabolites of salicylamide at 100°C for 16 hours. One or two ml of the hydrolyzed samples were extracted with 30 ml of ethylene dichloride. A ten-ml aliquot of the latter phase was then extracted with 5 ml of 20-fold diluted ferric nitrate stock solution (1% Fe(NO₃)₂ in 0.07 N HCO₃). The absorbance of the ferric nitrate reagent phase was determined at 530 μ against a water blank obtained by using water instead of urine samples and treating it as described above. When a 20 mg/dl solution of salicylamide was used, the absorbance was 0.13 in a 1-cm path length cell.

Free salicylamide (SAMF) in the urine was determined by adding 1 ml of 0.2 M sodium phosphate buffer (pH 7.0) to 2 ml of urine and extracting it with 15 ml of ethylene dichloride. Ten ml of the latter phase was then extracted with 9/5-fold diluted ferric nitrate stock solution. The absorbance of the ferric nitrate reagent phase was determined at 530 μ against a water blank. The absorbance of 20 mg/dl solution of salicylamide by this procedure was 0.32 in a 1-cm path length cell. Salicylamide glucuronide (SAMG) in the urine specimens was determined as SAMF by hydrolyzing SAMG. Beta-glucuronidase (Sigma Type I, 2000 units) and 2 ml of 0.4 M acetate buffer (pH 4.5) were added to 1 or 2 ml of the urine specimens, and the mixtures were incubated at 37°C for 16 hours. A water blank and the specimens were assayed as described for SAMF above. SAMG was expressed by subtracting SAMF from the content of salicylamide obtained in the last procedure.

In the following, the percent of SAMG was used as SAMG/SAMT × 100. SAMT, SAMG and SAMF of 0–12 hours-urine specimens were the sum of those from 0–6 and 6–12 hours-urine specimens. The percent of SAMG of 0–12 hours-urine specimens was calculated from that of 0–6 and 6–12 hours-urine specimens.

To observe the effect of drugs which were thought to influence hepatic drug metabolism, several methods were used. Nine-hundred mg