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Alteration of Hepatic 3'-Phosphoadenosine 5'-phosphosulfate and Sulfate in ICR Mice by Xenobiotics that are Sulfated

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Abstract—Phenol, acetaminophen (AA) and salicylamide are all known to be sulfated in rats and mice. We have previously demonstrated that capacity-limited sulfation of xenobiotics in rats is due to the reduced availability of hepatic 3'-phosphoadenosine 5'-phosphosulfate (PAPS), the cosubstrate for sulfation, which in turn is limited by the availability of its precursor, inorganic sulfate. Because species differences have been reported in the extent of sulfation, this study was conducted to determine whether these xenobiotics lower hepatic PAPS and sulfate in ICR mice. All three substrates decreased serum sulfate concentrations in a dose- and time-dependent manner. However, contrary to the observations in rats, phenol markedly increased hepatic PAPS concentrations in a dose-dependent manner, 1 hr after *ip* injection of 0~4 mmol/kg. Following *ip* injection of 4 mmol/kg phenol, hepatic PAPS concentraions were enhanced 2~3 fold, 0.5~2 hr after dosing and returned to control values 3 hr after dosing, whereas AA and salicylamide had little effect on hepatic PAPS concentraions. In summary, these studies demonstrate that phenol markedly enhances hepatic PAPS concentrations in mice, whereas hepatic PAPS levels are not affected by AA and salicylamide. Our data suggest that 1) hepatic sulfation for high dosages of xenobiotics in ICR mice is not limited by the availability of cosubstrate and 2) there are significant species differences in the regulation of PAPS between rats and mice.

Keywords □ xenobiotics, sulfation, PAPS, sulfate, species differences

Sulfation is an important metabolic pathway in the biotransformation of various xenobiotics and many endogenous compounds such as neurotransmitters, steroid hormones, peptides and bile acids (Mulder and Jakoby, 1990). Sulfation is catalyzed by the sulfotransferases, a group of soluble enzymes found primarily in liver, kidney, intestinal tract and lung. These enzymes catalyze the formation of sulfate esters with PAPS as the sulfate donor (Mulder, 1981; Sipes and Gandolfi, 1986). PAPS, the cosubstrate for sulfation, is formed in two-step reactions from ATP and inorganic sulfate, catalyzed by ATP sulfurylase and APS kinase.

Phenol, AA and salicylamide are all known substrates for sulfation (Mulder and Jakoby, 1990). The pathway of sulfate activation and sulfated metabolites of these compounds are illustrated in Fig. 1. Salicylamide is mainly metabolized to glucuronide and sulfate conjugates, and gentisamide, which is further sulfated to gentisamide 2- or 5-sulfate (Levy and Matsuzawa, 1967; Moris and Levy, 1983; Song *et al.*, 1971; Fielding *et al.*, 1984). Also 3-hydroxylation is a significant route of salicylamide biotransformation in mice after high doses (Howell *et al.*, 1988). The metabolite of 3-hydroxylation of salicylamide, 2, 3-dihydroxylbenzamide, can be further sulfated. Phenol was also chosen for the present investigation as it is biotransformed mainly to glucuronide and sulfate conjugates (Mehta *et al.*, 1987; Oehme and Davis, 1970; Weitering *et al.*, 1979; Williams, 1938).

Sulfate conjugation is considered to have a high affinity but low capacity (Caldwell, 1981). The important factors influencing sulfation are thought to be availability of PAPS, availability of inorganic sulfate, rate of PAPS synthesis via sulfate activation and activities of sulfotransferases.

Previously we have demonstrated that capacity-limi-

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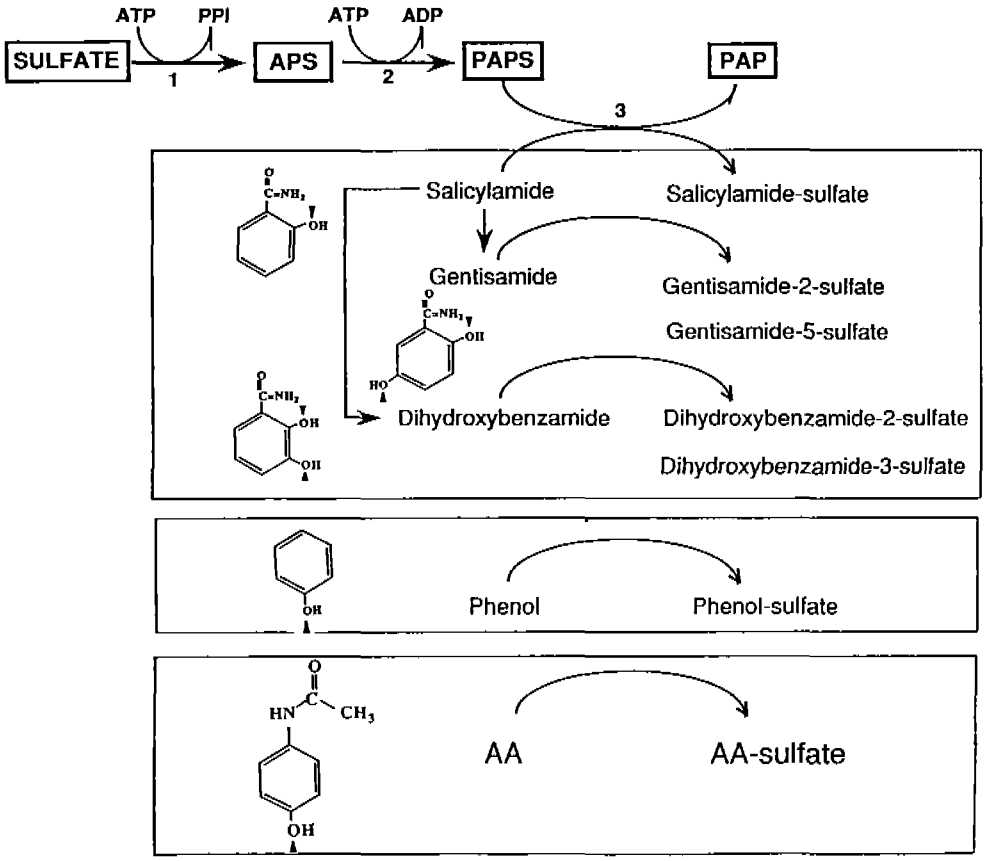

Fig. 1. Sulfate activation and sulfation of phenolic compounds: 1. ATP sulfurylase; .2. APS kinase; 3. Phenol sulfotransferase.

ted sulfation of xenobiotics is due to the reduced availability of hepatic PAPS, which in turn is limited by the availability of its precursor, sulfate (Kim *et al.*, 1995). These concepts concerning the capacity-limited characteristic of sulfation and the decreased availabilty of cosubstrate and its precursor during the process of sulfation were based on the experiments performed in rats, which are the main species used for studies in drug metabolism both *in vivo* and *in vitro*. As there are marked species differences in the sulfation of xenobiotics, including phenol, AA and salicylamide which have been used as model compounds for the study of conjugation reactions, this study aimed to determine the effects of phenol, AA and salicylamide on hepatic PAPS, as well as serum sulfate concentrations in ICR mice, another widely used experimental animal (Caldwell, 1981; Williams, 1974; Gregus *et al.*, 1988). Here we report striking species differences in the availability of PAPS during the sulfation of phenolic compounds.

MATERIALS AND METHODS

Animals and materials

Male ICR mice were obtained from National Institute of Safety Research (Seoul, Korea) and maintained on 23± 2℃ and 55± 10% humidity with a 12 hr light-dark cycle. Rodent chow (Shinchon Diet Co., Seoul) and tap water were provided *ad libitum*. [^{14}C]naphthol was obtained from Amersham (Amersham, U.K.). Phenol, AA, salicylamide and PAPS were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animal treatments and sample collection

For the time-course studies, mice were injected *ip* with either saline or 4 mmol/kg of phenol, AA or salicylamide. Animals were decapitated 0.5, 1, 2, 3 or 4 hr after administration of various xenoboitics. For the dose-response studies, mice were treated either saline or 0.5, 1, 2, 4 mmol/kg of phenol or AA, 1 hr prior to sacrifice. Salicylamide was administered at the same doses as phenol, 0.5 hr prior to decapitation. Liver was taken for the quantification of PAPS concentrations and PAPS samples were analyzed immediately after sample preparation. Serum obtained after coagulation and centrifugation of collected blood samples was stored at −70℃ for the analysis of sulfate.

Analysis of hepatic PAPS and sulfate

The hepatic PAPS values were determined by the

procedure of Hazelton *et al.* (1985), which measures the formation of [^{14}C]naphthyl sulfate from [^{14}C]naphthol and PAPS in the presence of rat liver phenol sulfotransferases. Sulfate concentrations in serum were determined by CIA(Capillary Ion Analysis) based on the procedure of Cole and Scriver (1981). Electromigration was used for sample injection. Sulfate samples were separated through a Fused Silica capillary with chromate as a mobile electrolyte.

Statistics

One-way analysis of variance was used to evaluate the statistical significance of changes in all indices as a function of time and dose, followed by Scheffe's multiple range test with $p<0.05$ set at the minimum level of significance.

RESULTS

The time-courses of phenol, AA or salicylamide on hepatic PAPS concentrations in mice are shown in Fig. 2. The PAPS concentrations in the liver of untreated mice were quantitated to be the range of 90~100 nmol/g liver. Contrary to observations in rats, hepatic PAPS concentrations following administration of 4 mmol/kg AA or phenol did not decrease, but were surprisingly increased after dosing of phenol. Administration of 4 mmol/kg phenol rapidly enhanced hepatic PAPS concentrations, with a maximal increase to 338% of controls at 0.5 hr after dosing. The hepatic PAPS levels returned to control values 3 hr after dosing of phenol. Also, AA treatment did not alter hepatic PAPS concentrations. Even though hepatic PAPS values were significantly decreased 0.5 hr after *ip* injection of 4 mmol/kg salicylamide, it did not decrease hepatic PAPS levels at any other time point.

The dose-response studies for hepatic PAPS values after administration of various dosages (0~4 mmol/kg) of compounds are shown in Fig. 3. Phenol increased hepatic PAPS levels to 184 and 204% of control mice, respectively, 1 hr after dosages of 2 and 4 mmol/kg. AA did not affect PAPS levels 1 hr after dosages of 0~4 mmol/kg. Liver PAPS concentrations were significantly reduced by administration of 4 mmol/kg salicylamide, but salicylamide did not alter hepatic PAPS levels, 0.5 hr after administration of 0~2 mmol/kg.

Fig. 4 illustrated the serum sulfate concentrations in control and treated animals. The mean serum sulfate concentration of control mice measured by CIA was 1.08 mM. As expected, all three phenolic compounds significantly depleted serum sulfate concentrations, which is considered to be the largest source of sulfate for sulfation. Serum sulfate values were significantly decreased to 56%, 74% and 71% of control values, 1 hr after administration of 4 mmol/kg phenol, AA and salicylamide, respectively.

DISCUSSION

Species differences in the metabolism of xenobiotics

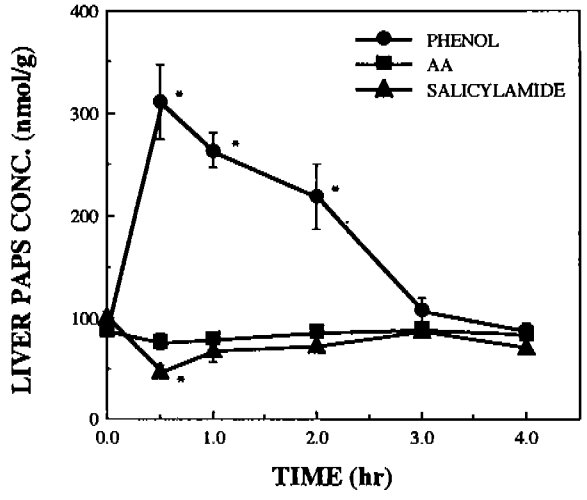

Fig. 2. Time courses of hepatic PAPS concentrations after administration of phenol, AA and salicylamide. Hepatic PAPS values were determined from ICR mice which were administered 4 mmol/kg phenol, AA and salicylamide *ip*, 0.5~4 hr prior to decapitation. Values are means ± SE for groups of 5~6 mice. An asterisk denotes a significant difference from the control group at $p<0.05$.

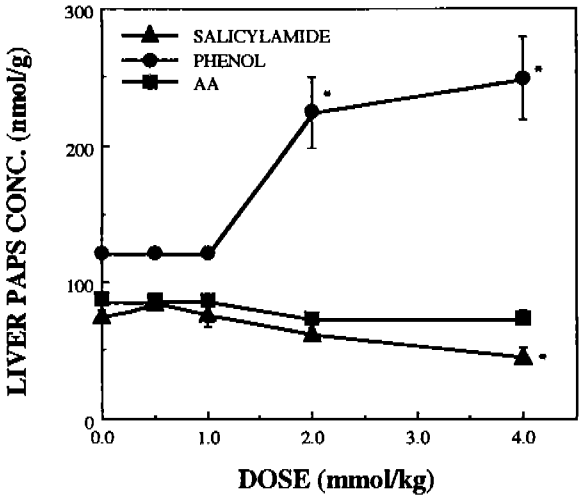

Fig. 3. Dose response of hepatic PAPS concentrations after administration of phenolic compounds. Hepatic PAPS levels were quantitated 0.5 or 1 hr after administration of various dosages of salicylamide, phenol or AA. Values are means ± SE for groups of 5~6 mice. An asterisk denotes a significant difference from the control group at $p<0.05$.

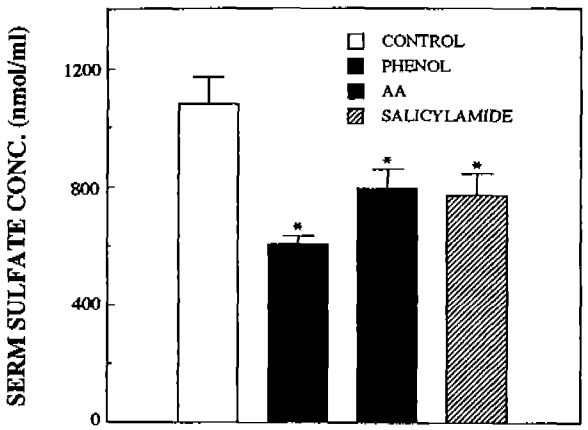

Fig. 4. Effect of administration of phenolic compounds on serum sulfate concentraions. Sulfate concentrations were determined by CIA in mice 1 hr after administration of 4 mmol/kg phenol, AA and salicylamide. Values are means ± SE for groups of 5~6 mice. An asterisk denotes a significant difference from the control group at $p<0.05$.

have been systematically elucidated by Dr. R. T. Williams and his colleague. Species differences in the metabolism of xenobiotics are sometimes due to a deficiency of a particular reaction peculiar to a certain species. For example, the cat is deficient in the ability to glucuronidate phenolic substrates, whereas the pig is deficient in sulfation (Caldwell, 1981). More often species variation in biotransformation is due to the competition of various enzymatic pathways for the same substrate (Caldwell, 1981). For example, most species have the capacity to biotransform phenol to both sulfate and glucuronide conjugates, although a large species variation in the relative rates of both conjugation reactions exit (Mulder, 1981). The rat and mouse, the most widely used experimental animals form similar amount of glucuronide and sulfate conjugates following a relatively low dose of phenol (266 μmol/kg, *po*) (Capel *et al.*, 1972).

Marked species differences have been noted in the ratio of glucuronidation and sulfation. For instance, after administration of a single dose of AA (150 mg/kg), rats excrete 64% of dose as the sulfate conjugate (Thomas *et al.*, 1974), whereas mice conjugated 10% of the dose with sulfate (Whitehouse *et al.*, 1977). A number of phenolic compounds are competitively biotransformed to glucuronide and sulfate conjugates. In most cases, sulfate conjugates predominate at low doses, while glucuronide conjugates are the major metabolites at high doses. Also, saturation of the sulfation pathway has been reported *in vivo* for phenol (Mehta *et al.*, 1987; Oehme and Davis, 1970; Weitering *et al.*, 1979), AA (Galinsky and Levy, 1981; Hjelle and Klaassen, 1984) and salicylamide (Levy and Matsuzawa, 1967; Williams, 1938; Waschek, 1985).

Generally, sulfation is considered to have a high affinity but low capacity for conjugation of phenols (Caldwell, 1981). We have already demonstrated that high dosages of substrates for sulfation such as phenol, AA, salicylamide and 1-naphthol markedly deplete hepatic PAPS concentrations, and significantly decrease sulfate concentrations in serum and liver in rats (Kim *et al.*, 1995; Kim *et al.*, 1992). These findings establish that cosubstrate depletion does occur after high dosages of all four phenolic compounds and indicate that the mechanism for the capacity-limited sulfation of high dosages for these compounds is due to the limited availability of hepatic PAPS, which in turn is limited by the availability of sulfate in serum and liver. However, the results of the present study suggest that this mechanism might not be appropriate in mice.

Our data clearly show that sulfated xenobiotics did not reduce hepatic PAPS concentrations in a dose- and time-dependent manner, in spite of these xenobiotics decreasing sulfate concentrations in the serum of mice. Based on the observation in rats, higher dosages of xenobiotics cause marked depletion of PAPS during the process of sulfation, because the utilization of PAPS is faster than its synthesis. Also, xenobiotics that are sulfated cause depletion of inorganic sulfate, as observed in rats and mice, due to the consumption by ATP sulfurylase to synthesize new PAPS. The depleted sulfate concentration in serum aggrevates the extent of the depletion of hepatic PAPS level in rats. However our findings suggest that sulfation for high dosages of xenobiotics in mice is not limited by the availability of the cosubstrate.

One of the interesting findings in these studies is that hepatic PAPS concentrations can be increased during the process of sulfation. Following *ip* injection of 2 or 4 mmol/kg phenol, hepatic PAPS concentraions were enhanced 2~3 fold, 0.5~2 hr after dosing. This phenomenon was confirmed repeatedly in dose-response studies. Theoretically, an increase in hepatic PAPS level could occur either by increased PAPS formation through sulfate activation or decreased utilization of PAPS through the inhibition of sulfotransferases.

Pentachlorophenol and 2, 6-dichloro-4-nitrophenol are very effective inhibitors of sulfotransferases (Camphell *et al.*, 1987), and inhibit the sulfation of many compounds both *in vivo* and *in vitro* (Koster *et al.*, 1982). It has been reported that hepatic PAPS levels increase after administration of the inhibitors of sulfotransferase (Dills and Klaassen, 1986). Therefore, the

effectively reduced utilization of PAPS by these compounds, because they decrease sulfate conjugations, results in the enhancement of hepatic PAPS levels. Thus, inhibition of sulfotransferases also appear to be one of possible explanation for the enhancement of hepatic PAPS level.

PAPS is present in tissues in very low concentrations (Brzeznicka *et al.*, 1987), but is synthesized very rapidly. The rate of PAPS synthesis has been estimated to be as high as 100 nmol/min/g liver in the perfused rat liver (Pang, 1981). This indicates that the synthesis of PAPS in liver can be accelerated to the extent that the steady-state hepatic PAPS pool can be replaced each minute. Although the turn over and synthesis rate of PAPS appear to be rapid, little definitive information is available on the synthesis and regulation of PAPS to explain how the generation of PAPS can enhance.

Because capacity-limited sulfation and cosubstrate availability are so important in toxicology, further investigation to understand the mechanisms of these major differences in rats and mice can reserve tremendous significance in toxicity testing as well as extrapolation of data from laboratory animals to humans.

In conclusion, these studies demonstrate that phenol markedly enhances hepatic PAPS concentrations in mice, whereas hepatic PAPS levels are not reduced by AA and salicylamide. All three phenolic compouds significantly decreased serum sulfate. Our data suggest that hepatic sulfation for high dosages of xenobiotics in ICR mice is not limited by the availability of cosubstrate, and there are significant species differences in the regulation of PAPS between rats and mice.

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