Effect of Coadministered Salicylamide on Terbutaline Metabolism in Rats

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Abstract □ Concomitant oral administration of salicylamide (200 mg/kg) and ^{3}H-terbutaline (1 mg/kg) to rats with ligated bile ducts decreased absorption of terbutaline from the gut from 73 to 56% as measured by urinary excretion of radioactivity in 48 hr. No increase in the fraction of terbutaline excreted unchanged was observed, suggesting that salicylamide does not substantially inhibit the conjugation of terbutaline with glucuronic acid. An increase in the fraction of terbutaline excreted unchanged observed in normal animals may result from enhanced excretion of terbutaline glucuronide into bile rather than from inhibition of conjugation.

Keyphrases □ Salicylamide—effect on absorption and metabolism of terbutaline, rats □ Terbutaline—absorption and metabolism, effect of salicylamide, rats □ Absorption—terbutaline, effect of salicylamide, rats □ Metabolism—terbutaline, effect of salicylamide, rats □ Bronchodilators—terbutaline, absorption and metabolism, effect of salicylamide, rats □ Analgesics—salicylamide, effect on absorption and metabolism of terbutaline, rats

The metabolic fate of the bronchodilator drug terbutaline in rats was reported following administration of 5-mg/kg doses by oral, subcutaneous, intraperitoneal, and intraportal routes (1). Regardless of the route of administration, only one metabolite, a glucuronide conjugate of terbutaline, was excreted in the urine along with unchanged drug. On oral administration, terbutaline was extensively conjugated by the intestinal mucosa during absorption from the gut lumen (1). In fact, the fraction of drug excreted unchanged in the urine was less than 3.5% of the dose at 10 mg/kg or less and rose to only 6.2% at 1000 mg/kg (2). Therefore, it was decided to study the possibility of competitively inhibiting glucuronide conjugation and thereby increasing the transfer of unchanged terbutaline across the intestinal epithelium by concomitant administration of salicylamide, another phenolic drug.

Salicylamide inhibited the glucuronide conjugation of salicylic acid in humans (3) and decreased the formation of acetaminophen sulfate and, to a less extent, acetaminophen glucuronide in humans (4). Salicylamide significantly inhibited the conjugation of isoproterenol in the dog intestine (5). Isoproterenol is structurally similar to terbutaline; on oral administration to dogs, it was extensively metabolized and mainly excreted in urine as a sulfuric acid ester (6, 7). Introduction of 1–2 g of salicylamide into surgically isolated gut loops of dogs prior to isoproterenol administration raised the fraction of radioactivity found in the venous effluent as unchanged isoproterenol to about 74% compared to about 4% in the absence of salicylamide (5).

Since, in rats, terbutaline is excreted in urine mainly as the glucuronide, salicylamide might be expected to inhibit the conjugation. The effect in rats of various doses of salicylamide administered concomitantly with terbutaline on the amounts of free and conjugated drug subsequently excreted in urine is reported in this paper.

EXPERIMENTAL

Aqueous solutions of salicylamide (4–72 mg/ml) were prepared in 1 *N* NaOH, and the pH was adjusted to 10.0 with 1 *N* acetic acid. Appropriate volumes of solutions of nonradioactive and purified ^{3}H-terbutaline were then added to provide solutions containing salicylamide equivalent to doses of 10, 100, and 200 mg/kg and terbutaline base equivalent to a dose of 1 mg/kg in 1 ml. Aliquots of 1 ml of these solutions containing approximately 70 μCi of tritium were administered orally to groups of three male Sprague–Dawley rats weighing approximately 250 g.

Rats in a control group were given 1 ml of a buffered solution (0.15 *M* $NaHCO_3$ adjusted to pH 10 with 1 *N* NaOH) containing 1 mg of terbutaline base/kg and approximately 70 μCi of tritium. All rats were fasted overnight prior to dosing and for 8 hr thereafter. Water was available *ad libitum* before and throughout the experiment. Urine was collected at intervals of 0–8, 8–24, and 24–48 hr postmedication.

An identical experiment was conducted using other groups of rats in which the common bile duct had been ligated with two sutures and then severed between the sutures immediately prior to drug administration.

Aliquots of 10–30 μl of 0–8- and 8–24-hr urine were examined by electrophoresis using the technique of Conway *et al.* (8). Electrophoretic runs were made in pH 3.0 phosphate buffer, which provided adequate separation of all metabolites (1).

The tritium content of urine was analyzed by liquid scintillation counting, and the radioactive zones on electrophoregrams were located and quantitated by scanning in a gas-flow counter as described previously (1, 2).

RESULTS AND DISCUSSION

Following oral administration of ^{3}H-terbutaline in a solution buffered at pH 10 to normal rats, radioactivity was rapidly excreted in urine, accounting for mean values of 31% of the dose in 8 hr and 35% in 24 hr. These results are in good agreement with the reported excretion in urine of 40% of a 1-mg/kg dose of ^{3}H-terbutaline administered in water (2). The excretion of radioactivity decreased with increasing doses of concomitantly administered salicylamide to 18 and 23% in 8 and 24 hr, respectively, with the highest dose (200 mg/kg) of salicylamide administered. Without or with coadministration of salicylamide, little additional radioactivity was excreted in the urine in the 8–24-hr period and a negligible amount was excreted thereafter.

In contrast, groups of rats with ligated bile ducts excreted 49, 70, and 73% of the administered radioactivity in 8-, 24-, and 48-hr periods, respectively; when salicylamide (200 mg/kg) was administered concomitantly, these values decreased to 17, 50, and 56%, respectively.

Ligation of the bile duct resulted in a more prolonged elimination of radioactivity in the urine whether or not salicylamide was administered.

Compositions of the 0–24-hr urines are summarized in Fig. 1. In animals with patent bile ducts, the total radioactivity excreted in urine decreased with increasing doses of salicylamide, although the decrease was significant only at the 200-mg/kg dose. At the same time, the small quantity of free terbutaline excreted increased about twofold in terms of percentage of dose or fourfold in terms of percentage of total radioactivity in urine. In animals with ligated bile ducts, the total radioactivity excreted in urine remained constant for doses of salicylamide up to 100 mg/kg but decreased with the 200-mg/kg dose. Since excretion was essentially complete in 24 hr, these results confirm that coadministration of high doses of salicylamide decreases terbutaline absorption from the gut.

The fraction of terbutaline excreted in unchanged (*i.e.*, free) form remained essentially constant in the bile duct-ligated animals, suggesting

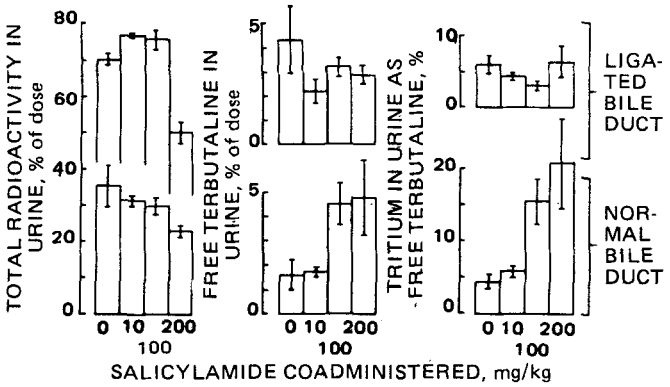

Figure 1—*Excretion of total radioactivity and free terbutaline in the 24-hr urine following oral administration of ^3H-terbutaline (1 mg/kg) coadministered with salicylamide to rats with either patent or ligated bile ducts. Values are the means ±1 SE for groups of three rats.*

that terbutaline in the intestinal wall and in the liver is not appreciably inhibited by coadministered salicylamide. A definitive explanation for the rise in the fraction of terbutaline excreted unchanged when salicylamide was coadministered to normal animals cannot be given. Nevertheless, the observation can be rationalized in terms of the reported finding (2) that unchanged terbutaline passing the gut and subsequently conjugated in the liver is preferentially excreted in the bile while the conjugate previously formed in the intestinal wall is preferentially excreted in urine. The simultaneous presence of salicylamide or its conjugate might influence the partitioning between bile and portal blood of terbutaline glucuronide formed in the liver and thereby influence the observed fraction of terbutaline excreted unchanged in the urine. Such a process would not influence the ratio of metabolites in bile duct-ligated animals since all conjugated drug, regardless of its site of formation, would be excreted in urine.

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Dehydrodiisoeugenol: A Naturally Occurring Lignan from *Aristolochia taliscana* (Aristolochiaceae)

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Abstract □ The ethanol–water extract of *Aristolochia taliscana* Hook and Arn (Aristolochiaceae) yielded a compound which was identified as dehydrodiisoeugenol by means of elemental analysis, IR, UV, NMR, and mass spectra, and direct comparison with a synthetic sample.

Keyphrases □ *Aristolochia taliscana*—ethanol–water extract of aboveground parts, dehydrodiisoeugenol isolated and identified □ Dehydrodiisoeugenol—isolated from ethanol–water extract of aboveground parts of *Aristolochia taliscana* □ Lignans—dehydrodiisoeugenol isolated from ethanol–water extract of aboveground parts of *Aristolochia taliscana*

During the search for tumor inhibitory constituents from plants, it was found that the ethanol–water extract of stems, leaves, flowers, and fruits of *Aristolochia taliscana* Hook and Arn (Aristolochiaceae)[1] contained a crystalline substance that did not show tumor inhibition activity against the P-388 lymphocytic leukemia test system[2] (3PS). However, to determine the nature of the potentially active constituents in the ethanol–water extract of this plant, it was decided to characterize the isolated compound. The plant was collected in Nayarit, Mexico, in June 1970.

RESULTS AND DISCUSSION

Elemental analysis and molecular weight determination of the compound, mp 130°, by mass spectrometry indicated the molecular formula $C_{20}H_{22}O_4$. IR, UV, NMR, and mass spectral data revealed that the compound corresponded to dehydrodiisoeugenol (I), an oxidation coupling product of *trans*-isoeugenol (1). This structure was verified by direct comparison of UV and IR spectra, co-TLC, and mixed melting point with a synthetic sample[3].

Dehydrodiisoeugenol, licarin A, was previously isolated for the first time from the trunk wood of *Licaria aritu* Ducke (Lauraceae) and characterized by IR, UV, optical rotatory dispersion, NMR, and mass spectral methods (2). Except for its melting point (114–116°) and optical activity, other data (UV, IR, and NMR) reported for licarin A[4] were identical with the dehydrodiisoeugenol isolated from *A. taliscana*.

[1] Identification was confirmed by Robert Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson, Ariz., where a reference specimen was also deposited.

[2] Data from the Drug Evaluation Branch, Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, MD 20014.

[3] Prepared in accordance with the procedure given in Ref. 1.

[4] Courtesy of Dr. O. R. Gottlieb, Laboratoio de Produtos Naturais da Fundacao de Amparo a Pesquisa do Estado de San Paulo, Instituto do Quimica, Universidade do Sao Paulo, Brazil.