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Intestinal first-pass metabolism of phenacetin in rabbits pretreated orally and intraperitoneally with 3,4-benzpyrene

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Phenacetin (PHT), used as an analgesic and an antipyretic, is metabolized extensively, the major metabolic route being O-de-ethylation to acetaminophen (NAPA). NAPA is subsequently conjugated to form a glucuronide (NAPA glucuronide, NAPAG) and a sulfate (NAPA sulfate, NAPAS).

It has been reported that an enzyme system present in the mucosal lining of the small intestine of rats is capable of metabolizing PHT and that the enzyme activity increases in rats pretreated with 3,4-benzpyrene [1, 2] and 3-methylcholanthrene [3]. The activity of this intestinal enzyme system is also increased in rats that have been exposed to cigarette smoke [1, 3, 4] or have been fed charcoal-broiled beef [5], rat chow [5] or vegetables [6]. Klippert *et al.* [7] found that 3-methylcholanthrene pretreatment results in enhanced PHT disposition as was shown from decreased plasma half-life time, decreased oral availability, increased clearance, and an increase in metabolite levels. In rats pretreated with 3-methylcholanthrene, the intestine contributes significantly, and predominantly over the liver, to PHT first-pass metabolism. In contrast, gut wall metabolism in control rats could not be demonstrated [7].

In a previous report [8], we demonstrated enhanced PHT metabolism after oral pretreatment of rabbits with 3,4-benzpyrene as shown by decreased levels of PHT and increased levels of NAPA and NAPAG in mesenteric

venous blood. However, no effect of 3,4-benzpyrene on the metabolism of NAPA was found. These results indicate enzyme induction for O-de-ethylation of PHT by pretreatment with 3,4-benzpyrene.

To investigate the effects of two different routes of administration of inducer on enzyme induction, we examined the intestinal first-pass metabolism of PHT in rabbits pretreated orally and intraperitoneally with 3,4-benzpyrene.

Materials and methods

Materials. PHT and 3,4-benzpyrene were of reagent grade and were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). β-Glucuronidase was obtained from the Tokyo Zohki Kagaku Co., Ltd. (Tokyo, Japan). β-Glucuronidase/arylsulfatase was purchased from Boehringer Mannheim GmbH (Mannheim, Federal Republic of Germany). Olive oil was obtained from the Katayama Kagaku Co., Ltd. (Osaka, Japan). All other reagents used in these experiments were of the finest grade available.

Animals. Male albino rabbits, weighing 2.0 to 2.5 kg, were used throughout the study. The animals were housed in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd., Tokyo, Japan). The rabbits were fasted for 48 hr prior to the absorption experiments but had free access to water. They were dosed orally and intraperitoneally with 3,4-benzpyrene (40 mg/kg) dissolved in olive oil; the control rabbits did not receive the olive oil vehicle. Twenty-four and 48 hr after the 3,4-benzpyrene treatment, experiments on the intestinal absorption of PHT were carried out.

Absorption studies. In situ rabbit intestinal sacs with complete mesenteric venous blood collection were prepared as reported by Barr and Riegelman [9]. The technique of collecting all venous blood draining from the region of absorption was developed to provide an *in vivo* preparation with intact circulation. The advantage of this preparation is that free drug and drug metabolites which are absorbed into the capillary blood can be collected in the venous effluent and not reach the general circulation. Animals were anesthetized with pentobarbital, via the marginal ear vein. Additional pentobarbital was administered as necessary during the experiments to maintain anesthesia. After complete anesthesia, a midline incision was made, and the mid-ileal portion of the intestine (5–8 cm) was cut. The intestinal lumen was washed with 0.9% NaCl, and both ends of the mid-ileal portion of intestine were ligated to prepare a closed sac. This portion was selected because of its accessibility and suitable vasculature to facilitate cannulation. The mesenteric vein was cannulated with polyethylene tubing (SP 45, i.d. 0.58 mm, o.d. 0.96 mm, Natsume Seisakusho Co., Ltd., Tokyo, Japan). The coagulation of blood was prevented by the intravenous administration of heparin (1000 I.U.). PHT was dissolved in pH 7.2 buffer solution reported by Schanker and Tocco [10], and 3 ml of this solution was administered by direct injection into the intestinal sac by syringe. All venous blood was collected in centrifuge tubes and assayed for PHT and its metabolites. The blood lost from the mesenteric vein was replaced continuously by intravenous infusion, via ear vein, of 0.9% NaCl. The isolated intestine was kept warm by a lamp and moist by frequent application of 0.9% NaCl to a paper covering the intestine. Statistical analysis of the results was carried out using Student's *t*-test.

Analytical methods. PHT was measured spectrophotometrically with an Hitachi 200-10 spectrophotometer (Hitachi Co., Ltd., Tokyo, Japan) [11]. NAPA, NAPAG and NAPAS were determined using a spectrofluorometric assay as previously described by Shibasaki *et al.* [12]. Glucuronide and sulfate were analyzed after hydrolysis of the sample with β-glucuronidase or β-glucuronidase/arylsulfatase at 37° for 24 hr.

Results and discussion

The effect of induction of intestinal drug-metabolizing enzymes on the absorption of PHT was examined in rabbits pretreated orally and intraperitoneally with 3,4-benzpyrene, using *in situ* intestinal sacs with complete mesenteric venous blood collection. The appearance of both PHT and its metabolites in the mesenteric blood was measured directly by cannulating the mesenteric vein of exposed rabbit intestine and collecting all venous blood draining from the absorbing region.

Table 1 shows the appearance of PHT, NAPA, NAPAG and NAPAS in the mesenteric venous blood after injection of PHT into the intestinal lumen in rabbits pretreated orally with 3,4-benzpyrene. In control rabbits, about 95 and 5% of the PHT absorbed appeared as unchanged drug and metabolites respectively. After oral pretreatment with 3,4-benzpyrene 24 and 48 hr before the absorption experiments, the total amount absorbed did not change compared to control. However, 3,4-benzpyrene pretreatment resulted in enhanced PHT metabolism as was shown from the decreased levels of PHT and the increased levels of NAPA and NAPAG in mesenteric venous blood. Pantuck *et al.* [2] reported that oral administration of 3,4-benzpyrene to rats increases the O-de-ethylation of PHT in subsequently prepared everted intestinal sacs, resulting in an increase in the amount of NAPA and a decrease in the amount of PHT transferred from the mucosal to the serosal side of the sacs *in vitro*. However, the amounts of NAPAG and NAPAS were not determined. Welch *et al.* [13] demonstrated, also in rats, that oral pretreatment with 3-methylcholanthrene decreases the plasma half-life of PHT after intravenous administration and reduces the systemic bioavailability of PHT after oral administration. By comparing the plasma levels of PHT in the portal circulation with those in the peripheral circulation, following the oral administration of PHT, it was concluded that the reduction in the bioavailability of PHT observed in 3-methylcholanthrene-treated rats was caused by a marked increase in the metabolism of PHT during its first pass through the liver. In the present study, enhanced metabolism of PHT in the intestinal wall was found in rabbits pretreated with 3,4-benzpyrene orally.

To investigate the difference in intestinal enzyme induction as a result of the route of administration of inducer, we examined the effect on PHT absorption of the induction of intestinal drug-metabolizing enzymes by intraperitoneal pretreatment with 3,4-benzpyrene. Table 2 shows the appearance of PHT, NAPA, NAPAG and NAPAS in the mesenteric venous blood after injection of PHT into the intestinal lumen in rabbits pretreated intraperitoneally with 3,4-benzpyrene. Following the intraperitoneal pretreatment with 3,4-benzpyrene 24 hr before the absorption experiments, no effect was found on the metabolism of PHT compared to control. 3,4-Benzpyrene pretreatment 48 hr before the absorption experiments resulted in enhanced PHT metabolism as shown by decreased levels of PHT and increased levels of NAPA and NAPAG in mesenteric venous blood.

Aitio *et al.* [14] reported that the intragastric administration of a single dose of 3,4-benzpyrene or 3-methylcholanthrene causes an increase in the activity of both arylhydrocarbon hydroxylase and UDP glucuronyltransferase in the intestine, liver and kidney. The fastest response was observed in the hydroxylase activity of the intestinal mucosa. Furthermore, Aitio [15] demonstrated that arylhydrocarbon hydroxylase activity of rat liver, kidney and intestinal mucosa increases rapidly after intragastric administration of 20-methylcholanthrene. Intraperitoneal administration of 20-methylcholanthrene has only a slight effect on the intestinal mucosal arylhydrocarbon hydroxylase. In addition, different effects on mutagenic activation and N-hydroxylation of 2-aminofluorene were also observed after the intraperitoneal and intragastric administration of 3-methylcholanthrene [16]. When injected intra-

Table 1. Appearance of PHT and its metabolites in mesenteric venous blood after administration of PHT in rabbits pretreated orally with 3,4-benzpyrene

Treatment	Time after treatment (hr)	Total amount absorbed (% of dose)	PHT	NAPA	NAPAG	NAPAS
				(μg)		
Control		79.8 ± 3.7 (4)	456.1 ± 24.8	7.8 ± 1.7	9.8 ± 2.4	5.4 ± 0.6
3,4-Benzpyrene	24	87.4 ± 1.2 (5)	356.5 ± 16.7*	98.5 ± 5.7†	60.8 ± 7.4‡	8.8 ± 3.5
3,4-Benzpyrene	48	86.9 ± 4.6 (4)	358.9 ± 32.5	87.8 ± 3.5†	68.6 ± 15.4§	6.0 ± 3.7

Dose: 3 ml of 200 μg/ml solution of PHT. Blood collection: 0–120 min. Results are means ± SE. Numbers in parentheses represent the number of experiments.

The amounts of NAPA, NAPAG and NAPAS were calculated as PHT.

*–§ Statistical significance: * $P < 0.02$; † $P < 0.001$; ‡ $P < 0.01$; and § $P < 0.05$.

Table 2. Appearance of PHT and its metabolites in mesenteric venous blood after administration of PHT in rabbits pretreated intraperitoneally with 3,4-benzpyrene

Treatment	Time after treatment (hr)	Total amount absorbed (% of dose)	PHT	NAPA	NAPAG	NAPAS
				(μg)		
Control		79.8 ± 3.7 (4)	456.1 ± 24.8	7.8 ± 1.7	9.8 ± 2.4	5.4 ± 0.6
3,4-Benzpyrene	24	81.4 ± 4.0 (5)	453.4 ± 20.8	15.4 ± 5.8	13.3 ± 5.4	6.3 ± 1.8
3,4-Benzpyrene	48	75.8 ± 3.6 (5)	327.5 ± 20.8*	69.0 ± 6.6*	47.6 ± 5.8†	10.8 ± 3.2

Dose: 3 ml of 200 μg/ml solution of PHT. Blood collection: 0–120 min. Results are means ± SE. Numbers in parentheses represent the number of experiments.

The amounts of NAPA, NAPAG and NAPAS were calculated as PHT.

*,† Statistical significance: * $P < 0.01$, and † $P < 0.001$.

peritoneally into rats, 3-methylcholanthrene did not modify either the N-hydroxylation or the mutagenic properties of 2-aminofluorene in the presence of postmitochondrial and microsomal fractions from small intestine. On the other hand, intragastric administration of the inducer significantly induced maximum velocity of *N*-hydroxylase. Moreover, the ability of the small intestinal fractions to activate 2-aminofluorene into a mutagenic intermediate(s) was enhanced after intragastric application. From these results, the mode of administration of inducer appears to be decisive in the induction of small intestinal enzymes.

In the present study, the responses to oral and intraperitoneal administration of 3,4-benzpyrene were profoundly different. The difference in the effect of 3,4-benzpyrene, depending on the route of administration, on intestinal enzyme induction was probably due to the difference in concentration of 3,4-benzpyrene reaching the intestine. The intestinal tract receives most of the inducer when it is given orally. Additional studies are needed to clarify the slow response to intraperitoneal administration of 3,4-benzpyrene in the intestine.

In summary, oral 3,4-benzpyrene pretreatment of rabbits 24 and 48 hr before the absorption experiments resulted in enhanced PHT metabolism as shown by the decreased levels of PHT and the increased levels of NAPA and NAPAG in mesenteric venous blood after injection of PHT into the intestinal lumen. Following the intraperitoneal pretreatment with 3,4-benzpyrene 24 hr before the absorption experiments, no effect was found on the metabolism of PHT compared to control. 3,4-Benzpyrene pretreatment 48 hr before the absorption experiments resulted in enhanced PHT metabolism as shown by the decreased levels of PHT and the increased levels of NAPA and NAPAG in mesenteric venous blood.

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