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Effects of Proton Pump Inhibitors on Thyroid Hormone Metabolism in Rats

A COMPARISON OF UDP-GLUCURONYLTRANSFERASE INDUCTION

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ABSTRACT. The effects of proton pump inhibitors on thyroid hormone metabolism in rats were examined. Pantoprazole, omeprazole, and lansoprazole were administered repeatedly to female SD rats at doses of 5, 50, and 300 mg/kg/day for 1 week, and changes in UDP-glucuronyltransferase activities were examined. Increases in *o*-aminophenol UDP-glucuronyltransferase activity, which was measured as that responsible for the glucuronidation of thyroxine, were evident following 7-day high-dose administration of all the proton pump inhibitors tested. Of the three proton pump inhibitors investigated, *o*-aminophenol UDP-glucuronyltransferase activity was greatest following the high-dose administration of omeprazole. Androsterone UDP-glucuronyltransferase activity in rats treated with the proton pump inhibitors increased significantly, but these increases were smaller than those of *o*-aminophenol UDP-glucuronyltransferase. Pantoprazole and omeprazole treatment did not affect plasma T_4 or T_3 significantly, whereas lansoprazole treatment produced marked reductions in plasma T_4 but did not affect plasma T_3 significantly. After administration of ^{125}I-labeled thyroid hormone to rats treated with the proton pump inhibitors, biliary excretion of radioactivity increased significantly in omeprazole- and lansoprazole-treated rats; these increases were attributed to induction of liver thyroxine UDP-glucuronyltransferase activities. The order of biliary excretion of radioactivity, as well as the *o*-aminophenol UDP-glucuronyltransferase activity, in the treated animals was: omeprazole > lansoprazole > pantoprazole. Therefore, repeated administration of the proton pump inhibitors increased thyroxine-metabolizing activity via induction of UDP-glucuronyltransferase, and this induction by pantoprazole was less pronounced than that by omeprazole or lansoprazole. BIOCHEM PHARMACOL **54**;11:1225–1231, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. proton pump inhibitors; pantoprazole; omeprazole; lansoprazole; UDP-glucuronyltransferase; thyroxine

PAN,† OM, and LAN are substituted benzimidazole derivatives (see Fig. 1) that have potent and long-lasting inhibitory effects on gastric acid secretion. They are classified as PPIs, as they covalently bind to the H^+/K^+ ATPase of parietal cells via the formation of a disulfide bond [1–3]. PPIs have potent clinical effects on gastric ulcer healing, superior to the H_2 blockers [3–5].

The interaction of PPIs with cytochrome P450 enzymes has been investigated extensively. Both OM and LAN are mixed inducers of CYP1A and CYP3A in human hepatocytes in primary culture [6, 7]. The metabolism of caffeine is increased after repeated administration of OM in humans [8]. A significant increase of antipyrine clearance [9] and an induction of theophylline metabolism by repeated treatment with LAN have also been reported in humans [10]. On the other hand, an absence of inductive effects on drug-metabolizing enzymes after repeated administration of PAN was shown in humans [11], although it induced hepatic xenobiotic-metabolizing enzymes in the rat [12].

LAN has been reported to have a toxic effect on the thyroid in carcinogenicity tests in rats [13]. Two causal factors have been suggested regarding the initiation of these effects: (1) a direct action of active intermediates of certain drugs on the thyroid, as in the case of *N*-methyl-*N*-nitrosourea and *N*-bis(2-hydroxypropyl)nitrosoamine [14]; and (2) an indirect action on the thyroid caused by the induction of UGT activity, resulting in the subsequent decrease in plasma levels of thyroid hormone and the increased secretion of thyroid-stimulating hormone, thereby eliciting thyroid hyperplasia, as in the case of phenobarbital and other drug-metabolizing enzyme inducers [15–17].

The two main thyroid hormones, i.e. T_4 and T_3, have differential physiological activities, with T_3 activity higher than that of T_4. In peripheral tissues, a portion of T_4 is deiodinated to T_3. Both hormones are metabolized by UGT

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† *Abbreviations*: PAN, pantoprazole; OM, omeprazole; LAN, lansoprazole; PPI, proton pump inhibitor; U, untreated; UGT, UDP-glucuronyltransferase; T_3, triiodothyronine; and T_4, thyroxine.

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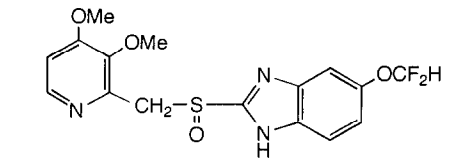
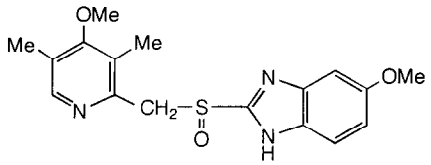


Pantoprazole (PAN)



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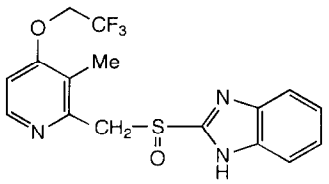

Lansoprazole (LAN)

FIG. 1. Chemical structures of PAN, OM, and LAN.

or sulfo-conjugation in liver, and these conjugated forms are excreted in bile. The decreases in plasma concentrations of thyroid hormones following the administration of xenobiotic-metabolizing enzyme inducers is thought to be due to increased glucuronic acid conjugation of the hormones [18, 19]. The generation of thyroid tumors, concomitant with the induction of xenobiotic-metabolizing enzymes, is typically observed in experimental animals, although such a mechanism is not thought to occur in humans [14]. In clinical studies, these PPIs show no effect on thyroid hormones [20–23].

To examine the mechanism of thyroid tumor generation in rats by PPIs, we investigated the induction of distinct UGT activities, changes in plasma concentrations of thyroid hormones, and the pharmacokinetics of the hormones themselves, as well as their glucuronic acid conjugates.

MATERIALS AND METHODS

Materials

PAN was supplied by Byk Gulden GmbH (Konstanz, Germany). OM and LAN were synthesized by the Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Triton X-100 and *o*-aminophenol were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{3}H]Androsterone (specific radioactivity: 1250 μCi/μg, 7.25 MBq/μg) and [^{125}I]T_4 (specific radioactivity: 1250 μCi/μg, 46.3 MBq/μg) were purchased from Du Pont/NEN Research Products (Boston, MA, U.S.A.). Androsterone was purchased from the Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Total and free T_4 and total and free T_3 radioimmunoassay kits were purchased from Kodak Japan Ltd. (Tokyo, Japan). UDP-glucuronic acid was purchased from the Oriental Yeast Co., Ltd. (Tokyo, Japan), and $MgCl_2$ was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were commercially available and of analytical grade.

Animals and Treatment

SD female rats (body weight: 150–211 g) were used and assigned to the following groups: (N = 3): control, PAN, OM, and LAN. The PAN-, OM-, and LAN-treated groups received oral doses with a carboxymethyl cellulose suspension (0.5%) of 5, 50, or 300 mg/kg/day for 1 week. The control group received orally a carboxymethyl cellulose suspension (0.5%) of 1 mL/kg/day for 1 week.

Assays for UGT Activities

Rats were killed by exsanguination from the carotid artery 24 hr following the final administration. Livers were excised, and hepatic microsomes were prepared. Protein concentrations in microsomes were determined by the method of Lowry *et al.* [24] using bovine serum albumin (Fraction V, Sigma) as the standard.

UGT activities toward *o*-aminophenol [25] or androsterone [26] were determined as described previously. The substrate concentrations of *o*-aminophenol and androsterone were 250 and 10 μM, respectively.

Assays for Plasma Concentration of Thyroid Hormones

Blood samples from the rats were centrifuged at 1500 × *g* for 10 min to obtain plasma. Total and free T_4 and total and free T_3 levels were determined by radioimmunoassay with the kits purchased from Kodak Japan Ltd.

Biliary Excretion of Thyroid Hormone

PAN, OM, and LAN were administered orally to female SD rats for 1 week at a dose of 300 mg/kg/day. One day following the final drug dose, [^{125}I]T_4 was administered to each animal at a dose of 25 μCi/rat. Five hours after administration of the [^{125}I]T_4, bile ducts were cannulated and samples were collected at 10-min intervals for 40 min. The amount of total bile excreted during this time period was measured by weight, and the radioactivity of the sample was quantitated using a gamma counter (Aloka Co., Ltd., Tokyo, Japan).

HPLC Analysis of Bile Samples

The bile samples collected from the rats following administration of [^{125}I]T_4 were diluted 2-fold with water, and subjected to HPLC analysis according to the method of Rutgers *et al.* [27]. The HPLC system (Beckman Instru-

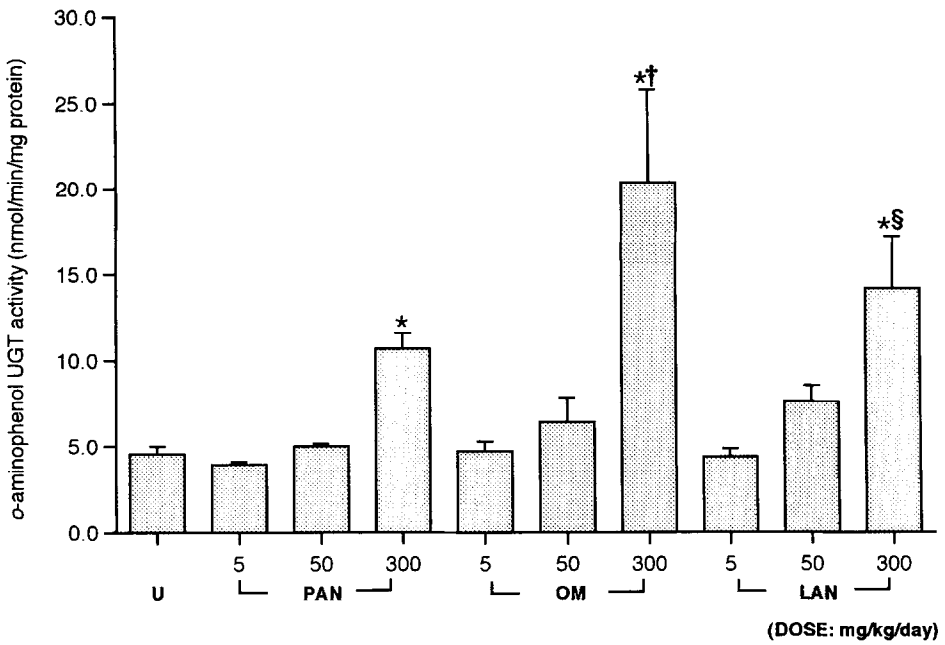

FIG. 2. UGT activity of o-aminophenol (250 μM) in rat liver microsomes following repeated oral administration of PAN, OM, and LAN at doses of 5, 50, and 300 mg/kg/day for 1 week. Values are means ± SD, N = 3. Groups were compared by ANOVA, followed by Fisher's PLSD method for multiple comparisons. Key: (*) significant difference from control (U) group, $P < 0.05$; (†) significant difference from PAN-treated group at the respective dose, $P < 0.05$; and (§) significant difference from OM-treated group at the respective dose, $P < 0.05$.

ments Inc., Fullerton, CA, U.S.A.) was as follows: column, Inertsil ODS2 (GL Science, Tokyo, Japan); UV detector, programmable solvent module 166; radioactivity detector, Radiodetector 171; solid cell, volume 125 μL; pump, programmable solvent module 126; autosampler, System Gold 507; computer program for analysis, System Gold 711. A detection wavelength at 254 nm was used with a flow rate of 0.8 mL/min. The mobile phase consisted of 0.02 M ammonium acetate (pH 4.0) and acetonitrile and was mixed by a gradient program (0–6 min, 84/16; 6–18 min, from 84/16 to 73/27; 18–22 min, 73/27; 22–27 min, from 73/27 to 55/45; 27–38 min, 55/45).

Data Presentation and Statistical Analysis

All values are presented as means ± SD of three rats. Groups were compared by ANOVA, followed by Fisher's PLSD method for multiple comparisons. A P value < 0.05 was considered statistically significant.

RESULTS

Activities of o-Aminophenol UGT and Androsterone UGT

Repeated administration of PPIs at the highest dose of 300 mg/kg/day caused a significant increase in o-aminophenol UGT activity when compared with activity in the control (untreated) group (Fig. 2). The increases due to PAN (2.4-fold) and LAN (3.1-fold) were significantly smaller than those observed with OM at the highest dose (4.5-fold). All three PPIs affected UGT activities toward androsterone. The activities increased significantly following administration of PAN at the high (1.3-fold) and middle (1.3-fold) doses and OM at the highest dose (1.3-fold), but these increases were much smaller than those of o-aminophenol UGT. LAN slightly reduced androsterone UGT activity at the low (88%) and middle (86%) doses (Fig. 3).

Plasma Concentration of Thyroid Hormone

Plasma concentrations of total and free T_3 24 hr after the last administration were not affected by repeated administration of PPIs (Table 1). In contrast, plasma concentrations of total T_4 significantly decreased in the LAN-treated group (45%). Administration of PAN (23%) and OM (20%) seemed to decrease total T_4 levels; however, these decreases were not statistically significant. The plasma concentrations of free T_4 decreased by PAN (20%) and OM (18%) treatment, and the decreases by the compounds were significantly smaller than those of LAN (46%).

Biliary Excretion of Thyroid Hormone

Thyroid hormones in the rat were radiolabeled with ^{125}I by injecting the animal with [^{125}I]T_4, and the radioactivity excreted in the bile was measured to determine the effect of repeated administration of each test substance on biliary excretion of thyroid hormones. Repeated administration of PPIs at a dose of 300 mg/kg/day resulted in an increase in the amount of total radioactivity excreted in bile during a 10-min interval, compared with the control group (Fig. 4). Rats administered OM and LAN showed significant increases of 2.7- and 2.2-fold in biliary excretion of [^{125}I]hormone, respectively, whereas a significant increase was not found in the treatment with PAN (Fig. 4). In agreement with the increases in the biliary excretion of radioactivity following treatment with the PPIs tested, the total volume of excreted bile also increased by 1.5- to 2.1-fold (data not shown). A correlation between the rate of biliary excretion

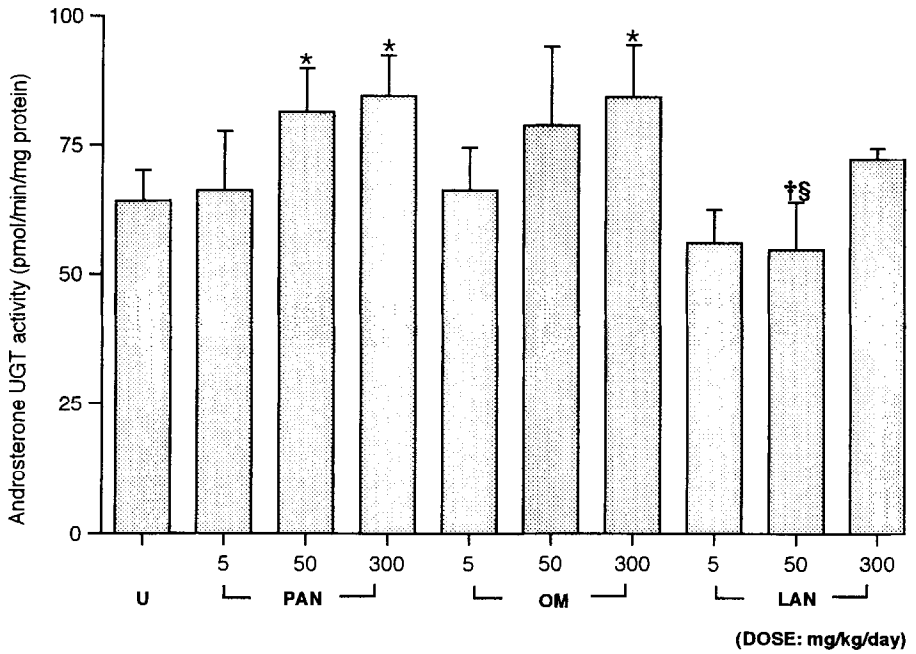

FIG. 3. UGT activity of androsterone (10 μM) in rat liver microsomes following repeated oral administration of PAN, OM, and LAN at doses of 5, 50, and 300 mg/kg/day for 1 week. Values are means ± SD, N = 3. Groups were compared by ANOVA, followed by Fisher's PLSD method for multiple comparisons. Key: (*) significant difference from control (U) group, $P < 0.05$; (†) significant difference from PAN-treated group at the respective dose, $P < 0.05$; and (§) significant difference from OM-treated group at the respective dose, $P < 0.05$.

of T_4 with the activities of *o*-aminophenol UGT (Fig. 2) was found to be good ($r^2 = 0.884$, $P < 0.0001$) (Fig. 5).

Profile of Biliary Radioactivity after Administration of Labeled Thyroid Hormone

To examine whether specific thyroid hormone metabolites excreted in bile were affected by treatment with these drugs, the HPLC profile of the biliary metabolites was determined utilizing a radioisotope detector. The chromatographic analysis of the bile demonstrated that the radioactivity excreted in bile was contained mainly in T_4 and T_4 glucuronide, in addition to other minor metabolites (Fig. 6). Treatment with PAN, OM, and LAN resulted in the excretion of labeled T_4 glucuronide, with negligible levels of excreted free T_4.

DISCUSSION

UGTs metabolize thyroid hormones by conjugation of the hormones with glucuronic acid. Induction of T_4 UGT activity results in enhanced conjugation with subsequent decreased plasma T_4 levels and increased secretion of thyroid stimulating hormone, thereby eliciting thyroid hyperplasia.

Typical xenobiotic-metabolizing enzyme inducers such as 3-methylcholanthrene and phenobarbital induce UGTs along with cytochrome P450s, and 3-methylcholanthrene

TABLE 1. Plasma thyroid hormeon levels following repeated oral administration of PAN, OM, and LAN at a dose of 300 mg/kg/day for 1 week

Treatment	Total T_3 (ng/mL)	Total T_4 (ng/mL)	Free T_3 (pg/mL)	Free T_4 (pg/mL)
U	0.62 ± 0.20	36.8 ± 8.5	3.43 ± 0.08	15.1 ± 3.4
PAN	0.71 ± 0.06	28.5 ± 8.6	3.21 ± 0.28	12.1 ± 1.0*
OM	0.51 ± 0.05†	29.3 ± 0.3	2.97 ± 0.17	12.4 ± 0.6
LAN	0.54 ± 0.07	20.1 ± 3.9*	2.95 ± 0.55	8.1 ± 1.1*†§

All values are presented as means ± SD, N = 3. Groups were compared by ANOVA, followed by Fisher's PLSD method for multiple comparisons.
* Significant difference from control group, $P < 0.05$.
† Significant difference from PAN-treated group, $P < 0.05$.
§ Significant difference from OM-treated group, $P < 0.05$

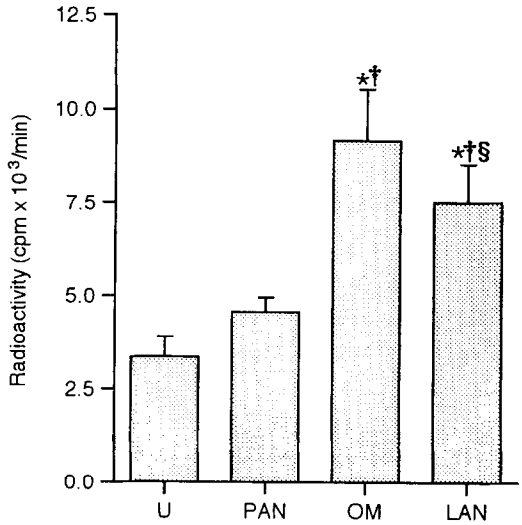

FIG. 4. Biliary excretion of $[^{125}I]T_4$ in PAN-, OM-, and LAN-treated rats. Rats previously exposed to PAN (300 mg/kg/day for 1 week), OM (300 mg/kg/day for 1 week), and LAN (300 mg/kg/day for 1 week) received $[^{125}I]T_4$ i.v. About 5 hr later, bile collection was started, followed by counting the radioactivity excreted in bile. Values are means ± SD, N = 3. Groups were compared by ANOVA, followed by Fisher's PLSD method for multiple comparisons. Key: (*) significant difference from control (U) group, $P < 0.05$; (†) significant difference from PAN-treated group, $P < 0.05$; and (§) significant difference from OM-treated group, $P < 0.05$.

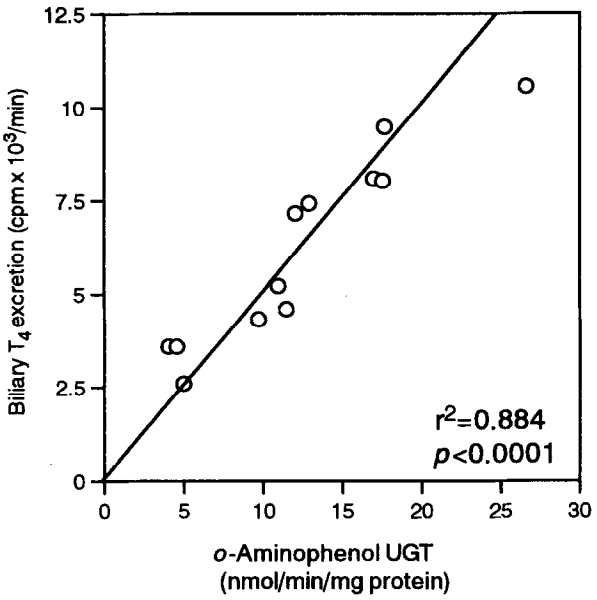

FIG. 5. Correlation between T_4 glucuronidation and o-aminophenol in rats treated with PAN (300 mg/kg/day for 1 week), OM (300 mg/kg/day for 1 week), and LAN (300 mg/kg/day for 1 week).

and phenobarbital are known to induce distinct UGT enzymes, similar to the cytochrome P450 situation [28]. Because OM and LAN were reported to induce cytochrome P450s, it was postulated that they may also induce UGT enzymes. The thyroid hormones, T_3 and T_4, are conjugated with glucuronic acid by different UGT forms [29, 30].

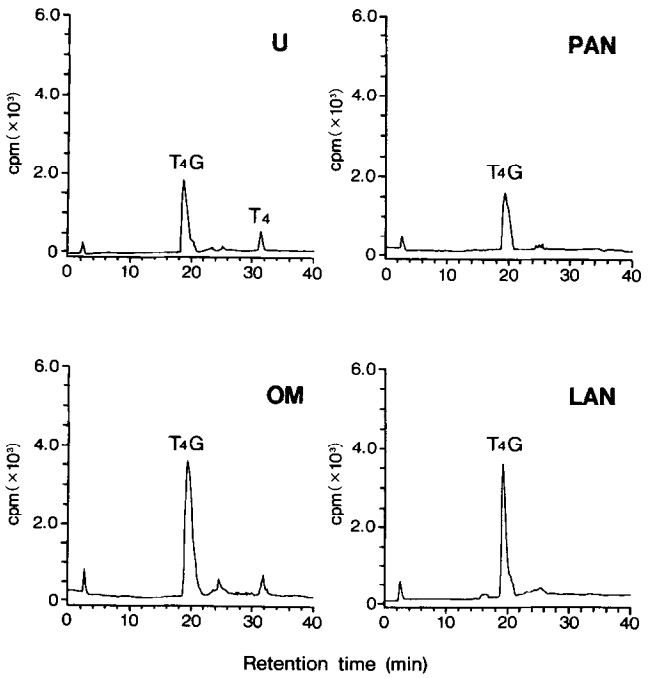

FIG. 6. HPLC analysis of bile from PAN-, OM-, and LAN-treated rats at a dose of 300 mg/kg/day for 1 week. Bile samples were analyzed by HPLC as described in Materials and Methods. T_4G, thyroxine glucuronide.

Recently, multiple forms of UGT have been reported, and the UGT enzyme(s), primarily in the UGT1 family, that catalyzes the conjugation of p-nitrophenol and o-aminophenol, and is inducible by inducers such as 3-methylcholanthrene, has been supposed to be the major UGT isoform(s) that catalyzes the glucuronic acid conjugation of T_4. On the other hand, the UGT enzyme(s), primarily in the UGT2B subfamily, that catalyzes the metabolism of androsterone has been supposed to be the major one that catalyzes the conjugation of T_3 [28, 31–33]. Thus, in this study, o-aminophenol and androsterone were used as surrogate markers for UGT activity toward T_4 and T_3, respectively, while more than one UGT enzyme has been proposed to be involved in the conjugation of T_4 and T_3.

In this study, different UGT activities in hepatic microsomes were measured following the repeated administration of three PPIs to examine the relative effects of these compounds on thyroid hormone-metabolizing enzymes. Of the three compounds tested, the o-aminophenol UGT activity, which is supposed to also catalyze the glucuronidation of T_4, was in the order of OM > LAN > PAN following the high-dose administration. In one of our recent studies, the repeated administration of PAN in rats resulted in the induction of CYP1A subfamily; however, the induction level by PAN was smaller than those by OM and LAN [34], which was similar to those of o-aminophenol UGT activities in the present study. In contrast, the induction of the UGT enzyme responsible for glucuronidation of T_3 was measured using androsterone as a marker substrate. The degrees of the enzyme activities that glucuronidated T_3 in the treatment of the PPIs were lower than those of T_4 UGT activities.

All of the PPIs induced o-aminophenol UGT activities to some extent following high-dose administration, and this induction varied among the compounds. As a result, the effects of this varied enzyme induction on total plasma levels of thyroid hormones were examined. As expected, T_4 comprised the majority of total circulating thyroid hormone, and changes in UGT activities responsible for the modification of T_4 affected total thyroid hormone levels. Of the three PPIs tested, the decrease in total and free T_4 levels was greatest following treatment with LAN; however, overall decreases in thyroid hormone levels were relatively small after treatment with the PPIs. In the body, there is a circulating pool of thyroid hormone, and this pool is maintained at constant levels to assure thyroid homeostasis. The mechanism responsible for maintaining this pool prevents abrupt decreases in plasma T_3 or T_4 levels, irrespective of the induction of particular hepatic-metabolizing enzymes [35]. Administration of 3-methylcholanthrene, on the other hand, induces UGT activity to such an extent that this pool of thyroid hormones is severely depleted. β-Naphthoflavone, a 3-methylcholanthrene-type inducer of xenobiotic-metabolizing enzymes, causes a marked decrease in plasma T_4 levels as well as an increase in p-nitrophenol UGT activity [36]. Treatment with this compound did not affect plasma concentrations of T_3,

consistent with our present observations that PPIs do not alter T_3 UGT activity significantly. Changes in the levels of T_3 cannot be explained solely by changes in the UGT activity [37].

Biliary excretion of ^{125}I-labeled thyroid hormones significantly increased with repeated administration of OM and LAN but not with PAN. The extent of increased biliary excretion for each compound correlated with the extent of *o*-aminophenol UGT induction. Therefore, the increases of biliary excretion were attributed to induction of liver T_4 UGT activities. Direct chromatographic analysis of the circulating hormones demonstrated that free T_4 levels observed in the untreated group essentially disappeared with the various treatments, and the T_4-glucuronide conjugate became the most detectable form. Concomitant with the increase in biliary excretion of thyroid hormones, the volume of total bile also increased; therefore, the increase in total bile volume would offset and maintain a constant concentration of thyroid hormone in bile.

In conclusion, the repeated dosing of PAN, OM, and LAN at 300 mg/kg/day increased both T_4 UGT activities and the biliary excretion of glucuronidated T_4 in the rat. The increases following PAN administration were smaller than those seen with the other PPIs. Thus, the possibility of thyroid hyperplasia induced by PAN was suggested to be less than that by other PPIs.

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