BISPHENOL A GLUCURONIDATION AND ABSORPTION IN RAT INTESTINE

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ABSTRACT:

Bisphenol A, an environmental estrogen, can be leached from plastic tableware and from the coating of food and drink cans, orally exposing human beings to the compound. The present study focuses on the absorption and metabolism of bisphenol A in the rat intestine, as elucidated experimentally by segmented everted intestine. One hour after the application of 2 μmol of bisphenol A to the mucosal fluid, the absorption of bisphenol A was slightly greater in the colon (48.6%) than in the proximal jejunum (37.5%). In the serosal side, unconjugated bisphenol A appeared in small amounts, increasing distally (maximal 1.6 nmol, colon). Large amounts of the bisphenol A glucuronide were then transported into the serosal side, also increasing distally (proximal, 80.4 nmol; distal, 478.4 nmol). The greatest amount of the glucuronide (573 nmol) was excreted into the mucosal side of the small intestine, whereas in the colon, mucosal excretion was minimal (67.2 nmol). On high-dose application of bisphenol A to the mucosal fluid, the transported unconjugated bisphenol A increased markedly throughout the intestine and colon. These results suggest that bisphenol A in the intestinal lumen is glucuronidated almost exclusively during its passage through the intestinal wall.

A growing number of industrial chemicals has been reported to act as endocrine disrupters in mammals and other animals (Hoyer, 2001). One of the prominent environmental hormones, bisphenol A 3 (2,2-bis[4-hydroxyphenyl]propane), has demonstrated estrogenic activity having adverse effects on the reproductive system (Kim et al., 2001; Chen et al., 2002). Bisphenol A is widely used in the manufacture of epoxy, polycarbonate, and polyester-styrene resins (National Toxicology Program, 1982), and traces of the compound can be easily leached from food containers and tableware made of such plastics and can be taken up by human beings through eating and drinking (Brotzons et al., 1995; vom Saal et al., 1998). In vitro, bisphenol A has stimulated cell proliferation as well as the induction of progesterone receptors on MCF-7 human breast cancer cells (Krishnan et al., 1993). In the reproductive tract of female rats, a single high dose of the compound (37.5–150 mg/kg) was reported to induce cell differentiation, and c-fos proto-oncogene expression (Steinmetz et al., 1998). Exposing pregnant CF-1 mice for 7 days to bisphenol A (2.4 mg/kg) evoked early puberty of the female offspring, significantly hastening vaginal opening and onset of estrous (Howdeshell et al., 1999). Bisphenol A introduced orally into the body must pass through the intestine and liver before arriving at the reproductive organs, where irreversible damage may be inflicted. To elucidate the mechanism governing the detrimental effects on the target organs, it is essential to clarify metabolism and disposition of the compound during its journey through the gastrointestinal tract. Previously, we found that bisphenol A in rat liver is extensively glucuronidated by UGT2B1, an isoform of UDP-glucuronosyltransferase (Yokota et al., 1999). Additional study showed that the compound is glucuronidated in rat perfused liver and is excreted exclusively into the bile (Inoue et al., 2001). In the intestine, however, which provides the foremost barrier against ingested toxicants, behavior of the compound has not been delineated.

In experiments applying 1-naphthol to rat-everted intestine, glucuronidation activity toward the phenolic compound was evident, and the resultant glucuronide was expelled from the enteric mucosal cells into the lumen (Inoue et al., 1999). These observations, together with our hepatic findings to date, led us to conduct the present work using everted intestine to determine the fate of bisphenol A that enters the intestine of the rat.

Materials and Methods

Chemicals. Bisphenol A was purchased from Kanto Chemical Co. (Tokyo, Japan); high-performance liquid chromatography (HPLC) grade acetoni-trile from Labscan Ltd. (Dublin, Ireland); β-glucuronidase (type B-1; from bovine liver) from Sigma-Aldrich (St. Louis, MO). Bisphenol A glucuronide purified from the bile after rat liver perfusion with 7.5 μmol of bisphenol A (Inoue et al., 2001) was quantified by HPLC by using the difference between β-glucuronidase-treated sample and untreated sample, and was used as a standard.

Animals. Male Sprague-Dawley rats (8-weeks old; 300–340 g) were used in all experiments. The rats were housed under standard conditions and given food and water ad libitum. The animals were handled according to the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which is based on the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health.

Preparation of Everted Intestine. Krebs-Ringer’s bicarbonate buffer (135.0 mM Na+, 5.0 mM K+, 2.5 mM Ca2+, 1.2 mM Mg2+, 122.4 mM Cl−, 25.0 mM HCO3−, 10.0 mM glucose) was used in all experiments. The buffer...
solution was aerated by 95% O₂, 5% CO₂ and the pH was adjusted to 7.4. The rats were euthanized by decapsulation, and the jejunum, ileum, and colon were flushed with cold Krebs Ringer’s buffer. The bowels of the animals were excised and prepared according to a modification of the segmentation and eversion method described previously (Inoue et al., 1999). Briefly, with the exception of the duodenum, the excised small intestine was lavaged and divided into four sections of equal length as quickly as possible. The distal portion of each section was excised and trimmed to 10 cm and designated I, II, III, and IV in distal order, with segment I being from the jejunum and segment IV from the distal ileum. In the same manner, the colon was excised, washed, and trimmed to a final segment of 10 cm taken from the distal end.

The five trimmed segments were turned inside out and fixed on a polyethylene tube in the mucosal buffer solution (40 ml). Serosal buffer solution (40 ml) was pumped through the everted bowels (tube pump MP-32N; EYELA, Tokyo, Japan) at 5 ml per min via polyethylene tubes. Bisphenol A was added to the mucosal buffer solution in concentrations of 10, 50, and 100 μM, and reaction products were collected independently from the serosal and mucosal sides at 0, 20, 40, and 60 min after each addition of the compound.

**HPLC Analysis of Reaction Products.** The mucosal and serosal samples were filtered by a disposable disk filter (HLC-DISK3) from Kanto Chemical Co. and stored at −80°C until analysis. The samples were analyzed by an HPLC system (Tosoh, Tokyo, Japan) based on the method described previously (Yokota et al., 1999). Briefly, the samples were eluted with a solution of acetonitrile/H₂O/acetic acid (37:63:0.1 v/v/v) on a constant flow rate at 1 ml/min. The eluted samples were analyzed with UV 222-nm detection using TSK-gel ODS-80Ts-reversed phase column (4.6 × 250-mm; Tosoh Tokyo, Japan). The results were recorded with C-R6A integrator from Shimadzu (Tokyo, Japan).

**β-Glucuronidase Reaction.** After being filtered, the various mucosal and serosal buffer solutions were allowed to react with the β-glucuronidase (Inoue et al., 2001), and the products were analyzed by HPLC to verify whether the metabolite was the glucuronide. Hardly any sulfatase activity of the β-glucuronidase was detected by HPLC under the same conditions as those for β-naphthyl sulfate, indicating that the metabolite was in fact the glucuronide.

**Statistical Analysis.** All data were presented as the mean ± S.E., and the means were compared by use of analysis of variance, with the p value of 0.05 as the level of significance.

**Results**

**High-Performance Liquid Chromatography of Buffer Solution.** Elution profiles obtained by HPLC are shown in Fig. 1. In mucosal buffer solution from the intestinal segments treated with 50 μM bisphenol A, three peaks (numbered as 1, 2, and 3) were observed. With increasing incubation time, the peak eluted at ~5.6 min (peak 1) increased gradually, and the last peak (peak 3) decreased (Fig. 1, A-C). In serosal buffer solution, a modest peak (peak 3) of the substrate appeared at ~19 min (Fig. 1D). In the solution containing β-glucuronidase, which cleaves the glucuronide, unconjugated bisphenol A appeared (Fig. 1E; peak 3), signaling that the first peak (peak 1) represented the bisphenol A glucuronide. An unidentified peak eluted at ~6.8 min (peak 2) did not change dynamically with incubation time.

**Bisphenol A Absorption and Transport.** On each addition of bisphenol A (10, 50, or 100 μM) to the mucosal side, bisphenol A concentration in the mucosal fluid decreased with incubation. As shown in Table 1, the rate of bisphenol A disappearance from the mucosal compartment was notable on a high dose of bisphenol A (100 μM). Although the distal intestine showed the greatest extent of bisphenol A disappearance, the data were not significant among the five segments of the intestine (p > 0.052).

Bisphenol A was absorbed from the mucosal side and transported to the serosal side, as evidenced by the appearance of small amounts of unconjugated bisphenol A. The appearance of serosal bisphenol A increased distally (Fig. 2) and accelerated markedly with the high-dose application (100 μM).

**Bisphenol A Glucuronidation.** Bisphenol-A glucuronide expelled into the mucosal side as well as that transported into the serosal side increased with the incubation period (Fig. 3). In contrast to the excretion of the glucuronide to the mucosal side, an ~10-min time lag was observed in the transport of glucuronide into the serosal side, suggesting that the intestinal wall may have interfered with the diffusion of the glucuronide in the everted intestine experimental system. Although both the excretion and transport of the glucuronide increased in relation to the administrative dose of bisphenol A, the amounts appeared to reach plateau with a high dose (100 μM) of the compound.

In the small intestine, the greatest amount of the glucuronide was secreted into the mucosal side, whereas in the colon, the mucosal secretion of the glucuronide was diminished (Fig. 4). Glucuronide secretion to the serosal side was minimal in the proximal small intestine and increased with progression distally to the colon.

**Fate of Bisphenol A at 60-min Postapplication.** In the small intestine at 60-min postapplication of low-dose bisphenol A (10 μM), the absorbed substrate was almost completely glucuronidated, and the
Bisphenol A was added to the mucosal buffer solution of each segment in concentrations of 10 μM, 50 μM, and 100 μM. The amount of bisphenol A disappearance (mean ± S.E.) was obtained by subtracting the final amounts of bisphenol A in the mucosal buffer solution after 60-min incubation. I, II, III, and IV indicate the intestinal sites in distal order from the ligament of Trietz.

TABLE 1
Bisphenol A disappearance from the mucosal compartment of rat everted intestinal segments within 60-min incubation

<table>
<thead>
<tr>
<th>Dose (μM)</th>
<th>I (nmol/10-cm bowel/60 min)</th>
<th>II (nmol/10-cm bowel/60 min)</th>
<th>III (nmol/10-cm bowel/60 min)</th>
<th>IV (nmol/10-cm bowel/60 min)</th>
<th>Colon (nmol/10-cm bowel/60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>175 ± 22</td>
<td>158 ± 14</td>
<td>163 ± 14</td>
<td>198 ± 18</td>
<td>244 ± 7</td>
</tr>
<tr>
<td>50</td>
<td>751 ± 92</td>
<td>996 ± 98</td>
<td>862 ± 111</td>
<td>1086 ± 105</td>
<td>1163 ± 98</td>
</tr>
<tr>
<td>100</td>
<td>1325 ± 324</td>
<td>1581 ± 282</td>
<td>1556 ± 143</td>
<td>1592 ± 220</td>
<td>2016 ± 114</td>
</tr>
</tbody>
</table>

Discussion

Results of this study affirm that, in the intestine of Sprague-Dawley rats exposed to bisphenol A, 1) most of the compound absorbed by the intestine is glucuronidated within the intestinal wall; 2) the resulting glucuronide is eliminated preferentially into the mucosal side in the small intestine and into the serosal side in the colon; and 3) on a high-dose exposure to bisphenol A, the relative absorption of unconjugated bisphenol A increases dramatically.

Bisphenol A Glucuronidation during Absorption. As suggested by the present results, the proximal intestine is seen as playing a highly protective role against ingested bisphenol A. Bisphenol A in the lumen of the rat intestine was highly glucuronidated during its passage through the intestine, with most of the compound excreted to the mucosal side as glucuronide, which is low in estrogenic activity (Matthews et al., 2001). This was particularly evident for the proximal jejunum, where mucosal excretion of the glucuronide greatly exceeded serosal excretion. Thus, it appears that the proximal jejunum defends the body against potential adverse effects of orally introduced bisphenol A by limiting entry of the free compound into the blood stream and by curtailing exposure to the middle and distal parts of the intestine. In line with these results, low exposure has been reported in association with oral intake of bisphenol A (Pottenger et al., 2000). Comparing the concentration-time profiles for bisphenol A in the blood of F344 rats exposed intraperitoneally and those exposed orally to the compound, Pottenger et al. (2000) found that oral administration results in a low exposure to unconjugated bisphenol A. In the light of our present findings, the diminution of exposure to unconjugated bisphenol A on oral administration may be ascribed to high glucuronidation of the compound in the proximal intestine, which is the foremost barrier to damage from oral administration.

Previously, we showed that bisphenol A glucuronidation in the liver is mediated by UGT2B1, an isofrom of UDP-glucuronosyltransferase, and that the isofrom is not expressed in rat intestine (Yokota et al., 1999). Generally, the UGT2B family glucuronidates steroid hormones (Turgeon et al., 2001). To our knowledge, the steroid UDP-glucuronosyltransferases are still unclear in rat intestine. In human intestine, however, UGT2B-family isofroms have been reported by Radomska-Pandya et al. (1998). This leads us to conjecture that one...
or more steroid UDP-glucuronosyltransferase isoenzymes of the UGT2B family other than UGT2B1 are responsible for catalyzing bisphenol A within the rat intestinal wall. Further studies are needed to identify the suspected isoenzyme that may catalyze glucuronidation of bisphenol A in rat intestine.

Excretion of the Resulting Glucuronide. Whereas in the intestine the bisphenol A glucuronide was excreted into the mucosal side, the direction of elimination was reversed in the colon, where transport was into the serosal side. Recently, ATP-dependent transporters have been described as mediating the transport of the glucuronide across the cell membrane (Oude Elferink et al., 1995). In rat liver a member of the ATP-binding cassette family, namely, multidrug resistance associated protein (MRP), is reported to be capable of mediating transmembrane excretion of a wide range of amphipathic compounds, including bilirubin-, estrogen- and xenobiotic-glucuronide (Yamazaki et al., 1996). In the rat intestine, MRP2, localized in the apical domain of the enterocyte, is distributed in the proximal intestine (Mottino et al., 2001) and MRP3, localized in the basolateral domain, is distributed mainly in the ileum and colon (Rost et al., 2002). Intriguingly, the apical and basolateral directions of bisphenol A glucuronide excretion in our study parallels the distribution patterns of MRP2 and MRP3, respectively. Thus the supposition may be made that the elimination direction of bisphenol A glucuronide is governed by the distribution of an organic anion transporter system such as MRP.

Appearance of Serosal Bisphenol A in the Colon. As the lumen was the site into which large amounts of the bisphenol A glucuronide were eliminated in our study, presumably the excreted glucuronide would flow with the luminal contents to the distal intestine. In the colon, most likely the glucuronide would be deconjugated by lumen bacterial β-glucuronidase, an enzyme known to generate toxic and carcinogenic substances (Reddy et al., 1992). Deconjugation by lumen bacterial β-glucuronidase is known to be involved in the reactivation of an antitumor chemical derived from Irinotecan (Kaneda and Yokokura, 1990). Furthermore, a deglucuronidated Irinotecan derivative (SN-38 glucuronide) is reabsorbed in the distal intestine, whereas it damages the mucosa (Takasuna et al., 1996). In the light of these reports, the notable absorption and transport of unconjugated bisphenol A to the serosal side in the rat colon in our study suggests that the deconjugated bisphenol A is eventually reabsorbed by the colon. This proposition concurs with Upmeier et al. (2000), who recently, in a toxicokinetic study of bisphenol A in female DA/Han rats, have shown the possibility of enterohepatic recirculation and protracted absorption of bisphenol A from the intestinal tract. These findings bear out that, for ingested bisphenol A, the metabolism and disposition of the compound in the intestinal tract play a pivotal role in mediating the degree of toxic damage by the compound.

Generally, the paramount issue in the study of adverse effects of bisphenol A has to do with oral exposure to the chemical in low doses (Feldman, 1997). Rubin et al. (2001) has described adverse effects in rat offspring after maternal administration. Conversely, other studies have shown no adverse effects (Cagen et al., 1999; Ema et al., 2001). Thus, the toxicity of low doses of bisphenol A remains controversial. We believe that the sensitivity of an animal to ingested bisphenol A reflects the condition inside the intestine (e.g., the luminal contents and the types of bacterial flora). Further studies are required to clarify the correlation between catalytic reactivation of bisphenol A glucuronide by luminal flora and adverse effects caused by bisphenol A.

Conclusion

Because the intestine absorbs environmental estrogens introduced orally into the body, it is important to trace the fate of such compounds before inflow into the bloodstream. Evidence is accruing that most bisphenol A that is ingested is glucuronidated during its absorption by the small intestine and by its passage through the liver (Inoue et al., 2001). The present study has established that bisphenol A is excreted into the intestinal lumen as a glucuronide, bearing out that the gastrointestinal tract is a strategic pathway against invasion of bisphenol A at target organs such as the gonads and the brain. Further
work is warranted to determine the fate of the enteroluminal glucuronide in its complete pathway before excretion.

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