Note

Down-Regulation of mdr1b mRNA Expression in the Kidneys of Mice Following Maternal Exposure to Tributyltin Chloride

Kazuo Kobayashi-Hattori, Takahiro Watanabe, Kimiko Kimura, and Yoshiko Sugita-Konishi

1Division of Microbiology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
2Division of Food, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
3Department of Veterinary Medical Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Received September 9, 2005; Accepted January 22, 2006

We investigated the change in renal mdr1b mRNA expression in offspring exposed to tributyltin chloride (TBTC) via the placenta and lactation or via lactation, using the real-time reverse transcription-polymerase chain reaction. Pregnant ICR mice were given water containing TBTC (0, 15, and 50 µg/ml) ad libitum from the start of pregnancy to weaning or from parturition to weaning. Exposure via the placenta and lactation significantly reduced the renal mdr1b level in offspring. Exposure to TBTC through the mother might impair the exclusion system of toxic compounds in offspring.

Key words: tributyltin chloride; mdr1b; offspring; placenta; lactation

P-glycoprotein (P-gp), a plasma membrane protein that belongs to the superfamily of ATP-binding cassette transporters, is a major cause of multidrug resistance in tumor cells.1,2) P-gp is encoded by the genes MDR1 and MDR2 in humans and mdr1a, mdr1b, and mdr2 in rodents, of which the MDR1, mdr1a, and mdr1b genes are known to confer multidrug resistance.1,2) P-gp has also attracted attention in the toxicological field.2,3) Besides tumor cells, P-gp is present in normal tissues such as the adrenal glands, the colon, small intestine, kidney, liver, placenta, and testis, the blood-brain barrier, and immune cells.1,3) It has been proposed that P-gp plays an important role in protecting the host from toxic compounds.2) Hence it is important to investigate the effects of xenobiotics on the levels of P-gp and its mRNA expression. Brady et al.4) showed that mdr1a and mdr1b mRNA levels increased in kidney and liver when certain chemical substances were administered to rats. Seree et al.5) reported that dexamethasone administered orally to mice down-regulated mdr1b mRNA expression in adrenal, kidney, and lung. Treatment with turpentine and lipopolysaccharide in mice has been shown to suppress hepatic expression of P-gp and mdr1 mRNA.6) However there has been no study on whether xenobiotics affect the expression of P-gp and its mRNA in the next generation. Among xenobiotics, we selected tributyltin chloride (TBTC), which is used primarily as a biocide to prevent the attachment and growth of marine organisms such as barnacles, plankton, and algae to ship hulls and fishing nets. Because TBTC passes through the placental barrier7) and transfers to milk,8) the effects of TBTC exposure on the next generation are of particular concern. The distribution of TBTC administered orally to rodents has been reported to be extensive in the liver and kidney.9,10) Because these organs are more susceptible to the influence of TBTC and the level of mdr1b mRNA expression was higher than that of mdr1a in the kidney of mice,11) we investigated renal mdr1b mRNA expression in mice maternally exposed to TBTC using real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) in the present study.

To examine the effect of the route of exposure on mdr1b mRNA expression, offspring were exposed to TBTC by the following pathways: (i) Exposure via the placenta and lactation. ICR mice one-day pregnant (Japan SLC, Tokyo) were divided into three groups and given water containing TBTC (0, 15, and 50 µg/ml; Wako Pure Chemical, Osaka, Japan) ad libitum up to weaning (for 39 d). (ii) Exposure via lactation. ICR mice 16-days pregnant (Japan SLC) were divided into three groups. After the natal day, the dams were given water containing TBTC (0, 15, and 50 µg/ml) ad libitum until the weaning of their pups (for 19 d). In both exposure groups, the kidneys of the offspring and the liver of the dams and offspring were removed after weaning. The

1 To whom correspondence should be addressed. Present address: Department of Nutritional Science, Faculty of Applied Bio-Science, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan; Tel/Fax: +81-3-5477-2443; E-mail: kikobaya@nodai.ac.jp
body weights of the offspring were also measured on the day of weaning. The animal experiments in this study were performed according to the Principle Law on Animal Experimentation of the National Institute of Health Sciences of Japan.

Quantification of mdr1b mRNA was performed by real-time RT-PCR. Total RNA from the kidney of the offspring was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA), Lysing Matrix D (Qbiogene, Carlsbad, CA), and a FastPrep FP120 Instrument (Qbiogene). Total RNA from the kidneys of normal ICR mice (UNITECH, Chiba, Japan) was used to prepare standard curves for mdr1b and 18s rRNA (as an internal control).

cDNA was synthesized from 1 µg of total RNA with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) according to manufacturer’s instructions. Reverse transcription was performed with the following reaction: 10 min at 25 °C, 1 h at 37 °C, and 5 min at 95 °C. Quantitative PCR was performed with an ABI PRISM 7700 (Applied Biosystems, Foster City, CA). The primer set and TaqMan probe for mdr1b were selected with Primer Express™ version 1.0 (Applied Biosystems), based on the cDNA sequence (Accession no. M14757) reported by Gros et al.12) (Table 1). As for the primer set and TaqMan probe for 18s rRNA, TaqMan Ribosomal RNA Control Reagent VIC probe (Applied Biosystems) was used. PCR amplifications for mdr1b and 18s rRNA were performed in duplicate wells in the same 96-well plate under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by 45 cycles of 30 s at 95 °C and 1 min at 59 °C.

TBTC concentrations in liver samples from the dams and the offspring, in milk samples from the stomach of the offspring, and in the whole body of the offspring were determined by gas chromatography (HP Model 5890 Series II gas chromatograph; Hewlett-Packard, Avonbale, PA) after solvent extraction. The detection limit for TBTC was 5 ng/g.

No influence was observed on the body weights of the offspring at weaning after exposure via the placenta and lactation or via lactation only (data not shown). Figure 1 shows the renal mdr1b mRNA levels in the offspring of both exposure groups. The renal mdr1b mRNA level of the offspring exposed to TBTC via the placenta and lactation decreased significantly as compared with the control, although no dose-dependent effect was observed (Fig. 1A). In the case of exposure via lactation alone (Fig. 1B), the renal mdr1b mRNA level showed a tendency to decrease in a dose-dependent manner. These results suggest that exposure to TBTC via the placenta and lactation might impair the ability of offspring to protect themselves from toxic compounds. The significant decrease caused by exposure via the placenta and lactation is presumably due to a high dose resulting from a longer exposure period than exposure via lactation only. In addition, there is a possibility that exposure via placenta leads to more TBTC being transferred to the offspring than via lactation. Noland et al.13) have reported that the majority of the tin in pups exposed to

Table 1. The Primer Set and Probe for mdr1b Used in Real-Time PCR

<table>
<thead>
<tr>
<th>Target</th>
<th>Accession no.</th>
<th>Primer/Probe</th>
<th>Sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mdr1b</td>
<td>M14757</td>
<td>forward</td>
<td>TTG GCA AAG CCG GAG AGA T</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reverse</td>
<td>GTC AGT GAG CCA GTG CTG TTC TTA T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>probe (VIC)</td>
<td>CTC ACC AAG CGA GTC CGA TAC ATG GTT T</td>
<td></td>
</tr>
</tbody>
</table>

---

A. Exposure via the placenta and lactation. B. Exposure via lactation only. Pregnant ICR mice were given water containing TBTC (0, 15, or 50 mg/ml) ad libitum from the start of pregnancy to weaning or from parturition to weaning. After removal of the kidneys from the offspring (n = 4–5), mdr1b mRNA levels were measured by real-time PCR. The average mdr1b mRNA level of each control group was defined to be 100%, and those of the TBTC-treated groups are relative values. Data are represented as the average ± standard deviation. Significant differences between the control groups and TBTC-treated groups were analyzed with Student’s t-test, indicated by an asterisk (p < 0.05).
dimethyltin dichloride, an organotin, was transferred during gestation rather than lactation.

Next, TBTC concentrations were measured in the livers of dams and offspring from both groups, because the level of TBTC (as tin) accumulated in the liver was similar to that in the kidney. As shown in Table 2, 7 ng/g and 10 ng/g of TBTC were detected in the livers of dams given TBTC (50 μg/ml) during pregnancy and lactation and during lactation respectively, whereas the level of TBTC was below the detection limit in the livers of TBTC (15 μg/ml) treated dams. The amount of TBTC in the livers of the offspring was below the limit of detection in both groups. Furthermore, we measured the TBTC concentration in milk collected from the stomach of the offspring exposed via lactation from TBTC (50 μg/ml) treated dams. The concentration was 30 ng/g (Table 2). Although the TBTC concentration transferred from TBTC (50 μg/ml) treated dams to offspring via the placenta and lactation was not measured, it was estimated to be more than 19 ng/g, because the TBTC concentration in the whole bodies of neonates (at 0 d from birth) was 19 ng/g (Table 2). These results suggest that TBTC was transferred to the offspring via both pathways. In fact, there is a report that TBTC underwent transplacental transfer. In addition, we recently reported TBTC transferred from the mammary gland to milk. Therefore, a trace amount of TBTC transferred from dams to offspring through the placenta and lactation is likely to affect the level of mdr1b mRNA expression.

There are three possibilities as to the reason the level of mdr1b mRNA decreased in the offspring exposed to TBTC. The first possibility is the influence of cytokines. It has been reported that IL-6, an inflammatory cytokine, down-regulated mdr1b mRNA expression and P-gp expression. Indeed, TBTC induced IL-6 mRNA expression in in vivo experiments. Whether renal IL-6 mRNA is induced in the offspring exposed to TBTC was examined by conventional RT-PCR, but no renal IL-6 mRNA was detected in the offspring of either group (data not shown). In conclusion, we found that the level of renal mdr1b mRNA decreased when offspring were exposed to TBTC during pregnancy and lactation. The present study suggests that exposure to TBTC during pregnancy and lactation might impair the exclusion system of xenobiotics in offspring. Under similar conditions of TBTC exposure, TBTC has been reported to modulate the level of neurotransmitters in the brains of offspring. TBTC also decreased the weights of thymus, spleen, and liver in offspring under similar conditions of TBTC exposure (personal communication). It is possible that TBTC affects the level of mdr1 mRNA in such organs. Some scientists warn that the prevalence of infectious disease increases in childhood when exposure to environmental contaminants takes place during the period of pregnancy and breastfeeding. Decreases in mdr1 mRNA expression by the inhibition of transcription factors. Some investigators believe that TBTC and its metabolites have a tendency to accumulate in the nucleus and bind to DNA. Hence accumulation of TBTC and its metabolites in the nucleus might inhibit the binding of transcription factors to DNA and suppress the induction of mdr1b mRNA, as curcumin did. The third possibility is a decrease in mdr1 mRNA stability due to TBTC. As far as we know, no information is available on the effect of TBTC on mdr1 mRNA stability, but there have been several reports on the relationship between decreases in mdr1 mRNA expression and mRNA stability. For instance, IL-6 and verapamil lowered the levels of mdr1 mRNA in hepatocytes and multidrug-resistant leukemic cells respectively, but did not affect mdr1 mRNA stability. On the other hand, there is a report that suppression of mdr1b mRNA expression by dexamethasone in primary hepatocytes was due to a decrease in mdr1b mRNA stability. Thus it cannot be ruled out that TBTC decreases mdr1 mRNA stability and thereby lowers the level of mdr1b mRNA expression. Further studies are required to clarify the mechanisms behind decreases in mdr1 mRNA expression following maternal exposure to TBTC.

### Table 2. TBTC Concentration in Mother and Offspring after Exposure

<table>
<thead>
<tr>
<th>Exposure pathways</th>
<th>TBTC exposure conc. (μg/ml)</th>
<th>TBTC conc. in liver (ng/g)</th>
<th>TBTC conc. in offspring (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam</td>
<td>Placenta and lactation</td>
<td>15</td>
<td>&gt;5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lactation</td>
<td>15</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Offspring</td>
<td>Placenta and lactation</td>
<td>15</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>Lactation</td>
<td>15</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

<sup>a</sup>The detection limit was below 5 ng/g.

<sup>b</sup>Analysis was carried out at 0 d from birth.
exposure to toxic compounds might be one of the reasons that such diseases are becoming more prevalent.

Acknowledgments

This work was supported by a research grant from the Ministry of Health, Labor, and Welfare of Japan.

References


