Expression and Regulation of Breast Cancer Resistance Protein and Multidrug Resistance Associated Protein 2 in BALB/c Mice

Ying HAN and Yuichi SUGIYAMA*

Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, University of Tokyo; Tokyo 113–0033, Japan. Received December 28, 2005; accepted February 13, 2006

Microsomal enzyme inducers are known to influence the expression of many transporter proteins and mRNA. In this study, we examined the effects of microsomal enzyme inducers on the mRNA expression of Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance Associated Protein 2 (MRP2) in BALB/c mice. mRNA expression in liver, duodenum, jejunum and ileum was examined in mice, which were treated with microsomal enzyme inducers—aryl hydrocarbon receptor (AhR) ligands 3-methylcholanthrene (3-MC) and pregnane-x-receptor (PXR) ligand pregnenolone-16α-carbonitrile (PCN) and compared with control vehicle. The results suggested that the expression level of bcrp mRNA in the ileum was twice that in the liver, duodenum and jejunum using both semi quantitative PCR and Real-time PCR. Mrp2 mRNA was significantly increased by both PCN and 3-MC treatment. In contrast, bcrp mRNA expression was not significantly affected by these inducers. In summary, this study demonstrated that the expression of mrp2 mRNA is regulated by PCN and 3-MC, however, bcrp mRNA expression was not significantly affected by PCN and 3-MC.

Key words induction; breast cancer resistance protein (BCRP); multidrug resistance protein 2 (MRP2)

MATERIALS AND METHODS

Chemical 3-MC was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). PCN was purchased from Wako Pure Chemical Industries (Osaka Japan). All other chemicals and reagents were of the highest grade and purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals and Surgery BALB/c mice (SLC, Tokyo Japan) were housed at 25°C with a 12 h light–dark cycle. Mice received water and food ad libitum and were allowed to acclimatize to their environment for 5 d before the study began. BALB/c mice (22—27 g) were given the following chemical treatment: PCN (400 mg/kg), 3-MC (40 mg/kg) or control vehicle (corn oil). PCN and 3-MC were dissolved in corn oil. All chemicals were injected i.p in a final volume of 5 ml/kg. Mice were injected i.p once daily for 4 d. On day 5, the mice were killed under ether anesthesia and the liver and intestine tissues were removed, snap-frozen in liquid nitrogen, and stored at −80°C (intestinal epithelia were obtained by scraping prior to freezing).

Isolation of Liver and Intestinal RNA Total RNA was extracted from liver and intestinal tissues using Isogen (Nippon Gene, Toyama Japan). Briefly, 50—100 mg of tissue samples was added to 1 ml Isogen and then homogenized for cell lysis. After standing the homogenates at room temperature for 5 min, 0.2 ml chloroform was added and the solutions were mixed thoroughly and centrifuged at 3000 g for

* To whom correspondence should be addressed. e-mail: yu-one.sugiyama@nifty.com © 2006 Pharmaceutical Society of Japan
RESULTS

Tissue Distribution of Bcrp mRNA Expression  
Bcrp mRNA expression levels in liver, duodenum and jejunum were similar. The expression of bcrp in the ileum was about twice as high as that in other tissues. The relative expression levels of bcrp mRNA normalized by G3PDH in the liver, duodenum, jejunum and ileum are shown in Fig. 1.

Effect of Microsomal Enzyme Inducer Treatment on Bcrp mRNA Expression  
Bcrp mRNA expression after treatment with microsomal enzyme inducers was determined by semi quantitative PCR assay and Real-time quantitative PCR assay (Table 1). No significant difference was observed in the expression of bcrp mRNA between treated mice and control mice.

CYP3A11 mRNA and Oatp2 mRNA Expression  
Because PCN treatment is known to induce expression of CYP3A11 mRNA and Oatp2 mRNA in mouse liver, we examined the effect of these inducers on the expression of CYP3A11 and Oatp2 mRNA. PCN significantly increased both CYP3A11 mRNA and Oatp2 mRNA expression in liver, however, 3-MC had no effect on the expression of CYP3A11 and Oatp2 mRNA (Figs. 2, 3).

### Table 1. Effect of Microsomal Enzyme Inducers 3-MC and PCN on Bcrp mRNA Expression

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>3-MC</th>
<th>PCN</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.28±0.07</td>
<td>0.33±0.12</td>
<td>0.29±0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.25±0.14</td>
<td>0.22±0.06</td>
<td>0.24±0.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.37±0.15</td>
<td>0.36±0.10</td>
<td>0.37±0.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.64±0.33</td>
<td>0.82±0.24</td>
<td>0.63±0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Statistics: Fisher’s LSD.

### Graphical Results of CYP3A11 mRNA Expression from Control and Inducer-Treated BALB/c Mice by Semi-Quantitative PCR

Results are expressed as the ratio of CYP3A11 signal to G3PDH signal. All data are given as mean±S.E. (n=4—11). *p<0.05.
Microsomal enzyme inducers are structurally diverse chemicals that upregulate hepatic enzymes. They are known to upregulate Phase I drug metabolizing enzymes called cytochrome P450s (CYP), which are important for the bioactivation and biotransformation of lipophilic chemicals to more water-soluble compounds. Transporter proteins are also targets of microsomal enzyme inducers. Some studies using human and rodent models have demonstrated that ABC transporter family transporter proteins, such as Pgp, Oatp2, and mrp2, are upregulated by microsomal enzyme inducers.\(^\text{10–11}\) In this study, we examined the effects of microsomal enzyme inducers on the mRNA expression of bcrp, which is also responsible for drug resistance and may have a pharmacological role. Bcrp mRNA expression was analyzed following treatment with PB and PCN in rat liver, while mrp2 mRNA expression is not.\(^\text{17,18}\) In addition, it was reported 3-MC did not show any effect on the expression of both protein and mRNA of mrp2 in rat.\(^\text{17,18}\) There appears to be a discrepancy between the effects of microsomal enzymes on mrp2 mRNA expression. To address this issue, further studies including detection of MR2 protein are needed. However, we wonder that, at least in part, this may be due to a species difference, or a difference in in vivo and in vitro experiment conditions since it is known that drug function is very complicated in vivo.

In conclusion, we have demonstrated that PCN and 3-MC could upregulate the expression of mrp2 but not bcrp at transcriptional level, suggesting that mrp2 and bcrp have different regulation mechanisms. However, further studies are needed to clarify whether the regulation effects could be achieved at protein level and what are the underlying mechanisms.

REFERENCES