ACCUMULATION, EXCRETION AND EFFECTS ON HEPATIC ENZYMES OF POLYCHLORINATED QUATERPHENYL CONGENERS IN RATS

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Six skeletal congeners of polychlorinated quaterphenyls (PCQs), namely polychlorinated o-quaterphenyl (2,2'-PCQ), 2,3'-diphenylbiphenyl (2,3'-PCQ), 2,4'-diphenylbiphenyl (2,4'-PCQ), m-quaterphenyl (3,3'-PCQ), 3,4'-diphenylbiphenyl (3,4'-PCQ) and p-quaterphenyl (4,4'-PCQ), were orally administered to Wistar rats at a dose of 10 mg/rat. On the 5th day after administration of PCQs, the rats were examined for accumulation of PCQ congeners, hepatic enzyme activities and organ weight changes. Accumulation of 3,3'-PCQ, 3,4'-PCQ and 4,4'-PCQ were 1.5–3.2% of dose in the liver, while those of 2,2'-PCQ, 2,3'-PCQ and 2,4'-PCQ were only 0.1 to 0.2%. The amount of 4,4'-PCQ accumulated in the mesenteric adipose tissue, 20 µg/rat, was much higher than those of other PCQ congeners. Large amounts of the PCQ congeners administered were excreted in the feces on the first day, accounting for 96 to 98% of dose for 2,2'-PCQ and 4,4'-PCQ, and 55 to 75% for the other PCQ congeners, and the daily excretions of PCQs after the second day were very small, less than 10% of the dose. Benzo[a]pyrene 3-hydroxylase activity was significantly depressed by the treatment with 3,3'-PCQ, 3,4'-PCQ and 4,4'-PCQ, contrasting to the toxic congeners of polychlorinated biphenyls and dibenzofurans which enhanced markedly this enzyme activity. DT-Diaphorase activity was also depressed by the treatment with 2,3'-PCQ, 2,4'-PCQ and 3,4'-PCQ. Significant atrophy of the thymus was observed by the treatment with 4,4'-PCQ.

Keywords — polychlorinated quaterphenyl congener; tissue accumulation; fecal excretion; benzo[a]pyrene 3-hydroxylase activity; DT-diaphorase activity; benzphetamine N-demethylase activity; cytochrome P-450 content

INTRODUCTION

A mass food poisoning called Yusho occurred in western Japan in 1968 by ingestion of the Kanemi rice oil contaminated with Kanechlor, a brand of polychlorinated biphenyls (PCBs) in Japan. The rice oil was found to contain not only PCBs but also polychlorinated dibenzofurans (PCDFs) and polychlorinated quaterphenyls (PCQs). As the toxicities of PCDFs are very high compared with those of PCBs, PCDFs are considered to participate a more important role than PCBs in causing Yusho.

The amount of PCQs was found to be 0.9–3.5 fold that of PCBs in the causal rice oil. However, the distribution and toxicity of PCQs in animals have not been well examined except the study of Hori et al. PCQs are dimers of PCBs and consist of more than 100 000 congeners being divided into six skeletons. All the six skeletal congeners were identified in the blood of Yusho patients.

We report here the accumulation of PCQs in the tissues of rats and their effects on enzyme activities in the liver of rats using six types of PCQ congeners.
MATERIALS AND METHODS

Chemicals — Six skeletal congeners of PCQs, namely polychlorinated o-quaterphenyl (2,2',PCQ), 2,3'-diphenylbiphenyl (2,3'-PCQ), 2,4'-diphenylbiphenyl (2,4'-PCQ), m-quaterphenyl (3,3'-PCQ), 3,4'-diphenylbiphenyl (3,4'-PCQ) and p-quaterphenyl (4,4'-PCQ), were synthesized by the method reported previously. 3-Hydroxybenzo [a] pyrene and benzphetamine hydrochloride were kindly donated by Dr. N. Kinoshita, School of Health Sciences, Kyushu University, Fukuoka, and by Dr. R. A. Neal, Department of Biochemistry School of Medicine, Vanderbilt University, Tenn., USA, respectively. All other chemicals used were of purest grade commercially available.

Animal Treatment — Four weeks old male rats (ca. 80 g) of Wistar strain (specific pathogen free) were purchased from Seiwa Co., Fukuoka, Japan. The rats were housed in stainless steel cages (4 rats/cage) and fed standard chow (MF-1, Oriental Yeast Co. Ltd., Tokyo, Japan) and water ad libitum. Each PCQ congener dissolved in corn oil (20 mg/ml) was orally given to rats in a single dose of 10 mg PCQ per rat. Control animals were given the vehicle alone. On the 5th day after administration of PCQs, rats were sacrificed by a stunning blow on the head after fasting overnight. All the liver and mesenteric adipose tissue of the sacrificed rats were immediately removed, weighed and then submitted to the enzyme assays and gas chromatographic analyses described later. The feces from the individual rats were collected daily and also subjected to the gas chromatographic analyses.

Enzyme Assays — The liver from the rats was perfused with an ice-cold 0.9% potassium chloride and homogenized with 3 volumes of ice-cold 0.25 M sucrose containing 0.1 mM ethylenediaminetetra acetic acid (EDTA) and 10 mM Tris-HCl (pH 7.5). The homogenate was centrifuged at 9000 x g for 20 min and the supernatant was preserved on ice. The pellet was resuspended in the initial volume of 0.25 M sucrose and centrifuged again at 9000 x g for 20 min. The resultant supernatant was combined with the first supernatant. The assays of benzo[a]pyrene (BP) 3-hydroxylase, benzphetamine (BZ) N-demethylase and DT-diaphorase in the 9000 x g supernatant were performed by the method of Nebert et al., 10 Yoshimura et al., 11 and Ernster et al., 12 respectively. Cytochrome P-450 content in the 9000 x g supernatant was determined by the dithionate-reduced difference spectrum of CO bubbled sample according to the method of Matsubara et al., 13 Protein was determined by the method of Lowry et al., 14 using bovine serum albumin as a standard. Total liver lipid was extracted from the perfused liver by the method of Folch et al., 15 and weighed after drying.

Gas Chromatographic Analyses — The method of cleaning up the sample for gas chromatographic analysis of PCQ congeners was essentially the same as that described previously. 16 The liver (ca. 1.0 g), mesenteric adipose tissue (ca. 0.5 g) and feces (0.5 - 20 g) were saponified with 1.5 N KOH–ethanol solution and extracted with n-hexane. The extract was diluted with n-hexane. An aliquot of the n-hexane solution was treated with fumic sulfuric acid and centrifuged at 3000 rpm for 5 min. The upper n-hexane layer was analyzed for PCQ congeners by gas chromatography. The gas chromatography was carried out on a gas chromatograph (GC-74AG, Shimadzu Corp., Kyoto, Japan) fitted with an electron capture detector (63Ni) and a glass column (2.6 mm x 2 m) packed with Gaschrom Q (80–100 mesh) coated with 1.5% Silicone OV-1. The temperature of the injector, column and detector were maintained at 300, 260 and 300 °C, respectively. Pure nitrogen (99.9999%) was used as the carrier gas at a pressure of 3 kg/cm². Amounts of PCQ congeners were quantified by comparing their gas chromatographic peak areas with those of corresponding authentic PCQ congeners.

Statistical Analysis — Statistical differences among measured values were determined using the Student’s t-test.
RESULTS AND DISCUSSION

Accumulation and Excretion of PCQ Congeners

Table 1 summarized the amounts of PCQ congeners in the liver and mesenteric adipose tissue of rats on the 5th day after the oral administration of PCQs. The amounts of 2,2'-PCQ, 2,3'-PCQ and 2,4'-PCQ in the liver were about 0.1–0.2% of the dose, while the amounts of 3,3'-PCQ, 3,4'-PCQ and 4,4'-PCQ in the liver were 1.5–3.2% of the PCQs administered, suggesting the latter PCQs are more accumulative in the liver than the former PCQs. In the mesenteric adipose tissue, 4,4'-PCQ was predominantly accumulated and 2,4'-PCQ was least retained among the PCQs tested as shown in Table I. The PCQs consisting of dimers coupled with PCBs at 2-position, such as 2,2'-PCQ, 2,3'-PCQ and 2,4'-PCQ, seem less accumulative in the liver and mesenteric adipose tissue, and the PCQs without coupling at the 2-position, such as 3,3'-PCQ, 3,4'-PCQ and 4,4'-PCQ, are found to be more retinable in the liver. However, accumulation of these PCQs in the liver (less than 4%) was much lower than that of toxic 2,3,4,7,8-PCDF (65–95%).

Daily excretion profiles of PCQ congeners in the feces of rats are indicated in Fig. 1. In all the PCQ congeners tested, most of the PCQ administered were excreted in feces on the first day (more than 59%). Daily excretion of PCQs was rapidly decreased after the second day (less than 10%). This excretion profile was similar to the data of Hori et al., who reported that 87.8% of the dosed PCQs was excreted to the feces on the first day after the oral administration to rats. Majority of the dosed PCQs was not absorbed in the alimentary tract of rats and the absorbed or retained PCQs were slowly excreted to the feces after the second day. The excretion rates of 2,2'-PCQ and 4,4'-PCQ were high, 96 and 98% respectively, as compared with those of other PCQ congeners. This may be related to the findings of Mochiike et al., that is, concentration of 2,2'-PCQ and 4,4'-PCQ were lower than other PCQ congeners in the blood of Yusho patients. These PCQ congeners were produced by chlorinating individual quaterphenyls to the chlorine contents (7–8 Cl) similar to the PCQs contained in the rice oil of Yusho incident. Although the components of PCQs are somewhat different from those of the PCQs in rice oil, the accumulation and excretion profiles of PCQs in Yusho patients would have been variable according to the skeletal types of PCQs like these results.

Gas chromatograms of the PCQ congeners in tissues and feces are shown in Fig. 2. Gas chromatograms of the PCQs excreted in feces were similar to those of the corresponding PCQs administered, suggesting most of the PCQs passed through the alimentary tract to feces. However, gas chromatograms of the PCQs excreted from the liver and mesenteric adipose tissue were somewhat different from those of the corresponding PCQs administered. Portions of the

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Liver (μg)</th>
<th>Mesenteric adipose tissue (μg)</th>
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<tbody>
<tr>
<td>2,2'-PCQ</td>
<td>13 ± 2.8</td>
<td>6.8 ± 1.7</td>
</tr>
<tr>
<td>2,3'-PCQ</td>
<td>24 ± 5.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>2,4'-PCQ</td>
<td>17 ± 8.7</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>3,3'-PCQ</td>
<td>150 ± 28</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>3,4'-PCQ</td>
<td>180 ± 38</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>4,4'-PCQ</td>
<td>320 ± 180</td>
<td>20 ± 2.0</td>
</tr>
</tbody>
</table>

*Each value represents the mean of four experiments ± S.D.*
2,2'-PCQ, 2,3'-PCQ and 2,4'-PCQ with shorter retention times were eliminated faster than the PCQs with longer retention times in the liver and mesenteric adipose tissue of rats. In contrast with this, portions of 3,3'-PCQ and 3,4'-PCQ with longer retention times were eliminated faster than the PCQs with shorter retention times in the liver of rats. Elimination profiles of PCQs from the liver and mesenteric adipose tissue in rats were variable on skeletal types of PCQs and also number and position of chlorine in the same types of PCQ congener.

**Biological Effects of PCQ Congeners**

In PCB and PCDF congeners, their toxic potencies are correlated with contents of cytochrome P-450 (448) and enzyme inducing activities of BP 3-hydroxylase and DT-diaphorase and phenobarbital (PB)-type congeners frequently induce BZ N-demethylase. 4,5,11) There-

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**FIG. 1. Excretion of PCQ Congeners in Rat Feces after the Oral Administration of PCQ Congeners (10 mg/rat)**
- ● 2,2'-PCQ, ○ 2,3'-PCQ, ▲ 2,4'-PCQ, △ 3,3'-PCQ, ■ 3,4'-PCQ, □ 4,4'-PCQ.
- Each point represents the mean of four rats.

**FIG. 2. Gas Chromatograms of PCQ Congeners in Rat Feces and Tissues**
- A, authentic sample; B, feses extract; C, liver extract; D, mesenteric adipose tissue extract.
- 1, 2,2'-PCQ; 2, 2,3'-PCQ; 3, 2,4'-PCQ; 4, 3,3'-PCQ; 5, 3,4'-PCQ; 6, 4,4'-PCQ
- Gas chromatographic condition: Injector temp., 300°C; Column temp., 260°C; Detector temp., 300°C; Column, 1.5% Silicone OV-1 on Gaschrom Q (80—100 mesh) 2.6 mm i.d. × 2 m glass column.
fore, cytochrome P-450 contents and these enzyme inducing activities were determined for elucidating toxic potencies of PCQ congeners.

The contents of cytochrome P-450, and activities of BP 3-hydroxylase, BZ N-demethylase and DT-diaphorase in the liver of rats pretreated with six PCQ congeners are summarized in Table II. The CO difference spectra exhibited absorption maximum at 450 nm by pretreatment with all the PCQ congeners. The content of cytochrome P-450 was significantly increased in the liver by pretreatment with 2,4'-PCQ and 3,4'-PCQ. Pretreatment with 3,3'-PCQ, 3,4'-PCQ and 4,4'-PCQ depressed BP 3-

hydroxylation, which are predominantly mediated by 3-methylcholanthrene (MC)-induced cytochrome P-448 and usually enhanced by treatment with MC-type chemicals. Pretreatment with 2,2'-PCQ significantly depressed BZ N-demethylation, which are increased by PB-type inducers. Activity of DT-diaphorase, another type of enzyme induced by MC-type inducers, was also decreased by 2,3'-PCQ, 2,4'-PCQ and 3,4'-PCQ pretreatments.

Table III shows the effect of pretreatment with PCQ congeners on gravimetric changes of the liver, spleen and thymus and on total lipid

**TABLE II. Effects of PCQ Congeners on Enzyme Activities of Rat Liver**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Contents of cytochrome P-450&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Activities of Benzo[a]pyrene&lt;sup&gt;b&lt;/sup&gt; 3-hydroxylase</th>
<th>Activities of Benzphetamine&lt;sup&gt;c&lt;/sup&gt; N-demethylase</th>
<th>Activities of DT-diaphorase&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.114 ± 0.025</td>
<td>9.95 ± 2.24</td>
<td>0.825 ± 0.130</td>
<td>0.231 ± 0.066</td>
</tr>
<tr>
<td>2,2'-PCQ</td>
<td>0.104 ± 0.021</td>
<td>5.39 ± 2.43</td>
<td>0.609 ± 0.072&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.192 ± 0.039</td>
</tr>
<tr>
<td>2,3'-PCQ</td>
<td>0.133 ± 0.032</td>
<td>6.24 ± 1.61</td>
<td>0.977 ± 0.192</td>
<td>0.136 ± 0.011&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2,4'-PCQ</td>
<td>0.152 ± 0.008&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.19 ± 2.08</td>
<td>0.942 ± 0.148</td>
<td>0.125 ± 0.019&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3,3'-PCQ</td>
<td>0.136 ± 0.027</td>
<td>3.84 ± 1.16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.807 ± 0.106</td>
<td>0.177 ± 0.053</td>
</tr>
<tr>
<td>3,4'-PCQ</td>
<td>0.161 ± 0.018&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.27 ± 2.44&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.064 ± 0.165</td>
<td>0.127 ± 0.019&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>4,4'-PCQ</td>
<td>0.161 ± 0.035</td>
<td>3.53 ± 0.68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.930 ± 0.096</td>
<td>0.142 ± 0.008</td>
</tr>
</tbody>
</table>

*Each value represents the mean of four experiments ± S.D.  a) nmol/mg protein, b) pmol/min/mg protein, c) nmol/min/mg protein, d) μmol/min/mg protein, e) significantly different from the control, p< 0.05.*

**TABLE III. Effects of PCQ Congeners on Organ Weights and Total Liver Lipids**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Organ weight (g/100 g body weight)</th>
<th>Total liver lipids (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>Control</td>
<td>3.78 ± 0.15</td>
<td>0.472 ± 0.073</td>
</tr>
<tr>
<td>2,2'-PCQ</td>
<td>3.47 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.416 ± 0.033</td>
</tr>
<tr>
<td>2,3'-PCQ</td>
<td>3.51 ± 0.21</td>
<td>0.453 ± 0.045</td>
</tr>
<tr>
<td>2,4'-PCQ</td>
<td>3.54 ± 0.10</td>
<td>0.401 ± 0.065</td>
</tr>
<tr>
<td>3,3'-PCQ</td>
<td>3.44 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.456 ± 0.022</td>
</tr>
<tr>
<td>3,4'-PCQ</td>
<td>3.63 ± 0.35</td>
<td>0.397 ± 0.027</td>
</tr>
<tr>
<td>4,4'-PCQ</td>
<td>3.32 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.404 ± 0.034</td>
</tr>
</tbody>
</table>

*Each value represents the mean of four experiments ± S.D.  a) Significantly different from the control, p< 0.05.*
content in the liver. Pretreatment with 2,2'-PCQ, 3,3'-PCQ and 4,4'-PCQ caused significant decrease in weight of the liver, and the thymus weight was significantly decreased by pretreatment with 4,4'-PCQ. The content of total lipid in liver was significantly decreased by pretreatment with 2,4'-PCQ.

All the PCQ congeners caused significant depression of activities of BP 3-hydroxylase or similar tendency in the liver. These results were contrary to the finding of Hori et al., who reported that BP 3-hydroxylation was enhanced in the liver of rats by treatment with PCQs which had been separated from the Kanecchlor 400 used as a heat transfer medium. This discrepancy would be explained by the difference of the rat strains, SD strain for their experiment and Wistar strain (specific pathogen-free) for us, and/or by the impurities in their sample which might include PCBs and PCDFs by incomplete separation from the used Kanecchlor 400.

PCBs and PCDFs are known as strong enzyme inducers and enhanced BP 3-hydroxylation, BZ N-demethylation and activity of DT-diaphorase in the liver of rats at the doses of 1–50 mg/kg for PCBs and 1–1000 μg/kg for PCDFs. On the other hand, however, all the PCQ congeners depressed BP 3-hydroxylation and activity of DT-diaphorase significantly or showed similar tendency at the dose of 10 mg/rat. Toxic PCB and PCDF congeners occasionally caused hypertrophy of the liver and atrophy of the thymus in the treated rat. However, the PCQ congeners caused no hypertrophy of the liver and no significant atrophy of the thymus in the rats except 4,4'-PCQ which showed significant atrophy of the thymus. The toxic potencies of these PCQ congeners would be much lower than those of PCBs and PCDFs.

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