A potent hepatocarcinogen, diethylnitrosamine (DEN), was orally administered at 50 ppm for 5 weeks to Wistar rats preexposed to polychlorinated biphenyls (PCB) in utero and via mother's milk. The numbers of liver tumors induced by administration of DEN were significantly reduced in the rats exposed to PCB in an early stage of life, compared with control rats. This tumor-inhibiting action was particularly clear in male offspring.

Key words: Inhibitory effect — Polychlorinated biphenyls, transplacental — Diethylnitrosamine — Hepatoma — Rat

It has been reported that some microsomal enzyme inducers such as phenobarbital and polychlorinated biphenyls (PCB) inhibit or reduce the production of hepatomas in rats when they are administered before or during carcinogen exposure.1,7) PCB is also known to be transferred from the mother to fetuses and sucklings via the placenta and mother's milk.2,3)

Thus, offspring exposed to PCB in utero and via mother's milk after birth may be less susceptible to chemical hepatocarcinogenesis. The present study was undertaken to investigate this point.

Materials and Methods

Ten-week-old female Wistar strain rats were divided into three groups consisting of 10 each. These rats were caged individually and mated. On days 5, 10 and 15 of gestation, groups 1 and 2 were given PCB (Kanechlor 500, Kanegafuchi Chemical Co., Osaka) dissolved in olive oil by gastric intubation at doses of 200 mg/kg/day and 40 mg/kg/day, respectively. Group 3 was given olive oil alone and served as a control group. The animals, dams and their offspring, were given normal water and a pelleted diet (NMF, Oriental Yeast Co., Tokyo) ad libitum.

One offspring from each litter was sacrificed at 28 days of age, for quantitative determination of PCB in the liver. Analysis of PCB was done with a gas chromatograph equipped with an electron-capture detector.2)

The remaining offspring of experimental and control groups were separated by sex and given 50 ppm diethylnitrosamine (DEN, Tokyo Kasei Chemical Co., Tokyo) in drinking water for 5 weeks. They were weighed weekly throughout the experiment. Consumption of food and drinking water was measured weekly during treatment. Six to eight rats in each group were sacrificed at 16, 20 and 24 weeks after the start of DEN exposure. The liver was weighed and fixed in 10% neutral formalin solution. After 3 to 5 days of fixation, the liver was sliced with a razor blade into sections 2 mm thick as reported previously,5) and the number of tumors was counted. Histological observation was carried out on paraffin-embedded sections stained with hematoxylin and eosin. Differences in the number of tumors between different groups were analyzed by means of the unpaired t-test.

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Results and Discussion

The dams showed no abnormality in appearance or behavior during gestation and for six months thereafter.

The average numbers of offspring produced by each dam were 6.2, 6.5 and 7.1 in these three groups. Although no external abnormality was detected in the offspring of experimental groups, a slight decrease of average body weight was observed, and several deaths before weaning occurred among the offspring of group 1. The extents of body weight gain during DEN exposure were similar among these three groups.

The numbers of small liver nodules (smaller than 5 mm in size) per male rat were 2.0 and 5.8 in group 1, 4.7 and 8.3 in group 2, 6.0 and 13.0 in group 3, at 20 and 24 weeks, respectively. However, some of those nodules were neoplastic nodules histologically, while almost all of the tumors (larger than 5 mm in size) were characteristic hepatocellular carcinomas. The numbers of large tumors (larger than 10 mm in size) were too few (less than one per rat on an average) to permit analysis of the data. Thus, the number of liver tumors larger than 5 mm in size was selected as an indicator of hepatocarcinogenic potency.

The liver tumor (larger than 5 mm in size) yields together with the liver-to-body weight ratios at 20 and 24 weeks are summarized in Table I. Although a few small nodules (smaller than 5 mm in size) were seen in all three groups at 16 weeks, no liver tumor (larger than 5 mm in size) was observed except for 1 tumor in a male rat [group 3]. The tumor yields in control group 3 for males were in good agreement with those given in a previous report.5) The average number of liver tumors per male rat of group 1 was significantly less (P<0.05) than that in control group 3 at both 20 and 24 weeks. Similarly, the average number of liver tumors in group 2 for male rats was significantly less (P<0.05) than that in control group 3 at 20 weeks. No

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Sex of offsprings</th>
<th>Offspring</th>
<th>20 w.k.</th>
<th>24 w.k.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCB 200 mg/kg</td>
<td>male</td>
<td>DNE 50 ppm</td>
<td>5.3±0.1</td>
<td>6.2±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>5.1±0.2</td>
<td>6.2±0.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PCB 40 mg/kg</td>
<td>male</td>
<td>DNE 50 ppm</td>
<td>5.3±0.1</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>5.5±0.2</td>
<td>6.3±0.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>male</td>
<td>DNE 50 ppm</td>
<td>6.0±0.4</td>
<td>7.5±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>5.6±0.3</td>
<td>6.1±0.4</td>
<td></td>
</tr>
</tbody>
</table>

The data are expressed as mean±SE. Numbers in parentheses are the total number of liver tumors per group, and the number of rats bearing liver tumors, 71(6) 1980
liver tumor (larger than 5 mm in size) was observed at 20 weeks in female rats of group 1, while 1 tumor per rat on average was observed in control group 3. There was a consistent decrease in the average numbers of liver tumors produced in groups 3, 2 and 1, in that order, for both males and females. Tumor-inhibiting action was more distinct in males than females, presumably due to the difference of sensitivity to PCB.4)

Since PCB concentration in the livers of offspring at 4 weeks old was 360 ± 30 ppm for group 1, 18 ± 7 ppm for group 2 and less than 1 ppm for control group 3, placental and mammary transfer of PCB to offsprings was evident, and this presumably resulted in the decrease of DEN-induced liver tumors. In the offspring of the rats exposed to PCB, the liver showed no remarkable change upon light microscopy, but an increase in smooth endoplasmic reticulum in hepatic cells was observed at 4 weeks of age by electron microscopy (unpublished data). It is generally considered that this ultrastructural finding suggests the induction of microsomal enzymes. In fact, the enzymes essential for the breakdown of PCB were already present in suckling mice less than 12 days after birth, as determined in an experiment involving oral exposure of pregnant female mice.6) Thus, a microsomal drug-metabolizing enzyme system may be involved in this tumor-inhibiting action induced by placental and mammary transfer of PCB.

Such a modification of tumor production due to pre- and neo-natal exposure to chemicals may be important in relation to human cancer development.

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REFERENCES