POLYCHLOROBIPHENYLS (PCB) INDUCED FETOPATHY

II. EXPERIMENTAL STUDIES: POSSIBLE PLACENTAL TRANSFER OF POLYCHLOROBIPHENYLS IN RATS

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(Received for publication September 30, 1971)

Four cases of polychlorobiphenyls (PCB) induced fetopathy were described in the previous paper as the part one of this report.

The following experimental studies were performed to confirm the possibility of placental transfer of polychlorobiphenyls in pregnant rats, and to elucidate the pathogenesis of the fetopathy. The incidence of maternal death and the abortion increased in proportion to the amount of chlorbiphenyls administered to the mother rat, and histological changes were observed mainly in the liver and in the kidney of the mother.

Although, neither external malformation nor abnormal histological findings were noted in the fetus of the rat, H-labeled polychlorobiphenyls administered in mother rats were detected in the placenta, amnion and in the whole body of the fetus.

The above experimental studies suggest us that the fetopathy observed in human could be caused by placental transfer of polychlorobiphenyls.

Polychlorobiphenyls poisoning, so called Yusho occurred frequently all over the Western Japan around Fukuoka Prefecture, since June 1968. After polychlorobiphenyls (Kanechlor - 400 : KC - 400) mixed accidentally in the edible rice oil used by the patients was found to be responsible, almost 3 years have already passed. However, no definite treatment has been established at present, and it is the major social problem.

The newborn case from Yusho mother in October 1968 at the first time, thereafter 3 more cases, giving the total of 4 cases, were experienced as described in the previous report. Experimental studies were also carried out along with clinical observation on the above cases.

The present studies were performed to confirm the possibility of placental transfer of polychlorobiphenyls in preg-
nant rats, and to elucidate the pathogenesis of fetopathy.

MATERIALS AND METHODS

Female Wistar rats were divided into 5 groups. In the control group, rice oil without polychlorobiphenyls (KC-400) was administered, while KC-400 containing 25, 75, 225 and 675 mg/kg in 1.0-1.5 cc was administered daily through a gastric tube from the first day of pregnancy in groups I-IV. The dose causing acute or subacute toxicity was selected based on the result of the preliminary experiment.

RESULTS

1. Influence on the course of pregnancy and delivery:

Among various groups, spermatozoa was confirmed in vaginal smear examination in animals shown in Table 1. Ten animals in the control group, and 10, 12, 12 and 10 animals in groups I-IV respectively were used in the experiments.

| TABLE 1 |
| Influence on pregnancy and course of delivery |
| Dose of KC | Spermatozoa was confirmed in vaginal smear | Sacrifice | Death | Delivery | Number of newborn ( ) Stillbirth? Postmatal death? |
| Control | Rice Oil KC(-) | 10 | 5 | 0 | 5 | 53 (4) |
| Group I | 25mg/kg | 10 | 5 | 0 | 5 | 53 (11) |
| Group II | 75mg/kg | 13 | 6 | 1 | 5 | 52 (11) |
| Group III | 225mg/kg | 12 | 6 | 6 | 0 | 0 |
| Group IV | 675mg/kg | 10 | 5 | 5 | 0 | 0 |

The course of pregnancy in the control group was favorable and normal delivery took place in all animals except for those sacrificed half way during the course of experiment for histological examination.

In animals treated with rice oil containing KC-400, on the other hand, piloerection and unrest in behavior were noted. Especially in group IV, as shown in Fig.1 and 2, hemorrhage from the intestinal canal and partial loss of hair were noted.

In groups I and II, delivery was possible in many cases, while all animals in group III and IV died around the 10th day.

The number of newborn delivered reached 53 in the control group, 53 in group I and 52 in group II. No great
difference was noted in number of newborn. But a tendency to abundant stillbirth and postnatal death was noted in animals treated with rice oil containing KC-400.

The newborn and youngs of stillbirth were studied as to the shape, autopsy findings and radiographic findings of skeletal bones. No obvious external malformation was noted. (Fig. 3, 4)
2. Influence on main organs of the mother:

1) Organs weight

Main organs of pregnant rat mother treated with KC-400 were weighed and body weight/organ weight ratio was calculated sacrificed by blood letting after 7 days of treatment.

Results are shown in Table 2. As compared with the control group, swelling of liver and kidney was frequently noted. Such tendency was more pronounced as the dose increased. In Fig. 5, the liver weight was compared between control group and group IV.

2) Histological findings

No particular changes of the liver was noted in group I. In groups II and III liver cells were mildly swollen, while swelling of liver cells and partial degeneration were noted in group IV. On some of these cases, electron microscopic examination was carried out. As the result, swelling of liver cells, increase of smooth surfaced endoplasmic reticulum, and imbibition of mitochondria were noted. (Fig. 6) In the kidney, mild tubular dilatation was noted in group I. In group IV, changes of tubular epithelium and congestion was noted. (Fig. 7) Scarcely any changes of the spleen were noted in groups I and II, and mild follicular atrophy was noted in groups III and IV. In the lung, no changes was noted in groups I and II, mild destruction of alveolae was noted in group III, and findings of pulmonary edema were noted in group IV.

As shown in Fig. 8, the histological findings of hypoplastic uterus with reference to the days of pregnancy, and the findings suggesting pregnant changes and abortion were observed. Such histological picture was seen in several cases.

3. Influence on the fetus and the postnatal growth:

No abnormal histological findings
As shown in Fig. 1, the rate of increase of body weight was not much different from the control group. Further pregnancy and delivery were possible in groups I and II.

4. Placental transfer of polychlorobiphenyls (KC-400) : radioactive study:

1) Method of administration

$^3$H-KC-400 was mixed in edible oil in approximate concentration of 760 µCi per 1 ml, and accurately administered through a gastric tube. Six pregnant rats shortly before delivery were selected for study. At the 18 and 48 hours after administration, each three of those were sacrificed to obtain the sample desired.

2) Measurement of radioactivity

Approximately 50 mg of tissue was minced and ground in a mortar, while 0.1 ml of blood or amniotic fluid was taken for radioactivity measurement. The samples were solubilized through addition of 2 ml hyamine hydroxyd. After decoloration with 30 % $\text{H}_2\text{O}_2$, 15 ml of toluene series scintillater and 1 drop of concentrated hydrochloric acid were added for measurement in Beckman Type LS 233 liquid scintillation counter. Each sample was measured 3 times and averaged value of radioactivity (cpm) per 100 mg was calculated.

3) Results

As shown in Table 3, radioactivity was found to distribute in all specimens. High concentration was found in the blood, liver and kidney of the mother rats and some activity was also found in the placenta, amniotic fluid and fetus.

DISCUSSION

Influence of Polychlorobiphenyls on each organ and property of this substance remain in the body has been studied using various animals, tissue of the
TABLE 3

Distribution of H3-KC-400 in the organs and passability through placenta.

Radioactivity (cpm)/100mg

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>hour after administration</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Placenta</th>
<th>Amniotic fluid</th>
<th>Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>6643.3</td>
<td>8150.8</td>
<td>3868.3</td>
<td>1811.0</td>
<td>1773.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>4888.8</td>
<td>5697.0</td>
<td>4036.0</td>
<td>2302.0</td>
<td>2059.5</td>
<td>1783.5</td>
<td>1011.3</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>444.00</td>
<td>4935.5</td>
<td>2502.8</td>
<td>1392.3</td>
<td>966.3</td>
<td>1636.0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>5270.0</td>
<td>8753.0</td>
<td>8035.5</td>
<td>2670.8</td>
<td>3206.8</td>
<td>1257.0</td>
<td>2435.8</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>-</td>
<td>1063.1</td>
<td>8018.5</td>
<td>4344.3</td>
<td>3480.5</td>
<td>-</td>
<td>1208.8</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>7483.3</td>
<td>10865.5</td>
<td>8335.3</td>
<td>4879.3</td>
<td>4639.0</td>
<td>3378.0</td>
<td>2639.0</td>
</tr>
</tbody>
</table>

patient and their babies. However, few reports of detailed study on the possibility of placental transfer are available.

Nishizumi et al. 1) found no influence on the course of pregnancy in the mice by the administration of moderate dose of KC-400. No skin changes of the newborn, malformation or findings worth while for special description in subsequent growth and development were noted.

In our studies, no influence is noted on the course of pregnancy and litter number with the use of small dose. No external malformation was noted. Mortality rate of the mother and the incidence of abortion increased as the dose was increased. However, the course of the growth of newborn was as uneventful as in the control group. Survived mother rats had the capacity for further conception, pregnancy and delivery.

Morphological findings of the liver in human were reported in detail by Hirayama et al. 2). According to these results, liver damage was not detected at the level of optical microscopy and only the swelling of Kupffer cells was found. Increase of smooth-surfaced endoplasmic reticulum seen in electron microscopy is not characteristic to chlorbiphenyls poisoning, but rather represents an adaptation phenomenon to drugs.

In our studies using pregnant rats, main changes were found in the liver and kidney. Histological changes were tend to be observed in all organs and the mortality increased in proportion to the dose. In cases with findings of abortion described above, influence was seen on almost organs, suggesting the abortion might be induced as the secondary phenomenon due to general weakness.

The presence of placental transfer is extremely important to discuss the influence of various drugs or substances on the fetus. Although these were reports of qualitative detect of chlorbiphenyls in the skin and subcutaneous tissue of the stillborn infants from the mother with Yusho 3), and in the placenta by means of gas-chromatography 3), no radioactive study was available.

Our results suggest that there was possible placental transfer of chlorbipheneyls (KC-400), even though no histological changes were observed in fetus in rat.

ACKNOWLEDGEMENT

The authors are indebted to Prof. Y. Nakagawa, Dean. School of Medicine, Kurume.
PCB INDUCED FETOPATHY

University for the offering the Grant for Aid for "Yusho" Research. The authors wish to express deepest appreciation to Professor Y. Masuda, former director of University hospital and former chief of "Yusho" Research Group in Kurume University and to Professors H. Urabe, N. Shujaku, S. Yamaguchi, R. Ogura and N. Nagasaki for the kind cooperation and the advices.

This investigation was supported by the Grant in Aid for "Yusho" Research of the Ministry of Health, Fukuoka-ken, Kitakyushu-shi and the Grant in Aid for "Yusho" Research of School of Medicine, Kurume University.

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