TUMORIGENESIS AND CYSTIC LESION OF THE LIVER BY N-BIS (2-HYDROXYPROPYL) NITROSAMINE IN DDY MICE

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Accepted August 9, 1985

Abstract……The tumorigenesis and cystic lesion by a single intraperitoneal administration (ip) of N-bis(2-hydroxypropyl)nitrosamine (DHPN) for 52 weeks were studied in ddY mice. The amount of DHPN was 1000 mg/kg in group I, 500 mg/kg in group II, 250 mg/kg in group III, 125 mg/kg in group IV and 0 mg/kg in group V. The tumorigenesis of DHPN was found in the lung and liver. However, cystic lesion was observed only in the liver. Lung tumors were adenoma, adenocarcinoma and squamous cell carcinoma. As liver tumors, adenoma, hepatocellular carcinoma, cholangioma and hemangioma were observed only in the mice treated with DHPN. Incidence of cystic lesion in the liver was detected in all groups treated with DHPN. Histologically, cystic lesion of the liver showed four patterns of bile duct-like, sinusoid-like, hepatocyte-like and mixed.
Key words: N-bis(2-hydroxypropyl)nitrosamine, tumorigenesis, cystic lesion, lung, liver, ddY mice.

INTRODUCTION

DHPN, an intermediate product in the metabolism of di-n-propyl nitrosamine, was first synthesized by Krüger et al. (1974) and tumorigenesis of this compound were clearly demonstrated by Pour et al. (1974). The tumorigenic actions of DHPN are dependent on the animal species, strain, route of administration and dose response (Shirai et al., 1984). The target organs for the tumorigenesis of DHPN were the nasal cavity, thyroid, esophagus, lung, pancreas, liver, kidney and urinary bladder (Mohr, et al., 1977; Konishi et al., 1978).

On the other hand, cystic lesion of the liver in experimental animals was frequently observed together with liver tumors induced by chemical carcinogens (Rao and Reddy, 1977), but such changes have been reported to occur in a small number of individuals. Investigation of the target organs of cystic lesion by ip or intramuscular injection of
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DHPN on mice has been first reported by the authors (Yamamoto and Imai, 1984; Yamamoto, 1984).

In this study, we have described in detail of induction and histological features of cystic lesion in the liver together with lung and liver tumors a high incidence by a single ip of DHPN in ddY mice.

MATERIALS AND METHODS

Animals : Two hundred SLC: ddY male mice of 5-week-old of age, purchased from Shizuoka Laboratory Animal Center, were housed individually to a aluminium cage, and kept in an air-conditioned animal room at 22±1°C with a relative humidity of 55±10%, and given diet (Oriental Yeast Co., MF) and water ad libitum.

Experimental Design : Experimental design is summarized in Table 1. Animals were divided into 5 groups of 40 mice. DHPN, obtained from Makarai Chemical Co., was dissolved in physiological saline.

Pathological Examination : General condition and behavior were observed every day, and body weight was examined twice a week. Mice that died during the experimental period were all necropsied. At the end of 52-week experiment, all the survived mice were sacrificed with ether and necropsied. All organs and tumors were fixed in 10% buffered formalin and subjected to the routine histological examination. Special stains, such as periodic acid-Schiff (PAS), elastica Van-Gieson, azan-Mallory and oil-red O employed when indicated. Analysis of results was performed only on the mice survived until 49 to 52 weeks after start of the experiment.

RESULTS

1. General behavior and mortality : As toxic manifestations, loss of body weight, roughness of hair and sedation were observed in small number of mice in group I to group III, and these mice were died within 5 days to 3 weeks after start of the experiment. As pathological findings of these mice, no tumors and cystic lesion were observed.

2. Incidence and Distribution of Tumors : The results are shown in Table 1. Tumors were observed in the lung, liver, spleen, lymph node and mammary gland. Tumors of the lung and liver were in high rates in groups treated with DHPN. The incidences of lung tumors were 77.5% in group I, 87.5% in group II, 80% in group III, 70% in group IV and 2.5% in group V. The incidences of liver tumors were 90.3% in group I and 100% in group II to group IV.

3. Incidence of cystic lesion : Cystic lesion was developed only in the liver of mice treated with DHPN. The incidences were 90.3% in group I and 100% in group II to group IV (Table 2).

4. Pathological Findings : Macroscopically, multiple tumor nodules (about 3 to 10 over/surface) were developed in the lung and liver. In the lung, grossly greywhite or yellowish white tumors with irregular surface were 2 to 10 mm in diameter. In the
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Table 1  Experimental design and incidence of tumors in ddY mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose$^{a}$ (mg/kg)</th>
<th>Number of mice</th>
<th>Incidence of tumors (%)</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lymph.</th>
<th>Mammary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial$^{b}$</td>
<td>Dead$^{c}$</td>
<td>Effective$^{d}$</td>
<td>Number</td>
<td>Alive</td>
<td>Tumor</td>
<td>Tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>I</td>
<td>1000</td>
<td>40</td>
<td>13</td>
<td>31</td>
<td>24(77.4)</td>
<td>28(90.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>500</td>
<td>40</td>
<td>3</td>
<td>40</td>
<td>35(87.5)</td>
<td>40(100)</td>
<td>1(2.5)</td>
<td>1(2.5)</td>
</tr>
<tr>
<td>III</td>
<td>250</td>
<td>40</td>
<td>1</td>
<td>40</td>
<td>32(80.0)</td>
<td>40(100)</td>
<td>2(5.0)</td>
<td>2(5.0)</td>
</tr>
<tr>
<td>IV</td>
<td>125</td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>28(70.0)</td>
<td>40(100)</td>
<td>1(2.5)</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0$^{e}$</td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>1(2.5)</td>
<td>0</td>
<td>2(5.0)</td>
<td>2(5.0)</td>
</tr>
</tbody>
</table>

a) DHPN were dissolved in physiological saline at levels 30%.
b) Six week-old SLC: ddY male mouse.
c) Number of mice died within 3 weeks after start of experiment.
d) Number of mice survived over 4 weeks after start of experiment.
e) Received only the physiological saline.

Table 2  Incidence and histological patterns of cystic lesion of the liver in ddY mice treated with DHPN.

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective No. of mice</th>
<th>Incidence (%)</th>
<th>Histological patterns (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bile duct-like</td>
<td>Sinusoid-like</td>
</tr>
<tr>
<td>I</td>
<td>31</td>
<td>28(90.3)</td>
<td>8(28.6)</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
<td>40(100)</td>
<td>10(25.0)</td>
</tr>
<tr>
<td>III</td>
<td>40</td>
<td>40(100)</td>
<td>15(37.5)</td>
</tr>
<tr>
<td>IV</td>
<td>40</td>
<td>40(100)</td>
<td>18(45.0)</td>
</tr>
<tr>
<td>V</td>
<td>40</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 3  Histological types of lung and liver tumors in ddY mice treated with DHPN.

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective No. of mice</th>
<th>Lung tumors</th>
<th>Liver tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adenoma (%)</td>
<td>Adeno ca. (%)</td>
</tr>
<tr>
<td>I</td>
<td>31</td>
<td>23(74.2)</td>
<td>1(3.2)</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
<td>29(50.0)</td>
<td>14(35.0)</td>
</tr>
<tr>
<td>III</td>
<td>40</td>
<td>27(67.5)</td>
<td>6(15.0)</td>
</tr>
<tr>
<td>IV</td>
<td>40</td>
<td>20(50.0)</td>
<td>7(17.5)</td>
</tr>
<tr>
<td>V</td>
<td>40</td>
<td>1(2.5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Adeno ca.: Adenocarcinoma, Squ. cell ca.: Squamous cell carcinoma, Hepato. ca.: Hepatocellular carcinoma
Photo. 1  Gross finding of cysts (black arrows) and tumor nodules (white arrows) of the liver in a mouse of group III.

Photo. 2  Histology of multiple cysts in a mouse of group II. H–E stain.  ×40
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liver, brownish yellow or whitish yellow tumors varied in size from 5 to 10 mm in diameter. In addition, multiple cyst formation of the liver was also observed. The cysts were round in shape, soft, blackish brown or dark red in color and measuring 1 to 5 mm (Photo. 1).

Microscopically, lung tumors were adenoma, adenocarcinoma and squamous cell carcinoma (Table 3). Adenoma or adenocarcinoma showed follicular/papillary structures and consisted of cuboidal or columnar cells. Squamous cell carcinoma showed hyperkeratosis and pearl formation, and tumor cell nuclei were hyperchromatic and irregular. Mitosis was frequently observed in both of adenocarcinoma and squamous cell carcinoma. However, no metastasis from these tumors was observed. Liver tumors were adenoma, hepatocellular carcinoma, cholangioma and hemangioma (Table 3). Adenoma consisted of basophilic, acidophilic or clear cells. Hepatocellular carcinomas were often composed of more confluent nodular areas separated by compressed hepatocytes and variable amounts of connective tissue. They contained portions arranged in pseudoglandular and trabecular patterns. The architecture of cholangioma was characterized by glandular pattern. The nuclei of these tumor cells were regular in size and shape and exhibit no mitotic activity and the stroma of mature connective tissue was not unduly vascular. Hemangioma showed a distinct proliferation of endothelial cells. The tumor cells were arranged in a glandular pattern, but differed from the cells derived from bile duct in respect to their similarity to the endothelial cells.

Cyst formation of the liver was characteristically observed. Histological features of cystic lesion in the liver were as follows: bile duct-lile, sinusoid-like, hepatocyte-like and mixed patterns. Cysts of bile duct-like pattern consisted of simple cystic wall lined by cuboidal or flattened epithelial cells derived from the bile duct (Photo. 2). Their watery contents in the lumens were pale-stained with eosin or PAS. Cysts of sinusoid-like pattern were large or very large, and lined by flattened endothelial cells or compressed hepatocytes. The cystic contents were composed of watery, bloody or cellular materials. Cysts of hepatocyte-like pattern were observed in a small number. The hepatocytes showed marked variation in size and fusion with remarkable swelling. Their cytoplasm was pale-stained with eosin, PAS or oil-red O, and nuclei were small, sometimes located excentrically in the cytoplasm. The mixed pattern was composed of three histological features: bile duct-like, sinusoid-like and hepatocyte-like. Their cystic contents were also composed of watery, bloody or cellular materials.

On the other hand, hemangioma and leukemia in the spleen, leukemia in the lymph nodes and adenocarcinoma in the mammary glands were also sporadically observed in each group.

DISCUSSION

In this work, the effects of a single ip of DHPN to ddY mice were examined. There were developed cystic lesion of the liver together with lung and liver tumors. Total
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lung tumors including adenoma, adenocarcinoma and squamous cell carcinoma were found in 78.8% of mice treated with DHPN, which were somewhat lower compared with the lung tumors (100%) including adenoma, adenocarcinoma, squamous cell carcinoma and comined carcinoma of the experiments by Konishi et al. (1976) and Kondo (1977).

In case of the liver, tumors and cystic lesion were characteristically observed. They were produced at a remakable high rate (98%) in mice treated with DHPN. As for liver tumors in mice developed by DHPN, hemangioma, hemangioendothelioma and hemangioendothelial sarcoma were observed at the experiment by Konishi et al. (1978), while in this study forms were variegated including adenoma, hepatocellular carcinoma, cholangioma and hemangioma. As mentioned above, comparison of report by Konishi et al. with that of the authors showed that the latter involved the various histological types of liver tumors.

The most noticeable finding in this study was cystic lesion in the liver. Cysts with various size were scatteringly found in all the liver lobules, aimingionly the liver as target organs. On the cysts induced by chemicals other than DHPN, solitary or multiple cysts were reported when N-methyl-N'-nitro-N-nitrosoguanidine was fed to Brown Norway strain rats for 6 months (Ohta and Matsubayashi, 1981). Cysts found in this study were those of multifocal origin and could be histologically classified into four patterns: bile duct-like, sinusoid-like, hepatocyte-like and mixed pattern. Rao and Reddy (1977) recognized the formation of cysts by sc of 2,2-dihydroxy-di-n-propyl-nitrosamine on guinea pigs at a rate of 78%, whose ratio was higher than that of liver tumors. However, it was not reported whether the cysts were of solitary or multiple type. In this study, the cysts were all classified into multiple type as mentioned above, and the result coincided with the production of tumors.

It has been recently reported that the production of tumors with DHPN is accelerated by different promoters (Hiasa et al., 1983), and the induction of new type tumors quite different from those reported hitherto has been also reported (Shirai, et al., 1984). These suggest the complexity of the mechanism of DHPN in organisms. Krüger et al. (1974) claimed the disorders of the metabolism of fatty acids as one of the mechanisms of the production of tumors developed by DHPN, but much still remains unknown. As for the production of cysts, it is interesting that the intramuscular injection as well as ip (Yamamoto et al., 1984) caused the production of them at a high rated and it should by investigated in future why DHPN produced cyst of multiaocal origin together with the production of tumors in the liver.

REFERENCES

Konishi, Y., Denda, A., Kondo, H., et al. (1976) : Lung carcinomas induced by oral administra-
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tion of N-bis(2-hydroxypropyl)nitrosamine in rats. Gann, 67, 773-780.

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