COMMUNICATION

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INDUCTION OF HYPERPLASTIC LIVER NODULES BY PHENOBARBITAL IN RATS INITIATED WITH N-BUTYL-N-(4-HYDROXYBUTYL)NITROSAMINE*1

Nobuyuki Ito, Keisuke Nakanishi, Akihiro Hagiwara, Michiko Shibata, and Shoji Fukushima
First Department of Pathology, Nagoya City University Medical School*2

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N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN) induces tumors only in the urinary tract, especially the urinary bladder, in several species of animals including rats and mice.4,5) There are no reports on the hepatocarcinogenicity of BBN. However, many investigators have recently shown that various non-hepatocarcinogens induce hepatic neoplasms in rats when given as initiators after partial hepatectomy followed by promoting agents.1,3,6,9)

Although benzo[a]pyrene has been proven to be a non-hepatocarcinogen in rats, like BBN, the enhancing effect of phenobarbital had been shown to induce preneoplastic liver nodules and hepatocellular carcinomas in rats subjected to partial hepatectomy and then benzo[a]pyrene administration as an initiator, followed by the prolonged administration of phenobarbital.3,6) The promoting effect of phenobarbital on hepatocarcinogenesis has been demonstrated extensively3, 6, 7, 8, 10) and this compound has been shown to be useful in detecting weak carcinogens or "pure" initiators.7) However, the promoting effect of phenobarbital has not been seen in other organs such as the skin2) and lung.10)

In the present experiment we examined whether BBN could induce preneoplastic liver nodules in rats without partial hepatectomy, upon the prolonged administration of phenobarbital, and whether phenobarbital could promote urinary bladder carcinogenesis.

A total of 120 male Fischer 344 rats (Charles River Japan Inc., Kanagawa), 6 weeks old, was divided into 4 groups (30 each). Rats in groups 1 and 2 were given 0.01% BBN (Izumi Chemical Co., Yokohama) and phenobarbital in drinking water at doses of 40 mg/kg/day. Rats in groups 3 and 4 were given the same dose of BBN and phenobarbital and partial hepatectomy was performed 14 days after the start of the experiment. All animals were killed 14 days after the hepatectomy.

Fig. 1. Hyperplastic nodule in rat liver induced by BBN and phenobarbital. H.E. ×100.

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*2 1, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467 (伊東信行, 中西敬介, 萩原昭裕, 菅田道子, 福島昭治).
HYPERPLASTIC NODULES BY BBN AND PB

Table I. Histopathological Changes of the Liver and Urinary Bladder in Rats Treated with N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) Followed by Phenobarbital (PB)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Effective No. of rats</th>
<th>Liver (%)</th>
<th>Urinary bladder (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hyperplastic nodule</td>
<td>Simple</td>
</tr>
<tr>
<td>1</td>
<td>BBN→PB</td>
<td>30</td>
<td>8 (26.7)*</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>2</td>
<td>BBN</td>
<td>28</td>
<td>0</td>
<td>19 (67.9)</td>
</tr>
<tr>
<td>3</td>
<td>PB</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Significantly different from the incidence in group 2 (P<0.01).

hama) in drinking water containing 0.01% Tween 80 (Katayama Chemical Co., Osaka) for 4 weeks as an initiating chemical and then rats in groups 1 and 3 were administered basal diet (Oriental Yeast Co., Tokyo) containing 0.05% phenobarbital (Iwaki Seiyaku Co., Tokyo) as a promoting chemical for 32 weeks. Group 4 rats (controls) were fed on basal diet. All the animals were sacrificed at the end of the 36th week. The liver and urinary bladder (inflated via the urethra with 10% formalin solution) were both removed and fixed with the same fixative solution. Serial sections of the liver and urinary bladder were stained with hematoxylin and eosin (H.E.) and examined histopathologically.

The results are shown in Table I. Hyperplastic nodules (Fig. 1) developed in the liver of rats in group 1 at a significant level (P<0.01). The size of nodules and the number per rat were very small. However, none were detected in the other groups. Two types of hyperplasias (simple, and papillary or nodular) and papilloma developed in the urinary bladder of rats in groups 1 and 2. However, there was no marked difference in the incidences between these groups.

These data clearly demonstrate that BBN acts as a weak initiator of hepatocarcinogenesis inducing preneoplastic lesions in rats in this experimental system with phenobarbital as a hepatopromoter. However, phenobarbital does not act as a bladder promoter.

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REFERENCES