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Possible Lack of Carcinogenic Potential of 9-(4’-Aminophenyl)-9H-pyrido[3,4-b]indole (aminophenylnorharman) for the Pancreatic Duct Epithelium in Hamsters

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Abstract: The carcinogenic potential of 9-(4’-aminophenyl)-9H-pyrido[3,4-b]indole (aminophenylnorharman: APNH), a newly identified heterocyclic amine, was evaluated in a rapid production model for pancreatic duct adenocarcinoma in hamsters. In experiment 1, the initiation potential of APNH was examined. Hamsters received corn oil, 30 mg/kg body weight N-nitrosobis(2-oxopropyl)amine (BOP) or APNH (totally 50 and 100 mg/kg) as initiators followed by two cycles of augmentation pressure for pancreatic duct carcinogenesis and were sacrificed 75 to 78 days after the beginning of the experiment. Ductal hyperplasia and atypical hyperplasias developed in hamsters receiving APNH but their numbers and incidences did not differ from the corn oil case. In experiment 2, promoting potential of APNH for pancreatic duct carcinogenesis was examined. Hamsters received 30 mg/kg body weight BOP as an initiator followed by augmentation pressures and thereafter diet containing 0, 25 or 50 ppm APNH for 50 days. All hamsters were sacrificed 100 days after the beginning of the experiment. The numbers and incidences of ductal lesions, including adenocarcinomas, did not differ among the experimental groups. The results suggest, APNH may not have initiation or promotion potential for pancreatic duct carcinogenesis in hamsters. (J Toxicol Pathol 2002; 15: 7–12)

Key words: aminophenylnorharman, heterocyclic amine, pancreatic carcinogenesis, hamster, nitrosamine

Introduction

Pancreatic duct adenocarcinoma is the most common pancreatic exocrine malignancy and the fifth leading cause of cancer mortality in both the United States and Japan1,2, more than 17,000 people dying of this disease each year in Japan3. Due to its silent clinical course, at the time of diagnosis the majority of pancreatic cancer cases are incurable, with a poor prognosis. Therefore, early detection and prevention offer the only hope for effective control of this deadly disease. In particular, identification of causal factors is of prime importance.

Previously, we established a rapid production model for pancreatic duct adenocarcinoma in hamsters and reported a sequence of well-characterized changes in ductal morphology4–8. This rapid production model was based on the principle of the resistance of carcinogen initiated cells to the cytotoxic and cytostatic effects of carcinogens, as reported for rat liver carcinogenesis9. The detail of the model was described elsewhere4–8. Using this experimental model, both initiation and promotion activity of pancreatic duct can be examined9,11.

Norharman (9H-pyrido[3,4-b]indole) is produced in the pyrolysis of tryptophan and reported to be present in alcoholic beverages like beer, wine, sake or whisky, as well as in cigarette smoke condensate, cooked meat and fish12–15. Previously, it has been reported that, norharman itself is not a mutagen in Salmonella typhimurium TA98 and TA100, either with or without a metabolic activation system (S9 mix)16, but becomes mutagenic to S. typhimurium TA98 with S9 mix when incubated with non-mutagenic aromatic amines, such as aniline or o-toluidine17. It was found that the
mutagenic compound produced is 9-(4'-aminophenyl)-9H-pyrido[3,4-b]indole (APNH) which is converted to N-hydroxyamino derivatives producing DNA adducts after esterification.

Norharman is widely distributed in our environment and the fact that, aniline is present in cigarette smoke condensate and some kinds of vegetables, suggests that exposure of humans to APNH is highly likely. In the present experiment, we synthesized APNH and studied its effects in terms of pancreatic duct carcinogenesis, in both initiation and promotion phases of using a rapid production model for pancreatic duct adenocarcinoma in hamsters.

Materials and Methods

Experimental protocol
A total of 70 female Syrian golden hamsters (Nihon SLC, Inc., Hamamatsu, Shizuoka, Japan) weighing approximately 90 g each at the commencement were used. The hamsters were housed, three or four to a plastic cage, in an air-conditioned (10–15 ventilations/h) room, with a constant temperature (25 ± 3°C) and relative humidity (55 ± 8%) with alternating 12-h periods of light and darkness.

Two experimental protocols were used for the detection of initiation and promotion potential for pancreatic duct carcinogenesis, as shown in Figs. 1 and 2. The structure of APNH, purchased from the Nard Institute (Osaka, Japan), was previously reported. Before experiments 1 and 2, toxicity test for APNH was performed. Animals were administered diets containing APNH for 1 week and APNH at a dose of 0.05% in diet was judged as maximum tolerant dose of 1 week-toxicity test. This dose of APNH induced degeneration of proximal tubulus of kidney. According to this result, the dose of APNH in experiments 1 and 2 were decided. In experiment 1, group 1 received 1ml corn oil twice (The Nisshin Oil Mills, Ltd, Tokyo, Japan) by gavage, group 2 received 30 mg/kg body weight BOP by subcutaneous single injection, groups 3 and 4 received 25 or 50 mg/kg body weight of APNH in 1ml corn oil by gavage twice. After the above treatments, all hamsters underwent two cycles of augmentation pressure and were sacrificed 75 to 78 days after the beginning of the experiment. In experiment 2, groups 1–3 received a single subcutaneous injection of 30 mg/kg body weight BOP followed by two cycles of augmentation pressure. Eighteen days after the end of the 2nd cycle of augmentation pressure, hamsters received 0, 25 and 50 ppm APNH diets for 50 days and were sacrificed 100 days after the beginning of the experiment. Details for the augmentation procedures were described previously.

Histopathological examination
At autopsy, the pancreas and liver from each animal were immediately removed. The pancreas tissue was divided into three lobes, and sections were also taken from each liver lobe and fixed in 10% buffered formalin for overnight at 4°C, and routinely processed for paraffin embedding.
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A horizontal tissue section from each lobe of pancreas was cut at 4 µm thickness and stained with hematoxylin and eosin (H & E) for histopathological examination. Diagnostic criteria for pancreatic ductal lesions were as previously described4–8,10,11.

Statistical analysis
Statistical analysis was performed using the χ² test and the Student’s t- test for incidence and multiplicity data, respectively.

Results

Initiating potential of APNH on pancreatic duct carcinogenesis

The results of experiment 1 are summarized in Table 1. Five hamsters of group 4 died within 9 days after APNH treatment and 2 hamsters died after the 1st cycle of augmentation pressures, caused by toxicity of APNH. The final average body weight of group 4 was decreased compared with group 1 (132 ± 27.5 g in group 1 vs 113 ± 19.7 g in group 4), but groups 3 and 4 showed no differences.

Table 1. Numbers and Incidence of Pancreatic Ductal Lesions in Hamsters from Experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. of hamsters</th>
<th>Effective no. of hamsters</th>
<th>Number per animal (incidence, %)</th>
<th>Hyperplasia</th>
<th>Atypical hyperplasia</th>
<th>C.I.S</th>
<th>Invasive carcinoma</th>
<th>Total lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Corn oil</td>
<td>10</td>
<td>10</td>
<td>0.70 ± 0.82</td>
<td>0.10 ± 0.31</td>
<td>0</td>
<td>0</td>
<td>0.80 ± 1.03</td>
<td>(50)</td>
<td>(10)</td>
</tr>
<tr>
<td>2 BOP</td>
<td>10</td>
<td>9</td>
<td>4.22 ± 2.39a</td>
<td>1.56 ± 1.51a</td>
<td>0</td>
<td>0.33 ± 1.00</td>
<td>6.11 ± 3.86a</td>
<td>(100)</td>
<td>(66.7)</td>
</tr>
<tr>
<td>3 APNH</td>
<td>10</td>
<td>10</td>
<td>0.60 ± 1.07</td>
<td>0.10 ± 0.32</td>
<td>0</td>
<td>0</td>
<td>0.70 ± 1.34</td>
<td>(33.3)</td>
<td>(10)</td>
</tr>
<tr>
<td>4 APNH</td>
<td>25 mg/kg × 2, i.g</td>
<td>10</td>
<td>10</td>
<td>1.33 ± 0.58</td>
<td>1.33 ± 2.31</td>
<td>0</td>
<td>0</td>
<td>2.67 ± 2.89</td>
<td>(100)</td>
</tr>
<tr>
<td>5 APNH</td>
<td>50 mg/kg × 2, i.g</td>
<td>10</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Numbers and Incidence of Pancreatic Ductal Lesions in Hamsters from Experiment 1

- Number of ductal lesions per animal (mean ± standard deviation).
- Significantly different from group 1 (P<0.001).
- Significantly different from group 1 (P<0.05).

Fig. 2. Experimental protocol for promotion potential of APNH in the rapid production model for pancreatic duct adenocarcinomas in hamsters. \( \text{\textbullet} \), 30 mg/kg BOP s.c.; \( \text{\textbullet} \), 500 mg/kg DL-ethionine i.p.; \( \text{\textbullet} \), 800 mg/kg L-methionine i.p.; \( \text{\textbullet} \), 20 mg/kg BOP s.c.;
in body growth curves from group 1 (Fig. 3). Hyperplasias and atypical hyperplasias developed in hamsters receiving APNH in the initiation phase but there were no statistically significant differences between group 1 and group 3 or 4. Pancreatic duct adenocarcinomas did not develop in groups 1, 3, and 4.

**Promoting potential of APNH on pancreatic duct carcinogenesis**

The results from experiment 2 are shown in Table 2.

Three hamsters of group 1 and two hamsters of group 3 died. The final average body weights of group 2 and group 3 showed a tendency for increase (161.6 ± 29.5 g in group 1 vs 181.8 ± 13.8 g in group 2 and 172.8 ± 21.2 g in group 3) despite a reduction in diet intake (Fig. 4). The numbers and incidences of ductal lesions including carcinomas were not significantly increasing in groups 2 and 3 compared with those values of group 1.

**Discussion**

In the present experiment, no involvement of APNH was apparent in either initiation or promotion phases of pancreatic duct carcinogenesis in hamsters. In experiment 1, the numbers of pancreatic ductal lesions of groups 3 and 4 were not significantly increasing compared with group 1, and pancreatic duct adenocarcinomas did not develop in groups 3 and 4. Furthermore, in experiment 2 the numbers of pancreatic ductal lesions in groups 2 and 3 were not significantly increasing compared with group 1. These results suggest that APNH have no initiation and promotion activity for duct epithelial cells under these experimental conditions.

Norharman and aniline are widely distributed in the environment. For example, norharman was found to be contained at levels of 12.3–14.1 µg/cigarette in cigarette smoke condensate and 39 ng/g broiled beef, 158 ng/g broiled sardine. Furthermore, norharman was present at levels of

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**Table 2. Numbers and Incidence of Pancreatic Ductal Lesions in Hamsters from Experiment 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of APNH</th>
<th>Initial no. of hamsters</th>
<th>Effective no. of hamsters</th>
<th>Total diet intake per hamster (g)</th>
<th>Total Chemical intake per hamster (mg)</th>
<th>Number per animal (incidence, %)</th>
<th>Total lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
<td>7</td>
<td>630.29 ± 2.54</td>
<td>0</td>
<td>7.14 ± 2.54 (100)</td>
<td>11.71 ± 3.45</td>
</tr>
<tr>
<td>2</td>
<td>25 ppm</td>
<td>10</td>
<td>10</td>
<td>486.60 ± 3.97</td>
<td>12.2</td>
<td>7.20 ± 3.97 (100)</td>
<td>12.60 ± 6.15</td>
</tr>
<tr>
<td>3</td>
<td>50 ppm</td>
<td>10</td>
<td>8</td>
<td>468.25 ± 5.55</td>
<td>23.4</td>
<td>10.0 ± 5.55 (100)</td>
<td>17.12 ± 8.64</td>
</tr>
</tbody>
</table>

2) Number of ductal lesions per animal (mean ± standard deviation).
9.3–33.5 ng in all 24-h urine samples from healthy volunteers eating a normal diet\textsuperscript{11}. Norharman was also found to be present in 24-h urine of patients receiving parenteral alimentation at levels of 6.9–14.7 ng\textsuperscript{21}. Norharman is a $\beta$-carboline, with a chemical structure related to those of heterocyclic aromatic amines\textsuperscript{22}. Aniline is carcinogenic in rodents, but not mutagenic to $S$. typhimurium strains except with an S9 mix in the presence of norharman\textsuperscript{23}.

Heterocyclic amines (HCAs) are produced when protein-rich foods such as meat and fish are heated\textsuperscript{24–26}. Carcinogenic forms have been detected in all urine samples examined from healthy volunteers eating normal diets but not in the urine of postoperative patients receiving parenteral alimentation\textsuperscript{27}. Thus, quantification of HCAs in cooked foods and in human urine has indicated that human beings are continuously exposed to low levels of HCA in the diet and from cigarette smoke\textsuperscript{28}. The calculated average intake of carcinogenic HCAs is about 0.4–16 $\mu$g/day, per capita\textsuperscript{29}. A total of about 20 mutagenic and carcinogenic HCAs have been identified so far\textsuperscript{29}, with example inducing tumors in the liver, lung, breast, small and large intestines, most are hepatocarcinogens\textsuperscript{29}. However, studies of the complete carcinogenic potential of HCAs in hamsters have been limited. Subcutaneous injection of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) produced fibrosarcomas\textsuperscript{30}.

We recently investigated the influence of eight HCAs on pancreatic duct carcinogenesis in a rapid production model in hamsters\textsuperscript{11}, and found increased numbers of invasive carcinomas in hamsters given 0.02% Trp-P-1 for 70 days. The number and incidence of invasive carcinomas were also elevated in hamsters given diet containing 0.06% 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) for 50 days, suggesting a possible involvement of Trp-P-1 and 4,8-DiMeIQx in pancreatic duct carcinogenesis\textsuperscript{11}.

In the present study, however no initiating or promoting activity of APNH on pancreatic duct carcinogenesis was detected in an rapid production model.

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References


16. Nagao M, Yahagi T, and Sugimura T. Differences in effects of norharman with various classes of chemical mutagens and...


20. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans; Aniline and Aniline hydrochloride. IARC Scientific Publications No. 27, IARC, Lyon, 39–61, 1982.


