Carcinogenic Activity of Quinoline on Rat Liver

Kazuya Hirao, Yoshitaka Shinohara, Hiroyuki Tsuda, Shoji Fukushima, Michihito Takahashi, and Nobuyuki Ito

First Department of Pathology, Nagoya City University Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan

SUMMARY

The effects of prolonged p.o. administration of quinoline or 2-chloroquinoline on rat liver were examined histologically. Hepatocellular carcinomas and hemangiendotheliomas were observed in the livers of rats fed a basal diet containing 0.05, 0.10, or 0.25% quinoline for about 16 to 40 weeks. However, no neoplastic changes were seen in the livers of rats fed a basal diet containing 0.05, 0.10, or 0.25% 2-chloroquinoline for 40 weeks. In groups that received low concentrations of quinoline, the incidences of hepatocellular carcinomas were higher than the incidences of hemangiendotheliomas. No remarkable changes were detected in the livers of rats treated with either quinoline or 2-chloroquinoline.

INTRODUCTION

The carcinogenic activity of quinoline derivatives has been reported by many observers (20–23, 28, 29), and the effects of 4-NQO (4, 14) and 4-hydroxyaminoquinoline-1-oxide (15) in particular have been studied at biological, biochemical, and molecular levels. However, there have been no reports on the chronic toxicity or carcinogenicity of quinoline or 2-chloroquinoline.

It was then found that liver tumors induced in rats by quinoline were similar to those induced by several hepatocarcinogens. This paper describes histopathological and clinicopathological analyses of hepatocarcinogenesis in Sprague-Dawley rats treated with quinoline or 2-chloroquinoline.

MATERIALS AND METHODS

A total of 126 male Sprague-Dawley rats (Clea Japan, Inc., Tokyo, Japan), weighing 160 to 185 g, were used. The rats were divided into 6 groups of 20 rats and a control group of 6 rats. Quinoline and 2-chloroquinoline were purchased from Nakarai Pure Chemical Co. Ltd., Kyoto, Japan. Both chemicals were proved to be over 99.8% pure by gas chromatography, and Chart 1 shows their chemical structures. The rats were fed on the semisynthetic basal diet described previously (12) composed of 75% polished rice powder, 10% casein, 4% salt mixture, 10% corn oil, and 1% vitamin mixture, with the following supplements: Group 1, 0.05% quinoline; Group 2, 0.1% quinoline; Group 3, 0.25% quinoline; Group 4, 0.05% 2-chloroquinoline; Group 5, 0.1% 2-chloroquinoline; and Group 6, 0.25% 2-chloroquinoline; Group 7, no addition. The diets were prepared once a week and were kept in a dark cold room.

Rats were housed individually in screen-bottomed cages in an air-conditioned room at 24°C and were weighed weekly. Rats were killed with ether after 40 weeks or when they became moribund; rats that died within 16 weeks were excluded from the effective numbers of animals. All effective rats were necropsied and examined macroscopically for tumors.

Histological Study. The liver of each animal was weighed and pieces were taken for histological studies. The tissues were fixed in 10% buffered neutral-formalin solution. All tissues were stained with hematoxylin-eosin, and selected tissues were treated with Mallory's, van Gieson's, periodic acid-Schiff, or Gomori's silver stain.

Blood Chemistry. Erythrocyte and leukocyte counts, the hematocrit, and the contents of hemoglobin, SGOT, SGPT, alkaline phosphatase, cholinesterase, cholesterol, total protein, and blood urea nitrogen were examined at 40 weeks in 6 of the rats treated with 0.05% quinoline, in 14 of those treated with 0.05% 2-chloroquinoline, and in 6 control rats. Erythrocytes, the hematocrit, hemoglobin, and leukocytes were counted with a microcell counter (TOA Medical Electronics, Kobe, Japan). SGOT, SGPT, alkaline phosphatase, cholinesterase, total protein, and blood urea nitrogen were measured with a Hitachi Type 400 automatic analyzer.

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2 The abbreviations used are: 4-NQO, 4-nitroquinoline-1-oxide; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

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RESULTS

Growth Curves. Rats treated with a high concentration of quinoline or 2-chloroquinoline gained their weight slowly. However, rats treated with 2-chloroquinoline gained more weight than did those treated with quinoline. Some animals treated with 0.25% quinoline died of massive intraabdominal hemorrhage due to rupture of vascular tumors in the liver.

Gross Findings in the Liver. A summary of the mean survival period and changes in the body and liver weights of rats in the 6 groups is shown in Table 1. Most rats treated with a high concentration of quinoline or 2-chloroquinoline died within 40 weeks due to the toxic effects of these chemicals or rupture of vascular tumors of the liver. In general, groups treated with quinoline or 2-chloroquinoline showed increased liver weight, which was especially marked in rats treated with quinoline. Many large and small nodules developed in the livers of rats treated with quinoline. Examinations were made on 6 of 11 rats (54.5%) treated with 0.05% quinoline, 12 of 16 rats (75.0%) treated with 0.1% quinoline, and 18 of 19 rats (95.0%) treated with 0.25% quinoline. Two types of liver tumors were distinguished macroscopically, a hemorrhagic type and a white or dark-yellowish type up to 2.0 cm in diameter (Fig. 1). No liver tumors were seen in any rats treated with 2-chloroquinoline.

Histological Findings in the Liver. Two kinds of malignant tumor and nodular hyperplasia developed in the livers of rats treated with quinoline. Malignant tumors were hepatocellular carcinomas and hemangiosarcomas. Areas of hepatocellular carcinoma were discrete and not encapsulated, and many showed invasion of surrounding liver tissue. Most of the cords in hepatocellular carcinomas were 2 or more cells thick. The nuclei of tumor cells were prominent and were often multiple, and most tumor cells showed greater cytoplasmic basophilia than did nonneoplastic parenchymal cells. Mitotic figures were frequent in the cells of tumor tissues (Fig. 2). Nodular hyperplasia was observed in 6 of 11 rats (54.5%) in Group 1 and 4 of 16 rats (25.0%) in Group 2 (Fig. 3), but not in the rats in other groups. Areas of hemangiendotheliomas or hemangiosarcomas were composed of newly formed irregular capillary structures, proliferating endothelial cells, and spindle-shaped mesenchymal cells. Tumor cells of hemangiosarcomas frequently showed mi-

Table 1

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of rats</th>
<th>Experimental period (wk)</th>
<th>Body wt (g)</th>
<th>Liver wt</th>
<th>% of body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05% quinoline</td>
<td>11</td>
<td>36.5 ± 5.0</td>
<td>168.0 ± 6.9</td>
<td>556.0 ± 64.0</td>
<td>21.3 ± 3.8</td>
</tr>
<tr>
<td>0.1% quinoline</td>
<td>16</td>
<td>27.3 ± 6.0</td>
<td>179.3 ± 8.0</td>
<td>451.0 ± 52.9</td>
<td>19.1 ± 5.2</td>
</tr>
<tr>
<td>0.25% quinoline</td>
<td>19</td>
<td>20.0 ± 3.8</td>
<td>178.3 ± 10.4</td>
<td>330.4 ± 62.6</td>
<td>16.1 ± 3.4</td>
</tr>
<tr>
<td>0.05% 2-chloroquinoline</td>
<td>15</td>
<td>40.0 ± 0.0</td>
<td>175.8 ± 6.1</td>
<td>679.7 ± 81.2</td>
<td>16.4 ± 2.5</td>
</tr>
<tr>
<td>0.1% 2-chloroquinoline</td>
<td>12</td>
<td>40.0 ± 0.0</td>
<td>175.0 ± 3.5</td>
<td>656.0 ± 93.7</td>
<td>17.2 ± 4.5</td>
</tr>
<tr>
<td>0.25% 2-chloroquinoline</td>
<td>15</td>
<td>31.4 ± 9.6</td>
<td>181.0 ± 9.6</td>
<td>498.4 ± 127.7</td>
<td>14.6 ± 3.2</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>40.0 ± 0.0</td>
<td>178.8 ± 8.2</td>
<td>677.5 ± 64.5</td>
<td>12.3 ± 1.3</td>
</tr>
</tbody>
</table>

* Rats dying within 16 weeks were not included.

Table 2

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of rats</th>
<th>Liver change</th>
<th>Tumor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bile duct proliferation</td>
<td>Fatty change</td>
</tr>
<tr>
<td>0.05% quinoline</td>
<td>11</td>
<td>± ± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>0.1% quinoline</td>
<td>16</td>
<td>+ + +</td>
<td>+ +</td>
</tr>
<tr>
<td>0.25% quinoline</td>
<td>19</td>
<td>+ + +</td>
<td>+ +</td>
</tr>
<tr>
<td>0.05% 2-chloroquinoline</td>
<td>15</td>
<td>± ± ±</td>
<td>± ± ±</td>
</tr>
<tr>
<td>0.1% 2-chloroquinoline</td>
<td>12</td>
<td>± ± ±</td>
<td>± ± ±</td>
</tr>
<tr>
<td>0.25% 2-chloroquinoline</td>
<td>15</td>
<td>± ± ±</td>
<td>± ± ±</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>± ± ±</td>
<td>± ± ±</td>
</tr>
</tbody>
</table>

* Rats dying within 16 weeks were not included.
totic figures and were irregular in size. Vascular spaces, occasionally filled with erythrocytes and hemorrhagic materials, were frequent in neoplastic lesions (Fig. 4).

In the nonneoplastic region of the liver, there appeared a slight to moderate degree of oval cell infiltration and proliferation of the bile ducts and also fatty degeneration of liver parenchymal cells. Fatty changes of liver cells were more severe in groups treated with 2-chloroquinoline than in those treated with quinoline (Fig. 5). Occasionally, small foci were seen showing dilated sinusoidal spaces and proliferated endothelial cells with a multilayered arrangement. No cholangiobiosis, fibrosis, or cirrhotic changes were seen in any groups. Histological findings in the livers of these rats are summarized in Table 2.

Other Organs. Two of the 16 rats treated with 0.1% quinoline had hemorrhagic metastatic foci in the lungs. These foci showed the same histological pattern as hemangiosarcomas with large irregular nuclei and many mitotic figures (Fig. 6). Animals treated with quinoline or 2-chloroquinoline did not develop primary neoplasms in any organs, including s.c. or retroperitoneal tissues, other than in the liver. Hemoperitoneum or hemotorax were not seen.

Hematology and Serum Chemistry. Changes in the erythrocyte and leukocyte counts, the hematocrit, and the hemoglobin content are summarized in Table 3. These changes in the cell counts and hemoglobin level were not significant.

The levels of SGOT and alkaline phosphatase increased slightly in rats treated with 0.5% quinoline, but the levels of SGPT, cholinesterase, total protein, and blood urea nitrogen did not change markedly. No changes were detected in rats treated with 0.05% 2-chloroquinoline. Studies were made on the hematology or serum chemistry of groups treated with high concentrations of these chemicals.

DISCUSSION

This study shows that hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas of the liver are induced in rats by quinoline. 2-Chloroquinoline was not found to have any tumorigenic action in the rats.

Two types of liver tumor induced by quinoline were distinguished histologically, namely, hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas. The hepatocellular carcinomas induced by quinoline were similar in historical appearance to those induced by diethylnitrosamine (9, 10, 17, 18, 25, 26), 3'-methyl-4-dimethylaminoazobenzene (6, 19, 27), N-2-fluorenylacetamide (23, 25), and n-toluylenediamine (12). The hemangiosarcomas were also similar to those induced by several nitroso compounds or cycasin (9–11, 16, 17). However, the vascular tumors induced by nitroso-compounds were reported to develop both in the liver and in several other organs, and s.c. administration of nitroso compounds was more effective than p.o. administration in inducing hemangioendotheliomas and hemangioendothelial sarcomas (3, 8, 24, 30–32). In the present study, vascular tumors were found only in the liver at high incidence by p.o. administration. This suggests that the carcinogenic actions of quinoline and nitroso compounds are quite different.
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It has been reported that it is often almost impossible to exactly classify vascular tumors induced by chemical carcinogens (8, 24, 30–32), because the differences between them are too slight. The vascular tumors induced by quinoline also showed borderline differences. However, in this work some rats showed metastatic changes in the lungs that had a typical malignant histological pattern.

With regard to preneoplastic changes of the liver, there is some evidence that hepatocellular carcinomas may originate from areas of nodular hyperplasia (5–7, 9, 12, 13). About preneoplastic lesions of vascular tumors, it may be suggested that some potent metabolite of quinoline may produce oncogenic changes in the sinusoidal lining cells or small capillary endothelial cells of the liver. Previously, it was observed that sinusoidal cells or small capillary cells in angiomatous lesions of dog liver treated with nitroso-compounds were irregular in size and shape and protruded into the luminal surface (8–10, 24, 30). Electron microscopically, the cells had many pinocytotic vesicles, the bottom of the cells invaded profoundly into the oridinal structure (10, 31, 32).

In the present observation, above-described proliferative vascular lesions might very likely progress and sinusoidal dilation might become prominent. These might lead to the development of irregular endothelial cell hyperplasia with varying vascularization, with or without atypical cell, and to the formation of tumor masses of the cells.

Several observers (8, 24, 30) described cystic dilation of the sinusoids simulating ill-defined hemangiomatous structures, focal hyperplasia, and localized proliferation of bizarre-shaped endothelial cells in the liver. In support of this observation, nodular hyperplasia and focal vascular proliferation showing sinusoidal dilation and endothelial cell proliferation were seen in groups treated with quinoline that developed carcinoma or sarcoma. However, no hepatocellular carcinomas or hemangiosarcomas were seen in groups without showing nodular hyperplasia or focal vascular proliferation.

The carcinogenic activities of quinoline derivatives were studied by Nakahara et al. (22) and many observers (1, 2, 4, 14, 15, 20, 21, 23, 28, 29). In particular the carcinogenicity of 4-NOO and its related compounds was demonstrated in rats, mice, guinea pigs, hamsters, and rabbits (20, 21, 28). It was also reported that 4-NOO and its related compounds induced such tumors as papillomas of the skin, lung carcinomas, and fibrosarcomas (29). However, there has been no report of the carcinogenicity of quinoline itself. The changes of the liver induced by quinoline in rats were very similar histopathologically to those induced by diethylnitrosamine or cyscin. There were no data on the possible formation of quinoline metabolite in the liver. However, in regard to the present study, quinoline may be activated only in the liver, perhaps by the formation of the N-oxide, as the different action of nitroso compounds was clearly related to the formation of tumor masses of the cells.

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Fig. 1. Multiple white or hemorrhagic nodules in the liver of a rat treated with 0.05% quinoline for 40 weeks. Large arrow, hepatocellular carcinoma: small arrow, hemangioendothelial sarcoma.

Fig. 2. Hepatocellular carcinoma in a rat treated with 0.05% quinoline for 40 weeks. Nuclear irregularities and mitotic figures of cancer cells are seen. \( \times 200 \)

Fig. 3. Nodular hyperplasia of the liver in rat treated with 0.1% quinoline for 36 weeks. The hyperplastic nodule is composed of acidophilic cells. \( \times 100 \)

Fig. 4. Hemangiosarcoma in the liver of a rat treated with 0.25% quinoline for 21 weeks. \( \times 100 \)

Fig. 5. Diffuse fatty changes of liver parenchymal cells in a rat treated with 0.1% 2-chloroquinoline for 36 weeks. No connective tissue or bile duct proliferation is seen in this periportal area. \( \times 100 \)

Fig. 6. Metastatic foci of the lung of a rat treated with 0.10% quinoline for 40 weeks. Growth of atypical endothelial cells is seen. \( \times 100 \)

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