Mitogenic and Carcinogenic Effects of a Hypolipidemic Peroxisome Proliferator, [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetate Acid (Wy-14,643), in Rat and Mouse Liver


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ABSTRACT

Long-term effects of Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetate acid), a potent hepatic peroxisome proliferator structurally unrelated to the clinically used drug clofibrate, were investigated in male acatalasemic C57B1/6 mice and F344 rats. Acatalasemic mice were fed Wy-14,643 at a dietary concentration of 0.1% (w/w) for 6 months and then at 0.05% (w/w) until the termination of the experiment at 14.5 months. F344 rats were fed this compound at a 0.1% (w/w) level in the diet for 16 months. Hepatocellular carcinomas developed in 18 of 18 acatalasemic mice and 15 of 15 F344 rats that survived chronic Wy-14,643 treatment. Metastases to lungs were observed in 5 of 18 mice and 6 of 15 rats with Wy-14,643-induced hepatocellular carcinomas. The primary liver tumors in rats contained numerous peroxisomes. The increase in the number of these organelles in tumor cells was associated with a significant elevation of carnitine acetyltransferase activity, suggesting that these intrahepatic tumors respond to the peroxisome proliferative effect of Wy-14,643. The catalase activity of these tumors, however, was not increased.

Short-term administration of Wy-14,643 induced DNA replication and cell division in the rat liver as determined by [3H]thymidine incorporation into DNA, autoradiography, and colchicine-arrested metaphases in liver cells. The stimulation of liver cell proliferation was not associated with hepatocellular necrosis. The elevations of serum α-fetoprotein concentration were associated with and proportional to liver cell proliferation.

The observation that nafenopin and Wy-14,643, 2 structurally unrelated hypolipidemic agents, induce "primary" liver cell proliferation and hepatocellular carcinomas prompts a concern over the potential carcinogenicity of hepatic peroxisome proliferators as a class.

INTRODUCTION

Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetate acid), which was originally synthesized by Santilli et al. (39), has been shown to possess both hypolipidemic and antiatherogenic properties (15). This compound was found to be 60 times more potent than the clinically used hypolipidemic drug clofibrate (ethyl-α-p-chlorophenoxyisobutyrate) in lowering serum lipid levels in experimental animals (49) but it did not enter into clinical trials, possibly due to suspected hepatotoxicity in experimental animals. Previous work by Reddy and Krishnakartha (31) showed that Wy-14,643 caused a significant hepatomegaly and produced a marked increase in the hepatic peroxisome population in rats and mice. Subsequently, we have demonstrated that structurally related analogs of Wy-14,643 that lacked hypolipidemic effect failed to induce hepatic peroxisome proliferation and peroxisome-associated enzymes (28, 34).

During the past 5 years, several chemicals with a hypolipidemic property have been identified as hepatic peroxisome (microbody) proliferators in rats and mice (29, 31, 33). Because of this association, a relationship between hepatic peroxisome proliferation and lipid metabolism was suggested (31). Recently, Lazarow and DeDuve (18) and Lazarow (17) presented evidence to indicate that peroxisomes catalyze the β-oxidation of long-chain fatty acids. The presence in peroxisomes of a fatty acyl-CoA-oxidizing system (17, 18) and carnitine acetyltransferase (20) and their increase in the livers of animals treated with peroxisome proliferators (11, 12, 18, 22, 23, 26, 48) appear to substantiate the relationship between peroxisome proliferation and lipid metabolism.

Because of the long-term administration of drugs such as clofibrate for the control of hyperlipidemic states in man (7), it is essential to investigate various aspects of the persistent hepatomegaly and peroxisome proliferation induced by these agents. Long-term feeding of nafenopin (2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid; Su-13,437), a closely related analog of clofibrate, has been shown to induce hepatocellular carcinomas in rats and acatalasemic mice (35, 38). The availability of several compounds with diverse chemical structures that induce peroxisome proliferation in liver cells (29, 31, 33, 47) provides an opportunity to investigate the relationship, if any, between peroxisome proliferation, hepatomegaly, and hepatocarcinogenesis. We now report that Wy-14,643 (Chart 1), a potent hypolipidemic peroxisome proliferator, which is structurally different from clofibrate, is a mitogen for liver and induces hepatocellular carcinomas in rats and mice.

MATERIALS AND METHODS

Long-Term Administration of Wy-14,643. Male F344 rats weighing 80 to 100 g were obtained from ARS Sprague
Hepatocarcinogenicity of a Peroxisome Proliferator

![Chart 1. Structure of nafenopin (2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid; Su-13,437) and Wy-14,643 ((4-chloro-6-(2,3-xylidino)-2-pyrindinylthio)acetic acid). Nafenopin is a structural analog of the clinically used hypolipidemic drug clofibrate, but Wy-14,643 is not. These 2 structurally unrelated peroxisome proliferators produce liver tumors in rats and mice.](image)

Dawley, Madison, Wis., and were housed in individual cages. Fifteen rats were fed Wy-14,643 (Wyeth Laboratories, Inc., Radnor, Pa.) at a concentration of 0.1% (w/w) in the ground chow until the termination of the experiment at 16 months; 10 male rats served as controls and were fed the same diet without Wy-14,643. The acatalasemic CS^a mice (5) used in these studies were derived from a colony maintained in this laboratory. Twenty male acatalasemic mice were fed Wy-14,643 at a dietary concentration of 0.1% (w/w) for 6 months. At the end of this period, the 18 surviving mice were fed this compound at a dietary concentration of 0.05% (w/w) until the termination of the experiment at 14.5 months. Ten male acatalasemic mice served as untreated controls (Table 1). Animals were killed under light ether anesthesia.

**Morphology and Transplantation.** Tissues for light microscopic examination were fixed in neutral buffered formalin and embedded in paraffin. Sections 4 to 6 μm thick were stained with hematoxylin and eosin. For electron microscopy, selected samples of liver tumors and uninvolved portions of the liver were fixed for 30 to 60 min in 2.5% glutaraldehyde, buffered to pH 7.4 with 0.1 M sodium cacodylate, and then postfixed in osmium tetroxide and processed as described previously (29). For cytochemical localization of peroxisome catalase, selected samples of liver and liver tumors were processed according to the procedure described by Novikoff and Goldfischer (25). The methodology for transplantation of rat liver tumors into syngeneic weaning rats has been previously described (35).

**Assay of Enzyme Activity.** Liver tumors and nontumorous portions of the liver of rats fed Wy-14,643 were obtained at the time of sacrifice (Table 2) for the determination of catalase and carnitine acetyltransferase activities by the methods described previously (10, 20, 23, 29). Total protein was measured by the method described by Lowry et al. (19).

**Subcellular Fractionation and Separation of Proteins.** Homogenates of rat liver tumors and of uninvolved portions of liver were prepared in 0.25 M sucrose and were fractionated by differential centrifugation (32). The postnuclear pellets were solubilized and separated by SDS*-polyacrylamide gel electrophoresis according to the method of Laemmli (16).

**Serum AFP Determination.** Male F344 rats were fed 0.2% Wy-14,643 (w/w) in ground chow for periods of up to 16 weeks. Groups of 4 to 6 rats were bled via the inferior vena cava at 1, 3, 6, 8, 12, and 16 weeks. Additional groups of rats were killed at 1, 2, 3, 5, 7, and 10 days of 0.2% Wy-14,643 feeding (Chart 2). The AFP concentrations were measured using the radioimmunoassay described previously (43). Serum samples from 7 rats bearing Wy-14,643-induced primary hepatocellular carcinomas were also assayed for AFP.

**Autoradiographic Localization of [³H]Thymidine Uptake.** In this experiment, the incorporation of [³H]thymidine by liver cells of rats fed Wy-14,643 was studied by light microscopic autoradiography. Male F344 rats weighing 100 to 125 g were maintained on 0.125% Wy-14,643 (w/w) in ground chow, and groups of 3 animals were killed at 24, 48, 72, 96, and 120 hr. [³H]Thymidine (specific activity, 2 Ci/ mmol; Research Products International Corp., Elk Grove Village, Ill.) was injected i.p. in a dose of 0.5 μCi/g body weight once every day after the commencement of Wy-14,643 feeding. Pieces of liver were fixed in neutral buffered formalin and processed for light microscopic autoradiography (30). The remaining liver tissue of rats killed at 120 hr was used for the measurement of total hepatic DNA concentration (1, 6) and of [³H]thymidine incorporation into DNA (Table 3). The radioactivity incorporated into DNA was measured in a Beckman LS-9000 liquid scintillation spectrometer.

In addition, the extent of incorporation of [³H]thymidine into rat liver DNA was measured after a single dose of Wy-14,643 (1000 μg/kg body weight) by gavage. [³H]Thymidine (2 μCi/g body weight) was injected i.p. 2 hr before sacrifice at 18, 24, 36, and 48 hr after the administration of Wy-16,643, and the amount of radioactivity incorporated into liver DNA was measured (Table 4).

**Measurement of Liver Mitosis.** Male F344 rats weighing 80 to 100 g were given a single dose of Wy-14,643 (200 μg/kg body weight) by stomach tube at the start of the experiment and were fed a diet containing 0.2% Wy-14,643 (w/w); they were killed at the time intervals indicated in Table 5. Each animal received a s.c. injection of colchicine (0.1 mg/100 g body weight) 6 hr before sacrifice. Segments of liver were processed for histological examination. For the mitotic counts, both interphase nuclei and those in metaphases were enumerated in 4-μm-thick hematoxylin- and eosin-stained sections (30) and presented as the average of 3 animals killed at each time interval.

**RESULTS**

**Liver Tumor Induction.** The incidence of liver tumors in male acatalasemic mice and F344 rats fed Wy-14,643 is....

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*The abbreviations used are: SDS, sodium dodecyl sulfate; AFP, α-fetoprotein.*

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shown in Table 1. Because 2 acatalasemic mice fed 0.1% Wy-14,643 died near the end of the first 6 months of the experiment, the surviving mice were fed this compound at a dietary concentration of 0.5% until the termination of the experiment at 14.5 months. All 18 acatalasemic mice that survived long-term administration of Wy-14,643 developed multiple tumors involving several lobes of the liver. The tumors measured 3 to 28 mm in diameter and appeared gray to gray-brown. The livers were greatly enlarged and revealed no evidence of cirrhosis or scarring. Microscopically, the cellular morphology of these mouse liver tumors ranged from well differentiated to anaplastic. Although the majority of these hepatocellular carcinomas were of the trabecular type (Fig. 1), several had a pleomorphic, poorly differentiated pattern. Vascular invasion was frequently encountered in these tumors. Pulmonary metastases were present in 5 of 18 mice with hepatocellular carcinomas.

Between 14 and 16 months, 15 of 15 male F344 rats fed 0.1% Wy-14,643 developed liver tumors. These tumors were multiple and measured 3 to 42 mm in diameter. The tumor-bearing livers were markedly enlarged. The tumors appeared gray, and sectioned larger tumors revealed areas of focal hemorrhage and softening. Histologically, all these tumors were hepatocellular carcinomas with trabecular patterns (Fig. 2). Some tumors were moderately to poorly differentiated and had broad trabeculae with central necrosis. The nuclei, in general, were very prominent in these hepatocellular carcinomas. Mitoses were frequently encountered, and an occasional tumor with a trabecular pattern showed evidence of fatty metamorphosis (Fig. 3). Metastases to the lungs were found in 6 rats (40%) bearing primary hepatocellular carcinomas. These occurred as either single or multifocal metastases, usually presenting as clusters of well-differentiated cells resembling the cells of trabecular hepatocellular carcinomas. Some of these metastases were very small, composed of few cells, and were detected only on careful microscopic examination.

The ultrastructural appearance of rat and mouse hepatocellular carcinomas was similar to that observed in our earlier studies with nafenopin (35, 38). Peroxisomes were prominent in these tumors, and several of these organelles in primary tumors, as well as transplants derived from these tumors, contained prominent nucleoids (Figs. 5 and 6).

**Peroxisome-associated Enzymes in Rats Bearing Primary Hepatocellular Carcinomas.** Since the animals were fed with the peroxisome proliferator Wy-14,643 until sacrifice, it appeared important to compare the levels of catalase and carnitine acetyltransferase activities in primary liver tumors with the levels in uninvolved portions of livers. The data on these 2 peroxisome-associated enzymes are presented in Table 2. Although peroxisomes in these primary hepatocellular carcinomas appeared abundant (Fig. 5), the catalase activity in these tumors was not inducible. The activity of this enzyme in uninvolved portions of liver was slightly elevated. In contrast, the carnitine acetyltransferase activity in Wy-14,643-induced primary hepatocellular carcinomas was markedly increased (Table 2).

**Mitogenic Effect of Wy-14,643.** The liver weights of both rats and acatalasemic mice increased significantly within a few days of Wy-14,643 administration in the diet. To analyze the early events, the effect of continuous administration of Wy-14,643 for 5 days on the liver weight, liver DNA, and uptake of [3H]thymidine by rat liver was investigated. Table 3 presents the data obtained from this experiment. A signif-

### Table 1

**Effect of long-term administration of Wy-14,643 on the development of liver tumors in male acatalasemic mice and F344 rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Animals with liver tumors</th>
<th>Activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acatalasemic mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wy-14,643 (0.1% for 6 mos.; 0.05% for 8.5 mos.)</td>
<td>20</td>
<td>18</td>
<td>100%</td>
</tr>
<tr>
<td>F344 rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wy-14,643 (0.1% for 16 mos.)</td>
<td>15</td>
<td>15</td>
<td>100%</td>
</tr>
</tbody>
</table>

* a Number of animals alive at appearance of first liver tumor.
  b In relation to the effective number.
  c Wy-14,643 was added to powdered chow at the concentrations (w/w) shown and fed ad libitum.
  d Pulmonary metastases were found in 5 mice and 6 rats with liver tumors.
  e Significant different (p < 0.001) from animals fed control diet, as determined by Fisher's exact test.

### Table 2

**Catalase and carnitine acetyltransferase activities in Wy-14,643-induced hepatocellular carcinomas in male F344 rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase</th>
<th>Carnitine acetyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-bearing rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinomas</td>
<td>31 ± 11 (9)</td>
<td>160 ± 46 (6)</td>
</tr>
<tr>
<td>Uninvolved liver</td>
<td>56 ± 7 (9)</td>
<td>193 ± 63 (8)</td>
</tr>
<tr>
<td>Normal rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>45 ± 3 (9)</td>
<td>2.9 ± 1.5 (4)</td>
</tr>
<tr>
<td>Wy-14,643 (0.05%) for 3 wk</td>
<td>86 ± 6 (5)</td>
<td>206 ± 14 (5)</td>
</tr>
</tbody>
</table>

* a Mean ± S.D.
* b Numbers in parentheses, number of animals studied.
* c Significantly different from controls (p < 0.001).
tcant increase in liver weight, liver DNA, and specific radio-
activity of the DNA occurred in animals fed Wy-14,643 for 5
days. The nuclear labeling of liver cells, as analyzed by light
microscopic autoradiography, was markedly increased
(Fig. 8) in rats fed Wy-14,643 for 5 days.

Table 4 presents the effects of time course on the change
in liver weight and the incorporation of \(^{3H}\)thymidine into
liver DNA of rats after a single dose of Wy-14,643 by

<table>
<thead>
<tr>
<th>Time of sacrifice (hr)</th>
<th>Mitotic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.15</td>
</tr>
<tr>
<td>30</td>
<td>7.81</td>
</tr>
<tr>
<td>36</td>
<td>9.12</td>
</tr>
<tr>
<td>42</td>
<td>11.19</td>
</tr>
<tr>
<td>54</td>
<td>10.34</td>
</tr>
<tr>
<td>60</td>
<td>8.86</td>
</tr>
<tr>
<td>66</td>
<td>8.17</td>
</tr>
<tr>
<td>72</td>
<td>8.8</td>
</tr>
</tbody>
</table>

The number of cells entering mitosis between 18 and 72
hr during the administration of Wy-14,643 was determined
by recording the colchicine-arrested metaphases (Fig. 9)
over a 6-hr period (Table 5). The total number of hepato-
cytes entering mitosis during 36 to 42 hr was approximately
11%. No evidence of hepatocellular necrosis was seen at
any time interval during the first week of Wy-14,643 feeding,
suggesting that the wave of DNA replication and mitosis
does not represent reparative hyperplasia.

Serum AFP. The serum level of AFP was determined in
rats fed 0.2% Wy-14,643 (w/w) for up to 16 weeks. The AFP
concentration was significantly elevated at 5 and 7 days and
declined thereafter (Chart 2). The serum AFP levels did not

Table 3
Effect of Wy-14,643 administration on liver weight, liver DNA,
\(^{3H}\)thymidine labeling of liver cell nuclei, and \(^{3H}\)thymidine
incorporation into liver DNA of male F344 rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver wt (g/100 g body wt)</th>
<th>Liver DNA (mg/100 g body wt)</th>
<th>Incorporation of (^{3H})thymidine (dpm/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.08 ± 0.31</td>
<td>10.85 ± 0.23</td>
<td>3.9 ± 1.12</td>
</tr>
<tr>
<td>Wy-14,643</td>
<td>7.33 ± 0.16</td>
<td>16.01 ± 1.27</td>
<td>27.3 ± 5.66</td>
</tr>
</tbody>
</table>

\( ^{a}\) Wy-14,643 was fed in powdered chow at a dietary concentra-
tion of 0.125% (w/w) for 5 days.

\( ^{b}\) \(^{3H}\)Thymidine (0.5 μCi/g body weight) was injected i.p. at 24,
48, 72, and 96 hr, and then the rats were killed at 120 hr. Three
animals were used in each group.

\( ^{c}\) Mean ± S.E.

\( ^{d}\) Significantly different from control (p < 0.001).

DISCUSSION

The present study demonstrates that long-term adminis-
tration of the hypolipidemic peroxisome proliferator Wy-
14,643 in the diet of male F344 rats and acatalasemic C58
mice results in the development of hepatocellular carcino-
mas, with a 100% incidence in both species. No tumors
were observed in control F344 rats and in acatalasemic mice
fed an identical diet minus Wy-14,643. Although the
number of animals used in these experiments is small, the
tumor incidence in Wy-14,643-fed animals, according to
Fisher's exact test or \( \chi^2 \) analysis, is significantly differ-
ent from the appropriate controls (p < 0.001). Pulmonary
metastases were found in 28% of acatalasemic mice and
40% of F344 rats bearing hepatocellular carcinomas. The
incidence of lung metastases in acatalasemic mice ob-
served in this study is comparable to that reported recently
by Vesselinovitch et al. (52) in C57BL × C3H F1 mice, and by
Reddy et al. (38) in nafenopin-treated mice with malignant
liver cell tumors.

The primary hepatocellular carcinomas induced by Wy-
14,643 in F344 rats in the present study provided an oppor-
tunity to delineate differences in the inducibility, between

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Table 5
Effect of Wy-14,643 administration on the mitotic activity in rat
liver parenchymal cells

Male F344 rats were given a single dose of Wy-14,643 (200 mg/
kg body weight) by gavage and were maintained on a diet contain-
ning 0.2% Wy-14,643 (w/w). Colchicine, 0.1 mg/100 g body weight,
was injected s.c. 6 hr before scheduled sacrifice. The mitotic index
is expressed as the cumulative percentage of cells entering mitosis
for each 6-hr period. Three animals were used for each interval.

<table>
<thead>
<tr>
<th>Time of sacrifice (hr)</th>
<th>Mitotic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
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<td>66</td>
<td>8.17</td>
</tr>
<tr>
<td>72</td>
<td>8.8</td>
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</tbody>
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increase in 7 rats bearing Wy-14,643-induced primary hep-
atocellular carcinomas.

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normal and neoplastic cells, of the peroxisome-associated enzymes catalase and carnitine acetyltransferase. As expected (14, 51), the catalase activity in Wy-14,643-induced rat liver tumors was decreased, whereas the carnitine acetyltransferase activity increased significantly in response to dietary Wy-14,643. It is now clearly established that Wy-14,643 and all other peroxisome proliferators increase the activity of carnitine acetyltransferase in rat and mouse liver (22, 28, 31, 32, 45, 48) and that this increase parallels the increase in peroxisome population. Electron microscopic examination of Wy-14,643-induced primary hepatocellular carcinomas in the present study revealed the presence of several peroxisomes in the cytoplasm, and these organelles stained positively for catalase when incubated in the alkaline 3,3'-diaminobenzidine medium (25). Accordingly, the increase in carnitine acetyltransferase activity in these liver tumors may, in part, be related to the number of peroxisomes, and it suggests that the intrahepatic primary tumors respond to the peroxisome-proliferative effect of Wy-14,643. An alternative explanation for the increased numbers of peroxisomes in primary tumor cells may be that peroxisome proliferation has already occurred in the hepatocytes from which the tumor cells arise, and the tumor cells merely retain these organelles. This, however, is unlikely since the initiated cells divide several times prior to becoming grossly visible. It is of particular interest, however, to note that the increase in peroxisome population in these primary hepatocellular carcinomas was not associated with an increase in catalase activity, which is the marker enzyme for this organelle (4). The increase in carnitine acetyltransferase but not of catalase activity in these Wy-14,643-induced tumors suggests that the cellular mechanisms regulating these peroxisomal enzymes differ considerably. It should be noted that the catalase activity is very low in many liver tumors and in the livers of tumor-bearing animals (14, 51). The lowering of liver catalase in tumors has been attributed to the presence of a tumor component, called toxohormone, that inhibits the synthesis or enhances the degradation of catalase (14). The presence of peroxisomes in primary as well as transplantable hepatocellular carcinomas in rats induced by Wy-14,643 in these studies (Fig. 6), however, suggests that these tumors are better differentiated (13, 51).

The mechanism by which Wy-14,643, a peroxisome proliferator, exerts its carcinogenic effect is not known. Recently, Reddy et al. (35, 36, 38) reported the development of hepatocellular carcinomas in rats and mice fed the peroxisome proliferator, nafenopin. The observation that nafenopin and Wy-14,643, 2 structurally unrelated hypolipidemic agents, induce hepatocellular carcinomas prompts a concern over the potential carcinogenicity of hepatic peroxisome proliferators as a class. Wy-14,643 causes marked liver enlargement in rats and mice (31, 34). The liver cells are hypertrophic and reveal proliferation of peroxisomes and smooth endoplasmic reticulum. It is evident from this study that Wy-14,643 induces liver cells to proliferate, as judged by the incorporation of [3H]thyimidine into rat liver DNA, [3H]thymidine autoradiography, and the analysis of colchicine-arrested metaphases during the 5-day treatment period. The increase in [3H]thymidine incorporation was also evident at 24 hr after a single dose of Wy-14,643 by stomach tube. The absence of any histological evidence for hepatocellular necrosis in these livers suggests that the mitogenic effect is a primary action of Wy-14,643. The fact that Wy-14,643 and nafenopin (21), 2 carcinogenic peroxisome proliferators, stimulate DNA replication [unlike most carcinogens, which strongly inhibit DNA replication both in vivo and in vitro (53)] appears to place the peroxisome proliferators in a different class of carcinogens. The fact that the dose of Wy-14,643 administered to rats and mice in these studies may be higher than the hypolipidemic dose used for humans should not detract from the potential implications of these findings.

The importance of the primary mitogenic effect of 2 carcinogenic peroxisome proliferators, Wy-14,643 and nafenopin (21), in the initiation or promotion of liver tumorigenesis (37) remains to be elucidated. It is conceivable that these agents may induce mitotic irregularities, as well as cause DNA damage. If these agents do cause DNA damage, their ability to induce DNA replication and cell proliferation may convert a transitory abnormality in DNA into an inheritable change, leading to liver tumorigenesis (2, 3, 50).

Wy-14,643 like nafenopin (29), is a potent inducer of peroxisomes. The liver growth, increase in peroxisome population, and increase of several peroxisomal enzymes in rats fed these agents appear analogous to adaptive changes occurring in livers of animals exposed to a variety of microsomal enzyme inducers, such as phenobarbital (40). Several of these xenobiotics, which induce drug-metabolizing enzymes in liver together with an increase of smooth endoplasmic reticulum, have been shown to possess hepatocarcinogenic activity, in addition to their ability to promote liver tumor induction by other chemical carcinogens (2, 24, 27, 40). The exact mechanism of these actions is not understood.

Recently, Feinstein et al. (6) presented evidence to suggest that catalase, a marker enzyme for peroxisomes, and H2O2, which is generated also by peroxisomal oxidases, may be relevant in carcinogenesis. They observed an increased incidence of liver tumors in acatalasemic mice fed aminotriazole, a potent inhibitor of catalase, and attributed this to a diminished rate of degradation of H2O2, which has been shown to be mutagenic (9). Although this hypothesis appears attractive in explaining the differences observed with aminotriazole (6) or nafenopin liver tumorigenesis (38) between substrains of mice differing only in the catalase-synthesizing gene locus, it would be difficult to explain the development of liver tumors in rats fed Wy-14,643 and nafenopin, in which catalase activity is markedly elevated. The increased catalase activity in these livers with peroxisome proliferation should lead to an enhanced rate of H2O2 degradation, thereby reducing its presumed mutagenicity and carcinogenicity. However, the possibility that the peroxisome proliferation-associated increase in catalase activity is not adequate to degrade the H2O2 produced by the oxidases present in numerous peroxisome profiles in these cells cannot be ruled out. It is not certain if the mutagenicity is due to H2O2 or to the nascent oxygen liberated as a result of H2O2 degradation.

The elevations of serum AFP concentration are most probably related to the proliferative events induced by Wy-14,643. AFP production follows proliferation induced in
vivo by partial hepatectomy or chemically induced liver injury (42, 44). AFP production is also associated with proliferation of hepatocytes in vitro (42). Of further interest is the fact that Wy-14,643 does not induce liver cell necrosis, but only proliferation (hyperplasia). Thus, AFP production is closely associated with and proportional to the hepatocyte proliferation induced by Wy-14,643. AFP production has been shown to be an early event following exposure to other hepatocarcinogens, but the relationship of this to proliferation or to carcinogenic events remains uncertain (42). In the case of N-2-fluorenylacetamide or ethionine, AFP is found in small transitional "oval" cells early after carcinogen exposure (41, 45). These cell types are not seen during exposure to Wy-14,643. On the other hand, neoplastic nodules do develop after prolonged exposure to Wy-14,643. The neoplastic nodules produced by N-2-fluorenylacetamide do not contain AFP (41, 50), and the nodules produced by Wy-14,643 exposure appear at a time when serum AFP concentrations are almost normal. We have obtained preliminary evidence that none of the hepatocellular carcinomas arising from Wy-14,643 produce AFP. These findings are consistent with the hypothesis that non-AFP-producing hepatocellular carcinomas may arise from a different precursor lesion (neoplastic nodules) than do AFP-producing tumors, which may be derived from oval cells or zones of atypical hyperplasia (41).

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Hepatocarcinogenicity of a Peroxisome Proliferator

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Fig. 1. Hepatocellular carcinoma with trabecular pattern from a male acatalasemic mouse fed Wy-14,643 for 14 months. × 70.
Fig. 2. Hepatocellular carcinoma showing a well-differentiated trabecular pattern from a rat fed 0.1% Wy-14,643 for 15 months. × 100.
Fig. 3. Hepatocellular carcinoma with trabecular pattern reveals fatty metamorphosis. Rat fed Wy-14,643 for 16 months. × 125.
Fig. 4. Multiple metastases in the lungs from a trabecular hepatocellular carcinoma of a rat fed Wy-14,643 for 14 months. × 100.
Fig. 5. Primary hepatocellular carcinoma of the rat. Several peroxisomes (p) are present in the tumor cells, suggesting that these primary liver tumors respond to Wy-14,643-induced peroxisome proliferation. × 18,000.
Fig. 6. Hepatocellular carcinoma transplant in F344 rat. These transplantable tumors are well differentiated and contain peroxisomes (p) with prominent nucleoids. × 12,500.
Fig. 7. SDS-polyacrylamide gel electrophoretic profile of postnuclear pellet (100-μg protein samples) of normal rat liver (B and 1), liver tumor (B and 2), and an uninvolved portion of the liver (B and 3) of a Wy-14,643-fed rat. Arrows, position of 80,000-molecular-weight polypeptide.
Fig. 8. Autoradiograph of liver from rat fed Wy-14,643 for 5 days and given [3H]thymidine injections at 24, 48, 72, and 96 hr. Note dense labeling of several hepatocyte nuclei (arrows). × 200.
Fig. 9. Colchicine-arrested metaphases (arrows) between 36 and 42 hr in the liver of a rat treated with Wy-14,643. Colchicine (0.1 mg/kg body weight) was given i.p. 36 hr after the start of Wy-14,643. The animal was killed 6 hr after colchicine injection. × 200.
Mitogenic and Carcinogenic Effects of a Hypolipidemic Peroxisome Proliferator, [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic Acid (Wy-14,643), in Rat and Mouse Liver


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