A non-bile duct origin for intestinal crypt-like ducts with periductular fibrosis induced in livers of F344 rats by chloroform inhalation

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To evaluate the toxic effects of prolonged exposure to chloroform vapors, female and male F344 rats were exposed to 0, 2, 10, 30, 90 and 300 p.p.m. chloroform by inhalation for 7 or 5 days/week for up to 13 weeks. The purpose of this study was to characterize a lesion that occurred in the livers of rats in the 300 p.p.m. exposure groups. Atypical glandular structures lined by intestinal-like epithelium and surrounded by dense connective tissue occurred in the livers of rats exposed to strongly hepatotoxic atmospheric concentrations of chloroform. Bile duct bromodeoxyuridine labeling indices as well as observations of the locations of the early lesions at the 3 and 6 week time points indicate that these lesions arose from a population of cells remote from bile ducts. We refer to these lesions as intestinal crypt-like ducts with periductular fibrosis to distinguish them from true cholangiofibrosis. Here, intestinal crypt-like ducts with periductular fibrosis were seen only in rats exposed to 300 p.p.m. chloroform, and the multiplicity and severity of the lesions were greater in the right liver lobe. The lesion only occurred in association with liver necrosis and dramatic increases in hepatocyte labeling indices, while labeling indices in bile ducts in the same animals were not significantly different from controls. There was a treatment-related increase of transforming growth factor-\(\alpha\) immunoreactivity in hepatocytes, bile duct epithelium, bile canaliculi and oval cells, and an increase in transforming growth factor-\(\beta\) immunoreactivity in hepatocytes, bile duct epithelium and intestinal crypt-like ducts. Thus, intestinal crypt-like ducts with periductular fibrosis appeared to develop from a population of cells unrelated to bile ducts. Also, they occurred only in animals exposed to chloroform concentrations that induced significant hepatocyte necrosis and regenerative cell proliferation and were associated with increased growth factor expression or uptake.

Introduction

Chlorination is commonly used to disinfect water throughout the United States, and trace amounts of chloroform develop in water as a by-product of this process (1,2). Industrial processes such as the bleaching of paper can also discharge chloroform into the environment (3), and chloroform is commonly volatilized from water solutions and has been measured in ambient air in shower stalls and in the air above swimming pools at concentrations as high as 0.001, 0.06 and 0.1 p.p.m. respectively (4–6). Chloroform has been shown to increase the incidence of liver tumors in male and female B6C3F1 mice and kidney tumors in male Osborne-Mendel rats when administered by gavage at high concentrations in rodent carcinogenesis bioassays (7). Chloroform is considered to be a carcinogen that is not directly genotoxic (8–10). Evidence indicates that chloroform stimulates tumor development secondary to events associated with continual cytotoxicity and subsequent regenerative cell proliferation (11).

One of the chloroform-induced lesions reported in rodents is a condition termed cholangiofibrosis (12). This lesion can be induced by a variety of chemicals and has been reported in rats (12,13), humans (14,15), mice (16), Syrian (golden) hamsters (17,18) and fish (19,20). The various reports of this lesion describe it as atypical ducts surrounded by fibrous connective tissue (12–20). In F344 rats, cholangiofibrosis is reported exclusively in association with exposure to chemical treatment (13). Some, but not all, of these reports noted similarities between the cells lining the atypical duct-like structures and those of intestinal crypt epithelium. Those researchers have suggested alternative terms for the lesion, including adenofibrosis (21,22) and intestinal-like metaplasia (23,24). Chemical treatments that have produced this lesion include polychlorinated biphenyls (21,22), furan (25–27) and chloroform (12), and most often they are pathogenetically associated with liver necrosis. The furan-induced lesions reportedly develop preferentially in the right and caudate liver lobes (25–27). Maronpot et al. reported that 100% of the animals treated for 90 days with furan and maintained without further treatment developed cholangiocarcinomas at 6, 12 and 18 months (25). In addition, Elmore and Sirica demonstrated intestinal cell differentiation in 26/27 hepatic adenocarcinomas (cholangiocarcinomas) induced in rat livers by furan (28). Thus, this lesion appears to be a preneoplastic lesion with potential to develop into cholangiocarcinoma.

The purpose of the 90 day study was to evaluate the effects of prolonged exposure to chloroform via inhalation. This paper characterizes a lesion which is extremely similar to lesions previously referred to as cholangiofibrosis. In most of the previous studies, this lesion was evaluated only after complete development. In the present study, we were able to examine early time points so that the origin and early development of the lesion could be studied. The findings in this study suggest a non-biliary origin for this lesion, and therefore we recommend discontinuing the use of the term 'cholangiofibrosis' when referring to this lesion. In the present study, intestinal crypt-like ducts with periductular fibrosis developed subsequent to prolonged cytotoxicity of hepatocytes and regenerative cell proliferation. The lesions probably originated from a population of cells that is sufficiently undifferentiated to have retained the ability to differentiate along intestinal lines and which are responding to the microenvironment that developed.
secondary to the chronic cytotoxicity, including circulating growth factors. A 2 year inhalation bioassay with chloroform produced no cancer in male or female F344 rats at the maximum tolerated dose (MTD*) of 90 p.p.m. (29). In our study, intestinal crypt-like ducts with periductular fibrosis was seen only at 300 p.p.m., a dose which is severely toxic and exceeds the MTD.

Materials and methods

Chemicals

Chloroform of >99.5% purity with 0.006% amylene as stabilizers was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and analyzed to verify its purity. Bromodeoxyuridine (BrdU) was obtained from Sigma Chemical Co. (St Louis, MO), prepared fresh as a 20 mg/ml solution in PBS, stirred and sterilized by filtration.

Transforming growth factor-α (TGF-α) antibody was obtained from Oncogene Science (Cambridge, MA), and vimentin was purchased as a prediluted antibody from Biotech Solutions (Santa Barbara, CA). TGF-β antibody was a gift from Dr Randy Jirtle of Duke University, Durham, NC. Normal sera and secondary antibodies were purchased from Vector Laboratories (Burlingame, CA), and horseradish peroxidase (HRP) streptavidin was purchased from Zymed Laboratories (San Francisco, CA). All other products were purchased from general laboratory suppliers.

Animals

This study was conducted under federal guidelines for the use and care of laboratory animals (30) and was approved by the CIT Institutional Animal Care and Use Committee. This work is one phase of a large chloroform inhalation toxicity study that will be reported in detail elsewhere (31). Animals were housed in humidity- and temperature-controlled HEPA-filtered, mass air-displacement rooms in facilities accredited by the American Association for Accreditation of Laboratory Animal Care. Briefly, 7 week old rats were obtained from the Charles River Breeding Laboratory (Raleigh, NC) They were quarantined for 2 weeks, randomized by body weight and individually identified by ear tag. Animals were housed individually in polyethylene shoebox cages with cellulose bedding (ALPHADri, Shepherd Specialty Papers, Kalamaazoo, MI) and filter-top lids. Wire cage flooring was added prior to exposures with test chemicals. The animals received food (NIH-07 rodent chow) and water ad libitum with a 12 h light/dark schedule. Animals were randomized by weight into treatment groups. Sentinel animals were housed in the animal facility as part of an ongoing surveillance program for parasitic, bacterial and viral infections, and were pathogen-free throughout the study.

Rats were weighed weekly throughout the study.

Methods

Male and female F344 rats were exposed to chloroform vapors for 6 h a day, daily for 4 days or 7 or 5 days/week for 1, 6, or 13 weeks. A stop group was included in which animals were exposed for 7 days/week for 6 weeks and then held without further exposure for 7 weeks (6 week stop group). After the indicated periods of exposure, animals were anesthetized, killed by exsanguination and necropsied. Animals were administered BrdU via an osmotic minipump (Alzet model 2ML1, 10 ml/h delivery rate, Alzet Corporation, Kalamazoo, MI) and filter-top lids. Wire cage flooring was added prior to exposures with test chemicals. The animals received food (NIH-07 rodent chow) and water ad libitum with a 12 h light/dark schedule. Animals were randomized by weight into treatment groups. Sentinel animals were housed in the animal facility as part of an ongoing surveillance program for parasitic, bacterial and viral infections, and were pathogen-free throughout the study.

Male rats had mildly pale livers without grossly visible foci. The chloroform-treated animals had histologic lesions in the liver that were time and dose dependent. These lesions ranged from minimal hepatocellular vacuolation at 90 p.p.m. to severe hepatocyte vacuolar degeneration with numerous severely vacuolated and necrotic hepatocytes, exclusively at 300 p.p.m. Hepatocytic changes were most severe in midzonal regions, slightly less severe in centrilobular areas, and least severe in perportal areas. In some of the more severely affected livers, mild centrilobular necrosis was present. Rats exposed for 6 weeks or more had centrilobular necrosis, often accompanied by fibrosis. Mild biliary duct hyperplasia, characterized by bridging anastomoses between portal triads, was present in many of the animals exposed 7 days/week for 13 weeks at 300 p.p.m. (Figure 1). Other consistent findings throughout the high-dose groups included binucleated hepatocytes, extensive hepatocellular karyomegaly and megaloacytosis.

Results

Exposure to 300 p.p.m. chloroform for up to 13 weeks was toxic to both sexes of rat, producing over a 10% depression in body weight gain, hepatic necrosis and inflammation, and a dramatic increase in regenerative hepatocyte proliferation (31). For longer studies, a concentration of 300 p.p.m. would exceed the maximum tolerated dose. In fact, a recent 2 year inhalation study with chloroform in F344 rats used 90 p.p.m. as the MTD (29). Intestinal crypt-like ducts with periductular fibrosis were observed only in rats exposed to 300 p.p.m. of chloroform, which was the highest exposure concentration used in this study. The next lower exposure concentration was 90 p.p.m. Therefore, unless otherwise noted, further references to lesions will only denote variations between sex and time course. All female rats treated for 7 days/week for 13 weeks had variably sized, firm, pale, slightly raised foci scattered throughout all liver lobes. These foci were much larger and more prominent in the right and caudate lobes. They were roughly spherical and ranged from 2 to 5 mm in diameter. Many had coalesced to form larger, irregular-shaped masses that effaced most of the affected lobe. Male rats had mildly pale livers without grossly visible foci.
Non-bile duct origin for intestinal crypt-like ducts with periductular fibrosis

Fig. 1. Intestinal crypt-like lesions in liver of female rat exposed to 300 p.p.m. chloroform, 7 days/week for 13 weeks. Evident are numerous intestinal crypt-like ducts containing intralumenal debris and surrounded by fibrous connective tissue. X100.

All 15 of the female rats and 13/15 male rats exposed 7 days/week for 13 weeks and 3/8 female and 2/8 male rats exposed 5 days/week for 13 weeks had single or multiple well-developed foci of adenofibrosis. In addition, 1/10 male rats exposed 7 days/week for 6 weeks had multiple well-developed foci of intestinal crypt-like ducts with periductular fibrosis; at 13 weeks, however, the multiplicity and severity of the lesion was much greater in females than males. The lesion was composed of ductular structures surrounded by mild to moderate amounts of fibrous connective tissues (Figure 1). These ductular structures were generally lined by a simple columnar epithelium having modest numbers of goblet cells. Occasionally, the epithelium in these structures was comprised of cuboidal cells. Ductular lumens contained varying amounts of debris, mucus, sloughed degenerate epithelial cells, and a few degenerate and non-degenerate neutrophils (Figure 2). Small numbers of inflammatory cells, principally neutrophils, were in the connective tissue stroma of these foci.

Early versions of the lesion consisting of three or four cuboidal epithelial cells surrounding a small lumenal opening and surrounded by two or three concentric layers of plump spindloid cells (Figure 3) were present in males exposed for 3 weeks or longer. These foci were not observed in females at 3 weeks of exposure, and females were not examined at the 6 week exposure interval. The early foci observed at 3 weeks occurred without evidence of significant bile duct proliferation, which was consistently present at later time points. In addition, many of these lesions developed near central veins and in other areas of the hepatic lobule remote from biliary structures. When treatment was stopped after 6 weeks and the animals examined at 13 weeks, the livers contained numerous small foci interpreted as resolving lesions scattered throughout the hepatic parenchyma. These foci were characterized by accumulations of macrophages and lymphocytes around a

Fig. 2. Intestinal crypt-like lesion with ducts lined by intestinal-like epithelium and surrounded by fibrous connective tissue. X200.
few spindloid cells accompanied by a virtual absence of early versions of the lesion.

Intralumenal material in the more well-developed intestinal crypt-like ducts reacted positively to PAS and alcian blue stains. The cytoplasms of goblet cells lining these ducts stained strongly positive with PAS and alcian blue. The early lesions did not react with PAS, and there was only faint bluish staining in the connective tissue stroma of these lesions with alcian blue. In the resolving lesions observed in the 6 week stop groups, there were variable numbers of PAS-positive granules in the cytoplasm of a few of the cells, and there was faint positive reactivity in the stroma of these foci with alcian blue. These staining patterns indicated the presence of both acidic and neutral mucopolysaccharides. Also, the negligible staining of this stroma with PAS suggests that the stroma surrounding the ductular structures developed via fibroplasia and does not merely represent deposition of basement membrane material.

In trichrome-stained liver sections, there was variably intense bluish staining in the connective tissue stroma of the adenofibrotic lesions. The cytoplasm of most of the epithelial cells lining the ductular structures stained intensely red. In the early foci, similar but less intense staining was evident in the stroma as well as the ductular epithelium. These staining patterns indicate that a considerable collagen content existed in the stroma of the lesion. Significant collagen content was only evident after the lesions became fairly well developed and was characterized by differentiation of the ductular epithelium into columnar cells with goblet cells. This occurred in groups exposed for 13 weeks.

In control female rats, only the cytoplasm of centrilobular hepatocytes was slightly to mildly immunoreactive for TGF-α (Figure 4). This staining was evident at 3 weeks, very intense at 6 weeks and slightly less intense at 13 weeks. It disappeared by 13 weeks when exposure was stopped after 6 weeks. Oval cells had marked TGF-α immunoreactivity (Table I), and intestinal crypt-like ducts had slight to moderate TGF-α immunoreactivity.

TGF-β immunoreactivity between control female and male rats was different. Control female rats had moderate, usually diffuse immunoreactivity in centrilobular and midzonal hepatocytes, which sometimes decreased slightly in periportal areas (Table II). Control male rats had minimal to mild immunoreactivity in centrilobular and midzonal hepatocytes and less in periportal areas (Table II). Treated males and females had a duration-dependent increase in TGF-β immunoreactivity in hepatocytes and bile duct epithelium (Figure 5). Adenofibrotic ducts were slightly to mildly reactive, but neither oval cells nor bile canaliculi were TGF-β immunoreactive (Figure 5) (Table II).

Fibroblasts and inflammatory cells in the stroma of the intestinal crypt-like lesions reacted slightly positive to vimentin. There was no immunoreactivity to vimentin in the ductular structures. The vimentin immunoreactivity in these cells indicates that they are of mesenchymal origin.

There was striking similarity between the cells of intestinal crypt-like ducts and intestinal mucosal epithelial cells. This included not only their physical appearance but essentially identical staining patterns with H&E as well as with PAS and alcian blue.

There were numerous variably sized cells, presumably macrophages, scattered throughout the liver sections. These cells had golden cytoplasm filled with cholesterol-like slits. Most of these cells were solitary; however, some were in small clusters. Though they were situated at various locations in hepatic lobules, many were in close proximity to central veins. Small numbers were present in livers from animals exposed to 90 p.p.m. chloroform, and this alteration was increased in animals exposed to 300 p.p.m. chloroform. These cells were often associated with dead or dying hepatocytes, and occasionally dead hepatocytes were evident within their cytoplasm.

Their cytoplasm stained moderately positive with PAS; however, the cleft-like material in the cytoplasm did not react to PAS. Neither the cytosol nor the cleft-like material stained significantly with alcian blue.

Labeling indices of hepatocytes as percentage of cells in S-phase was not different from control in rats exposed to 90 p.p.m. chloroform (31). Only animals exposed to 300 p.p.m. had statistically significant increases in hepatocyte regenerative proliferation. Only those rats with marked toxicity and increases in hepatocyte labeling developed cholangiofibrotic lesions. Following chloroform exposure, labeling indices were only slightly increased in bile ducts. Neither oval cells nor cells of the intestinal crypt-like lesions were present in appreciable numbers in the livers of control animals and therefore were not evaluated; however, significant labeling was seen in oval cells and cells of intestinal crypt-like ducts in the treated animals (Figure 6, Table III).

Discussion
The present study describes adenofibrosis, which is an important and controversial toxicant-induced hepatic lesion.
associated with the development of cholangiocarcinoma. This lesion is reported by others in response to treatment with furan (25–28) and other chemical exposures (18–22). The basic pathogenesis, gross and histologic appearance, and behavioral characteristics of the lesion appear to be the same regardless of inciting agent. In this report, adenofibrotic lesions were observed in rats exposed to chloroform, and they were morphologically similar to those reported for furan (25–28).

The origin and pathogenesis of these intestinal crypt-like ducts with periductular fibrosis have not been definitively determined because most previous reports only evaluated fully developed lesions. Elmore and Sirica reported observing sequential development of intestinal mucosal cell types in the right and caudate liver lobes of furan-treated rats (35). Subsequently, Sirica et al. suggested that these lesions arise from proliferating biliary duct cells and that metaplasia occurs giving them their phenotypic expressions (36). The possibility that these lesions arise from proliferating hepatocytes has also been examined. In a study in which rats were subjected to partial hepatectomies with hepatocyte proliferation inhibited by dietary administration of 2-acetylaminofluorene (2-AAF), lesions referred to as intestinal metaplastic ducts surrounded

<table>
<thead>
<tr>
<th>Cells or structures</th>
<th>7 days/weeks (3 weeks)</th>
<th>7 days/weeks (6 weeks)</th>
<th>7 days/weeks (13 weeks)</th>
<th>5 days/weeks (13 weeks)</th>
<th>6 week stop</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrilobular hepatocytes</td>
<td>+</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>Midzonal hepatocytes</td>
<td>−</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>Penportal hepatocytes</td>
<td>−</td>
<td>NE</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Bile duct epithelium</td>
<td>+/−</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Early versions of the intestinal</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>crypt-like lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal crypt-like ducts</td>
<td>LNP</td>
<td>NE</td>
<td>LNP</td>
<td>NE</td>
<td>LNP</td>
<td>LNP</td>
</tr>
<tr>
<td>Proliferating oval cells</td>
<td>LNP</td>
<td>NE</td>
<td>LNP</td>
<td>NE</td>
<td>LNP</td>
<td>LNP</td>
</tr>
<tr>
<td>Bile canaliculi</td>
<td>+/−</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
</tr>
</tbody>
</table>

NE, tissues from animals at this time point were not examined; LNP, these lesions were not present.

Fig. 4. TGF-α stain of liver from female rat exposed to 300 p.p.m., 7 days/week, for 13 weeks showing variable staining in intestinal crypt-like ducts (solid arrows), intense staining in proliferating biliary ducts (open arrows), and intense bile canalicular staining (arrowheads). ×400.

Fig. 5. TGF-β stain of liver from female rat exposed to 300 p.p.m., 7 days/week, for 13 weeks showing diffuse moderately intense staining in hepatocytes and variable staining in intestinal crypt-like ducts (arrows). ×200.
by fibroplasia developed (23). Thus the possibility that these lesions arose from proliferating hepatocytes was essentially precluded. These authors concluded that cholangiofibrotic lesions arose from a stem cell, probably the oval cell. Additional support for this hypothesis was the finding, in their model, that only oval cells had early increased labeling with a marker for DNA synthesis (23).

In the present study, early versions of the lesion were located in areas remote from portal triad regions of the lobules. They were seen as early as 3 weeks of treatment and often appeared prior to provide evidence of significant bile duct proliferation. This distribution pattern for the early lesions of this lesion has also been reported after treatment with 2-AAF (23). In addition, there were significant differences between the staining characteristics of the intestinal crypt-like ducts and proliferating biliary ducts and no evidence of progression from proliferating biliary structures to early versions of the lesion. These data suggest that this lesion does not arise from biliary cells. Further support for this contention is evident in the fact that cell proliferation indices in bile duct epithelium were only slightly increased in our study, while labeling indices in oval cells and intestinal crypt-like ducts were dramatically increased compared to those of bile duct epithelium. In fact, a correlation analysis failed to demonstrate any relationship between the labeling indices of the cells lining bile ducts, the proliferating oval cell populations, or the cells lining the intestinal crypt-like ducts.

Some authors report significant differences between the cholangiofibrotic lesions and the lesions arising from oval cell proliferation. Tatamatsu et al. reported that the columnar cells in early intestinal metaplastic ducts, induced by 2-AAF, reacted negatively to γ-glutamyltranspeptidase (GGT) staining, while proliferating oval cells in the same study stained positive (23). Elmore and Sirica demonstrated a strong positive GGT staining in proliferating bile ductule-like structures induced in rat liver by furan but metaplastic intestinal glands in the same livers had heterogeneous GGT staining (26). Another report notes a complete lack of a-fetoprotein expression in cholangiofibrotic foci, as well as the presence of well-developed basement membranes enriched in laminin (24). This finding seems to conflict with the results of others, which note that hepatocarcinogen-induced oval cell proliferation is characterized by heterogeneous expression of a-fetoprotein and a deficiency of basement membrane laminin (37–39).

In the present study and in rats treated with furan, the lesion was more severe and almost limited to the right and caudate lobes of the liver (24–28). In rats treated with furan, the lesion was more severe on the surfaces of the right and caudate lobes that were in direct contact with the stomach (25,27). In those studies, furan was administered by gavage, and those researchers hypothesized that the distribution of the lesion might be attributable to enhanced exposure via diffusion through the stomach wall or to increased exposure of those lobes from streamline flow in the portal circulation (25–28). In the present study, intestinal crypt-like ducts with periductular fibrosis were also more severe in the right and caudate lobes; however, the chloroform treatments were administered by inhalation, precluding the possibility that diffusion across the stomach wall was responsible. It is also very difficult to explain this distribution pattern in this study with the streamline blood flow theory. In this study, this distribution pattern may be due to an inherent enhanced susceptibility of those hepatic lobes to the toxic effects of chloroform. This mechanism for this phenomenon has also been suggested for the furan-induced lesion by Sirica and Elmore (26).

Many reports have noted a similarity between the cells lining the adenofibrotic ducts and intestinal mucosal epithelial

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**Table III. Labeling indices in bile ducts as well as in proliferating oval cell populations and intestinal crypt-like duct epithelial cells***

<table>
<thead>
<tr>
<th>Exposure groups</th>
<th>Bile ducts</th>
<th>Proliferating oval cells</th>
<th>Intestinal crypt-like ducts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 p.p.m.</td>
<td>300 p.p.m.</td>
<td>0 p.p.m.</td>
</tr>
<tr>
<td>Males treated for 6 weeks</td>
<td>1.9±1.1⁵</td>
<td>3.0±1.4</td>
<td>NRI</td>
</tr>
<tr>
<td>Males treated for 13 weeks</td>
<td>1.8±1.0</td>
<td>3±1.9</td>
<td>NRI</td>
</tr>
<tr>
<td>Females treated for 13 weeks</td>
<td>2.5±0.7</td>
<td>4.9±1.3</td>
<td>NRI</td>
</tr>
</tbody>
</table>

NRI, these cells were not readily identifiable in the sections examined; LNP, these lesions were not present in the sections examined.

*The preparation and staining of the tissues for BrdU immunohistochemical evaluation was done as described in (24).

⁵Mean ± SD.
cells (24, 26, 28). In those reports, lesions referred to as cholangiofibrosis were examined histologically and ultrastructurally, and the epithelium lining the cholangiofibrotic ducts was described as being mostly columnar epithelium with well-developed brush borders, less numerous mucin-secreting goblet cells, and occasional neuroendocrine cells. In addition, Elmore and Sirica observed that the metaplastic intestinal glands stained positively for mucin, which was primarily localized to the goblet cells, and to the inspissated material in the lumens of the lesions (24). Histologically, the cells lining the intestinal crypt-like ducts observed in this study are clearly columnar cells admixed with goblet cells, and the ducts bear the same histochemical similarities to intestinal crypts as reported with furan.

The abundant fibroplasia that develops in this lesion is probably a secondary response to the development of the intestinal crypt-like ducts. Tatamatsu et al. described intestinal metaplastic ducts induced in rats exposed to dietary 2-AAF and partially hepatectomized that also developed significant fibroplasia (23). They showed that the development of the fibroplasia followed the appearance of the intestinal metaplastic ducts by at least a 2-week interval. The positive reaction to TGF-β on the part of some of the epithelial cells lining the ductular structures may coincide with an attempt to reduce the rate of proliferation in these cells. TGF-β has been reported to stimulate collagen deposition (40), and its overexpression in the liver may have resulted in the excessive fibrosis.

The lack of TGF-α immunoreactivity in the intestinal crypt-like ducts was similar to that reported in furan-induced lesions (28). However, the intensely positive reaction with TGF-α in the proliferating biliary ducts suggests that this growth factor may be involved in biliary duct hyperplasia but not intestinal crypt-like duct proliferation. Since TGF-α is known to be mitogenic for hepatocytes (40), TGF-α staining and hepatocyte proliferation might have been predicted to correlate. Instead, TGF-α staining decreased, while hepatocyte proliferation increased.

Increased staining in bile canaliculi for TGF-α was seen at all time points in the 300 ppm exposure groups. This is a novel finding in that bile canicular staining with TGF-α has not been reported in hepatocytes other than in altered foci. Tanno and Ogawa described bile canicular staining with TGF-α in hyperplastic nodules induced in livers by the Solt–Farber regimen (41). Other researchers have also reported membranous staining in hepatocytes with TGF-α; however, they did not report a bile canicular orientation for this staining (42,43). A consensus of these reports suggests that the membranous immunoreactivity to TGF-α probably represents staining of pro-TGF-α molecules. Another plausible explanation for this unique staining pattern includes increased expression of epidermal growth factor receptor and subsequent binding of TGF-α (40,41); however, we have no data to support or refute any of these hypotheses.

Many similarities exist between the lesion reported herein and that reported with furan. In the furan studies, cholangiofibrosis was noted in animals treated with 30 mg/kg/day for 13 weeks; and 100% of the animals in that study, necropsied at 6, 12 and 18 months after termination of treatment, developed cholangiocarcinomas (25). The development of the lesions in the present study was not followed beyond 13 weeks, therefore the possibility that these lesions would have behaved similarly is uncertain. The likelihood seems great that they would have progressed in the same manner in that Reuber reported cholangiocarcinoma associated with chloroform given by corn oil gavage (12). The fact that furan and chloroform are chemically dissimilar but are both considered non-genotoxic hepatotoxicants suggests that this lesion may represent a common response to growth control signals associated with necrosis and a state of continual regenerative cell proliferation and circulating growth factors.

The lesion reported herein is clearly different from the one reported by Sirica et al. in a study in which rats were subjected to bile duct ligation followed by exposure to furan (36). That regimen resulted in the formation of well-differentiated bile ductules surrounded by a thin fibrovascular stroma which occurred throughout all lobes of the liver. The lesion in this study has a very different morphologic appearance. In one study, Elmore and Sirica reported a very high incidence of intestinal-type adenocarcinomas in rats treated with furan and the occurrence of one cholangiocarcinoma which was readily distinguishable morphologically as well as histochemically (28). They also noted that this distinctive cholangiocarcinoma was the only neoplasm in the study which was found to have metastasized.

In this study, the lesions were more numerous and severe in the female rats at 13 weeks, but they arose earlier in the males. This was evident in that early versions of the lesion were observed in males as early as 3 weeks but not in females at the same time point; and that well-developed lesions were found in one male as early as 6 weeks. The reason for this apparent paradoxical development of the lesion between sexes is unclear.

In conclusion, the lesion reported herein occurred in rats exposed to chloroform via inhalation only at overtly necrogenic exposure concentrations. This lesion most probably arises from some population of cells, possibly the oval cell, and not from bile ducts. In addition, the lesion is only seen in association with extensive hepatocyte toxicity and regenerative proliferation. We therefore suggest that the term ‘cholangiofibrosis’ should be reserved for biliary duct proliferations with fibrosis and that, until a specific cell of origin is confirmed, a descriptive term be used to distinguish this lesion from those actually developing from bile ducts.

References
