Effects of Chloroform and Bromodichloromethane on DNA Synthesis in Male F344 Rat Kidney

by Michael M. Lipsky, Mary Skinner, and Christine O’Connell

We have been investigating the actions of chloroform (CHCl₃) and bromodichloromethane (BDCM) in rat kidney after different routes of exposure. Male F344 rats were exposed by gavage with corn oil or water as the diluting vehicle. All experiments lasted 30 days with gavage exposures 5 days per week for 4 weeks (20 doses). All animals were injected IP with bromodeoxyuridine (BrdU) 3 times over a 6-day period at 50 mg/kg/injection. Kidney tissue was fixed and slides were stained with hematoxylin and eosin for routine viewing and by the PAP (peroxidase-antiperoxidase) technique using anti-BrdU to label cells in DNA synthesis. There were no significant changes in gross parameters evaluated between the control rats and the rats exposed to CHCl₃ or BDCM. Rats exposed via corn oil gavage to CHCl₃ displayed a segment-specific epithelial necrosis (6/6 high dose, 2/6 low dose). The lesions were primarily localized to the second segment of the proximal tubule, although some spread to cells in the first segment was occasionally observed. No histologic lesions were observed in the kidneys of rats exposed to BDCM. Preliminary results indicate a significant increase in DNA synthesis in the CHCl₃-treated rats and a slight increase in DNA synthesis in BDCM-treated rats with corn oil as the diluent. The increase in BrdU labeling was primarily in cells of the S2 segment of the proximal tubule and interstitial cells of CHCl₃-exposed animals and in cells of the S3 segment of BDCM-exposed animals. The rats exposed using water as the vehicle did not show significant pathology (except for one rat in the high-dose CHCl₃ group, which had mild necrosis of proximal tubule epithelium). There were no changes in DNA synthesis in the CHCl₃-treated rat kidneys when water was used as the vehicle. There was a slight increase in total DNA synthesis in the BDCM-treated rats, and the increase was mainly in the third segment of the proximal tubule. These data indicate differences in histopathologic alterations and levels of DNA synthesis that appear to depend on the vehicle that the chemicals were dissolved in and/or the route of administration. CHCl₃-induced pathologic alterations were more severe in the animals exposed by corn oil gavage. BDCM, an established renal carcinogen in rats, failed to induce any histopathologic alterations under the different experimental conditions of this study.

Introduction

Chloroform (CHCl₃) and bromodichloromethane (BDCM) are trihalomethanes that are commonly detected at low levels in public water supplies (1-3). CHCl₃ induces hepatic and renal toxicity in mice and renal toxicity in male rats (4-9). BDCM induces renal toxicity in rats and hepatic toxicity in mice (10-12). Both chemicals have been shown to be metabolized by cytochrome P450, although the active metabolite has only been identified for CHCl₃ (13-15). BDCM (but not CHCl₃) has been demonstrated to be mutagenic (16). CHCl₃ produces site-specific degeneration and necrosis of epithelial cells in the proximal tubules of rodent kidneys (17-19), although high doses may also induce damage in the distal nephron. BDCM administered by intragastric gavage at a dose of 10 mg/kg for 10 days did not induce any histopathologic changes in the kidneys of male F344 rats (10). Both CHCl₃ and BDCM were shown to be tumorogenic to male rat kidney (4, 11, 20, 21). CHCl₃ was also carcinogenic to male and female...
mouse liver (20,21), and BDCM was carcinogenic in female rat kidney, male and female rat colon/rectum, male mouse kidney, and female mouse liver (16). Little information is available on the effects of either chemical on DNA synthesis in the kidney. However, because CHCl₃ is nonmutagenic and nephrotoxic, one may speculate that compensatory cell replication due to renal cell necrosis may play a role in its carcinogenicity. The effect of BDCM on renal DNA synthesis has not been described. The purpose of the present study was to evaluate the effects of subchronic gavage administration of CHCl₃ and BDCM on DNA synthesis in male rat kidneys at doses that were carcinogenic in chronic bioassays. We also compared the effects of each chemical when administered by corn oil gavage and water gavage.

Materials and Methods

Animals

Male F344 rats (150-200) were purchased from Charles River Laboratories (Wilmington, MA). They were singly housed in polycarbonate cages with food and water available ad libitum. Temperature and humidity were within recommended ranges for the species, with 12 air changes/hr and a 12-hr light cycle. The animals were observed once a day for signs of morbidity. Before entry in the study, the animals were quarantined for 2 weeks.

The results reported here are a subset of a larger study. This work was performed as two experiments. For both experiments, four groups, each consisting of six rats, were evaluated for histopathologic alterations. A minimum of three rats from each group were also used to quantify DNA synthesis (as determined by labeling with bromodeoxyuridine (BrdU)) All animals were anesthetized with sodium pentobarbital (50 mg/kg, IP), the kidneys were rapidly removed and sectioned into 1-mm thick sections for fixation. The animals were killed by exsanguination while anesthetized.

Chemicals and Dosing

CHCl₃ and BDCM were purchased from Aldrich Chemical Company, Milwaukee, WI. In the first experiment, CHCl₃ (180 and 90 mg/kg) or BDCM (100 mg/kg) was administered by gavage in corn oil 5 days/week for 4 weeks. In the second experiment, both chemicals were administered by gavage in water for the same time and at the same doses as in the first experiment. Control animals for each experiment were gavaged with the appropriate solvent (equal volume). The last dose in each experiment was administered the day before sacrifice. BrdU (50 mg/kg) was administered by IP injection every other day over a 6-day period, the last dose being given the day before sacrifice.

Pathology and BrdU Labeling Data

A limited necropsy was performed on each animal with emphasis on the kidneys and liver. The kidneys were removed, weighed, cut in cross-section into 1- to 2-mm thick slices, and fixed in 4% phosphate-buffered formaldehde. Two sections from the same area of the left and right kidneys from each animal were mounted in the same paraffin block for sectioning. Sequential sections were taken for routine hematoxylin and eosin staining and for immunohistochemical staining using the peroxidase–antiperoxidase method with a monoclonal antibody to BrdU. For each experiment, all sections were stained at the same time for BrdU according to methods described by Reimschuessel et al. (22).

All sections were viewed under high power, and consecutive random fields from the cortex and the outer stripe of the outer medulla were reviewed. Labeled and unlabeled nuclei were counted in the following nephron segments: glomerulus, interstitium, distal tubule, collecting duct, the third segment (S3) of the proximal tubule, and the combined first and second segments (S1, S2) of the proximal tubule. Data are expressed as the percentage of total nuclei that stained for BrdU incorporation in each segment.

Results

Pathology

Animals exposed to CHCl₃ by corn oil gavage displayed acute cell injury and necrosis, primarily in the epithelial cells lining the S2 segment of the proximal tubule. Although the extent of the injury made it difficult to determine, it appeared that some necrosis also occurred in the cells of the S1 segment. The injury was present in six of six animals exposed to the high dose of CHCl₃ and in two of six animals exposed to the low dose of CHCl₃. The morphological appearance of the S3 segment of the proximal tubule and the other nephron segments was unaffected by CHCl₃ exposure. Animals exposed to BDCM by corn oil gavage did not display any significant renal pathology related to chemical treatment.

Animals exposed to CHCl₃ with water as the vehicle showed minimal pathologic alterations in the kidneys. There was mild injury and necrosis in cells of the S2 segment in one of six animals in the high-dose group. No lesions were seen in the animals in the low-dose group. Animals exposed to BDCM with water as the vehicle did not display any pathological alterations in the kidneys.

DNA Labeling

Table 1 summarizes the DNA labeling data for animals exposed by corn oil gavage. There was a dose-dependent increase in total labeling of nuclei in renal cells of the CHCl₃-treated animals compared to the
control animals. The BDCM-treated animals had a slight increase in labeled cells. In the CHCl₃-treated animals, the largest increase in labeling was in the cells of the S2 segment of the proximal tubule (43.5% and 19.9% compared to 2.5% for the control animals). There was also an increase in labeling in the interstitial cells. The 90 mg/kg CHCl₃-treated animals also had an increase in labeled cells in the S3 segment of the proximal tubule, but this was not seen in the high-dose animals. The BDCM-treated animals had an increase in labeling primarily in the cells of the S3 segment of the proximal tubule and a mild increase in labeling in the interstitial cells.

In the animals exposed by the water gavage (Table 2), there was little to no change in labeling of renal cells by the BrdU method. Small increases were seen in the labeling of cells in the S3 segment of the proximal tubule, collecting duct, and the interstitial cells in animals exposed to BDCM. In general, the control animals in the water gavage experiment had lower levels of DNA labeling compared to the control animals in the corn oil gavage experiment.

### Discussion

CHCl₃ and BDCM induced renal adenomas and carcinomas when tested in chronic rodent bioassays (4, 16, 20, 21). Although BDCM is mutagenic (16), CHCl₃ shows no mutagenic potential. CHCl₃ is nephrotoxic in rats and mice, although the extent of toxicity varies among different strains and sexes of animals (6–8). CHCl₃ induces renal neoplasms in male rats; however, it exhibits only hepatocarcinogenicity in mice (4, 20, 21).

The mechanism of toxic action of CHCl₃ has been correlated to its metabolism by cytochrome P450-dependent mixed-function oxidases (6,8). Inhibitors of mixed-function oxidase activity decreased the nephrotoxicity of CHCl₃ (7). CHCl₃ was carcinogenic to rat kidney when administered by corn oil gavage or in the drinking water. In the present experiments, CHCl₃ displayed potent nephrotoxic effects when given by corn oil gavage, but not when given by water gavage. Only one of six animals displayed any morphological evidence of toxicity. In all cases the cell degeneration and necrosis was focused in the cells of the S2 segment of the proximal tubule, with no toxicity in the S3 segment, distal tubules, or collecting ducts. Increases in DNA synthesis (as determined by immunostaining for BrdU incorporation) closely paralleled the pattern of toxicity, with increased labeling primarily in cells of the S2 segment of the proximal tubule. In addition, increases in DNA synthesis were evident only in animals exposed by corn oil gavage, with only minimal increases in labeling noted in animals exposed by water gavage. In the Fischer rats used in this study, it seems that the vehicle for dilution of CHCl₃ is directly related to the potential for nephrotoxicity. This was not the case with Osborne-Mendel rats, in which the production of renal neoplasms occurred when CHCl₃ was administered by corn oil gavage or in the drinking water (4,23). Tumasonis et al. (12), however, also found no significant increase in renal lesions in Wistar rats chronically exposed in the drinking water for up to 180 weeks. Strain differences may play a large role in the sensitivity to the toxic and carcinogenic potential of this trihalomethane.

BDCM is a classic carcinogen with mutagenic potential (16). The effect of corn oil and water gavage exposure on DNA synthesis was similar. A mild increase in DNA synthesis was observed in both experiments. The increase was centered in the S3 segment of the proximal tubule. The increase in DNA labeling in the S3 segment of the proximal tubule by BDCM is consistent with the site of origin of some chemically induced

### Table 1. DNA labeling—corn oil gavage.*

| Nephron segment | % Cells with labeled nuclei |
|-----------------|----------------------------|
|                 | Control/CHCl₃, 180mg/kg    | CHCl₃, 90mg/kg | BDCM, 100mg/kg |
| Glomerulus      | 2.1 (0.95)                 | 1.5 (0.8)    | 1.1 (0.2)      | 3.2 (0.25)    |
| P1 and P2       | 2.5 (0.6)                  | 43.5 (10.8)  | 19.9 (9.7)     | 3.1 (0.8)     |
| P3              | 3.0 (0.8)                  | 3.8 (1.1)    | 5.2 (0.9)      | 5.0 (0.98)    |
| Distal tubule   | 1.7 (0.3)                  | 1.9 (0.7)    | 1.1 (0.26)     | 1.8 (0.32)    |
| Collecting duct | 1.0 (0.06)                 | 0.9 (1.6)    | 0.7 (2.1)      | 1.1 (0.9)     |
| Interstitium    | 1.37 (0.3)                 | 5.8 (0.7)    | 4.9 (0.36)     | 4.2 (0.32)    |

### Table 2. DNA labeling—water gavage.*

| Nephron segment | % Cells with labeled nuclei |
|-----------------|----------------------------|
|                 | Control/CHCl₃, 180mg/kg    | CHCl₃, 90mg/kg | BDCM, 100mg/kg |
| Glomerulus      | 0.73 (0.45)                | 1.0 (0.47)    | 1.1 (0.57)     |
| P1 and P2       | 1.2 (0.73)                 | 1.0 (0.12)    | 1.0 (0)        |
| P3              | 2.0 (0.8)                  | 2.4 (2.3)     | 2.85 (0.2)     |
| Distal tubule   | 0.6 (0.1)                  | 0.73 (0.06)   | 0.85 (0.6)     |
| Collecting duct | 0.5 (0.3)                  | 0.4 (0)       | 1.0 (0.28)     |
| Interstitium    | 1.5 (0.73)                 | 1.2 (0.59)    | 2.0 (1.1)      |

Abbreviations: CHCl₃, chloroform; BDCM, bromodichloromethane.

*Chemical concentrations are in milligrams/kilogram body weight. Values are the means of three animals in each group. Approximately 1000–1800 cells were counted in each nephron segment from each animal. Numbers in parentheses are population SDs of the means.
renal carcinomas that we have previously proposed (24,25). It is interesting to note that the level of DNA labeling in the corn oil gavage was generally higher than in the water gavage. This was noted for the BDCM-treated animals as well as for the control animals. The additional studies under way in our laboratory will help to determine if this is, in fact, the case or if it was just an anomaly in these reported experiments.

This research was supported by a grant from the American Water Works Association Research Foundation.

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