主論文の要旨

Exposure of mice to 1, 2-dichloropropane induces CYP450-dependent proliferation and apoptosis of cholangiocytes

（1，2−ジクロロプロパン曝露はマウスにおいてCYP450依存的に胆管細胞の増殖とアポトーシスを誘導する）

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Introduction

1, 2-Dichloropropane (1, 2-DCP) has been used as a paint remover in the industry. The International Agency for Research on Cancer reclassified this compound recently to group 1 (carcinogenic to humans) based on epidemiological studies of cholangiocarcinoma among offset-color proof-printing workers exposed to 1, 2-DCP in Japan. Two-year rodent carcinogenicity bioassays demonstrated that 1, 2-DCP induced tumors in liver and lung, but not in bile duct. The present study was designed to determine the effects of 1, 2-DCP on the proliferation and apoptosis of cholangiocytes in mice. Using 1-aminobenzotriazole (1-ABT), a nonselective CYP450 inhibitor, the study also investigated the role of CYP 450 in the observed effects of 1, 2-DCP on cholangiocytes.

Materials and methods

Forty-two 10-week-old male C57BL/6JJcl mice were randomly allocated into seven groups of 6 mice each. Mice of four groups were injected subcutaneously with 1-ABT at 50 mg/kg body weight in normal saline twice a day at 9:00 AM and 7:00 PM every day from 3 days before the start of the 4-week exposure to 1, 2-DCP at 0, 50, 250 and 1250 ppm until the end of 4-week exposure. Those of the other three groups were injected with normal saline at 5 ml/kg body weight during the same time course as mice cotreated with 1-ABT, and exposed to 1, 2-DCP at 0, 50 and 250 ppm. Mice were exposed to 1, 2-DCP by an inhalation exposure system for 8 hour/day from 10:00 AM to 6:00 PM, 7 day/week, for 4 weeks. Within 15-23 hours after the end of the 4-week exposure, mice were weighed and euthanized using an overdose of pentobarbital.

Plasma biochemical tests were outsourced to SRL Inc. (Tokyo). Microsomal CYP2E1 activity was measured with HPLC analysis system and cytosolic GST activity was measured with the GST Activity Assay Kit with liver and lung samples. Paraffin-embedded tissue sections were processed for hematoxylin & eosin or immunohistochemical staining.

The proliferation of cholangiocytes and hepatocytes was evaluated by immunohistochemical staining for BrdU and Ki-67. The TUNEL assay was conducted to detect apoptotic cholangiocytes and hepatocytes. The expression of caspase-3 and γ-H2AX in liver was detected by western blot.

Results

In 1-ABT-treated mice, the body weight of mice exposed to 1, 2-DCP at 1250 ppm was significantly lower than the control mice at the end of the 4-week experiment. Liver weight increased significantly after exposure to 1, 2-DCP at 250 ppm in 1-ABT-untreated mice. Treatment with 1-ABT significantly increased liver weight of mice unexposed to 1, 2-DCP (Table 1).

In 1-ABT-untreated mice, alanine transferase and total bilirubin increased

significantly following exposure to 1, 2-DCP at 250 ppm. In 1-ABT-treated mice, aspartate aminotransferase and total bilirubin were significantly high after exposure to 1, 2-DCP at 1250 ppm (Table 2).

In the liver of 1-ABT-untreated mice, CYP2E1 activity increased dose-dependently with 1, 2-DCP, with a significant change at 250 ppm. In mice cotreated with 1-ABT, CYP2E1 activity did not change following exposure to 1, 2-DCP at 0, 50 and 250 ppm, though it significantly increased at 1250 ppm. Cotreatment with 1-ABT decreased CYP2E1 activity in mice exposed to 1, 2-DCP at 50 and 250 ppm, compared with mice untreated with 1-ABT and exposed to 1, 2-DCP at the same concentrations. In mice untreated with 1-ABT, GST activity also increased dose-dependently with 1, 2-DCP, with a significant change at 250 ppm. It is noteworthy that GST activity did not change following 1, 2-DCP exposure in 1-ABT-treated mice, although it increased at 0 and 50 ppm, compared with mice untreated with 1-ABT and exposed to 1, 2-DCP at the same concentrations, respectively. In the lung, 1, 2-DCP exposure neither changed CYP2E1 nor GST activity, regardless of treatment with 1-ABT (Tables 3 and 4 and Figure 1).

Focal necrosis with hemorrhage and inflammatory cell infiltration was observed in the liver parenchyma of two mice of the 1-ABT-untreated mice that were exposed to 1, 2-DCP at 50 ppm and three mice exposed to 250 ppm. In the 1-ABT-cotreated mice, focal necrosis was observed in one mouse exposed to 1, 2-DCP at 250 ppm and three mice at 1250 ppm, but was not observed at 50 ppm. Hepatocyte fatty changes were observed in 1-ABT treated mice, except for the mice exposed to 1, 2-DCP at 1250 ppm. The incidence of hyperplasia in intrahepatic bile duct correlated linearly with 1, 2-DCP exposure dose in mice untreated with 1-ABT (Table 5 and Figure 2).

In 1-ABT-untreated mice, the BrdU and Ki-67 indices of cholangiocytes increased significantly with the concentration of 1, 2-DCP. However, there were no significant differences in 1-ABT-cotreated mice exposed to 1, 2-DCP compared with the control. In addition, 1-ABT reduced the proliferation of cholangiocytes in mice exposed to 1, 2-DCP at 250 ppm (Figure 3). There were no significant differences in the density of BrdU-positive hepatocytes among the different groups. In 1-ABT-untreated mice, the density of Ki-67-positive hepatocytes was significantly higher in animals exposed to 1, 2-DCP at 250 ppm. Cotreatment with 1-ABT significantly decreased the density of Ki-67-positive hepatocytes in mice exposed to 1, 2-DCP at 250 ppm (Figure 4).

The TUNEL index of cholangiocytes tended to increase with the concentration of 1, 2-DCP; in particular, the difference between 1250 ppm 1, 2-DCP exposed mice and control mice treated with 1-ABT was significant. Intriguingly, cotreatment with 1-ABT reduced cholangiocyte apoptosis in mice exposed to 1, 2-DCP at 0 and 250 ppm (Figure 5). There were no significant differences in the density of TUNEL-positive hepatocytes between 1, 2-DCP exposed mice and control mice irrespective of 1-ABT treatment. Western blot

results did not show any change in the protein expression of Caspase-3 in the liver in animals exposed to 1, 2-DCP, regardless of 1-ABT treatment (Figure 6).

Discussion

Exposure of C57BL/6J mice to 1, 2-DCP induced 1) proliferation and apoptosis of cholangiocytes accompanied by bile duct hyperplasia, 2) proliferation of hepatocytes. Administration of 1-ABT significantly inhibited CYP2E1 activity in the liver and attenuated the effect of 1, 2-DCP on proliferation of cholangiocytes and hepatocytes and apoptosis of cholangiocytes. The CYP450-mediated oxidative metabolism of 1, 2-DCP was suggested to be responsible for not only proliferation but also apoptosis of cholangiocytes, which might be involved in the carcinogenic process.

Treatment with 1-ABT effectively reduced CYP2E1 activity in both the liver and lung, but it increased the GST activity in the liver. Considering the amelioration of the impact of the hepatobiliary system by 1-ABT, the results highlight the important role of CYP450 in the bioactivation and toxicity of 1, 2-DCP.

Cholangiocytes are normally mitotically quiescent, but proliferate in response to specific pathological stimuli or toxins, leading to biliary diseases associated with dysregulation of the balance between cholangiocyte proliferation and apoptosis. In the present study, less apoptotic cells were found in 1-ABT-treated mice that were not exposed to 1, 2-DCP, suggesting that cellular CYP450 might maintain homeostasis and background apoptosis. CYP450 levels might affect various signal transduction pathways that alter the cell cycle, causing apoptosis or aberrant cell growth and thereby tumorigenesis.

Conclusion

We demonstrated that the hepatobiliary toxic effects of 1, 2-DCP appear within 4 weeks of exposure and that these effects are mediated through CYP450 in mice. 1, 2-DCP also triggered cholangiocyte proliferation and apoptosis, which are considered the underlying mechanisms of carcinogenicity.