Light and electron microscopic study of the liver in paraquat poisoning

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ABSTRACT — Intrahepatic cholestasis in paraquat poisoning in man has been thought to be secondary to extensive bile duct injuries, though its exact mechanism remains unsettled. We have examined liver biopsy specimens from two cases of paraquat poisoning. Case 1 (fatal) presented severe intrahepatic jaundice, and liver biopsy showed centrilobular cholestasis with extensive bile duct loss. Ultrastructurally, dilation of bile canaliculi with decrease of microvilli and thickening of pericanalicular ectoplasm was found in the hepatocytes. Case 2 (alive) showed mild liver dysfunction without jaundice. While liver biopsy showed nonspecific reactive changes with intact bile ducts and ductules, electron microscopy disclosed dilation of bile canaliculi with decrease of microvilli and thickening of pericanalicular ectoplasm in the hepatocytes, suggesting that damage to the bile secretory apparatus in the hepatocytes develops irrespective of extensive bile duct loss. These findings suggest that bile secretory apparatus in the hepatocytes as well as biliary epithelial cells could be a target of paraquat or its metabolites.

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Paraquat (1,1'-dimethyl-4,4'-dipyridilium dichloride), a herbicide widely used throughout the world, is known as an epithelial toxin and causes injuries to the lungs, kidneys, liver, heart, adrenals and skin (1-5). It has been suggested that paraquat frequently produces cytotoxic and cholestatic changes (2-7), although its exact hepatotoxic mechanism remains unknown. The aim of this study was to examine the pathology of the liver, by light and electron microscopy, with emphasis on the bile secretory pathway, in two cases of paraquat poisoning.

Case report

Case 1 (fatal)

A 54-year-old farmer was admitted to the Takaoka City Hospital on March 5, 1985, because of anorexia and oliguria of 3 days’ duration. There was no past history of hepatic diseases. On physical examination, he was found to be very ill, and severe icterus and oral ulcerations were noted. The liver, which was elastic, firm and smooth, was palpable three finger breadths below the right costal margin. The main laboratory data were as follows: total bilirubin, 11.9 mg/dl with a direct of 10.4 mg/dl; SGOT, 108 IU/l; SGPT, 5 IU/l; ALP, 1076 IU/l (<280); y-GTP, 171 IU/l (<30); LAP, 160 IU/l (<71); BUN, 142 mg/dl; prothrombin time, 12.8 s (control <13.3); and PO2, PCO2 and HCO3 of arterial blood, 50 mmHg, 33 mmHg and 18 mEq/l, respectively.

A chest X-ray showed patchy consolidation at the base of both lungs. An abdominal echogram
showed no dilatation of the biliary system. After admission, hemodialysis was performed because of renal failure. On March 6 (4 days after the initial symptoms), a needle biopsy of the liver was performed for evaluation of hepatic dysfunction. Three days later, paraquat was detected in the
urine. He died of respiratory failure 4 days after admission.

Liver biopsy showed well-preserved lobular pattern and liver cells cords. There was prominent centrilobular bile stasis (Fig. 1). Although there was no hepatocellular drop-out nor fatty change, hepatocytes showed anisocytosis and a number of multinucleated cells and giant mitochondria (Fig. 2). Portal tracts were mildly edematous, and most of interlobular bile ducts and bile ductules were lost (Fig. 2). Ultrastructurally, hepatocytes frequently showed dilatation of bile canaliculi with decrease of microvilli, thickening of pericanalicular ectoplasm and focal blurring of canalicular plasma membrane (Fig. 3), dilatation of the endoplasmic reticulum, phagosomes, vacuoles with myelin figures, and giant mitochondria with dark matrices (Fig. 4). There was widening of the intercellular lateral spaces with development of microvilli and deposition of osmiophilic substances (Fig. 3). Kupffer cells showed many phagosomes. One remaining bile ductule showed coagulation necrosis with loss of nucleus, and the occurrence of apoptotic bodies and autophagosomes (Fig. 5).

**Case 2 (alive)**

A 19-year-old man was admitted to the Takaoka City Hospital on Jan. 5, 1987. Four hours earlier he had ingested about 50 ml paraquat in a suicide attempt. On admission he had no symptoms, but he was agitated. There were several small ulcerations in the mouth. Neither anemia nor jaundice was noted.

The main laboratory data were as follows: white blood cells count, 10,500 /cumm; total bilirubin, 1.4 mg/dl; SGOT, 24 IU/l; SGPT, 10 IU/l; LDH, 928 IU/l; ALP, 137 IU/l; γ-GTP, 9 IU/l; LAP, 55 IU/l; BUN, 16 mg/dl. Arterial blood gas analysis disclosed that pH was 7.52, PO₂ 116 mmHg, PCO₂ 27 mmHg and HCO₃⁻ 22 mEq/dl.

![Fig. 3. One bile canaliculus (curved arrow) shows dilatation with decrease of microvilli and focal blurring of the canalicular membrane. Intercellular lateral spaces are dilated with deposition of osmiophilic substances (straight arrows). Case 1. × 4000.](image-url)
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Fig. 4. Giant mitochondria (M) with crystal-like materials. Arrows show osmiophilic substances in dilated intercellular lateral spaces (arrows). Case 1. × 13000.

respectively. The paraquat concentration in the serum and urine was 1 μg/ml and 9 μg/ml, respectively.

After admission, vigorous gastric and intestinal lavage were carried out, and hemoperfusion was started. Although liver and renal function deteriorated transiently, hemoperfusion was continued up to 24 times for 2 weeks, and the patient finally recovered.

On Feb. 2 (28 days after the ingestion of paraquat), needle biopsy of the liver was performed. At this time, the main laboratory data were as follows: serum bilirubin, 0.5 mg/dl; ALP, 112IU/l; γ-GTP, 38 IU/l; LAP, 59 IU/l, GOT, 19 IU/l, GPT 9 IU/l.

Histologically, the hepatic sinusoids were slightly dilated with mild lymphoid cell infiltration. Neither centrilobular cholestasis nor hepatocellular necrosis was seen. Portal tracts were normal, and interlobular bile ducts and ductules seemed intact. Ultrastructurally, many bile canalicularis showed luminal dilatation with decreased microvilli and thickened pericanalicular ectoplasm (Fig. 6). There were a few giant mitochondria with dark matrices. The hepatocytes showed a few phagosomes and lipofuscin granules. The bile ductal cells showed minimal changes, such as a small number of vacuoles with myelin figures.

Discussion

Several hepatic lesions have been described in paraquat poisoning: congestion, hepatocellular damage, bile ductal injuries, and cholestasis (3–10). Intrahepatic cholestasis is common in paraquat poisoning. For example, Mullick et al. (3) reported that 8 of 13 cases of paraquat poisoning presented cholestasis. As to the ultrastructural features of the liver in paraquat poisoning, there have been only two reports to our knowledge: Borchard et al. (6) revealed a number of nonspecific changes in the hepatocytes such as giant mitochondria with paracrystalline inclusion bodies in two cases. (Mitochondria are considered as the target organelle of paraquat (6, 11, 12)). And Matsumoto et al. (7) briefly described dilatation
Fig. 5. One bile ductular cell (D) shows loss of nuclei and cell organelles. One apoptotic body (long straight arrow) and several vacuoles (curved arrows) are also seen. Short straight arrows denote basement membrane of bile ductule. Case 1. × 3200.

of bile canaliculi with thickening of the canalicular wall and degeneration of microvilli in three autopsy livers.

To our knowledge, there have been no ultrastructural studies on biliary epithelial cells in paraquat poisoning. The fatal case with extensive bile duct loss in this study showed coagulation necrosis, apoptotic bodies and autophagosomes. These changes, which are also known to occur in other biliary diseases (13), may have played an important role in biliary epithelial destruction in this case. The other case, with histologically intact bile ducts, showed a small number of vacuoles in the biliary epithelial cytoplasm.

As to the mechanism of cholestasis in paraquat poisoning, extensive injuries of the intrahepatic biliary tree, called “destructive cholangitis” (3, 4, 7), are hypothesised as being important. Such a mechanism of cholestasis has also been hypothesised in other drug-induced cholestasis, including alpha-naphthylisothiocyanate (14) and 1,4-phenylenediiisothiocyanate (15). In the present study, the fatal case showed prominent centrilobular bile stasis and marked bile duct loss, while there were only nonspecific histologic changes of the liver with intact bile ducts and ductules in the other case without jaundice. These findings seem to support the hypothesis mentioned above.

It is of interest that cholestatic hepatocellular changes were seen not only in the fatal case with extensive bile duct loss and cholestasis, but also in the live case with minimal bile duct injuries. Therefore, the cholestatic hepatocellular damage was not exclusively secondary to extensive bile duct loss. Instead, it seems more likely that paraquat and its metabolites directly damaged the hepatocellular bile secretory apparatus. Lipid peroxidation (16) might cause damage to cell membranes facing bile canaliculi in the hepatocytes, followed by cholestasis in paraquat poisoning, as speculated in cytochalasin B (17) or phalloidin (18) toxicity. The widening of intercellular spaces with the development of microvilli and deposition
Fig. 6. Bile canaliculi (C) are dilated with decrease of microvilli and thickening of pericanalicular ectoplasm. Case 2. × 16,000.

of osmiophilic materials in the fatal case, suggests severe damage to hepatocytes, probably rupture of bile canaliculi with regurgitation of bile. In fact, the canicular membrane was focally blurred in the fatal case.

Biliary epithelial cells might also be similarly damaged by paraquat and its metabolites. Extensive biliary epithelial injuries and bile duct loss might result in intrahepatic biliary obstruction, followed by damage to the hepatocellular bile secretory apparatus, as seen in extrahepatic obstructive jaundice (19).

References


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