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The Protective Effect of Allopurinol on Cholestatic Liver Injury Induced by Bile Duct Ligation

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To determine whether oxygen free radicals are responsible for the pathogenesis of the cholestasis induced by ligation of common bile duct (CBD) variables which reflect the hepatic function in the serum, the amount of superoxide radical production, and xanthine oxidase(XO) activity were studied. The activity of serum alanine aminotransferase, bilirubin level in the serum and the amount of superoxide radical production were lower in a CBD ligation with allopurinol treated group than in a CBD ligation without allopurinol treated group. Abnormalities of the microscopic structures were reduced in a CBD ligation with allopurinol treated group than in a CBD ligation without allopurinol treated group. Allopurinol, an inhibitor of XO, prevented the hepatic damage induced by CBD ligation through the inhibition of XO. These experiments demonstrate that oxygen free radicals are responsible for the pathogenesis of the cholestatic liver.

Key Words : *Allopurinol, Cholestasis, Oxygen free radicals, Xanthine oxidase*

INTRODUCTION

In human beings cholestasis in the liver occurs in various diseases such as late viral hepatitis, carcinoma of the bile duct, gallstones in the bile duct, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alcoholic hepatitis, and certain drugs(Eddleston, 1994). In rats, cholestasis can be induced by common bile duct (CBD) ligation. CBD ligation in rats causes known biochemical and morphological abnormalities in the liver such as inflammation, necrosis, fatty change, biliary hyperplasia, fibrosis, and cirrhosis(Kountouras et al.,1984 ; Chang et al.,1987 ; Kim et al., 1989). However, the mechanism causing these abnormalities in bile duct ligated rats is not clear, although increased biliary pressure, retention of biliary constituents, and impairment of hepatocellular transport have been suggested (Hardison et al., 1983).

Oxygen free radicals which are known to damage intracellular structures and membrane structures in the liver (Reynolds and Moslen, 1980 ; Parks et al., 1983 ; Dashti et al, 1992), have been implicated in a variety of inflammatory disorders (McCord, 1983 ; Parks et al., 1983) including hepatic damage induced by carbon tetrachloride (Parks et al., 1983 ; Dashti et al., 1992). Oxygen free radicals may also be involved in the pathogenesis of the cholestatic liver.

To evaluate the involvement of oxygen radicals in hepatic damage under cholestatic conditions, the activity of xanthine oxidase (EC 1.2.3.2, XO) which is the major source of oxygen radicals(Leibovitz and Siegel 1980), the amount of superoxide radical production, and the levels of variables which are known to reflect hepatic function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST),

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alkaline phosphatase (ALP), bilirubin, total protein, and albumin levels were measured in cholestatic rats after allopurinol injection. Light and electron microscopic studies were also done.

MATERIALS AND METHODS

Animals

Normal male rats of the Sprague-Dawley strain, weighing between 320 and 350 grams, were used in this experiment.

In the sham operated control group rats were sacrificed on the 2nd day after sham operation. In the CBD ligated group rats were sacrificed on the 2nd day after CBD ligation. In allopurinol treated group, according to the method of Yoon et al. (1991) a dose of 50mg of allopurinol per kg of body weight was administered intraperitoneally at the time of CBD ligation and 24 hours after the first administration. Then the rats were sacrificed 24 hours after the second injection with allopurinol.

Rats were anesthetized with ether for surgery and sacrifice, and were fasted prior to sacrifice for 12 hours.

The CBD was exposed through a middle line incision. After double ligation the mid point of the CBD was cut. A sham operation was performed in the same way without CBD ligation.

Chemicals

Allopurinol, sodium deoxycholic acid, xanthine sodium salt, oxidized nicotinamide adenine dinucleotide, trichloroacetic acid, uric acid and bovine albumin were purchased from Sigma (USA). All other chemicals were of the highest commercially available purity.

Cell fractionation

After rats were anesthetized with ether blood was collected from the abdominal aorta, and the liver was perfused through the portal vein with physiologic saline solution. The liver was rinsed in cold saline solution.

The surface was then wiped dry. Cytosol and mitochondria were obtained by centrifugation on density gradient made of sucrose (Kwak and Kwak, 1986).

All procedures were performed at 2 to 4℃.

Enzyme assay

The XO activity was measured with a spectrophotometer(DU 650, Beckman) according to the method of Rowe and Wyngaarden(1966) with xanthine as a substrate. The XO activity was expressed as the amount of uric acid formed per milligram of protein (or per milliliter of serum) per minute. The ALT, AST and ALP activities were measured using kits purchased from Sigma(USA).

Superoxide radical production assay

The amount of superoxide radical production was measured according to the method of Auchlar and Voisin(1984). The amount of superoxide radical production was expressed as the amount of SOD-inhibitable nitro blue tetrazolium reduction.

Statistical analysis

Values were expressed as mean ± SD. Statistical evaluation of the difference between means was performed with Student's t-test. P values of ≤ 0.05 were considered significant.

RESULTS

Serum XO, cytosolic XO, superoxide radical production, serum ALT, serum AST, serum total bilirubin, and serum ALP levels were higher in the CBD ligated group than in the sham operated group (Tables 1 and 2).

Serum XO, cytosolic XO, superoxide radical production, serum ALT, cytosolic AST, and serum total bilirubin levels were lower in the allopurinol treated group than in the CBD ligated group (Tables 1 and 2). However, mitochondrial ALT and AST levels were higher in the allopurinol treated group than in CBD ligated group (Table 2).

In sham operated control group, the hepatocytes were shown well developed rough endoplasmic reticulum, abundant glycogen granules and some mitochondria.

In the CBD ligated group, three of four rats were shown large areas of hepatic necrosis associated with moderate infiltration of inflammatory cells in the portal-periportal regions, degenerative changes of hepatocytes and associated several secondary lyso somes in many hepatocytes (Fig. 1).

In the allopurinol treated group, two of three rats

Table 1. Effect of allopurinol on xanthine oxidase(XO) level and the amount of superoxide radical production

	Sham operated control group	CBD ligated group	Allopurinol treated group
Serum XO	42.65±4.24	58.73±5.90^{c}	23.33±1.95^{c,f}
Cytosolic XO	5.90±0.86	7.74±1.12^{a}	1.56±0.18^{c,f}
Superoxide radical production(nmol NBT reduced *mg* protein^{-1}min^{-1})	15.09±2.77	22.61±1.51^{c}	18.04±3.88

Values are means ± SD with 5 rats in each group. Animal groups are described in text. NBT, nitroblue tetrazolium. Values significantly different from control values(a : P<0.05, c : P<0.001) and from CBDL values (f : P<0.001).

were shown focally small areas of hepatic necrosis associated with some infiltration of inflammatory cells in the portal-periportal regions, prominent dilatation of rough endoplasmic reticulum and some fat droplets in the hepatocytes (Fig. 2).

DISCUSSION

Oxygen free radicals are recognized to play significant roles in inflammatory disorders (McCord, 1983 ; Parks et al., 1983). In our study, inflammatory and necrotic changes were shown after CBD ligation (Fig. 1) with hepatic dysfunction including serum AST, ALT, ALP, and total bilirubin levels (Table 2). Cytosolic XO and the amount of superoxide radical production were higher in the CBD ligated group than in the sham operated group (Table 1). These results suggest that increased levels of variables which reflect the hepatic function in the serum and morphological abnormalities may be caused by the increased amount of superoxide radical production through the activation of XO.

Serum and cytosolic XO activities, and the amount of superoxide radical production in allopurinol treated group were lower than those in the CBD ligated group (Table 1). Liver function test such as serum ALT, AST and bilirubin levels in the allopurinol treated group were lower than those in the CBD ligated group (Table 2). The reduced severity of cellular damage was observed microscopically in the liver treated with allopurinol (Fig. 2). These results indicate that allopurinol prevented damage of the liver by inhibition of oxygen radical production through the inhibition of XO.

Mitochondrial ALT and AST levels in the allopurinol treated group were higher than those in the CBD ligated rats (Table 2). And cytosolic ALT and AST levels in the allopurinol treated group were lower than those in CBD ligated rats (Table 2). It is hard to determine the reason for the results from this experiment, but it is suggested that mitochondrial damage in CBD ligated rats is reduced by allopurinol treatment under the cholestasis thus flow of these enzymes from mitochondria to cytosol was decreased.

According to the results, allopurinol an inhibitor of the XO, prevented the hepatic damage induced by CBD ligation through the inhibition of XO. These experiments demonstrate that oxygen radicals play a role in the aggravation of inflammation and necrosis

Table 2. Effect of allopurinol on valuables which reflect hepatic function

	Sham operated control group	CBD ligated group	Allopurinol treated group
Serum ALT (Karmen unit ml^{-1})	32 ± 10	658 ± 201^{c}	352 ± 145^{b,d}
Serum AST (Karmen unit ml^{-1})	111 ± 28	1,472 ± 242^{c}	1,336 ± 158^{c}
Cytosolic ALT (Karmen unit *mg* protein^{-1})	644 ± 61	566 ± 73	479 ± 72^{b}
Cytosolic AST (Karmen unit *mg* protein^{-1})	680 ± 127	616 ± 87	447 ± 60^{b,e}
Mitochondrial ALT (Karmen unit *mg* protein^{-1})	88 ± 11	79 ± 16	130 ± 18^{b,e}
Mitochondrial AST (Karmen unit *mg* protein^{-1})	215 ± 39	195 ± 27	277 ± 25^{a,e}
Serum total bilirubin (*mg dl*$^{-1}$)	0.28 ± 0.05	10.20 ± 2.10^{c}	6.67 ± 1.90^{c,d}
Serum ALP (μmol phenol *ml*$^{-1}$ min^{-1})	2.61 ± 0.87	10.18 ± 2.06^{c}	9.60 ± 1.13^{c}
Serum total protein (*g dl*$^{-1}$)	8.11 ± 0.40	8.38 ± 0.53	8.27 ± 0.66
Serum albumin (*g dl*$^{-1}$)	3.74 ± 0.29	3.43 ± 0.43	3.61 ± 0.50

Values are means ± SD with 5 rats in each group. Animal groups are described in text. ALT, alanine aminotransferase ; AST, aspartate aminotransferase ; ALP, alkaline phosphatase. Values significantly different from control values (a : P<0.05, b : P<0.01, c : P<0.001) and from CBDL values (d : P<0.05, e : P<0.01).

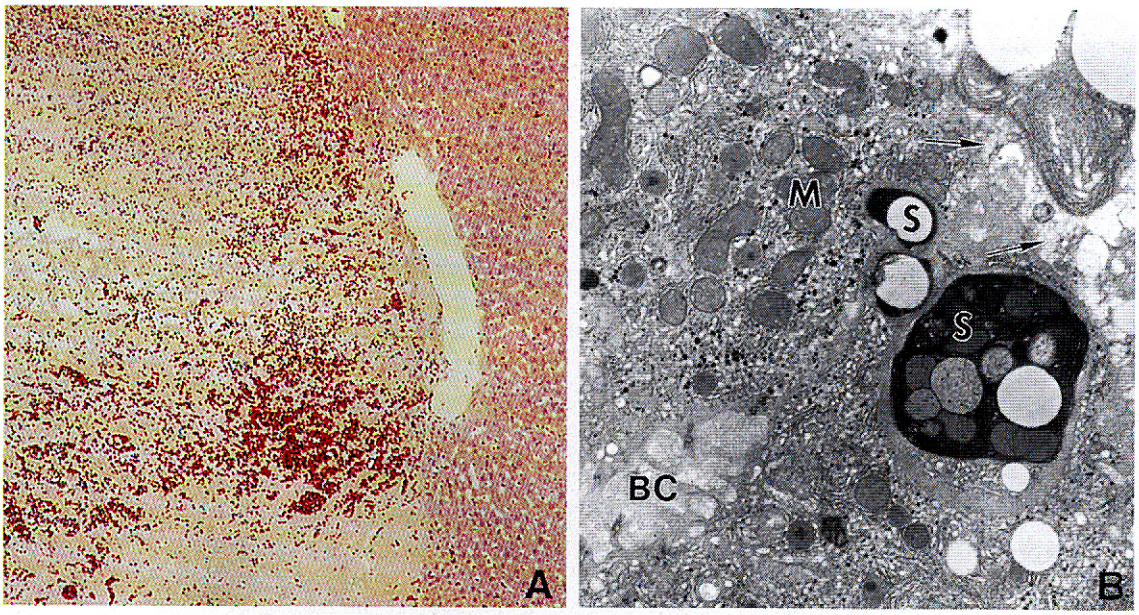

Fig. 1. Histological features of liver from rats with common bile duct ligation. (A) Note large area of hepatic necrosis associated with moderate infiltration of inflammatory cells in the portal-periportal regions (haematoxylin and eosin stain, original magnification×40). (B) Note degenerative changes of hepatocytes and associated several secondary lysosomes in many hepatocytes. BC: bile canaliculus, M: mitochondria, S: secondary lysosome, Arrows: necrotic degeneration of cytoplasm (uranyl acetate and lead citrate double stain, original magnification×10,200)

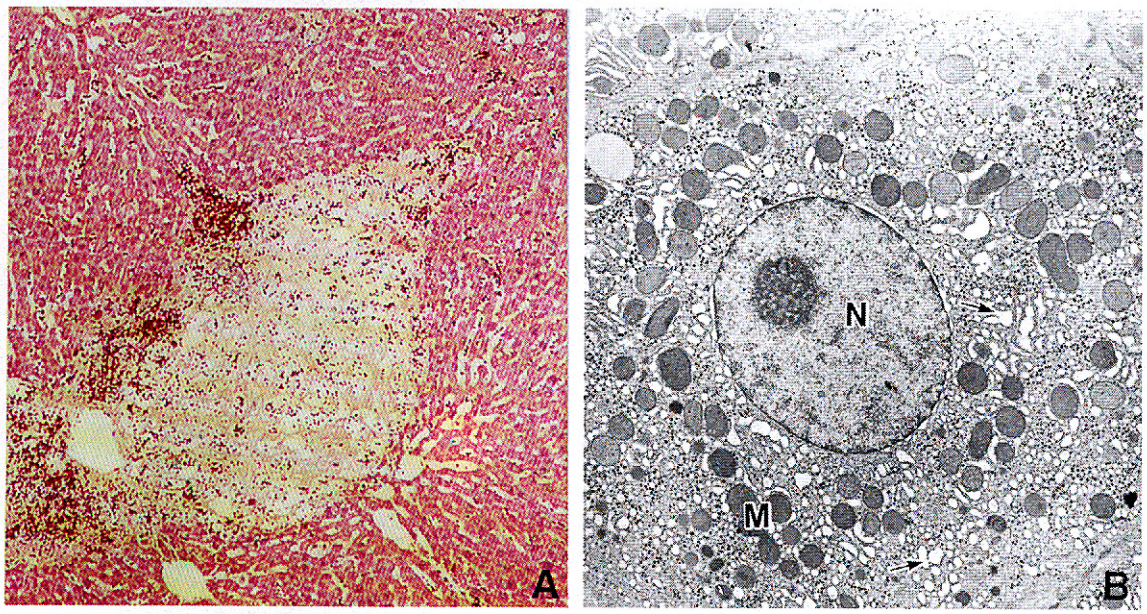

Fig. 2. Histological features of liver from rats treated with allopurinol after common bile duct ligation. (A) Note focally small area of hepatic necrosis associated with some infiltration of inflammatory cells in the portal-periportal regions (haematoxylin and eosin stain, original magnification×40). (B) Prominent dilatation of rough endoplasmic reticulum (RER), and some fat droplets in the hepatocytes are seen. N: nucleus, M: mitochondria, Arrows: endoplasmic reticulum (uranyl acetate and lead citrate double stain, original magnification×6,800)

in the liver under cholestasis induced by CBD ligation. So oxygen free radicals are suggested to be responsible for the pathogenesis of cholestatic liver.

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