Korean Biochem. J. (1994), Vol. 27, No. 4 pp. 346~349

Correlation Between Superoxide Radical Production and Hepatic Damage Induced by Bile Duct Ligation

Kyo-Cheol Mun

Department of Biochemistry, Keimyung University, School of Medicine, Taegu 700-310, Korea
(Received May 17, 1994)

Abstract: The correlation between superoxide radical production and hepatic damage under cholestasis induced by common bile duct (CBD) ligation was studied in rats. The amount of superoxide radical production in the cholestatic liver was significantly increased after CBD ligation compared to a sham operation control group. Serum and cytosolic xanthine oxidase (XO) activities were significantly increased after operation. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, and the bilirubin level of the cholestatic liver in the subject rats increased after CBD ligation. Severe morphological changes were also noted after CBD ligation. These parameters, which are known to reflect hepatic function in the serum, and the XO activity were significantly correlated with the amount of superoxide radical production. According to these results an increased XO activity includes an increased conversion from xanthine dehydrogenase in the liver, resulting in an increase in oxygen free radical production. These oxygen radicals may play a role in the aggrevation of inflammation and necrosis in the liver under cholestasis induced by CBD ligation.

In human beings cholestasis in the liver occurs in various diseases, such as congenital biliary obstruction, tumors of the bile duct, biliary stones, primary biliary cirrhosis, cholangitis, and cholestatic hepatitis (Halsted, 1976). Biliary obstruction in rats causes known biochemical and morphological abnormalities, such as inflammation, necrosis, fatty change, biliary hyperplasia, fibrosis, and cirrhosis (Moritz and Snodgrass, 1972; Chang *et al.*, 1987; Kim *et al.*, 1989). However the mechanism causing these abnormalities 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 have been implicated in a variety of inflammatory disorders (McCorrd, 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 involved in 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), the major source of oxygen radicals (Leibovitz and Siegel 1980), and the levels of parameters which are known to reflect hepatic function, such as ALT, AST, ALP, bilirubin, total protein, and albumin levels, were measured in cholestatic rats induced by common bile duct (CBD) ligation which is one of the most commonly used animal models to study abnormalities associated with hepatic cholestasis. The correlation between the amount of superoxide radical production and the above parameters was also studied.

Materials and Methods

Animals

Normal male Sprague-Dawley rats weighing between 320 and 350 g were used. All animals were maintained on a diet of commercial pellets purchased from Jin Yang Co., Limited.

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.

In the sham operated control group rats were sacrificed on the 2nd day after operation. In the CBD ligated group rats were sacrificed on the 2nd day after ligation.

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

Chemicals

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 and enzyme peparation

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 according to the method described by Kwak and Kwak (1986).

The cytosolic fraction was used as the cytosolic enzyme solution. The mitochondrial fraction was treated with 1% sodium deoxycholic acid containing 1% sodium bicarbonate, and was used as the mitochondrial enzyme solution.

All procedures were performed at 2 to 4℃.

Enzyme assays

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 and AST activities were measured according to the method of Reitman and Frankel (1957). The ALP activity was measured according to Kind and King (1954).

Superoxide radical production assay

The amount of superoxide radical production was measured according to the method of Auchlar and Voisin (1984) with modification according to Kim *et al.* (1993). The amount of superoxide radical production was expressed as the amount of SOD-inhibitable nitro blue tetrazolium reduction.

Determination of protein

Protein concentrations were determined by the biuret method (Gornall *et al.*, 1949) using bovine serum albumin as a reference protein.

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

The amount of superoxide radical production of the cholestatic liver was significantly increased after CBD ligation compared to the sham operated control group (Table 1). Serum and cytosolic XO activities were signif-

Table 1. Superoxide radical production and xnathine oxidase (XO) activity in common bile duct ligated rats

	Sham	CBDL
Superoxide radical production(nmol NBT reduced mg protein^{-1}·min^{-1})	15.09± 2.77	22.61± 1.51***
Serum XO (nmol uric acid ml^{-1}·min^{-1})	42.65± 4.24	58.73± 5.90***
Cytosolic XO (nmol uric acid mg protein^{-1}·min^{-1})	5.90± 0.86	7.74± 1.12*)

Values are means± SD with 5 rats in each group.
Values significantly different from control values(*p<0.05, *** p< 0.001).
Sham; the rats which were sacrificed at the 2nd day after sham operation, CBDL; the rats which were sacrificed at the 2nd day after common bile duct ligation.

Table 2. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activity and bilirubin, serum total protein and serum albumin levels in common bile duct ligated rats

	Sham	CBDL
Serum alnine aminotransferase (Karmen U ml^{-1})	32± 10	658± 201***
Serum aspartate aminotransferase (Karmen U ml^{-1})	111± 28	1,472± 242***
Cytosolic alanine aminotransferase (Karmen U mg protein^{-1})	644± 61	566± 73
Cytosolic aspartate aminotransferase (Karmen U mg protein^{-1})	680± 127	616± 87
Mitochondrial alanine aminotransferase (Karmen U mg protein^{-1})	88± 11	79± 16
Mitochondrial aspartate aminotransferase (Karmen U mg protein^{-1})	215± 39	195± 27
Serum total bilirubin (mg dl^{-1})	0.28± 0.05	10.20± 2.10***
Serum alkaline phosphatase (μmol phenol ml^{-1}·min^{-1})	2.61± 0.87	10.18± 2.06***
Serum total protein (g dl^{-1})	8.11± 0.40	8.38± 0.53
Serum albumin (g dl^{-1})	3.74± 0.29	3.43± 0.43

Values are means± SD with 5 rats in each group.
Values significantly different from control values (***p<0.001).
Other conditions were the same as in Table 1.

Table 3. Correlations between the amount of superoxide radical production and several parameters

Several parameters	Pearson correlation coefficients
Serum xanthine oxidase	0.83***
Serum alanine aminotransferase	0.75**
Serum aspartate aminotmasferase	0.77**
Serum total bilirubin	0.73**
Serum alkaline phosphatase	0.77**
Cytosolic xanthine oxidase	0.54*
Cytosolic alanine aminotransferase	−0.48
Cytosolic aspartate aminotransferase	−0.52
Mitochondrial alanine aminotransferase	0.43
Mitochondrial aspartate aminotransferase	0.24
Serum alkaline phosphatse	0.77**

Values significantly different from control values (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

icantly increased after operation (Table 1).

The serum ALT and AST activities of the cholestatic liver in the rats were increased approximately 10 to 20 fold after CBD ligation (Table 2). However, the hepatic cytosolic ALT and mitochondrial AST activities in the rats were decreased without significance (Table 2). The serum total bilirubin level was significantly increased approximately 35 fold after CBD ligation (Table 2). The serum ALP activity was significantly increased approximately 4 fold after CBD ligation (Table 2). The serum total protein and albumin levels were slightly changed, without significance (Table 2).

The parameters which are known to reflect hepatic function in the serum, and XO activity were significantly correlated with the amount of superoxide radical production (Table 3).

Microscopic study of the CBD ligated liver showed marked numerous and large focal necrosis of liver cells with moderate infiltration of inflammatory cells in the area of necrosis (Fig. 1).

Discussion

In cholestatic liver, serum ALT and AST activities were increased compared to sham operated controls. However, cytosolic and mitochondrial ALT and AST activities were decreased compared to controls. Cytosolic and mitochondrial ALT and AST may flow into the blood through damaged cell membranes caused by cmmon bile duct ligatation.

The serum bilirubin level increased after CBD ligaton, as did the serum ALT and AST activities. A liver function test of the serum revealed hepatic dysfuntion after CBD ligation with morphological change.

Fig. 1. Histological feature of liver from rats with common bile duct ligation. Note marked and large focal necrosis of the liver cells with moderate infiltration of inflammatory cells in the area of necrosis (Arrows) (haematoxylin and eosin stain, original magnification×40).

The amount of superoxide radical production was increased after CBD ligation, and several parameters which reflect hepatic function were significantly correlated with the amount of superoxide radical production. There may be some relation between hepatic dysfunction and the amount of superoxide radical production under cholestasis.

In spite of increased serum XO activity, cytosolic XO activity was increased compared to sham operated controls. The cause of increased serum XO is presumed to be enzyme which flowed into the blood from liver cells through damaged membranes, like ALT and AST. It also seems that cytosolic XO is induced by purine metabolites caused by cell necrosis, as shown in Fig. 1.

In this study the Pearson correlation coefficient was 0.54 ($p<0.05$) between superoxide radical production and cytosolic XO which is the major source of oxygen radicals, paticularly superoxide radicals. It is suggested that increased xanthine oxidase in the liver causes the increased amount of superoxide radical production.

According to these results increased XO activity includes increased conversion from xanthine dehydrogenase in the liver, resulting in an increase in oxygen free radical production. This increased oxygen radical production plays a role in aggrevation of inflammation and necrosis in the liver under cholestasis.

References

Auchlar, C. and Voisin, E. (1984) In CRC *Handbook of Methods for Oxygen Radical Research* pp. 123-132, CRC Press Inc., Boca Raton, Florida.

Chang, D. S., Kwak, J. S. and Sohn, T. J. (1987) *The Kyungpook Univ. Med. J.* **28**, 113-117.

Dashti, H. M., al-Sayer, H., Behbehani, A., Massa, J. and

Christenson, J. T. (1992) *J. R. Coll Surg. Edinb.* **37**, 23-28.

Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) *J. Biol. Chem.* **177**, 751-766.

Halsted, J. A. (1976) *The laboratory in clinical medicine. Interpretation and application* pp. 426-429, London, Sannders Co.

Hardison, W. G. M., Weiner, R. G., Hatoff, D. E. and Miyai, K. (1983) *Hepatology* **3**, 383-390.

Kim, H. S., Park, J. Y., Kim, E. Y., Kwak, K. S., Choi, Y. H. and Chung, J. M. (1989) *The Korean Journal of Internal Medicine* **36**, 459-470.

Kim, M. C., Lee, B. R. and Koh, K. S. (1993) *Korean J. Biochem.* **25**, 1-8.

Kind, P. R. N. and King, E. J. (1954) *J. Clin. Pathol.* **7**, 322-326.

Kwak, C. S. and Kwak, J. S. (1986) *The Keimyung Univ. Med. J.* **5**, 45-53.

Leibovitz, B. E. and Siegel, B. W. (1980) *J. Gerontol.* **35**, 45-56.

McCord, J. M. (1983) *Surgery* **94**, 412-414.

Moritz, M. and Snodgrass, P. J. (1972) *Gastroenterology* **62**, 93-100.

Parks, D. A., Bulkley, G. B. and Granger, D. N. (1983) *Surgery* **94**, 415-422.

Reitman, S. and Frankel, S. (1957) *Am. J. Clin. Path.* **28**, 56-63.

Rowe, P. B. and Wyngaarden, J. B. (1966) *J. Biol. Chem.* **241**, 5571-5576.