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Activities of Hepatic Antioxidant Enzymes in Bile Duct Ligated Rats

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= 국문초록 =

흰쥐 담즙울체간의 항산화 효소들의 활성도

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목적: 담즙울체성 간 손상에서 활성 산소에 대한 항산화 효소들의 방어 기능을 파악하고자 하였다. **대상 및 방법**: 흰쥐에서 총담관을 결찰한 후 xanthine oxidase, superoxide radical 생성량, 항산화 효소인 superoxide dismutase, catalase 및 glutathione peroxidase의 활성도를 측정하였다. **결과**: 총담관 결찰군에서 xanthine oxidase 활성과 이 효소의 반응 최대속도 및 superoxide radical 생성량은 증가하였다. Superoxide dismutase와 glutathione peroxidase의 활성은 의의있는 변화가 없었다. Catalase와 이 효소의 반응 최대속도는 감소하였다. **결론**: 담즙울체성 간 손상이 있을 때는 xanthine oxidase의 합성 증가로 인한 활성 증가로 활성 산소의 발생량이 증가되나 항산화 효소들은 생합성의 감소 등으로 인한 활성 감소로 활성 산소에 대한 방어적인 기능을 다하지 못하는 것으로 생각되며 이로 인해 담즙울체 간에서 활성 산소에 의한 간 손상은 더욱 촉진되리라 생각된다. **(대한소화기학회지; 30:66 - 71)**

색인단어: 활성산소, Catalase, Glutathione peroxidase, Superoxide dismutase, Xanthine oxidase

INTRODUCTION

The partial reduction of molecular oxygen in biologic systems produces the cytotoxic intermediates superoxide, hydrogen peroxide, peroxyl radicals and hydroxyl radical.[1,2] These oxygen free radicals are recognized to play significant roles in the pathogenesis of various disorders of the digestive system such as inflammatory disorders[1,3] and hepatic cirrhosis.[1,4] And the major source of these oxygen radicals appears to be the enzyme xanthine oxidase,[5] present in the largest amount in the liver,6 oxidizes hypoxanthine to xathine and xanthine to uric acid.[7]

Against damage by active oxygens, aerobic cells are protected by several antioxidants[2] include three well-known biological antioxidant enzymes such as

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superoxide dismutase, catalase, and glutathione peroxidase.[2,8,9] However, the role of these enzymes which protect the damage by active oxygens under the cholestasis is not clear.

To evaluate the protective role of three well-known biological antioxidant enzymes against oxygen free radicals under the cholestasis, the activity of xanthine oxidase, the amount of superoxide radical production, and the activities of antioxidant enzymes were measured under the cholestasis induced by common bile duct(CBD) ligation.

SUBJECT AND METHOD

1. Animals

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

All the experimental groups, with 5 rats in each group, were divided as follows: 1) 4 sham operated control groups: the rats were sacrificed at the 1st, 2nd, 3rd and 7th day after sham operation. 2) 4 CBD ligated groups: the rats were sacrificed at the 1st, 2nd, 3rd and 7th day after CBD ligation. All animals were maintained on a diet of commercial pellets purchased from Sam Yang Co., Limited. The rats were anesthetized with ether for surgery or being sacrificed, and they were fasted prior to sacrifice. The CBD was exposed through a middle line incision. And after double ligation, the mid point of the CBD was cut. The sham operation was performed in the same way without CBD ligation.

2. Chemicals

Xanthine sodium salt, trichloroacetic acid, uric acid, nitroblue tetrazolium, Tris(hydroxymethyl) minomethane hydrochloride, NADH, dimethylsulfoxide, hydrogen peroxide, sodium azide, p-phenylene diamine dihydrochloride, Tris(hydroxymethyl) aminomethane, reduced glutathione, NADPH, glutathione reductase were purchased from Sigma(USA). All other chemicals were of the highest purity commercially available.

3. Assays of enzyme activities and superoxide radical production

The xanthine oxidase activity was measured with spectrophotometer according to the method of Rowe and Wyngaarden[10] with xanthine as a substrate. The amount of superoxide radical production was assayed by the method of Auclair and Voisin.[11] Superoxide dismutase was assayed using alkaline dimethylsulfoxide as a superoxide anion-generating system in association with cytochrome C as a superoxide anion-ndicating scavenger by the method of Hyland et al.[12] Glutathione peroxidase activity was assayed by the method of Palgia and Valentine[13] in which the oxidation of glutathione is coupled to the glutathione reductase, thus promoting consumption of NADPH.

4. Statistical analysis

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

RESULTS

Cytosolic xanthine oxidase activity between the 1st day and the 7th day after operation was higher in CBD ligated group than in sham operated control group(Fig. 1).

Superoxide radical production between the 1st and 3rd day after operation was higher in CBD ligated group than in sham operated control group(Fig. 2).

Superoxide dismutase and glutathione peroxidase activities showed no significant changes throughout the experiments(Fig. 3, 4).

Catalase activity between the 3rd and 7th day after operation was lower in CBD ligated group than in

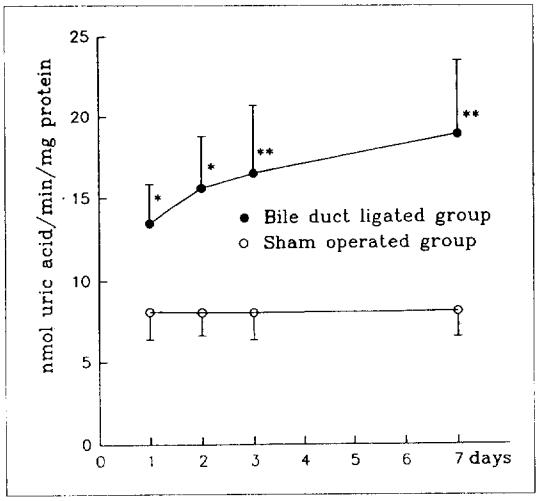

Fig. 1. Cytosolic xanthine oxidase activity in rats with bile duct ligation. Vertical bars are mean±SD with 5 rats in each groups. Values significantly different from sham operated control values(*; $p<0.05$, **; $p<0.01$).

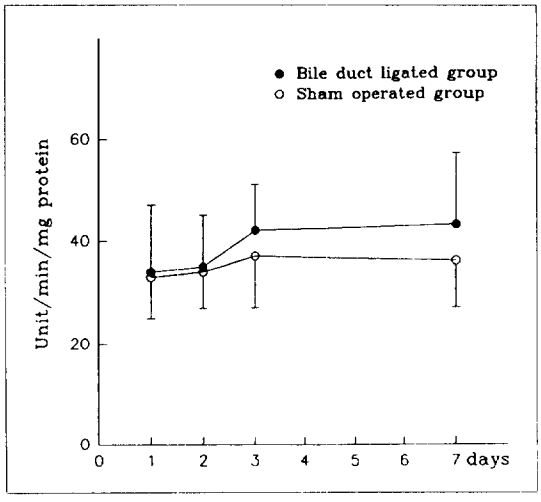

Fig. 3. Cytosolic superoxide dismutase activity in rats with bile duct ligation. Vertical bars are mean± SD with 5 rats in each group. One unit of superoxide dismutase activity was defined as the amount which inhibited the reduction of cytochrome C by 50%.

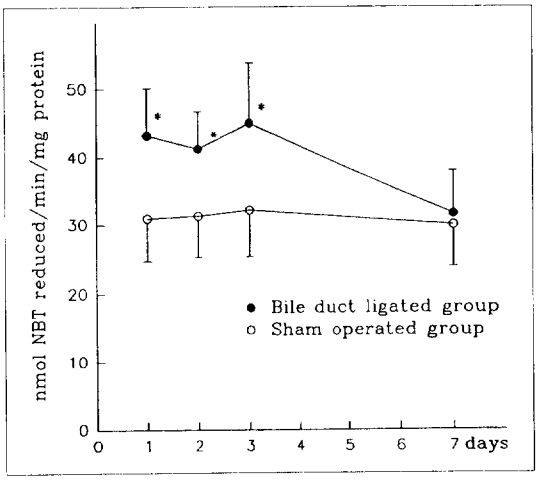

Fig. 2. The amount of superoxide radical production in rats with bile duct ligation. Vertical bars are mean ±SD with 5 rats in each group. NBT, nitro blue tetrazolium. Values significantly different from sham operated control values(*$p<0.05$).

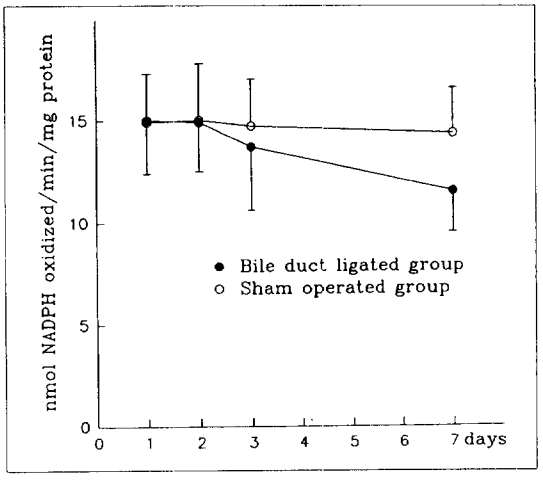

Fig. 4. Cytosolic glutathione peroxidase activity in rats with bile duct ligation. Vertical bars are mean± SD with 5 rats in each group.

sham operated control group(Fig. 5).

Km value of xanthine oxidase was not changed significantly in CBD ligated group, but Vmax value of this enzyme was higher in CBD ligated group than in sham operated control group(Fig. 6).

Km value of catalase was not changed significantly in CBD ligated group, but Vmax value of this enzyme was lower in CBD ligated group than in sham operated control group(Fig. 7).

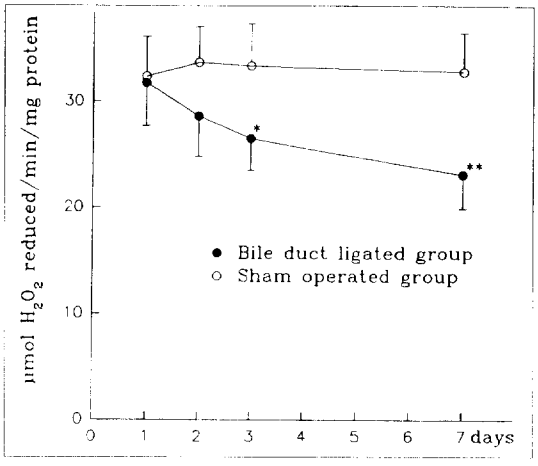

Fig. 5. Hepatic catalase activity in rats with bile duct ligation. Vertical bars are mean±SD with 5 rats in each group. Values significantly different from sham operated control values(*; $p<0.05$, **; $p<0.01$).

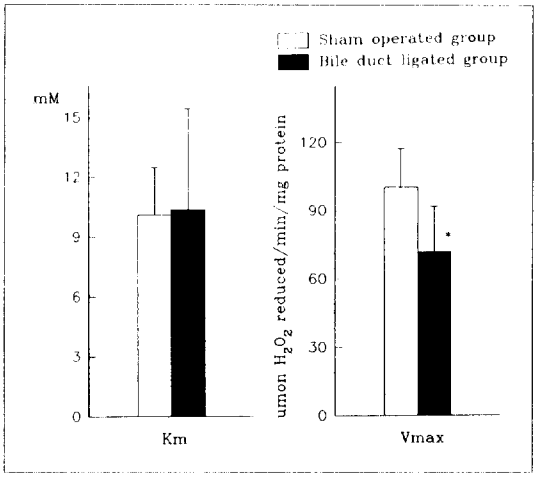

Fig. 6. Xanthine oxidase kinetic parameters determined with xanthine. Michaelis-Menten constants for xanthine oxidase were determined using xanthine at 7th day after bile duct ligation. Values are mean±SD with 5 rats in each group. Values significantly different from sham operated control values(**; $p< 0.01$).

DISCUSSION

CBD ligation in rats causes known biochemical and morphological abnormalities in the liver include

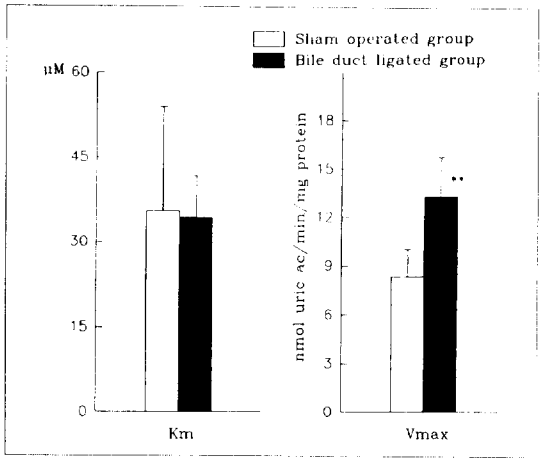

Fig. 7. Catalase kinetic parameters determined with hydrogen peroxide. Michaelis-Menten constants for catalase were determined using hydrogen peroxide at 7th day after bile duct ligation. Values are mean±SD with 5 rats in each group. Values significantly different from sham operated control values(*; $p<0.05$).

inflammation and necrosis.[14-16] From the CBD ligated liver which suffers inflammation and necrosis, purine nucleotides will be liberated thus xanthine oxidase which oxidizes hypoxanthine to xathine and xanthine to uric acid[7] will be induced. In this study, cytosolic xanthine oxidase activity and Vmax value were higher in CBD ligated group than in sham operated control group(Fig. 1, 6). These results indicate that the synthesis of xanthine oxidase in the liver is increased to oxidize nucleotides liberated from damaged hepatocytes under the cholestasis.

The amount of superoxide radical production was increased after CBD ligation(Fig. 2), like xanthine oxidase(Fig. 1). This result suggests that increased activity of xanthine oxidase which is the major source of oxygen free radicals[5] result in increased production of superoxide radicals under the cholestasis induced by CBD ligation.

In spite of increased production of superoxide radicals(Fig. 1), superoxide dismutase and glutathione peroxidase activities showed no significant changes (Fig. 3, 4). Catalase activity and Vmax value of this

enzyme was lower in CBD ligated group than in sham operated control group (Fig. 5, 6). These results indicate that although the production of oxygen free radicals is increased due to increased biosynthesis of xanthine oxidase, antioxidant enzymes do not play their protective role to oxygen free radicals due to decreased biosynthesis.

SUMMARY

Background/Aims: Protective role of antioxidant enzymes under the cholestasis was evaluated. **Methods:** The activity of xanthine oxidase, the amount of superoxide radical production, and the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase were measured under the cholestasis. **Results:** Xanthine oxidase activity, Vmax value of this enzyme and superoxide radical production were increased after CBD ligation. Superoxide dismutase and glutathione peroxidase activities showed no significant changes throughout the experiments. Catalase activity and Vmax value of this enzyme in the liver was significantly decreased after CBD ligation. **Conclusions:** Although the production of oxygen free radicals is increased due to increased xanthine oxidase, antioxidant enzymes do not play their protective role to oxygen free radicals due to decreased biosynthesis.

Key Words: Catalase, Glutathione peroxidase, Oxygen free radicals, Superoxide dismutase, Xanthine oxidase

REFERENCES

1. Parks DA, Bulkley GB, Granger DN. Role of oxygen-derived free radicals in digestive tract diseases. Surgery 1983; 94: 415-422.
2. Murray RK. Red and white blood cells. In: Murray RK, Granner DK, Mayer PA, Rodwell VW, eds. Harper's Biochemistry. 23rd ed. East Norwalk: Appleton and Lange, 1993:688-703.
3. McCord JM. The superoxide free radical: Its biochemistry and patho- physiology. Surgery 1983;94: 412-414.
4. Dashti HM, al-Sayer H, Behbehani A, Massa J, Christenson JT. Liver cirrhosis induced by carbon tetrachloride and the effect of superoxide dismutase and xanthine oxidase inhibitor treatment. J R Coll Surg Edinb 1992;37:23-28.
5. Leibovitz BE, Siegel BW. Aspects of free radical reactions in biological systems: aging. J Gerontol 1980;35:45-56.
6. Al-Khalidi USA, Chaglassion TH. The species distribution of xanthine oxidase. Biochem J 1965; 94:222-233.
7. Rodwell VW. Metabolism of purine and pyrimidine nucleotides. In: Murray RK, Granner DK, Mayer PA, Rodwell VW, eds. Harper's Biochemistry. 23rd ed. East Norwalk: Appleton and Lange, 1993;363-377.
8. Guemouri LG, Artur Y, Herneth B, Jeandel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin Chem 1991;37:1932-1937.
9. Ceballos-Picot I, Trivier JM, Nicole A, Sinet PM, Thevenin M. Age- correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. Clin Chem 1992;38:66-70.
10. Rowe PB, Wyngaarden JB. The mechanism of dietary alterations in rat hepatic xanthine oxidase levels. J Biol Chem 1966;241:5571-5576.
11. Auchlar C, Voisin E. Nitoblue tetrazolium reduction. In: CRC Handbook of Methods for Oxygen Radical Research Boca Raton, Florida: CRC Press Inc., 1984;123-132.
12. Hyland K, Voisin E, Banoun H, Auclair C. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generation system. Anal Biochem 1983;135:280-287.
13. Paglia ED, Valentine WN. Studies on the quantita-

tive and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70: 158-169.

14. Kountouras J, Billing BH, Scherer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rats. Br J Exp Path 1984;65:305-311.
15. Chang DS, Kwak JS, Sohn TJ. An ultrastructural study on the proliferative changes of bile ductules after ligation of common bile duct. The Kyungpook Univ Med J 1987;28:113-117.
16. Kim HS, Park JY, Kim EY, Kwak KS, Choi YH, Chung JM. Morphologic change of hepatocytes induced by common bile duct ligation. The Korean Journal of Internal Medicine 1989;36:459-470.