The Seoul Journal of Medicine
Vol. 35, No. 1: 19-28, March 1994

The Effects of Oxidant and Antioxidant Activity on Rat Peritonitis Induced by Cecal Ligation and Puncture†

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= Abstract = **The aims of this study were to determine the effects of oxidant and antioxidant defense activity on the degree and time course in peritonitis, and to define the relationship between biochemical and histologic changes induced by oxidative stress and antioxidant defense functions in the lungs and liver. Female Sprague-Dawley rats were divided into the control(sham operation) and CLP(cecal ligation and puncture) groups. Histologic examination, oxidative changes and antioxidant defenses presented as reduced(GSH) and oxidized tissue glutathione(GSSG) and malondialdehyde(MDA) content and catalase activity were studied in the lungs and liver at 6,12 and 24 hrs in each group. In the lung with CLP group, neutrophil with monocyte infiltration at 6 hrs, and interstitial edema and congestive findings at 24 hrs were shown in the pulmonary parenchymes. MDA was slightly increased gradually without significancy. GSSG was significantly elevated at 12 hrs. GSH and catalase were decreased at 6 hrs period, but significantly increased with time. In the liver, no pathologic findings except lymphocyte infiltration till 12 hrs, and mild destructive and congestive sinusoidal structures were noted at 24 hrs. MDA at 24 hrs and GSSG at 6hrs was significantly increased. Whereas, GSH and catalase at 6 hrs were significantly decreased but gradually increased above control level. These results suggest that histologic and biochemical changes in the lung and liver induced by peritonitis are caused by oxidant activities in parts. Also the extent of injury are closely related to the MDA levels. Initially, antioxidant defensive works are depressed, but activated gradually.**

Key Words: *Peritonitis, Oxidant, Antioxidant, MDA, GSH, Catalase*

INTRODUCTION

Multiple organ failure(MOF) associated with sepsis is presently the most common cause of death in severely injured patients and those with peritonitis admitted in an intensive care unit. Either bacterial or non-bacterial dependent peritonitis, an important cause of organ failure, has been known to be related to oxidant activity of the tissues and its lipid peroxidation(Garrison *et al.* 1982). Since the proposal of Gershman and Gilbert(1954) describing the damaging effects of oxygen free radicals or oxidants, there has been some evidence that oxidant damage contributes to the pathogenesis of several human diseases, including rheumatoid arthritis, immune injury to the lung and kidney, pulmonary emphysema,

Received in March 1994, and in a final form in March 1994.

† This study was supported by a grant No. 01-93-044 from the Seoul National University Hospital Research Fund

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cardiovascular disease, inflammatory disease, cataract, and cancer(Halliwell and Gutteridge 1986; Halliwell 1987; Machlin and Bendich 1987; Youn *et al.* 1991). The major oxidants include superoxide radical(O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical(OH^-), singlet oxygen ($^1O_2$), and hypochlorous acid(HOCl), which can originate endogenously from normal metabolic reactions and are responsible for phagocytosis. But, large amount of oxidants can be generated through the exposure of other states such as sepsis, ischemia-reperfusion injury, hyperbaric oxygen therapy, burn, smoking, pesticides, air pollutants, anesthetic agents, and radiation, resulting in endogenously harmful effects(Cross 1987; Heffner and Repine 1989). These harmful effects such as increased vascular permeability, impaired ATP production and cell membrane function, initiation and maintenance of local and systemic inflammtory responses, destruction of interstitium, impaired phagocytic action of macrophage, and intracellular DNA degeneration cause loss of celluar function, ultimately leading to organ failure (Cross 1987; Sugino *et al.* 1987; Youn *et al.* 1992). However, the relationship between oxidant activity and inflammatory response remains unresolved. Since oxidant change occurs immediately after inflammatory response and before systemic perfusion injury, and inactivates endogenous antioxidants(Kono and Fridovich 1982), it may be important to determine the time of antioxidant treatment. Moreover, little data are available on the correlation between biochemical assays including MDA, GSSG, GSH, and catalase activity and histologic changes in the lung and liver over time(Demling *et al.* 1992).

Accurate monitoring of tissue oxidative stress, therefore, is necessary to better define the pathophysiology of these inflammation-induced changes.

First, the degree of the organ injury caused by oxidants is assessed by determining the level of tissue or plasma lipid peroxide byproducts such as conjugated dienes, hydroperoxide, malondialdehyde(MDA), and fluorochrome. The conjugated dienes, the initial product formed from the oxidation of a polyunsaturated fatty acid, is subsequently converted to an endoperoxide and then to a variety of end products including MDA(Ohgawa *et al.* 1979; Sugino *et al.* 1987). Second, the level of tissue oxidized glutathione(GSSG), an oxidized form of a reduced glutathione(GSH), has been reported to be a sensitive marker of cell oxidant activity caused by H_2O_2 and lipid hydroperoxide release(White *et al.* 1986).

In the mammalian cell, endogenous antioxidant defense to oxidants is provided by superoxide dismutase(SOD), catalase, glutathione peroxidase, and GSH. A SOD dismutates superoxide radical to H_2O_2 and a catalase catalyzes the divalent reduction of H_2O_2 to H_2O and O_2, which prevents lipid peroxidation in the lung and inhibits release of prostanoid such as TxA_2 from endothelial cells or leukocytes. Glutathione peroxidase, a selenium-dependent enzyme, detoxifies H_2O_2 to H_2O through the oxidation of GSH to GSSG, which is then reduced to GSH by a second enzyme, glutathione reductase, with NADPH and glucose-6-phosphate dehydrogenase(= glutathione redox cycle)(Deneki *et al.* 1985; Heffner and Repine 1989).

Therefore, the resulting injury caused by inflammatory response is dependent on the balance between oxidant and antioxidant activities. Until now, most studies have been focused on the effects of oxidants and exogenous antioxidants in systemic sepsis by endotoxin or inflammation-inducing agents. The effect of exogenous antioxidants pretreatment including deferoxamine, 6-aminosteroid, ibuprofen, vit-E(α-tocoperol), β-carotene, glutathione, uric acid, and bilirubin has been reported in a number of acute lung injury models to prevent or ameliorate the oxidants changes(Sugino *et al.* 1987; Youn *et al.* 1991).

Our purpose was to determine the effects of oxidant and antioxidant defense activity on the degree and time course in peritonitis, and to define the relationship between biochemical and histologic changes induced by oxidative

stress and antioxidant defense functions in the lungs and liver.

MATERIALS AND METHODS

Animals

A total of 60 female Sprague-Dawley rats weighing 170 to 220g were housed individually in cages with free access to water and formula chow for 10 days. The animals were divided into two groups: a control group(n=30); and a peritonitis group(n=30) caused by cecal ligation and puncture(CLP). Ten rats in each group were killed at 6, 12, and 24 hrs.

Rat peritonitis model

The rats were fasted 8 hrs before operation and anesthetized with an intramuscular injection of 10mg ketamine hydrochloride/100gm of body weight. Peritonitis was produced by CLP as described previously(Chaudry *et al.* 1979). Through a 1cm midline incision, the cecum of the rats was exposed and ligated just below the ileocecal valve so that intestinal obstruction was not produced. The cecum was punctured twice with an 18 gauge needle and the abdominal wound was closed in layers. Control rats were given a sham operation without CLP. The rats were resuscitated with saline solution (10ml/100gm of body weight) administered subcutaneously at the time of operation, and fasted but allowed water after the operative procedure.

At 6, 12 and 24hrs, the animals were given an anesthetic dose of 7.5mg ketamine hydrochloride/100gm of body weight and killed. The peritoneal cavity and intraabdominal organs were inspected. The lungs and liver were then rapidly removed. The liver was perfused with ice cold 0.9% saline via the portal vein, and 0.5 gm pieces were rapidly frozen beteween two blocks of dry ice and stored at -70℃ for subsequent biochemical assays.

Histologic measurements

Samples of lung and liver tissue were fixed in formalin and sections stained with hematoxylin and eosin. Histologic changes were assessed with light microscopy.

Biochemical measurements

To determine the effects of oxidant activity and antioxidant defenses in the lung and liver, MDA, GSSG, GSH, and catalase activity were measured. The methods of biochemical assays were as follows:

Lipid peroxidation was measured as tissue MDA, using the thiobarbituric acid method of Ohkawa and coileagues(1979). MDA was reported as nmol per gram(nmol/g) of tissue.

GSH and GSSG of lung and liver homogenate were measured according to the DTNB-GSSG Reductase Recycling Assay of Griffith(1980), which provides a very sensitive assay for total tissue glutathione as described by equation.

GSH(reduced glutathione)
= total glutathione - 0.5 GSSG

Values were expressed as μmol/g of tissues.

Catalase activity was determined spectrophotometrically on the homogenate of the frozen lung and liver by measuring the consumption of H_2O_2 using the method of Beers and Sizer(1952). Catalase activity was presented as units/mg of tissues. The equation for determining catalase activity is as follows.

$$\text{Specific activity} = \frac{\text{O.D} \times 1000}{43.6 \times \frac{\text{mg protein}}{\text{ml reaction mix}}}$$

Statistical analysis

Data were analyzed using nonparamedical method. Within each group, Kruskal-Wallis one way ANOVA test was used to compare the individual time periods with baseline. Wilcoxon rank sum test was used to compare the difference between the control and CLP groups. The values were expressed in mean $\pm$ standard deviation. A $p<0.05$ was considered significant.

RESULTS

Histologic changes

In the control group, the lung appeared grossly and histologically normal(Fig. 1). In the

Fig. 1. Histology of the lung in the control group. Inflammatory cell infiltration is absent. (H & E stain, × 200).

CLP group, lung histology showed lymphocyte and neutrophil infiltration and interstitial thickening at 6 hrs(Fig. 2). These findings appeared progressively more severe with time and revealed mild vascular congestion and interstitial edema after 24 hrs(Fig. 3).

Fig. 2. Histology of the lung in the cecal ligation and puncture group at 6 hours. Lymphocyte and neutrophil sequestration and interstitial thickening are evident. (H & E stain, × 200).

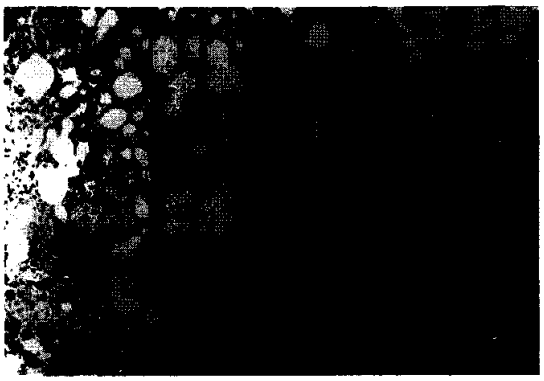

Fig. 3. Histology of the lung in the cecal ligation and puncture group at 24 hours. Lymphocyte and neutrophil sequestration and vascular congestion are evident. Interstitial accumulation of proteinaceous fluid is also noted. (H & E stain, × 200).

Liver histology in the control group grossly and histologically demonstrated no abnormal findings(Fig. 4). In the CLP group, liver histology revealed no abnormalities at 6 and 12 hrs, but showed lymphocyte infiltration, mild congestion, and slightly disrupted lobular structure and sinusoid after 24 hrs(Fig. 5).

Fig. 4. Histology of the liver in the control group. Lobular structure and sinusoid are well visualized. Inflammatory cell infiltration is absent. (H & E stain, × 100).

Fig. 5. Histology of the liver in the cecal ligation and puncture group at 24 hours. Lymphocyte infiltration and mild congestion are visualized. Lobular structure and sinusoid are slightly disrupted. (H & E stain, × 100).

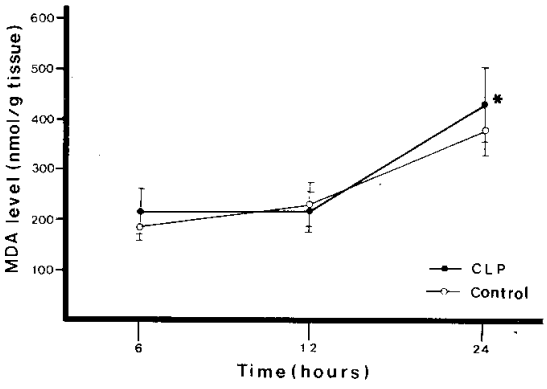

Fig. 7. The effect of peritonitis on liver lipid peroxidation measured as malondialdehyde(MDA). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

Biochemical changes

In the CLP group, lung MDA was modestly increased with time, but was not significant at any time compared with the control group(Fig. 6). At 6 and 12 hrs, liver MDA was not different compared with the control group, but liver MDA(438. 75 ± 79. 10 nmol/g) at 24 hrs was significantly increased compared with the control group(Fig. 7).

In the CLP group, there was a marked increase in lung GSSG compared with the control group at 12 hrs, but a decrease after then(Fig. 8). Liver GSSG(0.60 ± 0.78 mol/g) at 6 hrs in the CLP group was significantly increased compared with the control group. After then, liver GSSG was decreased(Fig. 9).

At 6 hrs, lung GSH(12. 38 ± 1.39 μmol/g) in the CLP group was significantly decreased

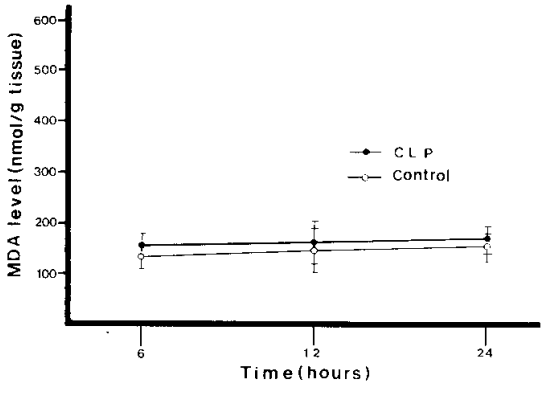

Fig. 6. The effect of peritonitis on lung lipid peroxidation measured as malondialdehyde(MDA). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group.

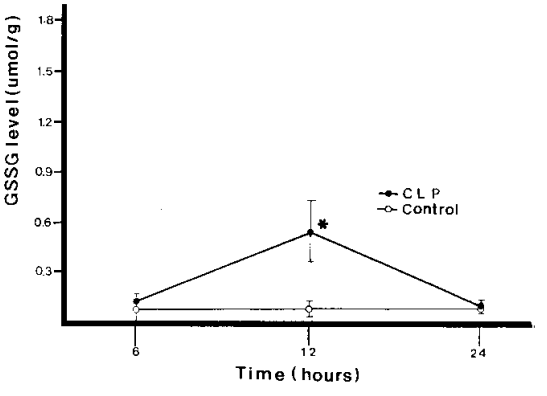

Fig. 8. The effect of peritonitis on lung oxidized glutathione(GSSG). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

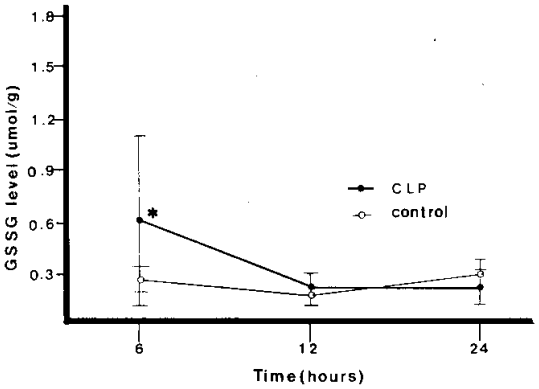

Fig. 9. The effect of peritonitis on liver oxidized glutathione(GSSG). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

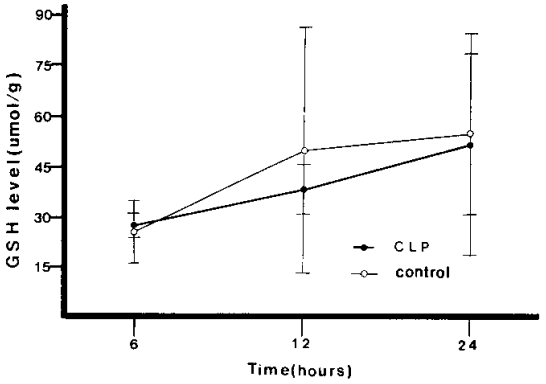

Fig. 11. The effect of peritonitis on liver reduced glutathione(GSH). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group.

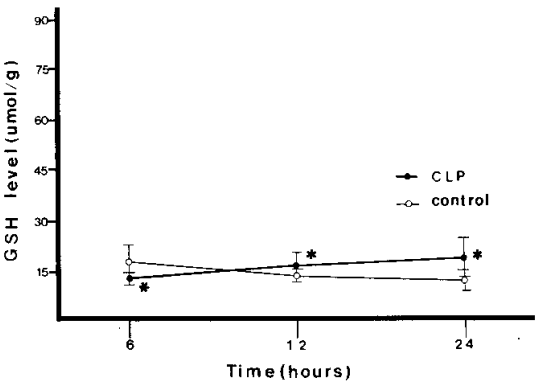

Fig. 10. The effect of peritonitis on lung reduced glutathione(GSH). Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

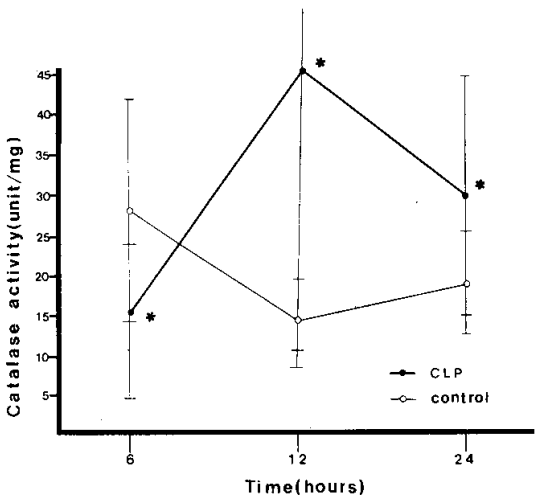

Fig. 12. The effect of peritonitis on lung catalase activity. Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

compared with the control group, but at 12 and 24 hrs, lung GSH was significantly increased compared with the control group during this period(Fig. 10). Liver GSH revealed a decrease at 6 hours but an increase with time compared with the control group(Fig. 11).

In the CLP group, there was a significant decrease at 6 hrs(15. 48±11.36) and significant increases at 12 and 24 hrs(45. 89±36.23 and 29.97±16.98, respectively) in lung catalase activity compared with the control group (Fig. 12).

There was a significant decrease in liver catalase activity at 6 hrs(8. 20±5.52) but a significant increase with time and at 24 hrs(17. 35±9.10) compared with the control group(Fig. 13).

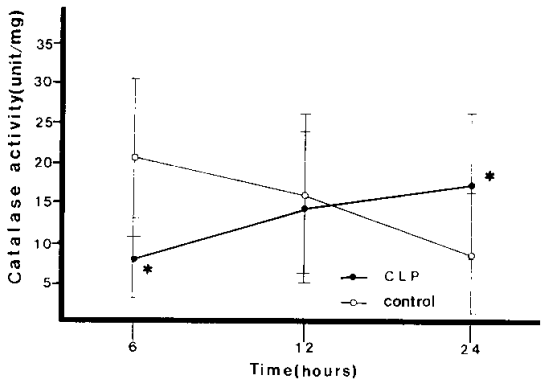

Fig. 13. The effect of peritonitis on liver catalase activity. Values are expressed as the mean ± SD. Note : CLP ; cecal ligation and puncture group. * ; significant change from control group.

DISCUSSION

In most animal studies, endotoxin, bacteria, or nonbacterial inflammatory agents have usually been used to produce sepsis. But these results were different according to the materials inducing sepsis. This study showed the effects of oxidant and antioxidant defense activity on the degree and time course in peritoritis. In the CLP group, the changes of MDA over time in the lung and liver relatively corresponded with the histologic changes. Lung MDA began to increase at 6 hrs and after then, was significantly increased compared with the control group. Lung histology revealed lymphocyte and neutrophil infiltration and interstitial thickening at 6 hrs and mild interstitial edema and congestion with time. Increased liver MDA at 24 hrs was also consistent with the histologic changes at 24 hrs showing inflammatory response. This increase of MDA, the product of lipid peroxidation, is considered to be caused by large amounts of oxidants.

Lung and liver GSSG were initially increased, but decreased with time. With increased intracellular oxidant stress, there is an increase in GSSG, which in turn is either rapidly converted back to reduced GSH or exported from the liver into the biliary system (Parks *et al.* 1988). Initially increased GSSG, therefore, is considered to be an effect of the severe oxidative changes, which means that the onset of lung and liver oxidant stress occurred very early after the onset of peritoneal inflammation, preceding the evidence of a systemic perfusion defect(Ishzaka *et al.* 1988). These findings suggest the use of MDA and GSSG in tissue as early indicators of oxidant-induced acute tissue injury.

The effect of oxidant on tissue is known to be varied according to the resistence of the organ to oxidant, and it has been reported that the liver is more sensitive to oxidant stress than the lung(Kornbrust and Marvis 1980). We also obtained similar results, that is, the levels of liver MDA were high compared with the lung.

We noted a significant increase in neutrophil and monocyte infiltratiion in the lung after CLP induced peritonitis, supporting other reports that oxidants causing lung injury are likely produced by neutrophil and monocyte (Tate 1983; Halliwell 1987). Another study reported that lung oxidant activity and tissue changes caused by systemic inflammation persist well after the systemic oxidant activity resolved and these oxidants are released from infiltrated monocyte(Demling 1992). We found an increase in monocyte infiltraton in the lung, but did not observe long term changes and demonstrate evidence supporting this report because of the high mortality rate of rats in peritonitis induced by CLP. In contrast, likely sources of liver oxidants are related to the increased liver xanthine oxidase activity. Liver ischemia due to a decrease in hepatic blood flow or products released by the activated neutrophil are possible contributors to the increased xanthine oxidase activity(Fantone and Ward 1982; Fried *et al.* 1989). An actual ischemic injury is not always necessary. Liver neutrophil and/or Kupffer cells activated by an initial cytokine release are also a likely source of the local oxidant release(Michie and Wilmore 1990).

We found an initial decrease and gradual

increase with time in GSH. Initial decrease in GSH appeared to demonstrate that GSH did not convert to GSSG but released into the biliary system, and gradual increase corresponded with the report that increased GSH with time is due to increased production of GSH from remaining available substrate.

We also noted a marked difference in the effect of antioxidant defenses in the lungs and liver over time. Lung GSH was preferentially restored relative to the liver even in the presence of ongoing oxidant activity. This antioxidant restoration may well attenuate the degree of subsequent lung injury. Glutathione activity in tissues, such as the liver, kidney, and red cells, has been documented to be decreased during oxidant release as GSSG is exported out of the cell into the biliary tract in the liver and into plasma from the red cell and kidney. GSH is also lost from injured cells (Machlin and Bendich 1987; Deneki *et al.* 1989; Maddaiah 1990). The rapid return of lung GSH, compared with the liver, is probably due to active lung uptake of GSH from plasma, with the GSH initially produced and exported by the liver. These findings suggest that antioxidants are used as a defense mechanism to oxidant stress. The degree of tissue lipid peroxidation by oxidant stress generally has been known to be inversely correlated with tissue GSH level (Maddaiah 1990).

Catalase activity was also initially decreased, but increased over time. The decrease in catalase activity is likely due to a combination of oxidant inactivation and increased turnover(Kono and Fridovich 1982). Lung catalase activity is known to be relatively low compared with systemic tissues such as liver and red cells(Perry 1982). But, our study showed different results, which suggest that oxidant activity is not severe, so MDA, a product of lipid peroxidation, is not significantly increased.

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