Korean J Physiol Pharmacol
Vol 13: 181-187, June, 2009

Ischemia/reperfusion Lung Injury Increases Serum Ferritin and Heme Oxygenase-1 in Rats

Yoon-Yub Park

Department of Physiology, School of Medicine, Catholic University of Daegu, Daegu 705-718, Korea

Intestinal ischemia/reperfusion (I/R) is one of common causes of acute lung injury (ALI). Early and accurate diagnosis of patients who are like to develop serious acute respiratory distress syndrome (ARDS) would give a therapeutic advantage. Ferritin and heme oxygenase-1 (HO-1) are increased by oxidative stress and are potential candidates as a predictive biomarker of ARDS. However, the mechanisms responsible for the increases of ferritin and HO-1, and their relationship to ALI, are unclear. In order to elucidate the interactions between ferritin and HO-1, we studied the changes in ferritin and HO-1 levels in serum and bronchoalveolar lavage (BAL) fluid after intestinal I/R injury in rats. Leukocyte number and protein contents in BAL fluid were elevated following I/R, and the increases were attenuated by mepacrine pretreatment. Both serum ferritin and HO-1 concentrations were progressively elevated throughout the 3 h observation period. Mepacrine pretreatment attenuated the increase of serum and BAL fluid ferritin concentrations, but did not suppress the increase of serum HO-1. Moreover, BAL fluid HO-1 levels did not change after I/R or after mepacrine pretreated I/R compared with sham rats. Unlike ferritin, HO-1 levels are not exactly matched with the ALI. Therefore, there might be a different mechanism between the changes of ferritin and HO-1 in intestinal I/R-induced ALI model.

Key Words: Ferritin, Heme oxygenase-1, Ischemia/reperfusion, Acute lung injury, ARDS

INTRODUCTION

Acute lung injury (ALI) and its most severe form, the acute respiratory distress syndrome (ARDS), are frequent complications in critically ill patients and responsible for significant morbidity and mortality (Repine, 1992; Ware and Matthay, 2000). Ischemia/reperfusion (I/R) frequently occurs in human pathological conditions, and it has been considered as a significant cause of ALI and ARDS (Otamiri et al., 1988; Grace, 1994). The pathogenesis of this injury appears to involve the generation of reactive oxygen species (ROS), which can be detected during both ischemia (Minamiya et al., 1998) and reperfusion (Kennedy et al., 1989).

Having been indicated as one of the earliest and most important components of tissue injury after reperfusion of the ischemic organ, ROS and the end-products of lipid peroxidation have been shown to up-regulate a number of cytoprotective genes, including heme oxygenase-1 (HO-1; Choi and Alarm, 1996). It has been suggested that the induction and maintenance of HO-1 expression was a key for protection in an intestinal I/R model (Hassoun et al., 1992; Willis et al., 1996; Attuwaybi et al., 2003). HO-1 induction before injury protects against oxidant- mediated cytotoxicity in cell culture (Lee et al., 1996) and prolongs survival after lipopolysaccharide (LPS) instillation in rats (Otterbein et al., 1995). HO-1 inhibition also resulted in a loss of endotoxin-induced protection against oxidant injury in vascular smooth muscle cells (Yet et al., 1997).

Heme oxygenase is a heme-cleaving enzyme that opens the porphyrin ring, producing biliverdin, carbon monoxide, and free iron (Tenhunen et al., 1968; Otterbein et al., 2003). HO-mediated release of iron has been shown to influence ferritin expression, such that an increase in HO-1 may lead to increased ferritin expression (Eisenstein et al., 1991; Harrison and Arosio, 1996; Ferris et al., 1999). This sequence of events has been proposed as one of the cytoprotective functions of HO-1 (Vile et al., 1994; Choi and Alarm, 1996).

Previous studies have reported simultaneous induction of ferritin and HO-1 following hyperoxia- and hemoglobin-induced lung injury in rats (Balla et al., 1995; Taylor et al., 1998).

Ferritin plays an important role as a fast-acting endogenous cytoprotectant in cellular antioxidant defense mechanisms by rapidly sequestering free cytosolic iron, which is the crucial catalyst of oxygen-centered radical formation via the Fenton reaction in biological systems (Balla et al., 1992; Cairo et al., 1995; Juckett et al., 1995; Kim et al., 1995). Therefore, the heme degradation products and the metabolic derivatives generated by HO-1 suppress toxic events in cells.

Received April 24, 2009, Revised May 11, 2009,
Accepted May 25, 2009

Corresponding to: Yoon-Yub Park, Department of Physiology, School of Medicine, Catholic University of Daegu, 3056-6, Daemyeong-dong, Nam-gu, Daegu 705-718, Korea. (Tel) 82-53-650-4475, (Fax) 82-53-621-4106, (E-mail) yypark@cu.ac.kr

ABBREVIATIONS: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; HO-1, heme oxygenase-1; BAL, bronchoalveolar lavage.

Serum ferritin levels were elevated in an animal model of ALI (Park et al., 2003, 2004; Park and Lee, 2006). Several clinical studies also have reported that serum ferritin level might be a useful predictor of the onset of severe acute respiratory distress syndrome (Connelly et al., 1997; Sharkey et al., 1999; Ji et al., 2007). If the increase of serum ferritin is solely dependent on HO-1 following ALI, HO-1 would be a better predictor for the early diagnosis of ARDS than ferritin. However, met-hemoglobin induces ferritin even after the inhibition of HO-1 activity in rat lung endothelium (Balla et al., 1993). This means there might induction of ferritin independent HO-1. Until now, relatively few studies have addressed the interactions between ferritin and HO-1 and the sequential changes of ferritin and HO-1 in serum and/or bronchoalveolar lavage (BAL) fluid following ALI at the same time (Mumby et al., 2004).

On the other hand, there is a limitation to use serum ferritin level as an indicator of lung injury. In a previous report, no close relationship was seen between serum and BAL ferritin increase in a severe hemorrhage-induced ALI model (Park et al., 2004). Therefore, it is very important to evaluate the reliability of biomarkers and to understand the mechanisms responsible for the changes of HO-1 and ferritin in serum or BAL fluid. In this study, the temporal pattern of the changes of ferritin and HO-1 in serum and BAL fluid following intestinal I/R were examined, while evaluating ferritin and HO-1 levels as a predictor of ARDS in rats subjected to intestinal I/R injury.

METHODS

Experimental protocols were approved by the Ethical Committee of Animal Experiments in Daegu Catholic University, School of Medicine. Male Sprague-Dawley (Hyo Chang Science, Daegu) rats weighing 300~450 g were used. Rats were anesthetized by intraperitoneal injection of mixed ketamine (80 mg/kg) and xylazine (16 mg/kg). The left femoral artery was incised, and a polyethylene catheter (PE-50, Clay-Adams, USA) filled with heparinized saline (100 U/ml) and inserted for blood sampling. The rats remained deeply anesthetized for the entire experiment, booster shots if needed.

Rats were randomly divided into different groups: Mepacrine-treated I/R groups (Mepa+I/R; injection of PLA_2 inhibitor mepacrine, 60 mg/kg i.p.), I/R groups (saline vehicle, i.p.). Thirty minutes after the pretreatment with saline or mepacrine, intestinal ischemia was achieved by clamping the superior mesenteric artery for 30 min using a non-traumatic artery clamp, and recirculation of blood flow was established by releasing the clip. Reperfusion was allowed for 3 hours. Sham-operated rats underwent identical surgical procedures, except that no artery clamp was applied.

For measurement of serum ferritin and HO-1 concentrations, 100 μl of arterial blood was collected through the catheter just before ischemia and 30, 60, and 180 min after the reperfusion. At the end the experiment, samples of lung and jejunal part of intestinal tissue were excised and frozen at $-70^{\circ}C$ for various assays.

Bronchoalveolar lavage was performed 3 h after the reperfusion by the method previously described (Park and Park, 2006). Total leukocytes in BAL fluid were counted with a hemocytometer. Protein concentrations in BAL fluid were measured using a bicinchoninic acid method (Sigma, St. Louis, USA). Ferritin and HO-1 concentrations were quantified with commercially available ELISA kits specific for rat ferritin (Panapharm Laboratories, Japan) and HO-1 (Takara Bio Inc., Japan) following the instructions.

Tissue samples of lung and intestine were homogenized separately in sodium phosphate buffer (100 mM, pH 7.4) and heated at $70^{\circ}C$ for 10 min. The homogenates were cooled to $4^{\circ}C$ and centrifuged at 12,000 g for 15 min. Subsequently, the supernatants were assayed for tissue ferritin and protein concentrations and the results expressed as ng/mg of tissue protein.

Data were presented in means±S.E.M. Unpaired t test was used to compare groups, and paired t test was used for paired observations within a group. A p value of <0.05 was considered statistically significant.

RESULTS

Effects of mepacrine on intestinal I/R-induced acute lung injury

ALI was induced following intestinal I/R in rats and effectively attenuated by mepacrine pretreatment (Fig. 1, 2). The number of leukocytes in BAL fluids were significantly increased following intestinal I/R (3.1±0.5 millions/two lungs) compared to the values obtained for sham-treated rats (0.9±0.1 millions/two lungs). Intestinal I/R treated rats also had significantly elevated lung lavage protein contents (5.7±0.1 mg/two lungs) compared to sham rats (4.0±0.4 mg/two lungs). Mepacrine treatment significantly attenuated the increase in leukocyte number and protein content in lung lavage fluids.

Changes of serum ferritin and HO-1 levels after intestinal I/R

The serum ferritin concentrations of all groups were the same before I/R (Fig. 3). From 30 min after I/R, serum ferri-

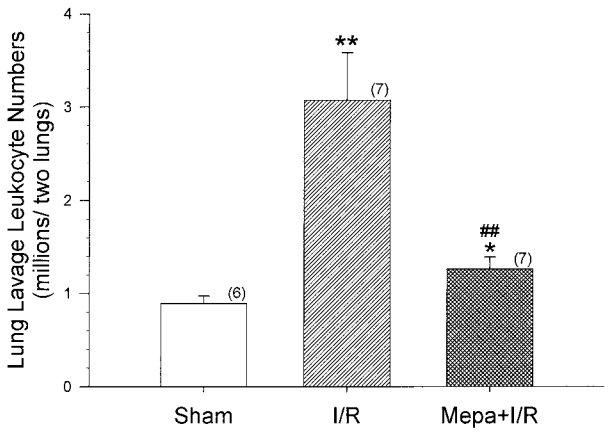

Fig. 1. Intestinal ischemia/reperfusion (I/R) significantly increased the number of leukocytes in lung lavage fluids. Mepacrine pretreatment (Mepa+I/R) effectively suppressed the increase. The data shown are means±S.E.M. for the number of rats shown in the parentheses. *$p<0.05$, **$p<0.01$, compared with Sham; $^{\#\#}p<0.01$, compared with I/R.

tin concentration in I/R rats started to sigzznificantly increase from the baseline value (from 27±8 to 45±9 ng/ml, $p<0.01$). After 1 h of reperfusion, serum ferritin levels of I/R rats were significantly higher than the values of mepacrine-treated I/R rats (Mepa+I/R) and sham-treated rats. Serum ferritin levels of mepacrine-treated I/R rats were significantly elevated compared with the levels of sham-treated rats only at 3 h after reperfusion. Serum ferritin concentrations of I/R rats at 180 min following reperfusion

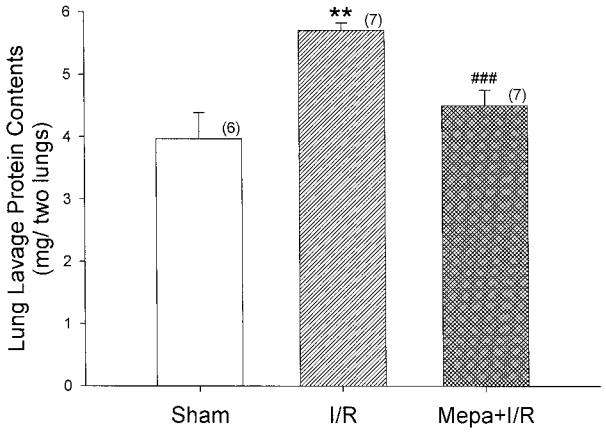

Fig. 2. Rats subjected to intestinal ischemia/reperfusion (I/R) had significantly increased protein contents in lung lavage fluids. This change was significantly attenuated by the pretreatment with mepacrine (Mepa+I/R). The data shown are means±S.E.M. for the number of rats shown in the parentheses. $^{**}p<0.01$, compared with Sham; $^{###}p<0.001$, compared with I/R.

(148±15 ng/ml) were significantly higher than the values obtained for Mepa+I/R (65±7 ng/ml, $p<0.001$) and sham rats (38±8 ng/ml, $p<0.001$). In contrast, sham rats did not show any significant changes of serum ferritin throughout the experiment.

Fig. 4 shows the changes of serum HO-1 concentrations. Until 30 min of reperfusion, there was no difference in HO-1 levels across groups. However, after 60 min of reperfusion, mepacrine treated I/R rats had increased HO-1 levels compared with I/R and sham-treated rats. At the end of 3 h of reperfusion, I/R treated rats (I/R, 9.8±1.2; Mepa+I/R, 12.4±2.3 ng/ml) showed a significant increase of serum HO-1 concentrations compared with sham rats (4.3±0.2 ng/ml). In comparison with the changes of serum ferritin, serum HO-1 levels of mepacrine-treated I/R rats were significantly higher than untreated I/R rats.

Changes of BAL fluid ferritin and HO-1 levels after intestinal I/R

Intestinal I/R significantly increased BAL fluid ferritin levels (Fig. 5). Mepacrine treated rats had a significant decrease in the lavage ferritin concentrations compared to I/R rats. However, HO-1 concentrations in BAL fluid were the same in all groups (Fig. 6).

Ferritin contents of lung and intestinal tissues after intestinal I/R

Ferritin contents of lung and intestinal tissues were elevated following intestinal I/R (Fig. 7, 8). Mepacrine-treated I/R rats showed a tendency to decrease in tissue ferritin contents compared to I/R rats.

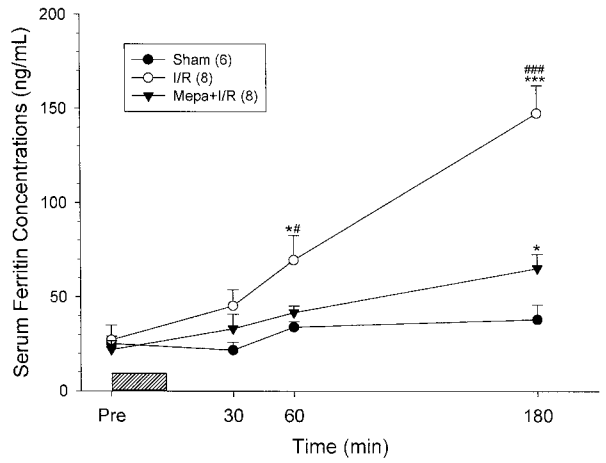

Fig. 3. Rats subjected to ischemia/reperfusion (I/R) had greatly increased serum ferritin concentrations after 60 min following I/R. Serum ferritin concentrations of I/R rats at 180 min of reperfusion were significantly higher than those of other rats. By comparison, mepacrine pretreatment (Mepa+I/R) significantly attenuated the increase following I/R. Serum ferritin concentrations of sham rats did not change through the observation period. Each point represents mean±S.E.M. for the number of rats shown in the parentheses. $^{*}p<0.05$, $^{***}p<0.001$, compared with Sham; $^{\#}p<0.05$, $^{###}p<0.001$, compared with Mepa+I/R.

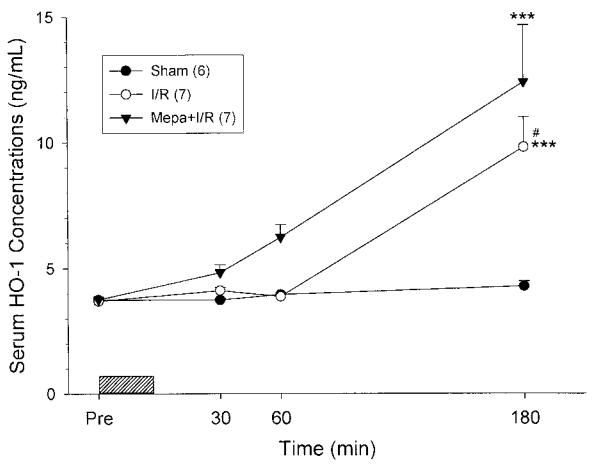

Fig. 4. Rats subjected to ischemia/reperfusion (I/R) had greatly increased serum heme oxygenase-1 (HO-1) concentrations after 3 hours of reperfusion compared to Sham rats. At 180 min following I/R, HO-1 levels of mepacrine-treated rats (Mepa+I/R) was significantly higher than the values of untreated I/R and sham rats. Serum HO-1concentrations of sham rats did not change over the course of the experiment. Each point represents mean±S.E.M. for the number of rats shown in the parentheses. $^{***}p<0.001$, compared with Sham; $^{\#}p<0.05$, compared with Mepa+I/R.

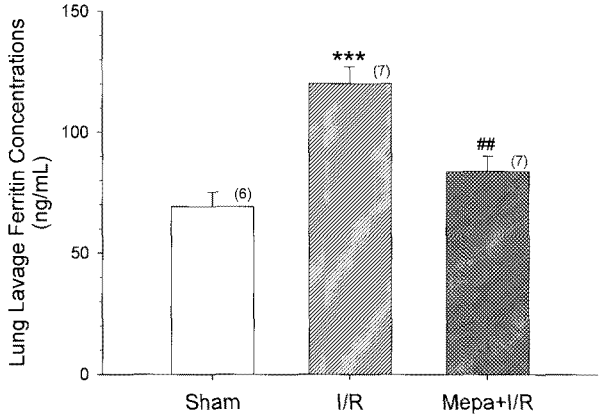

Fig. 5. Intestinal ischemia/reperfusion (I/R) significantly increased lung lavage fluid ferritin concentrations. Mepacrine pretreatment (Mepa+I/R) significantly reduced the increase. The data shown are means±S.E.M. for the number of determinations shown in the parentheses. ***$p<0.001$, compared with Sham; $^{\#\#}p<0.01$, compared with I/R.

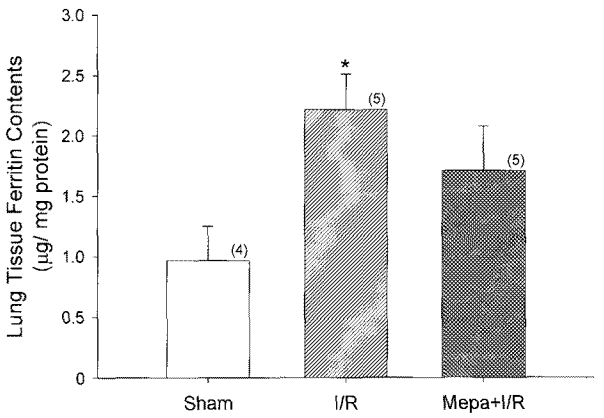

Fig. 7. Intestinal ischemia/reperfusion (I/R) significantly increased lung tissue ferritin content. Mepacrine pretreatment (Mepa+I/R) slightly reduced the increase. The data shown are means±S.E.M. for the number of determinations shown in the parentheses. *$p<0.05$, compared with Sham.

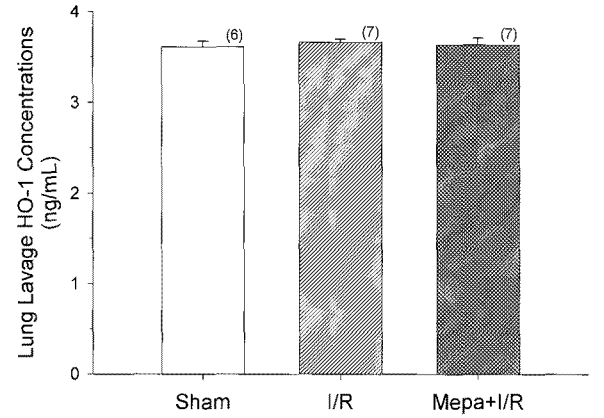

Fig. 6. There were no significant differences in lung lavage heme oxygenase-1 (HO-1) concentrations among all groups. Ischemia/reperfusion (I/R) did not change HO-1 levels compared to the values of sham rats. The data shown are means±S.E.M. for the number of rats shown in the parentheses.

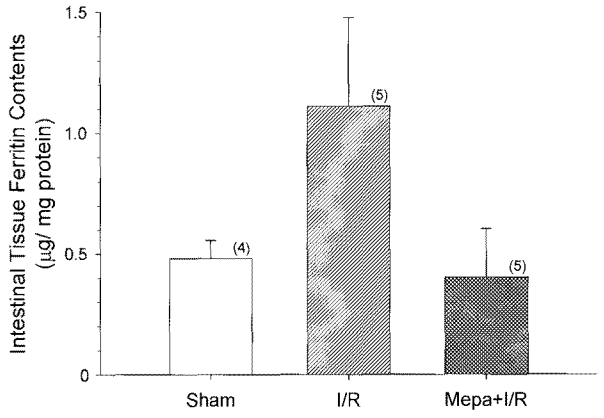

Fig. 8. Intestinal ischemia/reperfusion (I/R) had tendency to increase intestinal tissue ferritin contents. Mepacrine pretreatment (Mepa+I/R) attenuated the tendency. The data shown are means±S.E.M. for the number of rats shown in the parentheses.

DISCUSSION

The period of reperfusion after ischemia is thought to be a critical period of oxidant damage in many tissues, including heart, brain, gut, and other organs (Torti and Torti, 2002). Until now, however, there remains limited information about the injurious and protective mechanisms after I/R, and there have been no specific therapies for this disorder. Therefore, if there is a biomarker to predict progressing to ARDS, prophylactic therapy in at risk patients may get better results than administration to those with established disease.

Several previous studies (Park et al., 2003, 2004; Park and Lee, 2006; Park and Park, 2006) reported that mepacrine pretreatment significantly reduced ALI in severe hemorrhage- and intestinal I/R-induced ALI models. In this study, mepacrine pretreatment attenuated I/R-induced ALI. These results were useful in this study to evaluate predictability of biomarkers in three kinds of injury, including normal, I/R, and reduced I/R. In this study, serum and BAL fluid ferritin levels were well matched with the severity of ALI.

The antioxidant properties of ferritin are likely mediated by sequestration and storage of iron, thus preventing ferryl or hydroxyl radical generation (Klausner et al., 1993; Picard et al., 1998; Kakhlon et al., 2001), playing a key role in maintaining iron homeostasis. During postischemic reoxygenation of the rat liver, early ferritin degradation was counteracted by enhanced ferritin transcription. It was suggested that this might act to re-establish ferritin levels and limit reperfusion damage (Tacchini et al., 1997). Indeed, hypotransferrinemic mice, which have high levels of ferritin and lactoferrin, are resistant to hyperoxia-induced lung injury (Yang et al., 1999).

Although serum ferritin levels usually correlate with to-

tal body iron stores, several physiologic conditions such as inflammatory or infectious states (Harrison and Arosio, 1996) can increase serum ferritin as one of the acute-phase proteins (Gabay and Kushner, 1999). Rogers et al. (1990) also reported that the inflammatory induction of ferritin synthesis was different from iron-dependent ferritin gene expression. Serum ferritin levels may increase solely as a consequence of cellular necrosis or damage (Jacobs and Worwood, 1975). This possibility is supported by previous clinical findings of an association between serum ferritin levels and the injury severity score (Connelly et al., 1997; Sharkey et al., 1999; Ji et al., 2007).

In this study, serum and BAL ferritin had increased following I/R, and significantly decreased after mepacrine pretreatment. These results clearly matched with the findings of ALI in BAL fluid (Fig. 1, 2).

In accordance with previous studies, the concomitant increases of ferritin levels and severity of ALI in untreated I/R can suggest a possibility to use serum and/or BAL ferritin levels as a biomarker for predicting ARDS. These findings confirm previous *in vitro* (Balla et al., 1992) and *in vivo* (Park et al., 2003, 2004; Park and Lee, 2006; Park and Park, 2006) studies.

On the other hand, serum protein levels can be a mirror of the status of systemic inflammation, not the direct product of injured tissues. A previous report indicated that there was no close relationship between serum and BAL ferritin increases in a severe hemorrhage-induced ALI model. In spite of this limitation, serum ferritin concentration was well matched with the severity of lung injury (Park et al., 2004). Therefore, serum as well as BAL ferritin levels might be a useful predictor of the onset of severe ARDS.

In accordance with previous reports, serum HO-1 levels were greatly elevated following intestinal I/R. Unlike serum ferritin, serum HO-1 levels were elevated and higher than the values of untreated I/R rats in spite of reduced ALI caused by mepacrine pretreatment. In addition, the increase of serum HO-1 levels had a slower onset compared with ferritin levels. A potential explanation may be from a supporting report that intratracheal LPS rapidly induces ferritin protein in the lung independently of its mRNA synthesis or HO enzyme activity (Carraway et al., 1998). Therefore, there might be HO-1 independent induction of ferritin or different mechanism to induce these two proteins in an intestinal I/R injury model.

Mumby et al. (2004) reported that HO-1 concentrations were elevated in lung tissue and BAL fluid taken from patients with ARDS compared to controls. In addition, there was a significant correlation between BAL cellular HO-1 and BAL fluid ferritin concentrations in ARDS patients (Mumby et al., 2004). In this study, however, BAL fluid HO-1 levels were same in all groups, whereas serum HO-1 levels were greatly increased in both I/R injury groups. This might be explained by the difference of the severity of injury or measurement. However, there needs be more studies to elucidate the exact regulatory mechanisms between HO-1 and ferritin.

The present study also demonstrates that the elevations of ferritin in injured tissues, including intestines and lung, were parallel with the serum and BAL fluid levels. These results also support the reliability of serum and/or BAL ferritin levels as a predictive biomarker for the onset of severe ARDS in an intestinal I/R-induced ALI model. However, it remains unclear whether this would be constant in other models of ALI or over long-time observations.

It is known that HO-1 induction responds to common causes of oxidative stress to the airways, including hyperoxia, hypoxia, endotoxemia, heavy metal exposure, bleomycin, diesel exhaust particles, and allergen exposure (Lee et al., 2000; Li et al., 2000). Several studies suggest that HO-1, which is induced after ischemia or oxidant stress, may exert protective effects in I/R, oxidant injury, or endotoxic shock (Otterbein et al., 1995, 1997; Lee et al., 1996). In addition, HO-1 may contribute to ischemic preconditioning, a process of acquired cellular protection against I/R injury, as observed in transplanted lungs in guinea pigs (Soncul et al., 1999). Also, HO-1 overexpression provided potent protection against cold I/R injury in a rat model through an anti-apoptotic pathway (Katori et al., 2002).

Although there was some difference in the temporal pattern of inductions of ferritin and HO-1, both had similar cytoprotective functions against oxidative stress. Indeed, HO-1 overexpression by pharmacological or genetic engineering has exerted protective functions in an I/R injury model (Amersi et al., 1999; Kato et al., 2001; Ryter et al, 2006; Scott et al, 2007). In addition, maintenance of low free iron by increased ferritin levels plays an important role in antioxidant cytoprotection. Like HO-1, overexpression of ferritin heavy chain protected rat livers from I/R injury, and prevents hepatocellular damage upon transplantation into syngeneic patients (Berberat et al., 2003). Furthermore, Balla et al. (1992) already reported that ferritin, rather than heme oxygenase, was the ultimate protectant in oxidant mediated endothelial cell injury *in vitro*. These data means ferritin may be a good biological marker as well as a cytoprotectant in I/R injury patients. Therefore, these two stress induced proteins are important for not only as a diagnostic biological maker, but also as part of a new therapeutic regimen for wide inflammatory disorders.

In conclusion, these results suggested that the serum and lung lavage ferritin concentrations were correlated with intestinal I/R-induced lung injury. However, HO-1 levels were not exactly matched with the lung injury in intestinal I/R-induced ALI model. Therefore, there might be a different mechanism in the induction of ferritin and HO-1 following intestinal I/R injury. Further studies will be required to elucidate the precise mechanisms of the changes of these two proteins in the ALI model or other inflammatory diseases.

ACKNOWLEDGEMENTS

This work was supported by the grant of Research Institute of Medical Science, Catholic University of Daegu (2007).

REFERENCES

Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR, Kolls JK, Alam J, Ritter T, Volk HD, Farmer DG, Ghobrial RM, Busuttil RW, Kupiec-Weglinski JW. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest* 104: 1631−1639, 1999.

Attuwaybi BO, Hassoun HT, Zou L, Kozar RA, Kone BC, Weisbrodt NW, Moore FA. Hypothermia protects against gut ischemia reperfusion-induced impaired intestinal transit by inducing heme oxygenase-1. *J Surg Res* 115: 48−55, 2003.

Balla G, Jacob HS, Balla J, Rosenberg M, Nath KA, Apple F, Eaton

JW, Vercellotti GM. Ferritin: a cytoprotective antioxidant stratagem of endothelium. *J Biol Chem* 267: 18148−18153, 1992.

Balla J, Jacob HS, Balla G, Nath KA, Eaton JW, Vercellotti GM. Endothelial cell heme uptake from heme proteins: induction of sensitization and desensitization to oxidant damage. *Proc Natl Acad Sci USA* 90: 9285−9289, 1993.

Balla J, Nath KA, Balla G, Juckett MB, Jacob HS, Vercellotti GM. Endothelial cell heme oxygenase and ferritin induction in rat lung by hemoglobin in vivo. *Am J Physiol* 268: L321−327, 1995.

Berberat PO, Katori M, Kaczmarek E, Anselmo D, Lassman C, Ke B, Shen X, Busuttil RW, Yamashita K, Csizmadia E, Tyagi S, Otterbein LE, Brouard S, Tobiasch E, Bach FH, Kupiec-Weglinski JW, Soares MP. Heavy chain ferritin acts as an antiapoptotic gene that protects livers from ischemia reperfusion injury. *FASEB J* 17: 1724−1726, 2003.

Cairo G, Tacchini L, Pogliaghi G, Anzon E, Tomasi A, Bernelli-Zazzera A. Induction of ferritin synthesis by oxidative stress. Transcriptional and post-transcriptional regulation by expansion of the "free" iron pool. *J Biol Chem* 270: 700−703, 1995.

Carraway MS, Ghio AJ, Taylor JL, Piantadosi CA. Induction of ferritin and heme oxygenase-1 by endotoxin in the lung. *Am J Physiol* 275: L583−592, 1998.

Choi AM, Alam J. Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 15: 9−19, 1996.

Connelly KG, Moss M, Parsons PE, Moore EE, Moore FA, Giclas PC, Seligman PA, Repine JE. Serum ferritin as a predictor of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 155: 21−25, 1997.

Eisenstein RS, Garcia-Mayol D, Pettingell W, Munro HN. Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron. *Proc Natl Acad Sci USA* 88: 688−692, 1991.

Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK, Tysoe SA, Wolosker H, Baranano DE, Dore S, Poss KD, Snyder SH. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol* 1: 152−157, 1999.

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448−454, 1999.

Grace PA. Ischaemia-reperfusion injury. *Br J Surg* 81: 637−647, 1994.

Halliwell B. Oxidants and human diseases: some new concepts. *FASEB J* 1: 358−364, 1987.

Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1275: 161−203, 1996.

Hassoun HT, Kozar RA, Kone BC, Safi HJ, Moore FA. Intraischemic hypothermia differentially modulates oxidative stress proteins during mesenteric ischemia/reperfusion. *Surgery* 132: 369−376, 2002.

Jacobs A, Worwood M. Ferritin in serum. Clinical and biochemical implications. *N Engl J Med* 292: 951−956, 1975.

Ji YS, Kim NH, Jung HG, Ha DY, Jung KH. Siginificance of serum ferritin in multiple trauma patients with acute respiratory distress syndrome. *J Korean Soc Traumatol* 20: 57−64, 2007.

Juckett MB, Balla J, Balla G, Jessurun J, Jacob HS, Vercellotti GM. Ferritin protects endothelial cells from oxidized low density lipoprotein in vitro. *Am J Pathol* 147: 782−789, 1995.

Kakhlon O, Gruenbaum Y, Cabantchik ZL. Repression of the heavy ferritin chain increases the labile iron pool of human K562 cells. *Biochem J* 356: 311−316, 2001.

Kato H, Amersi F, Buelow R, Melinek J, Coito AJ, Ke B, Busuttil RW, Kupiec-Weglinski JW. Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. *Am J Transplant* 1: 121−128, 2001.

Katori M, Buelow R, Ke B, Ma J, Coito AJ, Iyer S, Southard D, Busuttil RW, Kupiec-Weglinski JW. Heme oxygenase-1 overexpression protects rat hearts from cold ischemia/reperfusion injury via an anti-apoptotic pathway. *Transplantation* 73: 287−292, 2002.

Kennedy TP, Rao NV, Hopkins C, Pennington L, Tolley E, Hoidal JR. Role of reactive oxygen species in reperfusion injury of the rabbit lung. *J Clin Invest* 83: 1326−1335, 1989.

Kim YM, Bergonia H, Lancaster JR Jr. Nitrogen oxide-induced autoprotection in isolated rat hepatocytes. *FEBS Lett* 374: 228−232, 1995.

Klausner RD, Rouault TA, Harford JB. Regulating the fate of mRNA: the control of cellular iron metabolism. *Cell* 72: 19−28, 1993.

Lee PJ, Alam J, Weigand GW, Choi AMK. Overexpression of heme oxygenase-1 in human pulmonary epithelial cells results in cell growth arrest and increased resistance to hyperoxia. *Proc Natl Acad Sci USA* 93: 10393−10398, 1996.

Lee PJ, Camhi SL, Chin BY, Alam J, Choi AM. AP-1 and STAT mediate hyperoxia-induced gene transcription of heme oxygenase-1. *Am J Physiol Lung Cell Mol Physiol* 279: L175−182, 2000.

Li N, Venkatesan MI, Miguel A, Kaplan R, Gujuluva C, Alam J, Nel A. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. *Immunol* 165: 3393−3401, 2000.

Minamiya Y, Tozawa K, Kitamura M, Saito S, Ogawa J. Platelet-activating factor mediates intercellular adhesion molecule-1-dependent radical production in the nonhypoxic ischemia rat lung. *Am J Respir Cell Mol Biol* 19: 150−157, 1998.

Mumby S, Upton RL, Chen Y, Stanford SJ, Quinlan GJ, Nicholson AG, Gutteridge JM, Lamb NJ, Evans TW. Lung heme oxygenase-1 is elevated in acute respiratory distress syndrome. *Crit Care Med* 32: 1130−1135, 2004.

Otamiri T, Lindahl M, Tagasson C. Phospholipase A2 inhibition prevents mucosal damage associated with small intestine ischemia in rats. *Gut* 29: 489−494, 1988.

Otterbein L, Chin BY, Otterbein SL, Lowe VC, Fessler HE, Choi AM. Mechanism of hemoglobin-induced protection against endotoxemia in rats: a ferritin-independent pathway. *Am J Physiol* 272: L268−275, 1997.

Otterbein L, Sylvester SL, Choi AMK. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. *Am J Respir Cell Mol Biol* 13: 595−601, 1995.

Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 24: 449−455, 2003.

Park SD, Park YY. Changes of serum ferritin in acute lung injury induced by intestinal ischemia/reperfusion. *Korean J Physiol Pharmacol* 10: 187−191, 2006.

Park YY, Lee YM. Effects of aspirin on the pathogenesis of acute lung injury in rats subjected to hemorrhage. *Tuberc Respir Dis* 60: 83−91, 2006.

Park YY, Hybertson BM, Wright RM, Fini MA, Elkins ND, Repine JE. Serum ferritin elevation and acute lung injury in rats subjected to hemorrhage: Reduction by mepacrine treatment. *Exp Lung Res* 30: 571−584, 2004.

Park YY, Hybertson BM, Wright RM, Repine JE. Serum ferritin increases in hemorrhaged rats that develop acute lung injury: effect of an iron-deficient diet. *Inflammation* 27: 257−263, 2003.

Picard V, Epsztejn S, Santambrogio P, Cabantchik ZI, Beaumont C. Role of ferritin in the control of the labile iron pool in murine erythroleukemia cells. *J Biol Chem* 273: 15382−15386, 1998.

Repine JE. Scientific perspectives on adult respiratory distress syndrome. *Lancet* 339: 466−469, 1992.

Rogers JT, Bridges KR, Durmowicz GP, Glass J, Auron PE, Munro HN. Translational control during the acute phase response. Ferritin synthesis in response to interleukin-1. *J Biol Chem* 265: 14572−14578, 1990.

Ryter SW, Alarm J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev* 86: 583−650, 2006.

Scott JR, Chin BY, Bilbana MH, Otterbein LE. Restoring HOmeostasis: is heme oxygenase-1 ready for the clinic? *Trends Pharmacol Sci* 28: 200−205, 2007.

Sharkey RA, Donnelly SC, Connelly KG, Robertson CE, Haslett C, Repine JE. Initial serum ferritin levels in patients with multiple trauma and the subsequent development of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 159: 1506−1509,

1999.

Soncul H, Oz E, Kalaycioglu S. Role of ischemic preconditioning on ischemia-reperfusion injury of the lung. *Chest* 115: 1672–1677, 1999.

Tacchini L, Recalcati S, Bernelli-Zazzera A, Cairo G. Induction of ferritin synthesis in ischemic reperfused rat liver: analysis of the molecular mechanisms. *Gastroenterology* 113: 946–953, 1997.

Taylor JL, Carraway MS, Piantadosi CA. Lung-specific induction of heme oxygenase-1 and hyperoxic lung injury. *Am J Physiol* 274: L582–590, 1998.

Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 61: 748–755, 1968.

Torti FM, Torti SV. Regulation of ferritin genes and protein. *Blood* 99: 3505–3516, 2002.

Vile GF, Basu-Modak S, Waltner C, Tyrrell RM. Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci USA* 91: 2607–2610, 1994.

Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 324: 1334–1349, 2000.

Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 2: 87–90, 1996.

Yang F, Coalson JJ, Bobb HH, Carter JD, Banu J, Ghio AJ. Resistance of hypotransferrinemic mice to hyperoxia-induced lung injury. *Am J Physiol* 277: L1214–1223, 1999.

Yet SF, Pellacani A, Patterson C, Tan L, Folta SC, Foster L, Lee WS, Hsieh CM, Perrella MA. Induction of heme oxygenase-1 expression in vascular smooth muscle cells: a link to endotoxic shock. *J Biol Chem* 272: 4295–4301, 1997.