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SHORT COMMUNICATION

Regenerative Response in Acute Renal Failure Due to Vitamin E Deficiency and Glutathione Depletion in Rats

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ABSTRACT. In this study, we investigated some factors contributing to renal regeneration after acute renal failure (ARF) induced by vitamin E (VE) deficiency and glutathione (GSH) depletion. Acute renal failure was induced by feeding rats a vitamin E-deficient diet for 6 weeks and then injecting buthionine sulfoximine (BSO), a glutathione-depleting agent. The level of hepatocyte growth factor (HGF), a renotropic factor for regeneration in the kidney, showed a transient increase at 5 hr after the BSO treatment. Subsequently, renal ornithine decarboxylase (ODC) activity, a marker of G_1 phase, and labeling index (LI) of proliferating cell nuclear antigen (PCNA), a marker of DNA synthesis (S phase), reached peaks at 10 and 53 hr after the injection, respectively. Thus, it appears that the increase in ornithine decarboxylase activity and subsequent elevation in proliferating cell nuclear antigen labeling index following the increase in the hepatocyte growth factor level in the kidneys are closely related to the renal regenerative response after acute renal failure. BIOCHEM PHARMACOL **56**;4: 543–546, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. acute renal failure; vitamin E; glutathione; hepatocyte growth factor; renal regeneration

It has been suggested that oxidative stress might be involved in the pathogenesis of ARF** in animal models [1, 2]. In fact, we clarified the above suggestion in an animal model of ARF [3, 4]. In these reports, depletion of GSH by treatment with BSO, a specific inhibitor of γ-glutamylcysteine synthetase, did not, by itself, cause acute tubular necrosis in rat kidney. However, depletion of GSH combined with VE deficiency caused severe necrosis in the proximal tubules of the kidney. In addition, we found that formation of lipofuscin, a cross-link compound of malonylaldehyde with amino groups of phospholipid, increased in this ARF model, implying that lipid peroxidation played an important role in the kidney injury [3, 4].

In general, renal tubular cells do not proliferate under normal intact conditions, whereas a drastic regeneration in the proximal tubules occurs with a few days after ischemic or nephrotoxic renal damage [5]. Currently, there are increasing lines of evidence that several growth factors play important roles in driving the renal regenerative process [6]. Of these factors, it has been reported that HGF can stimulate mitogenesis of rat hepatocytes and that it plays a key role in initiating the regenerative process of the liver after chemical liver injury [7]. Furthermore, recent studies suggest that ODC, the rate-limiting enzyme of polyamine biosynthesis and a marker of G1 phase, acts as a signal related to liver regeneration [8–10]. Similarly, the increase in renal HGF level precedes the first round of DNA synthesis during renal regeneration after ischemic or mercuric chloride-induced ARF [11]. These reports suggest that HGF acts as a trigger for renal regeneration as well as hepatic regeneration. However, the role of HGF in renal regeneration after ARF induced by VE deficiency and GSH depletion is not clear at present. In this context, the present study was undertaken to assess if HGF could play an important role in driving the renal regeneration after VE deficiency and GSH depletion-induced ARF, and if ODC could be a signal related to HGF-induced DNA synthesis.

MATERIALS AND METHODS

A VE-deficient diet was prepared as described previously [3]. Commercial safflower oil that had been treated with powdered activated charcoal was used as VE-deficient oil [3], and a mixture of vitamins without VE based on the

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** *Abbreviations:* ARF, acute renal failure; BSO, buthionine sulfoximine; BUN, serum urea nitrogen; GSH, glutathione; HGF, hepatocyte growth factor; LDH, lactate dehydrogenase; LI, labeling index; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen; and VE, vitamin E.

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composition of a defined mixture of vitamins (AIN-76) was prepared. The basal diet consisted of casein, 20%; sucrose, 50%; cellulose powder, 5%; mineral mixture (AIN-76), 3.5%; vitamin mixture, 1%; choline bitartrate, 0.2%; corn starch, 10.3%; VE-deficient safflower oil, 10%. A VE-sufficient diet was prepared by addition of VE to the basal diet. The contents of VE in the VE-sufficient and VE-deficient diets were 100 IU/kg diet and 0.5 IU/kg diet, respectively.

Four-week-old Wistar rats (Clea) were fed a VE-sufficient or a VE-deficient diet for 6 weeks and were given an i.p. injection of BSO solution, dissolved in 0.9% NaCl, at a dose of 0.1 mmol/kg of body weight. Control rats received the vehicle only. By feeding the VE-deficient diet for 6 weeks, the renal level of γ-tocopherol in the VE-deficient group was 95% lower than that in the normal group [3]. Fresh diets were given every day and water was provided *ad lib*. For a time course experiment of BSO administration to rats, the animals were anesthetized with ether, and sacrificed by the collection of blood from the hearts at 0, 5, 10, 29, 53 and 101 hr after BSO injection. At each time after the injection, LDH, BUN, and serum creatinine were quantified by standard methods with an autoanalyzer (model 705; Hitachi Ltd.). Three rats were used in each experimental group.

In order to prepare a sample for the assay of the GSH and HGF levels and ODC activity, the same kidney was used in each group. The separate 0.2-g samples of renal tissues were taken for the next analysis, that is, of total GSH level, ODC activity, and HGF level. In order to determine total GSH level, the renal tissue was homogenized at 4° for 1 min in 5 mL of 0.1 M phosphate buffer (pH 7.5) and 2.5 mL of 10% solution of trichloroacetic acid. Total GSH (reduced and oxidized) concentration was quantified as previously described [12]. The tissue assay of ODC activity was homogenized at 4° for 0.5 min in 0.8 mL of 0.05 M Tris-HCl (pH 7.5) containing 0.25 M sucrose. The homogenate was centrifuged at 100,000 *g* for 30 min, and the supernatant was assayed for ODC activity by measurement of the amount of radioactive CO_2 liberated from L-[1-^{14}C]ornithine [13]. The tissue for immunoblot analysis of HGF was homogenized at 4° for 0.5 min in 0.05 M Tris-HCl (pH 7.5) containing 2 M NaCl, 0.01% Tween 80, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The homogenate sample was centrifuged at 100,000 *g* for 30 min, and the supernatant was used as the sample for the assay of HGF level. The supernatant was boiled for 3 min in SDS-PAGE sample buffer under nonreducing conditions. The sample was subjected to 7.5% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Immunoblot analysis was performed with an anti-HGF antibody (IBL) using the ECL detection system (Amersham). The signal band was quantified using a densitometer (CS-9300PC, Shimazu).

The kidney was fixed with 10% buffered formalin. The tissue was embedded in paraffin, sectioned and stained with hematoxylin and eosin for general histologic examination.

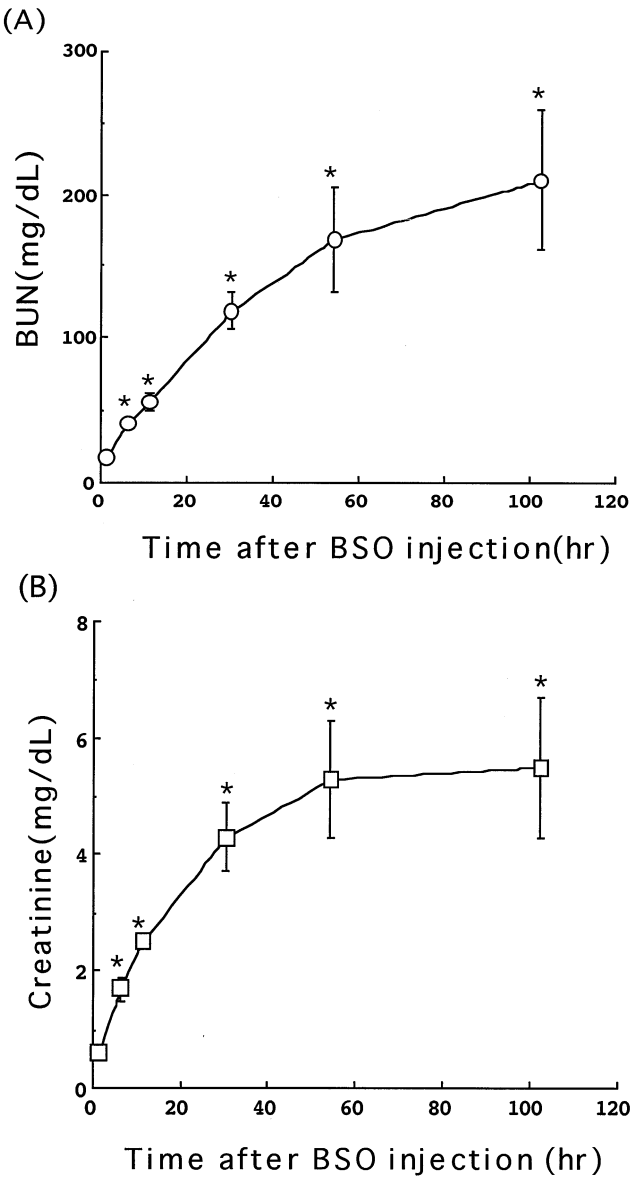

FIG. 1. Time course change in total GSH content of the kidney in VE-deficient rats after BSO injection. Three rats were used in each experimental group and each value represents the mean ± SE. *Significantly different from the value at 0 hr.

For PCNA staining, the deparafinized sections of the tissue were stained with a avidin-biotin-peroxidase method with an anti-PCNA antibody (Dakopatts). PCNA LI was determined by counting more than 1,000 nuclei in randomly selected microscopic fields.

Statistical comparisons between the value at 0 hr and that at each point were carried out using Student's *t*-test. $P < 0.05$ was used for significant difference.

RESULTS AND DISCUSSION

BUN and serum creatinine levels as markers of ARF were elevated from 5 to 101 hr (Fig. 1), indicating that ARF was caused by the combination of GSH depletion and VE

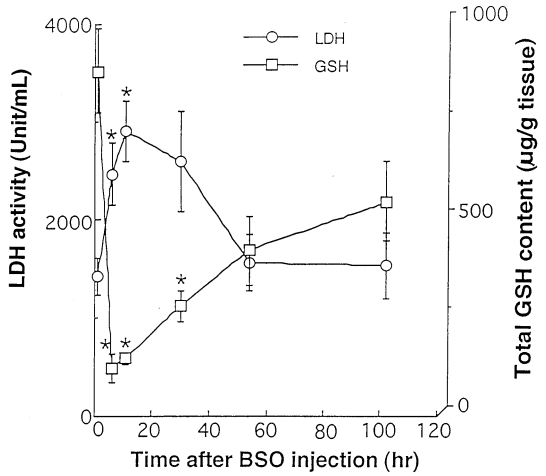

FIG. 2. Time-course changes in BUN (A) and serum creatinine (B) in VE-deficient rats after BSO injection. Three rats were used in each experimental group and each value represents the mean ± SE. *Significantly different from the value at 0 hr.

deficiency as previously reported [3]. As shown in Fig. 2, time-course changes in the serum level of LDH showed a reciprocal pattern compared to that in total GSH content of the kidney. After BSO injection, the LDH level was maximal at 10 hr, whereas the GSH content reached a minimum level at 5 hr, with both levels then gradually returning toward the basal level at 0 hr. Time-course changes in renal HGF level, ODC activity and PCNA LI are shown in Fig. 3. The HGF level at 5 hr after BSO injection transiently increased, and then returned to the basal level. Additionally, the HGF level in the VE-deficient group was similar to that in the normal group. The mean values for densitometrical analyses of the HGF bands (N = 2) were as follows: normal, 100%; 0 hr, 121%; 5 hr, 325%; 10 hr, 95%; 29 hr, 91%. Following the increase in the HGF level, the ODC activity showed a transient increase at 10 hr after the injection with a significant difference. After the ODC activity decreased below the basal level, the PCNA LI reached a maximal level at 53 hr after the injection, which was 20-fold higher than the basal level. Also, both levels at 0 hr were not different from those in the normal group (data not shown). In histologic analysis, severe necrosis in renal proximal tubules was observed at 5 hr, and regeneration in the renal tubules occurred from 53 to 101 hr after the injection (data not shown).

It has been reported that renal injury during reperfusion after ischemia is caused by the hydrogen peroxide produced, and that this injury is closely related to the GSH content in the kidney [14]. Our previous report showed that depletion of GSH by treatment with BSO did not, by itself, cause ARF [3]. However, severe necrosis was induced in the proximal tubules of the kidney by the combination of GSH depletion and VE deficiency [3]. This report suggests that the GSH level is a determining factor for the induction of ARF in the state of VE deficiency. In other words, the severity of the ARF can be reduced by attenuating the

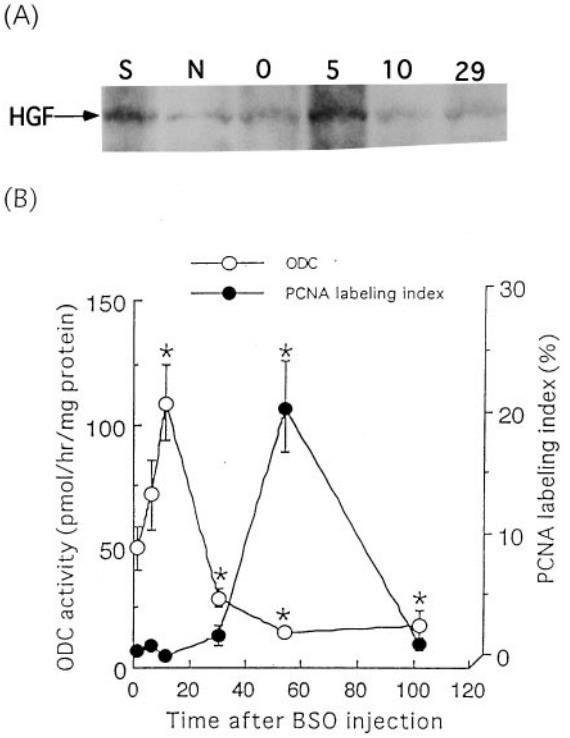

FIG. 3. Time-course changes in renal HGF level (A), ODC activity (B) and PCNA LI (B) in VE-deficient rats after BSO injection. In immunoblot analysis of HGF, three samples in each experimental group were combined, and 50 μg of the combined sample was applied to each lane. This experiment was carried out twice and the representative result is shown in Fig. 2A. S, purified rat HGF; N, HGF in the normal group; 0, HGF at 0 hr after BSO injection; 5, HGF at 5 hr; 10, HGF at 10 hr; 29, HGF at 29 hr. In analysis of ODC activity and PCNA LI, three rats were used in each experimental group and each value represents the mean ± SE. *Significantly different from the value at 0 hr.

decrease in the GSH level in the VE deficiency. In fact, the decrease in the GSH level by treatment with a BSO dose (0.1 mmol/kg) lower than the previous dose (1 mmol/kg) contributed to the reduction in the severity of the ARF, leading to the regenerative response after the ARF. In this study, we confirmed the VE deficiency and GSH depletion-induced ARF from the time course changes in BUN and serum creatinine levels. Also, we checked subsequent regenerative response based on histologic observation as well as the time-course changes in the serum level of LDH and total GSH content of the kidney.

Recovery from ischemic or toxic ARF is due to replicative repair and replacement of injured and necrotic tubular cells by cells that are actively dividing [15]. In general, regenerative cells that transfer from normal low (G_0 phase) to high rates (S phase) of DNA synthesis and mitosis (M phase) usually involve the activation of growth factor-related signal pathways [16]. In liver regeneration, HGF produced by nonparenchymal liver cells at an early stage of regeneration challenges hepatocytes, transduces growth signals such as ODC induction, and finally induces DNA

synthesis [7, 10]. In this study, we observed the increase in ODC activity and the subsequent elevation in PCNA LI following the increase in the HGF level in the kidney after VE deficiency and GSH depletion. Our present findings also indicated that PCNA positive cells were mainly restricted to renal tubular cells at the damaged areas (data not shown). In addition, an immunohistochemical study has shown that HGF was induced in renal interstitial cells after ischemic or nephrotoxic ARF, thereby challenging renal tubular cells [11]. Taking together our present findings and these reports, it appears that HGF stimulates the regenerative response of the renal tubular cells after VE deficiency and GSH depletion-induced ARF, and that the induction of ODC acts as an important signal related to HGF-triggered DNA synthesis. However, in order to further clarify the regenerative process after the ARF, it is necessary to estimate which signal-transduction pathway is activated by HGF in the course of the ODC induction.

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