

Angiotensin-converting enzyme inhibition by lisinopril enhances liver regeneration in rats

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Abstract

Bradykinin has been reported to act as a growth factor for fibroblasts, mesangial cells and keratinocytes. Recently, we reported that bradykinin augments liver regeneration after partial hepatectomy in rats. Angiotensin-converting enzyme (ACE) is also a powerful bradykinin-degrading enzyme. We have investigated the effect of ACE inhibition by lisinopril on liver regeneration after partial hepatectomy. Adult male Wistar rats underwent 70% partial hepatectomy (PH). The animals received lisinopril at a dose of 1 mg kg body weight⁻¹ day⁻¹, or saline solution, intraperitoneally, for 5 days before hepatectomy, and daily after surgery. Four to six animals from the lisinopril and saline groups were sacrificed at 12, 24, 36, 48, 72, and 120 h after PH. Liver regeneration was evaluated by immunohistochemical staining for proliferating cell nuclear antigen using the PC-10 monoclonal antibody. The value for the lisinopril-treated group was three-fold above the corresponding control at 12 h after PH (P<0.001), remaining elevated at approximately two-fold above control values at 24, 36, 48 (P<0.001), and at 72 h (P<0.01) after PH, but values did not reach statistical difference at 120 h after PH. Plasma ACE activity measured by radioenzymatic assay was significantly higher in the saline group than in the lisinopril-treated group (P<0.001), with 81% ACE inhibition. The present study shows that plasma ACE inhibition enhances liver regeneration after PH in rats. Since it was reported that bradykinin also augments liver regeneration after PH, this may explain the liver growth stimulating effect of ACE inhibitors.

Liver regeneration is a unique phenomenon in which the loss of hepatic tissue rapidly induces an orchestrated response involving sequential changes in gene expression, growth factor production, and morphologic structure (1,2). Several substances with potentially important roles in liver regenera-

tion have been recently identified (2). The kallikrein-kinin system exerts a variety of biological effects, including vasodilatation, increased vascular permeability and smooth muscle relaxation or contraction (3-5). Bradykinin has been reported to act as a growth factor for several cell types, such as mesangi-

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al cells (6), keratinocytes (7), and fibroblasts (8). We reported that bradykinin augments liver regeneration after partial hepatectomy in rats (9). Angiotensin-converting enzyme (ACE) is also known as kininase II, a powerful bradykinin-degrading enzyme (3). ACE inhibitors have two main mechanisms of action: the simultaneous prevention of angiotensin II production and bradykinin degradation (4).

In this study, we have investigated the effect of ACE inhibition by lisinopril on liver regeneration. Male Wistar rats (180-220 g) underwent 70% partial hepatectomy (PH) as described by Higgins and Anderson (10). The rats received lisinopril (MK 521; Merck, White House Station, NJ, USA) at a dose of $1 \text{ mg kg body weight}^{-1} \text{ day}^{-1}$, or saline solution in a proportional volume, intraperitoneally, for 5 days before hepatectomy, and daily after surgery. Plasma ACE activity was measured by radioenzymatic assay according to the method of Ryan et al. (11). Four to six animals from the lisinopril and saline groups were sacrificed under ether anesthesia at 12, 24, 36, 48, 72, and 120 h after PH. Sham operations consisting of laparotomy and manipulation of the liver were also carried out. Just before PH and 24 h after PH, blood samples (2 ml/kg) were collected for measurement of ACE activity, and mean arterial blood pressure was monitored for 30 min via a catheter placed into the left femoral artery (5 animals per group).

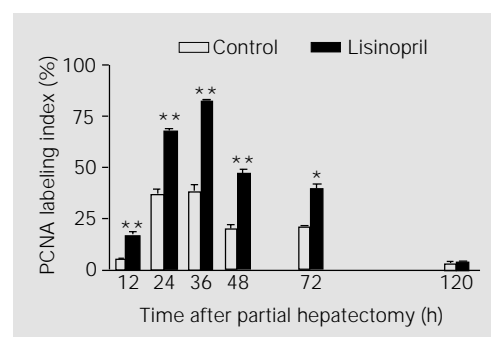
Liver regeneration was evaluated by immunohistochemical staining for proliferat-

ing cell nuclear antigen (PCNA) using monoclonal primary anti-PCNA antibody (PC-10; DAKO A/S, Glostrup, Denmark) on formalin-fixed and paraffin-embedded liver tissues (12-14). Sections were cut at $4 \mu\text{m}$, mounted on poly-L-lysine-coated glass slides, deparaffinized, rehydrated in an increasing alcohol series, placed in PBS, and treated with 2% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity.

Nonspecific protein binding was blocked by preincubation with 5% normal horse serum diluted in PBS for 30 min. This was followed by incubation with PC-10 monoclonal primary antibody, diluted 1:40 in PBS for 120 min at 25°C . The sections were then incubated for 35 min with a biotinylated horse antimouse immunoglobulin. The reaction product was detected with an avidin-biotin-peroxidase complex and diaminobenzidine was used as a chromogen substrate. Positive and negative controls were used to assess and control the staining procedure. Sections were examined blindly at high power (400X), and 10 fields were chosen at random. Nuclear labeling indices for PCNA (positive nuclei/total number of counted nuclei) were determined by evaluation of at least 1,000 hepatocyte nuclei. Data are reported as mean \pm SEM. The Student *t*-test was used for statistical analysis of the data.

The results concerning the PCNA labeling index were quite consistent (Figure 1). The value for the lisinopril-treated group was three-fold above the corresponding control at 12 h ($P < 0.001$), remaining elevated at approximately two-fold above control values at 24, 36, 48 ($P < 0.001$), and at 72 h ($P < 0.01$), but values were not significantly different at 120 h ($P = 0.47$). Plasma ACE activity was significantly higher in the saline group ($112.24 \pm 12.22 \text{ nmol mg protein}^{-1} \text{ h}^{-1}$) than in the lisinopril-treated group ($21.82 \pm 5.14 \text{ nmol mg protein}^{-1} \text{ h}^{-1}$; $P < 0.001$), with an 81% rate of ACE inhibition. The values of mean arterial blood pressure were not signif-

Figure 1 - Effect of lisinopril on nuclear labeling index by proliferating cell nuclear antigen (PCNA) immunostaining. Lisinopril- and saline-treated rats were sacrificed at 12, 24, 36, 48, 72 and 120 h after 70% partial hepatectomy. Each point represents the mean \pm SEM (4 to 6 animals per group). The nuclear labeling index for sham-operated rats was $1.08 \pm 0.15\%$ ($N = 5$). * $P < 0.01$, ** $P < 0.001$ compared to saline-treated group (Student *t*-test).



icantly different (106 ± 2 mmHg vs 104 ± 2 mmHg).

The present study shows that ACE inhibition enhances liver regeneration after PH. In addition to inhibiting the renin-angiotensin system, ACE inhibitors potentiate the biological actions of kinins (4). It was reported that bradykinin augments liver regeneration after PH (9), which may explain the liver growth stimulating effect of ACE inhibitors. Recent investigations have reported that ACE inhibition increases renal hepatocyte growth factor (HGF) mRNA and cardiac HGF con-

centration in experimentally hypertensive rats, and that angiotensin II downregulates HGF production in mesangial cells in a cultured model (15,16). HGF was initially identified as the most potent growth factor for hepatocytes (17). Another interpretation of these data is that angiotensin II may also downregulate hepatocellular HGF production, and that hepatic HGF concentration might be elevated in response to ACE inhibition, potentially resulting in the improvement of liver regeneration.

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