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Human C1 inhibitor attenuates liver ischemia-reperfusion injury and promotes liver regeneration

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ABSTRACT

Liver ischemia-reperfusion injury (IRI) is a well-known cause of morbidity and mortality after liver transplantation (LT). Activation of the complement system contributes to the pathogenesis of IRI. Effective treatment strategies aimed at reducing hepatic IRI and accelerating liver regeneration could offer major benefits in LT. Herein, we investigated the effect of C1-esterase inhibitor (human) [C1-INH] on IRI and liver regeneration. Mice were subjected to 60-min partial IRI, with or without 70% partial hepatectomy, or CCl_4-induced acute liver failure. Before liver injury, the animals were pretreated with intravenous C1-INH or normal saline. Liver IRI was evaluated using serum levels of alanine aminotransferase, serum interleukin-6, and histopathology. Liver samples were stained for specific markers of regeneration (5-bromo-2'-deoxyuridine [BrdU] staining and proliferating cell nuclear antigen [PCNA]). Histology, serum interleukin-6, and alanine aminotransferase release revealed that C1-INH treatment attenuated liver injury compared with controls. Improved animal survival and increased number of BrdU- and PCNA-positive cells were observed in C1-INH–treated animals which underwent IRI + partial hepatectomy or CCl_4 injection compared with control group. These data indicate that complement plays a key role in IRI and liver regeneration. C1-INH represents a potential therapeutic strategy to reduce IRI and promote regeneration in LT.

1. Introduction

Liver transplantation (LT) has evolved as the therapy of choice for patients with end-stage liver disease regardless of its etiology. Liver ischemia-reperfusion injury (IRI) is the leading cause of hepatocellular injury, mortality, and morbidity after LT [1]. IRI causes liver damage and impairs regeneration after LT. Pathogenic mechanisms involved in IRI have been extensively investigated [2–9]. The interaction among signaling pathways involved in IRI are highly complex and are not well described. IRI represents inflammatory process that includes activation of innate immunity and cytokine release followed by hepatocyte and sinusoidal endothelial damage.

Inflammatory agents known to potentiate hepatic IRI injury have been well described and include tumor necrosis factor, interleukin (IL)-1β, and IL-6 [3–5]. In particular, IL-6 induces adhesion molecule and chemokine expression leading to rapid infiltration of neutrophils, which are among the principal effectors of liver IRI injury [6]. Toll-like receptors (TLRs) are pattern recognition receptors that recognize conserved pathogen-associated molecular patterns. Activation of innate immunity through TLR ligation occurs in IRI [7–9].

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Studies in models of myocardial [2,3], intestinal [4], renal [5], and hepatic IRI have provided evidence for the role of complement system activation in the pathogenesis of IRI [9].

The effect of complement activation during liver IRI is diverse. Several complement components become localized in the ischemic liver [7,10–12]. Furthermore, plasma levels of activated complement components are increased after liver IRI in pigs and humans [10,12]. Complement activation products and complement membrane attack complex have deleterious effects on the liver and contribute to neutrophil chemotaxis, vasoconstriction, microcirculation failure, increased vascular permeability, and cell death [10–12].

Previous studies show that the activation of complement is activated in ischemic liver via the alternative and classical pathways and plays an important role in liver IRI [7,10,13,14]. C1-INH is a member of the serine protease inhibitor (serpin) family and is a major inhibitor of the classical complement pathway [14]. Because of its anti-inflammatory properties, C1-INH has been evaluated in various animal models for diseases such as sepsis and myocardial infarction [15–19]. These studies have yielded promising results, and initial studies with this compound in patients with these diseases have been done [20–22]. In the present study, the role of the human C1-INH in liver IRI and regeneration in a relevant preclinical model was investigated.

2. Materials and methods

2.1. Animals

This study was approved by the Institutional Animal Care and Use Committee at the University of Massachusetts Medical School. Wild-type (C57BL6), TLR-4−/−, and C3−/− mice were used. To further evaluate the mechanistic role of C1-INH, we used C3−/− and TLR4 knockout mice. C3 is downstream to C1 and a major component of complement system. TLR4 has also been shown to be involved in liver IRI.

All mice were used when aged 8–10 wk. Mice were fed a pellet diet with water and libitum and kept on a 12-h light/12-h dark cycle. For all procedures, mice were anesthetized with an intraperitoneal injection of 0.05 mL/10 g body weight of a "ketamine cocktail," consisting of ketamine (13 mg/mL), xylazine (2.6 mg/mL), and acepromazine (0.15 mg/mL) in sterile normal saline. All animals were allowed to adapt to the laboratory environment for 7 d with free access to water and standard laboratory chow. Animals were housed under standard environmental conditions with a 12-h light/12-h dark cycle.

2.2. Livery injury and regeneration models

To mimic the clinical scenarios relevant to LT and liver failure dealing with IRI and liver regeneration, animals were subjected to one of the following three different procedures:

(1) For procedures involving hepatic IRI, mice ($n = 7$) were subjected to partial warm hepatic IRI. The portal triad was dissected and a microvascular clamp placed, conferring ischemia to the median and the left lobes for 60 min, followed by reperfusion. In all IRI studies, C1-INH was administered intravenously (IV) (via tail vein) 30 min before ischemia at a dose of 100, 200, 400, 800, and 1000 U/kg or normal saline.

Mice were killed at predetermined time points after reperfusion for serum and liver sampling (6, 12, 18, and 24 h).

(2) For procedures involving combined IRI and 70% partial hepatectomy (PH), we incorporated both the above hepatic IRI and PH procedures. The portal triad was dissected and a microvascular clamp placed, conferring ischemia to the median and the left lobes. PH (70%) was done by resecting the right and caudate lobes and leaving only ischemic tissue in place. After surgeries, mice were killed, and livers and blood samples were collected at different time points after operation (6, 12, 18, and 24 h). A separate group ($n = 7$) was watched for survival analysis. Blood was also collected from the vena cava for serum preparation at the time of killing. The lowest and most effective (based on the above experiments) doses of C1-INH was administered IV (tail vein) 30 min before ischemia or normal saline.
(3) Acute liver failure model: C57BL/6 mice were administered carbon tetrachloride (CCl_4; Sigma) via intraperitoneal injection (4 mg/kg) as a chemical model of liver injury and regeneration. CCl_4 was dissolved in mineral oil and was administered by single intraperitoneal injection. For characterization of hepatic injury in response to CCl_4 injection, animals were killed daily for the measurement of serum alanine transaminases (ALTs) and for histopathologic analysis of liver injury. All experimental regimens were repeated for survival analyses, and animals were observed for up to 15 d. C1-INH was administered IV (tail vein) after CCl_4 or normal saline.

2.3. Markers of hepatocellular injury and IRI

Serum levels of ALT were determined (IDDEX Veterinary Services, Sacramento, CA). Serum IL-6 levels were determined using a commercially available enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

2.4. Histologic analysis

After euthanasia, livers were fixed with 10% buffered formalin for paraffin embedding or in optimal cutting temperature compound for frozen sections with no fixation. Five-micron paraffin-embedded sections were stained with hematoxylin and eosin for conventional morphologic evaluation. Suzuki classification [22], which consisted of three parameters of hepatic IRI: sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchymal necrosis. Each parameter was graded numerically as follows: congestion, 0 = none; 1 = minimal; 2 = mild; 3 = moderate, and 4 = severe. The same criteria were used in the graduation of the vacuolization, and for necrosis, the numerical graduation was as follows: 0 = nonnecrotic cells, 1 = single cell necrosis, 2 = <30% necrosis, 3 = <60% necrosis, and 4 = >60% necrosis. Two independent pathologists who were blinded to study did the histologic analysis.

2.5. *Assessment of liver regeneration*

Independent markers for hepatic regeneration were used. For assessment of hepatic proliferation, BrdU was injected (50 mg/kg intraperitoneally) 2 h before harvesting the liver. BrdU incorporation in liver sections was determined by immunohistochemical staining. Positive and negative cells were counted in 10 randomly selected fields by light microscopy using a ×40 objective lens. Liver specimen was also stained for PCNA as a marker of liver regeneration.

2.6. *Statistical analysis*

Results are expressed as mean ± standard deviation. A paired Student *t*-test for analysis of matched data and one-way analysis of variances followed by a Newman-Keuls posttest for analyses between groups were performed using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA). For the survival studies, Kaplan-Meier log-rank analysis will be performed. A *P* value <0.05 was considered significant.

3. Results

The increase of plasma ALT levels in mice treated with C1-INH was significantly less ($P < 0.001$) after 3 h of reperfusion in animals that received C1-INH at 100 U/kg (222 ± 56 U) and 400 U/kg (196 ± 34 U compared with control group (388 ± 56 U). However, ALT levels were lower after 6 and 24 h in all animals that received C1-INH compared with control (Fig. 1A). C1-INH at 400 U/kg was the most effective dose for preventing IRI. C1-INH was also able to attenuate IRI in C3−/−, which showed the effect of C1-INH is not exclusively through inhibition of classical complement pathway (Fig. 1B). C1-INH had no effect on liver IRI in TLR4−/− mice (Fig. 1D).

However, serum IL-6 after 6 h of reperfusion was significantly less ($P < 0.001$) in the animals that received C1-INH at 100 U/kg (52 ± 37 pg/mL) and 400 U/kg (44 ± 29 pg/mL)

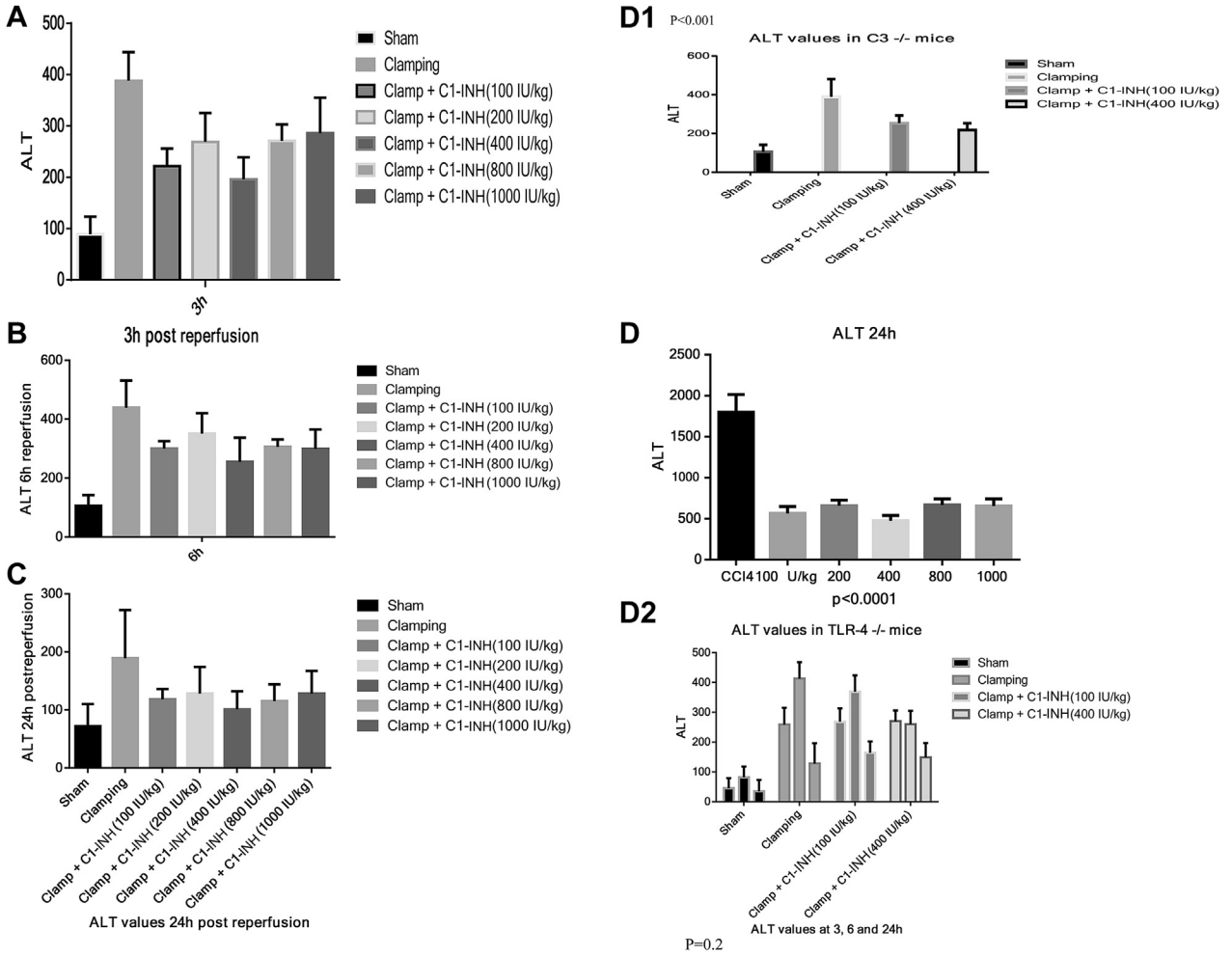

Fig. 1 – ALT levels in C1-INH–treated groups' mice (wild-type) were significantly lower than control at 3 h postreperfusion only at 100 and 400 U/kg ($P < 0.001$) (A). After 6 and 24 h postreperfusion C1-INH groups (all doses) had lower ALT level. C1-INH groups also have lower ALT in C3−/− (B) and CCl4-induced ALF groups (C) but not in TLR-4−/− (D). (Plasma ALT [mean ± standard deviation]) measured after reperfusion).

compared with control group (110 ± 43 pg/mL). We saw the same effect in C3−/− but not in TLR4−/− mice (Fig. 2B and C). These findings confirmed our first observation that the effect of C1-INH is through the TLR4 ligand and not exclusively by inhibition of classical complement pathway.

Histology showed that there was less congestion, vacuolization, and necrosis in the C1-INH group compared with control. Histology scores were lower in the animals that received C1-INH (Fig. 3). Treatment with C1-INH also led to less hepatocellular injury, decreased IL-6 level, and improved histology compared with control in the group that received CCl-4 (Figs. 1C, 2D, and 3D). For example, CCL4 induced >60% necrosis in the control animals that decreased to about 30% in the C1-INH–treated groups (Fig. 3) and improved animal survival (Fig. 4).

Animals that underwent IRI + PH or CCl_4-induced ALF and received C1-INH at 100 and 400 U/kg have improved survival compared with control animals (Fig. 4). All animals in the control groups (IRI + PH and CCL-4) died before d 5. However, C1-INH treatment was able to extend the survival and rescue the animals. There were more BrdU- and PCNA-positive cells in the animals that received C1-INH compared with control (Fig. 5).

4. Discussion

LT is the most efficient treatments available for various end-stage liver diseases [1]. LT does inevitably lead to hepatic IRI. Primary nonfunction or dysfunction, which occurs as a result of combined IRI and secondary tissue regeneration impairment, remains a serious complication after LT [1]. Complement activation is an important mediator of IRI after major surgery and transplantation [23]. Activation of the classical complement pathway in this type of tissue damage can occur via antibody-dependent as well as independent mechanisms, which in the latter case may involve the direct binding of C1q to damaged cells and *in situ* deposited acute phase proteins [11]. To prevent the effects of complement activation, the therapeutic application of complement inhibitor C1-INH, an inhibitor of the classical, alternative, and lectin pathways of complement activation has been preliminary tested in man [22–24]. Herein, human C1-INH was tested in a murine model of liver IRI at different doses. The biological activities of C1 inhibitor may be divided into two broad categories: the regulation of vascular permeability and anti-inflammatory functions. In our study, exogenous administration of C1-INH significantly attenuated liver IRI and promoted liver regeneration. C1-INH treatment in all models led to improved histology and decreased ALT and serum IL-6. IL-6 is a major cytokine involved in the pathogenesis of liver IRI and IL-6 knockout mice has been shown to be less prone to IRI [4,6–8]. With respect to the recommended dose of C1-INH, our study suggests that a dose as low as 100 U/kg was effective. Although, 400 IU/kg was the most effective dose as was shown to have the lowest levels of ALT and IL-6 and better histology scores. There was not a clear dose-dependent effect of

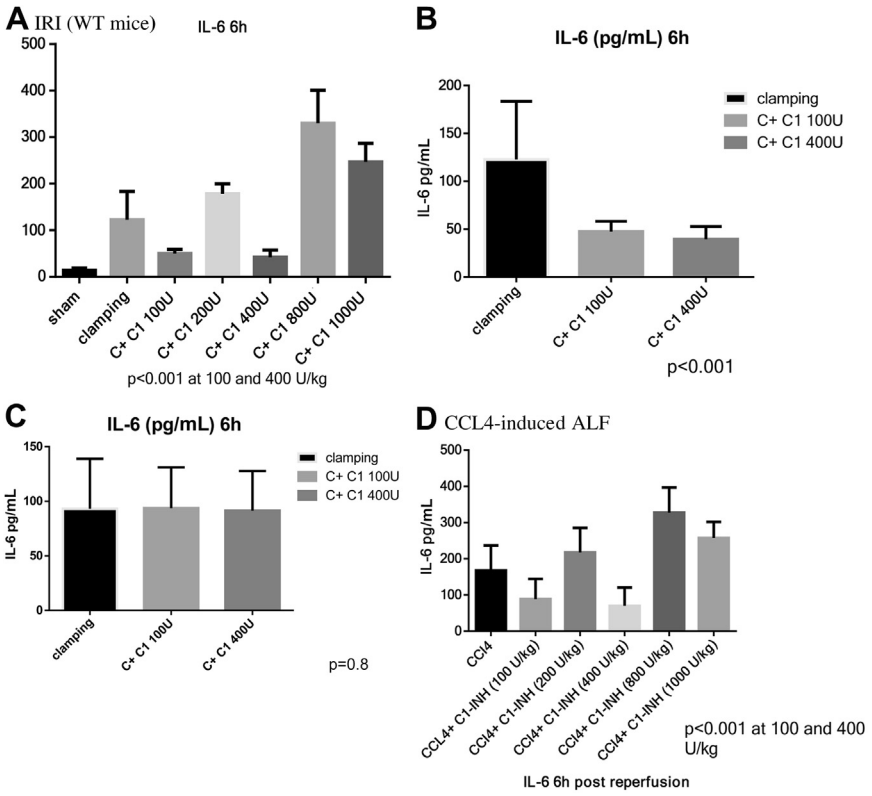

Fig. 2 – Serum IL-6 (mean ± standard deviation) after 6 h of reperfusion after IRI in (A), C3−/− (B), TLR-4−/− (C), and CCl4-induced ALF (D). IL-6 levels were significantly lower in C1-INH treated at 100 and 400 U/kg groups compared with other groups in WT and C3−/− and not in TLR-4−/− mice.

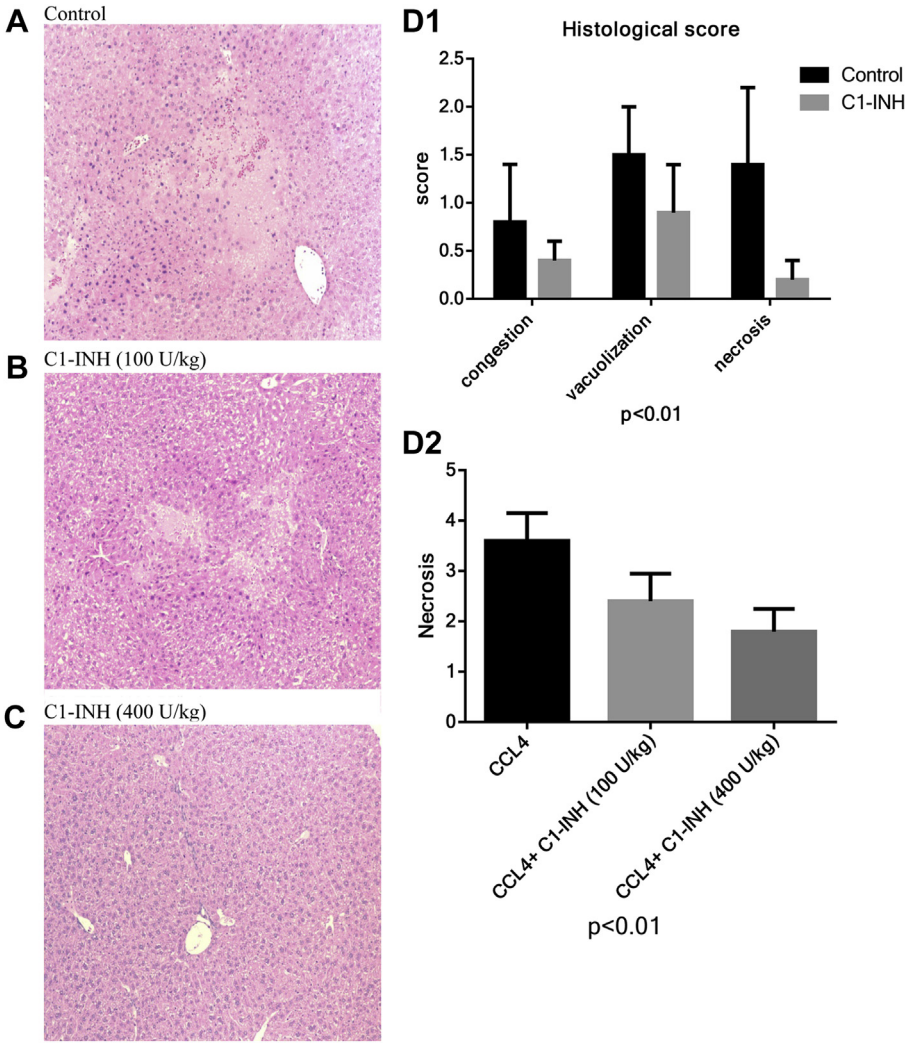

Fig. 3 – Hematoxylin and eosin staining of liver IRI (A, control, B, C1-INH 100 U/kg, and C, 400 U/kg) showed less necrosis, vacuolization, and necrosis in C1-INH groups than control, and (D) the lower level of congestion, vacuolization, and necrosis in C1-INH–treated groups both after IRI + PH (D1) and CCl4 (D2) ($P < 0.01$). (Color version of figure is available online.)

C1-INH. This could be possibly due to the effect of C1-INH on different pathways beside complement system.

Inhibition of an early step in the classical pathway is of relevance in view of the proinflammatory effects of early products of the complement activation cascade, such as C4a [23]. Although in this study the classical pathway of complement was blocked by C1-INH, hepatic IRI (i.e., ALT leakage from hepatocytes) was significantly attenuated also in C3−/− mice. This suggests that alternative pathways and contact system activation may also be involved in IRI. C1-INH exerts its effects not exclusively via reduction of classical complement activation. C1-INH is also a major inhibitor of the lectin pathway of complement activation, the contact activation system, and the intrinsic pathway of coagulation [23]. It, therefore, may have broader anti-inflammatory properties than single target agents. These additional effects of C1-inhibitor probably explain the therapeutic benefit of C1-INH independent of an effect on the classical complement activation [24]. C1-INH has been found to bind to sinusoidal endothelium or the sinusoidal pole of the liver trabeculae, linked to sinusoidal endothelium, after 8 h of cold storage in University of Wisconsin solution containing C1-INH and 2 h of reperfusion [12]. Deposition of the anti-C3 antibodies on liver cells shows marked heterogeneity, which is likely related to the mainly midzonal expression of IRI injury but perhaps also to variability in sensitivity to complement activation. We made no attempt to quantify the amount of C3 in hepatocytes after administration of C1-INH, considering the large variation of those results from assessment of liver sections [24]. Therefore, further definition of the contribution of the various complement pathways in liver IRI is of major importance for the development of an effective, specific, and safe treatment in LT.

TLRs are pattern recognition receptors that recognize pathogen-associated molecular patterns and facilitate innate immune responses for the initial host defense. The endogenous TLR4 has been shown to participate in acute tissue injury processes such as IRI. TLR4 ligand is released from damaged hepatocytes during liver IRI and subsequently

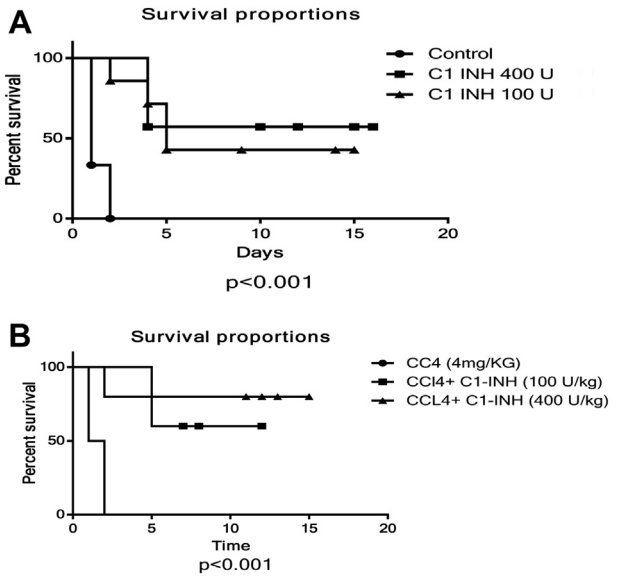

Fig. 4 – Animal survival improved in the C1-INH–treated groups ($P < 0.001$) which underwent IRI + PH (A) or CCl4-induced ALF (B).

stimulates nonparenchymal cells including Kupffer cells through TLR4 [25]. In this study, C1-INH has no effect on liver IRI in TLR4 knockout mice. Serum ALT and IL-6 levels in animals that received C1-INH were not different from control animals. It seems that C1-1INH works through TLR4 pathway.

The success of liver resection and LT especially living-donor split LT depends on the liver's ability to regenerate after major tissue loss [1]. Liver has the remarkable ability to regenerate and restore its anatomic and homeostatic integrity in response to PH, toxic exposure, or viral injury [26]. Liver parenchymal cells, including hepatocytes, and non-parenchymal cells such as endothelial, stellate, and Kupffer cells, respond to these stimuli by shedding their quiescent phenotype and synchronously entering the S phase of the cell cycle and proliferation [27].

Previous studies have shown that various parts of complement system, such as C3 and C5, are essential component in liver regeneration. Mice deficient in this complement protein were unable to mount a normal regenerative response after liver injury [28,29]. Our study demonstrated that C1-INH can also promote liver regeneration, in addition to its effect on IRI. The animals that underwent combined IRI and PH showed improved survival after receiving C1-INH. The number of regenerative cells (BrdU- and PCNA-positive cells) increased in C1-INH group.

We conclude that our results provide further support for the role of human plasma-derived C1-INH liver IRI and regeneration. Our study also demonstrates that pre-ischemic administration of C1-INH is effective in reducing liver IRI, providing an effective pharmacologic intervention to protect against IRI and promote regeneration in LT and surgery.

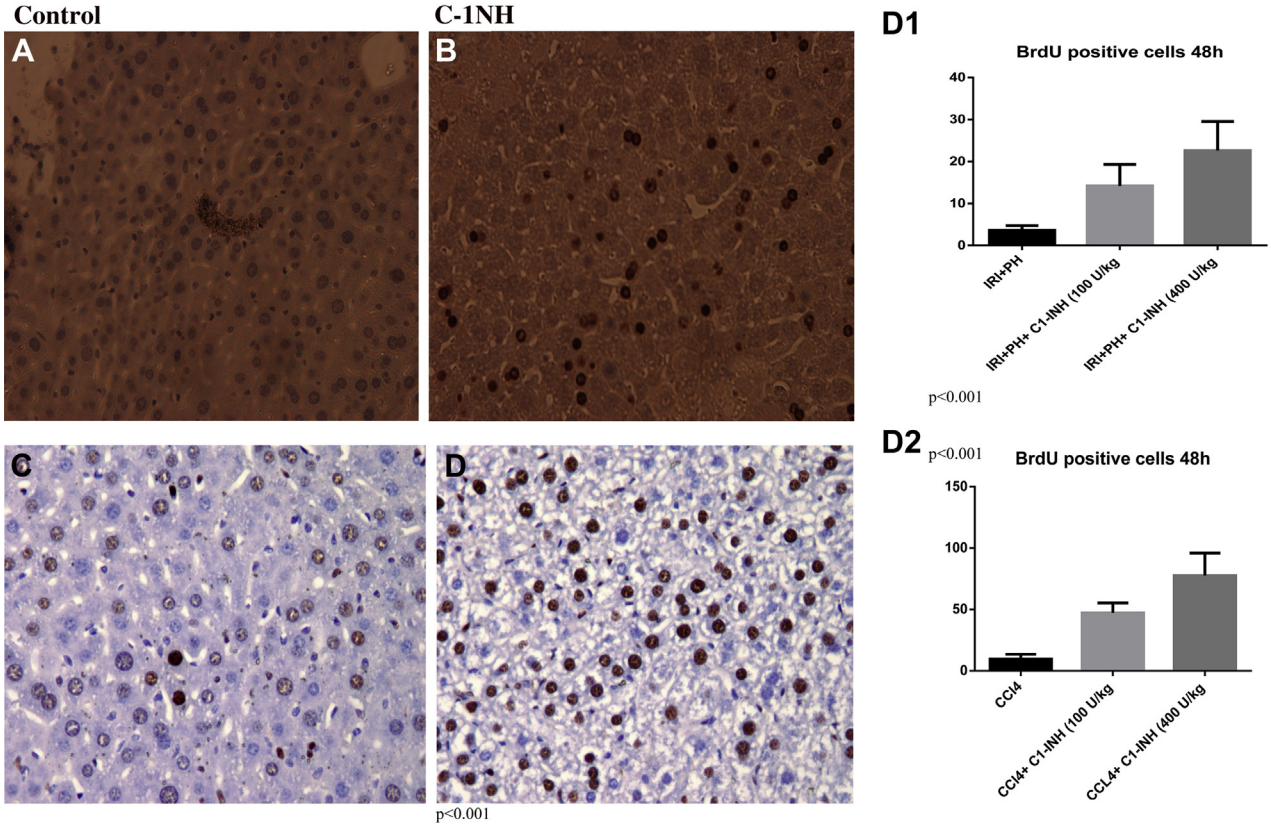

Fig. 5 – Liver regeneration was promoted in C1-INH group as showed by number of BrdU- and PCNA-positive cells (A, control and B, C-1NH) and (C) increased number of PNCA-positive cells in C1-INH group than control. (Color version of figure is available online.)

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REFERENCES

[1] Saidi RF. Current status of liver transplantation. Arch Iran Med 2012 Dec;15:772.
[2] Horstick G, Berg O, Heimann A, et al. Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. Circulation 2001;104:3125.
[3] Fu J, Lin G, Wu Z, et al. Anti-apoptotic role for C1 inhibitor in ischemia/reperfusion-induced myocardial cell injury. Biochem Biophys Res Commun 2006;349:504.
[4] Saidi RF, Chang J, Verb S, et al. Effect of methylprednisolone on warm ischemia-reperfusion injury in the liver. Am J Surg 2007;193:345.
[5] Hebert LA, Cosio FG, Birmingham DJ. The role of the complement system in renal injury. Seminars Nephrol 1992; 12:408.
[6] Heijnen BH, Straatsburg IH, Padilla ND, Van Mierlo GJ, Hack CE, Van Gulik TM. Inhibition of classical complement activation attenuates liver ischaemia and reperfusion injury in a rat model. Clin Exp Immunol 2006;143:15.
[7] Saidi RF, Chang J, Brooks S, Nalbantoglu I, Adsay V, Jacobs MJ. Ischemic preconditioning and intermittent clamping increase the tolerance of fatty liver to hepatic ischemia-reperfusion injury in the rat. Transplant Proc 2007;39:3010.
[8] Lehmann TG, Heger M, Munch S, Kirschfink M, Klar E. In vivo microscopy reveals that complement inhibition by C1-esterase inhibitor reduces ischemia/reperfusion injury in the liver. Transpl Int 2000;13(Suppl. 1):S547.
[9] Chan RK, Ibrahim SI, Verna N, Carroll M, Moore FD Jr, Hechtman HB. Ischaemia-reperfusion is an event triggered by immune complexes and complement. Br J Surg 2003;90: 1470.
[10] Straatsburg IH, Boermeester MA, Wolbink GJ, et al. Complement activation induced by ischemia-reperfusion in humans: a study in patients undergoing partial hepatectomy. J Hepatol 2000;32:783.
[11] Meyer zu Vilsendorf A, Link C, Jorns A, Nagel E, Kohl J. Preconditioning with the prostacyclin analog epoprostenol and cobra venom factor prevents reperfusion injury and hyperacute rejection in discordant liver xenotransplantation. Xenotransplantation 2001;8:41.
[12] Bergamaschini L, Gobbo G, Gatti S, et al. Endothelial targeting with C1-inhibitor reduces complement activation in vitro and during ex vivo reperfusion of pig liver. Clin Exp Immunol 2001;126:412.
[13] Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 1999;17:593.
[14] Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol 1993;264: G801.
[15] Tokuka A, Tanaka A, Kitai T, et al. Interrelationship of oxygen supply by hepatic artery and portal vein: rapid analysis of ischemia-reflow-induced changes in hepatic oxygenation in experimental and clinical subjects by tissue near-infrared spectroscopy. Eur Surg Res 1994;26:342.
[16] Wakabayashi H, Miyauchi A, Karasawa Y, Hamamoto I, Maeba T, Tanaka S. Effect of warm ischemia and reperfusion injury on inducing major histocompatibility complex antigens on hepatocytes and nonparenchymal cells in the rat liver. Transplant Proc 1993;25:3205.
[17] Thijs LG, Hack CE, Strack Van Schijndel RJ, et al. Activation of the complement system during immunotherapy with recombinant IL-2. Relation to the development of side effects. J Immunol 1990;144:2419.
[18] Rossen RD, Swain JL, Michael LH, Weakly S, Giannini E, Entman ML. Selective accumulation of the first component of complement and leukocytes in ischemic canine heart muscle. A possible initiator of an extramyocardial mechanism of ischemic injury. Circulation Res 1985;57: 119.
[19] Rossen RD, Michael LH, Kagiyama A, et al. Mechanism of complement activation following coronary artery occlusion: evidence that myocardial ischemia in dogs causes release of constituents of myocardial subcellular origin that complex with human C1q in vivo. Circulation Res 1988;62:572.
[20] Vogt W, Hesse D. Activation of the fifth component of human complement by oxygen-derived free radicals, and by methionine oxidizing agents: a comparison. Immunobiology 1992;184:384.
[21] Vogt W, von Damerau BZI, Nolte R, Brunahl D. Non-enzymic activation of the fifth component of human complement, by oxygen radicals. Some properties of the activation product, C5b-like C5. Mol Immunol 1989;26:1133.
[22] Suzuki S, Nakamura S, Koizumi T, et al. The beneficial effect of a prostaglandin I2 analog on ischemic rat liver. Transplantation 1991;52:979.
[23] Caliezi C, Wuillemin WA, Zeerleder S, Redondo M, Eisele B, Hack CE. C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. Pharmacol Rev 2000;52:91.
[24] Davis AE 3rd, Mejia P, Lu F. Biological activities of C1 inhibitor. Mol Immunol 2008;45:4057.
[25] Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology 2008;48:322.
[26] Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997;276:60.
[27] Guo JT, Zhou H, Liu C, et al. Apoptosis and regeneration of hepatocytes during recovery from transient hepadnavirus infections. J Virol 2000;74:1495.
[28] Schieferdecker HL, Schlaf G, Koleva M, Gotze O, Jungermann K. Induction of functional anaphylatoxin C5a receptors on hepatocytes by in vivo treatment of rats with IL-6. J Immunol 2000;164:5453.
[29] Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. J Immunol 2001;166:2479.