New Phenomenon

Elevated expression of hepcidin post-renal ischemia reperfusion injury

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Numerous studies have demonstrated that ischemia reperfusion (I/R) injury is the main cause of acute renal failure in both native kidneys and allografts, which had a profound influence on both early and late function of a transplanted kidney [1]. I/R injury causes the generation of high levels of free radicals composed mainly of reactive oxygen intermediates, and a sufficient concentration of free radicals is critical for the associated tissue damage. The mislocalized iron has been indicated to play an important role in this process [2,3], because these ions can mutagenize many types of molecules, including lipids, nucleotides, and the DNA backbone. Catalytic iron in urine, blood, and peroxidized lipids has been documented in acute renal failure mediated by hemoglobin and myoglobin [4]. Hepcidin is an iron regulatory hormone mainly produced by hepatocytes in response to inflammatory stimuli, iron overload and hypoxia, and plays a key role in maintaining iron homeostasis [5,6].

In this study, we assessed the expression of hepcidin in rat liver tissues and serum before and after renal I/R injury. Adult Sprague–Dawley (250–300 g) rats were randomly divided into I/R group and control group (n = 10). Rats in I/R group were further divided into three groups according to the time for tissue and specimen harvest after injury (6, 24, and 48 h). The I/R injury was made by a modification of a previously published protocol [7]. In all rats, a midline incision was made, the right renal vessels were ligated, and a right nephrectomy was performed. For I/R group, the left renal artery was completely occluded by clamping with a non-crushing microvascular clamp for 45 min. The presence of ischemia was visually confirmed by observing blanching of the kidney. After 45 min of ischemia, the clamps were removed, the wounds were closed and the animals were returned to their cages. The rats in the sham operation group underwent the same procedure except being clamped. Liver tissues and venous blood were collected at indicated time points to detect the expression of hepcidin and IL-6 using enzyme-linked immunosorbent assay and semi-quantitative reverse transcriptase-polymerase chain reaction (PCR) assay. Serum creatinine (Cr) index was examined in all of specimens.

Total liver RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to manufacturer’s instruction. The relative expression level was normalized to the reference gene GAPDH. The primers were as follows: hepcidin: 5'-AGGCAAGATGGCACAAGCCTG-3' (forward) and 5'-CTATTTATGCAACGAGAC-3' (reverse); GAPDH: 5'-CAAGGTCATCCCATGCAACATTG-3' (forward) and 5'-GTCCACCACTGTGTGCTTG-3' (reverse). The final PCR products of hepcidin and GAPDH were electrophoresed on the 1% agarose gel and imaged for semi-quantitation analysis with Image-Pro plus software. The serum hepcidin and IL-6 protein levels were determined using commercial EIA kit (RayBiotech, Norcross, USA) according to the manufacturer’s instruction. Serum Cr level was determined with automatic biochemistry analyzer (Hitachi 7600; Hitachi, Tokyo, Japan). Rat kidneys were isolated and fixed with 4% paraformaldehyde in phosphate buffered saline. The fixed tissues were paraffin embedded, sectioned (7 μm), and followed by hematoxylin and eosin staining. Data were analyzed using SPSS13.0 software. All values are presented as the mean ± SEM. The variance between two groups was analyzed using one-way analysis of variance method. Correlation coefficient was determined by the Pearson correlation analysis.

Renal tissue section from control rat shows normal tubule structure [Fig. 1(A)]. At 6 h post of I/R injury, epithelial cells at proximal convoluted tubule began to be swollen and granulized [Fig. 1(B)]. At 24 h post of IR injury, the vacuolar was degenerated, and cell necrosis began to appear at proximal convoluted tubule. The blood vessel appeared to be dilated. The destruction of the vessel basal membrane and inflammatory cells infiltration were also observed at this stage [Fig. 1(C)]. However, these lesions began to be alleviated at 48 h after of I/R injury [Fig. 1(D)]. Hepcidin expression in rat liver tissue increased significantly after renal I/R injury compared with control rats (P < 0.05) (Fig. 2). The highest expression is at 24 h after injury, and then decreased to the control level at 48 h after reperfusion injury. Consistently, serum IL-6 and Cr levels also increased in rats subjected to renal I/R injury.
when compared with the control rats ($P < 0.05$) (Table 1). The expression levels of hepcidin, serum IL-6 and Cr are positively correlated in I/R groups. Correlation analysis suggested that there was a positive correlation between serum hepcidin, IL-6 ($P = 0.013$), and Cr ($P < 0.001$) at 24 h post of renal I/R injury ($P < 0.05$), while no significant difference is in the group of control or 48 h post of renal I/R injury (Table 2).

This study showed the elevated expression of hepcidin in liver and serum after renal I/R injury, and the associated inflammatory responses post of renal I/R Injury, which provided the evidence that hepcidin was a relevant indicator for renal I/R injury diagnosis. These observations indicated that during renal I/R injury, kidney was subjected to ischemia, blood losing, and inflammation, which led to serum IL-6 level elevated and hepcidin expression induced. Our previous study demonstrated that the inflammation associated with chronic renal insufficiency promoted hepcidin expression, and the proinflammatory cytokine, IL-6, played a major role in hepcidin transcriptional induction [8]. As IL-6 is a well-known activator for Jak/Stat3 signaling cascade, it was assumed that Stat3 regulates the hepcidin expression [9]. Our study indicated that hepcidin could be used as one of the key indicator molecules for kidney I/R injury caused by iron metabolism alteration.

In conclusion, renal I/R injury causes the increase of hepcidin expression, which may be associated with in vivo micro-inflammatory or renal injury. Thus, hepcidin could be used as one of the indicators for detection of the degree of renal I/R caused injury. Moreover, it may have clinical
value to reflect change of iron metabolism due to the renal I/R injury.

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References