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• BASIC RESEARCH •

Effect of N-desulfated heparin on hepatic/renal ischemia reperfusion injury in rats

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Abstract

AIM: To investigate the effect of N-desulfated heparin on hepatic/renal ischemia and reperfusion injury in rats.

METHODS: Using rat models of 60 minutes hepatic or renal ischemia followed by 1 h,3 h,6 h and 24 h reperfusion,animals were randomly divided into following groups,the sham operated controls,ischemic group receiving only normal saline,and treated group receiving N-desulfated heparin at a dose of 12 mg/kg at 5 minutes before reperfusion. P-selectin expression was detected in hepatic/renal tissues with immunohistochemistry method.

RESULTS: P-selectin expression, serum ALT, AST, BUN and Cr levels were significantly increased during 60 minute ischemia and 1 h, 3 h, 6 h and 24 h reperfusion,while the increment was significantly inhibited,and hepatic/renal pathology observed by light microscopy was remarkably improved by treatment with the N-desulfated heparin. Furthermore,the heparin was found no effects on PT and KPTT.

CONCLUSION: P-selectin might mediate neutrophil infiltration and contribute to hepatic/renal ischemia and reperfusion. The N-desulfated heparin might prevent hepatic/renal damage induced by ischemia and reperfusion injury without significant anticoagulant activity.

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INTRODUCTION

Hepatic/renal ischemia-reperfusion injury is very common clinically. So far, no effective treatment for this pathological injury is available. It has been found that cell adhesion molecule P-selectin plays an important role in hepatic/renal ischemia-reperfusion injury by mediating the interactions of polymophonuclear neutrophils with endothelium. P-seletin monoclonal antibody has been demonstrated to prevent effectively reperfusion-induced hepatic/renal tissue damage[1-22]. Heparin, a highly sulfated proteoglycan, has anti-inflammatory activity besides its anticoagulant function. Recently reports suggested that heparin could prevent leukocyte adhesion through inhibiting the role of P-selectin and might be used to treat inflammatory diseases[23]. However, the clinical use of heparin for treatment of inflammation is impeded by its strong anticoagulant activity[24]. Chemically modified heparins which have been developed have relatively low anticoagulant activity remain their anti-inflammatory activities[25,26]. In this study, we investigated the effect of N-desulfated heparin (NNH) on hepatic/renal ischemia-reperfusion injury in rats.

MATERIALS AND METHODS

Animal model

Ninety male Wistar rats (Shanghai Experimental Animal Centers of Chinese Academy of Sciences), weighing 200±10 g, were given free access to food and water for three days before the experiments. The rats were anesthetized with 2.5 % sodium pentobarbital intraperitoneally, and randomly divided into 2 groups. In one group of rats, the ligament linking liver, diaphragm and abdominal wall were separated, portal vein and liver artery that drain blood to left hepatic lobe were freed by blunt dissection and then blocked with a microvascular clamp for 60 minutes, after that clamp was removed, and reperfusion was started; while in another group, the left renal artery was freed, blocked with a microvascular clamp for 60 minutes, then the clamp was removed and reperfusion was started, simutaneously, the right kidney was cut off. The two groups of rats were randomly divided into NNH-treated group (n=20) and non-treated group (n=20). They were divided into subgroups according to the indicated time: 1, 3, 6, 24 hours after reperfusion. NNH (12 mg/kg, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences), or saline were injeted by veins five minutes before reperfusion. A sham-operated group (n=5, anesthesia and opening celiac cavity, no blocking of hepatic or renal blood flow) served as control.

Collection and measurement methods of specimens

Blood, hepatic and renal tissues were harvested at the indicated time. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and blood urea nitrogen (BUN) and creatinine (Cr) were measured with a 747 automatic analyzer (Hitachi boehringer Mannhein, Mannhein, Germany). Hepatic and renal tissue samples were fixed in 10 % formalin and embedded in paraffin. Sections were cut 5 μm thick and stained with hematoxylin and eosin for light microscope examination. Expression of P-selectin in hepatic/renal tissue

was detected by an immunohistochemistry method with a labelled streptavidin biotin (LSAB) kit (Fujian Maixin Biotechnology Co., products of Biotechnology co. CA, USA). Activated partial thromboplastin time (APTT) and protothrombinogen time (PT) were also measured.

Statistical analysis

Data was presented with $\bar{x}\pm s$, and Student' s *t* test was used to determine changed between different groups. $P<0.05$ was considered significant.

RESULTS

Histopathologic evaluation

One hour after reperfusion, visual observation revealed that the left hepatic lobe was more swollen than the right lobe, and was dark in color. Under the light microscope, interstitial congestion and infiltration of inflammatory cells were observed. 1 hour after reperfusion, with the observation of naked eyes, the renal cortex was pale, the renal medulla displayed blood stagnation and was dark in color. Under the light microscope, edema, denaturation with different extent and necrosis of renal tubular epithelial cells were observed. Simultaneously, interstitial congestion, edema and infiltration of inflammatory cells were also observed. However, in the N-desulfated heparin-treated group. The outward apperance of the liver and kidney was similar to that of normal control. Hepatic cells and tubular cells showed less swelling and no denaturation or necrosis, and interstitial changes were not obvious.

Hepatic and renal function evaluation

Twenty four hours after hepatic reperfusion, the serum levels of ALT (628±91 μ/L) and AST (1608±199 μ/L) in the saline-treated group were much higher than the sham-operated group (52±11 μ/L and 80±17 μ/L respectively, $P<0.01$). The NNH-treated group revealed significantly lower levels of ALT (161±24 μ/L) and AST (360±49 μ/L) compared with saline-treated group ($P<0.01$).

Twenty four hours after renal reperfusion, the serum levels of BUN (14.54±0.67 mmol/L) and Cr (102.2±4.67 μmol/L) were much higher than the sham-operated group (7.88±0.57 mmol/L and 39.00±4.47 μmol/L, respectively, $P<0.01$). The NNH-treated group presented with significantly lower levels of BUN (10.60±0.80 mmol/L) and Cr (67.78±5.01 μmol/L) compared with saline-treated group ($P<0.01$).

P-selectin expression in hepatic and renal tissues

P-selectin was expressed widely within hepatic and renal tissues 1 hour after reperfusion, which was mainly distributed on small vessels of left hepatic lobe and tubular epithelium. In addition, it was also expressed on part of hepatic cellular membrane, glomerulomesangium, capillary loops, and interstitium. After treatment with the NNH, there were no obvious yellow-brown positive granules in the hepatic and renal tissue, suggesting that the P-selectin expression was not displayed.

PT and KPTT assays

Twenty four hours after hepatic reperfusion, PT (15.0±1.6 s) and KPTT (21.9±4.1 s) in the saline-treated group were similar to that of sham-operated group (13.4±1.7 s and 17.9±2.9 s respectively, $P>0.05$). PT(14.6±1.9 s) and KPTT (18.7±3.7 s) in NNH-treated group did not differ from those in saline-treated group ($P>0.05$). 24 hours after renal reperfusion, PT and KPTT were 13.7±1.4 s and 17.6±3.1 s respectively, while in NNH-treated group they were 13.3±1.6 s and 17.1±3.5 s respectively ($P>0.05$).

DISCUSSION

Recently, the role of cell adhesion molecules and neutrophil in orgen ischemia and reperfusion injury has attracted attention[27-48]. As a potential member of the selectin family, P-selectin is found in both weibel-plade body of epithelial cell of middle and small blood vessels and a-granule of platelet. It is expressed rapidly on the surface of these cells after their activation. P-selectin plays an important role in inflammation by initiating neutrophil rolling, adhesion and recruitment to injured tissue[27]. Blockade of P-selectin expression or interaction with its ligands can attenuate leukocyte adherence and infiltration during ischemia and reperfusion injury. And P-selectin monoclonal antibody was found to have protective effects on the injury[21,22].

Heparin and its analogue have been shown to bind to cell adhesion molecule P-selectin. The binding can inhibit adhesion of leukocytes mediated specifically by the adhesion molecules and are of anti-inflammatory activity[25,26]. Although heparin can be used to treat inflammatory diseases, their clinical potentials are limited due to their potent anticoagulant activity. Several chemical modifications of heparin that have reduced anticoagulant activities while preserving their anti-inflammatory activities have been reported. For example, the O-desulfated heparin derivatives have been shown to be of treatment for inflammation, while they still retained 5-30 % anticoagulant activities as compared with heparin[49]. Further, N-acetylheparin is known to have lower anticoagulant activity while preserving the anti-inflammatory activity[50]. N-desulfated heparin has been reported to have significantly reduced anticoagulant activity[51,52]. However, the anti-inflammatory roles of the heparin have not been extensively studied before.

The effect of N-desulfated heparin on ischemia and reperfusion injury was observed in this study based on the established rat model of hepatic/renal ischemia-reperfusion.

Hepatic and renal tissue displayed significant histopathologic damage after hepatic/renal ischemia-reperfusion while the serum levels of ALT and AST as well as BUN and Cr were increased. We showed that ischemia-reperfusion induced hepatic/renal injury was remarkably attenuated when NNH was given 5 min before reperfusion as shown by improved hepatic/renal function and less pathologic damage. The results suggest that the NNH has a protective effect on hepatic/renal reperfusion injury by inhibiting the interaction of neutrophils and endothelium.

After ischemia and reperfusion, P-selectin expression was up-regulated in hepatic and renal tissue, suggesting that P-selectin is related to hepatic/renal reperfusion injury. It has been found that leukocyte rolling and recuitment was delayed when deficient mice were infected,suggesting that P-selectin is involved in the early events of inflammation mediated by leukocytes[53]. Results from this study showed that P-selectin expression in hepatic and renal tissue was inhibited in NNH-treated group. This is consistent with down-regulated expression of sialyl lewis X, a ligand for P-selectin located mainly in neutrophils, as with anti-P-selectin therapy (unpublished data). These suggest that P-selectin might mediate neutrophil infiltration within the liver and kidney in the early stage of hepatic/renal reperfusion injury. Furthermore, blockade of P-selectin can attenuate inflammatory cell infiltration and pathological damage. N-desulfated heparin can be of protective role in the hepatic and renal injury caused by ischemia-reperfusion by inhibiting the adhesion and activation of neutrophils mediated by P-selectin. In addition, we found the heparin had no significant effects on PT and KPTT. It is

consistent with Bjornsson' s study[51]. Therefore, the N-desulfated heparin might be an efficient approach for the treatment of reperfusion injury with no obvious anticoagulant activity.

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