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World J Gastroenterol 2003;9(7):1563-1566
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Effect of tetramethylpyrazine on P-selectin and hepatic/renal ischemia and reperfusion injury in rats

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Received: 2002-12-30 **Accepted:** 2003-02-19

Abstract

AIM: To investigate the effect of tetramethylpyrazine on hepatic/renal ischemia and reperfusion injury in rats.

METHODS: Hepatic/renal function, histopathological changes, and hepatic/renal P-selectin expression were studied with biochemical measurement and immunohistochemistry in hepatic/renal ischemia and reperfusion injury in rat models.

RESULTS: Hepatic/renal insufficiency and histopathological damage were much less in the tetramethylpyrazine-treated group than those in the saline-treated groups. Hepatic/renal P-selectin expression was down regulated in the tetramethylpyrazine-treated group.

CONCLUSION: P-selectin might mediate neutrophil infiltration and contribute to hepatic/renal ischemia and reperfusion injury. Tetramethylpyrazine might prevent hepatic/renal damage induced by ischemia and reperfusion injury through inhibition of P-selectin.

Chen JL, Zhou T, Chen WX, Zhu JS, Chen NW, Zhang MJ, Wu YL. Effect of tetramethylpyrazine on P-selectin and hepatic/renal ischemia and reperfusion injury in rats. *World J Gastroenterol* 2003; 9(7): 1563-1566
http://www.wjgnet.com/1007-9327/9/1563.htm

INTRODUCTION

Hepatic/renal ischemia-reperfusion injury is common clinically. Up to now, there has been no effective treatment for this pathological injury. Cell adhesion molecules have been found to play an important role in hepatic/renal ischemia-reperfusion injury by mediating interactions of polymorphonuclear neutrophils with endothelium. P-seletin monoclonal antibody has been demonstrated to prevent effectively reperfusion-induced hepatic/renal tissue damage[1-23]. Tetramethylpyrazine (TMP), a traditional Chinese herb, has been widely used especially in the treatment of patients with cerebral and cardiac ischemic diseases in China. Experimental study has found that TMP could protect vascular endothelial cells, and inhibit respiratory explosion and free radicals of polymorphonuclear neutrophils[24-28]. In the present study, we investigated the effect of TMP and P-selectin on hepatic ischemia and reperfusion injury in rats.

MATERIALS AND METHODS

Animal model

Ninety male Wistar rats (Shanghai Experimental Animal Center 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, the 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, then the clamp was removed, and reperfusion was performed. While in the other group, the left renal artery was freed, and blocked with a microvascular clamp for 60 minutes, then the clamp was removed and reperfusion was performed, simultaneously, the right kidney was cut off. The two groups of rats were randomly divided into TMP-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. TMP or saline was intravenously injected five minutes before the 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 and 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 analyzor (Hitachi Boehringer Mannhein, Mannhein, Germany). Hepatic and renal tissue samples were fixed in 10 % formalin and embedded in paraffin. 5 μm thick sections were cut into and stained with hematoxylin and eosin for light microscope examination. Expression of P-selectin in hepatic/renal tissue was detected by immunohistochemistry method with a labeled streptavidin biotin (LSAB) kit (Fujian Maixin Biotechnology Co., products of Biotechnology Co. CA, USA).

Statistical analysis

Data were presented as $\bar{x}\pm s$, and Student' s t test was used to determine changes 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. One hour after reperfusion, the renal cortex was macroscopically 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 TMP-treated group, the outward appearance of the liver and kidney was similar to that of normal. 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 u/L) and AST (1 608±199 u/L) in the saline-treated group were much higher than those in the sham-operated group (52±11 u/L and 80±17 u/L respectively, $P<0.01$). The TMP-treated group revealed significantly lower levels of ALT (190±21 u/L) and AST (386±62 u/L) than those in the 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 in the TMP-treated group than those in the sham-operated group (7.88±0.57 mmol/L and 39.00±4.47 μmol/L, respectively, $P<0.01$). The TMP-treated group presented with significantly lower levels of BUN (11.21±0. 56 mmol/L) and Cr (70.61±4.95 μmol/L) than those in the 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 kidney. In addition, it was also expressed on part of hepatic cellular membrane, glomerulomesangium, capillary loops, and interstitium. After treatment with TMP, there were no obvious yellow-brown positive granules in the hepatic and renal tissues, suggesting that P-selectin expression was not displayed.

DISCUSSION

Recently, the role of cell adhesion molecules and neutrophils in ischemia and reperfusion injury has aroused attention[29-50]. Ischemia reperfusion liver injury is characterized by microvascular leukocyte accumulation and massive infiltration of postischemic tissues. Primary leukocyte endothelial cell interaction(rolling) is mediated by selectins, whereas firm adherence and transendothelial migration involve immunoglobulin superfamily(intercellular adhesion molecule-1, ICAM-1) with leukocyte β_2-integrins (CD11/CD18)[51]. As a potential member of the selectin family, P-selectin has been found in both Weibel-Palade body of epithelial cells of middle and small blood vessels and α-granule of platelets. It is expressed rapidly on the surface of these cells in seconds after their activation. Furthermore, P-selectin can be up-regulated by *de novo* synthesis in the ischemia-reperfusion injury in hours. P-selectin plays an important role in inflammation by initiating neutrophil rolling, adhesion and recruitment to injured tissue[15]. 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 has been found to have protective effects on the injury[21].

Tetramethylpyrazine (TMP) is an active ingredient of Ligustium Wallich Franch. It has been shown in animal models and clinical investigations that TMP is effective on ischemic diseases such as heart, brain and lung. TMP could block the calcium channel, reduce the bioactivity of platelets and platelet aggregation, and inhibit free radicals, and has inhibitory roles in platelets and arterial thrombus formation in dogs[52]. However, the roles and mechanisms of TMP in treatment of digestive diseases have not been extensively studied.

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

Hepatic and renal tissues displayed significantly histopathologic damages after hepatic/renal ischemia-reperfusion while the serum levels of ALT and AST as well as BUN and Cr were increased. It was showed that hepatic/renal injury induced by ischemia-reperfusion was remarkably attenuated when TMP was given 5 minutes before reperfusion as shown by improved hepatic/renal function and less pathologic damage. The results suggest that TMP 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 tissues, suggesting that P-selectin is related to hepatic/renal reperfusion injury. It was found that leukocyte rolling and recruitment were delayed when deficient mice are 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 tissues was inhibited in TMP-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. Wu found that TMP could reduce significantly the number of α-granule membrane protein (GMP140) of platelets and had inhibitory effects on platelets and arterial thrombus formation in dogs. TMP can play a protective role in hepatic and renal injury caused by ischemia-reperfusion by inhibiting the adhesion and activation of neutrophils mediated by P-selectin.

In an animal model of thioacetamide (TAA) induced acute hepatotoxicity, increase of serum SGOT and SGPT produced by TAA was decreased by TMP, and increase of malondialdehyde (MDA) produced by TAA was also prevented by *in vitro* addition of TMP to liver homogenates. A rise of serum interleukin-2 was similarly prevented. The results suggest that part of hepatocellular injury induced by TAA is mediated by oxidative stress caused by the action of cytokines through lipid peroxidation, TMP may act by preventing lipid peroxidation[54]. Another study showed that the hepatoprotective effect of TMP might be in part due to its inhibitory ability on membrane lipid peroxidation and free radical formation and its free radical scavenging ability[55]. Therefore, TMP might be effective on the treatment of on reperfusion injury.

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Edited by Xu XQ and Wang XL