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Ischemic preconditioning decreased leukotriene C4 formation by depressing leukotriene C4 synthase expression and activity during hepatic I/R injury in rats

Fenfang Hong, MB,[a,b] *and Shulong Yang, PhD*[a,*]

[a] *Department of Physiology, College of Medicine, Nanchang University, Nanchang, China*
[b] *Department of Experimental Teaching, Nanchang University, Nanchang, China*

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ABSTRACT

Background: Extensive experimental and clinical studies have shown that ischemic preconditioning (IP) can produce protective effects during hepatic ischemia reperfusion (I/R) injury. Our recent studies indicate that rat liver I/R injury is related to an abnormal increase in leukotriene (LT) C4 production. However, the mechanisms underlying IP actions on LTC4 generation during hepatic I/R injury remain to be explored.

Methods: We randomly divided adult male Sprague-Dawley rats into sham (control), I/R, and IP groups ($n = 6$). We subjected rat liver to 60 min partial hepatic ischemia followed by 5 h reperfusion with saline administered intravenously. We detected protein expression of LTC4 synthase (LTC4S) with Western blot, and measured LTC4 synthesis enzymes' activities and content by reverse-phase high-performance liquid chromatography. We assessed tissue injury using serum aspartate aminotransferase and aspartate aminotransferase activities and histologic changes. We examined liver tissue glutathione levels by a biochemical method.

Results: Ischemic preconditioning markedly decreased LTC4 content, reduced LTC4S protein expressions, and inhibited LTC4 synthesis enzymes' activities in rat liver compared with the I/R group ($P < 0.05$). We also observed a decline in serum alanine aminotransferase and aspartate aminotransferase activities ($P < 0.05$), together with hepatic tissue glutathione elevation ($P < 0.05$) in the IP groups. Positive expression of LTC4S on hepatocytes and sinusoidal endothelial cells in the IP group was significantly lower than that in the I/R group.

Conclusions: These findings demonstrate that reduced LTC4 production by IP treatment during hepatic I/R injury could partially result from the down-regulation of LTC4S protein expression and the depression of LTC4 synthesis enzyme activity. They suggest that the beneficial effects of IP may be involved in repression of LTC4 generation during hepatic I/R injury.

1. Introduction

Leukotriene (LT) C4 is a potent inflammatory mediator formed from arachidonic acid, and glutathione,5-lipoxygenase, 5-lipoxygenase activating protein, and leukotriene C4 synthase (LTC4S) participate in its biosynthesis [1]. Leukotriene C4 synthesis enzymes including LTC4S, microsomal glutathione S-transferase (mGST)2, and mGST3 directly catalyze LTA4

* *Corresponding author*. Department of Physiology, College of Medicine, Nanchang University, Bayi Road 461, Donghu District, Nanchang 330006, China. Tel/fax: 86 791 8601308.
E-mail address: yangshulong@yahoo.cn (S. Yang).
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and reduced glutathione (GSH) to generate LTC4, which is the first committed step in the synthesis of the cysteinyl leukotrienes (LTs), LTC4, LTD4, and LTE4 [2,3]. Increasing evidence shows that LTs are related to cholestasis, hepatic inflammation in metabolic disease [4], portal hypertension [5], acute and chronic cyclosporine A nephrotoxicity [6], endotoxemia [7], hepatorenal syndrome, fulminant hepatic failure [8], and primary graft nonfunction after liver transplantation [3,9,10].

Hepatic ischemia and reperfusion (I/R) injury is well known as an important clinical issue. It has been implicated in the pathogenesis of a variety of clinical conditions including trauma, thermal injury, hypovolemic and endotoxin shock, reconstructive vascular surgery, liver transplantation, and liver resectional surgery [11,12]. Several studies indicate the roles of LTs in hepatic I/R injury [12–14]. Takamatsu *et al* [13] observed that cysteinyl LT content in the hepatic tissue after 12 and 24 h reperfusion increased four- to fivefold compared with controls, and was accompanied by the enhancement of hepatic edema and plasma ALT elevation [13]. Our previous results also demonstrate that the mRNA and protein expressions of LTC4S enhanced in hepatocytes and sinusoidal endothelial cells and the activities of the LTC4 synthesis enzymes in liver microsomes increased after 5-h reperfusion in rats. More recently, montelukast, a cysteinyl leukotriene receptor antagonist, and MK-886, an inhibitor of 5-lipoxygenase, were shown to be effective in preventing liver and intestine injury by reducing apoptosis and oxidative stress in a hepatic I/R model [14].

Hepatic ischemic preconditioning (IP), a brief period of liver ischemia followed by reperfusion, is demonstrated to protect against prolonged I/R injury and improve the capacity of regeneration [15]. Since Lloris-Carsi *et al* [16] reported liver protection induced by IP treatment in a warm ischemic model in 1993, much research has confirmed the effects of IP in liver transplantation, including the reduction of blood loss in hepatectomy, marked diminished oxidant generation and oxidative reactions, intraoperative hemodynamic stability, and its significant role in liver regeneration [17–21]. However, some contradictory evidence is presented, as well [22,23]. Moreover, the mechanisms of IP defense against hepatic I/R injury are not fully elucidated [11,15,21,24]. We attempted the present experiments to investigate whether liver protection from IP might involve an influences on LTC4 generation during hepatic I/R injury, and if so, to explore the underlying mechanisms.

2. Materials and methods

2.1. Materials

We obtained male Sprague-Dawley rats (250–300 g) were obtained from the Experimental Animal Center, Nanchang University (Nanchang, China). We purchased LTA4 methyl ester, LTC4, and prostaglandin B2 from Cayman Chemical Co. (Ann Arbor, MI). The LTC4S rabbit polyclonal antibody was from Santa Cruz (CA). Enhanced chemiluminescence detection kit for horseradish peroxidase was from Biological Industries (Kibbutz Beit HaEmek, Israel). Polyvinylidene fluoride membranes were from Millipore (Bedford, MA). We obtained a polymer detection system for immunohistological staining, a 3, 3′-diaminobenzidine kit, and horseradish peroxidase–linked goat anti-rabbit and goat anti-mouse antibody from Zhongshan Biological Co. (Beijing, China). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and GSH detection kits were the products of Nanjing Jiancheng (Nanjing, China). All other chemicals were of the highest purity commercially available.

2.2. Animal model of hepatic I/R injury

We performed hepatic I/R injury as described in our previous report [12]. Briefly, we housed the rats and treated them in accordance with the Guidelines for the Care and Use of the Experimental Animal Center of Nanchang University. The local animal ethics committee approved the study. Rats were fasted for 12 h but allowed to drink water before the operation, and were randomized into three groups. In the I/R group, we anesthetized rats with pentobarbital 50 mg/kg intraperitoneally and created an external jugular vein catheter using a polyethylene tube 0.9 mm in inner diameter (Becton Dickinson Medical Devices, Suzhou, China). Then, we subjected the rats to midline laparotomy, exposed the liver, and rendered the left lateral and median lobes by clamping the hepatic arterial and portal venous blood supply using a microaneurysm clamp. After 60 min of hepatic ischemia (or sham), we reperfused livers for 5 h by removing the clamp and sutured the peritoneal cavity closed. We intravenously injected saline solution (3 mL/kg/min) by the external jugular vein at 15 min before the start of ischemia through the reperfusion period. For the sham group (control), we performed surgery on anesthetized rats in which hepatic blood flow was not occluded. For the IP group, we allowed a short ischemic period of 10 min followed by 10 min reperfusion before the start of 60-min ischemia and 5-h reperfusion according to the previous report [25,26]. After 5 h reperfusion, we collected serum from each animal for liver enzyme activity, removed the livers, prepared the middle lobe for microsomes, and fixed tem in 10% formalin for immunohistochemistry or staining with hematoxylin-eosin. Then we prepared the left lobule for 10% tissue homogenate and snap-froze it in liquid nitrogen and stored it at −70°C for reverse-phase high-performance liquid chromatography (RP-HPLC) detection.

2.3. Immunoblot analysis

We performed immunoblot analysis of LTC4S protein expression as described in our previous report [27].

2.4. Immunohistochemistry

We used the indirect immunoperoxidase method to localize LTC4S in paraffin-embedded liver sections from the control, I/R, and IP groups rats, and employed the polymer detection system for immunohistological staining and the DAB kit, according to the manufacturer's guidelines, as described in our previous report [27].

2.5. Measurement of LTC4 content

We measured the LTC4 content in liver tissue by HPLC, as described in our previous work [27].