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Mast cell degranulation promotes ischemia–reperfusion injury in rat liver

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ABSTRACT

Background: Mast cells (MCs) play a role in ischemia–reperfusion (I/R) injury in many organs. However, a recent study found that MCs are not involved in I/R injury in isolated rat livers that were perfused only for 1 h. The purpose of this study is to reevaluate the role of MCs in hepatic I/R injury in rat.

Materials and methods: A warm hepatic I/R injury model of 1 h ischemia followed by 24 h of reperfusion was used. MC modulation was induced *via* cromolyn injection or a method called MC depletion using compound 48/80. The effects of MC modulation were evaluated by toluidine blue staining and assessment of mast cell tryptase in sera. The role of MCs in I/R injury was evaluated by hematoxylin and eosin staining graded by Suzuki criteria, alanine aminotransferase and aspartate aminotransferase levels in sera, and malondialdehyde levels in liver homogenates.

Results: First, MC degranulation peaked after 2 h of reperfusion and liver damage peaked after approximately 6 h of reperfusion. Second, a method called MC depletion previously used in the skin with repeated injections of compound 48/80 worked similarly in the hepatic setting. Third, stabilization of MCs with cromolyn or depletion of MCs with compound 48/80 each decreased hepatic I/R injury. The most noticeable effects of cromolyn and compound 48/80 treatment were observed after approximately 6 h of reperfusion.

Conclusions: MC degranulation promotes hepatic I/R injury in rats.

1. Introduction

Mast cells (MCs) are well-characterized cells that harbor most factors responsible for allergic disease, such as histamine and inflammatory proteins [1]. During the past several years, our understanding of MC biology has rapidly expanded. MCs play a role not only in the innate immune system [2] but also in the adaptive immune system [3]. Furthermore, a large body of recent evidence has shown that MCs can participate in ischemia–reperfusion (I/R) injury in many organs, such as the intestine [4], heart [5], and brain [6]. I/R injury is a syndrome caused by ischemia and subsequent restoration of blood

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supply to an organ [7]. Organ transplantation, shock, and procedures to control bleeding during surgery can all lead to I/R injury. The effects of MCs during I/R are mainly mediated by chemicals stored within the granules of MCs that are released by degranulation. These chemicals include histamine [8], mast cell tryptase (MCT) [9], and chymase [10], among others. These molecules can promote injury by inducing physiological changes, such as increased tissue leakage and leukocyte infiltration [11]. There are many methods that can be used to study the effects of MC degranulation, such as stabilizing MCs to prevent degranulation [12], depleting MCs by degranulating most of the stored granules [13], and using MC-deficient animals [14]. Cromolyn is a well-known MC stabilizer. Compound 48/80, a well-known inducer of MC degranulation, can deplete MCs through repeated injections. The MC depletion method was previously used in skin [13].

The role of MCs in I/R injury of the liver has not been well characterized. A recent study [15] using an isolated perfused rat liver model concluded that MCs are not involved in hepatic I/R injury. However, this research was carried out *in vitro* and data were collected only 1 h after reperfusion. There are many experimental studies on hepatic I/R injury *in vivo* assessed at time point of 3 h after reperfusion or longer [16]. We therefore sought to clarify whether MCs play a role in hepatic I/R injury *in vivo*. First, we evaluated temporal changes in MC degranulation and hepatic function during reperfusion after ischemia in a warm I/R model. Second, we tested whether depleting MCs through repeated injections of compound 48/80, as previously used in the skin [13], could work similarly in the liver. Finally, we examined whether MC stabilization with cromolyn or depletion with compound 48/80 could reduce hepatic I/R injury *in vivo*. We observed that depletion of MCs with compound 48/80 to exhaust most granules before I/R insult or stabilization of MCs with cromolyn to prevent degranulation during I/R can protect the rat liver from I/R injury, indicating that MC degranulation plays a role in hepatic I/R injury.

2. Materials and methods

2.1. Ethics statement

All animal handling and experimental procedures were approved by the Animal Care and Use Committee of the Shanghai Jiao Tong University School of Medicine. Pentobarbital sodium anesthesia was used in every surgical procedure, and efforts were made to minimize suffering as much as possible.

2.2. Animals and reagents

All rats were purchased from Sino-British Sippr/Bk Laboratory Animal Ltd (Shanghai, China). They were maintained in standard conditions and fed with free water and standard laboratory chow food *ad libitum*. Animals fasted for 12 h before surgery. Cromolyn, compound 48/80, and toluidine blue were purchased from Sigma (St Louis, MO).

2.3. Surgical procedure

A warm I/R injury model was created in rats as previously described [17]. In brief, male Sprague-Dawley rats weighing 200–250 g were anesthetized by pentobarbital sodium (40 mg/kg). The abdomens were opened and the liver hila were exposed. The branches of portal veins and hepatic arteries that enter into the left-lateral and median lobes were occluded with a nontraumatic microvascular clip for 60 min, and then the clamp was removed to perfuse the ischemic liver lobes. This model of partial hepatic I/R injury can avoid splanchnic congestion and any confounding effects resulting from bowel ischemia or hemodynamic disturbances. Reperfusion was confirmed by an immediate color change before the abdomen was closed. Rats without color change during reperfusion after ischemia were excluded from furthermore analysis. The abdomens of sham-operated rats were left open for 60 min without I/R procedures. After closing the abdomen, 2 mL of normal saline was injected through the penile vein to compensate for fluid loss in each rat. During I/R, the body temperature of rat was maintained at 37 ± 0.3°C by a warm support. After surgery, the rats were allowed to recover with *ad libitum* access to food and water.

2.4. MC depletion with compound 48/80

Injection of compound 48/80 has previously been used to deplete MCs in the skin [13]. To determine whether this method can effectively deplete MCs in the liver, rats were assigned to a compound 48/80–no ischemia (CMP-N) group ($n = 5$) and a phosphate buffered saline–no ischemia (PBS-N) group ($n = 5$). Briefly, a 0.1% (w/v) solution of compound 48/80 in PBS was administered to rats intraperitoneally in the morning and evening for eight doses, starting with an evening dose. For the first six doses, 0.6 mg/kg was used, and 1.2 mg/kg was used for the last two doses. An equivalent volume of PBS was administered intraperitoneally to control animals. At 5–6 h after the last injection, rats were euthanized directly and samples were collected.

To determine the effects of MC depletion with compound 48/80 on hepatic I/R injury, rats ($n = 5$) were treated as described previously except that surgery was performed 5–6 h after the last injection and rats were euthanized *via* overdose of pentobarbital sodium (80 mg/kg) at various time points of reperfusion after ischemia for analysis.

2.5. MC stabilization with cromolyn

The MC stabilizer cromolyn was administered intraperitoneally (100 mg/kg) twice, once at 16 h and again at 40 min before surgery. An equivalent volume of PBS was administered intraperitoneally to control animals. Each group contained five rats.

2.6. Sample collection

After euthanasia, blood samples were obtained from the inferior vena cava. After coagulation, the samples were centrifuged to collect the sera and the sera were stored at −80°C until use. The median lobes of livers were harvested