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Preischemic transfusion of old packed RBCs exacerbates early-phase warm hepatic ischemia reperfusion injury in rats

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ABSTRACT

Background: Hepatic innate immune cells are considered to play a central role in the early phase of hepatic ischemia reperfusion (IR) injury. Transfusion of old red blood cells (RBCs) is known to prime immune cells, and transfusion before IR may exacerbate liver injury because of the expected hyperresponsiveness of immune cells.

Materials and methods: Twenty-four Sprague–Dawley rats were divided into four groups: sham operation (Sham); hepatic IR only (IR Control); and two transfusion groups, preischemic (Pre-T) and postischemic (Post-T), in which allogeneic RBCs stored for 2 weeks were transfused before hepatic IR or after reperfusion, respectively. Partial hepatic ischemia was induced for 90 min, and reperfusion was allowed for 120 min. Serum alanine transaminase levels, area of necrosis, and apoptotic cells were then assessed. Inflammatory (tumor necrosis factor alpha, interleukin 1 beta [IL-1β], IL-6, IL-10, and cyclooxygenase 2) and oxidative mediators (heme oxygenase 1, superoxide dismutase, and glutathione peroxidase 1) were assessed for elucidating the relevant mechanisms underlying the hepatic injury.

Results: Pre-T, but not Post-T, showed increased serum alanine transaminase levels than IR Control ($P < 0.05$). Area of necrosis was more severe in Pre-T than in IR Control or Post-T ($P < 0.01$), and apoptotic cells were also more abundant in Pre-T than in IR Control ($P < 0.01$). tumor necrosis factor alpha and IL-6 levels were higher in Pre-T than in IR Control or Post-T ($P < 0.05$), with no significant difference in cytoprotective protein levels.

Conclusions: Preischemic transfusion of old RBCs aggravated hepatic injury. Inflammatory cytokines seemed to play a crucial role in liver injury exacerbation. Our results indicate that transfusion before hepatic ischemia may be detrimental.

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Introduction

Hepatic ischemia reperfusion (IR) injury can occur in several clinical settings, including hepatic resection, a transplant, and trauma, potentially resulting in adverse outcomes. During the early phase (<2 h after reperfusion) of hepatic IR, Kupffer cells play a key role as the initial cytotoxic cell type and as a source of many proinflammatory mediators, thus being responsible for the initial warm hepatic IR injury.[1,2] During reperfusion, the injured hepatocytes leak damage-associated molecular patterns, which lead to the activation of antigen-presenting cells such as Kupffer cells and dendritic cells.[3] Hepatic IR injury also results in a local proinflammatory response mediated by numerous hepatic nonparenchymal innate immunity cells, including Kupffer cells, dendritic cells, T lymphocytes, natural killer cells, and neutrophils, and this response generates an immune cascade that sustains itself by recruiting peripheral immune cells from the circulation, ultimately causing hepatic reperfusion injury.[3] This overactivation of the immune system has now been recognized to be a critical factor in hepatic IR injury.[4]

Many clinical conditions implicated in hepatic IR injury may be complicated by substantial bleeding and eventually require multiple transfusions. In particular, preoperative red blood cell (RBC) transfusion is necessary in many situations such as severe anemia, chronic illness, and massive trauma. The current practice of blood transfusion services allows standard-issue RBCs to vary widely in age; this situation has resulted in a recently reported median storage duration of 15.3 days with a wide range[5] and the mean RBC age of 22.0 (standard deviation, 8.4) days[6] or longer for transfusion in critically ill patients. Besides, concerns about stored RBC lesions are a reality in clinical practice: Most RBCs that do not survive the first 24 h after transfusion are removed from the circulation within the first few hours after transfusion[7] by tissue macrophages in the liver, spleen, and kidneys, and this process leads to the acute delivery of large amounts of free iron to the monocyte–macrophage system and causes inflammation and injury via induction of proinflammatory cytokines.[8] Therefore, transfusion of old RBCs is known to prime hepatic macrophages through rapid delivery of substantial amounts of free iron to hepatic macrophages[9] or via the provision and induction of proinflammatory cytokines.[10] Recently, old packed RBC (OPRBC) transfusion was reported to prime the host innate immunity through a release of damage-associated molecular patterns generated throughout RBC storage or from endothelial-cell necropsies, thus potentiating subsequent injury.[11]

On the basis of the results of the aforementioned studies indicating that OPRBCs prime host innate immunity cells in the liver, thus rendering them hypersensitive to subsequent stimulation, we hypothesized that preischemic transfusion of old RBCs results in severe liver injury than does post-ischemic transfusion of OPRBCs during reperfusion. It is unknown whether preischemic transfusion of OPRBCs aggravates hepatic injury during the early phase of reperfusion.

Materials and methods

Animals

Male Sprague–Dawley rats weighing between 275 g and 325 g were used in this study. All the animal procedures were approved by the Institutional Animal Care and Use Committee (YUMC-AEC 2013-025). The rats were anesthetized by intraperitoneal injection of 30 mg/kg Zoletil and 10 mg/kg Rompun with additional doses administered as needed.

Collection and storage of OPRBCs

Four to six rats from each group were subjected to pooled collection of allogeneic blood. After midline laparotomy, blood samples from the rat hearts were drawn into an 8.5-mL BD Vacutainer (BD Diagnostics, Franklin Lakes, NJ) containing 1.5 mL of an acid citrate dextrose solution. Packed RBCs were prepared by centrifugation at 3500 rpm for 10 min, and hemoglobin concentrations were between 17 g/dL and 18 g/dL. The packed RBCs were stored at 4°C for 2 weeks and then used for transfusion. The storage duration of the RBCs was determined on the basis of other studies.[12,13] Blood cultures for bacterial infection and cross-matching were tested immediately before transfusion.

The surgical protocol

Partial hepatic ischemia was induced for 90 min, and reperfusion was allowed for 120 min. The rats ($n = 24$) were randomly subdivided into four equal groups: Sham, sham operation; IR Control, hepatic IR without transfusion of OPRBCs; Pre-T, transfusion of OPRBCs for 30 min before hepatic ischemia; and Post-T, transfusion of OPRBCs for 30 min begun at 30 min after reperfusion. The rats were placed under a heating lamp on a 37°C heating pad. Midline laparotomy without liver manipulation was performed on the rats in the Sham group. In groups IR Control, Pre-T, and Post-T, the portal vein and left branch of the hepatic artery were occluded using a microvascular clamp to induce ischemia of the median and left lateral lobes of the liver after midline laparotomy. OPRBCs were then infused into the tail vein, using a 24-gauge intravenous catheter. The transfused volume of OPRBCs was 15% of the estimated blood volume[14]: this value was arbitrarily selected according to a report indicating that transfusion of old RBCs induces dose-responsive proinflammatory-cytokine upregulation.[8] The rats were then placed in cages under warm conditions and allowed *ad libitum* access to water and chow. After reperfusion for 120 min, the rats were euthanized, and blood samples for the determination of liver enzyme levels were collected from the heart. Ischemic liver tissues were excised and prepared for histological analysis and reverse-transcription polymerase chain reaction (RT-PCR) for quantification of mRNA expression of inflammatory cytokines, antioxidant enzymes, and cyclooxygenase 2 (COX-2).