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Ribonuclease attenuates hepatic ischemia reperfusion induced cognitive impairment through the inhibition of inflammatory cytokines in aged mice

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ABSTRACT

Background: Elderly patients undergoing major surgery often develop cognitive dysfunction, and no optimum treatment exists for this postoperative complication. Ribonuclease, the counterpart of ribonucleic acid, has mostly been reported in terms of its use as a potential modality in anticancer therapy, and recent studies have demonstrated that ribonuclease can exert organ-protective effects in several pathological conditions. Our study also demonstrated that ribonuclease protects the liver against ischemia reperfusion injury. Nevertheless, it is unknown whether ribonuclease can attenuate the cognitive dysfunction that is induced by liver ischemia reperfusion. In this study, we aimed to evaluate the effect of ribonuclease on cognitive function after liver ischemia reperfusion.
Methods: Aged mice underwent sham surgery or 60 min of hepatic ischemia reperfusion, vehicle or ribonuclease, which were administered subcutaneously. The primary observation endpoint was the Morris water maze; following 24 h, 3 days, and 7 days of reperfusion, the levels of serum and hippocampus proinflammatory cytokines were measured to reveal the underlying mechanism.
Results: A probe test was conducted on day 3 and a reversal probe test was conducted on day 7 after surgery; the results demonstrated a reduction in cognitive function after liver ischemia reperfusion and that ribonuclease treatment attenuated cognitive impairment. The levels of serum and hippocampus proinflammatory cytokines (interleukin-6 and interleukin-1β) and extracellular ribonucleic acid were significantly increased at 24 h after reperfusion, but ribonuclease treatment markedly reduced the proinflammatory cytokine increase.
Conclusion: The results of the study suggested that hepatic ischemia reperfusion leads to cognitive impairment in aged mice and an increase in inflammatory cytokine expression in both serum and the hippocampus; more importantly, ribonuclease showed protective effects against cognitive impairment through inhibiting the release of inflammatory cytokines.

1. Introduction

Postoperative cognitive dysfunction (POCD), which is caused by brain impairment, is a frequent complication following major surgery in elderly people. This dysfunction is characterized by a progressive deterioration of cognitive function, reduction in self-care, increased hospitalization and delayed recovery. With advances in surgical and anesthetic techniques and in combination with increased life expectancy, POCD is becoming an area of focus in hospitals. Nevertheless, the definite mechanism of POCD remains unclear, and no optimum treatment yet exists for this postoperative complication. Increasing evidence has indicated that neuroinflammation plays an essential role in the pathogenesis of POCD [1]. High level of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), in serum and brain tissue are associated with the occurrence of POCD [2,3]. The effects of inflammation on cognition function may be mediated by different mechanisms; for example, direct effects on memory processes through cytokine-dependent

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signaling, alterations in neurogenesis, or epigenetic modifications [4].

A previous study demonstrated that extracellular RNA (exRNA) is enriched at sites of tissue damage and acts as a proinflammatory mediator [5]. In accordance with the damaging nature of exRNA, several proinflammatory cytokines (including TNF-α, IL-1β, and IL-6) are upregulated in macrophages [5]. Ribonuclease (RNase), the counterpart of exRNA, has mostly been reported as of potential use in anticancer therapy; however, recent studies have demonstrated that it can also exert potent organ-protective effects in several pathological conditions due to its mediation of an anti-inflammatory effect [6–8]. During cardiac ischemia reperfusion (IR) injury, RNase performs cardiac protection through the attenuation of myocardial cytokine production, leukocyte infiltration and apoptosis [8]; it also exhibits neuroprotective properties by reducing vessel occlusion, infarct volume [9], and preventing brain edema formation [6]. Our previous study demonstrated that RNase protects the liver against IR injury through the inhibition of inflammatory cytokines and apoptosis in mice (data not published). However, it is unknown whether RNase treatment can attenuate POCD that is induced by hepatic IR.

Therefore, based on the previous results and the contribution of the exRNA/RNase system to inflammation, we hypothesized that exRNA plays an important role in the IR-induced inflammation response and that POCD and RNase can protect against exRNA-mediated injury. Thus, in the present study, we aimed to evaluate the effect of RNase treatment on cognitive function and the expression of proinflammatory cytokines in a mouse hepatic IR model.

2. Materials and methods

2.1. Animals

Male wild type C57BL/6J mice (aged 15 months, 25–35 g) were used in the study. All animal experiments followed the guidelines published by the Ministry of Science and Technology of China. Care was taken to minimize discomfort, distress, and pain in the animals. The study protocol was approved by Animal Ethics Committee of West China Hospital of Sichuan University (Chengdu, China).

2.2. Experimental design

The mice were divided into four groups: vehicle-treated sham operation (Sham); RNase-treated sham operation (Sham + RNase); vehicle-treated mice subjected to IR (IR); and RNase-treated mice subjected to IR (IR + RNase). The first experiment was conducted to determine the effects of liver IR and RNase treatment on cognitive function in aged mice; a subsequent experiment was conducted to determine the effect of the RNase treatment on pro-inflammatory cytokine protein and mRNA expression in the serum and hippocampus at days 1 and 3. A schematic outline of the experimental protocol is presented in Fig. 1

2.3. Surgical procedure

We used a well-established nonlethal model of segmental (70%) hepatic warm liver IR [10]. Briefly, mice were anesthetized by an intraperitoneal injection of ketamine (120 mg/kg) and xylazine (4 mg/kg), and a midline laparotomy was performed. All structures in the portal triad (hepatic artery, portal vein, bile duct) to the left and median liver lobes were occluded with a microvascular clamp for 60 min; reperfusion was initiated by removal of the clamp. This method of segmental hepatic ischemia prevents mesenteric venous congestion by permitting portal decompression through

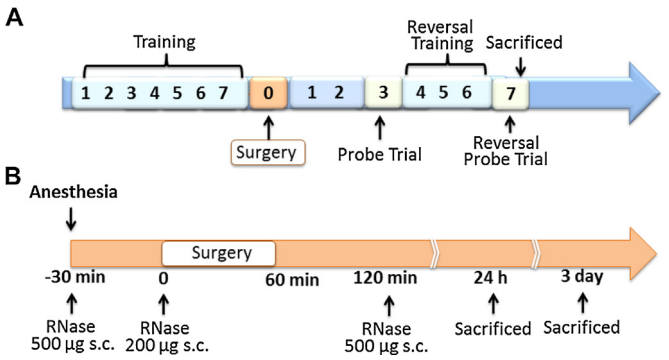

Fig. 1. A schematic outline of the experimental protocol and the timeline of RNase administration.
A, A schematic outline of the Morris water maze protocol (n = 12, except for the IR group, where n = 13). B, Timeline of RNase A administration and biochemical analyses (n = 5, except for the IR group, where n = 6).

the right and caudate lobes. The abdomen was covered to minimize evaporative loss. Throughout the ischemic interval, evidence of ischemia was confirmed by visualizing the pale blanching of the ischemic lobes. The clamp was then removed, and gross evidence of reperfusion based on an immediate color change was assured before closing the abdomen. The absence of ischemic color changes, lack of response to reperfusion, or death were criteria for exclusion from further analysis. Temperature was monitored and maintained at 36.5 to 37.5 °C. All mice received 50 μL of 0.2% ropivacaine subcutaneously for post-operative analgesia. At the end of the observation, the mice were anesthetized and killed by exsanguination. Sham-operated mice underwent the same procedure without portal triad occlusion. The operators were blinded to the study design and intervention.

2.4. Drug treatment

Bovine pancreatic RNase A (Invitrogen) was dissolved in normal saline (vehicle) and administered subcutaneously: 30 min before ischemia (500 μg/100 μL), immediately before ischemia (200 μg/200 μL), and 120 min after reperfusion (500 μg/100 μL). The dosage and time points of RNase administration were selected based on previous publications [6,8].

2.5. Morris water maze test

The Morris water maze (MWM) is the most widely accepted hippocampal-dependent test of spatial learning and memory for rodents [11]. Mice were released into the water facing the wall of the pool from one of four randomly assigned release points, if animals could not find the escape platform within 60 s, the experimenters gently put animals onto the platform and allowed them to rest there for 15 s. Each animal was trained for four trials per day for 6 consecutive days. Animals underwent surgery on day 7. To assess reference memory, a probe trial was given at postoperative day 3. The platform was removed, and the animals were placed in the opposite quadrant of the pool. On postoperative days 4, 5, 6 and 7, the mice were subjected to the reversal learning and reversal probe trial, in which the platform was relocated to the opposite quadrant of the pool. Reversal learning reveals whether animals can extinguish their initial learning of the platform's position and acquire a direct path to the new goal position [11]. Swimming distance, speed, latency to the platform, and percentage of time spent in the old quadrant were recorded by video tracking, and digital images were analyzed using water maze software (Panlab, Barcelona, Spain).