Resuscitation 81 (2010) 100–105

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Experimental paper

Effect of speed of rewarming and administration of anti-inflammatory or anti-oxidant agents on acute lung injury in an intestinal ischemia model treated with therapeutic hypothermia☆

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ARTICLE INFO

Article history:
Received 28 May 2009
Received in revised form 11 August 2009
Accepted 21 September 2009

Keywords:
Hypothermia
Rewarming
Acute lung injury
Cytokine

ABSTRACT

Aim of the study: Acute lung injury (ALI) develops in various clinical situations and is associated with high morbidity and mortality and therapeutic hypothermia (HT) has been studied to attenuate the ALI. However, the optimal method of rewarming has not been determined. We determined the effect of speed of rewarming and the administration of anti-inflammatory or anti-oxidant agents on ALI in an intestinal ischemia and reperfusion (I/R) model treated with HT.

Materials and methods: A Sprague–Dawley rat model of intestine ischemia and reperfusion was used. Two parallel animal experiments were conducted. In the survival study, rats ($n = 5$ per group) underwent normothermic intestinal ischemia (60 min, 36–38 °C) and then randomized into 7 groups with reperfusion: normothermia (NT), HT without rewarming (30–32 °C, HT), 2 h HT + rewarming for 1 h (RW1), 2 h HT + rewarming for 2 h (RW2), RW1 + N-acetyl cysteine (RW-NAC), RW1 + ethylpyruvate (RW-EP), and RW1 + dexamethasone (RW + Dexa). In the second experiment, we investigated the histological and biochemical effects on the lung 4 h after reperfusion ($n = 8$ per group).

Results: The survival rate was lowest after NT. The HT, RW2, and RW-Dexa groups survived longer than the RW1, RW-NAC, and RW-EP groups. ALI scores were lower in the HT, RW2, and RW-Dexa groups than RW1. Lung malondialdehyde content was also lower in these groups. Interleukin (IL)-6 was significantly higher in the RW1 group. Inducible NO synthase gene expression in lung was lower in the HT, RW2, and RW-Dexa than RW1, and serum NO was lower in the RW2 and RW-Dexa than RW1.

Conclusion: Gradual rewarming and administration of dexamethasone improved survival and attenuated ALI after intestinal I/R injury treated with HT in rats.

1. Introduction

Intestinal ischemia and reperfusion (I/R) injury occurs in the setting of various clinical situation, such as necrotizing enterocolitis, midgut volvulus, intussusception, mesenteric ischemic disease, hemodynamic shock, and sepsis.[1] Animal studies have shown that intestinal I/R results in a systemic inflammatory response that causes multiple organ failure.[2]

There is evidence suggesting that therapeutic hypothermia (HT) can attenuate multi-organ failure by various mechanisms including the slowing of metabolism,[3,4] apoptosis, neuroexcitatory cascade, immune response, inflammation, free-radical production, and altered membrane permeability.[5] Therapeutic HT has been evaluated in a variety of clinical situations including cardiac arrest, post-anoxic encephalopathy, traumatic brain injury, during surgery, stroke, subarachnoid hemorrhage, acute myocardial infarction, and ARDS.[6] In all cases, therapeutic HT is only induced temporarily after which the subjects need to be rewarmed. However the optimal method and rapidity of rewarming have rarely been investigated.[7] While gradual warming over a 6–8 h period is recommended after out of hospital cardiac arrest, there is little evidence to support this mode of rewarming.

In animal models of lethal hemorrhage or traumatic brain injury, rapid rewarming has been detrimental.[7–9] In these studies rapid rewarming has been associated with an inflammatory burst that is associated with worse outcomes. Clinical studies in patients with traumatic brain injury (TBI) or those undergoing coronary artery

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2009.09.020.

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doi:10.1016/j.resuscitation.2009.09.020

bypass grafting (CABG) patients revealed that rapid rewarming was also associated with worse outcomes.[10,11]

The current study was designed to investigate the effect of speed of rewarming and use of a variety of anti-inflammatory and anti-oxidant agents on the incidence and severity of acute lung injury in a rat model of intestinal ischemia and reperfusion (I/R) treated with therapeutic hypothermia. We hypothesized that gradual rewarming and addition of an anti-inflammatory or anti-oxidant would reduce mortality and acute lung injury.

2. Materials and methods

This study was approved by the Institutional Review Board of our hospital for the care and use of laboratory animals.

2.1. Animal preparation

Male Sprague–Dawley rats weighing 280–320 g were studied. The rats were housed in a controlled environment with free access to food and water before the experiment. The rats were anesthetized with ketamine/xylazine and underwent midline laparotomy and isolation of the superior mesenteric artery (SMA). A micro-bulldog clamp was applied at the aortic origin of SMA, and the incision was sutured. A 24 gauge catheter was inserted into the tail vein for drug or fluid infusion. All procedure was performed under a heating lamp to maintain the body temperature of 36–38 °C, that was measured with an indwelling rectal thermometer. De-clamping was done after 60 min of ischemia with the removal of micro-bullog clamp. Study drug (ethyl pyruvate 50 mg/kg iv push in RW-EP group and dexamethasone 10 mg/kg iv push in RW-Dexa group) or Hartman solution in other groups was administrated intravenously as loading dose and fluid resuscitation via a tail vein was started with NAC-mixed 5% dextrose (150 mg/kg/h, NAC group) or 5% dextrose (other groups) at a rate of 0.5 ml/100 g/h.

2.2. Experimental design

The study included two experiments. The first was a survival study while the second evaluated ALI. In the first experiment, all animals underwent normothermic ischemia for 60 min as described, and then were randomized into 7 groups with reperfusion: (1) normothermia (NT, maintaining a body temperature of 36–38 °C for the entire experiment); (2) HT (continuous hypothermia without rewarming, 30–32 °C); (3) RW1 (2 h HT + rewarming for 1 h); (4) RW2 (2 h HT + rewarming for 2 h); (5) RW-NAC (2 h HT + rewarming for 1 h + iv NAC); (6) RW-EP (2 h HT + rewarming for 1 h + iv ethyl pyruvate); (7) RW-Dexa (2 h HT + rewarming for 1 h + iv dexamethasone).

In the second experiment, we evaluated the histological and biochemical damage in the lungs 4 h after reperfusion in the above study groups ($n = 8$ per group) except for the NT group.

2.3. Methods of hypothermia and rewarming

HT was induced and maintained by covering the rats' bodies (excluding their heads) with ice packs. The target temperature was reached within 20 min without variation and this time was included total duration of HT. Rewarming was performed using a heating pad and adjusting the temperature of the heating pad to the desired temperature of the animals.

2.4. Tissue sample collection

After the observation period, blood samples were drawn to measure the plasma levels of nitric oxide and cytokines. The lungs were rapidly removed and the left lower lobe was fixed with 10% formaldehyde solution for histopathological evaluation and the rest of lung was cut into small pieces, and was snap frozen in liquid nitrogen and stored at −70 °C.

2.5. Acute lung injury (ALI) scoring

The ALI scoring was performed by a board certified pathologist masked to treatment assignment and classified into 4 categories based on the severity of alveolar congestion and hemorrhage, infiltration of neutrophils in the air spaces or vessel walls, and the thickness of alveolar wall/hyaline membrane formation. The severity of each category was graded from 0 (minimal) to 4 (maximal) and the total score was calculated by summing the individual scores. ALI score of each animal was calculated as the mean of 4 sections.

2.6. Cytokine measurements

Plasma concentrations of IL-6 and IL-10 were determined with commercially available enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN, USA).

2.7. Malondialdehyde (MDA)

MDA is a by-product of lipid peroxidation due to oxidative stress. MDA was measured according to the method of Ohkawa with thiobarbituric acid.[12] The level of MDA was presented as nmol/g of tissue.

2.8. iNOS gene expression

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) were used to determine the levels of iNOS expression. The total RNA from 100 mg of lung tissue was extracted using a commercial kit (TRI Reagent, Molecular Research Center, Inc., USA). The concentration of extracted RNA was determined at 260 nm by using a spectrophotometer (Beckman, USA). The extracted RNA (100 ng) was reverse-transcribed to generate first strand complementary DNA (cDNA). The reaction mixture (20 μl) for reverse transcription was composed of 5 mM $MgCl_2$, 1 mM dNTP, 2.5 mM Random Hexmer (Promega, USA), 1 U/μl ribonuclease inhibitor (Promega, USA), and Moloney Murine Leukemia Virus reverse transcriptase (GibcoBRL, USA). After generating cDNA, we obtained rat iNOS from the cDNA samples by using a PCR (PCT-200 Peltier Thermal Cycler, MJ Research, USA). The reaction mixture (20 μl) for the PCR contained 2 pmol of primer, 2.5 U/μl Taq polymerase (Takara, Japan), 0.8 mM dNTP, and 1.5 mM $MgCl_2$. The PCR was run for 30 cycles after an initial denaturation step at 94 °C for 3 min with the amplification profile for each cycle consisting of denaturation for 1 min at 94 °C, primer annealing for 1 min at 58 °C, and elongation for 2.5 min at 73 °C. After the last cycle of amplification, the samples were incubated for 10 min at 72 °C. The oligonucleotide primers for rat iNOS cDNA were 5′-CCCTTCCGAAGTTTCTGGCAGCAGG-3′ (sense) and 5′-GGCTGTCAGAGCCTTGTGCCTTTGG-3′ (antisense) (36). The PCR products were then visualized by UV illumination after electrophoresis through 1.5% agarose gels containing 0.5 μg/ml ethidium bromide. The sizes for the iNOS gene PCR products were 498 bp. The gel photographs were scanned using a bioimage processing system (Biomedlab, Korea). The band density of the iNOS cDNA was measured by an imaging densitometer (BIO-RAD, USA).