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Melanocortin-4 receptor agonists alleviate intestinal dysfunction in secondary intra-abdominal hypertension rat model

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ABSTRACT

Background: Intra-abdominal hypertension (IAH) is a potentially life-threatening disease. Melanocortin-4 (MC4) receptor activation exhibits life-saving properties. The aim of the present study was to examine whether treatment with the MC4 receptor agonist RO27-3225 ameliorates intestinal injury in IAH rats.

Methods: A total of 72 male Sprague–Dawley rats were randomized into six groups. Group 1 was the sham group. Group 2, the sham + RO group, received RO27-3225 (180 μg/kg, intraperitoneally). IAH was induced in group 3, the IAH group, by blood draw (mean arterial pressure = 30 mm Hg for 90 min) followed by shed blood and/or Ringer solution reinfusion. Intra-abdominal pressure was increased to 20 mm Hg by injecting air into the peritoneal cavity. Group 4, the RO group, was administered RO27-3225 at 5 min after blood draw. Groups 5 and 6 were the chlorisondamine (Chl) and HS024 groups, in which the rats were pretreated with the nicotinic acetylcholine receptor antagonist Chl or selective MC4 receptor antagonist (HS024), respectively, at 2 min before RO27-3225 was administered.

Results: RO27-3225 restored mean arterial pressure, reduced tumor necrosis factor-α, and interleukin-1β messenger RNA expression increased by IAH, alleviated histologic damage, and improved superoxide dismutase activity in the intestine. Compared with the IAH group, the levels of intestinal fatty acid-binding protein, intestinal edema and intestinal permeability were lower in the RO group. Furthermore, the RO27-3225 treatment increased the expression of Rho-associated coiled–coil-containing protein kinase 1 and phosphorylated myosin light chain. Chl and HS024 abrogated the protective effects of RO27-3225.

Conclusions: These data indicate that the MC4 receptor agonist counteracts the intestinal inflammatory response, ameliorating intestinal injury in experimental secondary IAH by MC4 receptor-triggered activation of the cholinergic anti-inflammatory pathway. It may represent a promising strategy for the treatment of IAH in the future.

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1. Introduction

Numerous studies have demonstrated that severe trauma, burns, and large-fluid resuscitation after noncontrolled surgery often lead to increased intra-abdominal pressure (IAP), which can result in intra-abdominal hypertension (IAH) [1–3]. If this condition is not promptly treated, it can progress to abdominal compartment syndrome (ACS). The World Society of the Abdominal Compartment Syndrome defined IAP, IAH, and ACS in 2006 and updated these definitions in 2013 [4–6].

IAH is divided into three categories, including primary, secondary, and recurrent. Secondary IAH refers to conditions that do not originate in the abdominopelvic region and could be produced by massive fluid resuscitation after hemorrhagic shock, affecting the abdominal cavity and solid organs and inducing intestinal ischemia, submucosal acidosis, increased capillary permeability, intestinal edema and bacterial translocation, and increased intestinal tract inflammation. Furthermore, large-fluid resuscitation increases IAP and results in a vicious cycle that ultimately leads to severe intestinal dysfunction [7].

There has been a recent increase in the study of endogenous melanocortins (MCs), which belong to the adrenocorticotropin and α-, β-, and γ-melanocyte-stimulating hormone (α-, β-, and γ-MSH) family [8,9]. These endogenous peptides and their synthetic analogs play roles in protecting the body against various diseases, such as hemorrhagic shock, intestinal ischemia, ischemic stroke, and traumatic brain injury [10–12]. Previous studies have shown that the melanocortin-4 (MC4) receptor can play an antishock role via the vagus nerve-mediated cholinergic pathway [11,13] in a self-defense anti-inflammatory mechanism that activates (as the main final step) the α7-acetylcholine receptor subunit on inflammatory cells [13,14].

In 2007, Giuliani *et al.* [15] found that MC can prevent morphologic and immunocytochemical changes to the heart, lung, and kidney after hemorrhagic shock. These effects are seemingly mediated by the rapid activation of the cholinergic anti-inflammatory pathway. Thus, this finding provides new insights in treating multiorgan damage in IAH.

Taken together, these observations prompted us to investigate the potential therapeutic value of the selective MC4 receptor agonist in a rat model of secondary IAH and to investigate the mechanism of its involvement.

2. Materials and methods

2.1. Animal preparation

This study was approved by the Animal Care Center of the Research Institute of Surgery and Daping Hospital (Third Military Medical University, Chongqing, China). Adult male Sprague–Dawley rats (weighing 220–250 g) were maintained in a pathogen-free environment with food pellets and tap water available *ad libitum* under a 12-h–12-h light–dark cycle. The rats were fasted at 12–16 h before surgery and were given free access to water. All the procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the Third Military Medical University and performed under the supervision of the veterinary staff.

The rats were anesthetized by an intraperitoneal injection of 30 mg/kg pentobarbital sodium and were spontaneously ventilated. They were placed on heating pads to maintain body temperature during anesthesia. The left femoral artery and vein were surgically prepared and catheterized (PE-50), respectively. The femoral artery was used for blood drawing and continuous blood pressure monitoring (mean arterial pressure [MAP]), and the femoral vein catheter was inserted into the inferior vena cava and used for fluid resuscitation and indirect IAP monitoring.

2.2. Experimental design and treatments

The rats were randomly allocated to the following groups ($n = 12$ for each group). Group 1, which served as the control group, in which the rats received only anesthesia, left femoral artery, and vein intubation without blood withdrawal and IAH induction. Group 2 (the sham + RO group), in which the rats underwent the same surgical procedures and received RO27-3225 (180 μg/kg, intraperitoneally). Group 3 (the IAH group), in which the rats were induced by a blood draw (MAP = 28–32 mm Hg for 90 min) followed by shed blood and/or Ringer solution (at twice the amount of the maximum blood volume drawn) via left femoral vein reinfusion. IAP was increased to 20 mm Hg by injecting air into the peritoneal cavity after hemorrhage and/or shock followed by resuscitation until the inferior vena cava pressure reached 20 mm Hg for 4 h. Group 4, the RO group, in which the rats were administered RO27-3225 at 5 min after blood collection. Groups 5 and 6, the chlorisondamine (Chl) and HS024 groups, respectively, in which the rats were pretreated for 2 min before administration of RO27-3225 with the nicotinic acetylcholine receptor antagonist (Chl, 250 μg/kg sc) or a selective MC4 receptor antagonist (HS024, 130 μg/kg, intraperitoneally), respectively [15–17] (Fig. 1).

2.2.1. Drugs

RO27-3225, which is a potent and selective MC4 receptor agonist [16,18,19], was supplied by Sigma–Aldrich (St. Louis, MO). HS024, which is a highly potent MC4 receptor antagonist [15,19], and Chl diiodide, which is an irreversible, long-lasting nicotinic acetylcholine receptor antagonist [10,20], were supplied by Tocris (Bristol, United Kingdom). All the substances were dissolved in saline and administered at a volume of 1 mL/kg.

2.3. Quantitative real-time polymerase chain reaction for messenger RNA expression analysis

Total RNA was isolated from the ileum near the ileocecal valve (sample size = 2–4-cm-long) using a Simple P Total RNA Extraction Kit (BioFlux, Tokyo, Japan) according to the manufacturer's instructions. Complimentary DNA was prepared using a PrimeScript RT reagent Kit with genomic DNA Eraser (Takara Bio Inc, Shiga, Japan) at 37°C for 15 min and 85°C for 5 s.