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Dexamethasone effects on vascular flow and organ injury in septic mice

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ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form
21 January 2014
Accepted 24 January 2014
Available online 29 January 2014

Keywords:
Steroid
Sepsis
Organ injury
Vascular hyporeactivity
Mesenteric blood flow

ABSTRACT

Background: To demonstrate the effects of low-dose dexamethasone treatment on mesenteric artery blood flow, oxidative injury, vascular reactivity, and survival in Swiss albino mice with intra-abdominal polymicrobial sepsis accomplished by cecal ligation and puncture (CLP).

Methods: Mice were allocated to CLP + saline, CLP + dexamethasone, sham + saline, and sham + dexamethasone subgroups to evaluate blood flow, organ injury, and vascular response to consecutive phenylephrine administrations at 24, 48, and 72 h. Survival rates were also evaluated in a different group of mice. Dexamethasone (1 mg/kg/d) and saline (4 mL/kg/d) were administered intraperitoneally to mice 2 h after CLP or sham procedure, whichever appropriate, and repeated once a day until evaluation time at 48 and 72 h. Relaparotomy was performed at the concerned time and mesenteric blood flow was measured, and liver, lung, and peritoneum samples were obtained. Alteration in mesenteric blood flow response to intravenous phenylephrine injections was recorded at the related time intervals in different mice groups. Survival group was followed up by 7-d administration of dexamethasone or saline for 18 d.

Results: The significant fall in mesenteric blood flow after CLP ameliorated with dexamethasone treatment at 48 and 72 h. Dexamethasone also diminished the malonyl dialdehyde level, which is an indicator of organ injury raised after CLP, at 24 h in liver, lung, and peritoneum samples. Dexamethasone therapy has significantly enhanced the vascular response to phenylephrine injections at all doses; however, no change was observed in survival rates.

Conclusions: Low-dose dexamethasone has beneficial effects on mesenteric blood flow and organ injury in experimental sepsis models.

1. Introduction

Sepsis, often presenting with multiple organ dysfunction syndrome and organ failure, is a major problem that causes the most challenging efforts in intensive care today [1]. Nitric oxide (NO) and a number of cytokines are known to be effective on common pathways of systemic inflammation, playing key roles on pathophysiology of vascular endothelial injury.

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0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.01.040

As a result, the vascular endothelium is known to become insensitive or oversensitive to many vasopressor or vasodilator agents [2–4]. Although high-dose corticosteroids are not recommended in severe sepsis, low doses of corticosteroids are preferred especially in the presence of vasopressor agents [5–9]. The demonstration of significant clinical changes *via* blockage of NO products by steroids in animal studies will bring new horizons for current approach to sepsis treatment.

In previous studies, it was shown that impaired mesenteric blood flow can be corrected or response to vasopressor agents can be augmented by steroids and NO synthase inhibitors through cytokine suppression in experimental sepsis models [2,4,10,11]. As it is known that steroids have suppressor effects during inflammatory processes not only because of suppression of NO but also through some other pathways, the question to be answered is when and at what dose steroid administration corrects vascular parameters, as well as organ injury and survival. Most studies that use 0.1–1 mg/kg dose of dexamethasone as low dose reported that parameters associated with sepsis have been recovered [12,13]. Also a dose of 1 mg/kg has been shown to inhibit inducible nitric oxide synthase production without affecting cyclooxygenase 2 synthesis in rat [13]. In most of cecal ligation and puncture (CLP)-induced sepsis models, the onset of late hypodynamic phase of sepsis is identified at 16–24 h [3,14].

The aim of this study is to evaluate the effects of low-dose dexamethasone on survival, mesenteric artery blood flow, organ injury, and impaired vasopressor response of the mesenteric artery in a CLP model in mice.

2. Materials and methods

A total of 110 female Swiss albino mice, weighing 20–40 g, were used in the present study. The mice were fed freely with standard laboratory food and water; they were accommodated at a controlled temperature of 22°C and humidity of 30–70% with 12-h dark and light cycle. Mice were divided into three main groups as CLP, sham, and survival, and mesenteric blood flow changes, organ injury, phenylephrine response, survival duration, and the effect of low-dose dexamethasone were investigated.

Seventy-two mice of the first group were divided into 24, 48, and 72 h subgroups (each subgroup $n = 24$) after CLP procedure. Each subgroup was further divided into CLP + saline ($n = 12$) and CLP + dexamethasone ($n = 12$) clusters. In all subgroups, mesenteric blood flow and organ injury ($n = 8$) and blood flow changes in response to phenylephrine administration ($n = 4$) were evaluated. A new group of 18 mice was created as sham group, and subdivided into subgroups where mesenteric blood flow changes and organ injury ($n = 12$) and the response to phenylephrine ($n = 6$) were evaluated. Normal saline was administered to half of each group of mice and dexamethasone to the other half. As phenylephrine response measurements were not shown to be influenced by time or dexamethasone in sham groups, the number of mice ($n = 6$) was not increased. The effect of low-dose dexamethasone on survival was evaluated in the third group on 20 mice with polymicrobial sepsis. To this end, CLP to 10 of 20 mice, CLP + dexamethasone to 10 other was applied, and 18-d survival rates were evaluated.

According to the methods described previously, polymicrobial sepsis was accomplished by CLP after the intraperitoneal injection of 400 mg/kg chloral hydrate (an anesthetic agent) [15]. Similar laparotomies for sham surgery groups were performed except the CLP procedure. All the animals were injected 40 mL/kg of normal saline subcutaneously immediately after the procedure. All animals in dexamethasone groups were given 1 mg/kg (in a volume of 4 mL/kg) of dexamethasone intraperitoneally after 2 h of CLP or sham procedure. This process was repeated after 24 h for the animals of 48- and 72-h groups. Thus, the 24-h groups received a single dose, 48-h groups received two doses, and 72-h groups a total of three doses of dexamethasone. Animals not receiving dexamethasone were administered 4 mL/kg of normal saline intraperitoneally. Mice monitored in survival groups received 1 mg/kg (in a volume of 4 mL/kg) of dexamethasone or 4 mL/kg of normal saline intraperitoneally similar to the corresponding subgroups, continuing for 7 d, and stopped gradually within 4 d. All groups of animals received 40 mL/kg/d of normal saline subcutaneously for 3 d after surgery. We established the presence or absence of sepsis in rats by evaluating each rat for the presence or absence of periorbital dark halo, the presence or absence of piloerection, and normal or decreased activity.

2.1. Mesenteric blood flow measurement

For mesenteric blood flow measurement and organ injury detection, 60 mice were prepared as described previously and were re-evaluated at 24, 48, or 72 h. As mentioned earlier, after anesthetizing with 400 mg/kg of chloral hydrate, abdomen was re-entered through the former midline incision extending between xyphoid and pubis. Each time the state of the intra-abdominal infection was evaluated by observing foul-smelling discharge, tissue destruction, and sometimes abscess formation, and then the mesenteric blood flow was measured over the isolated mesenteric artery using a Doppler flow-sensing probe (Perivascular Flow Probes; Transonic Systems, Ithaca, NY) and a flow rate measuring device (Ultrasonic Volume Flowmeter T-106X; Transonic Systems). The absolute blood flow values were measured in milliliters per minute according to the detailed guiding of our previous publication [2]. These absolute values were normalized for each mouse by dividing with the body weight of the individual animal and are expressed as milliliters per minute per kilogram of body weight (corrected mesenteric blood flow). After the mesenteric blood flow measurement, a sternotomy was added and the mice were sacrificed by exsanguination of the blood from the heart and simultaneously samples of the lung, liver, and peritoneum were taken to determine malonyl dialdehyde (MDA) levels to evaluate organ injury.

2.2. Determination of organ injury

There are many methods known to determine oxidative injury in tissues and lipid peroxides are one of those. MDA is a product formed during lipid peroxidation [3]. MDA and other aldehydes in laboratory conditions react with thiobarbituric acid to produce a colored mixture that allows the determination of lipid