Kidney Res Clin Pract 33 (2014) 79–88

Kidney Research and Clinical Practice

journal homepage: http://www.krcp-ksn.com
Contents lists available at ScienceDirect

Original Article

Development of intestinal ischemia/reperfusion-induced acute kidney injury in rats with or without chronic kidney disease: Cytokine/chemokine response and effect of α-melanocyte-stimulating hormone

Martin Skott [1,2], Rikke Nørregaard [2,3], Hanne Birke-Sørensen [3], Johan Palmfeldt [4], Tae-Hwan Kwon [5], Thomas Jonassen [6], Jørgen Frøkiær [2,3], Søren Nielsen [1,2,*]

[1] Department of Biomedicine, University of Aarhus, Aarhus, Denmark
[2] The Water and Salt Research Center, University of Aarhus, Aarhus, Denmark
[3] Institute of Clinical Medicine, University of Aarhus, Aarhus, Denmark
[4] Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Denmark
[5] Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, Korea
[6] Department of Pharmacology, University of Copenhagen, Copenhagen, Denmark

Article history:
Received 10 November 2013
Received in revised form
4 February 2014
Accepted 10 February 2014
Available online 29 April 2014

Keywords:
Acute kidney injury
Chronic kidney disease
Intestinal ischemia and reperfusion
α-melanocyte-stimulating hormone

Abstract

Background: The primary aim of the study was to investigate the cytokine/chemokine response in the kidney, lung, and liver following acute kidney injury (AKI). The secondary aim was to test whether α-melanocyte-stimulating hormone (α-MSH) could prevent a reduction in organ function, and attenuate the inflammatory cytokine/chemokine response within the kidney, lung, and liver following AKI in rats with or without preexisting chronic kidney disease (CKD).

Methods: A two-stage animal model, in which AKI was induced in rats with preexisting CKD, induced by 5/6 nephrectomy (Nx), was used. Six weeks later, AKI was induced by intestinal ischemia and reperfusion (IIR). Sham procedures [S(Nx) and S(IIR)] were also performed.

Results: Increasing levels of serum creatinine (sCr) demonstrated progressive development of CKD in response to Nx, and following IIR sCr levels increased further significantly, except in the S(Nx) group treated with α-MSH. However, no significant differences in the fractional increase in sCr were observed between any of the groups exposed to IIR. In kidney, lung, and liver tissue the levels of interleukin (IL)-1β were significantly higher in rats undergoing IIR when compared to the S(IIR) and control rats. The same pattern was observed for the chemokine monocyte chemoattractant protein (MCP)-1 in lung and liver tissue. Furthermore, kidney IL-1β and RANTES levels were significantly increased after IIR in the Nx rats compared to the S(Nx) rats.

Conclusion: Both the functional parameters and the cytokine/chemokine response are as dramatic when AKI is superimposed onto CKD as onto non-CKD. No convincing protective effect of α-MSH was detected.

* Corresponding author. The Water and Salt Research Center, Department of Biomedicine, Anatomy, University of Aarhus, DK-8000 Aarhus, Denmark.
E-mail address: sn@ana.au.dk (S Nielsen).

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http://dx.doi.org/10.1016/j.krcp.2014.02.002

Introduction

It is well accepted that preexisting chronic kidney disease (CKD) increases a patient's risk of developing acute kidney injury (AKI), moreover the risk increases in proportion to the respective CKD stage [1,2]. Furthermore, any episode of AKI in a patient with underlying CKD substantially increases the rate of transition to end-stage renal disease (ESRD), mostly because of the additional damage in the already compromised kidneys [3,4].

This association between CKD and AKI, has been widely supported epidemiologically, but only to a lesser extent biologically [5–9]. Therefore, we recently developed a two-stage animal model in which CKD was introduced prior to AKI. Briefly, CKD was induced by 5/6 nephrectomy (Nx) and AKI by intestinal ischemia and reperfusion (IIR). The latter leads to systemic inflammation and ultimately multiple organ failure (MOF) [10] and AKI [11,12]. This systemic inflammation initiates a release of different proinflammatory mediators (e.g., cytokines, chemokines, and leukocytes) into the systemic circulation [13]. Together these mediators induce generalized microvascular injury, which culminate in MOF, including acute pulmonary, hepatic, and renal injury [11,12,14,15]. This form of "hypoxic AKI" is fundamentally different from the often-studied warm ischemia and reperfusion (IR) AKI model. In this model, a complete interruption of renal blood flow, for various periods of time, by renal artery occlusion is used to induce AKI. Although this clamp approach is often used, it is clearly different from human AKI, in which most cases occur after systemic hemodynamic derangement rather than isolated renal artery IR [16–19].

We hypothesize that CKD in rodents exacerbates the inflammatory response within the kidney as well as remote organs after IIR. Moreover, we hypothesize that a single dose of the anti-inflammatory drug, α-melanocyte-stimulating hormone (α-MSH), administered intravenously, attenuates the inflammatory response after IIR.

α-MSH is a neuropeptide with broad anti-inflammatory properties. It has been shown to inhibit tissue injury in different experimental models of inflammatory organ failure. Specifically, it has been shown to protect against intestinal and renal IR injury [20,21] and liver injury during endotoxemia [22]. The mechanisms of action are wide-ranging: (1) inhibiting production and actions of proinflammatory cytokines and chemokines [22,23]; (2) inhibiting neutrophil migration and infiltration into the tissue [22,23]; and (3) increasing the production of the anti-inflammatory cytokine interleukin (IL)-10 [24]. Previous studies have reported the beneficial effects of α-MSH in a dose of 200 μg/g in rodents in various models of intestinal and renal IR [20,25,26].

Methods

Chemicals

α-MSH (500 μg) was purchased from Phoenix Pharmaceuticals (Phoenix Europe GmbH, Karlsruhe, Germany).

Animals

Male Wistar rats were purchased from Taconic (Eiby, Denmark). Animal experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health and approved by the Danish Ministry of Justice. All rats were housed in pairs at room temperature (21°C), with alternating 12:12-hour light-dark cycles, fed with standard rat chow (Altromin, Lage, Germany), and free access to tap water.

Experimental design and surgery

Rats were randomized according to their initial body weight into six groups (Fig. 1). Two groups underwent Nx 6 weeks prior to IIR, whereas two other groups underwent sham Nx [S(Nx)]. Another group underwent only sham IIR [S(IIR)] and a final group of untreated rats served as a control group. At "Before IIR" (Day 39), which refers to 3 days prior to IIR (Day 42), the rats in the two Nx groups were re-randomized into two new groups according to their serum creatinine concentrations (measured

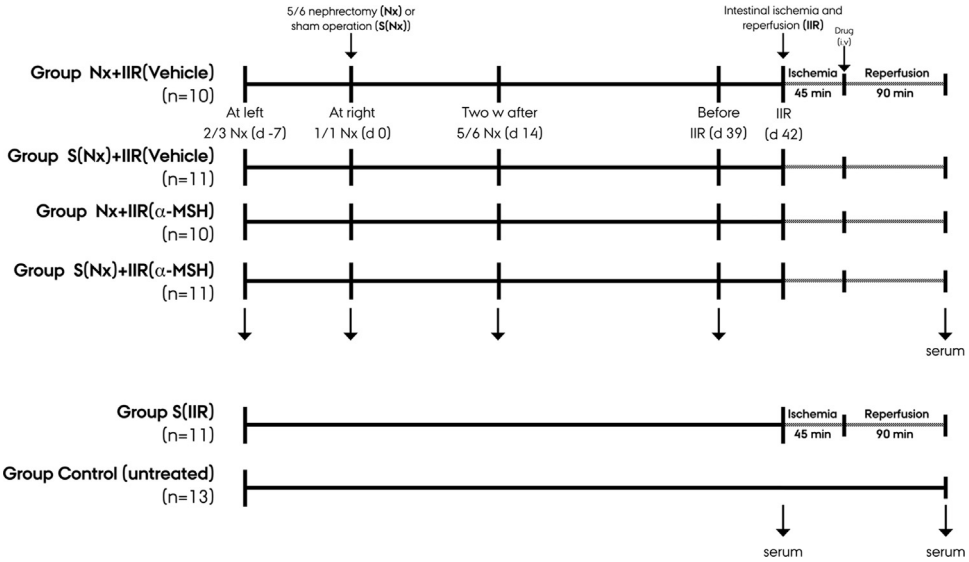

Figure 1. Experimental design. 5/6 Nephrectomy (Nx) was performed in two steps, 2/3 left Nx followed by right total (1/1) Nx 1week later. Six weeks after Nx, intestinal ischemia and reperfusion (IIR) were performed. Sham operations were performed for each surgical procedure as well. The control group was not subjected to any surgical procedures. The arrows indicate time of blood and urine sampling. d, day; IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.