Kidney Research and Clinical Practice

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

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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.

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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
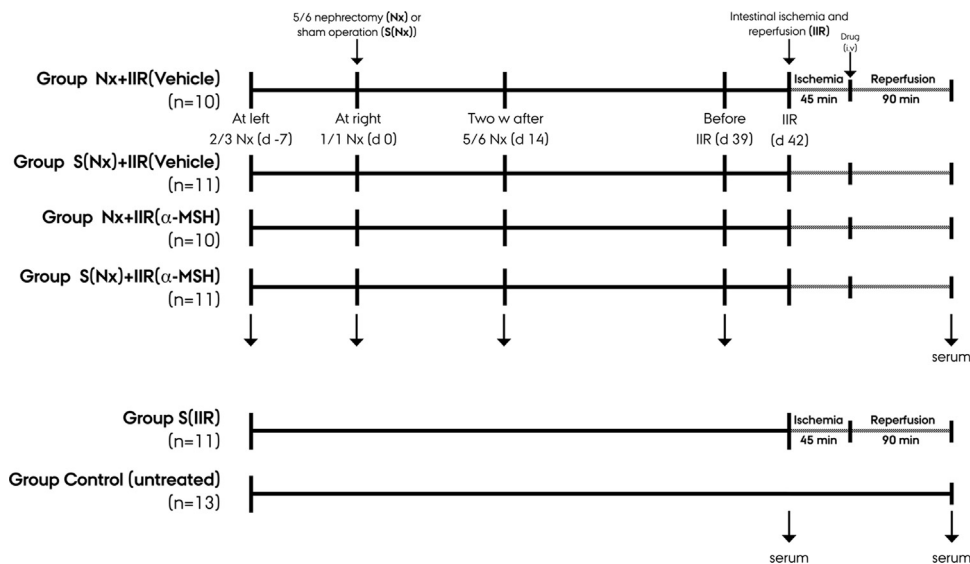

Figure 1. Experimental design. 5/6 Nephrectomy (Nx) was performed in two steps, 2/3 left Nx followed by right total (1/1) Nx 1week later. Six weeks after Nx, intestinal ischemia and reperfusion (IIR) were performed. Sham operations were performed for each surgical procedure as well. The control group 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.

from the tail vein). This ensured that an equal average impairment in kidney function within the two Nx groups was achieved, and the same procedure was performed on the rats in the two S(Nx) groups. At IIR, a group of Nx rats and S(Nx) rats received vehicle (0.5 mL 0.9% saline), whereas another group of Nx rats and S(Nx) rats received α-MSH (200 μg/kg), administered through the penile vein immediately after intestinal ischemia.

At "Initial" (Day-7), "After 5/6 Nx" (Day 0), "Two weeks after 5/6 Nx" (Day 14), and "Before IIR" (Day 39), blood was obtained from the tail vein in all vehicle and α-MSH groups. Prior to this, rats were housed in metabolic cages for 3 days to collect urine.

The rats in the Control and S(IIR) group had their blood taken at "Before IIR" (Day 39) and at "Euthanasia" (Day 42). These rats were not housed in metabolic cages at any time point during the study.

On the day of surgery animals were anesthetized with 2% isoflurane (Abbot Scandinavia, Solna, Sweden) and 2 L/minute atmospheric air. Nx was performed by excision of two thirds of the left kidney, and 1 week later total nephrectomy of the right kidney. Sham Nx [S(NX)] was performed without any removal of the renal mass. The amount of kidney tissue removed was calculated based on the assumption that both kidneys had the
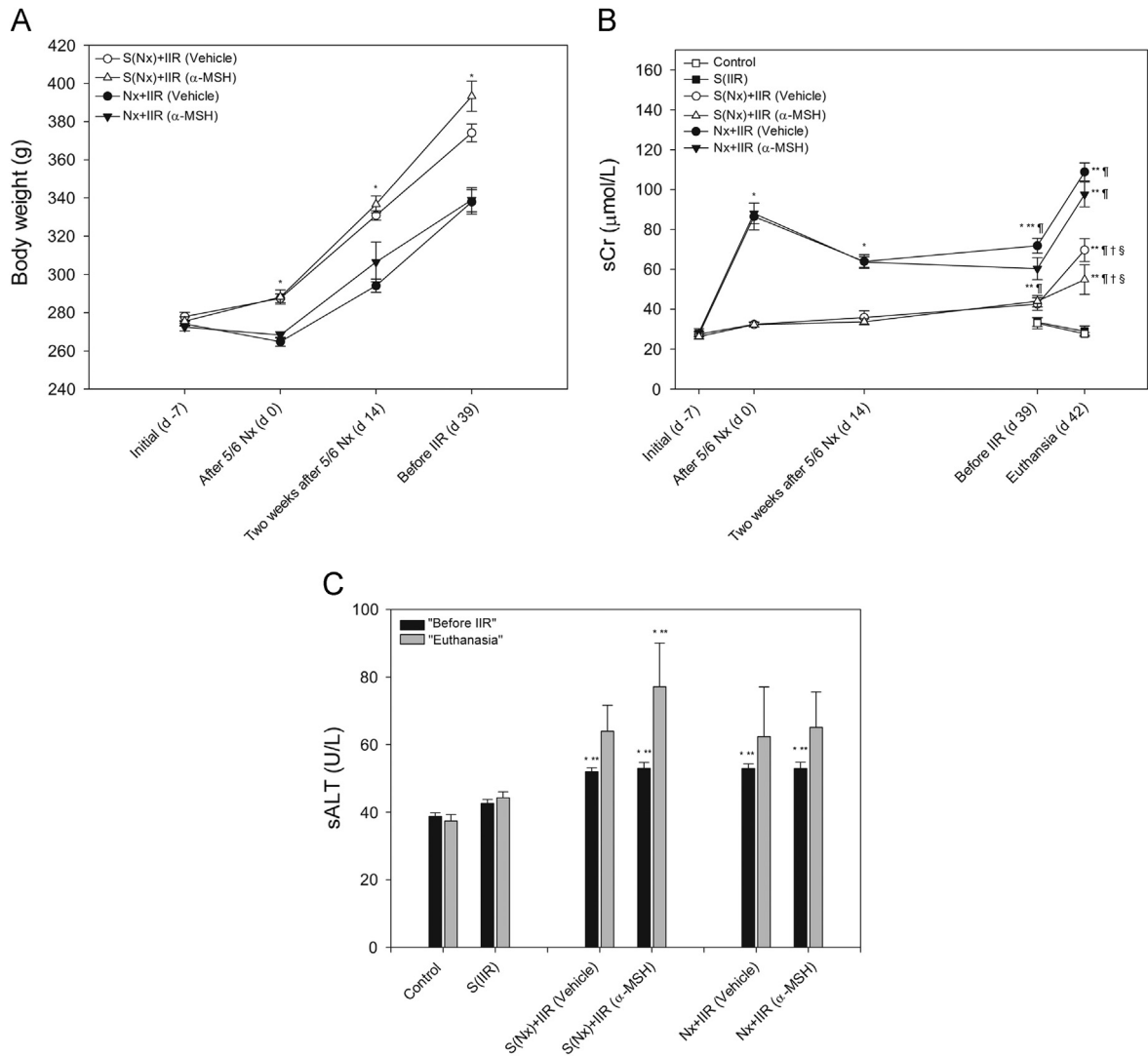

Figure 2. **Body weight and organ injury following Nx and IIR**. (A) Rats were weighted at each surgical procedure, blood sampling and stay in metabolic cages during the study. By repeated-measures analysis of variance, all groups had significant time × body weight interactions. Until "Before IIR" *post hoc* comparisons were performed on pooled data (all Nx+IIR groups vs. all S(Nx)+IIR groups). At "Before IIR" multiple (all 4 groups) comparisons (Tukey-Kramer) were performed. (B) Renal function was determined by serum creatinine (sCr). Until "Before IIR" comparisons were performed on pooled data (all Nx+IIR groups vs. all S(Nx)+IIR groups), thereafter multiple comparisons were performed (Tukey-Kramer). (C) Liver function before and after IIR was determined by measurement of serum alanine transaminase (sALT).

All values are presented as mean ($\pm$ standard error).

*$P < 0.05$ vs. S(Nx)+IIR (pooled data).

[†] $P < 0.05$ vs. Nx+IIR (Vehicle).

[‡] $P < 0.05$ vs. S(Nx)+IIR (Vehicle).

[§] $P < 0.05$ vs. Nx+IIR (α-MSH).

[||] $P < 0.05$ vs. S(Nx)+IIR (α-MSH).

[¶] $P < 0.05$ vs. S(IIR).

** $P < 0.05$ vs. Control.

d, day; IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

same weight, using the following equation:

$$[(\text{Total nephrectomy weight} + \text{Partial nephrectomy weight})$$
$$/(2 \times \text{Total nephrectomy weight})] \times 100. \qquad (1)$$

IIR was performed 6 weeks after Nx, as previously described [7]. Briefly, the small intestine was exteriorized through a midline laparotomy and the superior mesenteric artery (SMA) was

Table 1. Urine and serum analyses*

| Group	Period	SSodium (mmol/L)	$S_{Potassium}$ (mmol/L)	U_{Osm} (mosmol/kg H_2O)
S(Nx)	Initial (d −7)	137.5 ± 0.5	5.9 ± 0.1	1,747 ± 72
	After 5/6 Nx (d 0)	137.3 ± 0.3	5.3 ± 0.1	2,023 ± 71
	2 w after 5/6 Nx (d 14)	138.3 ± 0.3	5.9 ± 0.1	1,863 ± 113
Nx	Initial (d −7)	137.4 ± 0.4	6.1 ± 0.2	1,574 ± 123
	After 5/6 Nx (d 0)	137.2 ± 0.5	5.6 ± 0.1	632 ± 28[†]
	2 w after 5/6 Nx (d 14)	138.5 ± 0.4	5.8 ± 0.1	700 ± 65[†]
S(Nx)+IIR (Vehicle)	Before IIR (d 39)	138.8 ± 0.4	6.2 ± 0.3	1,821 ± 137
S(Nx)+IIR (α-MSH)	Before IIR (d 39)	138.0 ± 0.5	6.2 ± 0.3	1,763 ± 160
Nx+IIR (Vehicle)	Before IIR (d 39)	136.4 ± 0.5[‡]	6.4 ± 0.2	763 ± 102[§]
Nx+IIR (α-MSH)	Before IIR (d 39)	136.5 ± 0.5[‡]	6.4 ± 0.3	757 ± 72[§]

* Blood samples were taken at each of the surgical procedures and following 5/6 nephrectomy. Urine was collected during housing in metabolic cages for 24 hours. Values are shown as mean (± standard error). Until "Before IIR" comparisons were performed on pooled data [all Nx+IIR groups vs. all S(Nx)+IIR groups]. At "Before IIR" multiple comparisons (Tukey-Kramer) were performed.
[†] $P < 0.05$ vs. S(Nx)+IIR (pooled data).
[‡] $P < 0.05$ vs. S(Nx)+IIR (Vehicle).
[§] $P < 0.05$ vs. S(Nx)+IIR (Vehicle).
d, day; IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.
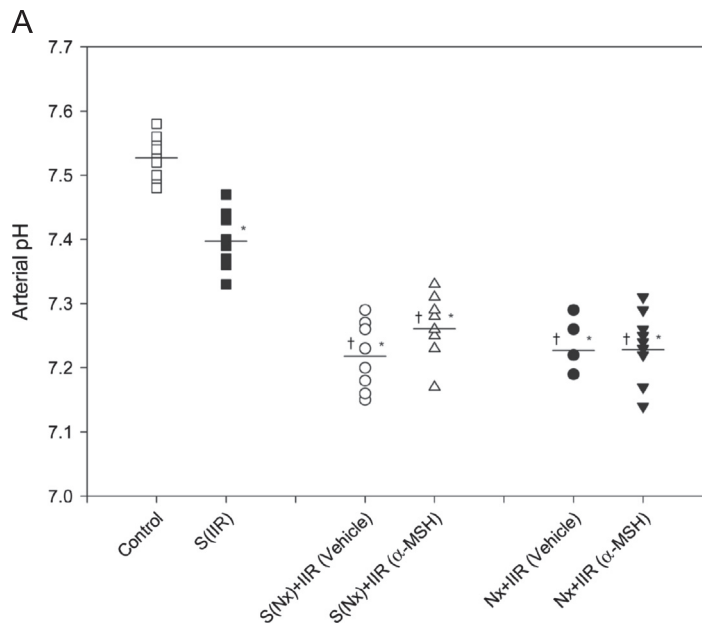
B

Group	n	pCO_2 (mmHg)	HCO_3^- (mmol/L)	pO_2 (mmHg)	ABE (mmol/L)
Control	13	38.1 ± 1.1	31.5 ± 0.6	100.4 ± 5.0	8.4 ± 0.4
S (IIR)	11	53.7 ± 1.5[*]	32.4 ± 1.0	61.5 ± 4.9	6.3 ± 0.9
S (Nx)+IIR (Vehicle)	10	44.5 ± 4.0	17.4 ± 1.5[*,†]	94.4 ± 9.3	-9.9 ± 1.4[*,‡]
S (Nx)+IIR (α-MSH)	9	50.7 ± 2.7[*]	21.1 ± 1.3[*,†]	94.9 ± 9.1	-5.1 ± 1.3[*,†]
Nx+IIR (Vehicle)	7	43.4 ± 5.1	17.1 ± 1.6[*,†]	122.3 ± 23.2[*]	-10.1 ± 1.3[*,†,‡]
Nx+IIR (α-MSH)	10	49.1 ± 3.4	19.6 ± 1.1[*,†]	107.1 ± 15.1[†]	-8.2 ± 0.9[*,†]

Figure 3. Blood gases. (A) Arterial blood pH. (B) In the table: pCO_2, partial pressure of carbon dioxide; HCO_3^-, concentration of bicarbonate; ABE, arterial base excess. In the dot plot individual values and mean (horizontal line) are shown. In the table values are presented as mean (± standard error).
[*] $P < 0.05$ vs. Control.
[†] $P < 0.05$ vs. S(IIR).
[‡] $P < 0.05$ vs. S(Nx)+IIR (α-MSH).
IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

occluded at its origin from the abdominal aorta for 45 minutes. Prior to closing the abdominal wall, 1 mL of saline (37°C) was infused in the peritoneal cavity and 1 mL was administered subcutaneously. The rats were allowed to awaken and were monitored in cages until 90 minutes after ischemia, following which euthanasia was performed. Sham IIR [S(IIR)] was performed by a midline laparotomy and manipulation of the SMA without occluding it.

During each surgical procedure, rats were placed on a heated table to maintain rectal temperature at 37.5°C. Initially, at each surgical procedure buprenophium 0.3 mg/kg (Temgesic; Reckitt Benckiser, Berkshire, UK) was injected subcutaneously to relieve pain, and later administered in the drinking water at the same dose.

Assessment of renal function

Serum creatinine was determined colorimetrically (Vitros 5.1, Johnson & Johnson, New Brunswick, NJ, USA). Urine osmolality was measured using a vapor pressure osmometer (Osmomat 030, Genotec, Berlin, Germany). Blood was obtained from the tail vein, apart from at euthanasia, when the blood sample was obtained from the abdominal aorta. AKI was defined as a statistical significant increase in serum creatinine level.

Measurement of proinflammatory cytokines and chemokines in the lung, kidney, and liver

The proinflammatory cytokine IL-1β, anti-inflammatory cytokine IL-10, and the chemokines monocyte chemoattractant protein (MCP)-1 and RANTES were measured in the kidney, lung, and liver homogenates using Procarta Cytokine Assay kit (Invitrogen A/S, Taastrup, Denmark) according to the manufacturer's instructions. The samples were analyzed on a Luminex 100 IS flow system (Luminex Corporation, Austin, TX, USA). Briefly, the color code of each bead identifies the target protein being assayed and the fluorescent intensities on the beads measure target concentration. The tissue samples were diluted in the assay buffer and measured in duplicate. Total system raw median fluorescence intensity (MFI) values were used for calculations of concentrations (ρg/mL) from four-parameter logistic fitted standard curves using the StarStation version 2.3 software (Applied Cystometry, Sheffield, UK). The fits expressed by the R-squared value were all > 0.995. To adjust measurements for differences in total protein content of the samples, values are expressed in picograms per gram of protein. Total protein concentration was determined by the Pierce BCA protein assay kit (BIO-RAD, Hercules, CA, USA).

Lung wet/dry ratio

At the completion of the experiment, the left lung was excised and immediately weighed. After storage at $-20°C$ for a month it was dried at $-60°C$ in a vacuum dryer for 48 hours and reweighed. Tissue water content was then calculated as:

$$\text{(Wet weight–Dry weight)/(Wet weight)} \times 100$$

and expressed as a percentage. (2)

Arterial blood gas

At euthanasia, arterial blood drawn from the abdominal aorta was analyzed using an ABL 5 blood gas analyser (Radiometer, Copenhagen, Denmark).

Liver enzyme

Serum alanine aminotransferase (ALT) was determined by the Vitros 5.1 system (Ortho Clinical Diagnostics, New Brunswick, NJ, USA) using slide technology.

Statistical analysis

Data were presented as mean $\pm$ standard error of the mean. Differences between the groups were examined for statistical significance by analysis of variance. *Post hoc* multiple comparisons were performed with the Tukey-Kramer method. Furthermore, a comparison was performed on pooled data from the Nx+IIR groups and S(Nx)+IIR groups, respectively, until "Before IIR". Longitudinal measurements of body weight were analyzed by repeated-measures analysis of variance to test time dependent interactions. When the statistical model was significant, further *post hoc* analyses were performed with the Tukey-Kramer method for multiple comparisons using STATA version 10.1 (Stata Corporation, Lakeway Drive College Station, TX, USA). A two-sided $P <$ 0.05 was accepted as statistically significant.

Results

Physiological data

Body weight increased in all four groups during the 6 weeks of follow-up after Nx or S(Nx) (Fig. 2A). Already after 5/6 nephrectomy, the body weight of the rats in the Nx groups was significantly lower compared to the rats in the S(Nx) groups. This difference remained significant during the 6 weeks of follow-up after Nx (Fig. 2A).

During the 6 weeks after Nx, 10% of the Nx rats and 13% of the S(Nx) rats were excluded. Exclusion criteria were wound infections, extreme weight loss, and spontaneous death. The
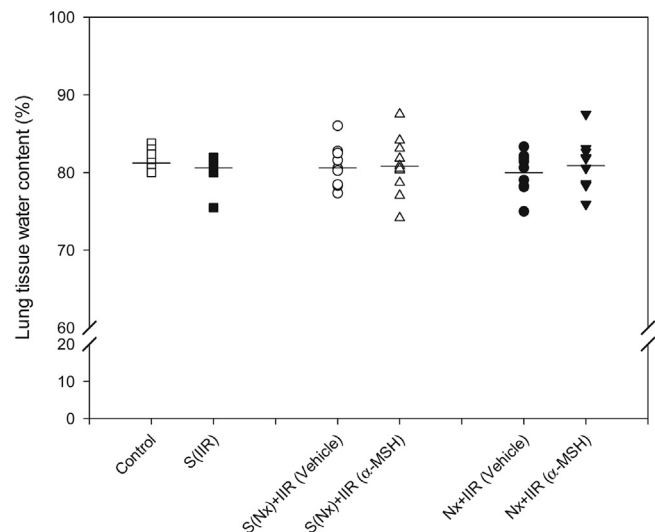

Figure 4. Lung edema. At the completion of the experiment, the left lung was excised and immediately weighed. After having been frozen at –20°C for 1 month, the lung was dried at –60°C in a vacuum dryer for 48 hours and reweighed. Tissue water content was then calculated. Individual content and mean (horizontal line) within each group are shown. IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

excluded rats were not included in the data analysis at any time point. On average, 76% (data not shown) of the kidney mass was removed in the rats that had Nx.

Renal function after Nx and S(Nx) respectively

Urine output and water intake were measured in metabolic cages; "After 5/6 Nx" (Day 0) both urine output and water intake were significantly higher in the Nx rats compared to the S(Nx) rats (Table 1). Consistent with these changes was a significantly lower urine osmolality in the Nx rats compared to the S(Nx) rats (Fig. 2B).

After Nx, initial indications of impaired renal function in the Nx rats were shown by dramatically elevated serum creatinine (sCr) levels, followed by a drop to a more steady, but significantly higher, level than in the S(Nx) rats (Fig. 2B).

Elevated organ specific biomarkers in serum confirmed the development of MOF, induced by IIR

A significant increase [from "Before IIR" (Day 39) to "Euthanasia" (Day 42)] of 54% (± 6%), 73% (± 20%), and 73% (± 21%) in sCr after IIR was observed in the Nx+IIR (Vehicle), S(Nx)+IIR (Vehicle), and Nx+IIR (α-MSH) rats, respectively, indicating that AKI was ongoing until euthanasia (Fig. 2B). No significant increase, 28% (± 17%) and –9% (± 10%) in sCr was observed in the S(Nx)+IIR (α-MSH) and S(IIR) groups, respectively in the same period after IIR. There were no significant differences in the fractional increase in sCr between any of the groups exposed to IIR ($P > 0.05$). At "Euthanasia" (Day 42) sCr was significantly higher in the groups exposed to IIR compared to S(IIR) (Fig. 2B). Moreover sCr was significantly higher at "Euthanasia" (Day 42) in the groups exposed to Nx compared to S(Nx) in response to IIR (Fig. 2B). There were no significant differences in sCr at "Euthanasia" (Day 42) between the groups treated with α-MSH compared to vehicle irrespective of Nx or
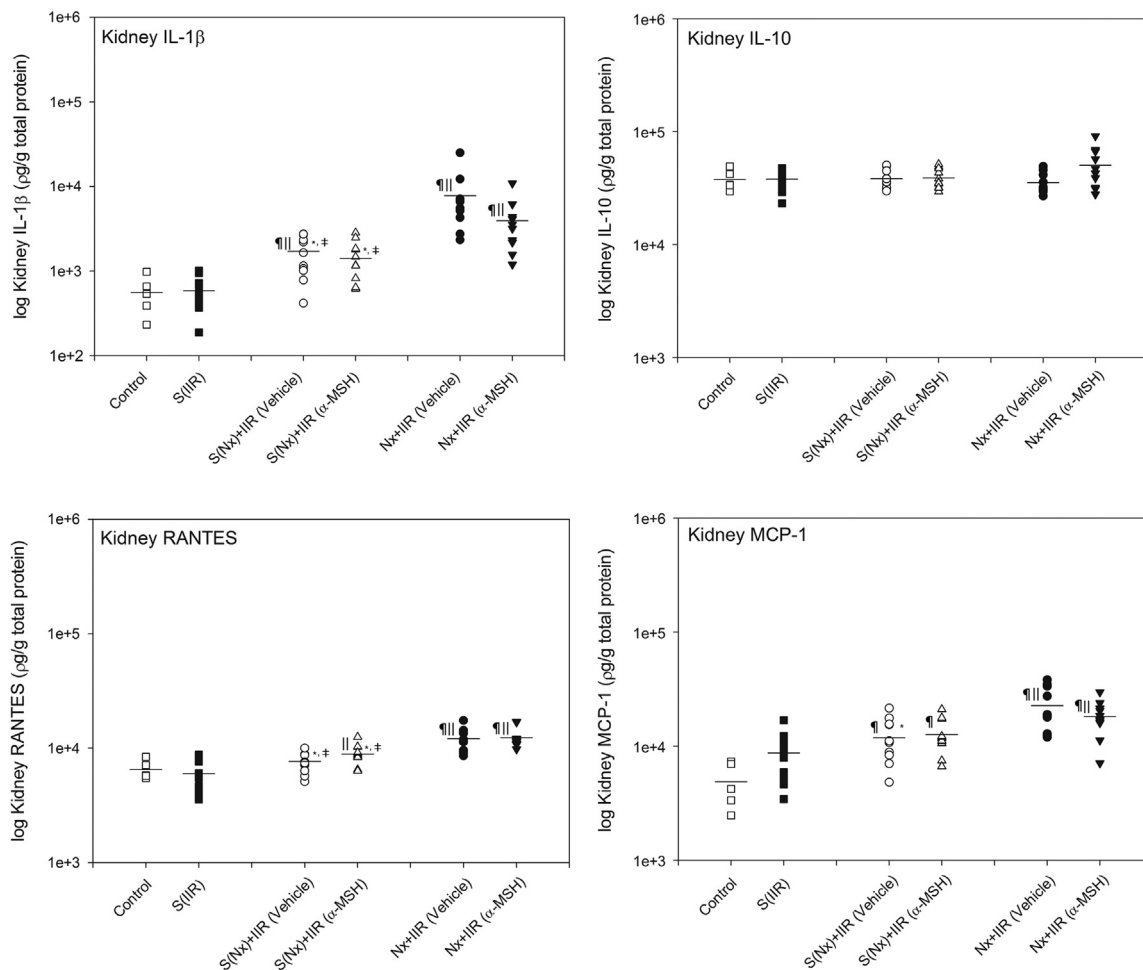

Figure 5. Kidney cytokines. Proinflammatory cytokines (IL-1β), anti-inflammatory cytokine (IL-10), and chemokines (RANTES, MCP-1) were measured in whole kidney tissue by multiplex assays. Individual tissue concentrations and mean (horizontal line) within each group are shown.
* $P < 0.05$ vs. Nx+IIR (Vehicle).
† $P < 0.05$ vs. S(Nx)+IIR (Vehicle).
‡ $P < 0.05$ vs. Nx+IIR (α-MSH).
§ $P < 0.05$ vs. S(Nx)+IIR (α-MSH).
‖ $P < 0.05$ vs. S(Nx).
¶ $P < 0.05$ vs. Control.
IL, interleukin; IIR, intestinal ischemia and reperfusion; MCP, monocyte chemoattractant protein; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

S(Nx) prior to IIR. The aforementioned changes in sCr between "Before IIR" (Day 39) and "Euthanasia" (Day 42) actually took place because of IIR on the day of IIR surgery, and not during the 3 days between "Before IIR" (Day 39) and "Euthanasia" (Day 42). Animal ethics demanded that we did not redraw blood just prior to the IIR procedure.

There was a tendency for an increase in the liver enzyme ALT after IIR, although not significantly. At "Before IIR" (Day 39) ALT was significantly higher in the Nx and S(Nx) groups, compared to the S(IIR) and Control groups, respectively (Fig. 2C).

Metabolic acidosis was achieved after IIR

After IIR, the pH of the arterial blood was significantly lower in the groups exposed to IIR compared to the S(IIR) and the Control groups. In the S(IIR) group, pH was significantly lower than in the Control group, reflecting that even laparotomy and anesthesia had an effect on blood pH (Fig. 3A). The groups exposed to IIR had significantly lower levels of HCO_3^- and arterial base excess (ABE) compared to the S (IIR) and Control groups, respectively (Fig. 3B). Because pH, HCO_3^-, and ABE were significantly lower in the groups exposed to IIR compared to S(IIR), the data indicated that metabolic acidosis occurred when rats were subjected to IIR.

Administration of α-MSH did not significantly change any of the blood gases.

Lung edema

As a surrogate for lung edema, lung tissue water content was calculated. There was no difference in the tissue water content between any of the groups (Fig. 4).

Changes in cytokines and chemokines in kidney, lung, and liver tissue

In the kidney (Fig. 5), lung (Fig. 6), and liver (Fig. 7) tissue the levels of IL-1β were significantly higher in the rats undergoing IIR when compared to S(IIR), except in the S(Nx)+IIR (α-MSH) group in the kidney. The same pattern was observed for chemokine MCP-1 in the lung and liver tissue. Furthermore IL-1β and RANTES were significantly higher in the kidney tissue in the rats, which prior to IIR were exposed to Nx compared to sham Nx (Fig. 5).

No differences in the levels of IL-10 within the kidney, liver, and lung tissue were observed among any of the groups.
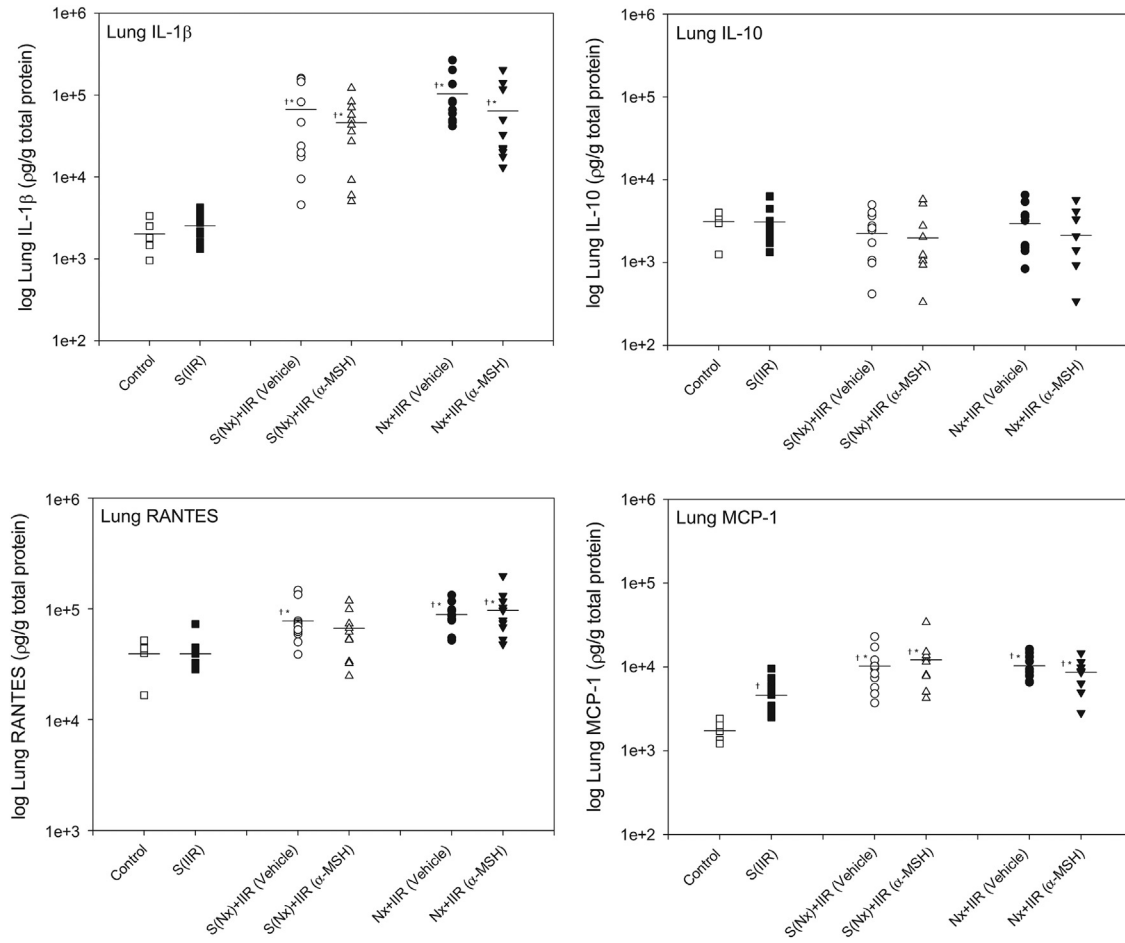

Figure 6. Lung cytokines. Proinflammatory cytokines (IL-1β), anti-inflammatory cytokine (IL-10), and chemokines (RANTES, MCP-1) were measured in whole lung tissue by multiplex assays. Individual tissue concentrations and mean (horizontal line) within each group are shown.
* $P < 0.05$ vs. S(Nx).
† $P < 0.05$ vs. Control.
IL, interleukin; IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

Discussion

In the current study, we evaluated the effects of an intravenous bolus of α-MSH on IIR-induced AKI, and found no significant protective effect of α-MSH.

Furthermore, we examined the effect of CKD on the severity of the inflammatory cytokine/chemokine response in the kidney, lung, and liver tissue induced by IIR. The main finding was that Nx (CKD) induced an increased cytokine/chemokine response within the kidney tissue, when compared to S(Nx) (non-CKD).

Preexisting CKD may predispose to a more severe inflammatory response

In previous work (unpublished data using a similar animal model and experimental setup) we found that Nx induced an increased cytokine/chemokine response within the kidney tissue, when compared to sham Nx. Additionally, the IL-1β response within the kidney tissue was further increased in the Nx rats when they were exposed to IIR compared to Nx rats exposed to sham IIR. In the current study we found that the IL-1β and

RANTES response within the kidney tissue was significantly higher in the Nx rats when they were exposed to IIR, compared to the S(Nx) rats exposed to IIR (Fig. 5). Similar findings have been observed in plasma IL-6 levels by Leelahavanichkul et al [6] in an alternative AKI onto CKD model in mice. Others have reported increased levels of IL-6 and MCP-1 in kidney tissues after AKI induced by renal IR [13,27–29] in rodents with normal kidney function prior to AKI.

As mentioned in the Introduction, the renal IR model is fundamentally different from the AKI model used in the current study. Thus, we cannot exclude that the intrarenal inflammatory cytokine/chemokine responses found in the current study could be caused by CKD secondary to Nx, and not solely by IIR.

Metabolic acidosis after IIR

During IIR profound cardiovascular instability occurs, marked by hypotension and increasing levels of lactic acid [7,30]. As a result of the shift to a more anaerobic metabolism after IIR, metabolic acidosis occurs. In the current study (Fig. 3) a pH between 7.21 and 7.26 and HCO_3^- between 17.1 and
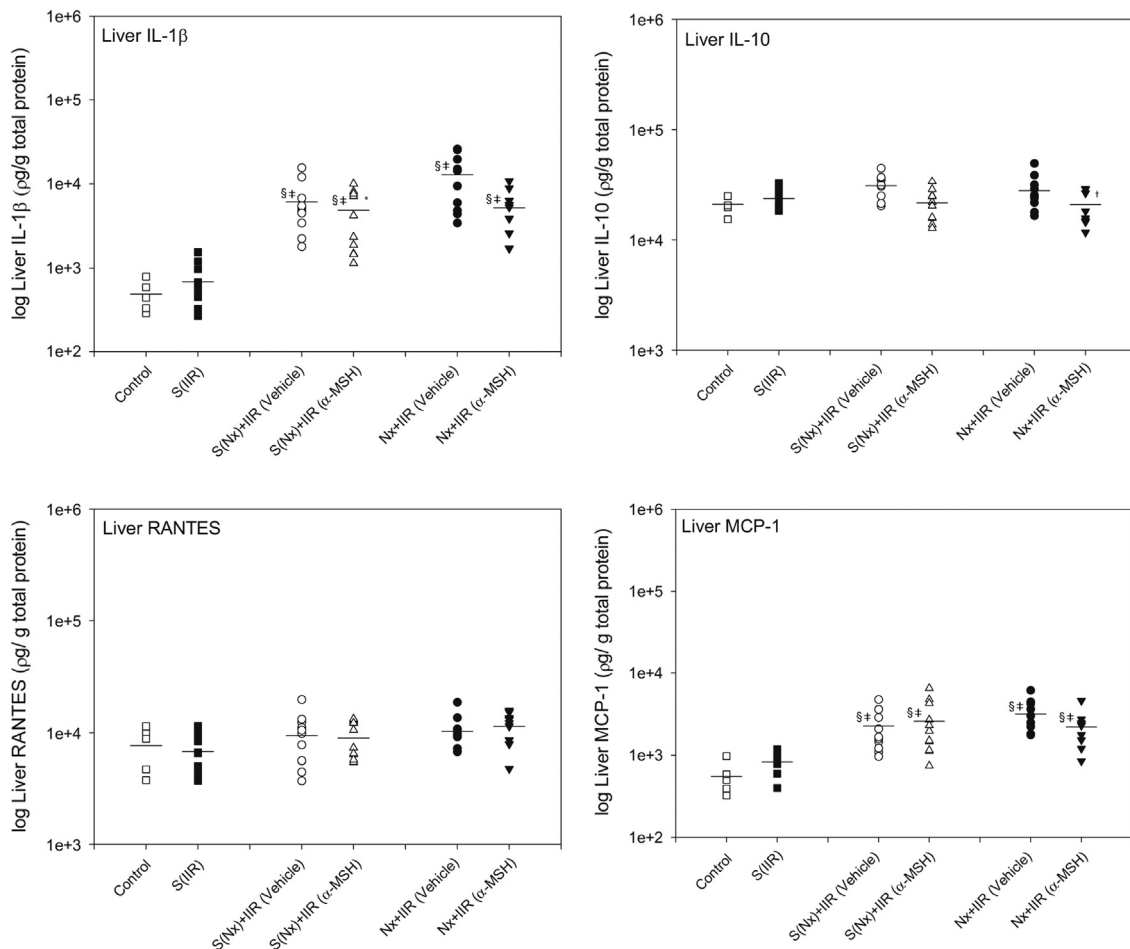
Figure 7. Liver cytokines. Proinflammatory cytokines (IL-1β), anti-inflammatory cytokine (IL-10), and chemokines (RANTES, MCP-1) were measured in whole liver tissue by multiplex assays. Individual tissue concentrations and mean (horizontal line) within each group are shown.
* $P < 0.05$ vs. Nx+IIR (Vehicle).
† $P < 0.05$ vs. S(Nx)+IIR (Vehicle).
‡ $P < 0.05$ vs. S(Nx).
§ $P < 0.05$ vs. Control.
IL, interleukin; IIR, intestinal ischemia and reperfusion; MSH, melanocyte-stimulating hormone; Nx, nephrectomy; S(Nx), Sham nephrectomy.

22.1 mmol/L was observed in the groups exposed to IIR. This confirms the findings of previous studies of IIR in rats with varying time periods of ischemia and reperfusion, in which a pH between 7.11 and 7.31 [31,32] and HCO_3^- between 11.0 and 16.8 mmol/L are presented [31,32]. Additionally, a significantly low level of ABE was found in the groups exposed to IIR compared to S(IIR). In conclusion, when comparing the IIR groups with the S(IIR) group, IIR induces metabolic acidosis.

Both anesthesia [33] and laparotomy [31,32] have been described to profoundly reduce pH. In the current study we found a lower pH and a higher pCO_2 in the S(IIR) group compared to the Control group (Fig. 3A).

Remote organ injury following IIR

Remote organ failure follows IIR. We observed increasing levels of serum creatinine (Fig. 3B) and alanine transaminase (Fig. 3C), reflecting acute kidney injury and acute liver injury, between "Before IIR" and at "Euthanasia". Unfortunately, we did not observe any differences in lung tissue water content at "Euthanasia" as a surrogate for pulmonary edema. Pulmonary edema is a common finding in acute lung injury [34], and occurs because of increased pulmonary microvascular permeability, as described in the IIR model [14,35]. The lack of differences between the rats in the current study exposed to IIR, compared to S(IIR) or the untreated Control, might be explained by the length of the ischemia period. In previous studies with increasing levels of pulmonary vascular permeability, intestinal ischemia periods between 60 minutes and 120 minutes are reported [14,35]. As mentioned, we used an ischemia period of 45 minutes, because of the increasing mortality reported in studies with $\geq$ 60 minutes [36,37].

α-MSH had no protective effect on AKI induced by IIR

In the current study, we found no significant organ protective effect of an intravenous bolus of α-MSH (200 μg/kg) administered prior to intestinal reperfusion. This is in contrast to other studies of primary and secondary AKI in rodents. In renal ischemia and reperfusion (primary AKI), α-MSH (150–200 μg/kg) has been shown to decrease renal injury, through neutrophil-independent pathways [23,38,39]. In a model of secondary AKI, induced by lethal cecal ligation and puncture (CLP) in mice, α-MSH (6.8 μg) prevented a rise in serum creatinine after CLP compared to vehicle [40].

Furthermore, α-MSH has been shown to protect against secondary lung injury following renal ischemia and reperfusion [21]. In the cytokine/chemokine measurements in the lungs, we did not observe any significantly acute protective effect of α-MSH. Additionally, in a murine study, α-MSH (50 μg) has been shown to prevent lipopolysaccharide (LPS)-induced hepatic inflammation and inhibit production of chemokine MCP-1 in liver tissue [22]. In the current study we did not observe differences in liver MCP-1 in the α-MSH treated groups compared to the vehicle. This may be explained by the differences in which acute liver injury is induced and the different methods of measuring MCP-1, Northern blotting (messenger RNA) versus Multiplex enzyme-linked immunosorbent assay (proteins) in the current study.

The discrepancy in the findings between the current and prior studies of the possible organ protective effect of α-MSH may be explained by the regimen in which α-MSH was administered: α-MSH was only injected intravenously as a bolus of 200 μg/kg through the penile vein immediately after reperfusion. This was because the aim was to investigate the acute response of α-MSH treatment in a model of acute MOF in rodents with or without preexisting CKD.

Limitations of the study

The current study is not without its limitations. First, we measured cytokines in whole tissue homogenates instead of plasma, which is used in clinical studies of AKI. The reasons for this are that a significant proportion of the cytokines act via paracrine mechanisms, and they have a short half-life in plasma, making them very difficult to detect. Moreover, the release patterns of each of the individual cytokines are different, and we only measured cytokines at a single time point. Late and more "downstream" releases of cytokines are not detected or evaluated in the current study.

Second, in addition to measuring cytokines/chemokines in whole tissue homogenates, evaluation of tissue inflammation, for example, by using immunohistochemical detection of neutrophils and macrophages, could have strengthened the evaluation of a CKD-exacerbated inflammatory response within the kidney after AKI. Unfortunately, we were not able to harvest any of the remnant kidney mass for immunohistochemistry.

Conflicts of interest

The authors have declared that no competing interest exists.

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