Acute Kidney Injury after Hemorrhagic Shock and its Relation to IL-10 and HSP70

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Abstract

Acute kidney injury after hemorrhagic shock resuscitation in relation to IL-10 and HSP 70 was examined. IL-10 and IL-6 elevated at 2h after hemorrhagic shock and resuscitation at wild type. IL-6 at 2h in KO type showed higher level compared to wild type at 2h. On the contrary, histological section showed that renal tubule was more damaged at wild type at 48h compared to IL-10 knockout type at 48h. HSP 70 was expressed more at renal tubule in KO type at 48h than that of wild type at 48h. From these results, we speculate that 1) IL-10 may aggravate the renal tubule, or 2) IL-6 may work as anti-inflammatory effect or 3) coexisting of IL-6 and IL-10 may produce the kidney injury at renal tubule region, or 4) HSP 70 may ameliorate the acute kidney injury at renal tubular region.

ABBREVIATIONS

IL-10: Interleukin-10; HSP: Heat Shock Protein

INTRODUCTION

Hemorrhagic shock induces systemic organ ischemia and fluid resuscitation results in reperfusion injury to all organs due to endothelial dysfunction. Hemorrhagic shock and resuscitation induces organ damage of lung, kidney and small intestine, or sometimes may result in multiple organ failure [1-6]. Kidney is the target organ for ischemia reperfusion injury; however its mechanism is not fully understood in spite of recent advances in research [7]. Interleukin-10(IL-10), a cytokine with anti-inflammatory and immuno-regulatory functions, regulates the biology of B and T cells. IL-10 plays an important role in acute kidney injury as well as chronic kidney disease. Studies have shown that IL-10 is protective against SLE-induced renal damage due to down-regulation of pathogenic Th1 responses [8]. In experimental mesangial proliferative glomerulonephritisIL-10 diminishes inflammatory cell recruitment and mesangial cell proliferation [9]. On the other hand, elevated IL-10 levels were found in diabetic patients. Moreover, increased IL-10 concentrations in serum predict albuminuria and correlate with the severity of diabetic nephropathy [10]. IL-10 can promote mesangial disposition of immune complexes, thereby contributing to the progression of glomerular injury [11].

From these results, IL-10 may work benefically for kidney injury, and unfavorably in some other clinical conditions. Heat shock proteins (HSPs) are highly conserved whose expression was induced by different kinds of stress. HSPs express molecular chaperones that help maintain and restore normal function and also express anti-apoptotic effects. Induction of HSP70 by nonlethal insults in vitro or in vivo is associated with acquired cytoprotection [12,13].

In this study, we tested whether IL-10 works beneficially or unfavorably in acute kidney injury after hemorrhagic shock and resuscitation, and what are the effects of HSP70.

MATERIALS AND METHODS

Animals

Care and handling of the animals were carried out in accordance with the National Institutes of Health Guidelines. Male inbred C57 BL6/J mice (JAX Stock Number 000664, Charles River Laboratories, Inc. Tsukuba, Japan) were used as wild type, and (B6.129P2- IIL10tm1Cgn/J mice JAX Stock Number 002251), as control animals;(JAX Stock Number 000664 C57BL/6J, Jackson Laboratory, Bar Harbor, MA, USA) were used as IL-10 gene knockout mice. Age ranged from 8 to 12 weeks old. Animals were housed at least 3 days under controlled light/dark conditions and the light period was from 8:00 AM to 8:00 P.M under specific pathogen free (SPF) condition. They were allowed access to ordinary pellet diet and tap water, ad libitum. All experimental
procedures were reviewed and approved by the Animal Experimentation Committee of Kindai University.

**Hemorrhagic shock model**

Hemorrhagic shock model was induced as previously described by Murao et al [1,2]. Briefly, mice were lightly anesthetized using isoflurane (1-chloro-2, 2, 2-trifluoroethyl difuoroethyl ether) by inhalation. The left femoral artery was cannulated with a medical grade polyethylene no.10 catheter and connected to a pressure transducer via a stopcock. Heparin (100U/kg body weight) was administered, and blood was withdrawn over a period of 5 min until a mean arterial pressure of 40 ± 5 mmHg was reached. The level of hypotension was maintained for 60 min by further withdrawal or infusion of small amount of blood as necessary and resuscitation was performed over approximately 5 min as follows: LR; resuscitation with 2 times the volume of lactated Ringer’s solution of the Shed Blood, and SB.

**Experimental design**

Animals were randomly assigned to the following resuscitation groups: 1) wild LR 2) IL-10 knockout LR (KO LR). Untreated groups for wild and KO mice were designated as control groups (control animals were anesthetized but not cannulated). After exsanguinations by withdrawing blood from the abdominal vena cava from the animals of each group (n=6), blood sample and histological sample were obtained aseptically. For cytokine analysis at 2h, 4h after hemorrhagic shock and resuscitation blood samples were kept frozen at -80 until analyzed by immunofluorescence technique. Kidney samples were kept as paraffin blocks until analyzed for histology and immunohistochemical stain. Histological samples were stained with Hematoxylin and eosin stain (H&E stain). Histological damage was assessed by the degree of vacuolation of renal tubular region as follows: 0: <5%, 0.5: 5 - 10 %, 1: 10 – 20 %, 2: 20 – 30 %, 3: 30%<, and necrosis : 0: <1%, 0.5: 1 - 5 %, 1: 5 - 10 %, 2: 5 – 10%, 3: 20% <. Total histology score was estimated as accumulation of these scores.

HSP 70 was assessed as positive cell % of renal tubular cells in the microscopical fields as follows: 0: <5%, 0.5: 5 - 10 %, 2: 20 – 30%, 3: 30% <.

**Statistical Analysis**

Data were reported as mean +/- SEM throughout. Data were analyzed according to one-way analysis of variance (ANOVA) followed by Bonferroni/Dunn’s test for differences between groups. Differences in mean values were considered statistically significant at p<0.05.

**RESULTS**

Based on reference No.4, total amount of blood loss following hemorrhage was about 1 ml, or 40.0 ml/Kg on average. In this model, most of the animals survived after hemorrhagic shock and fluid resuscitation.

Figure 1 shows IL-10 levels after hemorrhagic shock and resuscitation at 2h and 4h. IL-10 in wild type at 2h expressed significantly higher compared to other groups, and decreased at 4h.

Figure 2 shows IL-6 after hemorrhagic shock and resuscitation at 2h and 4h. IL-6 increased at 2h both wild type and KO group, and decreased at 4h. KO LR 2h showed higher level than wild type LR 2h.

Figure 3 shows kidney histological section of wild type at 48h. Histological damage was seen at renal tubular region as vacuolation and necrosis.

Figure 4 shows total histology score after hemorrhagic shock and resuscitation at 2h, 48h in both wild type and KO type, and controls with no treatment. Wild LR 48h showed higher histological damage compared to KO LR 48h (P<0.05).

Figure 5 shows HSP70 expression at renal tubular region of wild LR 48h and KO LR 48h and controls. HSP 70 expression of KO LR at 48h shows trend to higher expression compared to wild LR 48h.

![Figure 1 IL-10 of the serum. IL-10 of Wild LR at 2h showed higher than other groups.](image1)

![Figure 2 IL-6 of the serum. IL-6 of KO type at 2h showed higher trend than Wild type at 2h.](image2)
DISCUSSION

IL-10 is a cytokine with anti-inflammatory and immunomodulatory functions; regulates the biology of B and T cells. However, IL-10 may work beneficially for acute kidney injury, or unfavorably in some other clinical condition. Interleukin-6 (IL-6), a pro-inflammatory cytokine is increased in the serum of patients with acute kidney injury and predicts mortality [14]. However IL-6 may induce anti-inflammatory responses in some conditions [15,16].

In our experiment, IL-10 levels in wild type after hemorrhagic shock and resuscitation increased at 2h, and KO type showed no increase. IL-6 had a tendency to increase at 2h in KO type. As IL-10 is anti-inflammatory cytokine, IL-6 of KO type increased compared to wild type after hemorrhagic shock and resuscitation. Even though the higher IL-6 and absence of IL-10 was noticed in the group of KO type, histology score of KO type showed less damage compared to wild type at 48h in the renal tubules. We examined whether HSP expression was seen or not in immunohistochemical stain. Expression of HSP 70 at KO LR 48h in renal tubules showed higher than that of HSP70 at 48h in wild type.

From these results, we speculate that 1) IL-10 may contribute to histological damage after hemorrhagic shock and resuscitation, or 2) IL-6 may show the effect of amelioration for histological damage, or 3) coexistence of IL-6 and IL-10 may cause histological damage. 4) HSP 70 may reduce the damage of acute kidney injury in IL-10 knockout mice. Further investigation should be performed to analyze these mechanisms to reduce acute kidney injury including apoptosis [17].

CONCLUSION

IL-10 deficiency shows less damage compared to wild type in the mice model of hemorrhagic shock and resuscitation. HSP 70 may have the effect of recovery from damage of renal tubular injury after hemorrhagic shock and resuscitation.

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REFERENCES

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