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Use of soluble complement receptor type 1 to prevent local and distant organ injury in a rat intestinal ischemia reperfusion model

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ABSTRACT

Introduction: In this experimental study we aimed to examine the in vivo effect of soluble complement receptor type 1 (sCR1) in preventing local and distant organ injury in an ischemia reperfusion model via the superior mesenteric artery (SMA). Using these data, it may be possible to determine the clinical usage of sCR1.

Material and Methods: 24 male rats, weighing between 200 and 250 g, were classified into four groups. In group 1, the SMA was clamped for 60 minutes. In group 2, intravenous (IV) sCR1 was given after laparotomy. In group 3, the SMA was clamped for 60 min, at the 60th minute IV sCR1 was administered, and then 1 min later reperfusion was carried out. Group 4 was the laparotomy group. To investigate organ injury, liver function tests (serum AST and ALT levels) and kidney function tests (serum BUN and creatinine levels) were carried out. To evaluate the systemic and local effects of inflammation, total serum levels of protein, albumin, tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) were tested. In tissue samples, glutathione (GSH), malondialdehyde (MDA), and myeloperoxidase (MPO) positive neutrophil counts were identified.

Results: According to the statistical analysis, sCR1 was shown to reduce the ischemia-reperfusion injury and have anti-inflammatory effects. In addition, distant organ injury due to reperfusion was prevented by sCR1.

Conclusion: sCR1 was verified to decrease both mortality and morbidity.

Key words: *Complement system, SMA, liver, intestine, kidney*

Introduction

The effect of local ischemia-reperfusion (IR) on the intestine and other organs has been widely investigated, but more recently interest has increasingly focused on the systemic effects of localised IR. IR elicits a substantial systemic inflammatory response caused by the temporary absence of blood flow, followed by the recovery of blood flow [1]. The majority of IR-associated pathophysiology stems from induced microvascular dysfunction. Importantly, IR injury consists of more than ischemic injury alone [2]. Free oxygen radicals, leucocyte migration and activation, sinusoidal endothelial injury, microcirculatory irregularities, and activation of the coagulation and complement systems can all contribute to the initiation of systemic injury [3].

Increasing evidence supports the possibility that complement inhibitors have beneficial effects in the treatment of IR injury. In a rat IR model, both soluble complement receptor 1 (sCR1) and C5aR inhibitor

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therapy significantly decrease IR-induced neutrophil migration and injury [4]. Other studies have demonstrated that using sCR1 to treat IR injury decreases the area of infarct, polymorphonuclear leucocyte distribution, leucocyte adhesion receptor secretion, and chemotaxis via inhibition of C5a production. The production of the C5b-9 complex, which causes direct endothelial injury, is also inhibited by administering sCR1 to treat IR injury [5]. The present study investigated the role of sCR1 in minimising local and distant organ injury, and in preventing fatal organ injury via inhibition of IR-induced activation of the complement system.

Materials and Methods

This experimental study was performed at the Başkent University Experimental Research Center and was approved by the University's Animal Research Ethics Committee. All surgical procedures were performed according to the guidelines described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals [6]. Twenty-four male Wister albino rats (200 to 250 g each) were used in the study. The rats were divided into 4 groups (n = 6 rats each): (1) IR, (2) sCR1, (3) IR plus sCR1, and (4) control.

Surgery

Group 1 rats underwent laparotomy and then the superior mesenteric artery was clamped for 60 minutes to induce IR. In Group 2, sCR1 was administered intravenously (IV) immediately after laparotomy. In Group 3, 60 minutes after clamping the superior mesenteric artery (SMA), sCR1 was injected into the tail vein, and then reperfusion was initiated. In Group 4, laparotomy was performed and no treatment was administered. All rats were sacrificed four days after treatment, and then blood, liver, kidney, and small intestine were collected for biochemical and immunohistochemical analyses. Before both laparotomy and sacrifice, rats were anaesthetised using aseptic IP 50 mg/kg ketamine hydrochloride (Ketalar, EWL Eczacibasi Warner Lambert Ilaç Sanayi ve Ticaret A.S., Istanbul, Turkey) and 7 mg/kg xylazine hydrochloride (Rompun, Bayer Sisli, Istanbul, Turkey).

Soluble receptor type 1 treatment

sCR1 (Celldex Therapeutics, Inc., Needham, MA, USA) was administered as a 15 mg/kg IP infusion in Ringer's lactate solution into the rat tail vein either directly after laparotomy or 60 min after ischemia and 1 min before reperfusion.

Biochemical analysis

After sacrifice, blood samples were obtained from the vena cava and were analysed by the Başkent University Biochemistry Department to determine serum concentrations of creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, and the cytokines tumour necrosis factor (TNF-α) and interleukin (IL-6). Serum levels of TNF-α and interleukin-6 were quantified using an enzyme-linked immunosorbent assay kit (Invitrogen, Camarillo, CA, USA). Serum concentrations of blood urea nitrogen (BUN), creatinine, albumin, total protein, AST, and ALT were determined using the Abbott Architect C800 (Abbott Diagnostics, Lake Forest, IL, USA). Creatinine concentration was measured using the Jaffe reaction; total protein and albumin concentrations were measured using colourimetric methods; and blood urea nitrogen, AST, and ALT concentrations were measured using ultraviolet methods.

Immunohistochemistry

The left lobe of the liver, the right kidney, and a 20 cm segment of the jejunum were obtained from each rat after sacrifice and preserved in formaldehyde. A pathologist with Wellborn and Associates performed all microscopic evaluations. Tissue neutrophil counts were analysed separately for each group using strong cytoplasmic myeloperoxidase staining visible on an object slide at ×10 magnification as a cell marker.

Tissue malondialdehyde concentration

Tissue malondialdehyde (MDA) concentrations were evaluated as an indicator of lipid peroxidation according to the method defined by Beuge and Aust.

Tissue glutathione concentrations

Homogenous tissue glutathione (GSH) concentrations were evaluated by sulfhydryl group analysis, as described by Elman.

Statistical analyses

Statistical analyses were performed using SPSS version 10.0 software (IBM, Armonk, NY, USA). Data are reported as the mean and standard deviation. In nonparametric data analyses, group differences were determined using Kruskal-Wallis variance analysis, with probability values < 0.05 regarded as statistically

significant. Subgroup comparisons were performed using the Mann-Whitney U test, with probability values < 0.008 regarded as statistically significant.

Results

Immunohistochemical analysis

Evaluation of the number of myeloperoxidase-positive neutrophils in the neutrophil group showed significantly greater numbers ($p < 0.008$) in all three tissues (liver, kidney, and small bowel) than in all the other groups (Table 1). There were no other statistically significant differences among the other groups ($p > 0.008$).

Biochemical analysis

At four days after each procedure, samples were collected for evaluation of serum concentrations of ALT, AST, BUN, creatinine, total protein, albumin, TNF-α, and IL-6. No statistically significant treatment group differences were detected ($p > 0.008$), although ALT, AST, BUN, TNF- α, and IL-6 were highest in Group 1 (Table 2; $p < 0.008$). No statistically significant differences were seen among the other groups ($p > 0.008$).

Tissue concentrations of malondialdehyde and glutathione

When concentrations of MDA in liver, kidney, and small bowel tissue were compared, levels were highest in Group 1 (Table 3; $p < 0.008$) but were not significantly different among Groups 2, 3, and 4 ($p > 0.008$). Tissue GSH concentrations in liver and small bowel tissue were again highest in Group 1 ($p < 0.008$), with no statistically significant differences detected among

Table 1. Neutrophils positive for myeloperoxidase.

Organ	Group 1	Group 2	Group 3	Group 4	P Value*
Liver	29.83 ± 5.3	8.3 ± 4.1	10.5 ± 1.4	9.8 ± 3.5	< .008
Kidney	24.16 ± 5.7	7.156 ± 2.8	11.33 ± 2.1	11.8 ± 3.4	< .008
Small bowel	59.5 ± 11.3	12.66 ± 4.3	25.53 ± 5.5	17.8 ± 4.1	< .008

Data are the mean ± SD number of neutrophils in each tissue. * P values are the results of Fisher's exact test

Table 2. Biochemical parameters.

	Group 1	Group 2	Group 3	Group 4	P Value
AST	71. 7 ± 35.8	72.2 ± 9.6	71.8 ±5.9	110. 7 ± 6.7	< .008
ALT	75.67 ± 17.24	39.17 ± 5.49	32.33 ± 7.17	38.50 ± 5.54	< .008
BUN	31.83 ± 2.04	23.50 ± 2.25	22.67 ± 3.73	26.13 ± 4.61	< .008
Creatinine	0.56 ± 0.06	0.53 ± 0.081	0.49 ± 0.06	0.46 ± 0.03	> .008
Total protein	6.61 ± 0.34	7.05 ± 0.45	6.45 ± 0.23	6.50 ± 0.28	> .008
Albumin	3.33 ± 0.21	4.05 ± 0.20	3.65 ± 0.16	3. 66 ± 0.13	> .008
TNF-α	15.7 ± 0.5	5.5 ± 0.4	5.4 ± 0.7	5.6 ± 0.3	< .008
IL-6	59.8 ± 8.5	33.3 ± 5.6	38.6 ± 8.6	30.3 ± 2.3	< .008

Data are the mean ± SD number of neutrophils in each tissue. * P values are the results of Fisher's exact test

Table 3. Tissue malondialdehyde and glutathione concentrations.

	Group 1	Group 2	Group 3	Group 4	P Value
Malondialdehyde					
Liver	40.21 ± 1.7	32.3 ± 1.4	32.7 ± 2.8	34.1 ± 0.9	< .008
Kidney	32.58 ± 1.5	26.6 ± 1.8	26.8 ± 1.4	24.9 ± 1	< .008
Small bowel	36.6 ± 1.7	28.98 ± 1.2	29.58 ± 2.2	29.08 ± 0.9	< .008
Glutathione					
Liver	3.37 ± 0.51	4.55 ± 0.39	4.43 ± 0.40	4.42 ± 0.20	< .008
Kidney	2.83 ± 0.45	3.8 ± 0.33	3.68 ± 0.46	4.28 ± 0.33	> .008
Small bowel	2.56 ± 0.44	6.39 ± 0.27	6.44 ± 0.46	6.78 ± 0.21	< .008

Data are the mean ± SD number of neutrophils in each tissue. * P values are the results of Fisher's exact test

Groups 2, 3, and 4. When kidney tissue GSH levels were compared among groups, no statistically significant differences were found (Table 3; $p > 0.008$).

Discussion

Inhibition of the complement cascade reduces local and systemic inflammatory reactions caused by the revascularisation that occurs in postischemic reperfused tissue. In the present study, mucosal injury in IR was evaluated using neutrophil values, MDA, and GSH. Serious mucosal and end organ (i.e., liver and kidney) injury were evaluated by assessing tissue neutrophil counts, MDA and GSH concentrations, and serum markers of organ function. Myeloperoxidase staining was used to determine neutrophil infiltration and activation parameters [7]. Analyses revealed no significant group differences in myeloperoxidase staining for neutrophils in the liver, kidney, and small intestine. Neutrophils showed strong cytoplasmic myeloperoxidase staining that was markedly increased in the group that underwent IR (Group 1) in all tissues, indicating inflammation. Although there was also an increase in the group treated to IR and sCR1 compared with the control group, this increase was significantly less than the increase in the IR group. As a result, the complement inhibitor sCR1 was shown to inhibit neutrophil infiltration and activation, and to prevent tissue damage secondary to reperfusion in kidney, intestine, and liver tissue [7].

There were no statistically significant differences in MDA concentrations in liver, kidney, or small intestine tissue among Groups 2 (sCR1), 3 (IR and sCR1), and 4 (control). This finding indicates that sCR1 administration and complement inhibition prevent the lipid peroxidation normally caused by increases in free radical concentrations, both in local tissue and systemically, secondary to IR [8].

Consumption of GSH by conjugation during IR injury depletes intracellular GSH concentrations, with decreasing GSH levels normally replenished by biosynthesis within hepatocytes or by intake of exogenous GSH [9]. Decreased intracellular GSH concentrations during the early stages of IR are countered by adaptive responses that increase GSH biosynthesis [10, 11]. High GSH concentrations are an indicator of cell function and viability [11]. Contrary to these defences, decreases in GSH concentration indicate a weakened intracellular defence and mitochondrial injury [12].

The present study identified a significant difference in GSH concentrations of liver and small intestinal tissue between Group 1 (IR) and the other treatment groups (IR and sCR1, IR and sCR1, and control). No statistically significant differences in liver and small intestinal GSH concentrations were detected among Groups 2 (sCR1), 3 (UR and sCR1), and 4 (control), and there was no statistical difference among all groups in relation to kidney GSH concentrations. The low GSH concentrations measured in the IR group intestinal tissue may have been due to an increase in the concentration of intracellular reactive oxygen metabolites during the late period of reperfusion and thus may be considered as a marker of mitochondrial injury. Moreover, low GSH concentrations are an early indicator of mitochondrial and cytoplasmic GSH depletion and apoptotic cell death. The presence of high GSH concentrations in the IR plus sCR1 group may indicate a protective effect of sCR1 on cells and mitochondria against IR injury through decreases in neutrophil-induced oxidative stress and lipid peroxidation. Furthermore, liver tissue GSH concentrations were lower in the IR group than in the other treatment groups, but no statistically significant difference was detected between the sCR1 and control groups after IR injury. This lack of a significant difference in kidney GSH concentration may be due to the fact that the renal blood supply comes directly from the aorta and thus is affected later in IR through exposure to toxic substances and oxidative stress. Administration of sCR1 just before postischemic reperfusion can prevent injury to the hepatocytes caused by oxidative stress and lipid peroxidation. Thus sCR1 acts to prevent systemic neutrophil-induced lipid peroxidation and protects the entire organism from systemic reperfusion injury [13].

Evaluation of liver and kidney functional indicators as measures of systemic organ injury showed that concentrations of the liver injury indicators AST and ALT were higher in the IR group compared with the other groups. Concentrations of BUN and serum creatinine, indicators of kidney function, were similar in all groups, however. Thus, the kidneys were less susceptible than the liver to local IR-induced injury in our

rat model. This difference may have been due to direct venous blood flow from the affected intestinal tissue to the liver. There was no statistically significant difference in ALT and BUN concentrations between groups that were administered complement inhibitors after IR and the control group [14].

Serum albumin levels are known to decrease as a result of catabolism secondary to tissue injury and inflammation. Serum albumin concentrations were significantly lower in the IR group relative to the other treatment groups. There were no significant differences in serum albumin concentrations among the IR and sCR1 groups in comparison with the control group. This finding demonstrates that sCR1 prevents tissue injury and inflammation. There was also no significant difference among groups in total protein concentrations [15].

Cytokines also have important roles in facilitating inflammatory responses secondary to ischemia. Even with short periods of ischemia, TNF-α is secreted as a first step in a circulatory proinflammatory reaction that induces important metabolic and haemodynamic changes, and as a trigger for the later stages of cytokine activity. In turn, IL-6 is secreted in response to TNF-α and serves as a sensitive early marker of the extent of correlative, IR-induced tissue damage. In the present study, the IR group showed a statistically significant increase in TNF-α and IL-6 concentrations relative to the other groups. Tumour necrosis factor-α and IL-6 concentrations were also significantly higher in the IR plus sCR1 group than in the control group. Compared to group 1 (IR), however, sCR1 lowered TNF-α and IL-6 levels, which are both well-established indicators of inflammation and level of tissue damage; therefore, we consider sCR1 to effectively prevent local and systemic IR [16].

Conclusion

Ischemia is a localised event; however, after revascularisation, mediators released from ischemic tissue enter the systemic circulation and induce changes in other organs. IR injury is thus a systemic phenomenon that can cause dysfunction in multiple organs. The complement system plays an important role in local and distant organ injury, and inhibition of the complement signalling cascade via sCR1 can prevent both local and distant organ injury secondary to IR. The present study demonstrated that sCR1 has anti-inflammatory effects and that sCR1 administered prior to reperfusion decreases the organ injury induced by IR. sCR1 inhibition of the complement system can therefore be used clinically to decrease patient morbidity and mortality by preventing local and distant organ injury.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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