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ScienceDirect
Journal of Molecular and Cellular Cardiology 42 (2007) 620–630
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

# Anti-rat soluble IL-6 receptor antibody down-regulates cardiac IL-6 and improves cardiac function following trauma–hemorrhage

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Received 8 November 2006; accepted 11 December 2006
Available online 16 December 2006

**Abstract**

Although anti-IL-6-mAb down-regulates cardiac IL-6 and attenuates IL-6-mediated cardiac dysfunction following trauma–hemorrhage, it is not known whether blockade of IL-6 receptor will down-regulate cardiac IL-6 and improve cardiac function under those conditions. Six groups of male adult rats (275–325 g) were used: sham/trauma–hemorrhage + vehicle, sham/trauma–hemorrhage + IgG, sham/trauma–hemorrhage + anti-rat sIL-6R. Rats underwent trauma–hemorrhage (removal of 60% of the circulating blood volume and fluid resuscitation after 90 min). Vehicle (V), normal goat IgG or anti-rat sIL-6R (16.7 μg/kg BW) was administered intra-peritoneally in the middle of resuscitation. Two hours later, cardiac function was measured by ICG dilution technique; blood samples collected, cardiomyocytes isolated, and cardiomyocyte nuclei were then extracted. Cardiac IL-6, IL-6R, gp130, IκB-α/P-IκB-α, NF-κB, and ICAM-1 expressions were measured by immunoblotting. Plasma IL-6 and cardiomyocyte NF-κB DNA-binding activity were determined by ELISA. In additional animals, heart harvested and cardiac MPO activity and CINC-1 and -3 were also measured. In another group of rats, cardiac function was measure by microspheres at 24 h following trauma–hemorrhage. Cardiac function was depressed and cardiac IL-6, P-IκB-α, NF-κB and its DNA-binding activity, ICAM-1, MPO activity, and CINC-1 and -3 were markedly increased after trauma–hemorrhage. Moreover, cardiac dysfunction was evident even 24 h after trauma–hemorrhage. Administration of sIL-6R following trauma–hemorrhage: (1) improved cardiac output at 2 h and 24 h ($p<0.05$); (2) down-regulated both cardiac IL-6 and IL-6R ($p<0.05$); and (3) attenuated cardiac P-IκB-α, NF-κB, NF-κB DNA-binding activity, ICAM-1, CINC-1, -3, and MPO activity ($p<0.05$). IgG did not significantly influence the above parameters. Thus, IL-6-mediated up-regulation of cardiac NF-κB, ICAM-1, CINC-1, -3, and MPO activity likely contributes to altered cardiac function following trauma–hemorrhage. Since IL-6R blockade after trauma–hemorrhage down-regulates cardiac IL-6 and improves cardiac functions, blockade of IL-6R following trauma–hemorrhage appears to be a novel and effective adjunct for improving organ and cell function under those conditions.


*Keywords:* IL-6 receptor; Cardiomyocyte; Cytokine; NF-κB; Adhesion molecule; ICAM-1; Chemokines; Myeloperoxidase

## 1. Introduction

Previous studies have shown that trauma–hemorrhage produces cardiac dysfunction, which was associated with increased circulating IL-6 levels; the sustained elevation in plasma IL-6 levels correlates with poor outcome following trauma–hemorrhage [1–3]. It has also been demonstrated that stress conditions such as hypoxia [4], trauma–hemorrhagic shock [3,5], and septic shock [6,7] induce cardiomyocyte-derived IL-6. Furthermore, IL-6 has been implicated as a significant factor in heart failure [8,9] and in ischemic heart disease [10]. Our previous studies have shown that the increase in cardiomyocyte IL-6 levels and IL-6 gene expression following trauma–hemorrhage is correlated with cardiac dysfunction following trauma–hemorrhage [3]. More recently, we have shown that anti-IL-6-mAb neutralized cardiac IL-6 and attenuated IL-6-mediated cardiac dysfunction following trauma–hemorrhage [5].

The 21-kDa cytokine IL-6, originally identified as a B cell differentiation factor (BCDF) in 1981 [11,12], has been shown

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doi:10.1016/j.yjmcc.2006.12.004

to be a pleiotropic cytokine with a wide range of biological activities. IL-6 is produced by various types of lymphoid and non-lymphoid cells including T and B lymphocytes, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells [13], neutrophils and hepatocytes [4], macrophages [14], and cardiomyocytes [15]. IL-6 is a key molecule in the immune response, the acute phase reaction, and hematopoiesis [16]. It is a multifunctional cytokine [13,17] and elevated IL-6 levels are involved in the pathogenesis of many disorders and inhibition of IL-6 function has induced resistance to many disorders [18]. IL-6 mediates its pleiotropic functions through the IL-6R which comprised of two functional different membrane proteins: an 80 kDa ligand-binding chain (IL-6R, IL-6R α-chain, CD126) and a 130 kDa non-ligand-binding but signal transducing chain (gp130, IL-6R β-chain, CD130) [19,20]. IL-6, when bound to either a membrane anchored or a soluble form of IL-6R (sIL-6R), induces the homodimerization of gp130, resulting in a high affinity functional receptor [21].

Several approaches have been proposed to block IL-6 signal transduction which include: (1) inhibition IL-6 production or neutralization of IL-6; (2) blockade of IL-6 binding to IL-6R; (3) blockade of IL-6/IL-6R complex binding to gp130; and/or (4) blockade of the intracytoplasmic signal through gp130 [22]. Specific polyclonal antibody against the rat soluble IL-6R was generated in rabbit from Jack Gauldie's laboratory, and it has been shown that anti-rat sIL-6R stimulates B9 hybridoma cell proliferation [23]. Recently, we have shown that administration of anti-IL-6-mAb during resuscitation after trauma–hemorrhage down-regulated cardiac IL-6 and improved cardiac function after trauma–hemorrhage [5]. Furthermore, we have demonstrated that up-regulation of cardiac NF-κB, ICAM-1, CINC-1, -3, and MPO activity likely contributes to IL-6-mediated cardiac dysfunction following trauma–hemorrhage [5]. In this study, animals were treated with anti-rat sIL-6R antibody during resuscitation after trauma–hemorrhage and the effect of this treatment was measured on cardiac NF-κB, NF-κB DNA-binding activity, ICAM-1, CINC-1, -3, and MPO and cardiac function following trauma–hemorrhage.

## 2. Materials and methods

### 2.1. Rat trauma–hemorrhagic shock model

Trauma–hemorrhagic shock was induced in male adult Sprague–Dawley rats (275–300 g, Charles River Laboratories, Wilmington, MA) as described previously by us [24].

### 2.2. Antibodies used in this study

A specific antibody against rat soluble IL-6 receptor was generated in rabbits immunized with a replication deficient recombinant adenovirus encoding the soluble form of the rat IL-6R antibody (anti-rat sIL-6R) [23], which was a gift from Dr. Jack Gauldie. Other antibodies were purchased from various companies: normal goat IgG (R&D Systems, Minneapolis, MN); anti-rat IL-6 (Biosource International, Los Angeles, CA), IL-6 receptor, gp130, NF-κB, IκB-α, and ICAM-1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA); GAPDH (AbCam, Cambridge, MA); and histone-1 (Upstate, Charlottesville, VA).

### 2.3. Administration of anti-rat IL-6R Ab or normal goat IgG

Anti-rat sIL-6R (16.7 μg/kg BW) or normal goat IgG (16.7 μg/kg BW) was administered intra-peritoneally in the middle of resuscitation.

### 2.4. Determination of cardiac function

At 2 h after trauma–hemorrhage or sham-operation, cardiac output (CO) was determined by ICG dilution technique; MAP, heart rate, positive and negative d$P$/d$t$ were measured, stroke volume (SV), and total peripheral resistance (TPR) were calculated as previously described by us [3]. Left ventricular performance parameters including the maximal rate of pressure increase ($+\mathrm{d}P\mathrm{d}t_{\max}$) and decrease ($-\mathrm{d}P\mathrm{d}t_{\max}$) were determined with a heart performance analyzer (Digi-Med, Louisville, KY) [3,25]. In some experiments, we used $^{85}$strontium radioactive microspheres for the measurement of cardiac output following trauma–hemorrhage. Both ICG and $^{85}$strontium microspheres procedures are routinely used in our laboratory for cardiac function measurement [25].

### 2.5. Cardiomyocyte isolation

Rat cardiomyocytes were isolated following cardiac function measurement as previously described by us [3]. In brief, the heart was quickly removed from the chest and perfused in a retrograde manner via the aorta at 37 °C and at a consistent rate (12 ml/min/g tissue) for 5 min with a calcium-free Krebs buffer containing (in mM) 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.2 $KH_2PO_4$, 1.2 $MgSO_4$, 10 HEPES, and 11 glucose, gassed with 95% $O_2$–5% $CO_2$. Following the wash-perfusion, the calcium-free Krebs buffer was replaced by the enzymatic digestion buffer containing collagenase type II (Worthington, Lakewood NJ), 0.1% fat-free BSA, 100 μM $CaCl_2$, and 10 mM taurine and perfused at 37 °C at a consistent rate (5 ml/min/g tissue) for 9 min. When the heart became swollen and hard, left ventricle was removed and cut into small chunks ($\sim 1 \times 1$ mm$^3$) and further digested with the incubation buffer containing the enzymatic digesting buffer and 2% fat-free BSA in a shaker (60–70 rpm) water bath at 37 °C for 10 min. The supernatant containing the dispersed cardiomyocytes was filtered through a 300-μl filter into a 50-ml sterilized tube and gently centrifuged at 500 rpm for 1 min. The upper portion of the supernatant was discarded and ~30 ml BSA-free buffer (calcium-free Krebs buffer +50 μM $CaCl_2$) was added and centrifuged at 480 rpm for 1 min. The upper portion of the supernatant was discarded again, and 10 ml cell suspension was carefully layered onto high BSA (4%) medium and centrifuged (480 rpm, 1 min). The number of cardiomyocytes was then counted by suspending cardiomyocytes in 0.02% trypan blue under light microscope. All the buffers were filtered (0.2-μM filter) and equilibrated with 95% $O_2$–5% $CO_2$ for at least 20 min before use. To reduce