Pharmacological Reports
2009, 61, 504–513
ISSN 1734-1140

Effect of interleukin 6 deficiency on the expression of Bcl-2 and Bax in the murine heart

Karol A. Kamiński[1], Marcin Kożuch[2], Tomasz A. Bonda[1,3], Maria M. Stepaniuk[1,4], Ewa Waszkiewicz[1], Lech Chyczewski[4], Włodzimierz J. Musiał[1], Maria M. Winnicka[3]

[1]Department of Cardiology, Medical University of Białystok, M. Curie-Skłodowskiej 24A, PL 15-276 Białystok, Poland

[2]Department of Invasive Cardiology, Medical University of Białystok, M. Curie-Skłodowskiej 24A, PL 15-276 Białystok, Poland

[3]Department of General and Experimental Pathology, Medical University of Białystok, A. Mickiewicza 2C, PL 15-222 Białystok, Poland

[4]Department of Clinical Molecular Biology, Medical University of Białystok, Waszyngtona 13, PL 15-276 Białystok, Poland

Correspondence: Maria M. Winnicka, e-mail: mmw@amb.edu.pl

Abstract:
Interleukin 6 (IL-6) is a pleiotropic cytokine that is highly expressed in response to ischemia and reperfusion. It has dichotomous roles in the heart, functioning both as an inflammatory mediator as well as a protective agent. The aim of this study was to evaluate the effect of IL-6 deficiency on the expression of apoptotic regulatory proteins under both baseline conditions and following induction of ischemia and reperfusion in the mouse heart. C57BL/6J IL-6–/–^{TMKopf} (IL6KO) and C57BL/6J mice (WT) were subjected to 30 minutes of local reversible myocardial ischemia *in vivo* or a sham operation. The expression of Bcl-2, Bax and STAT3 in the heart was assessed by western blotting. Under both baseline conditions and following the sham operation, IL-6 deficiency was associated with reduced expression of Bcl-2 and Bax. The TUNEL-FITC, Evans blue and tetrazolium chloride staining of the hearts following ischemia and reperfusion revealed similar injury in operated IL6KO and WT animals. There was increased STAT3 phosphorylation in operated mice regardless of the genotype. Bcl-2 and Bax expression was also comparable between the mouse strains following ischemia and reperfusion. In summary, these results indicated that IL-6 deficiency affected the basal expression of apoptotic regulators, but this did not profoundly alter the extent of reperfusion injury or apoptosis in the mouse heart following ischemia and reperfusion.

Key words:
heart, ischemia and reperfusion, interleukin 6, apoptosis, mice

Abbreviations: CNTF – cilliary neurotrophic factor, DAPI – 4',6-diamidino-2-phenylindole, IL-1β – interleukin 1 beta, IL-6 – interleukin 6, IR – ischemia and reperfusion, JAK – Janus kinase, LIF – leukemia inhibitory factor, KO – knock-out, LCA – left coronary artery, LV – left ventricle, MAPK – mitogen activated protein kinase, PAGE – polyacrylamide gel electrophoresis, PBS – phosphate buffered saline, RIPA – radioimmunoprecipitation assay, RNA – ribonucleic acid, SDS – sodium dodecyl sulfate, STAT – signal transducer activator of transcription, STEMI – ST segment elevation myocardial infarction, TNF-α – tumor necrosis factor alpha, TTC – 2,3,5-triphenyltetrazolium chloride, TUNEL – terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling, WT – wild type

Introduction

Interleukin 6 (IL-6), cardiotrophin-1, leukemia inhibitory factor (LIF) and cilliary neurotrophic factor (CNTF) belong to a family of cytokines acting *via* the common receptor subunit gp130. IL-6 is a prototypic pleiotropic cytokine produced by various cell types, including leukocytes, fibroblasts, macrophages and endothelial cells. This cytokine is involved in mediating inflammation, antibody production, hormonal secretion, glucose metabolism, fatty acid turnover and many other physiological processes [23]. IL-6 is induced in the myocardium in response to harmful stimuli including ischemia [4], which is thought to contribute to the inflammatory reaction [16]. Interestingly, induction of IL-6 expression is more rapid and more pronounced in the previously ischemic area where blood flow has been restored as compared to the non-reperfused tissue [4, 16].

Reperfusion therapy has become a gold standard treatment for ST-segment elevation myocardial infarction (STEMI) and has saved thousands of patients, but there is still room for improvement. Restoration of blood flow in the ischemic myocardium causes a series of events associated with rapid "oxidative burst", which is characterized by activation of the inflammatory process resulting in leukocyte infiltration and apoptosis [10]. Thus, reperfusion may, to some extent, aggravate the injury by increasing the rate of apoptosis of cardiac cells. Previous studies have suggested that the expression of pro-inflammatory cytokines, such as IL-6 and TNFα, may be responsible for this activation of the inflammatory cascade and subsequent aggravation of the injury [10, 16]. On the other hand, IL-6 has been shown to inhibit the expression of pro-inflammatory cytokines (e.g., IL-1β) and promote secretion of the anti-inflammatory cytokine IL10 [13]. Moreover, IL-6 exerts cytoprotective effects in other situations, such as hemorrhagic shock or ischemic preconditioning, by promoting the expression of the anti-apoptotic protein Bcl-2 [1, 21]. Gp130 is the major player that transduces IL-6-mediated signals from the extracellular environment to the intracellular environment. Gp130 is a membrane bound receptor that has no intrinsic kinase activity but has binding affinity for JAK kinases and proteins containing src-homology domains. Thus, JAK-STAT, Akt and MAPK signaling pathways are activated in response to IL-6 stimulation [23]. The first two pathways are vital for protecting myocardial tissue during reperfusion [6]. It has been reported that restoration of blood flow to the previously ischemic myocardium evokes a rapid increase in the rate of cardiac cell apoptosis [10]. It is widely accepted that this is the primary mechanism for persistent myocardial dysfunction following successful treatment of myocardial infarction [10]. The recent advances in understanding of the molecular mechanisms underlying ischemia and reperfusion injury have created excitement, since they have proposed that delivery of therapeutic agents against components of the apoptotic cascade can inhibit cell death and prevent injury [14]. IL-6 is one of the cytokines that is highly expressed in the myocardium following ischemia and reperfusion, so it may significantly influence the apoptosis of cells within the heart.

The aim of this study was to examine the effects of IL-6 deficiency on the regulation of apoptosis under baseline conditions and following myocardial injury in the murine model of regional cardiac ischemia and reperfusion.

Materials and Methods

Male C57BL/6J IL-6–/–^{TMKopf} mice (IL6 KO) (body weight 27.8 g ± 2.1 g) and C57BL/6J (WT) control animals (body weight 27.9 ± 2.1 g) that were 12–16 weeks old were used in this study.

Eight non-operated mice of each genotype were sacrificed by cervical dislocation, and their left ventricles were dissected to assess protein expression and apoptosis under baseline conditions.

A total number of 75 animals were subjected to sham operation (SH) or to 30 minutes of regional reversible myocardial ischemia induced by ligation of the left anterior descending artery (IR group). The experimental model of ischemia and reperfusion was performed as previously described [6], with minor modifications. Briefly, mice were anesthetized with ketamine and xylazine *ip* (120 mg/kg and 3 mg/kg, respectively), placed on a heating pad at a constant temperature of 37°C, intubated and ventilated with a mouse respirator (Minivent, Harvard Instruments) using oxygen-enriched air (volume of 0.25 ml and frequency of 200/min). A left-sided lateral thoracotomy in the 5th intercostal space was performed, which was followed by exposure of the anterior wall of the left