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Short communication

Alteration of cytokine profile following hemorrhagic shock

Sumin Lu, Alex Aguilar, Kumar Subramani, Ninu Poulose, Ahmar Ayub, Raghavan Raju *

Georgia Regents University, Augusta, GA, United States

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ABSTRACT

Hemorrhage is one of the leading causes of death in patients with trauma. We recently demonstrated that resveratrol can improve cardiac function and prolong life following severe hemorrhagic injury (HI) in a rat model. The present work is focused on determining changes in NF-κB dependent gene expression in the heart and the systemic cytokine milieu following HI and the effect of resveratrol treatment. The results indicate an increase in phosphorylated NF-κB in the heart with a concomitant increase in the expression of NF-κB dependent genes following HI. There was also a significant increase of systemic cytokine levels, both pro and anti-inflammatory, following HI and resolution when treated with resveratrol. This study demonstrates the potential role NF-κB has in the physiological response to HI and the effectiveness of resveratrol in reducing immune activation.

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1. Introduction

Trauma is the number one cause of death in the United States, in the age group of 1–46 years and third cause of death worldwide [1]. Hemorrhage accounts for approximately 40% death associated with trauma [2]. Understanding the mechanisms following hemorrhagic shock is critical in reversing compromised molecular pathways and to prolonging survival.

Hemorrhagic shock is characterized by a strong inflammatory response that conceivably may be critical for survival following the insult [3]. However a sustained and exacerbated inflammatory response may be deleterious to the outcome following hemorrhagic injury (HI). NF-κB plays a major role in promoting transcription of a number of inflammatory genes [4].

Recent studies from our laboratory and other laboratories have demonstrated a profound salutary effect of resveratrol on organ function in experimental models of HI [5–8]. Resveratrol is a naturally occurring polyphenol found in various plants and fruits, including grapes. Resveratrol is an antioxidant and has been shown to improve mitochondrial function and reduce inflammation. Studies have shown that resveratrol activates SIRT1, a sirtuin family of proteins, which deacetylates a number of critical proteins including NF-κB [9,10]. Phosphorylation and acetylation of the p65 subunit are known to regulate the function of NF-κB [10].

Our continued studies demonstrated that resveratrol can improve survival and prolong life following HI even in the absence of resuscitation fluid [11]. However the role of resveratrol on the inflammatory response following HI or the role of NF-κB regulated genes is not well defined. In this study we determined the cytokine expression changes in the plasma and heart following HI, and with resveratrol treatment.

2. Methods

2.1. Animals

Male Sprague Dawley rats (250–350 g) were purchased from Charles River Laboratory (Wilmington, MA, USA) and housed in GRU animal facility. HI procedure and hemodynamics were described before [11]. Briefly, HI was induced by bleeding 60% of the circulating blood volume in 45 min and maintaining the animals at low blood pressure (40 ± 5 mm Hg) for another 45 min followed by resuscitation as described below. Resveratrol (10 mg/kg body weight) or vehicle (DMSO) was administered 10 min after the start of resuscitation, intravenously. All the experiments were in accordance with Institutional Animal Care and Use Committee at Georgia Regents University.

2.2. 2-hour study

In this group of animals, following HI, resuscitation was carried out with Ringers Lactate, two times the volume of shed blood. After resuscitation, the animals were observed for two hours, left

Abbreviations: HI, hemorrhagic injury; MOF, multiple organ failure; RSV, resveratrol.

* Corresponding author at: Georgia Regents University, CB 2601, Sanders Building, 1479 Laney Walker Blvd., Augusta, GA 30912, United States.

E-mail address: RRaju@GRU.edu (R. Raju).

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ventricular function was measured, sacrificed and heart tissue and blood samples collected for molecular analysis.

2.3. 3-hour study

The animals in this group were resuscitated with Ringers Lactate, two times the volume of shed blood. However, after resuscitation, the animals were observed for 3 h, sacrificed and heart tissue and blood were collected for further study.

2.4. Real time polymerase chain reaction

(PCR): Total RNA was isolated from heart tissue using Total RNA isolation mini kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol (Qiagen Inc, Valencia, CA) and cDNA synthesized (Agilent Technologies, CA). The sequence of the primers used were: NFkB p65: Forward: CCTCATCTTTCCCTCAGA GC, Reverse: CGCACTTGTAACGGAAACGC 3″; IL-2: Forward: ACTTC AAGCCCTGCAAAGGA, Reverse: GTTTCAATTCTGTGGCCTGCTT; IL-6: Forward: GAGCCCACCAGGAACGAAA, Reverse: AACTGGCTGGAAGT CTCTTGC; IL-10: Forward: TGCGACGCTGTCATCGATTT, Reverse: GTAGATGCCGGGTGGTTCAA; MIP-1α, Forward: CTGCCAAGTAGCC ACATCCA, Reverse: GGAATGTGCCCTGAGGTCTT; TNF-α: Forward: ACGTCGTAGCAAACCACCAA; Reverse: GCAGCCTTGTCCCTTGAAGA; β-actin: Forward: AGTACCCCATTGAACACG; Reverse: AATGCCAGT GGTACGACC. Quantitative SYBR green (Biorad, MA) real time PCR was performed using Stratagene Mx3000P (Agilent Technologies) real time PCR instrument with primer sets for each gene and normalized to beta actin. The results are expressed after normalizing to the values obtained for samples in sham group.

2.5. Western blot analysis

The heart tissues were homogenized and the proteins resolved on a 10% SDS polyacrylamide gel, transferred to PVDF membrane, blocked using 5% (w/v) non-fat dried milk and then incubated with respective antibodies overnight at 4 °C or for 1 h at room temperature (RT). The membranes were probed with antibodies to NF-κB (Cat # 8242; Cell Signaling, MA), and p-NF-κB (Cat # 3033; Cell Signaling Technology, Danvers, MA). The membranes were subsequently washed and incubated with horseradish peroxidase conjugated secondary antibody for 1 h at RT and developed using enhanced chemilumiscence (Cat # NEL113001EA, Perkin Elmer, MA). Protein bands developed on X-ray films were quantified using the ImageJ software (Wayne Rasband, NIH).

2.6. Plasma cytokine analysis

Cytokine levels in plasma were quantified by Rat Cytokine I Array (Aushon Biosystem, Billerica, MA, USA) by a multiplex method.

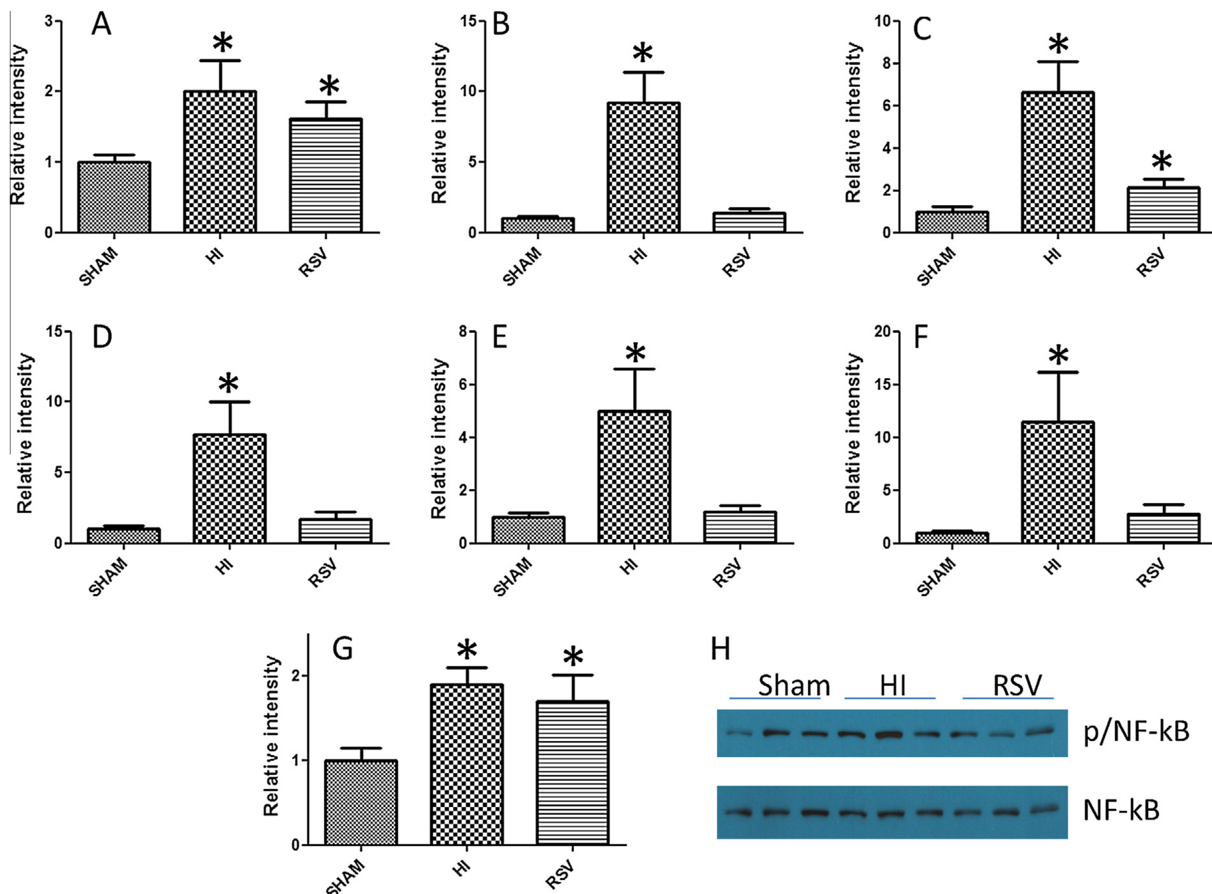

Fig. 1. NF-κB and NF-κB regulated cytokine expression in the heart following HI. NF-κB and NF-κB dependent cytokine gene expression changes following HI and with resveratrol treatment were determined by SYBR green real time PCR amplification of NF-κB p65 (A), IL-2 (B), IL-6 (C), IL-10 (D), TNF-α (E), and MIP-α (F). Panel G shows densitometric data on the ratio of band intensities of p-NF-κB/NF-κB as obtained by Western blot (Panel H). All quantitative results are averages of three sets of experiments. $n = 5–7$; $^{*}p < 0.05$; bars indicate mean ± SEM.

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