Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Functional role of inflammation in the surgical injury induced vascular remodeling of male albino rats

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Received March 7, 2017; **Accepted** August 19, 2017; **Published** September 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.9.2

Abstract: Our present study investigates the cellular and molecular inflammatory events in male albino rat arterial injury. Male albino rats were subjected to longitudinal incision and carotid artery clamping for the duration of 45 days. Heat shock protein (HSP) 27, HSP70, HSP47 and Nuclear Factor kappa B (NF-κB) expressions were determined by qPCR and Western blot method. The morphology of vessel wall alteration was studied by the light microscopy. The expression of NF-κB was found to be increased after ten days of carotid artery injury. The qPCR and Western blot analysis showed elevation in HSP47, HSP27, and HSP70 expression, ten days following the surgical injury. The neointima-formation and the media layer discontinuity were evidenced by light microscopy. The dendritic-like cells were in close contact with the lymphocytes. Our study reports that the surgical injury induces an inflammatory response through the increased NF-κB and HSPs expression.

Key words: Inflammation; Rats; Vascular remodeling; Protein.

Introduction

Angioplasty, bypass grafting, and stenting have been associated with endothelial dysfunction of mechanical artery injury (1, 2). The vessel wall injury induced restenosis causes activation of the inflammatory response (3, 4). Thus, association between restenosis and inflammatory response is yet to be investigated (5, 6). The smooth muscle cell proliferation and migration have been induced by the inflammatory response, which forms an intermediate lesion. Steffens and Mach (7) have reported the remodeling such as thickening of artery wall without unaltered lumen due to prolonged inflammatory response. There are several intracellular signaling promotes mitogenic smooth muscle cell proliferation. NF-κB plays a vital role in the regulation of multiple factors involved in proliferative and inflammatory events (8, 9).

Pockely (10) have reported that the several stress factors including, toxic materials, heat, injury, behavioral stress and surgery may induce heat shock proteins (HSPs). Yoshimura et al., (11) have reported the NF-κB have activated in atherosclerotic lesions and arterial injury of mammals. Snoeckx et al., (12) have reported the HSPs synthesis has been associated environmental stress in the blood vessel. The HSPs and NF-κB play a significant role in arterial injury vasculopathy induced animal model. Whereas, Kong et al., (13) have reported the cells derived bone marrow has been associated with the repairment of the vascular process. Takahashi and Lee (14) have reported the dendritic cells have been linked to the formation of neointima. Our present study investigates the expression of NF-κB activation and HSPs after carotid surgical injury induced male albino rats.

Materials and Methods

Materials

Primary antibodies, secondary antibodies, and primers were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA, USA). Assay kit was obtained from the Bio-Rad, CA, USA.

Animals

Rats were obtained from Shangai Animal House. Animals were placed in a proper cage with standard condition (25± 0.5 °C and 60 ±5%). The male albino rats were divided into 4 groups of six rats each.

Induction of carotid injury

Rats were anesthetized with diethyl ether, and rats were given an antibiotic. The incision was created in the anterior neck region of rats. The plastic made scanlom clamp was used for the bypass grafting coronary artery on carotids for 20 seconds to induce crushing based lesion. Simultaneously, the longitudinal incision was created via carotid wall thickness. Male albino rats were grouped into five groups, such as, control, 1 day, 15 days, 30 days and 45 days.

qPCR

The mRNA expression of NF-κB, HsP27, HsP47, and HsP70 were determined with use of qPCR. GAPDH was used internal control. SYBR Green Master Mix was used according to the manufacturers' instructions (15).

Western blot analysis

Rat carotid arteries were removed at fixed time. The

membranes were probed with NF-κB, HsP27, HsP47 and HsP70 antibodies for overnight. Tissues were washed with TBST buffer. HRP conjugated goat anti-rabbit IgG was incubated with the membrane for 1 h. The protein levels of NF-κB, HsP27, HsP47 and HsP70, were determined (16).

Morphology observation

The morphology of injured and non-injured carotids was investigated by the light microscope. The carotid tissues were prepared for the light microscope investigation (17) (Shangai, China).

Statistical analysis

Experimental data were expressed as mean ± SD. The statistical difference between injured and non-injured carotids was calculated using ANOVA. A $p<0.05$ was taken statistically significant.

Results

NF-κB mRNA and protein expression

The mRNA and protein expression of NF-κB was determined in the carotids of male albino rats using qPCR and Western blot analysis. NF-κB mRNA expression occurred in non-injured carotids nuclear extracts at a basal level. However, the mRNA expression gradually increased and attained peak at 45 days upon carotid injury (Figure 1). The protein expression was calculated at two different time points (15 and 30 days). NF-κB protein expression occurred at basal level in non-injured carotids nuclear extracts. The protein expression gradually increased and attained peak at 30 days upon carotid injury (Figure 2).

HsP27 mRNA and protein expression

Expression of HsP27 was determined in the carotids of male albino rats using qPCR and Western blot analysis. HsP27 mRNA expression occurred at basal level in non-injured carotids nuclear extracts. However, the mRNA expression gradually increased and attained peak at 45 days upon carotid injury (Figure 3).

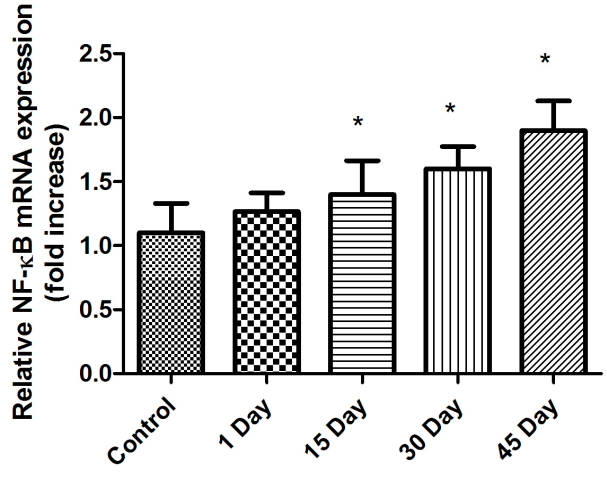

Figure 1. Up-regulation of mRNA expression of NF-κB after carotids injury (1, 15, 30 and 45 days). Expressions of NF-κB mRNA were determined with reference to GAPDH. Changes were expressed as fold changes and expressed as mean ± SD, n=6, *P<0.05.

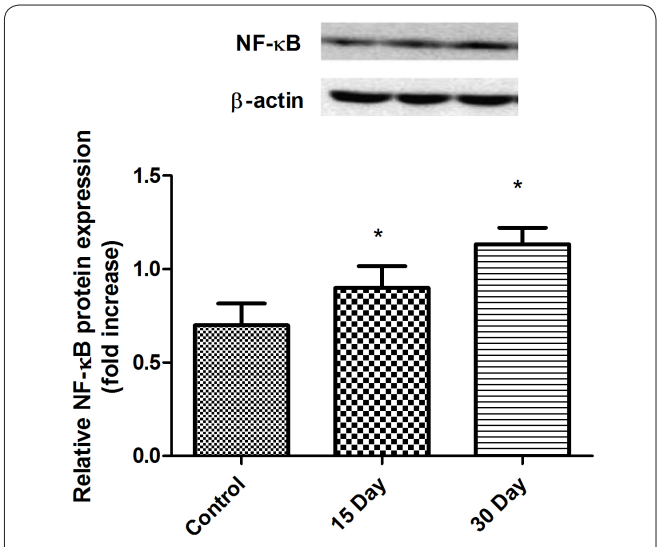

Figure 2. Up-regulation of protein expression of NF-κB after carotids injury (15 and 30 days). Anti-NF-κB was used to probe with extracts of carotids in Western blot analysis. Densitometry was used to quantitate (A), and obtained values were given as means ± SD (B), n=6, *p<0.05.

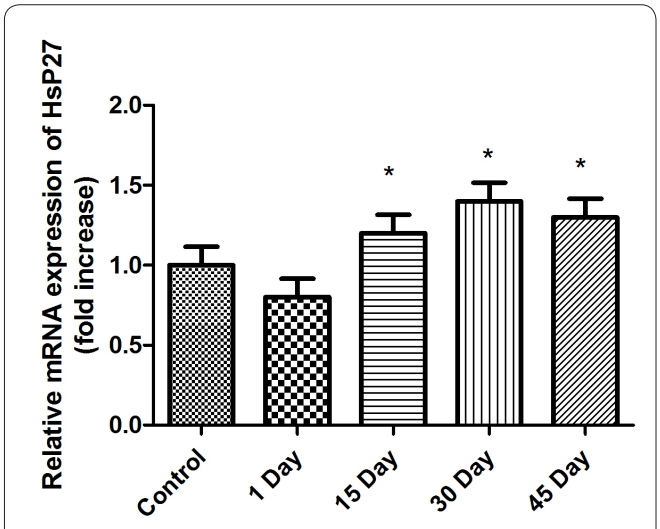

Figure 3. Up-regulation of mRNA expression of HsP27 after carotids injury (1, 15, 30 and 45 days). Expressions of HsP27mRNA were determined with reference to GAPDH. Changes were expressed as fold changes and expressed as mean ± SD, n=6, *P<0.05.

The protein expression was calculated at two different time points (15 and 30 days). HsP27 protein expression occurred at basal level in non-injured carotids nuclear extracts. The protein expression gradually increased and attained peak at 30 days upon carotid injury (Figure 4).

HsP47 mRNA and protein expression

Expression of HsP47 was determined in the carotids of male albino rats using qPCR and Western blot analysis. HsP47 mRNA expression occurred at basal level in non-injured carotids nuclear extracts. However, the mRNA expression gradually increased and attained peak at 45 days upon carotid injury (Figure 5). The protein expression was calculated at two different time points (15 and 30 days). HsP47 protein expression occurred at basal level in non-injured carotids nuclear extracts. The protein expression gradually increased and attained peak at 30 days upon carotid injury (Figure 6).

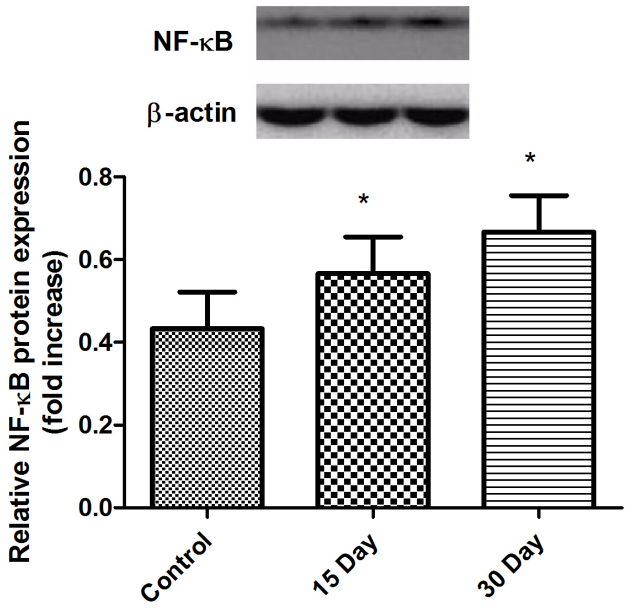

Figure 4. Up-regulation of protein expression of HsP27 after carotids injury (15 and 30 days). Anti-HsP27 was used to probe with extracts of carotids in Western blot analysis. Densitometry was used to quantitate (A), and obtained values were given as means ± SD (B), n=6, *P<0.05.

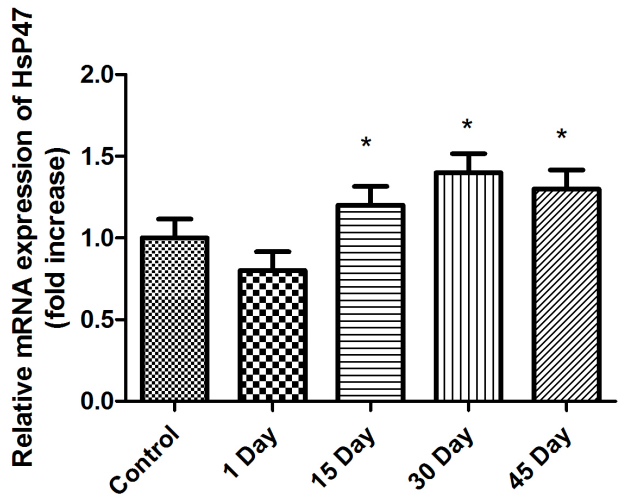

Figure 5. Up-regulation of mRNA expression of HsP47 after carotids injury (1, 15, 30 and 45 days). Expressions of HsP47mRNA were determined with reference to GAPDH. Changes were expressed as fold changes and expressed as mean ± SD, n=6, *P<0.05.

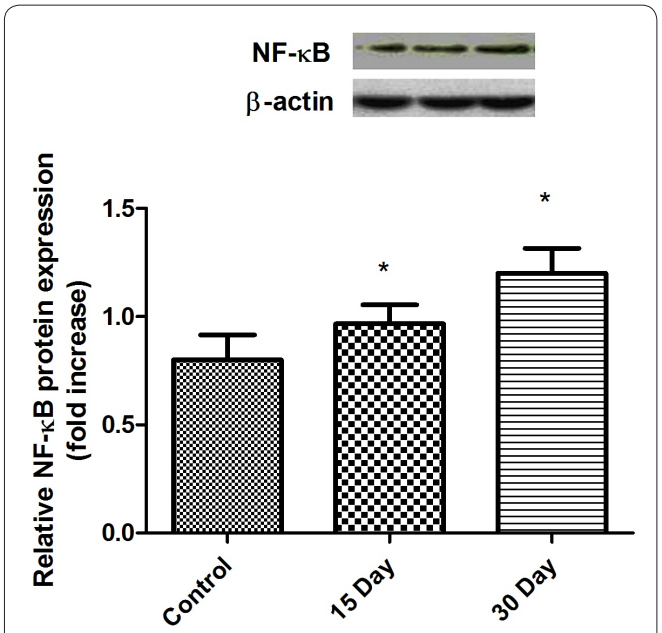

Figure 6. Up-regulation of protein expression of HsP47 after carotids injury (15 and 30 days). Anti-HsP47 was used to probe with extracts of carotids in Western blot analysis. Densitometry was used to quantitate (A), and obtained values were given as means ± SD (B), n=6, *P<0.05.

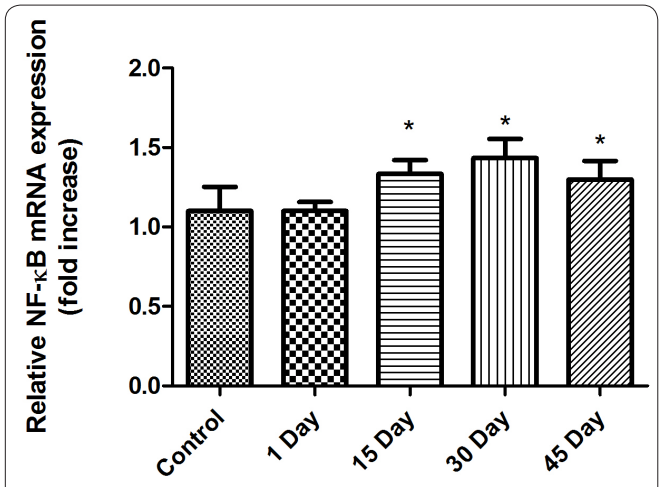

Figure 7. Up-regulation of mRNA expression of HsP70 after carotids injury (1, 15, 30 and 45 days). Expressions of HsP70 mRNA were determined with reference to GAPDH. Changes were expressed as fold changes and expressed as mean ± SD, n=6, *P<0.05.

HsP70 mRNA and protein expression

The expression of HsP70 was determined in the carotids of male albino rats using qPCR and Western blot analysis. HsP70 mRNA expression occurred at basal level in non-injured carotids nuclear extracts. However, the mRNA expression gradually increased and attained peak at 45 days upon carotid injury (Figure 7). The protein expression was calculated at two different time points (15 and 30 days). HsP70 protein expression occurred at basal level in non-injured carotids nuclear extracts. The protein expression gradually increased and attained peak at 30 days upon carotid injury (Figure 8).

Morphology Observation by Light Microscope

The formation of neointima and discontinuity of the media layer were observed in our microscopical observation. Neointima and media layer discontinuity that leads to transmigration of the cell. Usually, the adventitia and vascular lumen produce cell transmigration. Lumen and neointima seem to have the organelle-rich and immature cells. The endoplasmic reticulum and Golgi apparatus present in these cells. Very few lysosomes were present and few infiltrating cells found in the neointima region (Figure 9).

Discussion

Our study investigated the possible inflammatory events after surgical injury by quantitating NF-κB and HsPs mRNA and protein expression. Also, the morphological changes in the carotids were examined by Light Microscope using male albino rats as a mammalian model. The TNF-α and IL-1, growth factors, and T-cell activation signals activate rapidly the transcription fac-

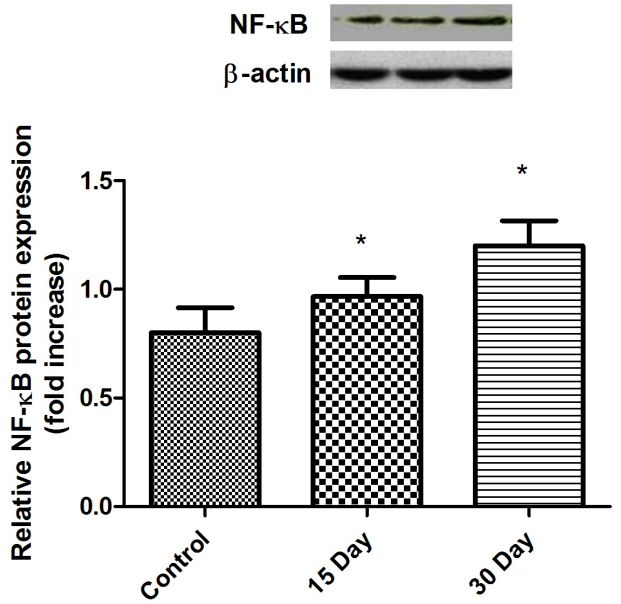

Figure 8. Up-regulation of protein expression of HsP70 after carotids injury (15 and 30 days). Anti-HsP70 was used to probe with extracts of carotids in Western blot analysis. Densitometry was used to quantitate (A), and obtained values were given as means ± SD (B), n=6, *p<0.05.

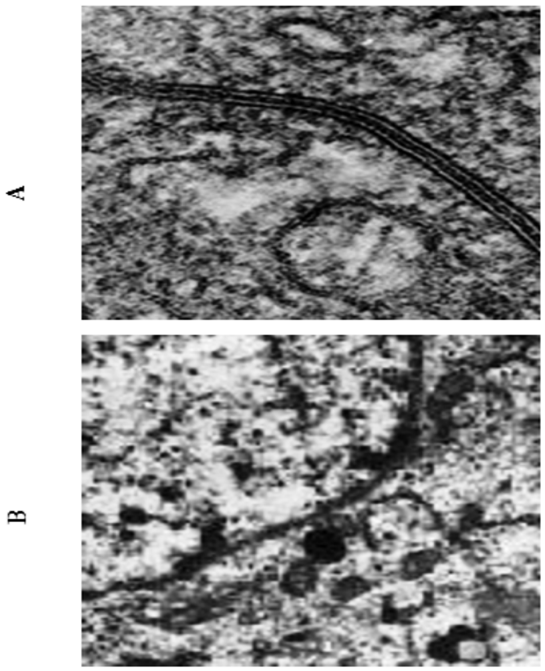

Figure 9. Light microscopy of injured carotids vessel wall 15days following injury. The occurrence of neointima formation, discontinuity and cell infiltration of cells were noted (Mag: 40x).

tor NF-κB (18). The NF-κB was activated rapidly in the injured carotids of male albino rats.

The inflammation is so stubborn and persistent in the wounded carotids than the non-injured carotids of male albino rats. The inflammation is systemic which leads expression of NF-κB even in non-injured carotids of male albino rats. Our experimental findings are agreed with the findings of Liu et al., (19) have stated that the NF-κB expression in the distal organs from the inflammation area. However, the injured carotids of male albino rats only showed tissue remodeling and cell infiltration. The marginal increase in the expression of NF-κB not necessarily associated with disuse damage and other pathogenesis.

The role of HsPs on atherosclerosis has been widely studied (20). However, the role of HsPs in the inflammatory response after surgical injury in the carotids yet to be investigated. The expression of HsP27, HsP47, and HsP70 has been increased immediately after the carotid injury in male albino rats. The mRNA and protein expression was correlated positively with the level of infiltrating cells. Greenberg et al., (9) have reported the expression of HSP70 limits the carotids damage significantly following a vascular injury. Xu et al., (21) have reported the induced HSP70 expression provides protection for vasculature from the carotid damage during severe hemodynamic stress.

Expression of HsP27 has been increased after the carotid injury in the male albino rats. Connolly et al., (22) have reported that the induced HSP27 expression could prevent restenosis. HSP70 and HSP27 expression did not increase in the non-injured carotids of rats, whereas it was increased significantly in the carotids. This indicates the expression of HsPs was related to protection from vessel damage. HsP47 was also found to be increased in the injured carotids. Sauk et al., (23) have reported the HSP47 have associated with procollagen in the region of the endoplasmic reticulum.

Sluijter et al., (24) have reported the overexpression of HsP47 in the carotids of smooth muscle has been associated with overproduction of collagen and intimal thickening. HsP47 expression was transient in our model, and it starts to decline after 30 days. Schell et al., (25) have reported the induced expression of HSPs protects carotids damage by reducing the NF-κB activation. The induced NF-κB and HsPs expression indicate their protective role in the injured carotids of male albino rats. However, the further experimental studies are to be carried out to define the molecular mechanism of these proteins against vascular damage.

The light microscopical investigation confirmed the presence of inflammatory cells in the injured carotids of male albino rats. The inflammatory could differentiate into macrophage, endothelial and dendritic cells whereas infiltrating cells differentiate into smooth muscle cells. The microfilament bundles of several types of cells express the filamentous actin that enters the arterial wall during injury. Sata et al., (26) have reported the participation of endothelial cells in the blood vessels repair following tumor angiogenesis, ischemic injury, and atherosclerosis. Bailey and Fleming (27) have reported the dendritic cells contact with lymphocytes throughout the immune response, and it is believed that the dendritic cells may involve in the regulation of arterial wall remodeling.

In conclusion, our data showed the sequence of inflammatory events in the injured carotids of male albino rats. NF-κB and HsPs expression believed to exert a protective role in remodeling and vascular injury. Also, our study showed the presence of dendritic cells, and their contact with lymphocytes might be crucial finding in the inflammatory events.

Conflict of Interest Statement

Authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animals

were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Authors' contributions

Song X. performed design of experiment and Li L. performed the animal experiments and Wu Y. performed the qPCR and Zhang L. performed the western blot analysis and Li X. performed the statistical analysis and preparation of manuscript.

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