The Cardioprotective Effect Of Selenium In Myocardial Ischemia Reperfusion Injury

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Abstract

The objective of the current study is to assess the possible cardioprotective effect of selenium in myocardial ischemia reperfusion injury induced by ligation of coronary artery in a male rat model. 24 adult male Sprague Dawley rats were randomized into 4 equal groups: (1) Sham group, rats underwent the same anesthetic and surgical procedures as the control group except for LAD ligation; (2) Active control group, rats subjected to regional ischemia for 30 min by ligation of LAD coronary artery and reperfusion for 2 hours; (3) Control vehicle group, rats received D.W (vehicle of selenium) via IP route and subjected to ischemia for 30 minutes before ligation of LAD coronary artery & reperfusion for 2 hr; (4) Selenium treated group, rats pretreated with selenium5mg/kg via IP injection 30 minutes before ligation of LAD coronary artery & then subjected to reperfusion for 2 hr. In control group, as compared with sham, tissue TNF-α, IL-6, IL-10, caspase-3 and BAX, plasma cTn-T and serum MDA significantly increased (P<0.05), while serum GSH significantly decreased (P<0.05). Histopathologically, control group showed a significant cardiac injury (P<0.05) compared with sham group. Selenium significantly counteracted (P<0.05) the increase of TNF-α, IL-6, caspase-3 and BAX and counteracted the increase in plasma cTn-T and serum MDA. Selenium produces a significant elevation (P<0.05) in cardiac IL-10 and serum GSH with significant reduction in (P<0.05) cardiac injury. In Conclusions, Selenium minimizes myocardial I/R injury in male rats via interfering with inflammatory reactions and apoptosis which were induced by I/R injury.

Key words: Myocardial ischemia, reperfusion, Selenium, inflammatory reactions, Apoptosis.

Abbreviations:
LAD (Left Anterior Descending artery), I.P (intraperitoneal), D.W (distilled water), MDA (malondialdehyde), GSH (reduced glutathione), TNF-α (Tumor Necrosis Factor alpha), IL-6 (Interleukin 6), IL-10 (Interleukin 10), caspase-3 (cysteine aspartic acid-protease 3), BAX (bcl2 associated X protein), cTn-T (cardiac troponin T), MI/R (myocardial ischemia reperfusion).

Introduction

Ischemia and reperfusion (I/R) is a condition in which blood flow and oxygen to the organ is decreased by either partial or complete obstruction of an artery that
carries blood to this organ, while the restorations of blood flow to an ischemic heart refer to myocardial reperfusion (Aragon et al., 2011). Prolonged lack of oxygen resulted in hypoxia which causes a decline in the cellular oxidative phosphorylation, so this will guide to decline in the biosynthesis of the ATP and phosphocreatine (Kaminski et al., 2002). Ischemia declines the activity of the sarcolemmal Na/K-ATPase, increases the activity of the Na–H exchanger, and promotes the activation of the Na–Ca exchanger in a reverse mode (Elmoselhi et al., 2003). Intracellular Ca2-overload influences the mitochondrial K channels opening and mitochondrial permeability transition pores, which lead to the activation of the apoptotic pathways (Minezaki et al., 1994). Ischemia resulted in an activated leukocytes liberate toxic reactive oxygen species (ROS), proteases and elastases, resulting in increased micro vascular permeability, edema, thrombosis and parenchymal cell decrease (Carden and Granger, 2000). Neutrophils synergize with platelets (Lefer, 1999) to amplify injury. TLRs deliver signals via a specific intracellular signaling pathway involving distinctive adaptor proteins and protein kinases, and ultimately initiate transcriptional factors resulting in inflammatory responses (Fang and Hu, 2011).

Method

Materials

Pure selenium powder as (sodium selenite 99%) (Santa crus, USA), normal saline (KSA) ketamine (Hikma, Jordan), Xylazine (RompunTM, 2% vials, Bayer AG, Leverkusen, Germany). Rat tumor necrosis factor-α (TNF-α), (IL-6), (IL-10), caspase3, BAX and cTnT (ELISA) kits were purchased from Biotangusa, USA. Trichloroacetic acid (TCA) Merck-Germany, Ethylene diaminetetraacetic acid disodium (EDTA) BDH, U.K. Thiobarbituricacid (TBA) Fluka company, Switzerland 5,5-Dithiobis (2-nitrobenzoic acid) DTNB Sigma company Ltd. Reduced glutathione Biochemical, USA and Methanol Fluka company, Switzerland. regarding instruments, High Intensity Ultrasonic Liquid Processor (Sonics & materials Inc., USA), Digital Spectrophotometer EMCLAB/ Germany, Bio-Elisa Reader, BioTek Instruments, USA and ventilator (Harvard. USA).

Animal

After the approval that has been established by the Institutional Animal Care and Use Committee (IACUC) and submission the required applications, 28 male albino rats weighting (200-300 g) were purchased from Animal Resource Center. They were housed in the animal house (for one week) in a temperature-controlled (25°±1C) room (humidity was kept at (60–65%) with alternating 12-h light/12-h dark cycles and were allowed to access freely regarding water and chow diet until the time of starting the experimental study.

Study design

After the 1st week of accommodation, the 24 rats were randomly divided into 4 groups (6 rats in each) as follow:

1- (Sham group): Rats underwent the same anesthetic and surgical procedures but without ligation for the LAD.

2- Active control (MI/R) group: rats followed surgical operation for LAD ligation and they were subjected to 30 min of ischemia and 120 min of reperfusion.

3- (MI/R) + Vehicle pretreated group: rats were pretreated with D.W via intraperitoneal injection 30 minutes before ligation of LAD, then underwent surgical LAD ligation, and subjected to 30 min of ischemia followed by 120 min of reperfusion.

4- (MI/R) + selenium pretreated group: rats of this group take a single I.P injection of selenium a concentration of 1 mg/kg dissolved in D.W 30 minutes.
immediately before ligation of LAD, then subjected to surgical LAD ligation with 30 minutes of ischemia followed by 120 min of reperfusion (Fatima and Mahboob, 2013).

Surgical ligation of the LAD

Rats were anesthetized with (IP) injection of 100 mg/kg ketamine and 10 mg/kg Xylazine (Wiedemann et al., 2010). After intubation of the trachea by a 20 G cannula and the endotracheal tube was connected tightly to the ventilation machine. The ventilation rate was fixed from 120-135 breath/minute with tidal volume 20 ml/kg body weight, with 100% oxygen. Pericardial layer incision was made by administration round end scissors to open the space. The LAD coronary artery was transient ligated 1 to 2 mm below the tip of the left auricle using a tapered needle and a 8-0 polypropylene ligature. Tightening the ligature could then occlude the artery for a 30-minute ischemic period [18]. The chest cavity was closed by bringing together the fourth and fifth ribs with one 2-0 silk suture. Cardiac reperfusion was achieved by releasing the tension applying to the ligature for 120 minutes [19]. The rats were euthanized after reperfusion via injection high dose of anesthesia and the chest was re-opened then the right ventricle was punctured with a syringe needle so that about 3 ml of blood was aspirated for later blood analysis. After that, the heart was isolated and divided into 2 pieces, the apical part used for histological examination and the basal was used for measuring the tissue parameters.

Blood sampling for measurement of plasma cTn-T, serum MDA and serum reduced GSH

At the end of experiment, about 2-3 ml of blood sample was placed in a tube containing disodium ethylene diamine tetra acetic acid (EDTA) (22 mg/mL) as anticoagulant and mixed thoroughly and then centrifuged at 3000 rpm for 15 min then the supernatant was used for determination of plasma cTn-T level, whereas the remaining blood was allowed to clot in an ordinary tube at 37 °C then it was centrifuged at 3000 rpm for 15 minutes then the supernatant was taken for MDA and GSH serum levels determination.

Tissue preparation for TNF-α, IL-6, IL-10, caspase 3 and BAX measurements

The upper parts of the ventricles were washed with cold normal saline to remove any blood, stored in deep freeze (-20°C), and then homogenized with high intensity liquid processor in 1:10 (w/v) phosphate buffered saline that contain 1% triton X-100 and protease inhibitor cocktail [17]. The homogenate was centrifuged at 14000 rpm 4°C for 20 min. The supernatant was collected for determination of TNF-α, IL-10, IL-6, Bax, and Caspase- by ELISA with a commercially available ELISA kit (Literature of kit by life Diagnostic, USA) according to the manufacturer’s instructions.

Preparation for Histopathology

The apical parts of the heart were excised immediately, rinsed using ice-cold 0.9% saline and fixed in 10% formalin solution pH 7.4 [18] embedded in paraffin wax. The paraffin-embedded tissues were sectioned (4-µm thick), stained with hematoxylin and eosin (H&E). Damage scores were evaluated according to the following morphological criteria that have been used to evaluate the histopathological damage [19] as follow: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling and necrosis; score 3 (severe), necrosis with presence of contraction bands and neutrophil infiltrate; score 4 (highly severe), widespread necrosis with
presence of contraction bands, neutrophil infiltrate, and hemorrhage.

**Statistical analyses**

Data were expressed as mean ± SEM. An expert statistical advice was considered for data analysis which were aided by computer. Statistical analysis were done using SPSS version 20.0 computer software (Statistical Package for Social Science). ANOVA (analysis of variance) had been used for measurement (numerical data). Mann-Whitney test had been used for myocardial damage score. P value <0.05 regarded as significant.

**Results:**

![Graph 1: Mean of myocardial TNF-α](image1)

*Figure 1: The mean of myocardial TNF-α (pg/mg) in the four experimental groups at the end of the experiment. *P*<0.05 vs. sham; **#** *P*<0.05 vs. Ctrl group.*

![Graph 2: Mean of myocardial level IL-6](image2)

*Figure 2: The mean of myocardial level IL-6 (pg/mg) in the four experimental groups at the end of the experiment. *P*<0.05 vs. sham group; **#** *P*<0.05 vs. Ctrl group.*

![Graph 3: Mean of myocardial IL-10](image3)

*Figure 3: The mean of myocardial IL-10 (pg/mg) in the four experimental groups at the end of the experiment. *P*<0.05 vs. sham; **#** *P*<0.05 vs. Ctrl group.*

![Graph 4: Myocardial mean of BAX](image4)

*Figure 4: The myocardial mean of BAX (pg/mg) in the four experimental groups at the end of the experiment. *P*<0.05 vs. sham group; **#** *P*<0.05 vs. Ctrl group.*
Figure 5. The myocardial mean of Caspase-3 (pg/mg) in the four experimental groups at the end of the experiment. *P<0.05 vs. sham group, # P<0.05 vs. Ctrl group.

Figure 6. The mean of plasma cTn-T level (pg/ml) in the four experimental groups at the end of the experiment. *P<0.05 vs. sham group, # P<0.05 vs. Ctrl group.

Figure 7. The myocardial mean of MDA (μmol/L) in the four experimental groups at the end of the experiment. *P<0.05 vs. sham group, # P<0.05 vs. Ctrl group.

Figure 8. The myocardial mean of GSH (μmol/L) in the four experimental groups at the end of the experiment. *P<0.05 vs. sham group, # P<0.05 vs. Ctrl group.
Table 1.
Comparison according to Mann-Whitney test for scoring regarding histopathological changes.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sham</td>
<td></td>
</tr>
<tr>
<td>2. Control</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>3. Selenium</td>
<td>&lt;0.05#*</td>
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</tbody>
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* vs. sham group, # vs. control group

Figure 9.
Representative photomicrograph of a section of the heart tissue section stained with Haematoxylin and Eosin (X 40). A) The control group showing hemorrhage, interstitial edema, necrosis and neutrophil infiltration. B) The sham group showing normal architecture. C) The vehicle group showing severe hemorrhage and extravasation of RBC, presence of severe interstitial edema, presence of neutrophil infiltration and necrosis. D) The selenium pretreated group showing near normal cardiac tissue with absence of edema, absence of neutrophil infiltration, absence of necrosis, and only congested capillary structure.
Biochemical results

Effect on Pro-inflammatory cytokines (TNF-α and IL-6). Results revealed a significant increase ($P<0.05$) in (TNF-α and IL-6) cardiac tissue levels in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant decrease ($P<0.05$) in the (TNF-α and IL-6) cardiac tissue levels as compared with the MI/R group as shown in table 1 and figures 1 and 2.

Effect on anti-inflammatory cytokine (IL-10). Results revealed a significant increase ($P<0.05$) in (IL-10) cardiac tissue level in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant elevation ($P<0.05$) in the (IL-10) cardiac tissue level as compared with all other groups (sham group, the MI/R group and MI/R + vehicle group as shown in table 1 and figure 3.

Effect on apoptotic markers (caspase-3 and BAX). Results revealed a significant increase ($P<0.05$) in (caspase-3 and BAX) cardiac tissue levels in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant reduction ($P<0.05$) in the (caspase-3 and BAX) cardiac tissue levels as compared with the MI/R group as shown in table 1 and figures 4 and 5.

Effect on Plasma Level of Troponin T (cTnT). Results revealed a significant increase ($P<0.05$) in (cTnT) plasma level in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant reduction ($P<0.05$) in the (cTnT) plasma level as compared with the MI/R group as shown in table 1 and figure 6.

Effect on the serum level of oxidative stress markers (MDA and GSH). Results revealed a significant increase ($P<0.05$) in the serum level of MDA in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant reduction ($P<0.05$) in MDA serum level as compared with the MI/R group. Concerning GSH, results revealed a significant decrease ($P<0.05$) in the serum level of GSH in the MI/R group as compared with the sham group, while in the MI/R + selenium pretreated group, selenium produce a significant increase ($P<0.05$) in GSH serum level as compared with the MI/R group as shown in table 1 and figures 7 and 8.

Histopathological Findings

Histologically, the MI/R group revealed a significant cardiac tissue injury ($P<0.05$) compared with the sham group, and this injury was showing sever hemorrhage, presence of interstitial edema, necrosis and neutrophil infiltration in contrast with the cross section of the sham group which showed a 100% normal structure of cardiac tissue with no interstitial edema, no diffuse myocardial cell swelling and necrosis, no neutrophils infiltration, no hemorrhage, no capillary compression and no evidence of apoptosis. Treatment of rats with selenium significantly decrease ($P<0.05$) the injury of cardiac tissue and cross section from this group (MI/R+selenium) showed near normal cardiac tissue with absence of edema, absence of neutrophil infiltration, absence of necrosis, and only congested capillary structure while there was no significant difference between the MI/R and MI/R + vehicle groups as shown in figures 9 A, B, C, D.

Discussion

The common origin of myocardial infarction is occlusion of the coronary artery as a result of the embolization of an unstable coronary plaque [20]. Activation of Polymorphonuclear cell (PMN’s), eicosanoids, cytokines, ROS and complement products have been shown to be involved in the initial ischemic period
The intracellular and extracellular accumulation of these products triggers homeostatic pathways involving necrosis, apoptosis and inflammation that initially occur during acute myocardial infarction. The apoptotic response may then lead to potential permanent tissue or end organ dysfunction. Restoration of blood flow to ischemic myocardium is the current therapy, yet is associated with ischemia/reperfusion injury[22].

Conclusion
It can be concluded that pretreatment with selenium modulates myocardial ischemia reperfusion injury via interfering with inflammatory, oxidative pathways and apoptosis.

Reference


