Original Article

Effect of betaine supplement on isoprenaline induced myocardial infarction and serum cathepsin G level in rat model

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Abstract

Background & Aims: Myocardial infarction is one of the most common life threatening diseases in worldwide. Betaine is a safe and well tolerated compound that shows beneficial antioxidant and anti-inflammatory properties. Previous studies demonstrated, betaine reduce cardiovascular diseases but molecular mechanism of action did not known completely. Cathepsin G play pivotal role in tissue injury and inflammation. Hence, we hypothesized betaine protective effects mediated by cathepsin G enzyme.

Materials & Methods: To examine this hypothesis, an animal model of 48 Albino rats weighing 200 ± 10 g was used. Light – dark cycle, temperature, humidity of cage were controlled. Rats divided into G1, G2, and G3 Groups and received betaine in dosage 50, 150 and 250 mg/kg via gavage respectively. Deionized water administrated for control group in same conditions. After 60 days treatment, isoproterenol (100 mg/kg) used for induction of myocardial infarction and then anesthesia and sampling performed. Serum level of cardiac troponin I and cathepsin G were measured via ELISA test. Serum homocysteine level measured by auto analyzer. Statistical analyses were done using SPSS 23.

Results: Our results shows, homocysteine level in control, G1, G2, and G3 are 9.98 ± 3.27, 7.29 ± 1.79, 6.69 ± 2.55 and 2.88 ± 1.4 µmol/L respectively that reduced dose dependently. Betaine protect heart against isoproterenol induced myocardial infraction. Cardiac troponin level in control, G1, G2, and G3 are 285.59 ± 49.87, 159.4 ± 66.94, 199.15 ± 78.33 and 209.31 ± 86.66 respectively. Cathepsin G level did not changed significantly between groups.

Conclusion: These results demonstrated betaine have protective effects on isoprenaline-induced myocardial infraction but cathepsin G is not underlay molecular mechanism.

Keywords: Betaine, Isoprenaline, Myocardial infarction, Cathepsin G, Rat

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Introduction

Myocardial infarction is one of the most common type of clinical cardiovascular disease (1). About 10 million people have heart attacks every year worldwide (2). After myocardial, cardiac troponin T increases in serum that correlated with infarct size. Micronutrients such as methyl-donors involved in cardiovascular diseases and further Myocardial infarction occurrence (3). Betaine is a methyl donor and has been considered as an antioxidant and anti-inflammation agent (4). Betaine

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supplement reduces homocysteine level and so prevents myocardial infarction (5). Homocysteine is a metabolite in derived from methionine, and can be further degraded to cysteine via vitamin B6-dependent reactions(6). Alternatively, it can be remethylated into methionine, which requires a methyl group obtained from 5-methyltetrahydrofolate or from betaine(6). On the other hand, antioxidant and anti-inflammatory properties of betaine are important factors in cardio-protective effects (7). Otherwise the molecular mechanism of betaine on cardiovascular system and myocardial infarction is not understood completely.

Cathepsin G is a serine protease of polymorph nuclear leukocytes that hydrolyses several types of proteins (8). Cathepsin G exhibit pro-inflammatory properties (9). Cathepsin G is enzyme with potent elastolytic activity that affects coagulation process and tissue remodeling at injury sites that proposed as molecular mechanism of cathepsin G involvement in cardiovascular diseases (10). Cathepsin G also reduced ERK-1/2 phosphorylation and attenuated apoptosis (11). On other hand apoptosis is key cellular event in cardiovascular diseases specifically myocardial infarction (12). Oxidative stress is another common point between myocardial infarction and Cathepsin G. Starodub et al study shows oxidative stress decrease serum cathepsin G levels in rats (13). Since inflammation, oxidative stress and apoptosis affect myocardial infraction outcome and these variable also are related to Cathepsin G. So we hypothesis that betaine protective effects on cardiovascular system mediated by Cathepsin G protein.

Materials and Methods

Forty-eight adult male wistar rats weighting 200 ± 10 g were randomly divided into four groups (n=12). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%), 12 hours light/dark photo cycle and received food and water ad libitum. Animal protocols were approved by the Animal Care and Use Committees of Urmia University of Medical Sciences. Betaine was prepared in sterile water and was administered by gavage at 0, 50, 150 and 250 mg/kg BW/day for 60 days. Myocardial infarction was induced by subcutaneous injection of 100 mg/kg isoprenaline (or isoproterenol) 2 times with 24-h intervals both in case (betaine pretreated) and control (received H_2O) G groups. A reduction in blood pressure considered as induction of myocardial infarction.

The serum was separated by centrifugation at room temperature and aliquoted and stored at -70°C until needed. Rat Serum cTnI and Cathepsin G levels was determined in serum using a Rat Cardiac CTnI (cTn-I) ELISA kit (CSB-E16443r, CUSABIO, china) and Rat cathepsin G (CTSG) ELISA Kit (MBS942938 MyBioSource USA) respectively. Both assays were performed according to the manufacturers' instructions. Briefly standards and sample were prepared and added to the wells, conjugated antibody addition, HRP enzyme and TMB substrate used for final visualization that detected by ELISA reader.

Statistical analysis:

One-way analysis of variance (ANOVA) was used to compare the activities of Homocysteine, cardiac troponin I and Cathepsin G levels among groups. In each test, the data are expressed as the mean ± S.E.M. and $p < 0.05$ is accepted as statistically significant.

Results

Serum homocysteine level:

Statistical compression of betaine treated group with control shows betaine (150 and 250 mg/kg)reduced the level of significantly ($p<0.05$) but lower dose (50mg/kg) of betaine did not shows significant change in Homocysteine levels (Figure1).

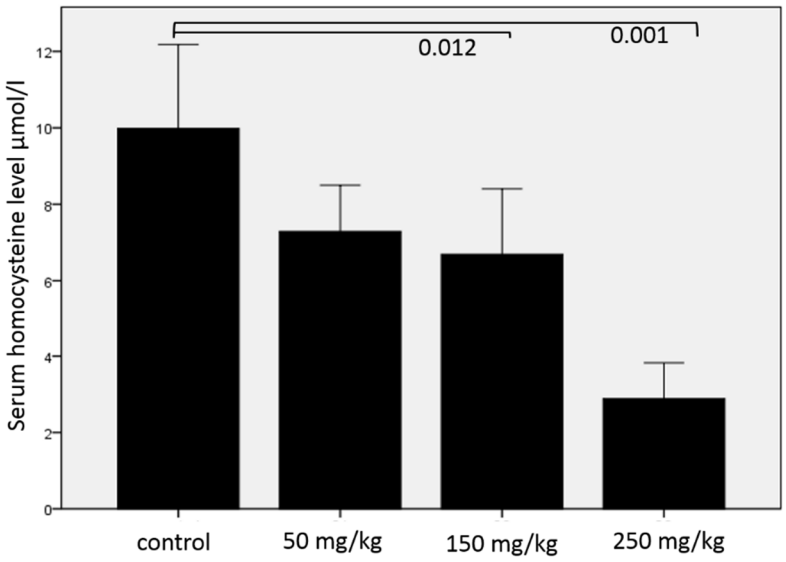

Figure 1: Effects of betaine (control: 0, case, 50, 150, 250 mg/kg) on serum Homocysteine level after isoproterenol-induced myocardial infarction.

Serum cardiac troponin I level:

Figure 2 shows the effects of betaine supplement on the cardiac troponin I level. Betaine (50 and 150 mg/kg) prevent cardiac damage and reduced cardiac troponin I release significantly. Betaine in higher dose (250 mg/kg) did not affect cardiac troponin I level significantly.

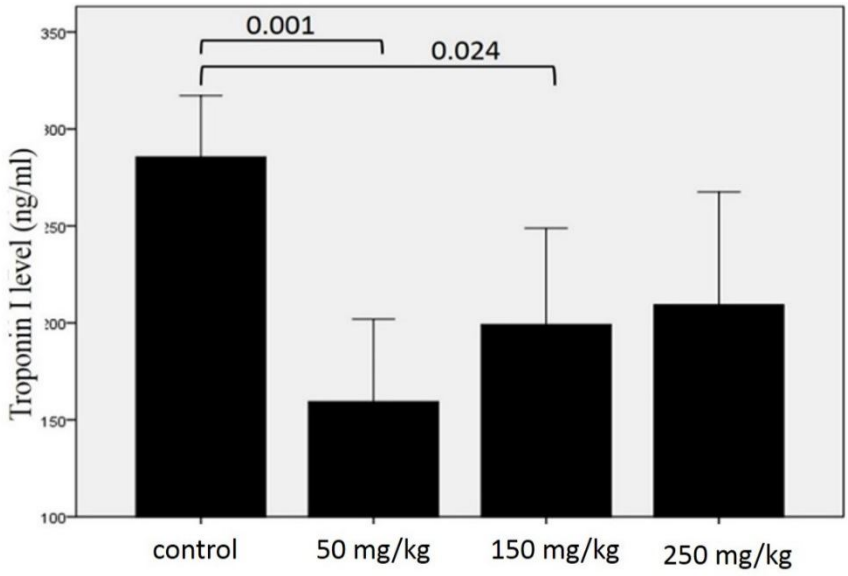

Figure 2: Effects of betaine ((control: 0, case, 50, 150, 250 mg/kg) on serum cardiac troponin I level after isoproterenol-induced myocardial.

Serum cathepsin G level:

In percent study, betaine supplement did not affect serum cathepsin G level after isoproterenol-induced myocardial infarction. Figure 3 illustrates mean of cathepsin G level in rats.

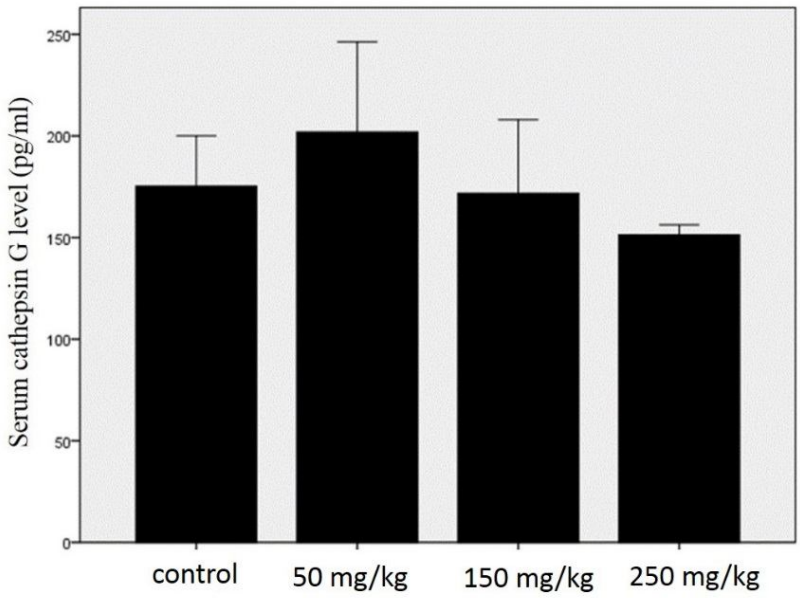

Figure 3: Effects of betaine on (control: 0, case, 50, 150, 250 mg/kg) serum Cathepsin G level after isoproterenol-induced myocardial. No statistical difference observed between groups.

Discussion

Cardio protective properties of betaine deduced from serum cardiac Troponin I level because cardiac Troponin I level is a biomarker for cardiac injury that associated with clinical presentation (14). Similar to our data, Zheng et al shows betaine prevent isoproterenol-induced myocardial damage via Regulation of signal transducer and activator of transcription 3 and apoptotic pathways that confirmed by cardiac Troponin I level and histopathological analysis (15).

Our results showed betaine reduced homocysteine level that consists with previous studies. Steenge et al study shows betaine is effective supplement that preventing a rise in plasma homocysteine concentration (16). Olthof et al demonstrated betaine acutely reduces the increase in homocysteine after methionine loading but author give awareness about betaine adverse effects on lipid profile (17). An outstanding property of betaine is active methyl donation to methyl acceptor. Thus, it is logic to consider betaine as anti-hyperhomocysteinemia agents. There is also several human clinical trial and animal model that conclude betaine potency in reduction of Homocysteine levels (18-20). Reduction in Homocysteine level not necessarily reduce cardiovascular disease risks (21). Homocysteine could considered as indicator for prediction of cardiovascular disease or a pathologic metabolite that cause to oxidative stress and inflammation (22-23). Homocysteine effect on myocardial infarction a final and important cardiovascular events, may mediated by endothelial damage, anabolic steroids alteration, nitric oxide metabolism and myocardial remodeling (24-27). Likewise, betaine protective role against myocardial infarction may mediate via other molecular mechanism such as Cathepsin G. in 1991, Ravichandran et al shows Cathepsin enzymes activity increased after isoproterenol-induced myocardial infarction and reduced in recovery stage (28). Mäntylä et al study shows saliva Cathepsin G level increased after myocardial infarction (29). Arterial thrombotic status an important factor that promote myocardial infarction which could modulated by Cathepsin G (30). Our results

shows betaine could not affect Cathepsin G serum levels. To the best of our knowledge, there are no studies evaluating betaine effects on Cathepsin G level. Only Ganesan et al study shows the activities of lysosomal enzymes were increased in plasma with a concomitant decline in the activities of these enzymes in heart tissue of isoprenaline-administered rats (31). Lysosome is sub cellar organelle that contains Cathepsin G and other lysosomal enzymes. Ganesan et al, concluded betaine protective effects isoproterenol-induced myocardial damage may occur via stabilizing membrane. Our results propose betaine effect is independent from Cathepsin G but more studies need to final conclusions.

Conclusion

These results demonstrated betaine have protective effects on isoprenaline-induced myocardial infarction but Cathepsin G is not underlay molecular mechanism. Reduction in homocysteine level signifies methyl donor properties play crucial role for betaine protective effects that is time dependent. Betaine in 250 mg/kg dose as effective dose and measurement of other inflammatory factors proposed for further studies.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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