B-type natriuretic peptide and adiponectin releases in rat model of myocardial damage induced by isoproterenol administration

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Abstract

B-type natriuretic peptide (BNP) and adiponectin play important role in the cardiovascular homeostasis regulation. We investigated BNP and adiponectin serum levels followed by isoproterenol (ISO) administration to rats and explored the relationship between them. Cardiac troponin I (cTnI) blood level was used as biochemical evidence of myocardial damage development. Adult male Wistar rats (average body weight 273.33± 21.63 g) were distributed into groups: control group received saline (n=6) and ISO groups (n=12) treated with ISO (subcutaneous single dose 100 mg/kg of rat body weight). ISO group was divided into two groups according to the time of BNP, adiponectin and cTnI determination: ISO I (n=6; 2 hours after ISO administration); ISO II (n=6; 4 hours after ISO administration). Blood for determination of parameters was taken from rat abdominal aorta. BNP, adiponectin and cTnI were determined by ELISA method. Data were statistically analysed by using SPSS version13 computer program. P value less 0.05 was considered statistically significant. Blood BNP and adiponectin were lower at 2 hours after ISO administration in comparison with control group (p=0.004 for BNP and p=0.174 for adiponectin). Four hours after ISO administration, we have noted significant elevation of both parameters compared to ISO I group (p=0.004 for BNP; p=0.02 for adiponectin). Test of correlation have showed significant relation between their blood levels during experimental period (rho=0.577; p=0.01). BNP and adiponectin are not simple indicators of myocardial damage development. They have possible associated and additive effects in cardiovascular homeostasis regulation.

KEY WORDS: B-type natriuretic peptide, adiponectin, blood, isoproterenol, rat

INTRODUCTION

It has not been established laboratory markers linking clinical and preclinical testing of heart functional disturbance followed by myocardial damage. Incorporation of new biochemical parameters in animal model contributes to elucidation of mechanisms of myocardial damage induction. B-type natriuretic peptide (BNP) is hormone secreted from cardiomyocytes in response to ventricular wall stretch and has been used as marker of ventricular dysfunction. Cardiac fibroblasts also produce BNP but its circulated amounts are released from cardiac myocytes as main source [1]. Small amounts of BNP are stored in granules and increased secretion depends on BNP gene activation. Increasing of diuresis, vasodilatation, inhibition of renin and aldosterone production and growth of cardiac and vascular myocyte are biological effects of BNP action [2]. BNP is released into circulation after ischemia and necrosis of myocardial cells, systolic and diastolic dysfunction and increased wall stress of the left ventricle in acute myocardial infarction [3]. Adiponectin is protein member of adipokines family. Significance of adiponectin is in its protective function in cardiovascular and metabolic disease development. Lower adiponectin blood level is connected to greater disease severity and poor outcome in acute coronary syndrome and congestive heart failure. Based on these facts, circulating adiponectin concentractions may represent both a protective or it should be harmful instead of harmuful signal [4]. Cardiac isoform of troponin I (cTnI) is specific biochemical marker of cardiomyocytes damage but its blood elevation could not explain mechanism of cardiac damage [5]. Troponins are powerful markers in laboratory animals for detection cardiac injury [6]. Isoproterenol (ISO)

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is synthetic catecholamine widely used in animal models for induction of myocardial damage. High dose of ISO induces rat myocardial necrosis development as consequence of hemodynamic, morphological and functional alterations in the heart. In the present study we intended to ascertain whether BNP and adiponectin blood level could be indicators of myocardial injury and functional alterations associated with isoproterenol administration. Besides that we examined the existence of relationship between their blood levels.

MATERIALS AND METHODS

Animals

Adult, male Wistar rats (Rattus norvegicus) n=18 (273,33 ± 21,63 g body weight) were used in the study. Animal had free acess to water and commercial food during the experimental period. They raised in humidity controlled and air-conditioned cages. The Ethical Committe of Faculty of Medicine, University of Sarajevo approved the study protocol. Rats were distributed into control group treated with saline (0.95% NaCl) and two groups treated with ISO (single subcutaneous dose 100 mg/kg body weight dissolved in saline). Isoproterenol hydrochloride used in the study was manufactured by Sigma Chemical Company, USA. Each group consisted of 6 rats. Dependently on biochemical markers analysis time, we created ISO group I (blood sampling was performed 2 hours after ISO administration) and ISO group II (blood sampling was performed 4 hours after ISO administration). Blood for laboratory analysis was taken from abdominal aorta under ether anesthesia after scheduled time for groups. After that, rats were euthanised by decapitation. After centrifugation, serum samples were stored at -20° C until assayed.

Procedures

BNP, adiponectin and cTnI serum levels were measured by enzyme linked immunosorbent assay (ELISA) using RAT BNP-32 (Phoenix Pharmaceutical Inc.), RAT ADIPONECTIN (Phoenix Pharmaceutical Inc.) and HIGH SENSITIVITY RAT CARDIAC TROPONIN-I (Life Diagnostics Inc. West Chester PA). All measurements were performed according to the manufacturer's instructions. Absorbance was measured at wavelength of 450 nm using ELISA reader (STAT FAX 2100, USA). Values of cTnI and adiponectin were expressed in ng/ml but BNP in pg/ml.

Statistical analysis

For statistical analysis of data, we used The Statistical Package for Social Sciences (SPSS) version 13. Data are presented as mean ± standard error of mean or as median with 25th and 75th percentiles dependent on normality of variables distribution. Difference between groups was tested by non parametric Kruskal-Wallis followed by Mann Whitney U test for BNP. Difference between groups for normally distributed variables was tested by One-way-ANOVA followed by Bonferroni test or Dunnet post hoc test. Correlation between adiponectin and BNP was tested by Spearman test. *P* value less than 0.05 was considered as significant.

RESULTS

The mean values of body weight, adiponectin, cTnI and median BNP values obtained in experiment were given in Table 1. The mean body weight of all rats were 273.33±21.63 g. There was no significant difference in rat body weight among experimental groups (p=0.301). Control group of rats were treated with saline and 4 hours after that we noted circulated values of adiponectin 3.1±0.19 ng/ml, BNP 0.73(0.72-0.85) pg/ml and 0 ng/ml for cTnI. Kruskal Wallis test for difference testing between groups have showed significant level of difference (p=0.004) for BNP. Difference between groups tested by One-way Anova for adiponectin was p=0.003 and p<0.0005 for cTnI. Values of adiponectin 2.27±0.23 ng/ml and BNP 0.29 (0.24-0.60) pg/ml were lower in ISO group I than their values in control group. There was statistical significant difference for BNP p=0.004, but difference between two groups in adiponectin blood level was insignificant (p=0.174). We have noted significant difference comparing ISO I adiponectin and BNP values with their values in ISO II for both markers. Value of adiponectin in ISO II group was higher (3.98±0.38 ng/ml) in relation to ISO I (p=0.02) and control group (p=0.143). BNP in ISO II was 0.81(0.73-1.35) pg/ml. There was significant differences between ISO II and ISO I groups (p=0.004). The relationship between adiponectin and BNP obtained in the experiment is presented in Figure 1.We have noted significant positive correlation between their blood levels (rho=0.577; p=0.01).

TABLE 1. Serum values of adiponectin, BNP and cTnI in rat during experimental period.

Variables	Groups			*p* value
	Control group (n=6)	ISO I group (n=6)	ISO II group (n=6)	
Rat body weight (g)	273±20.4	262.5±20.9	283.3±25.4	0.301
Adiponectin (ng/ml)	3.1±0.19	2.27±0.23	3.98±0.38	†0.02
BNP (pg/ml)	0.73 (0.72-0.85)	0.29 (0.24-0.60)	0.81 (0.73-1.35)	*0.004 †0.004
cTnI (ng/ml)	0	6.36±0.64	7.51±0.18	°0.000 *0.000

BNP-B-type natriuretic peptide; cTnI- cardiac troponin I: Data are presented as mean±standard error of mean or as median with 25th and 75th percentiles; *difference between control and ISO I group; °control versus ISO II difference; †difference between ISO I and ISOII group.

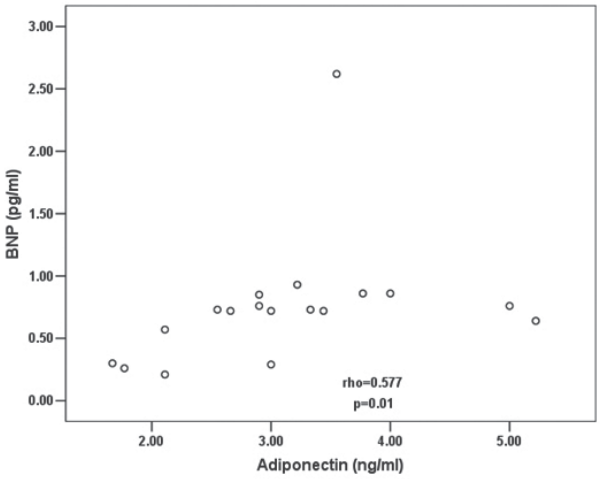

FIGURE 1. Correlation of BNP and adiponectin serum values in rats myocardial damage model induced by ISO. *rho=Spearman'correlation coefficient; p value-level of statistical significance*

DISCUSSION

In the present study, we have used rat model of myocardial damage induced by ISO administration. ISO administration to rats leads to hypoxia development in the least perfused area of subendocardial layer as result of diastole shortening [7]. Infarct-like myocardial lesions induced by ISO administration are results of increased oxygen demands and free radicals production [8]. We have attended to examine BNP and adiponectin blood levels as consequence myocardial damage induced by ISO and explore the relationship between them. According to blood elevation of cardiac specific marker cTnI in both ISO groups we confirmed rat cardiac damage. Values of cTnI in control group were undetectable in spite of saline administration to rats. Our results are in accordance with Kurata and associates study in which they investigated the correlation among clinicopathological parameters of rats myocardial damage treated with ISO. In their study, cTnI was undetectable before and after saline application to rats [9]. Bertischant and associates have noted insignificant elevation of cTnT in rats treated with saline pointed at stress tachycardia induced minimal release of cTnT into circulation [10]. Significant elevation of cTnI during 2-4 hours compared to control rats (p=0.000) pointed on rat cardiac damage induced by ISO. Except of necrosis, cardiomyocytes cTnI releasing could be caused by ischaemia and mechanical stretch of myocardial wall during volume overload [11]. We have noted significantly lower (p=0.004) median BNP value 2 hours after ISO administration compared to control (0.73(0.72-0.85) versus 0.29 (0.24-0.60) pg/ml). In the rat heart, experimental ischaemia leads to an immediate increase of BNP releasing, which correlates with the duration of ischaemia [12]. We consider that lower BNP in ISO I group compared to control is caused by rapidly excretion or metabolism of small amounts BNP released from store in cardiomyocytes. BNP has short half-life in circulation about 1.2 minutes [13]. In rats of ISO II group (4 hours after ISO administration), median value of BNP (0.81(0.73-1.35) pg/ml) was significantly elevated (p=0.004) in comparison with ISO I group (0.29 (0.24-0.60) pg/ml). The study of BNP release in human acute myocardial infarction has described two pattern of BNP release: monophasic and biphasic. In biphasic pattern concentration of BNP in the first measurement is lower than in the second one [14]. Increased BNP in rats with myocardial necrosis could depend on production, conversion as well as decreased degradation. The release of BNP reflects the alterations in left ventricular potency as a response to β-adrenergic stimulation [15]. Serum BNP levels were shown to increase in ISO-induced MI models, but we used short time model and there are no published data for this period [16]. The study of Magga and co-workers has investigated influence of acute cardiac overload (30 minutes to 4 hours) after arginin vasopressin injection. This study showed that pressure overload stimulates BNP gene expression in normal and hypertensive rats. One of possible mechanisms of elevated BNP values 4 hours after ISO administration is rapid stimulation of gene expression in heart [17]. Except that, tachycardia, glucocorticoids, thyroid hormones and vasoactive peptides angiotensin II and endothelin -1 influence on rapid BNP gene expression [18]. Adiponectin is adipokine with antiatherogenic and cardiac protective properties. Obtained data in our study have revealed that in this ISO model adiponectin kinetic is similar to BNP. In this case, we have noted lower values of adiponectin of ISO I group (2.27±0.23 ng/ml) compared to control (3.1±0.19 ng/ml); (p=0.174). Four hours after ISO administration adiponectin values (3.98±0.38 ng/ml) were elevated significantly (p=0.02) in comparison with ISO I group (2.27±0.23 ng/ml). Adiponectin levels rapidly decline in human acute myocardial infarction as result of its sequestering at site of vascular injury [19]. Adiponectin has been detected in the injured vessels but not in the intact ones in humans and rodents [20]. Shibata and coworkers found in their study that adiponectin levels decline transiently in mice following myocardial ischemia-reperfusion injury and abundant levels in injured myocardium were detected [21]. Fu and associates have considered that biphasic serum adiponectin level is sign of transition from diastolic to systolic disfunction [22]. Adiponectin have a cardioprotective effect against ischemia-reperfusion injury and hemodynamic stress in mice [23, 24]. Previous investigations pointed at possible BNP influence on stimulation of adiponectin secretion via cyclic guanosine monophosphate (cGMP)-dependent pathway [4, 25]. Blood elevation of adiponectin is its possible cardioprotective response

on myocardial damage development. The protective action of adiponectin against myocardial ischemia is mediated by its ability to activate cyclooxygenase-2 in cardiac cells that have been shown to play an important cardioprotective role [26]. Investigation of BNP and adiponectin in healthy subject has showed elevation of adiponectin in accordance with BNP reflecting cardiac function independently from other influences [27]. In our study we have noted significant positive correlation between their blood levels during experimental time (rho=0.577; p=0.01). Adiponectin elevation can be caused by BNP influence via type A guanil cyclase receptor (GC-A) which are expressed in blood vessels, kidneys, adrenal gland and heart [28]. In this way, BNP is not marker of haemodynamic disturbance and myocardial lesion development only, but cardioprotective molecule through influence on adiponectin secretion. Except of possible BNP influence on adiponectin secretion, adiponectin blood elevation can be induced by lipolytic effects of ISO. According to Mohan and coworkers, one of mechanisms of ISO action is its lipolytic action [29]. Two hours after ISO injection is critical period for lipolysis, but there is indication, that adiponectin suppresses lipolysis [30]. Except of ISO lipolytic action, it is known that natriuretic peptides has the same lipolytic effect also and can be one of causes of adiponectin blood elevation.

CONCLUSION

As conclusion we consider that BNP and adiponectin elevations in rat circulation following ISO administration are not only simple indicators of myocardial damage in this animal model. They have possible associated and additive effects in cardiovascular homeostasis regulation.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

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