CARDIOLOGY

Advanced glycation end products in isoproterenol-induced acute myocardial infarction

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Abstract

Background. Isoproterenol is a synthetic catecholamine that can produce diffuse myocardial necrosis at high doses. Advanced glycation end products (AGEs) are involved in the development and progression of cardiovascular disease, including acute coronary syndrome. The purpose of the study was to determine the changes of serum and tissue AGE content in isoproterenol-induced acute myocardial infarction and to assess their informational character in the early diagnosis, risk stratification and prognosis of disease.

Methods. Forty adult male rats were divided into 5 groups: sham (L1=11), control 0.9% NaCl (L2=11), and with experimental myocardial infarction (L3=6, L4=6; L5=6), induced by the subcutaneous injection of Isoproterenol Hydrochloride solution 100 mg/kg, and sacrificed over 6 hours, 24 hours and 7 days post infarction. The results were presented by median and interquartile range. The groups were compared using Kruskal-Wallis and Mann-Whitney nonparametric tests, and the Spearman correlation coefficient was calculated (SPSS 23.0).

Results. A decrease of AGE serum levels in L3 were identified, followed by a significant increase in L4, the trend maintained in L5, which significantly exceeded the values in sham and control groups. In the homogenate, AGEs presented an elevation in L3, with a relevant decrease in L4, and an inconsistent increase in L5 compared to sham and control groups.

Conclusion. The collected data suggest the utility of AGE assessment in early diagnosis and risk stratification in acute myocardial infarction.

Keywords: isoproterenol, advanced glycation end products, oxidative stress, hyperglycaemia, acute myocardial infarction

Introduction

Cardiac disorders remain the leading cause of death, most of which are due to acute coronary syndrome. In 2017 the European Cardiovascular Diseases Statistic Report presented a 45% mortality rate in Europe and 37% in the European Union [1].

Catecholamines are beneficial in regulating heart function by exerting positive chronotropic and inotropic effects on the myocardium [2]. Isoproterenol ($C_{11}H_{17}NO_3$) [1–(3,4–dihydroxyphenyl)–2–isopropyl-aminoethanol hydrochloride (ISO)] is a synthetic catecholamine and powerful non-selective β-adrenergic agonist that regulates myocardial contractility and metabolism [3]. This drug is used in the treatment of allergic emergencies, ventricular bradycardia and cardiac arrest [4]. At high doses (within 85–300 mg/kg range) ISO causes severe stress, inducing diffuse myocardial necrosis and fibrosis [2,4].

ISO-induced cardiotoxicity is explained by the generation through the autoxidation of highly cytotoxic free radicals [3] that alter tissue defense systems including chemical scavengers and antioxidant enzymes [4]. Myocardial injury induced by isoproterenol is due to various alterations of energy metabolism, caused by hyperglycemia, acidosis and excessive oxidative stress [2,3].

Glycemia represents a continuous risk factor for heart diseases [5], comparable to dyslipidemia, smoking and high blood pressure [6]. Raposeiras-Roubin et al. have shown that persistent hyperglycemia has a deleterious effect on

DOI: 10.15386/mpr-1348

Manuscript received: 22.04.2019
Accepted: 30.05.2019

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the myocardium, and suggested advanced glycation as one of the main mechanisms of action [7].

Advanced glycation end products (AGEs) represent a heterogeneous group of compounds derived from the interaction of reducing sugars and/or other α-carbonyl compounds with amino groups [8] within proteins, lipids, and nucleic acids [6]. The driving force of AGE formation is Millard reaction. The first reversible step consists of the condensation of reducing sugars with a free amino group [9]. The obtained Schiff´s base (aldimin) undergoes Amadori rearrangement to form ketosamine structure. Then the Amadori product undergoes further rearrangements, oxidations and elimination, and finally forms AGE [8]. This reaction is fast and depends on the concentration of available carbohydrates, being enhanced in hyperglycemia [5,6,10].

The deposition of glycated products is due to both an increased AGE production and an impaired degradation of modified proteins. Glycated intracellular proteins are cleaved slowly by the ubiquitin-proteasome system [9]. Partial proteolysis and/or intracellular capture mediated through AGER-1 receptor [8] are among the involved mechanisms. AGE degradation in macrophages generates soluble low-molecular peptides, named second generation AGEs [8].

Ott et al. suggested that AGE accumulation is possible in long-lived proteins, e.g. collagen of extracellular matrix [9]. Some factors, such as the concentration and reactivity of glucose, the availability of precursors of AGE [9], and oxidative stress [10] can accelerate AGE formation, resulting in structural and/or functional changes affecting short-lived substrates [8].

AGEs significantly contribute to tissue and organ dysfunction [9], triggering oxidative stress, inflammation and apoptosis due to stimulation of specific receptors (RAGE) [8].

The mechanisms underlying the biological effects are accounted for the following hypotheses: *glycation modifies the structure and inhibits the function of proteins [5]; **glycation induces protein cross-linking and leads to tissue stiffness [5,7]; ***soluble AGEs bind to RAGE-receptors and activate the intracellular signaling pathways (p21ras/MAPK, NADPH-oxidase/ ROS/ NF-kB; Jak/Stat), resulting in the expression of inflammatory cytokines (IL6, TNFα) and tissue inflammation [9].

AGEs also alter lipoproteins contributing to the development of atherosclerosis [7,8,10]. Both glycated and oxidized LDL and HDL reduce glutathione peroxidase-1 activity, and induce production of reactive oxygen species (ROS), endoplasmic reticulum stress, and apoptosis [8]. In 2014 Stirban et al. established that glycation and oxidation of LDL amplify their atherogenic effects, whereas oxidized and glycated HDL partially loses its antioxidant properties and protective role [8].

The experimental studies have demonstrated that AGEs produce complex vascular lesions. Circulating AGEs induce the cross-linking of connective tissue proteins in the arterial wall, increase vascular permeability and atherosclerotic lesions of the aorta [8].

Small sample clinical trials have reported the involvement of AGEs in acute coronary syndrome (ACS). The study performed by Kiuchi et al. has shown that AGE values correlate with the extent of coronary lesions in patients with ischemic heart diseases [11]. Raposeiras-Roubin et al. assumed the utility of plasma AGE levels as long-term predictors of mortality, reinfarction and risk of heart failure development in non-diabetic patients with ACS [7].

There are insufficient data in literature regarding the impact of isoproterenol on the synthesis of AGEs in vivo. Concomitantly there is a lack of conclusive evidence about the correlation between serum and tissue levels of AGE in experimental cardiac ischemia.

The aim of the research was to assess serum and homogenate modifications of advanced glycation end products in isoproterenol-induced acute myocardial infarction in order to evaluate the AGE potential as an early diagnosis and prognosis marker of disease.

Material and methods

Study design

The study included 40 white adult male rats (Rattus albicans) weighing 180-230 g. The animals were kept in the vivarium of Nicolae Testemitanu State University of Medicine and Pharmacy under standard conditions: in polypropylene cages, housed at 24 ± 2°C, alternating light/ dark cycle every 12 hours, daily-performed examination and cleaning of animal cages, and free access to food and water. The animals were fasted and no water 12 hours prior to the sacrifice. The experimental part of the research was carried out in the Laboratory of Biochemistry of Nicolae Testemitanu State University of Medicine and Pharmacy. The animals were maintained and used in accordance with the Animal Welfare Act. The study protocol was approved by the Research Ethics Committee of Nicolae Testemitanu State University of Medicine and Pharmacy (23.03.2015).

Myocardial infarction was induced by subcutaneous injection of a single dose of 100 mg/kg Isoproterenol Hydrochloride (Sigma Aldrich Chemie GmbH) dissolved in NaCl 0.9% solution. The rats were randomly divided into five groups: sham (L1=11) - no intervention; control (L2=11) - animals that were administered NaCl 0.9% solution; L3 (n=6), L4 (n=6) and L5 (n=6) included the animals with experimental myocardial infarction. The rats were anesthetized, and sacrificed under sterile conditions over 6 hours (L3 AMI6h), 24 hours (L4 AMI24h) and 7days (L5 AMI7days) post infarction.

Sample collection

The cardiac tissue (0.3 g) was homogenized in ice with 3 mL of sucrose buffer 0.25 M (pH=7.4). The obtained homogenate was treated with 30 µL of Triton X-100, being

placed in the refrigerator for 30 minutes, and centrifuged at 4°C at 3000 rpm for 10 minutes. The supernatant was stored at -40°C until analysis.

The collected blood samples were placed for 30 minutes into test-tubes allowing clotting, and then centrifuged for 10 minutes at 1500 rpm. Serum was stored in Eppendorfs at -40°C until analysis.

Biochemical analysis

Glucose and AGE levels were determined in serum and tissue samples. For glucose measuring, the ELITech assay kit (France) and manufacturer's instructions were used, the results being expressed in mM/L (serum) and mM/g*protein (tissue). AGEs levels were assessed according to Sero Luc method (12). The AGEs content was expressed in µg/mL (serum) and µg/g*prot (tissue). The markers were measured in five groups, including sham, control and experimental animals.

Statistical analysis

The obtained data were processed using SPSS 23.0 software. Descriptive statistical methods were applied for median and interquartile range (IQR) calculation. Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to analyse data distribution. The homogeneity of variance was assessed by Levene's test. The groups were compared using Kruskal-Wallis and Mann-Whitney nonparametric tests. The correlation coefficient was calculated by the Spearman correlation test. The $p<0.05$ value was considered statistically significant.

Results

The groups showed a statistically significant difference in serum glucose (p=0.0002***) and AGE levels (p=0.018*). A decrease of glucose and AGE serum levels in L3 was identified, followed by a significant increase in L4, the trend maintained in L5 by AGEs, while glucose values declined slightly compared to sham and control groups (Table I).

Table I. Serum levels of glucose and AGE.

Group	Glucose (mM/L)	AGE (µg/mL)
Sham	6.25 (IQR 0.45)	314.20 (IQR 58.43)
Control	6.53 (IQR 0.94)	328.20 (IQR 153.22)
AMI6h	4.56 (IQR 0.55)	262.20 (IQR 160.85)
AMI24h	7.00 (IQR 1.56)	354.30 (IQR 81.43)
AMI7d	6.02 (IQR 0.75)	392.50 (IQR 110.97)

There were no differences, either in glucose (p=0.113), or in AGE content (p=0.267) in the homogenate. Both glucose and AGEs presented an elevation in L3, with relevant decrease in L4, and inconsistent increase in L5 compared to sham and control groups (Table II).

Table II. Glucose and AGE values in cardiac homogenate.

Group	Glucose (mM/g*prot)	AGE (µg/g*prot)
Sham	4.94 (IQR 1.09)	25.14 (IQR 15.10)
Control	4.43 (IQR 1.02)	23.63 (IQR 15.65)
AMI6h	4.97 (IQR 0.84)	31.62 (IQR 6.66)
AMI24h	4.37 (IQR 0.56)	22.27 (IQR 23.01)
AMI7d	4.72 (IQR 0.35)	24.96 (IQR 8.91)

We identified a significant positive correlation between glucose and AGE values in the tissue (ρ=0.008**). No correlations were found between serum glucose and AGE levels (ρ=0.283).

Discussion

Stress hyperglycemia induced by cathecolamines has a deleterious effect on the myocardium and enhances AGEs formation. AGEs are involved in many cardiovascular and non-cardiovascular diseases through both direct (cross-linking) and indirect (via specific receptors) mechanisms [7,8,13]. Kitano et al. show that hyperglycaemic state and oxidative stress can stimulate AGE production that in turn increases the generation of reactive oxygen species, resulting in antioxidant systems impairment [14].

The AGE molecules are identified in plasma, cells and tissues. They accumulate in the arterial wall [10] and basal membranes, reducing collagen and/or connective tissue elasticity [9]. AGE-linked collagen accumulates in the arterial matrix in a disorganized and dysfunctional manner (6) and is resistant to enzymatic proteolysis [9]. The research performed by De Souza R. established that the deposition of AGE in collagen has detrimental cardiovascular effects [15].

In our study AGE values in the ***homogenate*** have initially increased by 26% in AMI6h compared to sham animals, and then dynamically decreased in AMI24h, declining by 37% the values of AMI6h group, and inconclusively elevated by 10% in AMI7d. Our results are similar to those reported by Nozynski et al., who have identified that in ischemic cardiomyopathy AGEs accumulate diffusely in the cytoplasm [16]. Experimental data suggested the interrelation between AGEs, oxidative stress and endoplasmic reticulum stress. The study conducted in 2013 by Gul et al. showed a significant correlation between AGE and malone dialdehyde values in patients with acute myocardial infarction, and demonstrated that AGE elevation was associated with oxidative stress [5].

Won et al. established the utility of serum levels of AGE as independent predictors, which reflects severity of coronary artery disease in diabetic patients [6]. In 2018, Qiu et al. noted that in STEMI patients the highest serum levels of AGE were found on admission, then decreasing on the second day and increasing again on the fifth day [10]. The results published by Rasool et al. indicated a significantly

higher AGE level in patients with ischemic heart diseases compared to control individuals [13].

Our results were similar to those reported in the literature, presenting a decrease in serum AGEs by 16% in AMI6h group, then an increase in AGE concentration by 29% in AMI24h group, the tendency maintained in AMI7d group, exceeding the values of AMI24h group by 12%. These variations can be a consequence of excessive AGE accumulation in the tissue in early ischemia. Both oxidative stress amplification and cell membrane lesion exacerbation lead to a fast elimination of AGEs from the tissue and elevation of serum AGE within 24 hours. The described changes initially reveal the development of ischemic injury, which subsequently worsens and turns into necrotic lesions.

Both the lack of correlation between glucose and AGEs levels in serum and the positive correlation between the above mentioned parameters in the homogenate suggest the need for a careful interpretation of increased AGE levels in blood considering the clinical context.

The assessment of AGE in ISO-induced cardiac ischemia indicates the imbalance of glucose metabolic state, which contributes to the development of oxidative stress [5]. Recent experimental studies have shown that elevated AGE values are associated with the instability [6] and progression [13] of coronary atherosclerotic plaque, and can be used as markers of acute ischemic heart lesions, and/or as a prognostic factor [10]. It is conclusive that the regularly found changes clearly express a degree of sensitivity and specificity facilitating the identification of acute myocardial infarction, being also a good predictor of disease prognosis.

Conclusions

The study findings confirm serum and tissue variations of AGE in isoproterenol-induced myocardial infarction, highlighting biochemical mechanisms underlying the detrimental cardiovascular effects. The presence and expression of advanced glycation end products are directly dependent on the degree of oxidative stress, reflecting the severity of myocardial lesions and the risk for developing major complications. The assessment of serum and tissue values of AGE is useful for the diagnosis and risk stratification in acute myocardial infarction.

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