

EXPERIMENTAL STUDY

Effect of apelin-13 on erythrocyte deformability during ischaemia–reperfusion injury of heart in diabetic rats

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ABSTRACT

OBJECTIVES: Erythrocyte deformability and plasma viscosity are of crucial importance for the perfusion of tissues and organs. The aim of this study was to evaluate the effect of apelin-13 on erythrocyte deformability during IR heart injury in diabetic rats.

METHODS: Eighteen Wistar Albino rats were included in the study after streptozotocin (55 mg/kg) treatment for four weeks of observation for diabetes existence. The animals were randomly assigned to one of five experimental groups. In the Group C, DC (sham-control group) and DCA (sham-control group–apelin-13), the coronary artery was not occluded or re-perfused. In the Group DIR, a branch of the left coronary artery was occluded for 30 minutes followed by 90 minutes of re-perfusion to produce IR. In the Group DIRA, a branch of the left coronary artery was occluded for 30 minutes followed by 90 minutes of re-perfusion to produce IR, and apelin-13 was administered via 10 µg.kg⁻¹ IP route 30 minutes before ligating the left coronary artery.

Deformability measurements were performed in erythrocyte suspensions containing Htc 5% in a PBS buffer.

RESULTS: The deformability index was significantly increased in diabetic rats; however, it was similar in Group DC, DCA and DIRA. It was significantly increased in the Group DIR when compared to the Group C, DIRA, DCA and DC. The relative resistance was increased in IR models.

CONCLUSION: Erythrocyte deformability was decreased in rats having diabetes and IR injury. This injury might lead to further problems in microcirculation. It was shown that apeline-13 may be useful in enhancing the adverse effects of this type of injury (Fig. 1, Ref. 35). Text in PDF www.elis.sk.

KEY WORDS: Erythrocyte deformability, myocardial ischaemia reperfusion, experimental diabetes, apelin-13, rat.

Introduction

In last two or three decades, the prevalence of diabetes mellitus (DM) has rapidly increased throughout the world, and experts estimate that it will increase by 200 % in the next several decades (1). Erythrocyte deformability (ED) facilitates blood flow through the circulation in vessels of variable diameter and enables effective exchange of gas and metabolic products in capillaries (2). ED is a function of; 1) red cell geometry, 2) viscosity of intracellular fluid, 3) erythrocyte membrane (3). Several clinical studies showed that ED decreases in diabetes (4–6). Diabetes affects erythrocyte metabolism and function through different ways. Metabolic changes in erythrocytes lead to oxidative stress, which is shown to affect erythrocyte shape in in vitro studies (7).

A 50 % increase in early mortality following coronary artery bypass grafting has been described in diabetic patients (8). One study demonstrated that the rate of unrecognised myocardial infarction was 39 % in diabetic patients and 22 % in non-diabetic patients (9). Cardiac surgery with cardiopulmonary bypass inevitably causes a systemic inflammatory response and ischaemia–reperfusion (IR) injury affecting multiple organs (1).

Haemorheological parameters that include (but are not limited to) haematocrit, plasma proteins, erythrocyte aggregation and erythrocyte deformability in DM are often disturbed (10). Several drugs have been used to prevent IR injury, including Vitamin C, levosimendan, dexmedetomidine (1, 11, 12).

Apelin is a recently discovered peptide encoded by the APLN gene in humans and is the endogenous ligand of the human G-protein coupled apelin receptor (APJ) (13). The APLN gene encodes a 77-amino acid prepropeptide divided by shorter mature peptides such as apelin-36, apelin-17 and apelin-13 (14). The most studied types of apelin are apelin-13 and apelin-36 (15). Increasing evidence suggests that apelin regulates multiple physiological functions, including fluid homeostasis, food intake, cell proliferation, blood pressure regulation, angiogenesis, and glucose utilization (15–17) Therefore, may be associated with diabetes, obesity, hypertension (HT) and / or cardiovascular diseases (17, 18).

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Despite its increased clinical use, the effects of the apelin-13 on myocardial injury induced by left coronary artery (LAD) IR have not yet been investigated.

The primary aim of this study was to investigate deformability changes and the preventive role of apelin-13 against these changes in erythrocytes of diabetic rats during an experimental model of myocardial IR injury.

Materials and methods

Animals and experimental protocol

This study was conducted in the GUDAM Laboratory of Gazi University with the consent of the Experimental Animals Ethics Committee of Gazi University. All the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals.

In the study, 30 female Wistar Albino rats weighing between 180 and 220 g, raised under the same environmental conditions, were used. The rats were kept at 20–21 °C in cycles of 12 hours of daylight and 12 hours of darkness and had free access to food until two hours before the anaesthetic procedure. The animals were randomly separated into five groups, each containing six rats.

Diabetes was induced by a single IP injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA), at a dose of 55 mg.kg⁻¹ body weight. The blood glucose levels were measured 72 hours following this injection. Rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 mg.dl⁻¹, and only animals with FBGs of > 250 mg.dl⁻¹ were included in the diabetic groups (diabetes only, diabetes-apelin-13, diabetes plus ischaemia–reperfusion and diabetes plus apelin-13–ischaemia–reperfusion). The rats were kept alive for four weeks after streptozotocin injection to allow the development of chronic diabetes before they were exposed to ischaemia–reperfusion (19). The rats were weighed before the study.

Rats were anesthetized with an IP injection of 100 mg.kg⁻¹ of ketamine. The trachea was cannulated for artificial respiration. The chest was shaved and each animal was fixed in a supine position on the operating table. The chest was opened by a left thoracotomy followed by sectioning the fourth and fifth ribs about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with a room air, using a volume of 1.5 ml/100 g body weight at a rate of 60 strokes/min. Sodium heparin (500 IU/kg) was administered through the peripheral vein in the tail.

After the pericardium was incised, the heart was exteriorized with gentle pressure on the right side of the rib cage. An 8/0 silk suture attached to a 10-mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest and the animals were allowed to recover for 20 minutes.

There were five experimental groups. Group C (control; n = 6), Group DC (diabetes–control; n = 6), Group DCA (diabetes–control–apelin-13; n = 6), Group DIR (diabetes–ischaemia–reperfusion; n = 6) and Group DIRA (diabetes–ischaemia–reperfusion–apelin-13; n = 6) underwent left thoracotomy and received IP apelin-13 (Apelin-13 trifluoroacetate salt, Sigma Aldrich) ad-

ministrated via 10 µg.kg⁻¹ IP route 30 minutes before ligating the LAD (13). A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery could then be occluded by applying tension to the ligature (30 minutes), and reperfusion was achieved by releasing the tension (90 minutes). However, after the above procedure, the coronary artery was not occluded or re-perfused in the control, diabetic control and diabetic control–apelin-13 rats.

All the rats were given ketamine 100 mg.kg⁻¹ IP and intracardiac blood samples were obtained. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were performed using erythrocyte suspensions with 5 % haematocrit in a phosphate buffered saline (PBS) buffer.

Deformability measurements

Blood samples were carefully taken and the measurement process was as fast as possible to avoid haemolysis of erythrocytes. The collected blood was centrifuged at 1000 rpm for ten minutes. Serum and buffy coat on erythrocytes were removed. An isotonic PBS buffer was added to the collapsing erythrocytes and this was centrifuged at 1000 rpm for ten minutes. Liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process, which was repeated three times. Erythrocyte packs were mixed with the PBS buffer to generate a suspension with a value of 5 % Htc. These erythrocyte suspensions were used for the measurement of deformability. Collection and deformability measurements of erythrocytes were performed at 22 °C.

The constant-current filtrometer system was used in the measurement of erythrocyte deformability. Samples to be measured were prepared with 10 ml of erythrocyte suspension and PBS buffer. The flow rate was held constant at 1.5 ml/min with an infusion pump. A 28 mm nucleopore polycarbonate filter with a 5 µm pore diameter was preferred. Pressure changes while the erythrocytes passed through the filter were detected by the pressure transducer, and the data were transferred to the computer with the help of an MP30 data equation system (Biopac Systems Inc, Commat, USA). The necessary calculations were performed with related computer programs by measuring the pressure changes at various times. Pressure calibration of the system was performed before each sample measurement. The buffer (P_f) and the erythrocytes (P_e) were passed through the filtration system and the changes in pressure were measured. The relative refractory period value (Rrel) was calculated by relating the pressure value of the erythrocyte suspension to the pressure value of buffer. Increasing Rrel in the deformability index was interpreted to adversely affect the erythrocytes' deformability (22, 23).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 12.0 program was used for the statistical analysis. Variations in blood glucose levels, erythrocyte deformability and rat weights between study groups were assessed using the Kruskal–Wallis test. The Bonferroni-adjusted Mann–Whitney U test was used after significant Kruskal–Wallis to determine, which groups differed from the others. Results were expressed as the

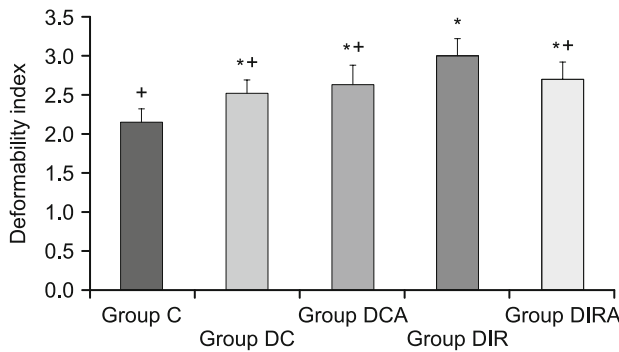


Fig. 1. Erythrocyte deformability values of the groups. Each bar represents the mean \pm SD. * $p < 0.05$ compared to Group C, + $p < 0.05$ compared to Group DIR.

mean \pm standard deviation (Mean \pm SD). Statistical significance was set at a p value of < 0.05 for all analysis and $p < 0.033$ (0.1/3) for Bonferroni-adjusted Mann–Whitney U.

Results

Blood glucose measurements were 86.7 ± 9.5 , 494.5 ± 145.3 , 537.5 ± 161.7 , 493.3 ± 168.5 and 506.2 ± 151.2 mg/dL for Group C, DC, DCA, DIR and DIRA, respectively. Serum glucose was detected to be significantly lower in the Group C when compared to the Groups DC, DCA, DIR and DIRA ($p < 0.0001$).

The deformability index was significantly increased in the diabetic rats ($p < 0.0001$); however, it was similar in Group DC, DCA, and DIRA (DC-DCA, $p = 0.326$; DC-DIRA, $p = 0.122$; DCA-DIRA, $p = 0.555$). It was significantly increased in the Group DIR when compared to the Group C, DC, DCA, and DIRA ($p < 0.0001$, $p = 0.001$, $p = 0.007$, $p = 0.026$, respectively) (Fig. 1). Relative resistance was increased in IR models.

Discussion

Diabetes mellitus (DM) is a metabolic disorder characterised by abnormally high blood sugar (hyperglycaemia) resulting from either low insulin levels or insulin resistance in most of the body cells. Diabetes mellitus has a high social and economic importance, as the number of diabetes patients continues to grow at an unprecedented rate throughout the world (10).

Haemorheological parameters, such as: haematocrit, plasma proteins, erythrocyte aggregation and erythrocyte deformability are often disturbed in DM (20). For migration of oxygen and vital molecules to the final organ capillaries and clearance of metabolic wastes, erythrocytes must be able to extend and curve and have the capability to move in these areas. This capacity, called “deformability”, becomes more important in microcirculation. Altered erythrocyte deformability not only changes the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (21–23).

Additionally, it has been suggested that the impaired perfusion at the tissue level observed as a complication of DM is primarily

due to reduced erythrocyte deformability (24, 25). In addition, metabolic changes and tissue perfusion due to cardiovascular problems may lead to inadequate recovery in plasma viscosity (26).

Cho et al (10) demonstrated that blood viscosity is significantly increased in diabetes. These results suggest that the consequent elevation of glucose in blood plasma primarily affects RBCs and the vascular endothelial cells, including the walls of capillaries. The impaired glucose tolerance or uncontrolled blood glucose levels often result in microvascular complications in diabetes. Moreover, the impairment of erythrocyte deformability is attributed to the specific changes in the membrane structure. The oxidative stress due to high glucose concentrations causes damage to the erythrocyte membrane proteins, even with a relatively short exposure time (27).

Barnes et al (28) showed that erythrocyte deformability was lower in the 14 diabetes patients with the most extensive microangiopathy than in the controls or the 22 diabetes patients with slight or no complications. They suggested that hyperviscosity and reduced erythrocyte deformability may be important and potentially treatable factors in the aetiology or progression of microcirculatory disease in diabetes. We also found that erythrocyte deformability was decreased in rats with induced diabetes.

In this study, for the first time to our knowledge, we have reported that IR of the diabetic rat heart resulted in significant negative changes that can be observed in erythrocyte deformability and that apelin-13, apelin signalling mediated important events in cardiovascular homeostasis. It has also, shown to have a positive effect on myocardial contractility by promoting a potent positive inotropic effect, which, administered at the beginning of heart ischaemia can provide varying degrees of protection against negative effects of variations in erythrocyte deformability.

Recent studies suggested that apelin signalling mediated important events in cardiovascular homeostasis and has been shown to have a positive effect on myocardial contractility by promoting a potent positive inotropic effect (29, 30). Apelin is a vasodilator both in *in vivo* (31) and *ex vivo* models employing human arteries, veins (32). Accordingly, intravenous apelin administration in rodents reduces the mean arterial pressure (33), systemic venous tone (34), and cardiac preload and afterload (29).

Apelin signalling may have an important role in the pathophysiology of diseases such as: hypertension, heart failure, cardiovascular disease, type 2 diabetes, and obesity, although their effects and functions are still unclear. The physiological effects of apelin on diabetes are not fully known.

Kursunluoglu-Akcilar and his friends in the experiment they done, after *i.p.* injection of apelin showed that the apelin reduced plasma insulin and blood glucose levels in diabetic and hypertensive diabetic rats and lowered blood pressure. In cases such as: hypertension, type 2 diabetes and hypertension + type 2 diabetes, apelin is a candidate agent for treatment (34).

Kursunluoglu et al demonstrated that apelin administration induced increased RBC aggregation in hypertensive rats. Results showed positive effects of apelin on RBC deformability in control animals, but not in hypertensive rats (15).

We also found that apelin-13 had positive effects on erythrocyte deformability in ischemia–reperfused diabetic rats.

In conclusion, the results of this study clearly demonstrated that erythrocyte deformability is significantly altered in experimental myocardial IR injury in the diabetic rat. This might lead to further problems in microcirculation. Thus, measurement of erythrocyte deformability might have an important impact on the follow-up for IR injury. Additionally, apelin-13 administered before induction of ischaemia was observed to have protective effects on these alterations in myocardial IR injury. Other aspects of these findings, including clinical significance and practical applications, merit further experimental and clinical investigation.

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