ORIGINAL ARTICLE

Apoptosis kinetics at reperfusion period in patients with acute ST-Segment Elevation Myocardial Infarction undergoing primary percutaneous coronary intervention and treated with thrombolytic therapy

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Abstract

Objective: To evaluate the kinetics of cardiomyocyte apoptosis in patients undergoing primary percutaneous coronary intervention and thrombolytic therapy in order to elucidate the dark side of reperfusion injury.

Methods: The prospective descriptive study was conducted at Istanbul University Cardiology Institute, Istanbul, Turkey, between June 2010 and December 2012. It comprised patients with persistent ST-segment elevation myocardial infarction who were divided into two groups. Patients in group 1 were treated with percutaneous coronary intervention, while those in group 2 received thrombolytic therapy. Cell death detection enzyme-linked immunosorbent assay kit was used for the analysis of cardiomyocyte apoptosis. Venous blood samples were collected to determine the apoptotic activity from the patients at the beginning of thrombolysis in myocardial infarction grade 3 of reperfusion in infarct-related artery according to thrombolysis in myocardial infarction classification, and after reperfusion provided at 6, 12, 24 and 72 hours. Creatine kinase, peak creatine kinase myocardial band and troponin levels were determined on admission and during 24hours of ST-segment elevation myocardial infarction . SPSS 15 was used for statistical analysis.

Results: There were 92 patients in the study; 48(51.6%) in group 1 and 44(48.4%) in group 2.There was no significant correlation between peak apoptotic activity levels at 72 hours of reperfusion and peak creatine kinase myocardial band (r=0.05;p=0.66) or the troponin (r=0.10;p=0.38) levels at 24 hours of ST-segment elevation myocardial infarction. Apoptotic activity levels increased at 72 hours compared to the baseline both for group 1 (p<0.001) and group 2(p<0.001).

Conclusions: Reperfusion injury was not primarily related to apoptosis and it was a slowly progressive benign event in patients with ST-segment elevation myocardial infarction-acute coronary syndrome. Also, the negative impact of percutaneous coronary intervention was not available on reperfusion injury.

Keywords: Apoptosis kinetics, ST-segment elevation, Acute coronary syndrome, Primary percutaneous coronary intervention, Thrombolytic therapy. (JPMA 66: 808; 2016)

Introduction

After acute ST-Segment Elevation Myocardial Infarction (STEMI), early and successful myocardial reperfusion is the most effective strategy for reducing the size of myocardial infarct (MI). Primary reperfusion therapies, including primary Percutaneous Coronary Intervention (PCI) and thrombolysis are the standard of care for the treatment of acute coronary syndrome (ACS). However, the return of blood flow to the heart can cause additional cardiac damage and complications, and reperfusion injury, which manifests as myocardial, vascular, or electrophysiological dysfunction. The main clinical symptoms of reperfusion injury include arrhythmias, endothelial cell damage leading to microvascular dysfunction, myocardial stunning, myocyte death and the no-reflow event.[1,2]

Apoptosis is defined as the programmed cell death which requires energy, and ensures the removal of damaged cells without inducing inflammation. Apoptosis can be induced through either cytochrome c, Fasligand (FASL) or tumour necrosis factor (TNF) receptor 1-2, and endoplasmic reticulum activation. Apoptosis promoting proteins are: c-myc, p53, and the bcl-2 family (bcl-2 and bcl-x). Enzymatic cleavage of key cytoplasmic and nuclear proteins, deoxyribonucleic acid (DNA) fragmentation, chromatin condensation, and cytoplasmic reorganisation represent the final stage of apoptosis.[3-11] DNA fragment residues of apoptotic cells not undergoing phagocytosis by neutrophils are intact and determined in blood during apoptosis process.[6,8]

Growing evidences from both animal experiments and clinical observations indicate that apoptosis, plays a key role in myocardial reperfusion injury.[7-11] The role of

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mitochondrial ion channels such as Bcl-2 in apoptosis is well-documented and, it is also likely that additional ion channels formed by the connexin 43 (Cx43) protein may play a similar role in cardiac myocyte apoptosis.[12-25]

Serially measuring creatine kinase myocardial band (CK-MB) is an established criterion for myocardial cell injury when electrocardiogram (ECG) is inconclusive with levels reaching twice the unaffected concentrations within five to six hours after the onset of chest pain and peaking in 12 to 24 hours. Troponin I (TnI) kinetics following acute MI are similar to CK-MB in that it takes four to eight hours to increase above the upper reference limit, peaks between 14 and 36 hours and may remain elevated three to seven days after the event. Troponin t (TnT) mimics the early release kinetics of TnI, but may remain elevated for as long as three weeks.[1,2]

The current study was planned to evaluate the kinetics and the association of some markers of myocardial necrosis and apoptosis in patients during reperfusion injury who underwent either primary PCI or received thrombolysis therapy with STEMI-ACS. Possible negative effect of PCI on reperfusion injury were also investigated.

Patients and Methods

The prospective descriptive study was conducted at Istanbul University Cardiology Institute, Istanbul, Turkey, between June 2010 and December 2012. It comprised patients with persistent ST-segment elevation myocardial infarction who were divided into two groups. Patients in group 1 were treated with percutaneous coronary intervention, while those in group 2 received fibrinolytic therapy (tPA) according to European Society of Cardiology (ESC) guideline.[1]

Patients excluded from the study were those aged <18 years, advanced valvular disease (aortic and/or mitral valvular insufficiency), or presence of heart failure with concomitant renal failure (serum creatinine>2.0 mg/dl or Glomerular Filtration Rate [GFR]<30 ml/min/1.73m^2), chronic hepatic failure, except for hypertension (HT) and diabetes mellitus (DM). PCI patients were excluded who had longer time than 6 hours past the start of chest pain. Also, the thrombolytic therapy patients were excluded who had longer time than 12 hours past the start of chest pain.

At the time of the study, there was no round-the-clock availability of invasive facilities for all patients with STEMI-ACS. There was no invasive team available on some nights.

The estimated number of study subjects in both groups was calculated by the formula: $n= Z1^2 1\text{-}\alpha/2S^2 \div d^2$ ($n=86=1.96^2 \times (1-0.55/2) \times 0.50^2 \div 0.05^2$) (N=Population Size; n= Sample Size; S= Standard Error; 0.05(5%) (Confidence Level 95%); Confidence Interval: upper =95%; Lower: 5%; The Z-values for confidence levels were: 1.645=90 percent confidence level; 1.96 = 95 percent confidence level; 2.576 = 99 percent confidence level (Z for p= 0.05, 0.01, 0.001 are 1.96, 2.58 and 3.28 Z values respectively); d= Relative Standard Error).

The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all the participants.

Patients with ischaemic-type chest pain lasting longer than 20 minutes, at least two consecutive precordial electrocardiographic >2mm or limb in lead >1mm ST-segment elevation, were diagnosed with persistent STEMI-ACS. Precordial ST-segment elevations in patients with anterior STEMI and the others with non-anterior STEMI were evaluated. The diagnosis of acute myocardial infarction (AMI) was confirmed by biochemical markers.[1,2] Demographic characteristics of the patients were noted. Chest pain onset time, pain duration, and the time of admission to the hospital were also recorded. Information about HT, DM, hyperlipidaemia, smoking, family history, and previous myocardial infarction, previous procedures, such as PCI and coronary artery bypass grafting (CABG), and laboratory finding of the patients were also recorded.

Following routine anti-ischaemic therapy guidelines, acetylsalicylic acid (ASA), clopidogrel 75mg, enoxaparin, angiotensin-converting-enzyme inhibitors (ACEIs), statins, beta-blockers and nitrates were administered in thrombolytic therapy study group.

Patients were administered ASA (162 to 325 mg orally), a thienopyridine loading dose (clopidogrel 300 to 600mg), enoxaparin and glycoprotein IIb/IIIa inhibitors (tirofiban) during PCI.

The tPA protocol was administered to patients receiving thrombolytic therapy with 15mg intravenous (IV) bolus followed by 0.75mg/kg (maximum 50mg) IV 30 minutes and then in 60 minutes. The rest of the 0.50 mg/kg (maximum 35mg) was performed according to the clinical status of the patients.

The starting and ending times of reperfusion treatment were recorded. After 90 minutes of reperfusion, the ECG-ST-elevation (reduction 50%) and pain reduction was used as criteria of reperfusion (zero hour) in the thrombolytic therapy status. In addition, coronary angiography (CAG) was performed after therapy initiation to determine infarct-related coronary artery (IRA) patency within the first 24 hours. Also, criteria for reperfusion in all patients after thrombolysis, number of patients with

thrombolysis in myocardial infarction (TIMI) grade 3 coronary reperfusion and coronary artery lesions were recorded.

In patients undergoing PCI, TIMI classification was used to define the appearance of IRA injury. As zero hour of reperfusion of the patients was considered TIMI grade 3 of IRA patency, which was achieved as soon as balloon catheters were introduced during the PCI process.[1]

Cell Death Detection kit (ELISA plus; Roche Diagnostics; Cat no.11774425001) was used for the determination of apoptosis.[6] Venous blood samples were obtained at the beginning of reperfusion (hour zero) and after reperfusion therapy at 6, 12, 24, 48, and 72 hours. Furthermore, creatine kinase (CK), CK-MB and troponin levels were measured on admission, and after 24 hours of STEMI. All reagents required for the study were prepared according to the instructions of the producer.

Blood samples were centrifuged (+4°C, 1500 rpm, 5 minutes) and the upper phase (plasma) was collected. Following the producers' instructions, 20µl of plasma samples were applied to the wells of the ELISA plate followed by adding 80µl of immune-reagent solution. The plates were kept for 2 hours on a shaker at 15-25°C. The plates were washed three times with 300µl incubation buffer. When this washing process was ended, the washing liquid in the wells' serum sample was determined by dividing the value obtained from each well sample by the negative control value. Each well was previously prepared according to the kit package insert. It was placed and kept in the shaker for about 20 minutes until the expected colour for the photometric analysis emerged. To stop the reaction, 100µl of 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) stop solution was added to each well. Each plate was read two times at a wavelength of 405nm. The blank value (incubation buffer + stop solution), was subtracted from all other values obtained.

All data was collected on admission and along 72nd hours of reperfusion in the patients with STEMI-ACS. All variables were analysed using SPSS 15. Descriptive statistics were computed and presented as means and standard deviation (SD) which were calculated for continuous variables like age and left ventricle ejection fraction (LVEF). Frequencies and percentages were computed for gender, DM, HT, drugs used. For normality analyses of data, inter-group independent Student t-test, and for the comparison of the means of two dependent groups, paired samples t-test was used. Pearson's linear correlation analysis was used for correlation between peak apoptotic activity levels at 72nd hours of reperfusion and peak CK-MB ortroponin levels, glycosylated haemoglobin (HbA1c). Level of significance was accepted at $p \leq 0.05$.

Results

Of the 590 patients with ACS who were referred to the emergency department, 92(15.5%) patients with STEMI-ACS comprised the study population. Of the selected patients, 48(51.6%) were treated with PCI and 44(48.4%) received tPA. Demographic clinical characteristics (Table-1) and baseline laboratory parameters (Table-2) of all patients were noted.

On transthoracic echocardiography (TTE) in PCI group patients, mean LVEF was 44±6.6% and in thrombolytic therapy it was 42±7.5% within the first 24 hours, with no

Table-1: Patient characteristics.

Characteristics	PCI n =48(%51.6)	Thrombolytic n=44 (%48.4) Therapies	P value
Age (years)	58.4±11.8	54.4±11.8	0.117
Gender (F/M)(n,%)	11/37 (23/77)	13/31 (29.5/70.5)	0.469
Hypertension (n, %)	25 (52.1)	12(27.3)	0.015
Diabetes mellitus (n, %)	14 (29.2)	9 (20.5)	0.335
Hyperlipidaemia (n, %)	10 (20.8)	5 (11.4)	0.219
Family history (%	12 (25.0)	18 (36.4)	0.237
Smokers (n, %)	20 (41.7)	26 (59.1)	0.095
Previous MI (n, %)	7 (14.6)	4 (9.3)	0.440
Pre-infarction Angina (%)	25 (52.1)	19 (43.2)	0.393
MI Localisation (An/N-An) (%)	26/22 (54.2/45.8)	26/18 (59.1/40.9)	0.634
Systolic BP (mmHg)	124±31	132±23	0.174
Diastolic BP (mmHg)	76±17	81±12	0.129
Heart rate (bpm)	78±16	77±15	0.772
Pain duration (minutes)	209±117	179±97	0.208

MI: Myocardial Infarction. An: Anterior. N-An: Non-anterior. PCI: Primary Percutaneous Coronary Intervention. STEMI: ST-Segment-Elevation Myocardial Infarction.
ACS: Acute Coronary Syndrome. BP: Blood Pressure. F: Female. M: Male.

Table-2: Laboratory findings of the patients at admission in both study groups of STEMI-ACS (mean ± SD; Student's t test; p<0.05).

Laboratory tests	PCI n =48 (%51,6)	Thrombolytic n=44 therapies (%48,4)	P value
Fasting blood glucose(mg/dl)	131±64	133±64	0.897
Total Cholesterol (mg/dl)	184±40	197±49	0.154
LDL (mg/dl)	120±37	128±39	0.335
HDL (mg/dl)	41±14	39±10	0.301
Triglyceride (mg/dl)	155±140	201±160	0.176
Uric acid (mg/dl)	5.3±1.6	5.1±1.4	0.704
BUN (mg/dl)	16±4	17±5	0.208
Creatinine (mg/dl)	0.95±0.17	0.96±0.2	0.807
HbA1C (%)	6.4±2.1	6.3±1.7	0.849
Na (mmol/L)	137±3.2	138±3.4	0.063
K (mmol/L)	4±0.6	4.1±0.5	0.424
ALT (U/L)	36±24	38±38	0.707
AST (U/L)	86±99	100±192	0.645
CRP (mg/dl)	13.5±19	28±32	0.096
WBC (C/mL)	12.2±4.2	12.5±3.1	0.746
Haemoglobin	13.3±2	13.9±1.5	0.141
Haematocrit (%)	39.8±5.6	41.9±4.4	0.057
Thrombocytes	230±52	253±66	0.085
GFR (ml/minute/m^2)	79.7±19	78.5±17	0.753
Peak CK-MB (U/L)*			
*(during first 24th hour of STEM?)	318±218	272±203	0.332
Peak troponin T*	13.25± 9.38	14.56±9.56	0.56

*(during first 24th hour of STEM?)

PCI:Primary percutaneous coronary intervention. LDL:Low-density lipoprotein. HDL:High-density lipoprotein. BUN:Blood urea nitrogen. HbA1C:Glycosylated haemoglobin. ALT:Alanine transaminase. AST:Aspartate amino transferase. CRP:C-reactive protein. WBC:White blood cell. GFR:Glomerular filtration rate. CK-MB:Creatine kinase-MB isoenzyme STEMI:ST-segment elevation myocardial infarction. ACS:Acute coronary syndrome.

meaningful difference between the two groups (p>0.05).

Only C-reactive protein (CRP) levels (r=0.439; p=0.008) were statistically significant and correlated with number of lesions in CAG. A significant negative correlation was determined for GFR (r=-0.246; p=0.027).

There was no correlation between apoptosis activity and patient characteristics at any time point investigated. A positive correlation was only found for the apoptotic activity at 72nd hours of reperfusion and the levels of HbA1c (r=0,327; p=0,012).

There was no statistically significant correlation between apoptotic activity levels, maximum degree of ST elevations and echocardiographic findings (p>0.05). There was also no significant correlation between peak apoptotic activity levels at 72nd hour of reperfusion and peak CK-MB (r=-0.05; p=0.66) values. Also, there was no significant correlation with troponin (r=0.10; p=0.38) levels at 24 hour of STEMI in both groups (Table-3).

There was no significant difference in apoptosis levels between the two groups at any time point (0, 6, 12, 24, 48, 72 hours) of reperfusion (p>0.05). But the apoptotic activity values increased gradually after PCI or thrombolytic reperfusion therapy along 72 hours compared to hour zero (primary PCI group 0.83±0.24 vs 3.57±2.43 cells/ml; p<0.001 and thrombolytic group 0.88±0.24 vs 3.96±3.1 cells/ml; p<0.001) (Table-4).

For comparison of the peak CK-MB levels and duration of ischaemic pain, the maximum ST elevation levels and left ventricular diastolic dysfunction (LVDD) had positive correlations (r=0.303; p<0.01; r=0.520; p<0.01; r=0.269; p<0.026 respectively). In contrast, negative correlation was observed for LVEF (r=-0.615; p<0.01) in all STEMI-ACS patients. Peak CK-MB level was significantly higher in patients with anterior MI compared to non-anterior MI (p<0.03).

For comparison of the peak troponin levels and maximum ST elevation levels, the duration of ischaemic pain had positive correlations (r=0.610; p<0.01; r=0.39; p<0.01 respectively). In contrast, negative correlation was observed for LVEF (r=-0.402; p<0.01) in all STEMI-ACS patients.

Apoptosis levels of patients undergoing reperfusion were

Table-3: Pearson correlations of peak CK-MB, troponin levels at 24th hour of STEMI between peak apoptosis levels at 72ndhour of reperfusion, maximum ST elevation> 2 mm ,duration of ischemic pain, LVDD,LVEF in all patients with STEMI-ACS**(Correlation is significant at the 0.01 level (2-tailed).

Pearsoncorrelation	R	p
Peak CKMB(during first 24th hour of STEMI)-Pain duration(minutes)	0,239	0.042
Peak CKMB(during first 24th hour of STEMI)- Maximum ST elevation levels	0,251	0.043
Peak CKMB (during first 24th hour of STEMI)- LVDD	0,384	< 0.01
Peak CKMB (24th hour)-LVEF	-0,615	< 0,01
Peak CKMB (during first 24th hour of STEMI)- peak apoptosis(72ndhour)	-0.05	0.66
Peak troponin(during first 24th hour)- Maximum ST elevation levels	r=0.610	< 0,01
Peak troponin (during first 24th hour of STEMI)-LVEF	-0.402	<0,01
Peak troponin (during first 24th hour of STEMI)-LVDD	0.14	0.22
Peak troponin (during first 24th hour of STEMI)-Pain duration	0.39	< 0.01
Peak troponin (during first 24th hour of STEMI)-peak apoptosis (72ndhour of reperfusion)	0.10	0.38

Correlations of peak CK-MB levels between duration of ischemic pain, maximum ST elevation and LVDD were determined a positive correlation in all groups (r=0.303;p<0,01;r=0.520;p<0.01; r=0.269;p<0.026 respectively), but they were negatively correlated with LVEF (r=-0.615;p<0.01).For comparison of peak troponin level between duration of ischemic pain, maximum ST elevation and LVDD,maximum ST elevation, duration of ischemic pain were found a positive correlation(r=0.610; p<0.01;r=0.39; p<0.01). In contrast a negative correlation was observed for LVEF (r=-0.402;p<0.01) in all STEMI-ACS patients.

CK-MB:Creatine kinase-MB isoenzyme. STEMI:ST-segment elevation myocardial infarction. LVDD:Left ventricle diastolic diameter. LVEF:Left ventricular ejection fraction. ACS:Acute coronary syndrome.

Table-4: Apoptosis kinetics according to the reperfusion methods and time course of reperfusion in both study groups with STEMI-ACS (mean ± SD; Student's t test; p<0.05).

Time course of reperfusion(hour)	PCI	Thrombolysis	P value
0	0.835±0.241 bp cells/ml	0.886±0.241 bp cells/ml	0.211
6	1±0.33	0.917±0.30	0.092
12	1.415±1.04	1.45±0.77	0.715
24	1.862±1.16	1.70±0.91	0.417
48	2.598±1.58	2.644±1.86	0.895
72	3.579±2.43	3.961±3.13	0.680

There was no significant difference comparing apoptosis levels between the two patient groups at any time point (0, 6, 12, 24, 48, 72nd hours) of reperfusion .But, the apoptotic activity values of two patient groups increased up gradually after PCI or thrombolytic reperfusion therapy along 72 hours compared to hour zero (primary PCI group 0.83±0.24 vs 3.57±2.43 cells/ml, p<0.001 and thrombolytic group 0.88±0.24 vs 3.96±3.1 cells/ml, p<0.001).

STEMI: ST-segment elevation myocardial infarction. ACS: Acute coronary syndrome. PCI: Primary percutaneous coronary intervention.

not significantly different comparing PCI versus thrombolytic therapy (p>0.05). There was also no significant difference of apoptosis levels between two groups at any investigated time point (p>0.05).

Discussion

The mechanism and prevention of myocardial ischaemia/reperfusion injury are complex and still have a dark side.[12-25] The hexameric Cx43 protein complex connexon, both as a gap junction and as a hemichannel, forms large-conductance ion channel with chemical gating similar to the Bcl-2 channels. Cx43 channels are voltage gated, perhaps, allowing sensing of the mitochondrial membrane potential in addition to the chemical environment. The role of mitochondrial ion channels is well-documented such as Bcl-2 in modulating apoptosis; it is highly probable that the ion channels formed by the Cx43 protein may also play a role in modulating apoptosis, ischaemic preconditioning and cell memory by contributing to the protection of the perinecrotic cells against reperfusion injury. However, no evidence exists to indicate that the mitochondrial Cx43 forms an ion channel.[12-19] Another such mechanism could be epigenetic modifications, such as methylation of so-called C-phosphate-G (CpG) sites in the genomic DNA sequence.[24,25] Our study is the first modestly sized, prospective, comparative, descriptive study of human beings investigated between thrombolysis and PCI groups to treat STEMI-ACS to compare necrosis versus cardiac apoptosis in myocardial ischaemia/ reperfusion injury.

Our study design showed that biochemical markers of necrotic cell death such as the peak CK-MB and troponin levels are increased in the early hours of STEMI as shown in previous studies.[1,2]

There were no significant correlation between peak apoptotic activity levels at 72 hour and peak CK-MB (r=-

0.05; p=0.66) values and troponin (r=0.10; p=0.38) levels at 24 hour in our study groups (Table-3). However, intact DNA fragments detected evidence of apoptotic cell death, and it was found that their residues were gradually increasing from the beginning of reperfusion up to 72 hours in our groups (primary PCI group 0.83±0.24 vs 3.57±2.43 bp cells/ml, p<0.001 and thrombolytic group 0.88±0.24 vs 3.96±3.1 bp cells/ml, p<0.001) (Table-4).

There were also no correlation with levels of maximal ST elevation, MI localisation, LVEF versus peak apoptotic activity levels at 72 hours of reperfusion in each group, respectively.

Apoptotic activity levels were not different between the groups according to reperfusion methods and negative impact of PCI was not available (Table-4).

Determining the cell death type by inducement of reperfusion and relationship between hours of cell death with start of reperfusion are important. Researchers investigated the impacts of ischaemia (I) and reperfusion (R) in two studies performed on dogs.[10,11] Their results indicate that permanent ischaemia without reperfusion did not induce apoptotic cell death, while two types of cell death, necrosis and apoptosis, were found after I/R, the Bcl-2 family may participate in early R-induced myocardial apoptosis.[10]

Our study suggests that apoptosis and necrosis/necroptosis (a specialised pathway of programmed necrosis) in reperfusion injury in early phase of STEMI are independent phenomena in patients with STEMI-ACS during the first 24 hours.

Also, as shown by previous studies, ischaemic reperfusion injury causes rapid dephosphorylation of hexameric structure on cardiomyocyte membrane gap junctions of cardiomyocytes and leads the dominant form of Cx43 degradation within first few hours. In this instance, decrease in pH causes Ca2+ influx into cardiomyocytes, and increasing reperfusion arrhythmias in the first hours as dependent of the action potential (AP) transmission.[12-19]

On the other hand, within hours and days, Cx43 was shown to be a significant variation in their spatial location and phenotype.[12-19] It is probably the impaired mitochondrial Cx43 that induces mitochondria/cytochrome c-mediated apoptosis pathways. The mitochondrial Cx43 over-expression of dominant-negative mutants (eg L160-mutant) leads to the formation of impaired molecular chaperones by means of microribonucleic acid (RNA).[12-19,24,25] These chaperones with the translocated Cx43 impair the nascent polypeptide chain structure of Cx43 with post-translational modifications such as glycosylation, phosphorylation, sulfation, acetylation, ubiquitination, ribosylation, C-terminal (glycosyl phosphatidyl inositol [GPI] membrane anchors) methylation.[24,25]

Also, epigenetics modifications of mitochondrial Cx43 can participate in the development of the property of AP transmission from cell to cell, and affect cardiac memory. So their bystander effect on long-term viable myocardial adjacent cells in perinecrotic region could cause probably a kind of successive numerous cell's suicide so that Cx43 would contribute numerous jeopardised cardiomyocytes to undergo apoptosis and/or necroptosis during reperfusion.[12-19,24,25]

For these reasons, all anti-apoptotic treatment modalities such as a Ca-sensitiserinotropic agent levosimendan, caspase and/or Poly (ADP-ribose) polymerase (PARP) inhibition, inhibition of Bax using antisense technology, adenine nucleotide transporter (ANT) antibodies, gap junction and microtubule inhibitors in the treatment of myocardial ischaemia/ reperfusion injury can individually exert beneficial therapeutic effects. Some studies have demonstrated anti-apoptotic, and bad remodelling corrective effects of PARP inhibitors and gap junction inhibitors.[6,21,22,24,25]

Also, in our study, positive correlation between the values of HbA1c and apoptosis were found. There was no relationship between fasting blood sugar levels and apoptosis that were measured at admission. Glycosylated end products rather than the accumulation of blood sugar elevation to acute more intensive stimulation of apoptosis observed in the chronic and uncontrolled hyperglycaemia was thought to be responsible.[20]

In terms of study limitations, modestly-sized samples were used. Our reperfusion tracking time was limited to 72 hours. Also, an indirect method was applied in the determination of apoptotic activity.

Conclusions

Reperfusion injury was not primarily related to apoptosis and it was a slowly progressive benign event. Moreover, apoptotic activity levels were not significantly different between the two groups. Also, the negative impact of PCI was not available on reperfusion injury. For these reasons, a great deal of research will be necessary to elucidate the dark side of reperfusion injury, the molecular mechanisms involved especially Cx43, and in order to take measures via microRNA silencing, epigenomics, transcriptomics and proteomics technologies.

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Conflict of Interest: None

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