Study of the possible cardioprotective effects of insulin, ATP, and L-arginine against isoproterenol-induced myocardial infarction

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Background/Aim

Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide. The present study was carried out to investigate the possible protective effects of insulin, ATP, and L-arginine on cardiac dysfunction in experimental isoproterenol (ISO)-induced myocardial infarction (MI), aiming at achieving useful means for protection and therapy against MI.

Materials and methods

Wistar rats of both sexes were allocated into five groups: the control group, the untreated MI group, and MI groups treated with insulin, ATP, or L-arginine. All rats were subjected to ECG recording, and plasma levels of troponin I and triglycerides were determined. The isolated perfused hearts, according to Langendorff's preparation, were studied; the left ventricular weight (LV) was determined, and the LV per body weight ratio (LV/BW) was calculated.

Results

The percentage mortality and total arrhythmia were significantly reduced upon treatment with ATP and L-arginine. The ST segment elevation was significantly reduced in insulin-treated rats. The QRS duration and QT_o intervals were significantly decreased in ATP-treated and L-arginine-treated rats, and the QT_c interval was significantly shortened in all three treated groups. The levels of plasma triglycerides significantly reduced on treatment with insulin and ATP. In the three treated groups, the peak developed tension baseline value and maximal response were significantly increased when compared with the untreated group. In addition, the half-relaxation time baseline value was significantly decreased in the treated groups when compared with the control group. The myocardial flow rate baseline value and maximal response were significantly increased on L-arginine treatment. The LV weights and LV/BW ratios were significantly increased in all three treated groups.

Conclusion

Insulin, ATP, and L-arginine were variably effective in partially modifying the ISO-induced MI insults and offered partial protection against ISO-induced myocardial damage.

Keywords:
ATP, insulin, L-arginine, myocardial infarction

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Introduction

Clinical studies have shown that acute myocardial infarction (AMI) is a leading cause of death worldwide. In a previous study, we demonstrated that rats subjected to isoproterenol (ISO)-induced myocardial infarction (MI) developed a high incidence of mortality and arrhythmia, together with bradycardia, systolic dysfunction, relative ischemia, and hyperlipidemia [1].

Insulin has been reported to induce a dose-dependent increase in the myocardial flow rate (MFR) [2] and to significantly suppress ISO-elicited cardiac injury, which may contribute to insulin-induced cardioprotection in myocardial ischemia reperfusion (I/R) [3]. Ma *et al.* [4] proposed that insulin, together with its metabolic modulation, antiapoptotic effect, and vasculoprotection, might be an effective drug against AMI.

Further, it has been stated that during ischemia, cellular stores of ATP are depleted, together with an increase in membrane permeability for Ca^{2+} and Na^{+}. Mitochondria exhibit altered respiration, and decreased respiratory control ratios that affect mitochondrial membrane integrity [5,6]. Yogeeta *et al.* [7] reported that increased mitochondrial Ca^{2+} concentrations will disturb the proton gradient across the mitochondrial membrane, thereby depleting ATP production in ISO-treated rats. Thus, treatment with this high-energy substrate (ATP) could prove to be a suitable candidate for cardioprotection against MI.

Moreover, arginine was proposed to improve cardiovascular function and reduce myocardial ischemia [8]. More recently, Piñeiro *et al.* [9] reported that L-arginine supplementation significantly reduced myocardial injury in normotensive, hypertensive, and hypercholesterolemic

rats, stressing its cardioprotective effect in physiological and pathological conditions. L-Arginine administration could thus be helpful in alleviating symptoms of AMI.

The present study was carried out to investigate the possible protective effects of insulin, the high-energy substrate ATP, and the nitric oxide (NO) precursor L-arginine on cardiac dysfunction in rat hearts with ISO-induced MI, aiming at achieving an efficient therapeutic means against MI.

Materials and methods

Animals

The present study was performed on Wistar rats of both sexes (initially weighing 150–270 g) that were purchased from the Research Institute of Ophthalmology (Giza). Rats were maintained at the Physiology Department Animal House under standard conditions of boarding. They were administered a regular diet consisting of bread, vegetables, and milk, with free access to water.

Experimental protocol

Rats included in this study were allocated into the following groups.

Group I

Control rats ($n = 18$) who received subcutaneous injection of distilled water (the solvent of ISO) for 2 successive days (at a 24-h interval).

Group II

Untreated MI rats ($n = 31$) who were subjected to induction of MI by subcutaneous injection of ISO (Egy-Drug, Cairo, Egypt) (85 mg/kg BW/day) for 2 successive days (at a 24-h interval) [10].

Group III

Insulin-treated MI rats ($n = 21$) who received subcutaneous injections of insulin (Sanofi-Aventis U.S. LLC) (0.5 IU/kg BW/day) [11] for 2 days and were subjected to induction of MI with an injection of ISO administered 1/2 h after the insulin injection on both days (at a 24-h interval).

Group IV

ATP-treated MI rats ($n = 18$) who received ATP (Park, UK) by intramuscular injections for 4 days (1.875 mg/kg BW/day) [12] and were subjected to induction of MI on the third and fourth days (at a 24-h interval) by injection of ISO 1/2 h after an ATP injection.

Group V

L-Arginine-treated MI rats ($n = 18$) who received L-arginine (Sigma Chemical Co., St. Louis, Missouri, USA) by gavage for 5 days (250 mg/kg BW/day) [13] and were subjected to induction of MI on the fourth and fifth day (at 24-h interval) with injection of ISO 1/2 h after L-arginine administration.

At the end of the experimental period, the rats were sacrificed and studied 24 h after the second ISO injection.

Experimental procedures

On the day of the experiment, overnight fasted rats were weighed and injected with 5000 IU/kg of heparin sodium (Nile Pharmaceutical Co., Cairo, Egypt). Half-an-hour later, the rats were anaesthetized with intraperitoneal injections of pentobarbital (40 mg/kg). The ECG was recorded using cardimax FX-121 (Fukuda Denshi Co. Ltd, Japan). A midline abdominal incision was made, and the abdominal aorta was exposed and cannulated. Blood samples were collected and centrifuged at 4000 rpm for 15 min. The separated plasma was stored frozen at -20°C for subsequent determination of troponin I and triglyceride (TG) levels. Finally, the heart was exposed, dissected, and immediately placed in ice-cold Krebs–Henseleit bicarbonate buffer solution for isolated heart studies and determination of cardiac weight.

Study of isolated hearts

Isolated hearts were perfused according to the technique of Langendorff [14], as modified by Ayobe and Tarazi [15]. The hearts were left to stabilize for 15 min until stable baseline recordings were obtained. The MFR was assessed by collecting the fluid for 3 min.

Infusion of isoproterenol

ISO was infused through a catheter tube (PE-50; Clay Adams, New Jersey, USA), connected to an opening just above the aortic cannula using a Sage-355 infusion pump (Orion Research Mfg., Cambridge, Massachusetts, USA) at rates of 0.15, 0.2, and 0.3 ml/min (physiological range). ISO was infused for 3 min, the cardiac activity was recorded, and the myocardial flow was collected and measured. From these recordings, the values of the heart rate (HR), peak developed tension (PT), half-relaxation time (½ RT), and MFR were determined. Baseline preinfusion values, responses to each dose of ISO, maximal responses (the highest response to three doses of ISO), and delta changes (calculated as the difference between maximal response and baseline preinfusion value) were measured.

Determination of cardiac weights

After perfusion, the hearts were washed with normal saline and kept frozen at -20°C until weighed. At the time of weighing, the hearts were left to defrost and fat and fibrous tissues were removed. Both atria were separated, and the right ventricular wall was peeled evenly. The left ventricle was blotted dry with filter paper and weighed on a five-digit precision Mettler balance (AE163). The left ventricular (LV) weight (in mg) was recorded, and the LV weight per body weight ratio (LV/BW) (mg/g) was calculated.

Biochemical techniques

Plasma troponin I levels were estimated by the immunoenzymometric assay [16], using kits supplied by Monobind Inc. (Lake Forest, California, USA). Plasma

TG levels were estimated according to the enzymatic colorimetric technique [17] using kits supplied by Stanbio Laboratory (San Antonio, Texas, USA).

Statistical analysis

All statistical data, statistical significances, correlations, and lines of regression were analyzed using statistical package for social science (SPSS Inc., Chicago, Illinois, USA) version 16. Statistical significance for differences between the two groups was determined using Student's *t*-test for parametric unpaired data and the Mann–Whitney test for nonparametric unpaired data. Pearson's χ^2 was used for comparison between different groups as regards the incidence of mortality and arrhythmias. A P value less than 0.05 was used to indicate statistical significance.

Results

Mortality

As shown in Table 1, the percent mortality was significantly decreased in ATP-treated and L-arginine-treated MI groups compared with the untreated MI group but was insignificantly decreased in the insulin-treated MI group.

ECG changes

The elevation of the ST segment was significantly decreased only in insulin-treated rats. The HR and QRS duration showed significant reduction on treatment with ATP and L-arginine. The QT_c interval was significantly decreased in the three treated groups, and the QT_o interval was significantly decreased in the insulin-treated and ATP-treated groups (Table 2).

Plasma troponin I and triglyceride level

Plasma troponin I levels were significantly increased in the ATP-treated and L-arginine-treated MI groups when compared with the control group but were insignificantly increased on comparison to the MI group; in addition they were insignificantly decreased in the insulin-treated MI group when compared with both the control and the untreated MI group (Table 3). Plasma TG levels were significantly decreased in the insulin-treated and ATP-treated MI groups when compared with both the control and the untreated MI group, and the levels in the L-arginine-treated MI group were insignificantly different from those in the other two treated groups.

Arrhythmia in isolated hearts

Table 4 shows that the percentage of total arrhythmia was significantly decreased in hearts of ATP-treated and L-arginine-treated MI rats but was insignificantly changed in hearts of insulin-treated MI rats when compared with those of the untreated MI group.

Studies on isolated perfused hearts

As shown in Table 5, the HR baseline value, maximal response, and delta changes were all insignificantly changed in insulin-treated, ATP-treated, and L-arginine-treated MI groups when compared with the untreated MI group.

In the insulin-treated MI rats, the PT baseline value and maximal response were significantly increased when compared with untreated MI rats. The ½ RT baseline value and maximal responses were significantly decreased in the insulin-treated rats when compared with control rats; however they were insignificantly different when compared with values in the untreated MI rats.

In ATP-treated MI rats, the PT baseline value and maximal responses were significantly increased when compared with those of the untreated MI group. The baseline ½ RT value was significantly decreased in ATP-treated rats compared with the control group, but was insignificantly changed when compared with value in the untreated MI group.

In the L-arginine-treated MI group, the PT baseline value maximal responses and delta changes were significantly increased when compared with the untreated MI group. The baseline ½ RT value was significantly decreased on comparison with that of the control group but was insignificantly changed when compared with those of the untreated MI group. The MFR baseline values and maximal responses were significantly increased in the L-arginine-treated rats when compared with the untreated MI group.

Body weight, left ventricular weight, and LV/BW ratio

As shown in Table 6, the BW, LV, and LV/BW ratios were all significantly increased in the insulin-treated, ATP-treated, and L-arginine-treated MI groups when compared with both the untreated MI and the control group.

Discussion

Our results show that each of the treatments offered only partial improvement of the deranged cardiac function

Table 1 Incidence of mortality in the control group, untreated myocardial infarction group, and groups of MI treated with insulin, ATP, and L-arginine

	Control	Untreated MI	Insulin-treated MI	ATP-treated MI	L-Arginine-treated MI
Total number of rats	18	31	21	18	18
Number of dead rats	0	7	2	0	0
Percent ratio of mortality	0	22.58[a]	9.52	0[b]	0[b]

MI, myocardial infarction.
[a]Significance of differences from control group calculated by χ^2-test at $P<0.05$.
[b]Significance of differences from MI group calculated by χ^2-test at $P<0.05$.

Table 2 Electrocardiographic parameters of the control group, untreated myocardial infarction group, and MI groups treated with insulin, ATP, and L-arginine

	Control (18)	Untreated MI (24)	Insulin-treated MI (19)	ATP-treated MI (18)	L-Arginine-treated MI (18)
ST segment (mm)	0.53 ± 0.14	1.23 ± 0.13[a]	0.86 ± 0.08[a,b]	0.9 ± 0.1[a]	1.00 ± 0.08[a]
HR (bpm)	419.2 ± 17.2	464.2 ± 11.8[a]	455 ± 12	419.6 ± 9.79[b]	406.9 ± 9.97[b]
QRS duration (ms)	52.2 ± 5.63	35 ± 4.21[a]	30.5 ± 2.35[a]	21.1 ± 1.11[a,b]	21.1 ± 1.11[a,b]
QT_o (ms)	96.7 ± 4.35	96.7 ± 3.74	80 ± 0.00[a,b]	81.1 ± 1.96[a,b]	91.1 ± 2.41
QT_c (ms)	255.4 ± 13.6	267.3 ± 11.3	219.5 ± 2.69[a,b]	215.8 ± 6.40[a,b]	237.2 ± 6.22[b]

Values are represented as mean ± SEM. The number of observations is given in parentheses.
HR, heart rate; MI, myocardial infarction; QT_c, QT interval corrected; QT_o, QT interval observed.
[a]Significance of differences from control group calculated by Student's *t*-test for unpaired data at $P < 0.05$.
[b]Significance of differences from MI group calculated by Student's *t*-test for unpaired data at $P < 0.05$.

Table 3 Plasma levels of troponin I and triglycerides in the control group, untreated myocardial infarction group, and MI groups treated with insulin, ATP, and L-arginine

	Control	Untreated MI	Insulin-treated MI	ATP-treated MI	L-Arginine-treated MI
Troponin I (ng/ml)	(17)	(18)	(18)	(16)	(17)
Median	0.6250	0.8333[a]	0.41665	1.06250[a]	1.0000[a]
25th percentile	0.3333	0.5000	0.23958	0.58307	0.8125
75th percentile	0.7500	1.4375	1.13548	2.13525	1.6250
TG (mg/dl)	(18) 102.1 ± 10.5	(18) 108.4 ± 9.60	(19) 69.5 ± 6.48[a,b]	(18) 61.6 ± 5.82[a,b]	(18) 101.5 ± 6.33

The number of observations is given in parentheses. Values for TG are represented as mean ± SEM.
MI, myocardial infarction; TG, triglycerides.
[a]Significance of difference from control group calculated by the Mann–Whitney test in case of troponin I and Student's *t*-test for unpaired data in case of TG at $P < 0.05$.
[b]Significance of difference from MI group calculated by Student's *t*-test for unpaired data in case of TG at $P < 0.05$.

Table 4 Incidence of arrhythmias (basal, isoproterenol-provoked, and total) in perfused isolated hearts of the control group, untreated myocardial infarction group, and MI groups treated with insulin, ATP, and L-arginine

	Control	Untreated MI	Insulin-treated MI	ATP-treated MI	L-Arginine-treated MI
Total number of rats	18	24	19	18	18
Number of rats with basal arrhythmia	2	6	3	0	1
Percent ratio	11.11	25	15.79	0[a]	5.56
Number of rats with ISO-provoked arrhythmia	1	2	0	1	0
Percent ratio	5.56	8.33	0	5.56	5.56
Total number of rats with arrhythmia	3	8	3	1	1
Percent ratio	16.67	33.33	15.79	5.56[a]	5.56[a]

ISO, isoproterenol; MI, myocardial infarction.
[a]Significance of difference from MI group calculated by χ^2-test at $P < 0.05$.

associated with ISO-induced MI. Insulin treatment protected against MI, though not completely, as indicated by a significant reduction in the ST-segment elevation and plasma levels of troponin I in group III rats compared with untreated rats. This protective effect of insulin supports previous observations that have demonstrated that glucose–insulin–potassium (GIK) or insulin alone reduced infarct size (IS) in myocardial ischemia-reperfusion rat hearts [18,19]. The insulin component in GIK also induced protection and minimized IS in dogs subjected to a 60-min coronary artery occlusion and a 3 h reperfusion by activating K_{ATP} channels [20]. Zhang *et al.* [21] reported that GIK and insulin alone reduced myocardial IS and improved cardiac recovery in rabbits after myocardial I/R *in vivo*. They suggested that insulin plays the predominant role in GIK-elicited myocardial protection [21]. Insulin cardioprotection during I/R appears to be due to its significant antiapoptotic effect mediated through inositol trisphosphate kinase, Akt, and phosphorylation of endothelial nitric oxide synthase [19].

The ECG changes, other than ST-segment elevation reduction, showed a significant reduction in the QT_o and QT_c intervals, which indicates that insulin pretreatment improves the proarrhythmic status induced by ISO. In addition, insulin treatment caused a strong significant reduction in the levels of plasma TG. This strong hypotriglyceridemic action of insulin could be accounted for by insulin-induced reduction of levels of circulating free fatty acids (FFA) and by cardiac uptake of FFA in animals [22] and in humans [23]. Supporting this fact, GIK was found to significantly decrease ventricular arrhythmia in MI rats, and this effect was explained in part by a decrease in circulating levels of FFA [24].

Data on the activity of isolated perfused hearts revealed that insulin pretreatment did not influence intrinsic chronotropy or maximal chronotropic responsiveness, thus the chronotropic compensatory cardiac mechanism was preserved. The ability to generate force, measured as PT, was significantly augmented both under basal conditions and in response to β adrenergic stimulation. Thus, the inotropic reserve, which represents an important cardiac reserve mechanism, was significantly enhanced.

The improved inotropic function upon insulin treatment was shown in patients with dilated cardiomyopathy [25]. Insulin was also shown to have a rapid and direct positive

Table 5 Heart rate, peak tension, half-relaxation time, myocardial flow rate baseline values, maximal responses, and delta changes of perfused isolated hearts from the control group, untreated myocardial infarction group, and MI groups treated with insulin, ATP, and L-arginine

	HR (bpm)	PT (g)	½ RT (ms)	MFR (ml/min)
Control				
Baseline values	184.06 ± 14.88 (16)	3.78 ± 0.31 (16)	49.38 ± 2.95 (16)	8.13 ± 0.54 (16)
Maximal responses	218.13 ± 14.09[c] (15)	4.68 ± 0.38[c] (15)	38.67 ± 3.22[c] (15)	8.04 ± 0.64 (15)
Delta changes	29 ± 9.83 (15)	0.82 ± 0.26 (15)	−9.33 ± 2.48 (15)	0.02 ± 0.32 (15)
Untreated MI				
Baseline values	167.06 ± 15.53 (18)	1.98 ± 0.29[a] (18)	41.11 ± 3.78 (18)	7.19 ± 0.49 (18)
Maximal responses	182.69 ± 17.43 (16)	2.54 ± 0.41[a,c] (16)	34.38 ± 3.16[c] (16)	7.73 ± 0.54 (16)
Delta changes	23.06 ± 13.52 (16)	0.46 ± 0.19 (16)	−7.50 ± 2.81 (16)	0.38 ± 0.25 (16)
Insulin-treated MI				
Baseline values	192.81 ± 18.98 (16)	3.37 ± 0.46[b] (16)	35.63 ± 3.29[a] (16)	8.54 ± 0.86 (16)
Maximal responses	206.06 ± 18.86 (16)	4.29 ± 0.39[b,c] (16)	26.88 ± 2.18[a,c] (16)	9.27 ± 0.98[c] (16)
Delta changes	13.25 ± 9.90 (16)	0.92 ± 0.26 (16)	−8.75 ± 3.40 (16)	0.73 ± 0.30 (16)
ATP-treated MI				
Baseline values	170.39 ± 9.92 (18)	3.71 ± 0.46[b] (18)	40 ± 3.13[a] (18)	8.20 ± 0.66 (18)
Maximal responses	178.29 ± 11.97[a] (17)	4.06 ± 0.52[b] (17)	38.24 ± 2.14 (17)	8.65 ± 0.68 (17)
Delta changes	12.12 ± 10.33 (17)	0.21 ± 0.46 (17)	−2.94 ± 2.39 (17)	0.49 ± 0.37 (17)
L-Arginine-treated MI				
Baseline values	188.41 ± 9.02 (17)	3.08 ± 0.25[b] (17)	40 ± 3.43[a] (17)	9.20 ± 0.63[b] (17)
Maximal responses	218.47 ± 14.71[c] (17)	4.81 ± 0.36[b,c] (17)	34.71 ± 2.99 (17)	9.45 ± 0.63[b] (17)
Delta changes	30.06 ± 11.28 (17)	1.72 ± 0.32[a,b] (17)	−5.29 ± 3.22 (17)	0.25 ± 0.12 (17)

Values are represented as mean ± SEM. The number of observations is given in parentheses.
HR, heart rate; MFR, myocardial flow rate; MI, myocardial infarction; PT, peak tension; ½ RT, half-relaxation time.
[a]Significance of difference from control group calculated by Student's *t*-test for unpaired data at $P<0.05$.
[b]Significance of difference from MI group calculated by Student's *t*-test for unpaired data at $P<0.05$.
[c]Significance of the maximal response to baseline value of the same group calculated by Student's *t*-test for paired data at $P<0.05$.

Table 6 Body weight, left ventricular weight, and left ventricular body weight ratio in the control group, untreated myocardial infarction group, and MI groups treated with insulin, ATP, and L-arginine

	Control (18)	Untreated MI (24)	Insulin-treated MI (19)	ATP-treated MI (18)	L-Arginine-treated MI (18)
BW (g)	173.6 ± 4.16	192.2 ± 6.30[a]	214.0 ± 5.78[a,b]	243.1 ± 3.36[a,b]	238.1 ± 2.33[a,b]
LV (mg)	290.1 ± 19.3	417.1 ± 31.2[a]	547.4 ± 24.6[a,b]	657.6 ± 22.7[a,b]	593.8 ± 18.9[a,b]
LV/BW (mg/g)	1.70 ± 0.12	2.15 ± 0.13[a]	2.57 ± 0.09[a,b]	2.70 ± 0.08[a,b]	2.50 ± 0.08[a,b]

Values are mean ± SEM. The number of observations is given in parentheses.
BW, body weight; LV, left ventricular weight; LV/BW, left ventricular body weight ratio; MI, myocardial infarction.
[a]Significance of differences from control group calculated by Student's *t*-test for unpaired data at $P<0.05$.
[b]Significance of differences from MI group calculated by Student's *t*-test for unpaired data at $P<0.05$.

inotropic effect on the postischemic rat heart [26]. Antiapoptotic effects of insulin mediated by NO may contribute to the augmented systolic function shown upon insulin treatment [19]. It has been reported that insulin suppresses ISO-elicited cardiac injury, including myocardial contractile dysfunction and cell apoptosis and necrosis in isolated I/R hearts [2], which supports our findings.

Left ventricular hypertrophy (LVH) observed in insulin-treated rats represents another compensatory mechanism for inotropic abilities. Moreover, other compensatory mechanisms were maintained, such as the relaxation rate, measured as ½ RT, and myocardial coronary flow. Insulin induces coronary vasodilatation through an endothelial-dependent mechanism releasing NO [27]. Therefore, insulin treatment of ISO-treated rats prevented the deterioration in functional performance of infarcted hearts, with all compensatory mechanisms preserved or enhanced.

The present study also showed that pretreatment with ATP was only partially effective in the protection against ISO-induced MI, as evidenced by the significant reduction in ST-segment elevation and by the data on mortality and arrhythmias. Mortality was actually abolished (0%), and arrhythmias were kept at a minimum, which was even lower than that of matched controls. These effects of ATP treatment could be explained by the assumption that ATP activates mitochondrial K_{ATP} channels. Actually, selective K_{ATP} channel openers have been reported to significantly reduce mortality in ISO-treated rats [28,29]. Furthermore, activation of mitochondrial K_{ATP} channels protected against ventricular fibrillation induced by postinfarction cardiac sclerosis [29,30] and also protected against ischemia-induced arrhythmia [31].

The protective effect of ATP treatment on arrhythmia could also be related to the significant reduction in the QT interval, both observed and corrected, as prolongation of the QT interval is known to be proarrhythmic. The present results indicate a significant reduction in plasma levels of TG; high plasma levels of TG are known to be deleterious to the heart [32].

Treatment with ATP led to a significant reduction in HR *in vivo*, recorded using ECG, whereas the HR of isolated perfused hearts, that is, *in vitro*, was maintained, both as intrinsic chronotropic activity under baseline conditions and as maximal chronotropic responses to β-adrenergic

stimulation. These data are at variance with other studies that reported a positive chronotropic effect upon intravenous administration of ATP [33] as well as in isolated hearts of control rats [34]. Such positive chronotropic effects were ascribed to activation of type 2 purine receptors (P2) localized on the membrane of vagal nerve fibers [35].

Moreover, ATP treatment augmented the force generation, measured as PT, both under basal conditions and in response to β-adrenergic stimulation. These effects are supported by previous findings stating that ATP improved contractility and increased the coronary flow rate of isolated hearts [35]. In addition, ATP was found to stimulate L-type Ca^{2+} channels in the myocardium [36].

The augmented inotropic intrinsic and maximal responses observed in ATP-treated rats with ISO-induced MI represent a major compensatory cardiac reserve mechanism. This favorable inotropic mechanism is further supported by significant LVH, which provides another compensatory mechanism in ATP-treated rats.

L-Arginine pretreatment of ISO-treated rats, in contrast, did not offer sufficient protection against myocardial damage in ISO-induced MI, as shown by the changes in the ST segment and the plasma levels of troponin I that were significantly higher in arginine-treated rats compared with matched controls and were not different from the results of ISO-treated rats. However, the findings on mortality and arrhythmias provide good evidence for the protection imparted by L-arginine pretreatment on ISO-induced MI, which prevented any death due to ISO-treatment. Moreover, arrhythmias were kept at a minimum by L-arginine pretreatment, being even less than that of matched controls. As an explanation of these contradictory data, it could be suggested that L-arginine treatment protected against mortality of rats treated with ISO through its antiapoptotic effects. Endothelial-derived NO, produced by action of endothelial nitric oxide synthase on L-arginine, protects against MI through inhibition of endothelial cells and cardiomyocyte apoptosis [37,38]. In ISO-induced MI, on using high doses of ISO, the incidence of myocardial necrosis was reported to be significantly higher than that of myocardial apoptosis [39]. According to Zhang *et al.* [40], the higher number of necrotic but not apoptotic cardiomyocytes appears to be the predominant source of elevated plasma levels of cardiac troponins.

The protective action of L-arginine treatment against arrhythmia induced in hearts of ISO-treated rats could be explained by NO-activated protein kinase C-delta-dependent activation of mitochondrial K_{ATP} channels, which protects against arrhythmias [29–31]. The significant reduction in the QT_c interval may also be related to the protective actions of L-arginine treatment against arrhythmias, as prolongation of the QT interval was shown to be proarrhythmic.

The present results also showed that the HR recorded by ECG *in vivo* was significantly decreased in L-arginine-pretreated ISO-treated rats. In contrast, the HR recorded in isolated perfused hearts was not different than that in L-arginine treated and untreated ISO-infarcted hearts, indicating that intrinsic chronotropic activity and maximal chronotropic responses as well as chronotropic reserves were maintained in L-arginine-treated rats.

Inotropic activity, measured as PT, was maintained under basal conditions and in response to β-adrenergic stimulation, whereas the inotropic reserve, measured as delta-PT, was augmented. Such inotropic reserves represent a major compensatory mechanism. This is supported by LVH, which represents another important compensatory mechanism. These data are supported by a previous study that showed that L-arginine significantly improved recovery of cardiac output, coronary flow, and peak aortic pressure in an I/R model of a working isolated heart [41].

However, the relaxation rate measured as ½ RT, which is an important diastolic function representing an important compensatory mechanism was maintained at its enhanced rate observed in untreated hearts with MI.

MFR, which is increased in L-arginine-pretreated hearts under basal conditions and during maximal response to adrenergic stimulation, is another important compensatory mechanism. Previous studies have shown that activation of the L-arginine/NO pathway reduces coronary vascular tone [42]. Maxwell and Cooke [8] used L-arginine to treat coronary artery disease associated with impaired NO synthesis and endothelial dysfunction. Such a compensatory mechanism of augmented MFR in L-arginine-pretreated hearts with ISO-induced MI may underlie its enhanced ability to generate force that enables proper functioning of the heart and may contribute to the survival of rats treated with ISO, despite the damage to their hearts.

Conclusion

Insulin, ATP, and L-arginine were variably effective in partially modifying the ISO-induced MI insults and offered partial protection against ISO-induced myocardial damage. Yet, all treatment modalities favorably produced conservation of cardiac performance and prevention of ischemia. In addition, ATP and L-arginine reduced mortality and were antiarrhythmogenic, and a hypolipidemic effect was achieved with insulin and ATP. Despite the beneficial effects of the agents used, none of them were successful in offering complete reversal of all ISO-induced disturbances, nor could any of them alone provide complete protection against the dysfunction inflicted by MI. Therefore, further studies are recommended using a combination of two or more of these agents, which could help pave the way toward achieving an efficient therapeutic means against MI.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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