ORIGINAL ARTICLE

COMPARISON OF ENOXIMONE, AMRINONE, OR LEVOSIMENDAN ENRICHED ST. THOMAS' HOSPITAL CARDIOPLEGIC SOLUTIONS USED FOR MYOCARDIAL PRESERVATION IN ISOLATED GUINEA PIG HEARTS

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Summary: Myocardial contractile function after cardioplegic arrest is often depressed and an ideal cardioplegic solution has not been developed yet. The aim of this study was to assess the efficacy of phosphodiesterase III inhibitors, amrinone and enoximone, and levosimendan, a novel Ca2+ sensitizing agent, on recovery of hearts after normothermic cardioplegic arrest when added to the St. Thomas' hospital cardioplegic solution. In the control group, isolated guinea pig hearts were perfused in Langendorff apparatus and arrested with standard St. Thomas' solution. In other groups, amrinone (10^{-5} mol.L^{-1}), levosimendan (10^{-5} mol.L^{-1}), or enoximone (10^{-4} mol.L^{-1}) were added to the cardioplegic solution. In all hearts, intraventricular pressure, $+dp/dt_{max}$, $-dp/dt_{max}$, area under pressure-time curve, heart rate, coronary flow, lactate dehydrogenase and creatine kinase enzyme leakage, and oxygen consumption were measured. In the enoximone group, contractility force and $+dp/dt_{max}$, were found to be significantly high in the reperfusion and inotropic periods in comparison with other groups ($p<0.05$). $-dp/dt_{max}$ and area under contractility-time curve values were significantly high in inotropic period in enoximone group ($p<0.05$). No statistically significant difference was noted in other groups. Cardioplegic solution enrichment with enoximone augmented mechanic functions in reperfusion period. No positive effect of amrinone or levosimendan was observed in this study.

Key words: *Enoximone; Amrinone; Levosimendan; St. Thomas hospital cardioplegic solution; Ischemia-reperfusion*

Introduction

In patients with poor cardiac function, low cardiac output syndrome is the most serious problem following cardiac surgery. Thus, many research have been conducted in the field of myocardial protection and different additives to cardioplegic solutions have been tested.

It is well documented that enoximone, levosimendan and amrinone improve myocardial contractility after cardiopulmonary bypass and in the postoperative period (2,11). However, the role of addition of these drugs to the cardioplegic solutions has not been investigated yet. This study is devised to investigate the effects of these drugs to ischemia-reperfusion injury and mechanical dysfunction in reperfusion period, when added to St. Thomas hospital cardioplegic solution. The hypothesis was that pre-treatment with these drugs might be beneficial as they may store more Ca^{++} in the sarcoplasmic reticulum before cardiac contractility restarts in the reperfusion period.

Material and Methods

All experiments were conducted in compliance with the "Principles of Laboratory Animal Care" and the Guide for Care and Use of Laboratory Animals.

Heart isolation and perfusion

Male and female guinea pigs weighing 500 to 800 g were used in this study (n=6 for each group). The animals were killed by a blow to the head without the use of anaesthetic agents. The hearts were excised and immersed immediately in cold (4 °C), heparinized (1000 IU L^{-1}) modified Krebs Henseleit solution, then mounted on a stainless steel cannula of the Langendorff perfusion apparatus, and the hearts were put in a heart chamber in order to prevent drying and keeping the temperature constant. The whole procedure beginning from decapitation to the initiation of perfusion after aortic cannulation was kept less than

60 seconds and otherwise hearts were discarded. Antegrade coronary perfusion was initiated and maintained at a constant perfusion pressure of 85 cm H_2O by a modified Krebs Henseleit solution (NaCl 137 mM, KCl 4.5 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 15.5 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.2 mM, and glucose 11.5 mM) and gassed with a mixture of 95% O_2 and 5% CO_2 to achieve a final oxygen tension above 500 mm Hg. The pH of Krebs Henseleit solution was adjusted to 7.4 when necessary. St. Thomas Hospital cardioplegic solution (NaCl 105 mM, KCl 16 mM, $CaCl_2$ 1.2 mM, $NaHCO_3$ 10 mM, and $MgCl_2$ 10 mM) was filled in the second column of Langendorff apparatus and was not gassed with carbogen. The perfusion solution and cardioplegic solutions were prepared from distilled and deionised water and filtered from a 5 (m membrane. The temperature of the heart and solutions were kept constant at 37 °C by means of a heat exchanger.

A latex balloon inserted to the left ventricle and left intraventricular developed pressure was recorded via P23XL pressure transducer (Grass Instruments). The balloon was filled with 30 mm Hg pressure. The transducer output was displayed on a Grass Model 5 polygraph (Grass Instruments Co. Quincy, Mass. USA). A simultaneous electrogram monitoring was made via two electrodes; one attached to the aortic cannula and the other to the apex of the left ventricle. Coronary effluent was collected for the measurement of coronary flow.

All of the hearts were compared for their pre-ischemic and post-ischemic left ventricular developed pressure, $+dp/dt_{max}$, $-dp/dt_{max}$, area under pressure-time curve on beat-by beat-basis heart rate, time to peak pressure, pressure-rate product, rhythm, LDH and CK enzyme leakage, myocardial oxygen consumption, and coronary flow values.

Experimental protocol

After a stabilization period of 20 minutes, baseline measurements were performed. The hearts in the control group were arrested with St. Thomas hospital cardioplegic solution for 5 minutes, then perfusion was totally stopped and the hearts were kept in cardioplegic solution for 30 minutes at 37 °C. Afterwards the hearts were reperfused for 10 minutes with Krebs Henseleit solution.

In the positive inotropic agent groups, the experimental protocol was initially the same but either enoximone (10^{-4} mol.L^{-1}), or levosimendan (10^{-5} mol.L^{-1}), or amrinone (10^{-5} mol.L^{-1}) was added to the St. Thomas Hospital cardioplegic solution. These doses were found to be maximum concentrations not deteriorating diastolic function for this preparation in our preliminary experiments. We also had a dose response study in normal and ischemia-reperfusion applied hearts for amrinone and levosimendan that helped us to find the maximum harmless concentrations for this study (16,17). These groups were further perfused with the same concentrations of the inotropic agents in Krebs Henseleit solution for another 5 minutes after the 10^{th} minute of reperfusion.

Enzyme levels in coronary effluent

The coronary effluent was collected during the course of the experiment for measurement of the coronary flow rate, and biochemical determination of creatine kinase (CK) (EC 2.7.3.2 Boehringer Mannheim GmbH) and lactate dehydrogenase (LDH) (EC 1.1.1.27 Boehringer Mannheim GmbH) levels as tissue damage markers. Hitachi System 717 automated analyzer was used for CK and LDH assays.

Oxygen consumption

For the measurement of oxygen consumption, simultaneous inflow and outflow perfusion solution was collected and measured by Ciba Corning 860 blood gas analysis apparatus.

Drugs used

Heparin (Liquemine® Roche, Turkey), amrinone (Sigma, USA), levosimendan (Orion Pharmaceuticals, Finland), enoximone (Merrel, United Kingdom) were used in the study.

Data analysis

All data were expressed as the mean (standard error of the mean. Statistical analysis among groups was performed by one way ANOVA and within groups for initial and reperfusion values by repeated measures ANOVA. Tukey's Honestly Significance test was used for post hoc comparisons. A p value <0.05 was considered statistically significant.

Results

There was no difference between groups in baseline values in any criteria evaluated ($p>0.05$ for every variables; Table 1-11). All of the hearts arrested within 3 minutes of cardioplegic solution administration and returned spontaneously to sinus rhythm.

Tab. 1: Peak systolic pressure values at the inital, reperfusion and inotropic periods.

Peak systolic pressure (mm Hg)	Initial	Reperfusion	Inotropic
St. Thomas	71±6	69±6	
Enoximone	69±10	83±10*	100±9*
Levosimendan	60±6	66±10	72±9
Amrinone	59±4	56±5	64±4

* $p<0.05$

Tab. 2: $+dp/dt_{max}$ at the inital, reperfusion and inotropic periods.

$+dp/dt_{max}$ (mm Hg.s^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	488±168	728±249	
Enoximone	791±279	1160±284*	1411±181*
Levosimendan	541±176	672±205	738±159
Amrinone	497±95	411±99	568±116

* p<0.05

Tab. 3: $-dp/dt_{max}$ at the inital, reperfusion and inotropic periods.

$-dp/dt_{max}$ (mm Hg.s^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	353±112	663±260	
Enoximone	402±81	418±80	997±185*
Levosimendan	280±72	428±127	562±135
Amrinone	362±76	310±75	401±54

* p<0.05

Tab. 4: Area under contraction time curve values.

Area under contraction time curve (mm Hg.s)	Initial	Reperfusion	Inotropic
St. Thomas	0.039±0.004	0.038±0.004	
Enoximone	0.032±0.007	0.042±0.008	0.055±0.007*
Levosimendan	0.028±0.005	0.031±0.007	0.034±0.007
Amrinone	0.022±0.003	0.022±0.004	0.025±0.003

* p<0.05

Tab. 5: Time to peak pressure.

Time to peak pressure(s)	Initial	Reperfusion	Inotropic
St. Thomas	0.12±0.01	0.13±0.01	
Enoximone	0.09±0.01	0.08±0.01	0.09±0.01
Levosimendan	0.12±0.01	0.10±0.01	0.10±0.00
Amrinone	0.09±0.01	0.09±0.02	0.09±0.01

Tab. 6: Pressure-rate product.

Pressure rate product (mm Hg × × beat.min^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	99939±38122	141986±51558	
Enoximone	173808±61894	273152±72125	327715±52297
Levosimendan	113893±46214	151670±52966	167098±40654
Amrinone	118114±24125	84783±22440	124838±28661

Tab. 7: Heart rate.

Heart rate (beat.min^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	193±11	185±9	
Enoximone	218±8	225±11	228±10
Levosimendan	187±27	197±25	212±19
Amrinone	237±10	167±34	217±7

Tab. 8: Creatine kinase enzyme leakage.

CK (IU.min^{-1} × × g heart^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	0.02±0.01	0.53±0.38	
Enoximone	0.03±0.01	0.47±0.14	0.43±0.16
Levosimendan	0.09±0.03	0.44±0.15	0.35±0.08
Amrinone	0.08±0.02	1.37±0.41	0.92±0.35

Tab. 9: Lactate dehydrogenase enzyme leakage.

LDH (IU.min^{-1} × × g heart^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	0.03±0.01	0.19±0.13	
Enoximone	0.02±0.01	0.11±0.03	0.10±0.03
Levosimendan	0.05±0.02	0.12±0.03	0.10±0.01
Amrinone	0.06±0.01	0.31±0.07	0.24±0.06

Tab. 10: Oxygen concumption.

Oxygen consumption (ml.min^{-1} × × g heart^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	1.52±0.12	1.49±0.15	
Enoximone	1.42±0.10	1.48±0.09	1.68±0.06
Levosimendan	1.48±0.21	1.96±0.33	1.97±0.43
Amrinone	2.09±0.45	1.89±0.47	2.05±0.41

Tab. 11: Coronary flow values.

Coronary flow (ml.min^{-1} × × g heart^{-1})	Initial	Reperfusion	Inotropic
St. Thomas	4.4±0.3	4.2±0.5	
Enoximone	3.6±0.3	3.5±0.2	3.9±0.2
Levosimendan	5.0±0.9	6.6±1.3	5.5±1.5
Amrinone	5.5±1.0	5.0±1.0	5.1±1.1

Peak systolic pressure and $+dp/dt_{max}$ values for enoximone group was significantly higher than other groups in the reperfusion and inotropic periods in comparison with other groups (Table 1,2). Also, $-dp/dt_{max}$ and area under contraction time curve values for enoximone group was significantly higher than other groups in the inotropic period in comparison with the other groups (Table 3,4). There was no significant difference between groups for time to peak, pressure rate product, and heart rate values in the reperfusion and inotropic periods (Table 5,6,7). There was also no significant difference between groups in CK, and LDH leakage, oxygen consumption, coronary flow values in the reperfusion and inotropic periods (Table 8,9,10,11).

Discussion

We investigated the effects of enoximone, amrinone and levosimendan on ischaemia-induced changes in myocardial function in isolated guinea pig hearts. Enoximone and amrinone selectively inhibit phosphodiesterase enzyme type III in cardiac and vascular smooth muscle cells and results in higher levels of cAMP that promotes Ca^{++} deposition in sarcoplasmic reticulum by activating Ca^{++} pump (3). The amount of Ca^{++} stored in sarcoplasmic reticulum is directly proportional to the amount released to myocardial cytoplasm; thus cardiac contractility increases as the amount of Ca^{++} released to cytoplasm with action potential increases (4). Levosimendan is a novel inodilator. Its inotropic mechanism is based on calcium sensitization of myofilaments and its vasodilator action is related to the opening of ATP-dependent K-channels (7).

A recent multicenter study clearly showed that enoximone improves exercise capacity in patients with heart failure without increasing adverse events (8). In ischemic human hearts with significant left anterior descending stenoses, hemodynamic effects of intracoronary enoximone injection evaluated with cardiac catheterisation, echocardiography and myocardial perfusion analyses. Shortening of echocontrast dye washout and $+dp/dt_{max}$ increase was found to be significant, which implies the improvement of systolic cardiac functions after intracoronary enoximone injection (10). Rechtman et al. suggested that another phosphodiesterase III inhibitor, amrinone, may be useful in reducing ischaemia-reperfusion injury and speculate that this involves altering ischaemia-induced changes in intracellular Ca (2+) in the myocytes (12). In isolated perfused rabbit hearts, amrinone reduces both the incidences of ventricular fibrillation and infarct size following coronary occlusion (13). Recently, the effect of different PDE-III inhibitors, including amrinone, was investigated on human neutrophil functions. Chemotaxis, phagocytosis, reactive oxygen species production, intracellular calcium ion concentration, and cyclic adenosine monophosphate levels in neutrophils were measured. Amrinone was shown to scavenge reactive oxygen species at clinically relevant concentrations (9).

The majority of clinically used inotropic drugs act by increasing cytosolic calcium levels, which may hypothetically worsen reperfusion stunning further and cause arrhythmias. Du Toit et al. tested the calcium sensitizer levosimendan in the Langendorff-perfused guinea pig heart, subjected to 40-min low-flow ischemia. Levosimendan improved reperfusion function without promoting arrhythmias in this model. This effect was attributed to opening the K (ATP) channels during ischemia and sensitizing myocardial contractile apparatus rather then elevating cytosolic calcium levels in reperfused hearts (18). Another experimental study showed perfusion of post-ischemic guinea pig hearts with levosimendan was associated with dose- and time-dependent increases in both contractility and speed of relaxation (6).

In this study, we encountered the mechanical contraction parameters; peak systolic pressure and $+dp/dt_{max}$, to be significantly higher in reperfusion and inotropic periods in enoximone group when compared with other groups (Table 1,2). $-dp/dt_{max}$ and area under curve values were significantly high in the inotropic period (Table 3,4). However, the comparison of drugs according to the other parameters showed no significant changes. In enoximone group O_2 consumption was found to be less than other groups but the difference was not statistically significant (Table 10).

Shimada et al., using an isolated working heart model studied the effects of dopamine, adrenaline, and noradrenaline pretreatment on ischemia/reperfusion injury. Dopamine and adrenaline had a harmful effect at normothermia (14). We were also worrying about the possibility of harmful effects of positive inotropic agents on myocardial protection. However, our results, including enzyme levels, revealed the harmless impact of enoximone, levosimendan and amrinone on reperfusion injury.

We were unable to find any other study concerning the effects of enoximone, amrinone and levosimendan on reperfusion injury when administered before ischemic arrest. Komai et al. used milrinone and isoproterenol in an isolated working rat heart model. Enrichment of St. Thomas' hospital solution with milrinone and isoproterenol aggravated reperfusion damage in this study (5). Contrary to this, another phosphodiesterase III-inhibitor (E-1020) was added to Bretschneider's HTK cardioplegic solution and exerted better myocardial protection in rabbit hearts (19). Similarly, adenosine monophosphate phosphodiesterase inhibitor, DN-9693, was examined in an isolated rabbit heart cardioplegic arrest model and was shown its myocardial protective effect with possible inhibition of leukocyte aggregation (1).

In stunned myocardium, oxygen consumption is relatively high and inotropic agents which act via intracellular increased calcium result in a higher oxygen demand. Theoretically, Ca-sensitization might be a favorable alternative. Sunderdiek et al. compared a Ca (2+)-sensitizer (EMD 60263) with enoximon on rabbit hearts during reperfusion after global ischemia. It is concluded that Ca-sensitization

may potentially be a superior method for inotropic support in the postischemic heart (15). However, our study revealed no better preservation with levosimendan rather than enoximone.

In conclusion, the addition of enoximone to St. Thomas hospital cardioplegic solution enhanced myocardial contractility without causing a harmful effect on diastolic function. Our results and previous studies do not clarify the reason why enoximone is superior to other phosphodiesterase inhibitor amrinone and levosimendan with this respect.

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