EFFECTIVENESS OF GLUTAMATE-ASPARTATE SOLUTION FILLED IN PERICARDIAL CAVITY ON DECREASING MYOCARDIAL DAMAGE (EXPERIMENTAL STUDY)

M. H. US, MD,
T. OĞUŞ, MD,
K. ÇAĞLI, MD,
K. İNAN, MD,
T. YEŞİLDERE,* MD,
G. ŞENNAZLI,* MD,
E. DURAN, MD,
Y.Ö. ÖZTÜRK, MD

From:
Department of Cardiovascular Surgery GATA Haydarpaşa Training Hospital Istanbul, Turkey
*Department of Pathology Istanbul Univercity Veteriner Faculty Istanbul, Turkey

Adress for reprints:
Dr.Melih Hulusi US
GATA Haydarpaşa Training Hospital
Department of Cardiovascular Surgery
KADIKÖY/İSTANBUL
Tel: +90 216 4147718
Fax: +90 216 3029929
Email: melihus@usa.net

Our aim in this study was to decrease the myocardial damage due to substrate deprivation between the time period from cross clamping until maintaining the homogeneous diastolic arrest.

In GATA Haydarpaşa Training Hospital Animal Laboratory in July 2000 30 Wistar Albino rats mean weight 300 grams were divided in to three groups (Group A, Group B and Group C). In Group A (control group); after opening the pericardial cavity all vasculature inlets and outlets were cross clamped. After 60 seconds all hearts were excised. In Group B and Group C; after opening the pericardial cavity, pericardial cavity was filled with 2 cc glutamate-aspartate solution and waited for 2 minutes. Afterwards in Group B; all inlet and outlet vasculature were cross clamped for 60 seconds and in Group C for 90 seconds and 2cc of blood was aspirated from right atrium. All hearts were excised and then were sent for pathological examination and blood samples were sent for biochemical assays.

In these 30 rats; in three groups the best pathological and biochemical outcomes were obtained in Group B. In Group A and Group C the results were the worst.

Result: In open-heart surgery as an extraphysiologic method, during the time period from cross-clamping until maintaining diastolic arrest, pericardial glutamate-aspartate solution applied topically maintains energy supply. By this way, better myocardial protection can be maintained.

Applying a cross clamp in open heart surgery is an extraphysiologic procedure and all studies stated that, even infusion of antegrade and retrograde cardioplegia solutions and hypothermia maintaining diastolic arrest needs a 3-5 minutes period(1,2,3,4).

In this period; aerobic metabolism still works and as coronary flow is blocked, myocardium gets deprived of aerobic substrates and oxygen, which causes ischemic-hypoxic myocardial damage (3,5). In order to prevent this damage some substrates were added to cardioplegic solutions (3,4,5,6). The most popular substrates are glutamate and aspartate aminoacids (7,8,9). However, the ischemic damage risk -even by addition of there substrates- cannot be absolutely excluded (10,11,12). That is why there is a big need for investigation of alternative energy supplying and lowering energy demanding methods after application of the cross clamp.

Key words: Glutamate-aspartate, myocardial damage, experimental

Our aim in this study was to decrease the myocardial damage due to substrate deprivation between the time period from cross clamping until maintaining the homogeneous diastolic arrest.

MATERIAL AND METHODS

Experimental Groups

In GATA Haydarpaşa Training Hospital Animal Laboratory in July 2000, 30 Wistar Albino rats mean weight 300 grams were divided in to three groups (Group A, Group B and Group C). Group A was the control group.

Experimental Analysis:

In laboratory operation room all rats were anesthesized for surgery by 20-40 mg/kg cethamine (Ketalar Eczacıbaşı Medical Industry) without depressing their respiration. Skin incision was made from jugulum to xiphoid process by 21 no scalpel. Sternum was divided by scissors beginning from xiphiod process. By the help of a skin retractor, the mediastinum was opened and was entered to the pericardial cavity. The pericardium was retracted by stay sutures. All these procedure were the same for all three groups. In Group A (control group); after opening the pericardial cavity all inlet and outlet vasculature were cross clamped. After 60 seconds all hearts were excised. In Group B and Group C; after opening the the cavity, pericardial cavity was filled with a 2 cc of glutamate-aspartate solution (solution formula: L-monosodic monohydrate glutamate 30 mM, L-Monosodic monohydrate aspartate 30 mM) at room temperature and then waited for 2 minutes. Afterwards in Group B; all inlet and outlet vasculature were cross clamped for 60 seconds and in Group C for 90 seconds and 2cc of blood sample was aspirated to a heparinized injector from right atrium which we thought to be the coronary sinus blood. All hearts were excised, put in cold saline solution for 15 seconds for maintaining arrest and then put in to 10% formaline solution and they were sent for pathologic examination and blood samples were sent for biochemical assays. For pathologic examination the hearts were prepared by paraffin and 5mm cross sectional slides were cut and dyed by hematoxylene-eosine. All these pieces were evaluated by light microscope semiquantitatively for grades of coagulation necrosis parameters; vascular congestion, neutrophil-macrophage infiltration of myocytes, eosinophilic pyknosis and loss of striation of cytoplasm, karyopyknosis of cytoplasm. Also all these pieces were evaluated for the grade of inflammatory response. For statistical analysis, two different scorings were used. For first scoring, the grade of inflammatory changes were scored as: no response: 0, minimal response: 1, moderate response: 2, maximal response: 3. For second scoring: no response: 0, existence of neutrophils: 1, existence of monocytes: 2, existence of macrophage: 3, necrosis: 4.
Blood samples aspirated from right atrium were analysed for; pH, pO2, pCO2, hematocrit, hemoglobin, sodium, potassium, chloride, calcium, glucose, bicarbonate, base excess and osmolarity. (Table 1)

Statistical Analysis:

All parameters were evaluated by Kruskall Wallis and Mann Whitney-U Test. P values less than 0.05 were statistically insignificant.

RESULTS

Due to pathologic scoring:
Group A: 1st scoring:10, 2nd scoring: 3.8
Group B: 1st scoring:7.8, 2nd scoring: 2.2
Group C: 1st scoring:11.4, 2nd scoring: 4

Table 1. Blood sample analyses

	Group A	Group B	Group C
pH	7.11*	7.28*	6.80*
pCO2	63.3	54.7	116.6
pO2	29.3	31.5	19.2
Htc	37	31	44
Hb	12.2	10.3	14.8
Na+	135*	139*	101*
K+	5.9	4.2*	6,5*
Ca+2	0,26*	0,47	0,21*
Glu	66*	89*	67*
HC03 -	20.3	28.7*	13.7*
BE	-9.4	-1,2*	-16,0
0sm:	270	273*	203*
CI-	93	91	91

*statistically significant values ($p<0.05$).

According to scoring; Group B's findings was good but for Group A and Group C findings were poor ($p<0.05$) (Figure 1).
There were no statistically significant differences between Group A and Group C ($p>0.05$).
In all three groups there were intramyocardial bleeding focuses both macroscopically and microscopically.
Biochemical assay values of blood samples aspirated from right atrium which were thought to be the coronary sinus blood (mean values):
In Group A and Group C; lower pH, lower sodium levels, higher potassium levels, lower calcium levels, lower glucose levels, lower osmolarity, lower bicarbonate levels and increase in base excess were found to be statistically significant ($p<0.05$). pCO2 was increased and pO2 decreased in all three groups but this was statistically insignificant ($p>0.05$). Htc and Hb levels were normal in all three groups ($p>0.05$).
Although the rats were not heparinized before cross clamping, there were no coagulation detected in hearts.

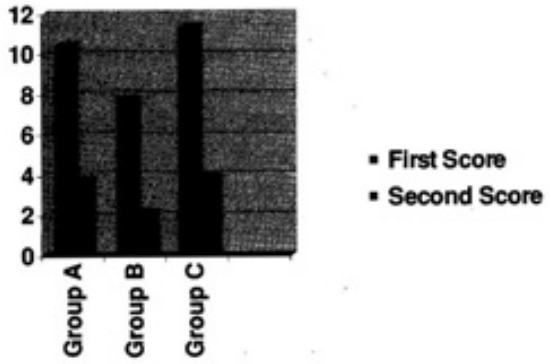

Figure 1. Pathological scoring.

DISCUSSION

In open heart surgery, many studies showed that after cross clamping aerobic metabolism works for a 60-80 second period and nearly all energy stores were consumed at that time (5, 8). Beginning from this period until maintaining diastolic arrest for 3-5 minutes ischemic-hypoxic damage occurs in myocytes (1, 3, 5). In order to prevent this myocyte damage, as an alternative solution we assumed to supply the necessary substrate and energy demand of myocardium by topically application of a glutamate- aspartate solution before cross clamping (8, 9, 11, 12, 13).
The justification for topical application of solutions to the heart are:
1* Histologically; the serous inner layer of the double layered visceral pericardium is in a very close contact with the epicardium and is composed of a mesothelial layer whose surface is covered with microvillous cubic epithelium. This microvilli structure can secrete and, while there is a negative intracardiac cavity pressure, it can also perform absorption (14, 15).
2* A noncoronary reflow phenomenon which is described as; the hearts collateral collaboration with the pericardial, plevural, brachial, mediastinal and diaphragmatic structures after than the coronary flow (14, 15, 16). After opening the pericardial cavity and manipulating the heart for freeing from the environmental structures, the formation of especially petechial hemorrhages on the

surface of the lipid layer results from the damage to the integrating of the vasculature. When this is destructed and the open orificed capillaries are examined microscopically, fenestration between the endothelial cells which permits diffusion can easily be detected. So absorption by these methods from the fenestrations can be possible (14, 17). Depending on these two histologic justifications and studies considering the benefits of different solutions for preserving hearts for transplantation, we can consider the benefit of topical substrate supplementation (14, 17, 18, 19, 20).

Myocardial tissues supply 90% of their energy demands from Krebs cycle, 10% from Embden-Meyerhoff pathway. These pathways supply their substrates 70% from fatty acids, 20% from carbohydrates, 6% from ketoacids and 4% from aminoacids (20, 21, 22, 23). Some substrates which can enter these metabolic pathways from any step, were used in cardiac surgery for metabolic support (5, 8, 20). The most popular ones are glutamate-aspartate.

Glutamate-aspartate are acidic aminoacids and they enter the Krebs cycle by transforming to α-ketoacids. Glutamate transforms to α-ketoglutarate by oxidative deamination and aspartate transforms to oksaloacetic acid by transamination and then they enter the Krebs cycle (24, 25, 26). The metabolic effect of these aminoacids are: (1) that they are direct substrates for aerobic metabolism, (2) indirectly a potential source for anaerobic substrate phosphorylation, (3) innovator of step products for malate-aspartate pathway which is very important in forming mitochondrial redoxpotential, (4) glutamate binds free ammonia in order to prevent the side effects of ammonia on metabolism. As it is concerned free ammonia decreases the nicotinamide dinucleotide (NAD) source which is a very important energy source for the mithocondrium and has a direct inhibitory effect on Krebs cycle enzymes (27, 28, 29, 30).

Some aminoacids can enter the Krebs cycle by transforming to glutamate-aspartate (31, 32, 33, 34, 35).

All studies performed by glutamate-aspartate showed striking beneficial effects on cardiac metabolism. Thomassen and colleague; stated that glutamine uptake was increased in CAD and this increase represented a direct correlation to the severity of disease (7, 8, 35). They also indicated an increase in regional contractility and cardiac output in aminoacid infused hearts (8,14,16). Pisarenko and colleagues reported an increase in myocardial performance in glutamine perfused patients postoperatively. They suggested that this increase in performances as a result of decreased ammonia excretion and increased lactate consuming. Mela and colleagues reported an increased contractility by infusing exogenous glutamate after global ischemia (25, 31, 35, 36).

As in our study; Group B's pathological and biochemical results were better so we suggested that there is topical absorption and it has a beneficial effect on metabolism. But in Group C, the reason that the results were poor, was associated with the long periods of cross clamping (90 seconds), and the increase in demand but not the enough supply by topical absorption. The decrease in blood pH, increase in pCO2, high potassium levels, decreased bicarbonate levels were the results of metabolic and respiratory acidosis. The reason for the respiratory acidosis was the opening of the pleural cavity with pericardial cavity and the lack of ventilatory support.Another reason for the high levels of the pCO2 was the increased substrate utilisation by both aerobic and anaerobical metabolisms and the result of these increased metabolit release (37, 38, 39). The reason for the pH decrease, calcium decrease, potassium increase, sodium decrease and the decrease of osmolarity in Group A and Group C was the destruction of the membrane integrity because of the ischemic-hypoxic damage and also the destruction of the Na-K ATPase and Na-Ca ATPase enzyme systems (36, 37, 38). Histochemical studies stated that sodium and calcium accumulates intracellulary and as potassium is transferred to extracellular area, it decreases intracellularly. This decrease in osmolarity is the lack of extracellular sodium and glucose levels. The glucose decrease is the result of increased consumption of the glucose by the myocardium under hypoxic stress (35, 39, 40, 41).

Light microscopic examination demonstrated every step in high degree coagulation necrosis in Group A and Group C. As it is widely known, cellular swelling and intratissue pressure increases which causes congestion in neighbouring vascular structures are the initial insults after hypoxic damage. In addition to this statement, in our study, the clamping all inlet and outlet vasculature on a beating heart caused stasis and distension in all vasculature which resulted vascular congestion. Due to the effects of toxic and acidic metabolites, cellular membrane integrity becomes damaged which causes the activation of phospholipase enzymes producing prostaglandins. Both prostaglandins and acidic metabolites are strongly chemotactic agents for the neutrophil-macrophage system (39). Proteolytic enzymes secreted by these inflammatory cells causes destruction in intracytoplasmic organelles, eosinophilic piknosis and loss of striation. As an end result of these destructive stimuli, there will be karyopiknosis of the nucleus. During this inflammatory process first neutrophils and then mononuclear cells and lastly macrophages migrate to damaged sites (36, 37, 42, 43, 44).

Extravasation of erythrocytes from the damaged sites, erosion of the surrounding vasculature by the proteolytic enzymes excreted during inflammatory process and trauma performed by the surgical instruments were the causes of the hemorrhagic focuses seen in pieces under light microscope (36, 37). We put every heart into cold saline as soon as possible in order to maintain the arrest and to stop the catabolic state to prevent the production of the toxic metabolites which are strongly destructive on cardiac metabolism. As considered before, these metabolites also initiate the inflammatory process which causes neutrophil and macrophage chemotaxis to the surrounding vasculature. By this process we excluded the side effects of all these factors and we were able to evaluate the pure effect of cross clamping (44).

In order to support our hypothesis for topical absorption for practical application there must be further in vivo and in vitro studies by increased number of experiments (14, 17, 18, 45). Electromicroscopic examination, histochemical experiments, and more detailed biochemical assays supporting this consideration will be the further challenges (14, 36, 44).

CONCLUSION

We suggest that after application of an extraphysiologic process like cross clamping, in order to supply the energy demend during the time period from cross clamping until maintaining diastolic arrest, filling the pericardial cavity by glutemate aspartete solution is benegicial. This procedure may help to decrease morbidity and mortality in an important rate.

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