ADDITIVE PROTECTION OF APROTININ, PROTEASE INHIBITOR TO COLD CARDIOPLEGIA FROM ISCHEMIC MYOCARDIUM

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Protective action of aprotinin from ischemic myocardial damage was evaluated in 9 patients compared to non-treated 18 patients, who underwent open heart surgery (22 ACB, 3 AVR and 2 MVR) with respect to the alterations of beta-glucuronidase, acid-phosphatase, MB-CPK and m-GOT. Cold cardioplegia with glucose-insulin-potassium solution was used in this investigation. Average arrest time was 78.6 ± 4.9 minutes associated with hypothermia between 25 and 28°C in rectal temperature. Aprotinin was administered in 9 patients intravenously with 5,000 KIU/Kg 30 minutes prior to CPB and then 5,000 KIU/Kg in the prime solution. Activity of beta-glucuronidase was significantly suppressed in the aprotinin-treated group compared to the non-treated group following cardioplegia and in the reperfusion period up to 6 hours, however, acid-phosphatase failed to demonstrate significant difference among two groups. Serum MB-CPK and m-GOT levels in the aprotinin-treated group did not elevate the beginning of reperfusion following cardioplegia. These data suggest that aprotinin add myocardial protection to cold cardioplegia.

INTRAOPERATIVE myocardial protection is critically important as myocardial functional capacity at the end of open heart surgery is the major determinant for the future prognosis of the heart.¹⁻³ Extensive investigation has been focussed on the myocardial protection from ischemia although one can not reach the goal to obtain the ideal method for myocardial protection.⁴ Systemic⁵ or topical hypothermia⁶ has been widely accepted in the clinical open heart

surgery. Pharmacological cardioplegia⁷⁻¹² associated with hypothermia has been recently emphasized with respect to better preservation of cardiac function and ideal intracardiac exposure due to the relaxation of cardiac muscle. Also, coronary perfusion has been consistently supported by many cardiac surgeons.¹³

Pathophysiology of myocardial damage subsequent to ischemia has been extensively investigated, and it is known that myocardial mitochondria is the most sensitive subcellular organelle to ischemia.¹⁴⁻¹⁶ Myocardial mitochondria is responsible to cardiac metabolism and hemodynamic functional capacity. No-reflow phenomenon is, sometimes, observed in the ischemic sub-endocardium following ischemic cardioplegia.¹⁷

Key Words:

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secondary to coronary arterial thrombosis, coronary endothelial injury, endothelial cell swelling in capillaries and extrinsic compression by interstitial edema. Whalen et al.¹⁸ clearly demonstrated the disturbance of cell volume regulation due to abnormality of cell membrane permeability subsequent to ischemia. These accumulated evidence supports the idea that the preservation of normal function of cell membrane is critically important for protection of myocardium from ischemia.

Activity of protease is increased following ischemia. Lysosomal enzymes have been well investigated in the ischemic condition associated with coronary artery ligation¹⁹ or cardiopulmonary bypass (CPB).²⁰ Aprotinin (Trasylo[®]) is known as one of the protease inhibitors, also Diez and Maroko²¹ and others²² demonstrated that aprotinin is effective to decrease ischemic injury of the myocardium when given immediately after coronary artery ligation. Mode of action of aprotinin is inhibition of protease activity; inhibition of tyrosine protease by aprotinin resulted in decrease in production of myocardial depressant factor;²³ lysosomal enzymes seem to be inhibited by aprotinin, furthermore, vasoactive substances such as plasmin, and kallikrein were affected by aprotinin, in which aprotinin plays a role as antifibrinolytic²⁴⁻²⁸ or an inhibitor of kinin activated following cardiopulmonary bypass.²⁹

Thus, this investigation is aimed to characterize the effect of aprotinin upon the global ischemic myocardium subjected to cardiopulmonary bypass. Lysosomal enzymes, isoenzymes of creatine phosphokinase and glutamic-oxaloacetic-transaminase were evaluated preoperative and postoperatively to assess myocardial injury in the patients undergoing open heart surgery with cardioplegia between one and two hours.

MATERIALS AND METHOD

27 patients who underwent aorto-coronary bypass procedure (ACB) 22 patients, aortic valve replacement (AVR) 3 patients, and mitral valve replacement (MVR) 2 patients were selected in this investigation.

Operations were conducted using cardiopulmonary bypass with cold cardioplegic solution associated with systemic hypothermia (25–28°C of rectal temperature). Cardioplegic solution contained 50 grams of glucose, 10 units of regular insulin and 40 mEq of potassium chloride in 1,000 ml of solution. The pH of solution was

adjusted at 7.40 by sodium bicarbonate. Average amount of cardioplegic solution injected was 727 ± 72 ml. Duration of ischemic arrest ranged between 53 and 92 minutes (average 78.6 ± 4.9 minutes). The ischemic period was strictly considered in this investigation being without significant difference among the treated and non-treated group. The prime solution of cardiopulmonary bypass contained 1,000 ml of Ringer's lactate solution, 300 ml of 25% of Mannitol, 60 ml of sodium bicarbonate, 50 grams of Albumin and whole blood to obtain the hematocrit level around 20–25%.

A protease inhibitor, aprotinin (Trasylo[®]), 5,000 KIU/Kg was administered intravenously 30 minutes prior to institution of CPB, then 5,000 KIU/Kg of aprotinin was given into the prime solution. Effect of aprotinin was evaluated in 9 patients of 7 CAB and 2 AVR.

Serum beta-glucuronidase, acid-phosphatase and isozymes of creatine-phosphokinase and glutamic-oxaloacetic-transaminase were measured before CPB and 0, 1, 2, 4, 6 and 24 hours in the period of reperfusion following cardioplegia. Mixed venous blood was withdrawn for these determinations.

RESULT

No operative mortality or perioperative infarction was noted in aprotinin-treated and non-treated groups.

Changes in beta-glucuronidase activity were shown in Figure 1. Aprotinin depressed significantly the activity of beta-glucuronidase compared to non-treated group for 6 hours following cardioplegia; beta-glucuronidase activity in aprotinin-treated group was from 65 to 85% of control level for 4 hours of reperfusion, then returned to control level afterwards.

Alterations of acid-phosphatase were demonstrated in Figure 2. Values of acid-phosphatase during 6 hours following cardioplegia were lowered than control, however, no significant difference was shown among two groups.

Changes of MB-CPK activity were shown in Figure 3. MB-CPK at the beginning of reperfusion was 1.1 ± 1.1 IU in aprotinin-treated group, and 9.4 ± 2.2 IU in non-treated group ($p < .05$). As shown in Figure 3, aprotinin-treated group showed the lower activity of MB-CPK compared to non-treated group following cardioplegia.

Increment of m-GOT compared to control value were shown in Figure 4. m-GOT at the

BETA-GLUCURONIDASE

Fig. 1. Changes of beta-glucuronidase were shown in aprotinin-treated group (●, n=8) and non-treated group (△, n=8). Values are expressed as % of control obtained before CPB. * indicates significant difference to non-treated group ($p < 0.05$).

ACID PHOSPHATASE

Fig. 2. Changes of acid-phosphatase were plotted in aprotinin-treated group (●, n=7), and non-treated group (△, n=7). Values were expressed as % of control obtained before CPB.

beginning of reperfusion following cardioplegia was significantly depressed in aprotinin-treated group compared to non-treated group.

DISCUSSION

Myocardium subjected to hypothermic cardioplegia with glucose- potassium- insulin has been well protected from ischemia with respect to lysosomal enzymes, isozymes of creatine-phosphokinase and glutamine-oxaloacetate-transaminase. Aprotinin prevented myocardium from

MB-CPK

Fig. 3. MB-CPK activity in the serum was shown in this figure in aprotinin-treated group (●, n=6), and non-treated group (△, n=17). * indicates significant difference to non-treated group ($p < 0.05$).

m-GOT

Fig. 4. Changes of m-GOT were shown in aprotinin-treated group (●, n=9), and non-treated group (△, n=18). * indicates significant difference compared to non-treated group ($p < 0.05$).

ischemic damage significantly compared to non-treated myocardium.

Effect of aprotinin upon lysosomal enzyme has been well documented.³⁰ Glenn et al²³ showed that aprotinin suppressed production of myocardial depressant factor, however, release of lysosomal enzymes to plasma was not inhibited by aprotinin in hemorrhagic shock. Therefore, aprotinin inhibits tyrosinase activity which links with lysosomal enzymes in the pancreas to produce myocardial depressant factor. While, Spilberg et al³¹ reported that the protective action of aprotinin is dependent upon the inhibitory mechanism to lysosomal enzymes. Suemori and

Yoshitake³² using rat in shock model supported the same mechanism of aprotinin as Spilberg et al.

It is agreed that CPB operated for long period of time results in the injury of capillary endothelial cells, disturbance of capillary permeability and tissue damage secondary to increased production of protease released from lysosome.³³ Suemori and Yoshitake³² demonstrated that release of lysosomal enzymes such as beta-glucuronidase and acid-phosphatase were significantly depressed following two hours of CPB by aprotinin which was administered with 12,000 KIU/Kg before CPB. With respect to lysosomal enzymes, only beta-glucuronidase was significantly depressed in aprotinin-treated group following cardioplegia in this investigation.

Regarding changes of acid-phosphatase, aprotinin did not demonstrate significant difference in protective effect compared to non-treated group. However, the value of acid-phosphatase was lowered than control level within 6 hours following cardioplegia. This result suggests that cold cardioplegia with hypothermia plays a protective role in the myocardium. Suemori et al.³² who used higher dose of aprotinin than ours as pretreatment, showed that aprotinin significantly suppressed the release of acid-phosphatase compared to non-treated group. They also demonstrated that acid-phosphatase level was lowered compared to control in early reperfusion following ischemic arrest both in aprotinin- and non-treated groups. It is consistent with our result.

Pretreatment of aprotinin was selected in order to protect the myocardium from ischemia because the hemodynamic support by CPB was at most several minutes before initiation of cardioplegia, and coronary circulation is ceased during ischemic arrest.

MB-CPK and m-GOT which are sensitive indicators to myocardial cellular injuries were obtained immediately after unclamping aorta. Their values in the serum were significantly depressed in aprotinin-treated group compared to non-treated group. Therefore, aprotinin prevents myocardium from ischemic injury during cardioplegia. These enzymatic levels from two to six hours following reperfusion were lowered in aprotinin-treated group than non-treated group, however, it failed to demonstrate significant difference among two groups. Furthermore, alterations of serum MB-CPK and m-GOT were minimal following hypothermic cardioplegia used in this inves-

tigation. This result may suggest that hypothermic cardioplegia protected myocardium from ischemic injury during open heart surgery. Diez and Maroko²¹ and Massion et al.²² have shown the significant protection of aprotinin upon ischemic myocardium in the experimental model of coronary artery ligation. They demonstrated that aprotinin was effective to reduce the severity of ischemic myocardial injury or was beneficial for the reduction of the size of the myocardial infarct. Our data obtained in open heart surgery have demonstrated that aprotinin has additive protection of myocardium to cold cardioplegia.

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