EFFECT OF DEFIBRROTIDE ON ENDOTHELIN LEVELS OF RABBITS IN MYOCARDIAL ISCHEMIA-REPERFUSION MODEL

Defibrotide is an antithrombotic and profibrinolytic drug which modulates endothelial function. A cardioprotective role of defibrotide on myocardial ischemia also has been proposed. In the present study we aimed to determine the effect of the drug on endothelin secretion, and measured the basal endothelin (ET1) levels of rabbits after 2 days of intravenous administration of defibrotide (50 mg/kg) and compared with control rabbits. Furthermore, to investigate the mechanism of cardioprotective actions of defibrotide during myocardial ischemia and reperfusion, endothelin I levels of rabbits were measured after myocardial ischemia induced by coronary artery ligation and reperfusion. The results have shown that defibrotide has no significant effect on endothelin levels of rabbits.

Key words: Defibrotide, endothelin 1, myocardial ischemia-reperfusion.

D efibrotide (Prociclide, Crinos) is the sodium salt of a single stranded DNA prepared from bovine lungs by controlled depolymerization. Defibrotide is a drug which has antithrombotic, profibrinolytic and antischismic effects without anticoagulant activity. The antithrombotic and fibrinolytic activity of defibrotide is due to its induction of tissue plasminogen activator (tPA) and PGI2 release from the endothelium and inhibition of plasminogen activator inhibitor (PAI). The drug is used in treatment of vascular disorders like peripheral obliterative arterial diseases and acute thrombophlebitis or prevention of deep vein thrombosis. Furthermore, it has been shown that the drug has cardioprotective effects during myocardial ischemia (MI), and is able to protect ischemic myocardium from early reperfusion damage. The beneficial effects of the drug has been attributed to stimulated profibrinolytic activity and prostacyclin generation. Considering that the drug plays an important role in modulation of endothelial functions, there is possibility of involvement of other
factors released from the vascular endothelium such as endothelins which play important role in regulation of blood flow. Three isoforms of endothelins have been characterized (ET-1, 2, 3). ET1 is the most potent vasoconstrictor and inotropic substance known. Although ET1 produced throughout the vascular endothelium participates in normal maintenance of vascular smooth muscle tone by paracrine functions, considerable evidence suggest a pathophysiological role for increased circulating endothelins. Several studies have reported that ET1 level increases during myocardial ischemia. To determine the effect of defibrotide on ET1 secretion, and to investigate the mechanism of cardioprotective effect of defibrotide in pathophysiological conditions like myocardial ischemia and reperfusion, ET1 levels of rabbits were measured after 2 days of intravenous administration, before and after a coronary artery ligation and reperfusion.

### MATERIALS AND METHODS

1. Animals:
Experiments were performed on 1.5-2 kg male albino rabbits which were kept in light and temperature controlled room and were fed with a standard diet (Istanbul Yem Sanayi, Topkapı), and had free access to water. Defibrotide (50 mg/kg) was administered intravenously to rabbits for 2 days. Sterile saline were given to control rabbits instead of defibrotide.

2. Experimental procedure:
Rabbits of either group were fasted overnight, anesthetized with 1.25 mg/kg of urethan given intraperitoneally. The right carotid arteries were catheterized for withdrawal of blood. After fentanyl citrate (3 μg) administration, animals were endotracheally intubated and mechanically ventilated with room air. Their chests were opened by median sternotomy and pericardium was opened vertically. The proximal portion of left anterior descending coronary artery (LAD) was ligated. After 60 min of occlusion, ligation was removed and after 60 min of reperfusion animals were sacrificed. Blood samples were collected, (a) just before median sternotomy, (b) after 60 minutes of ischemia produced by left anterior descending coronary artery ligation, (c) after 1 hour of reperfusion period. The endothelin1 concentrations were measured in collected blood samples. During the experiments the animals were monitored and ECG's were taken at various intervals.

3. Endothelin-1 determination:
Endothelin-1 concentrations were measured in rabbit plasma by radioimmunoassay (Amersham). The blood from rabbits were collected into tubes containing 7.5 mM EDTA and aprotinin (500 KIU/ml). Blood was centrifuged immediately at 2000g for 10 minutes at 4°C to remove cells and the plasma stored below -15°C prior to analysis. Endothelins in plasma samples have been extracted by using Amprep minicolumns (Amersham International). RIA is based on the competition between unlabelled ET-1 and a fixed quantity of 125I-labelled ET-1 (synthetic) for a limited number of binding sites on an ET-1 specific antibody.

4. Statistics:
Statistical analysis of the differences between the groups were performed by Mann-Whitney Test. Time related differences within the groups were also analyzed by Friedman Non-parametric Reapeted Measures Test. The values are expressed as median±SEM, n represents

<table>
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<th>TABLE 1: Plasma endothelin concentrations (fmol/ml) of control and defibrotide administered rabbits measured before coronary artery ligation, after myocardial ischemia (MI) and after reperfusion. Statistical analysis of data were performed by Mann-Whitney Test. The values are expressed as median±SEM, n represents the number of animals used in the experiments. A probability value of p&gt;0.05 represents a nonsignificant difference.</th>
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<td>Baseline</td>
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<tr>
<td>Control</td>
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<td>n=9</td>
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<td>Defibrotide</td>
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<td>P value</td>
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the number of animals used in the experiments. A probability value of P>0.05 represents a nonsignificant difference.

RESULTS

Endothelin levels of the plasma samples taken from the rabbits treated with defibrotide a) before LAD ligation b) after ischemia, and c) after reperfusion period were a) 13.43±3.29, b) 15.80±5.92, and c) 14.75±5.96 fmol/ml respectively. Similarly endothelin levels of control rabbits were a) 16.25±3.61, b) 27.37±4.11, c) 12.87±4.42 fmol/ml respectively. The results are shown in Table 1. The differences between the two groups are not significant. Also time related differences within the groups were also found insignificant.

DISCUSSION

Defibrotide is a drug which modulates endothelial function and it is proposed to be the first drug in endothelial cell supporting therapy. The drug has antithrombotic and profibrinolytic activity due to its stimulation of synthesis and release of plasminogen activator and prosta...


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