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

H. Moini, Pharm
Ç. Erşahin, MD
S. Bilsel, PhD
K. Emerk, PhD
M.Süngün, MD,
N. Doğan, MD

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.

From: Departments of Biochemistry, Marmara University, Sclool of Medicine and , Cardiovascular Surgery, Gülhane Military School of Medicine, İstanbul TÜRKİYE

Adress for reprints:
Serpil Bilsel, PhD
Marmara University,
School of Medicine, 81326,
İstanbul, TÜRKİYE

Defibrotide (Prociclide, Crinos) is the sodium salt of a single stranded DNA prepared from bovine lungs by controlled depolymerization[1]. Defibrotide is a drug which has antithrombotic, profibrinolytic and antiischemic effects without anticoagulant activity[2-5]. The antithrombotic and fibrinolytic activity of defibrotide is due to its induction of tissue plasminogen activator (tPA) and PGI_2 release from the endothelium and inhibition of plasminogen activator inhibator (PAI)[6-9]. The drug is used in treatment of vascular disorders like peripheral obliterative arterial diseases and acute thrombophlebitis or prevention of deep vein thrombosis[4]. 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[10]. The beneficial effects of the drug has been attributed to stimulated profibrinolytic activity and prostacyclin generation[8,10]. 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 subtance known[12]. Although ET1 produced throughout the vascular endothelium participates in normal maintenance of vascular smooth muscle tone by paracrine functions, considerable evidence suggest a pathophy- siological role for increased circulating endothelins. Several studies have reported that ET1 level increases during myocardial ischemia[13]. To determine the effect of defibrotide on ET1 secretion, and to investigate the mechanism of cardioprotective effect of defibrotide in pathological 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.

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 analyisis of data were performed by Mann-WhitneyTest. The values are expressed as median±SEM., n represents the number of animals used in the experiments. A probability value of p> 0.05 represents a nonsignificant difference.

	Baseline	After MI	After reperfusion
Control	16.25±3.61 n=9	19.76±4.11 n=9	16.16±4.42 n=8
Defibrotide	16.15±3.29 n=10	22.15±5.92 n=9	19.93±5.96 n=9
P value	0.8868	1.0000	0.9537

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 standart diet (İstanbul 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 intraperitonally. The right carotid arteries were catheterized for withdrawal of blood. After fentanyl citrate (3µg) 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 decending coronary artery (LAD) was ligated. After 60 min of occlusion, ligature 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 monitorized 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 minicolums (Amersham International). RIA is based on the competition between unlabelled ET-1 and a fixed quantity of ^{125}I-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 Nonparametric Reapeted Measures Test. The values are expressed as median±SEM, n represents

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[14]. The drug has antithrombotic and profibrinolytic activity due to its stimulation of synthesis and release of plasminogen activator and prostacylin from vascular endothelial cells[4,6-9]. Defibrotide has been shown to prevent biochemical and haemodynamic alterations caused by acute myocardial ischemia (AMI) induced by coronary occlusion in various animal models[15,16]. In experimental models of MI, the drug significantly attenuated ischemia induced cardiac dysfunction[8], reduced area of infarcted myocardium[17], attenuated increase in creatine kinase levels[18], and prevented ischemia induced ECG deviations[16]. It is suggested that the cardioprotective effects of defibrotide in animal models of cardiac ischemia may be due to increased PGI_2 [8,10], and decreased leukotriene B4 formation[19], but the complete mechanism of action is not completely understood. Since improved functional recovery after ischemia and reperfusion can be attained by increased coronary blood flow, release of vasoactive factors other than PGI_2 from endothelium may be involved in the mechanism of action of the drug. One of the factors which this agent could effect to change the blood flow is endothelin 1. ET1 is the most potent vasoconstrictor and inotropic agents known[11,12]. It is reported that endothelin1 level increases during MI[13]. Experimental data suggest that ET1 may contribute indirectly to regulation of regional and systemic vascular resistance by modifying the secretion of mediators including renin, angiotensin, aldosterone, atrial natriuretic peptide, prostaglandins and endothelium derived relaxing factor[11]. Although, it was shown that Defibrotide, by enhancing prostacycline generation, prevents endothelin-1 induced contraction in human saphenous veins[9], the direct effect of defibrotide on endothelin levels has not been investigated. To elucidate the possible role of defibrotide on endothelin levels in physiological conditions like myocardial ischemia and reperfusion, ET1 levels of rabbits were measured after 2 days of intravenous administration, before and after coronary artery occlusion and reperfusion. According to our findings, defibrotide does not effect endothelin 1 secretion from vascular endothelium under basal and pathological conditions. Our results showed slight increase in ET 1 levels after myocardial ischemia in both control and defibrotide treated groups but the increase was not statistically significant. Lack of significant increase in plasma endothelin levels of rabbits after MI may be due to use of healthy animals in our MI model. It should also be remembered that endothelins are local paracrine and autocrine mediators secreted in a polar fashion and circularing levels of ET1 may represent only a fraction having escaped local binding. Further studies are necessary to determine the precise mechanism of the cytoprotective action of defibrotide in ischemic-reperfused myocardium and endothelium.

REFERENCES

1- Prociciledi, U.S. patent no. 3, 770, 720(1973); British patent no. 1, 367, 655(1975)

2- Niada R, Mantovani M, Prino G, Pescador R, Nardi G: Antithrombotic activity of polydeoxyribonucleo

tides of mammalian origin (laboratory code: fraction P) in experimental animals. Thromb Haemost 1979; 42: 474-478

3- Niada R, Mantovani M, Prino G, Pescador R, Berti F, Omini C, Folco G: Antithrombotic activity of a polydeoxynucleotidic substance extracted from mammalian organs: A possible link with prostacycline. Thromb Res 1981; 23: 233-246

4- Palmer K, Goa K: Defibrotide: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in vascular disorders. Drugs 1993; 45:. 260-294

5- Niada R, Porta R, Pescador R, Mantovani M, Prino G, Berti F: Cardioprotective effect of defibrotide in acute lethal and nonlethal myocardial ischemia in the cat. Haemostasis 1986; 16 (suppl 1) 18-25

6- Davi G, Catalano I, Belvedere M, Amato S, Mogavero A: Effects of defibrotide on fibrinolytic activity in diabetic patientts with stable angina pectoris. Thromb Res 1992; 65: 211-220

7- Violi F, Ferro D, Saliola M, Quintaralle C, Basili S: Effect of oral defibrotide on tissue plasminojen activator and tissue plasminogen activator inhibitor balance. Europ J Clinical Pharmacol 1992; 42: 379-383

8- Hohfeld T, Strobach H, Schror K: Stimulation of prostacycline synthesis by defibrotide: Improved contractile recovery from myocardial stunning. J. Cardiovas Pharmacol 1991; 17: 108-115

9- Berti F, Rossoni G, Biasi G, Buschi A, Mandelli V: Defibrotide, by enhancing prostacycline generation, prevents endothelin-1 induced contraction in human saphenous veins. Prostaglandins 1990; 40: 337-350

10- Thiemermann C, Lobel P, Schror K: Usefulness of defibrotide in protecting ischemic myocardium fromerly reperfusion damage. Am J Cardol 1985; 56: 978-982

11- Leppaluoto J, Ruskoaho H: Endotelin peptides: biological activities, cellular signalling and clinical significance. Ann Med 1992; 24: 153-161

12- Kramer BK, Nibhida M, Ralph AK, Smith TW: Endothelins. Myocardial actions of a new class of cytokines. Circulation 1992; 85(1): 350-356

13- Miyauchi T, Yanagisawa M, Tomizawa T: Increased plasma concentrations of endothelin and big endothelin in acute myocardial infarction. Lancet 1989; 11: 53-4

14- Ulutin ON: Antithrombotic effect and clinical potential of defibrotide. Semin Thromb Hemost 1993; 19: 186-191

15- Niada R, Porta R, Pescador R, Mantovani M, Porino G: Cardioprotective effects of defibrotide in acute myocardial ischemia in the cat. Thromb Res 1985; 38: 71-81

16- Hohlfold T, Thiemermann C, Schror K: Protection from myocardial ischemic injury in cats and pigs by defibrotide-different mode of action: Prostaglandins in clinical research. Cardiovascular System 1989; 137-141

17- Thiemermann C, Thomas GR, Vare JR: Defibrotide reduces infarct size in a rabbit model of experimental myocardial ischemia and reperfusion and regional myocardial blood flow on the ischemic reperfused rabbit heart. Br J Pharmacol 1989; 97: 401-408

18- Milazzotto F, Carelli M, Citone C, Di Marcotullio G, Giampaolo P: Use of defibrotide in the treatment of acute myocardial infarction. Semin Thromb Hemost 1989; 15: 464-469

19- Biagi G, Legnani C, Rodrigo G, Coccheri S: Modulation of arachidonate metabolite generation in human blood by the oral defibrotide. Arzneimittel—Forschung/Drug Research 1991; 41: 511-514