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PROFIBRINOLYTIC ACTIVITY OF DEFIBROTIDE IS PROSTACYCLIN-INDEPENDENT. R. Porta, R. Pescador, R. Niada, M. Mantovani and G. Prino. Crinos Biological Research Laboratories, Piazza XX Settembre 2, 22079 Villa Guardia, Como, Italy.

The profibrinolytic, antithrombotic and thrombolytic activities of Defibrotide, a polydeoxyribonucleotide obtained from mammalian lungs, have been previously described. Defibrotide is also able to promote the generation and release into the circulation of prostacyclin and of plasminogen activator factor (tPA) from vascular walls. These two last activities, i.e., PGI_2 and tPA stimulation, could be of importance in the profibrinolytic activity of Defibrotide. In an attempt to clarify the profibrinolytic mechanism/s of Defibrotide, the role of PGI_2 generation in this activity was investigated in rabbits in which the cycloxygenase pathway was blocked with Indomethacin. Male rabbits were treated with Indomethacin, 10 mg/Kg, i.v., 20 minutes or 2 hours before Defibrotide administration. Defibrotide was injected i.v. into rabbits at two dose levels (16 or 64 mg/Kg). Rabbits treated with Defibrotide only were used as controls. The profibrinolytic activity was evaluated measuring the lysed area of normal human fibrin plates induced by euglobulin fraction obtained from plasma. Results are reported in the Table.

Treatment	lysis areas (mm^2) basal	5'after Defibrotide
Vehicle + D, 12.5 mg/Kg, i.v.	146.4 ± 10.4	219.4 ± 14.2 ●
Indo* + D, 12.5 mg/Kg, i.v.	168.5 ± 22.2	237.5 ± 21.1 ● ▲
Indo** + D, 12.5 mg/Kg, i.v.	131.1 ± 15.5	206.0 ± 22.8 ● ▲
Vehicle + D, 50 mg/Kg, i.v.	144.5 ± 6.9	337.1 ± 11.0 ●
Indo* + D, 50 mg/Kg, i.v.	130.8 ± 5.3	358.1 ± 10.2 ● ▲
Indo** + D, 50 mg/Kg, i.v.	148.7 ± 14.1	308.5 ± 6.9 ● ▲

Indomethacin (Indo) administered 20 min (*) or 2h (**) before Defibrotide.
ANOVA: ▲ N.S. vs. Vehicle + D 12.5 or D 50 mg/Kg i.v.; ● $P<0.01$ vs. basal.
The results reported here demonstrate that the profibrinolytic activity of Defibrotide is independent of PGI_2 generation: Indomethacin pretreatment was unable to suppress or to reduce the fibrinolytic response of the rabbit to Defibrotide.

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DEFIBROTIDE IN EXPERIMENTAL MYOCARDIAL ISCHEMIA IN THE CAT: EFFECTS ON HEMODYNAMICS, ENERGY METABOLISM AND INFARCT SIZE. R. NIADA, R. Porta, R. Tettamanti, R. Pescador, M. Mantovani and G. Prino. Crinos Biological Research Laboratories, Piazza XX Settembre 2, 22079 Villa Guardia, Como, Italy.

Defibrotide was able to prevent the hemodynamic and biochemical alterations caused by acute myocardial ischemia (AMI) induced by coronary occlusion in the cat when infused 3.5 h before and 5 h after left anterior descending coronary artery (LAD) occlusion. In the platelet perfused heart, Defibrotide was a selective stimulator of coronary vascular PGI_2 but not of platelet thromboxane formation. The present study was designed both to investigate the effects of Defibrotide injected 30 min after the induction of acute myocardial ischemia (AMI) in the cat and to evaluate the ability of this drug to reduce infarct size. In the first set of experiments a permanent ligature (5 hours) was placed around LAD. ST segment from ECG, mean aortic pressure (MAP), heart rate (HR) and the pressure-heart rate index (PRI) were considered. Plasma and tissue creatine phosphokinase activity (CPK), tissue lactate and ATP were measured by enzymatic kits from Boehringer Biochemia. 30 min after coronary occlusion a loading dose of Defibrotide (32 $mg\,Kg^{-1}$) was administered i.v. immediately followed by an infusion (32 $mg\,Kg^{-1}\,h^{-1}$ 4.5 h) MAP, HR and PRI were not modified either by AMI or by the infusion of Defibrotide. AMI-ST segment increases were reduced by Defibrotide from 0.5 h after the beginning of the treatment (-49% vs. AMI control) to the end of experiments (-83% vs. AMI control after 5 h occlusion period). Plasma CPK was reduced from 2.5 h after the beginning of the treatment (-29%) till the end of experiments (-52%). Ischemic tissue CPK, lactate and ATP were normalized by Defibrotide. In the second set of experiments the animals were infused with Defibrotide (50 or 200 $mg\,Kg^{-1}\,h^{-1}$, i.v.) starting 2 hours before coronary ligature. The infusion was maintained throughout the 5 h occlusion period. The risk and infarct areas were measured by Evans blu and nitroblue tetrazolium staining. The 51 ± 3% of risk area was infarcted in AMI control cats. Defibrotide at the two tested doses significantly reduced these infarct areas to 42 ± 4% and 34 ± 2% of risk areas respectively. The beneficial effects of Defibrotide observed in AMI could be attributed both to its ability to enhance PGI_2 release from vascular walls and to improved local tissue oxygenation and energy supplies. However it could be taken into account a direct cytoprotective action.

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DEFIBROTIDE DECREASES CHOLESTEROL CONTENT IN HYPERCHOLESTEROLEMIC RABBIT AORTA, WITH NO MODIFICATION OF PLASMA OR LIPOPROTEIN CHOLESTEROL. R. Pescador, R. Porta, R. Niada, M. Mantovani and G. Prino. Crinos Biological Research Laboratories, Piazza XX Settembre 2, 22079 Villa Guardia, Como, Italy.

Defibrotide was shown to stimulate the production of endogenous PGI_2 from rat and hamster aortic tissue, as well as the production of PGI_2 from the coronary vascular bed in the platelet perfused heart model. This effect was only seen in presence of platelets. During the infusion period with Defibrotide thromboxane release remained unaffected while platelet cAMP rised. Defibrotide, was also able to reduce the secretion of ATP from platelets as well as to deaggregate platelet clumps. It has been postulated that long-term administration of stable PGI_2 metabolites or analogues could be a more useful means of enhancing cholesterol and cholesteryl ester mobilization out of the arterial "foam" cell during early stages of cardiovascular disease. These observations prompted us to administer Defibrotide i.v. to cholesterol fed rabbits to verify if it could cause a decrease in cholesterol content of aortas. Aorta cholesterol was evaluated by gas chromatography. Plasma or lipoprotein lipids were assayed by enzymatic kits from Boehringer Biochemia. Plasma lipoproteins were separated by density gradient ultracentrifugation. Platelet aggregation was carried out with ADP using an optical aggregometer to find out the effective aggregatory concentration fifty (EC_{50}). Histology of the rabbit hearts was performed by optical microscopy on heart sections coloured with hematoxylin-eosine. Defibrotide caused a decrease (-49%, $P < 0.05$, vs. cholesterol fed rabbits treated with placebo) in cholesterol content of aortas with no modification of total plasma cholesterol, triglyceride and phospholipid. The cholesterol, triglyceride, phospholipid and protein of plasma lipoproteins were not affected too. The EC_{50} of rabbits treated with Defibrotide was normalized (-9%, N.S. vs. control animals fed the normal diet and treated with placebo; cholesterol fed animals treated with placebo: -32%, $P<0.05$ vs. control animals fed the normal diet). Vascular lesions in the hearts of animals treated with Defibrotide had a lower rate (33%) in comparison to that (87%) of animals fed with cholesterol and treated with placebo. It is concluded that the ability of Defibrotide to stimulate vascular PGI_2 formation and to reduce platelet sensitivity could be helpful in reducing the amount of cholesterol in the cardiovascular system in atherosclerotic prone situations.

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