0211

EFFECTS OF FIBRIN,FIBRINOGEN AND FIBRIN(-OGEN) DEGRADATION PRODUCTS ON THE GROWTH OF AORTIC SMOOTH MUSCLE CELLS IN CULTURE. K.Tanaka, T.Ishida and K.Watanabe. Department of Pathology, Kyushu University, Fukuoka, Japan.

Importance of smooth muscle cell proliferation has been emphasized as a key event in atherogenesis. A number of factors have been shown to stimulate cell proliferation in vitro. These include platelet, lipoprotein, insulin and so on. We studied the effects of fibrin, fibrinogen and fibrinogen degradation products on the growth of aortic smooth muscle cells in culture. Smooth muscle cells in the 7th-15th subculture were grown from the explants of the media of rabbit thoracic aortas.

Growth behavior of the cultured smooth muscle cells in response to fibrin clot was observed with phase-contrast microscope. Fibrin clot stimulated the cell proliferation during the period of 24 hours cultivation.

Effects of fibrin,fibrinogen and fibrinogen degradation products on the incorporation of [^{3}H] thymidine by the cultured smooth muscle cells were studied in replicate culture method. Both fibrin and fibrinogen stimulated the incorporation of [^{3}H] thymidine during the period of 24 hours cultivation. But they inhibited the incorporation after 48 hours, and smooth muscle cells were degenerated and detached from the tissue culture dish. Fibrinogen degradation products inhibited the incorporation of [^{3}H] thymidine by smooth muscle cells.

The cultured smooth muscle cells showed fibrinolytic activity by Todd's fibrinolysis autography.

These results suggest that both fibrin and fibrinogen have the stimulatory effect, while fibrinogen degradation products have the inhibitory effect on the proliferation of smooth muscle cells, and that fibrinolytic activity of arterial wall might play a role in smooth muscle cell proliferation that leads to the development of atherosclerotic lesions.

0212

RETRACTION OF FIBRIN CLOTS BY CULTURED ENDOTHELIAL CELLS. B. Barbieri, G. Balconi, E. Dejana and M.B. Donati. Istituto di Ricerche Farmacologiche "Mario Negri, Milan, Italy.

The interactions between endothelial cells (EC) and fibrin are still poorly understood. We approached this problem by studying the ability of cultured EC to induce in vitro the retraction of fibrin clots. EC were obtained from bovine aorta and human umbilical veins by collagenase treatment and grown in Eagle MEM. At the time of the test the cells were harvested from the flask by a short trypsin-EDTA treatment and resuspended in tyrode solution. The test system involved incubation of the cell suspension in a water-bath at 37°C in the presence of cell-free plasma which was clotted by thrombin. The course of retraction was followed by measuring the diameter of the clot with a microcaliper. Retraction values were expressed after calculation of percent activity by an appropriate formula. EC were found to induce the retraction of the fibrin clot to an extent which increased with the time (1-24 h) and with the number of cells in the system ($1\text{-}4\times10^6$/ml f.c.). Fibrin clot retractile (FCR) activity of EC could not be detected at 22°C or in presence of Na_2-EDTA or using mechanically disrupted cells. Moreover, using batroxobin instead of thrombin as a clotting agent, no retraction occurred; FCR of EC thus showed many characteristics in common with platelet- and fibroblast-induced clot retraction.

FCR activity of bovine EC increased with the number of subcultures, being very low in cells harvested from primary cultures. In contrast, human EC had high activity in primary cultures. Similarly to fibroblasts, EC with higher density in culture showed lower FCR, suggesting that confluency inhibits the cell contractile capacity.

FCR could thus represent a simple in vitro test to further characterize the biology of EC and to evaluate their role in the development of fibrin thrombi.

0213

THROMBIN-INDUCED RELEASE OF ADENINE NUCLEOTIDES FROM CULTURED HUMAN ENDOTHELIAL CELLS. P. Lollar and W.G. Owen. The Cardiovascular Center and Departments of Internal Medicine, Pathology, and Biochemistry, University of Iowa College of Medicine, Iowa City,Iowa,USA.

The effect of thrombin on the release of adenine nucleotides from cultured human umbilical vein endothelium was studied. When endothelial cell monolayers were incubated with ^{3}H-adenosine for two hours at 37° C, fifty percent of the radioactivity was incorporated into the cell. Thin-layer radiochromatography revealed that greater than 90% of the tritium was associated with ADP and ATP.

Addition of thrombin to the monolayers resulted in the release of tritium into the medium. This effect was dose-dependent and saturable, with a maximal response seen at 1×10^{-8} M thrombin. Diisopropylphosphoryl thrombin(DIP-thrombin), which is enzymatically inactive, did not cause a release of tritium. Blocking the high-affinity thrombin binding sites which are present on the endothelial cell surface was done with DIP-thrombin. Saturating these binding sites by greater than 95% with a fifty-fold excess of DIP-thrombin had no effect on thrombin-induced release, indicating that the high-affinity binding sites are not involved in the release of adenine nucleotides by thrombin.

0214

THROMBIN-INDUCED REFRACTORINESS OF PGI_2 AND ADENINE NUCLEOTIDE RELEASE FROM THE VASCULAR ENDOTHELIUM. A.F. Adams Brotherton and J.C. Hoak. Department of Medicine, University of Iowa, Iowa City, IA.

Primary monolayer cultures of human umbilical vein endothelium release both PGI_2 (measured by radioimmunoassay of 6-keto-$PGF_{1\alpha}$) and adenine nucleotides (AN) (measured as release of ^{3}H from monolayers prelabeled with [^{3}H]adenosine) when incubated with thrombin (0.5 U/ml) or A23187 (10 μM). The release of PGI_2 and AN in response to A23187 was greater than that observed in response to thrombin by an average of 2- and 3.5-fold, respectively. Preincubation of the endothelium with aspirin (100 μM) for 30 min resulted in a total inhibition of thrombin or A23187 induced release of PGI_2, but had no significant effect on the release of AN. Thus, AN release does not appear to be dependent on PGI_2 biosynthesis. After an initial exposure to thrombin, the endothelium became refractory to both PGI_2 and AN release. Prior exposure of the endothelium to thrombin also decreased A23187 induced release of PGI_2 by an average of 50%, but had no effect on AN release. Preincubation of the endothelium for 10 min with the cyclic nucleotide phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) (4 mM) increased the intracellular concentration of cyclic AMP (measured by radioimmunoassay) by an average of 3.5-fold, and caused a total inhibition of thrombin and A23187 induced release of PGI_2 and AN. Although PGI_2 (400 nM) and ADP (10 μM) increased cyclic AMP levels by several-fold in IBMX-treated endothelium, no significant increase was observed in the absence of IBMX. These findings suggest that the phenomenon of thrombin-induced refractoriness is not the result of a simple negative feedback mechanism involving the activation of adenylate cyclase by PGI_2 and AN released from the endothelium. Complex mechanisms involving the thrombin binding mechanism, depletion of Ca^{2+} gradients or inactivation of Ca^{2+}-dependent reactions are more likely to be responsible.