1277

THE EFFECT OF TICLOPIDINE ON CULTURED HUMAN ENDOTHELIAL CELLS: POSSIBLE INFLUENCE ON EXTRACELLULAR MATRIX FORMATION. F. Piovella, L. Piovella°, P. Almasio, M.M. Ricetti, E. Ascari°°. Istituto di Clinica Medica I°e °°Istituto di Patologia Medica I° dell Universita di Pavia, 27100 Pavia e °Medical Department "Crinos" S.p.A. Villaguardia, Como, Italy.

Endothelial cells derived from human umbilical cord vein were seeded and grown to confluence in culture medium TC 199 with 20% foetal calf serum without other growth factors supplement. Cells were then detached utilizing a trypsin-EDTA solution, harvested in fresh culture media containing different concentrations of Ticlopidine, poured on glass coverslips placed on 35 mm Petri dishes and cultured for six days without further medium replacement. Control dishes were prepared using fresh medium without Ticlopidine.

Treated cells revealed a decreased tendency to adhere to the substratum as well as morphological changes in comparison with controls when examined by phase contrast microscopy. This effect was more evident with higher concentration (0.33 mg/ml) of Ticlopidine, while lower concentrations (0.05 mg/ml) caused morphological changes only. Indirect immunofluorescence for fibronectin utilizing monospecific antibodies, revealed a different fluorescent pattern if compared with controls. In particular a less pronounced intracellular granular fluorescence and a delay in the appearance of the fibrillar extracellular matrix.

1278

DIFFERENCES IN CELL CYCLE BETWEEN HUMAN ADULT AORTIC AND VENA CAVA SMC IN CELL CULTURE.A IMPULS-CYTOPHOTOMETRIC STUDY.

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Human aortic and vena cava SMCs show different growth pattern and proliferation rates in cell culture.We further tried to substantiate these differences by examination of the cell cycle phases with the impuls-cytophotometry(ICP).As reported in the previous abstract SMCs were established from explant cultures of human adult aortic and vena cava.After sufficient outgrowth the SMCs were subcultivated and used for cell cycle analysis at different intervals.Acridine orange(AO), ethidium bromide(Eb) and mithramycin(M) were used for nucleic acid staining followed by fluorescence microscopy.For ICP a nucleus suspension was prepared and stained with Eb/M.DNA-histogramms of SMC were recorded in a ICP 22 in combination with a 500-channel distribution analyzer..Results:Clear DNA-histogramms of SMC-suspension could not be obtained because of the large cell size and their tendency to form aggregates in suspension.Therefore, a nucleus suspension of the appropiate culture was prepared.The AO-stained SMCs show a green and red staining of the nucleus. In Eb/M-stained SMCs a uniformly staining was observed. Histogramms of proliferating art.SMC show a peak at 4N(about 50%), a second peak at 2N (about 40%) and a minor one at 8N (about 10%).In contrast we found only two peaks in the vena cava SMCs at 2 N (about 85%) and at 4N (about 15%).

Summary: Again these results show that there a marked differences between the art. and venous SMCs.These results fit with the microscopic examinations and the growth curves for both types of SMCs.These differences have to be considered of the effect of drugs on functions of SMCs are examined.

1279

CHARACTERISATION OF VACCINIUM MYRTILLUS ANTHOCYANOSIDES EFFECT ON CULTURED HUMAN ENDOTHELIAL CELLS. F. Piovella, P. Almasio, M.M. Ricetti, E. Ascari*. Instituto di Clinica Medica I°, *Instituto di Patologia Medica I° - University of Pavia, Pavia 27100, Italy.

Vaccinium myrtillus anthocyanosides (Vma) have been demonstrated as significantly effective substances in promoting wound repair and the formation of new capillaries in experimental animals. Their effect have been also studied in clinical trials, demonstrating a significant anti-haemorrhagic action in patients with defective platelet-endothelium axis (primary haemostasis). We report the results of an extensive study performed on human endothelial cells (h.e.c.) derived from umbilical cord vein and cultured in vitro in the presence of various Vma concentration. As controls non-treated h.e.c. as well as dexamethasone-treated h.e.c. were used. H.e.c. growth rate as well as their morphology, factor VIII related antigen (FVIII R:Ag) content and release and fibronectin extracellular matrix, were the parameters studied in our experiments. Vma cause an increased h.e.c. growth in comparison with controls. This action is similar to the effect described for dexamethasone, but it is not mediated by variations of the fibronectin extracellular matrix. Vma-treated cells do not show any variations of FVIII R:Ag immunofluorescent pattern nor significant morphological changes. The stimulatory action on growth rate is extended to the other cell types of the vessel wall as demonstrated by the cultures of suspensions of h.e.c. highly contaminated by fibroblasts and smooth muscle cells.

1280

ARTERIAL THROMBOSIS IN HEPARIN-INDUCED THROMBOCYTOPENIA IS NOT ACCOMPANIED BY A DECREASED PGI2 STIMULATING ACTIVITY IN PLASMA. R.Kulzer, G. Schäfer, U. Budde, S. Popov. Institut für experimentelle Hämatologie und Bluttransfusionswesen der Universität Bonn, 5300 Bonn, FRG.

A patient developed severe thrombocytopenia and acute arterial occlusion 6 days after onset of heparin therapy. Heparin was administered because of venous thrombosis in the lower limbs. Aggregometry showed the typical signes of heparin-induced thrombocytopenia with all evaluated heparins of different origin. PGI2 stimulating activity of plasma was in the range of normal plasma samples. No aggregating activity of the plasma could be shown without heparin(Method of Lian et al.). Heparin activity was determined before and after aggregation with a chromogenic substrate.

After cessation of heparin therapy the platelets raised into the lower normal range within a few days.