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VASCULAR DAMAGE IN PATIENTS ON CHRONIC HEMODIALYSIS. K. Izumi (1), M. Noguchi (1), E. Iwasaki (1), K. Deguchi (2), S. Shirakawa (2). Department of Internal Medicine, Matsuzaka-chuou-Hospital, Kouseiren of Mie, Matsuzaka-city (1) and 2nd Department of Internal Medicine, School of Medicine, Mie University, Tsu-City (2), Mie, Japan.

Calcification in the abdominal artery and endothelial cell function of the peripheral veins were studied in the patients recieving maintenance hemodialysis for the chronic renal failure.

The grade of calcification was expressed by the aortic calcification index (ACI) calculation from the abdominal CT scan films. The endothelial cell function was estimated from the release capasity for tissue plasminogen activator (t-PA) and von Willebrand factor (vWF), and the fibrinolytic capacity (by ELT and fibrin plate lysis area) during 10-min venous occlusion (VO) of the cubital vein. The dialysed patients were divided into 4 groups according to the duration of hemodialysis : group A ; under 1 year (n=7), group B ; 1-4 years (n=14), group C ; 4-7 years (n=15) and group D ; 7-10 years (n=8).

The level (X + SD) of vWF : Ag, vWF act. (RCoF) and t-PA in patients before VO were 439 ± 255(%),171 ± 42(%), 4.2 ± 0.6(ng/ml) in group A, 292 ± 157, 151 ± 35, 4.1 ± 1.2 in group B, 174 ± 114, 133 ± 47, 3.7 ± 0.8 in group C and 130 ± 27, 66 ± 21, 3.1 ± 0.7 in group D, respectively. With the increase in the duration of hemodialysis, the release capacity for vWF and t-PA, and the fibrinolytic capacity during VO decreased regardless of the aggravation of fibrinolytic activity before VO, When the activity was estimated by ELT, Cl-inactivator resistant fibrinolytic activity and fibrin lysis area. These findings are thought to reflect the exhaustion of endotherial cells. In elder patients (60-80 years old), the release capacity for vWF and t-PA during VO decreased with the increase in the ACI level.

It is conclude that the patients on chronic hemodialysis have a greater incidence of calcification of abdominal artery and lower function of endothelial cells in the cubital vein than non-dialysed control.

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HUMAN ENDOTHELIAL CELLS MODIFICATIONS DURING LONG TERM CULTURE. M.P. Wautier, J.L. Wautier. U. 150 INSERM, LA334 CNRS Hôpital Lariboisière, Paris, France.

The culture of human endothelial cells is largely used for vascular research. The possibility of developping long term culture of human endothelial cells (EC) raised the question regarding the identity after several passages. To further investigate this aspect we have cultured human umbilical vein EC until the 12^{th} passage on fibronectin coated dishes supplemented with ECGF. We have studied the EC morphology by light and electron microscopy, the reactivity with ^{51}Cr labelled platelets, and prostacyclin synthesis. Until the 6^{th} passage no major change could be noted, except the occurence of rare large EC and a reduction in the doubling time between 2^{nd} and 5^{th} passage. After the 7^{th} passage up to the 10^{th} EC became more elongated and did not grow in strict monolayer. The number of vacuoles and mitochondria increased as well as the doubling time. After the 12^{th} passage the EC were still viable but proliferated very slowly. The adhesion of radiolabelled platelets dramatically increased (150%) and PGI_2 production significantly decreased (6 Keto $PGF_{1\alpha}$: 1st passage 13±2.5 ng; 6^{th} passage 0.33±0.27 ng/10^6 EC). In our culture conditions EC kept most of their original characteristics up to the 6^{th} passage but then lost some of them. At any passage EC contained Weibel Palade bodies and von Willebrand factor. We can conclude that after the 7^{th} passage EC in culture are different from the original cells and could possibly represent an in vitro model of EC ageing.

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MICROCARRIER CULTURE OF HUMAN ENDOTHELIAL CELL TYPES – A SOURCE OF METABOLITES. N.R. Hunter (1), I.R. MacGregor (1), J. Dawes (2) and D.S. Pepper (1). SNBTS HQ Laboratory (1) and MRC/SNBTS Blood Components Assay Group (2), 2 Forrest Road, Edinburgh, EH1 2QN.

The production of human endothelial cell secretory products in amounts sufficient for biochemical studies is largely restricted by the culture growth area. Conventional flat bed systems yield at best 20-30 x 10^6 cells per 180cm^2 culture flask. To overcome this problem, cells may be grown on Cytodex 3 microcarriers allowing large numbers of cells to be grown and conditioned in small culture volumes. A typical microcarrier unit will contain 200-300 x 10^6 cells and may be expanded in excess of 1000 x 10^6 cells at confluence. High viability (95%) and recovery (70-80%) in sub-culturing of microcarrier to microcarrier culture can be achieved with careful management of culture conditions and brief exposure to enzymes.

Human umbilical artery and vein, and saphenous vein endothelial cells were prepared and grown on microcarrier cultures to cell populations of 200-450 x 10^6 cells and conditioned for 14 day periods in serum-free media.

The production profiles of several endothelial cell proteins including thrombospondin (TSP), von Willebrand Factor (vWF) and issue plasminogen activator (t-PA) were measured by radioimmunoassay under these conditions, and demonstrate the use of microcarrier cultures in producing milligram quantities of endothelial cell protein. For example, a HUVEC culture of 200 x 10^6 cells conditioned with serum-free media for 14 days yielded a total of 6.9mg TSP, 0.7mg vWF and 48.9ug t-PA. In this laboratory one such application of the system was the purification of endothelial proteins in amounts sufficient for immunisation of mice prior to the production of monoclonal antibodies and for subsequent characterisation.

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HUMAN OMENTAL TISSUE MICROVASCULAR ENDOTHELIAL CELLS (HOTMEC): ISOLATION AND NEW ASPECTS OF CHARACTERIZATION. E. Anders (1), J.U. Alles (2), U. Delvos (1), B. Pötzsch (1), K.T. Preissner (1), W. Speiser (1) and G. Müller-Berghaus (1). Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen (1) and Department of Pathology, Justus-Liebig-Universität, D-6300 Giessen (2), FRG.

To study the function of microvascular endothelial cells in comparison to large vessel endothelial cells, HOTMEC were enzymatically isolated from human omental tissue and plated on petri dishes precoated with an extracellular matrix prepared from isolated fibroblasts of the same tissue or precoated with fibronectin. The culture medium was supplemented with 10% fetal calf serum; growth factors were not needed. HOTMEC were subcultured in a split ratio of 1:3 and maintained in culture for up to 3 month. Cultured HOTMEC were identified and discriminated from other non-endothelial cells by different characteristics and functions. 1. The cells demonstrated the typical polygonal shape as known for endothelial cells isolated from umbilical veins. In comparison to human umbilical vein endothelial cells (HUVEC), however, HOTMEC showed prominant nuclei with several nucleoli and presented a pronounced granulation of the perinuclear cytoplasm. 2. A monoclonal antibody specific for endothelial cells was bound to cultured HOTMEC. 3. Von Willebrand Factor (vWF) antigen was demonstrated within the cells by immunofluorescence staining; measurable amounts of vWF were only found in HUVEC in contrast to HOTMEC using an ELISA. 4. The addition of purified human protein C to HOTMEC preincubated with thrombin led to the activation of the zymogen as demonstrated by a chromogenic assay system. The kinetics of protein C activation were identical for HOTMEC and HUVEC. Tissue plasminogen activator (tPA) as well as plasminogen activator inhibitor (PAI) activity were detected in the culture supernatant of HOTMEC. After incubation period of 12 h in serum-free medium, the conditioned medium of confluent HOTMEC contained 100-fold higher levels of tPA than that of HUVEC. The data demonstrate that the cells isolated from human omental tissue have morphological as well as functional characteristics typical for endothelial cells. Furthermore, the study indicates that HOTMEC and HUVEC present quantitative differences in coagulant and fibrinolytic activities.