The HKU Scholars Hub The University of Hong Kong 香港大學學術庫

Title	Cytokine profiles in patients with lower limb ischaemia
Author(s)	Chan, YC; Gulati, V; Shukla, N; Okonko, DO; Abdus-Samee, M; Stanford, J; Mansfield, AO; Stansby, G
Citation	British Journal of Surgery, 1999, v. 86 n. suppl 1, p. 15-16
Issued Date	1999
URL	http://hdl.handle.net/10722/148730
Rights	British Journal of Surgery. Copyright © John Wiley & Sons Ltd.

Vascular 007

Platelet activation after bypass for critical limb ischaemia and the role of pharmacotherapy to increase graft survival

I.V. Mohan, C.S. Berwanger, M. Mireskandari, D. Okonko, N.J. Cheshire, A. Mansfield, J. Wolfe, D. Mikhailidis* and G. Stansby *Imperial College School of Medicine, Academic Surgical Unit, St Mary's Hospital, QEQM Wing, and *Royal Free Hospital, London*

Background: Irrespective of the initiating agent, the final common pathway leading to the formation of the platelet plug is platelet aggregation. The activated platelet glycoprotein IIb/IIIa receptor has been identified as the pivotal mediator of platelet aggregation. The aim of this study was to investigate the status of platelet activation after peripheral bypass and the effect of adjuvant therapy.
Method: We retrospectively investigated 15 patient, 11 male and four female, median age 70 (range 54–80) years, who had undergone bypass surgery for critical ischaemia attending the outpatient department, and 10 matched vascular controls with claudication. Whole blood platelet aggregometry was performed with spontaneous platelet aggregation (SPA), adenosine di-phosphate (ADP), and 5-hydroxytryptamine (5-HT) stimulation. Flow cytometric analysis was performed using monoclonal antibodies: PAC-1 for activated GPIIb/IIIa, and CD62P for P-selectin. Seven patients were on aspirin, and five warfarin; three had no adjunctive treatment. The aspirin and warfarin groups were compared to each other. Data were expressed as mean (± SEM) and statistical analysis was performed using Mann–Whitney U-test.
Results: Three min SPA was similar for patients and controls, 31·5 (± 3·65) per cent vs 36·5 (± 5·21) per cent, ($P = 0·7321$). However after ADP stimulation there was markedly increased platelet aggregation in patients 43·6 (± 6·07) per cent vs 87·88 (± 2·78) per cent, flow cytometric analysis for activated glycoprotein IIb/IIIa complex (PAC-1), 10·58 (± 1·88) per cent vs 26·08 (± 4·18) per cent; and P-selectin, 3·71 (± 0·95) per cent vs 7·02 (± 1·91) per cent, demonstrated increased resting platelet activation ($P < 0·001$). A subgroup analysis of all parameters between patients taking aspirin and warfarin was not significant ($P > 0·2020$), Mann–Whitney U-test).
Conclusion: Increased resting platelet activation occurs after bypass grafting in peripheral vascular disease, this continues despite the use of aspirin or warfarin. Alternative pharmacotherapy should therefore be considered for the high-risk graft.

Vascular 008

Identification of a 75kDa glycoprotein ligand for the endothelial specific tyrosine kinase receptor, tie-1

M.J. McCarthy, P.R.F. Bell and N.P.J. Brindle *Department of Surgery, University of Leicester, Leicester*

Background: Tie-1 is an endothelial cell-specific tyrosine kinase receptor that is essential for the development and stabilization of newly developed vessels and maintenance of endothelial cell integrity in the latter stages of angiogenesis. There is presently no known ligand for the tie-1 receptor.
Methods: Tie-1 extracellular domain was cloned using reverse transcription polymerase chain reaction from human umbilical vein endothelial cells. The tie-1 extracellular domain was then expressed as a fusion protein that could be used to probe cell lines for tie-1 extracellular domain binding and hence for the presence of tie-1 ligand.
Results: Using this molecular technique and additional purification strategies we have been able to isolate a 75kDa glycoprotein from concentrated conditioned malignant melanoma media. Furthermore, incubation of this media with human endothelial cells induces autophosphorylation of the tie-1 receptor. Pre-incubation of this medium with the fusion protein failed to induce autophosphorylation of the tie-1 receptor in endothelial cells.
Conclusion: An activating 75kDa tie-1 ligand is produced by a malignant melanoma cell line. Isolation and cloning of this ligand will allow further investigation into the function of this receptor and may possibly be used as a therapeutic agent in conjunction with vascular endothelial growth factor to enhance the growth of mature blood vessels in critically ischaemic limbs.

Vascular 009

Efficient gene transfer in vein grafts by improved adenovirus vector offer exciting prospects for gene therapy

P. Yiu, S. Stevenson*, J. Marshall-Neff*, W. Pugsley, J. McEwan and I. Taylor *Cardiovascular Repair and Remodelling Group, Department of Surgery, UCL Medical School, London and *Genetic Therapy Inc., A Novartis Company, Massachusetts, USA*

Background: Vein graft stenosis secondary to neointimal hyperplasia remains an obstacle to the successful outcome of arterial bypass surgery. Gene therapy using replication-deficient adenoviral vectors is a potentially powerful approach to vein graft disease. We have evaluated gene transfer using an Av3, a new generation of adenovirus vector within an improved safety profile (deletions in E1, E2a and E3).
Methods: The lumen of intact rabbit jugular veins were exposed *in vitro* to Av3nBg, (carrying marker gene nuclear localizing B-galactosidase), at four doses ($n = 5$ for each), $3·5 \times 10^8$ to 7×10^9 plaque forming units (pfu)/mL for 30 min. Rings of vein were maintained in culture for 49 h before exposure to X-gal substrate. Endothelial cells expressing the transgene were counted. The optimal viral dose was used *in vivo* using a rabbit carotid artery vein bypass model. Av3null (empty virus, $n = 2$), Av3Bg exposed vein grafts ($n = 5$) and control grafts ($n = 2$) were removed at 3 days for histology and quantification of transgene expression.
Results: In organ culture, exposure to $3·5 \times 10^9$ pfu/mL resulted in detectable expression of the transgene in 50·6 ± 5 per cent of endothelial cells. Below and above this dose, the number of endothelial cells positive for the B-gal transgene declined. Arterial vein grafting alone caused oedema and infiltration of leucocytes after 3 days and neointimal thickening at 4 weeks. Exposure of the vein lumen *in situ* to Av3Bg, $3·5 \times 10^9$ pfu/mL, prior to its use as a bypass graft resulted in gene transfer confined to the endothelium. At 3 days 70 ± 5 per cent of endothelial cells expressed the transgene, significantly higher than gene transfer in organ culture ($P = 0·004$). Importantly, contact with virus vector did not aggravate neointimal response at 4 weeks.
Conclusion: Endothelial gene transfer to vein grafts was highly efficient *in vivo* using the new, improved Av3 vector.

Vascular 010

Cytokine profiles in patients with lower limb ischaemia

Y.C. Chan, V. Gulati*, N. Shukla, D.O. Okonko, M. Abdus-Samee, J.C. Stanford†, A.O. Mansfield and G. Stansby *Academic Surgical Unit, Imperial College School of Medicine at St Mary's, *Department of Medicine, Imperial College School of Medicine at St Mary's, †Department of Microbiology, University College Hospital, London*

Background: Differential production of cytokines by T lymphocytes determines the nature and extent of the cell-mediated immune response. Previous studies have demonstrated that Th_1 cytokines such as IFN-γ may inhibit vascular smooth muscle cell proliferation, thereby preventing arterial stenosis. The aim of this study was to quantify intracellular T-cell cytokines in vascular patients.
Method: We studied healthy controls ($n = 11$, age: 60 ± 6·1 [mean age ± SEM]. Patients with intermittent claudication ($n = 11$, age 70 ± 2·8 years), and critical ischaemia ($n = 6$, age 74 ± 4·5 years). Peripheral blood mononuclear cells (PBMC) were isolated using a Ficoll density centrifugation technique. We stained for surface markers (CD4+, CD8+) and intracytoplasmic cytokines [interleukin-2

(IL-2), interleukin-4 (IL-4), gamma-inteferon (IFN-γ)] with monoclonal antibodies. Single cell analyses were performed with flow cytometry, which offers the advantage of representing results as a proportion of cells expressing the specific cytokine rather than of the total amount of cytokine produced.
Results: Results are represented as medians (interquartile ranges):

	IL-2 (CD4+ and CD8+)	IL-4 (CD4+ and CD8+)	IFN-γ (CD4+ and CD8+)
Controls	3·53 (1·01–7·99)	0·16 (0·00–0·65)	5·15 (3·59–7·24)
Claudicants	1·41 (0·62–3·68)*	0·22 (0·18–0·36)	3·66 (1·72–5·08)*
Critical ischaemics	0·31 (0·02–1·10)†	0·13 (0·10–0·20)	2·75 (1·20–5·64)*

Mann–Whitney U-test: *$P < 0·05$, †$P < 0·01$.

Patients with critical ischaemia also had lower levels of IL-2 than claudicants (*). Subgroup analysis demonstrated that production of CD4-IL-2, CD8-IL-2, CD4-IFN-γ, and CD8-IFN-γ were all decreased in vascular patients, while there were no significant changes in CD4-IL-4 or CD8-IL-4 production.
Conclusion: This is the first study to suggest that patients with lower limb ischaemia may have impaired Th_1 cytokines (such as IL-2, IFN-γ) production. Deficiency in these cytokines may be one of the risk factors for postoperative complications in vascular patients, and immunomodulation may have preventive or therapeutic potential.

Vascular 011

Homocysteine induces vascular smooth muscle proliferation

D. Byrne, C. Condron, C. Kelly, A. Hill, D. Bouchier-Hayes and A. Leahy *Department of Surgery, Beaumont Hospital, Dublin, Ireland*

Background: Hyperhomocysteinaemia has been identified as an independent risk factor for the development of atherosclerosis. The mechanisms by which this thiol-containing amino acid causes atherosclerosis is unknown. Much of the experimental work has been done with pharmacological levels of homocysteine only. Vascular smooth muscle cell (VSMC) proiliferation is central to atherosclerotic plaque formation. It is probable that VSMC secretion and altered apoptosis are also involved in plaque development. The aim of this study was to evaluate the effects of homocysteine on VSMC proliferation and apoptosis, using doses consistent with moderate to severe clinical hyperhomocysteinaemia.
Method: Embryonic rat aortic smooth muscle cells was used in all proliferation and apoptotic studies. Proliferation was measured colorimetrically, at homocysteine doses of 5 uM, 50 uM, 100 uM, 250 uM, 500 uM and 1000 uM vs. control. Apoptosis was measured using flow cytometry for the same homocysteine concentrations.

	Proliferation (%)	Apoptosis (%)
Homocysteine concentrations		
Control	100	1·9 ± 0·29†
5 uM	106 ± 5·818*	3·1 ± 0·83†
50 uM	113·1 ± 2·21*	2·6 ± 0·20
100 uM	113·5 ± 3·5	2·3 ± 0·36†
250 uM	122 ± 4·668*	3·1 ± 0·53†
500 uM	129·1 ± 5·87	2·9 ± 0·56†
1000 uM	157·8 ± 6·77*	3·2 ± 0·6†

*$P < 0·05$ vs control, †P = n.s.

Results: Results are expressed as mean ± SEM. ANOVA was used to determine statistical significance
Conclusion: These data demonstrate that VSMC proliferation was significantly upregulated in a dose-dependent fashion for all homocysteine concentrations ($P < 0·05$). There was no significant alteration in the apoptotic rate at any concentration used compared with control. The results suggest that increased vascular smooth muscle cell proliferation may be one explanation for homocysteine-induced atherosclerotic disease.

Vascular 012

Respiratory muscle dysfunction secondary to aortic cross-clamping and reperfusion is attenuated by thermal preconditioning

R. McLaughlin, C.J. Kelly, E. Kay* and D. Bouchier-Hayes *Royal College of Surgeons in Ireland, Departments of Surgery and *Pathology, Beaumont Hospital, Dublin, Ireland*

Background: Respiratory failure after aortic cross-clamping and reperfusion is in part due to a systemic inflammatory response, however the role of respiratory muscle dysfunction is unknown. The 72kDa heat shock family of protein, which can be induced by thermal preconditioning, act as molecular chaperones protecting cellular proteins from stresses such as ischaemia reperfusion (IR). This chaperone effect is of particular significance in preserving the structural and functional myofilamental protein activities necessary for muscle contraction. The aims of this study were to evaluate: (i) whether IR injury causes diaphragm muscle dysfunction; and (ii) whether thermal preconditioning would confer protection against diaphragmatic IR injury.
Method: Male Sprague–Dawley rats (250–350 g) were randomized into three groups ($n = 8$ per group): (a) control b) IR and c) IR + thermal preconditioning. The rat model of abdominal infrarenal aortic cross-clamping fo 1 h of ischaemia followed by reperfusion was used. Thermal preconditioning by temperature elevation to 41°C ± 0·5°C, maintained for 15 min, was induced 18 h prior to IR injury. Animals were sacrificed at 24 h, 48 h and 7 days of reperfusion, with diaphragm muscle contractile function being assessed using electrical field stimulation in a tissue bath.
Results: Results are expressed as peak tension achieved in grams. ANOVA was used to determine statistical significance.

Group	Mean twitch function (SEM) (g)	Mean tetanus function (SEM) (g)
Control	367·36 (21·7)	757·04 (45·4)
24 h IR	204·92 (17·2)†	282·74 (19·2)†
24 h IR + thermal	270·4 (25·1)*	552·04 (35·2)*
48 h IR	148·94 (14·5)†	322·34 (28·5)†
48 h IR + thermal	309·92 (23·6)*	529·8 (29·6)*
7 days IR	186·51 (10·5)†	442·46 (34·8)†
7 days IR + thermal	274·5 (23·9)*	517·3 (35·9)*

*$P < 0·05$ vs. IR, †$P < 0·05$ vs. control.

Conclusion: These data demonstrate that lower torso ischaemia reperfusion causes diaphragmatic muscle dysfunction with significant decreases in mean diaphragm twitch and tetanic contractile function ($P < 0·05$). Thermal preconditioning significantly preserved muscle mean twitch and tetanic contractility at all timepoints ($P < 0·05$). These data demonstrate that thermal preconditioning protects respiratory muscle electrophysiological function after systemic ischaemic reperfusion injury.