forms at 50kDa. Protein application to human vascular endothelial cells leads to a specific phosphorylation of the Tie2 receptor, maximal at 1 μg/ml for each.
Conclusion: Using rational design we have engineered the first small molecular mass ligands for the Tie2 receptor. Two of these ligands have been shown to activate the receptor. Studies are currently underway to test the functional effects of these novel ligands on vascular endothelial apoptosis, leakage and inflammation.

Intracellular accumulation of pro-atherogenic lipid particles is dependent on a novel cytoplasmic motif within the LOX-1 scavenger receptor

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Objective: Cellular accumulation of pro-atherogenic oxidised low-density lipoprotein (OxLDL) is a key step in atherogenesis. This is mediated by scavenger receptors such as the lectin-like oxidised low-density lipoprotein scavenger receptor-1 (LOX-1). Here we test the role of cytoplasmic determinants within LOX-1 that regulate endocytosis.
Method: Human native lipid particles were purified, chemically modified in vitro into OxLDL, and incorporated with a fluorescent dye for use in receptor-ligand assays. Conserved residues within the human LOX-1 cytoplasmic domain were substituted with a non-polar, neutral amino acid (alanine) within a tagged LOX-1 (LOX-1) or a LOX-1 hybrid protein fused to a reporter molecule. Gene transfection and expression of the different LOX-1 proteins in cultured human cells were used to identify regulatory sequences in receptor-mediated endocytosis.
Results: Alanine-scanning mutagenesis of the LOX-1 cytoplasmic domain revealed, when compared with human LOX-1, the most significant inhibition on OxLDL uptake was caused by mutations at D4A (40% reduction), D5A (92% reduction) and L6A (43% reduction). Other substitutions within this domain had little effect on LOX-1-mediated OxLDL internalisation. This aspartate-aspartate-lysine (DDL) cytoplasmic motif could independently confer similar endocytic properties to a LOX-1 hybrid protein where an equivalent region within another cell surface receptor (human transferrin receptor-1) was replaced with the DDL motif from LOX-1.
Conclusion: LOX-1-mediated uptake of pro-atherogenic OxLDL is regulated by recognition of this novel and transplantable DDL tripeptide motif. These studies reveal new determinants in LOX-1 for intracellular trafficking and accumulation of OxLDL particles that promote atherogenesis.

Platelet inhibition by nitric oxide is reduced with increasing disease severity in peripheral arterial disease

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Objective: Patients wiith peripheral arterial disease (PAD) exhibit platelet hyperactivity despite antiplatelet medication, which may represent reduced inhibition of platelet function and contribute to high cardiovascular morbidity and mortality. Platelet function may therefore be used to stratify these patients and identify those at high cardiovascular risk, allowing tailoring of aggressive antiplatelet regimens to reduce mortality.
Method: PAD patients (intermittent claudication [IC] and critical limb ischaemia [CLI]) and age- sex-matched controls were recruited. Flow cytometric analysis of basal, adenosine diphosphate (ADP)-induced and nitric oxide-inhibited (using the NO donor S-nitrosglutathione [GSNO]) platelet-fibrinogen binding (PFB) and platelet-leucocyte aggregate (PLA) formation was performed. Numbers recruited: PFB assay C = 13, IC = 20, CLI = 7; PLA assay C = 13, IC = 17, CLI = 5.
Results: PFB at low-dose ADP concentrations and PLA formation (platelet-monocyte and platelet-neutrophil), both basally and at all ADP concentrations, were significantly increased with increased disease severity (PFB: C v CLI $p = 0{\cdot}044$, IC v CLI $p = 0{\cdot}008$; PLA: for example, ADP 0·1 μM C v CLI $p < 0{\cdot}001$, C v IC $p < 0{\cdot}001$). Inhibition of PFB and PLA formation by NO was decreased with increasing disease severity (PFB: C v CLI $p = 0{\cdot}03$, IC v CLI $p = 0{\cdot}046$ for GSNO 10 μM, C v CLI $p = 0{\cdot}007$, IC v CLI $p = 0{\cdot}001$ for GSNO 100 μM. PLA: C v CLI $p = 0{\cdot}038$, IC v CLI $p = 0{\cdot}047$).
Conclusion: Patients with PAD exhibit increased platelet activity in response to ADP and decreased platelet inhibition by NO. Increased PLA formation and reduced inhibition of platelet function by NO may serve as markers for disease progression or prediction of cardiovascular morbidity/mortality, permitting more aggressive pharmacological management of cardiovascular risk in these patients.

The effects of N-acetylcysteine on host inflammatory response and renal function in patients undergoing infra-inguinal bypass surgery

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Objective: The aim of this prospective, randomised controlled trial is to investigate the effects of oral N-acetylcysteine (NAC) on the systemic inflammatory response and renal function in patients undergoing lower limb bypass surgery.
Method: Patients undergoing lower limb bypass surgery were randomised to receive either 600mg of oral NAC or the usual oral intake without NAC pre- and postoperatively. Urinary 11-dehydrothromboxane B2, plasma IL6, urinary p75TNF receptors, absolute neutrophil count, C-reactive protein, intestinal permeability, and systemic inflammatory response score along with renal function, were measured before surgery (PO) and daily until day 5 postoperatively (D1-5). While a p value of $< 0{\cdot}05$ was considered significant, Bonferroni correction was used in the presence of multiple comparisons.
Results: Fourteen patients were randomised into each group. The control group had a higher concentration of urinary 11-dehydrothromboxane B2 concentrations on D1 and D3 compared with pre-operative concentration. No significant change was observed in the NAC group. Absolute neutrophil count in the control group was significantly higher on D1–D3, with no difference in the NAC group. A significant reduction in serum creatinine concentration was found in the NAC group postoperatively. This was associated with a corresponding increase in estimated glomerular filtration rate. In the control group, no significant difference was observed in postoperative renal function. No significant change was demonstrated within or between the groups in the IL6, p75TNFr, lactulose-mannitol ratio, systemic inflammatory response score or morbidity and mortality.
Conclusion: The results show that NAC may reduce neutrophil activation and improve renal function in patients undergoing infra-inguinal arterial bypass surgery.

Do novel risk biomarkers reflect the severity of peripheral arterial disease?

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Objective: To determine the association between novel atherosclerotic risk biomarkers and severity of peripheral arterial disease (PAD).
Method: Stable claudicants were recruited from outpatient vascular clinics. All patients were taking antithrombotic and lipid-lowering therapy. Traditional and novel atherosclerotic biomarkers were measured. Traditional biomarkers were total cholesterol, low-density and high-density lipoprotein cholesterol (LDL, HDL), total cholesterol/HDL ratio, triglycerides and blood pressure. Novel biomarkers were C-reactive protein (CRP), von Willebrand Factor (vWF), interleukin-6 (IL6), red cell folate (RCF), vitamin B12, homocysteine (Hcy) and Hcy genotypes: MTHFR 677-CT, MTHFR 1298-AC, MTR 2576-AG, MTRR 66-AG. The severity of PAD was evaluated using ankle-brachial pressure index (ABPI), brachial-knee and brachial-ankle pulse wave velocity (bk and ba-PWV) measurements. The correlation between biomarkers and PAD severity was assessed using the Spearman correlation test and results were adjusted for the other biomarkers.
Results: One hundred and thirty-three patients with PAD were recruited. Hcy and systolic blood pressure had a positive independent correlation with bk-PWV