SA1

Dynamics of shear stress-induced remodelling

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Chronic changes in wall shear stress lead to vascular remodelling, characterized by increased vascular wall diameter and thickness, to restore wall shear stress values to baseline. Release of nitric oxide (NO) from endothelial cells exposed to excessive shear is a fundamental step in the remodelling process, and potentially triggers a cascade of events, including growth factor induction and matrix metalloproteinase (MMP) activation, that together contribute to restructuralisation of the vessel wall. MMPs, which are secreted as inactive zymogens (pro-MMPs), are rapidly cleaved and activated in in vivo models of chronic increased blood flow, and remain active until shear stress is normalized. Enhanced production of NO in high flow conditions, along with generation of reactive oxygen species through NADPH oxidase, combine to form peroxynitrite, which is important for MMP cleavage in the early phase of arterial remodelling. However, the later phase of this process implicates not only the activation of MMPs but also their ongoing synthesis. In this respect, we have uncovered a role for NF-kappaB as a key factor regulating the expression of MMP-9 and thus participating to the remodelling of vessels.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA2

Stem cell differentiation into vascular cells induced by mechanical stress

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It was established that stem cells could repair lost endothelial cells and participate in the formation of neointimal lesions, because stem cells can differentiate into a variety of cells to replace dead cells or to repair damaged tissues. In this process, stem cells homing to the surface of injured vessels have to differentiate into vascular cells to exert their repairing functions (Xu Q. Circ Res. 2008;102:1011). Obviously, microenvironment where stem cells are attached play a crucial role in cell differentiation, although the spectrum of the initiators/stimulators responsible for such a differentiation remain to be clarified. It is well known that atherosclerotic lesions in the arteries are localized in some areas where blood flow is disturbed resulting in endothelial dysfunction/death in the presence of hyperlipidemia. Since recent findings suggest the potential role of stem cells in endothelial regeneration, it can be hypothesized that mechanical stress induced by blood flow can influence the differentiation process of stem cells (Xu Q. Nature Clin. Pract. Cardiovasc. Med. 2006;3:94). Support this hypothesis is recent findings that shear stress can induce differentiation of stem cells towards endothelial cell phenotype (Zeng et al. J Cell Biol. 2006; 174:1059), while stretch stress leads to differentiate into smooth muscle cells. It indicates that "good" blood flow (laminar shear stress) promotes endothelial differentiation from stem cells that tethering the surface of the vessel wall. How the stem cells sense and transduce the extracellular physical stimuli into intracellular biochemical signals is a crucial issue for understanding the mechanisms of stem cell differentiation. Collecting data derived from our and other laboratories showed that many kinds of molecules in the cells such as receptors, G proteins, cell cytoskeleton, kinases and transcriptional factors could serve as mechanoceptors directly or indirectly in response to mechanical stimulation implying that the activation of mechanoreceptors existing on the surface of stem cells is a crucial event. The sensed signals can be further sorted and/or modulated by processing of the molecules both on the cell surface and by the network of intracellular signalling pathways resulting in a sophisticated and dynamic set of cues that enable stem cell responses. The new findings indicate that signal pathway VEGF-Akt-HDAC-p53/p21 is crucial for stem cell differentiation into endothelial cells. The present presentation will summarize the data on shear stress-induced stem cell differentiation and the impact of such a differentiation on the pathogenesis of vascular diseases.

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SA3

Stretch-dependent growth and differentiation in vascular smooth muscle

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Vascular adaptation to pressure and flow involves an intricate interplay of the endothelium, the smooth muscle layer and the extracellular matrix. Shear stress on the endothelium causes vasodilatation, which in turn increases tension in the vascular wall and causes stretch-induced growth (outward remodelling). Reduced endothelial function causes instead increased vascular tone, which leads to inward remodelling of the vascular wall and an increased wall-to-lumen ratio. Vascular smooth muscle cells exposed to physiological levels of stretch grow in a maintained contractile phenotype, in contrast to the

membrane were separated by 1 mm from the SMCs plated on an outer chamber (EC/M/SMC).

RESULTS AND DISCUSSION

Pre-exposure of ECs to HSS, but not LSS, for 24 h inhibits SMC-induced E-selectin expression in ECs. EC/SMC co-culture induced an increase in E-selectin mRNA expression in ECs within 1 h. Separation of ECs from SMCs by 1 mm retarded the E-selectin expression. Pre-shearing of ECs at HSS for 24 h inhibited the co-culture-induced E-selectin expression; this was not seen with LSS. Thus, SMCs induced EC expression of E-selectin via a paracrine effect that can be inhibited by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are mediated by the JNK and p38 pathways. The phosphorylation of ERK, JNK, p38, and Akt in ECs showed transient increases after co-culture with SMCs. The co-culture-induced E-selectin expression was inhibited by inhibitors for only JNK and p38. Pre-shearing at HSS, but not LSS, for 24 h inhibited the co-culture-induced JNK and p38 phosphorylation. JNK- or p38-specific siRNA caused significant inhibition of the co-culture-induced E-selectin expression. The increase in E-selectin-Luc promoter activity in ECs by SMC-co-culture was prevented by pre-shearing at HSS, but not LSS. Thus, the SMC-induction of EC expression of E-selectin is mediated by JNK and p38 and blocked by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are dependent on NF-κB. Inhibition of NF-κB abolished the co-culture-induced E-selectin promoter activity. Co-culture with SMCs increased the NF-κB-DNA binding activity in EC nucleus, which was inhibited by HSS (but not LSS). Thus, the co-culture induced E-selectin expression is mediated by NF-κB, and this effect is inhibited by HSS.

IL-1β and IL-6 produced by EC/SMC are the major factors contributing to the SMC-induced signaling and E-selectin expression in ECs. Using a human cytokine array system, we identified IL-1β and IL-6 as the proteins released from EC/SMC at significantly higher levels than EC/EC (>4-fold). Neutralizing antibodies against IL-6 and/or IL-1β inhibited the co-culture-induced increases in E-selectin mRNA, JNK and p38 phosphorylation, and NF-κB-DNA binding activity.

IRAK and gp130 are involved in regulatory effects of SMC-co-culture and shear stress on EC E-selectin expression. The SMC-induced E-selectin expression in ECs was suppressed by siRNAs against gp130 (IL-6 receptor) and IRAK (complex with the IL-1β receptor upon its stimulation). The co-culture-induced phosphorylations of gp130 and IRAK were inhibited by pre-shearing at HSS (but not LSS) for 24 h.

These results indicate that the SMC-induction of E-selectin in ECs involves the paracrine action of IL6 and IL-1β on their receptors to activate the JNK, p38 and NF-κB, and that this effect can be inhibited by high shear stress.

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SA13

Shear stress, inflammation and atherosclerosis

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Atherosclerosis is the disease with the highest mortality in the western world. Despite its large socio-economical impact, the underlying mechanisms are only partially known. It has been accepted for decades that atherosclerosis is a lipid driven disease, despite the fact that risk factors related to lipid metabolism only partially explain atherogenesis. Furthermore, new therapies specially focussed upon lipid metabolism only partially reduce plaque size. Recently two concepts – inflammation and blood flow/shear stress – have undergone a renaissance and gained a lot of interest as complementary explanations for plaque formation and these concepts will be the topic of the present manuscript.

The role of inflammation became apparent from a series of mouse studies where systematically parts of the immune system were knocked down, before the induction of atherosclerosis. These studies identified inflammation as an independent mechanism attributing to plaque formation, and based upon these results and further studies atherosclerosis is considered a lipid driven inflammatory disease. The effect of blood flow in atherosclerosis is based upon the observation that plaques are not evenly distributed over the arterial system. These predilection sites are at or near side branches, i.e. where blood flow is non-uniform, or at the lesser curvature of bends, i.e. where blood velocity is relatively low. The effect of blood flow on the vessel wall is through shear stress which alters the physiology of endothelial cells. Shear stress (τ N/m2 or Pascal (Pa)) arises from the friction between two virtual layers in a fluid, and is induced by the difference in movement of the two layers (dv/dr s-1; in case of a cylindrical tube) and the "roughness" (or viscosity Pa.s) between these layers ($\tau = dv/dr * \eta$). Shear stress also arises at the interplay between blood and the endothelial layer, where it induces a shearing deformation of the endothelial cells. This shearing deformation affects the phenotype of the endothelial cells and thereby the inflammatory component and plaque progression/composition.

This paper describes the interaction between shear stress and inflammation. We will first describe recent findings on the sensing mechanism of shear stress by the endothelium. Subse-

quently, pro-inflammatory pathways modulated by shear stress in endothelial cells, followed by the effect of shear stress on plaque progression and plaque composition. At the end we will discuss new findings related to longitudinal plaque heterogeneity.

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SA14

Engineering vascular grafts

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Coronary artery and peripheral vascular disease are associated with significant morbidity and mortality. In these patients, surgical intervention including small diameter bypass grafting with autologous veins or arteries is a common treatment. However, many patients lack suitable autologous vessels, either because these vessels are diseased themselves or because of previous surgery, and in these cases, synthetic grafts are often used. Unfortunately, many of these grafts fail because of the low number of endothelial cells and the proportion of the endothelialised surface remaining after exposure to flow, which results in acute thrombosis and subsequent occlusion of the vessel.
At the University of Manchester, we are developing small calibre vascular grafts for coronary or peripheral bypass and vascular access grafts for haemodialysis. These grafts are based on electrostatically spun polyurethane and polycaprolactone with controlled porosity and biodegradability and are coated with specific vascular matrix molecules to regulate cell adhesion, migration, and growth factor bioavailability. This talk will focus on the approaches we are using to improve both the initial adhesion of endothelial cells to the graft surface and the retention of these cells to this surface following restoration of flow. Our studies have revealed significant new insights into the biology of endothelial cell attachment to surfaces coated with specific vascular matrix molecules, with important implications for the design of the next generation of vascular grafts.

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SA15

Waveform analysis and microcirculatory function

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Risk factors for cardiovascular disease mediate their effects by altering the structure and function of wall and endothelial components of arterial blood vessels. Pathological change in the microcirculation plays a pivotal role in promoting end-organ dysfunction that not only predisposes to further organ damage but also increases the risk for future macrovascular events. The microcirculation is recognised as the site where the earliest manifestations of cardiovascular disease occur that may play a pivotal role in driving the atherosclerotic process in conduit vessels(1).
Ultrasound and the Doppler effect have been long used to measure blood velocity and its temporal and spatial variation within the vascular tree in order to diagnose and monitor vascular disease. Changes in morphology of the linear flow velocity spectral envelope is not representative of any single vessel but is determined by changes in the properties and total cross-sectional area of downstream vascular networks(2). Quantitative analysis of Doppler-time velocity waveforms that reflect measures of flow pulsatility (eg resistive index, pulsatility index) can mirror changes in downstream vascular resistance and may predict future adverse clinical outcomes(3). In a series of studies in different patient groups we have shown these derived indices often provide misleading information in relation to the haemodynamic actions of drug interventions and are not sensitive in detecting early microvascular dysfunction in different vascular beds in humans(4,5). Novel algorithms that enable quantitative analysis of the Doppler velocity spectral envelope over the duration of the cardiac cycle provides more sensitive information in relation to the haemodynamic action of drugs and identification of early microvascular abnormalities in humans. Data will be presented showing the superiority of this approach in identifying early microvascular abnormalities in waveforms obtained from different arterial territories in different disease states associated with increased cardiovascular risk.
Techniques capable of detecting microvascular damage and monitoring response to therapeutic interventions, especially in vulnerable target organs of interest, may improve risk stratification and could represent a valuable surrogate for future cardiovascular outcome.

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SA5

Mechanotransduction and the glycocalyx

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The surface of endothelial cells (ECs) is decorated with a wide variety of membrane-bound macromolecules that constitute the glycocalyx (GCX). As the most apical structure on the EC, the GCX senses the force (shear stress) of flowing blood and transmits it via the cytoskeleton throughout the cell to sites where transduction of force to biochemical response (mechanotransduction) may occur. In this presentation the structure of the GCX and many of the experiments that demonstrate its role in mechanotransduction and vascular remodeling will be reviewed. Experiments with enzymes that degrade specific glycosaminoglycan components have been used to show that the GCX mediates the shear-induced production of nitric oxide, a central process in cardiovascular control, while the same enzyme treatments do not affect shear-induced production of prostacyclin, another hallmark of EC mechanotransduction. These experiments reinforce the concept of distributed sites of mechanotransduction in EC. The characteristic remodeling of the EC cytoskeleton and intercellular junctions in response to shear stress are dependent on the GCX as well, and the experiments that support the role of the GCX in these processes will be reviewed as well.

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SA6

Modulation of inflammatory responses of endothelial cells by changes in local shear stress

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Stable local haemodynamic microenvironments may determine the phenotype of endothelial cells (EC) in different regions of the circulation, but acute changes in flow might also modulate functional responses. We aim to understand how different levels or patterns of shear stress applied to endothelial cells regulate inflammatory responses, and in particular, leukocyte recruitment. For this purpose, we developed models in which human EC of various types (HUVEC from umbilical veins; HUAEC from umbilical arteries; HCAEC from coronary arteries) were cultured in glass capillaries coated with desired substrates. These constructs were conditioned by different levels of shear stress for different periods, or exposed to abrupt changes in shear. Conditioning could be combined with treatment with cytokines such as tumour necrosis factor-α (TNF) and interleukin-1β (IL-1), and adhesion and migration of flowing neutrophils analysed as an 'inflammatory' readout.

Initial studies showed that conditioning of HUVEC for 24h at increasing shear stress acted to powerfully suppress responses to TNF, but not IL-1, judged by neutrophil recruitment (Sheikh et al., 2003; 2005). However, in subsequent studies, responses to both cytokines were suppressed by shear conditioning for HUAEC and HCAEC. Studies in which culture medium constituents, such as basic fibroblast growth factor, were swapped, indicated that this difference between the endothelial cells arose from culture conditions rather than from an in vivo imprinted phenotype. The fact that the original 'static' cultures of each cell type showed similar abilities to support adhesion and migration of neutrophils also indicated that the phenotypes of EC were plastic and could be re-set by conditioning in vitro. Taking this further, we analysed expression of selected genes in HUVEC immediately after digestion from veins, after standard culture in vitro and then after shear conditioning. Changes were induced by initial culture, which were reversed in part at least by the return to a shear environment. Thus it seems that endothelial phenotype is highly pliable, with enviromental factors, such as shear stress and growth factors, modifying responses in an interlinked but reversible manner.

We thus investigated whether the less responsive state induced in vitro by shear stress would change when flow was ceased. This might be relevant to ischaemic conditions in vivo (for instance linked to thrombo-embolism, surgical interventions or organ transplantation), where an inflammatory response typically follows reperfusion. We found that response of EC to TNF only increased slowly over 24-48h after cessation of flow, and that if a very low level of shear stress was retained, then the response remained suppressed (Matharu et al.,2008). In all of the above, functional