313P REMODELLING OF TISSUES: IMPLICATIONS FOR AGEING AND NOVEL TREATMENT REGIMES

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Acute inflammation is a beneficial, non-specific response of tissues to injury and generally leads to repair and restoration of the normal structure and function In contrast, asthma represents a chronic inflammatory process of the airways followed by healing whose end-result is an altered structure referred to as a remodelling of the airways. Repair usually involves two distinct processes: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type, and replacement by connective tissue and its eventual maturation into scar tissue. In many instances both processes contribute to the healing response and inflammation.

In asthma the processes of cell dedifferentiation, migration, differentiation and maturation, as well as of connective tissue deposition, are frequently associated with altered restitution of airways structure and function. There are several features indicating that asthma is characterised by altered healing and repair processes, such as the presence of myofibroblasts and the increased number of fibroblasts, the hyperplasia of smooth muscle cells and mucous glands, and the altered homeostasis of the extracellular matrix leading to collagen deposition and elastolysis. Most of these features are seen in almost all asthmatics whatever the severity or duration or etiology of asthma, and their reversibility under anti-inflammatory treatments is still not completely known. In this regard, while the increased thickness of the reticular layer of the basement membrane may be reduced by steroids, smooth muscle hypertrophy and mucous gland enlargement appear to be permanent abnormalities.

The end result of all these structural alterations is a tremendous increase of airway wall thickness which, particularly in the more severe forms of the disease, causes a markedly and permanently reduced airways caliber. These features result in an increased resistance to airflow, particularly when there is bronchial contraction and bronchial hyperresponsiveness. The effect on airflow is compounded by the presence of increased mucous secretion and inflammatory exudate, which not only blocks the airway passages but causes an increased surface tension favouring airway closure.

Although the clinical consequences of airway remodelling are not fully understood, it is conceivable that this process can directly contribute to lung senescence as well as to the progressive lung function loss over the years.

314P ENDOTHELIUM-DERIVED NITRIC OXIDE

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The finding that the biological actions of endothelium-derived relaxing factor are due to the endogenous release of nitric oxide (NO) revealed the existence of a ubiquitous biochemical pathway. Nitric oxide is formed from the amino acid L-arginine by a family of enzymes, the NO synthases (NOS), of which several isoforms are now known to exist. The constitutive NOS that was first discovered in the vascular endothelium has been designated as eNOS, whereas that present in the brain, spinal cord and peripheral nervous system is termed nNOS. A third form, induced by immunological or inflammatory stimuli, is known as iNOS.

The discovery of the L-arginine:NO pathway has led to many new insights into cardiovascular physiology and pathophysiology. The synthesis of NO by the vascular endothelium is a vasodilator mechanism which plays a role in the physiological regulation of blood flow and pressure in animals and man. Inhibition of its generation by arginine analogues such as NG-monomethyl-L-arginine (L-NMMA) leads to marked regional vasoconstriction and a hypertensive response. Mice in which the eNOS gene has been disrupted and which specifically lack eNOS have an elevated blood pressure compared with their wildtype counterparts. Nitric oxide also contributes to the control of platelet aggregation and the regulation of cardiac contractility. These physiological effects of NO are all mediated by the action of a constitutive NO synthase and subsequent activation by NO of the soluble guanylate cyclase. Other actions of endothelium-derived NO include attenuation of white cell adhesiveness and inhibition of smooth muscle cell proliferation. Impaired production of NO by eNOS has been implicated in several cardiovascular disorders, including hypertension, vasospasm and atherosclerosis.

Immunological stimuli such as endotoxin lipopolysaccharide and cytokines induce iNOS in many cells and tissues. This enzyme, which was originally identified in macrophages, contributes to the cytotoxic actions of these cells. The NO produced by this enzyme in the cardiovascular system contributes to the profound vasodilatation of septic shock, the hyperdynamic state of cirrhosis and to some inflammatory conditions of the heart. L-NMMA, when used at low doses in animals and man, reverses the hypotension and the hypereactivity to vasoconstrictors characteristic of shock. Selective inhibitors of iNOS will undoubtedly prove beneficial for the treatment of the hypotension of shock or cytokine therapy as well as dilated cardiomyopathy, cirrhosis and other vascular conditions in which this enzyme is induced.

Thus NO generated by endothelial cells can act both as a physiological mediator and as a pathophysiological entity. One way in which this dual action may be achieved is through the actions of NO on mitochondrial function. At low physiological concentrations NO inhibits cytochrome c oxidase in a reversible manner which is competitive with oxygen; this would allow cells to adapt to acute changes in their environment. At higher concentrations NO irreversibly inhibits other enzymes in the respiratory chain, either directly or via the formation of peroxynitrite. This inhibition contributes to the pathological actions of NO.

315P ENDOTHELIAL CELL GENE EXPRESSION AND OXIDATIVE STRESS

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Atherosclerosis is associated with an elevated level of lipid peroxidation and increased formation of reactive oxygen species with the vascular wall. Vascular endothelial and smooth muscle cells respond to oxidative stress with adaptive increases in the expression of endogenous antioxidant defences to counteract oxidative damage. Exposure of vascular cells to oxidative stress results in an initial depletion of intracellular glutathione (GSH) and subsequent adaptive increases in GSH synthesis (Jornot & Junod, 1993; Siow *et al.*, 1998). Increased expression of stress response proteins, such as heme oxygenase-1 (HO-1), in human atherosclerotic lesions and vascular cells exposed to oxidative stress agents may serve a multi-purpose role, via metabolism of heme to the antioxidants biliverdin/bilirubin, carbon monoxide and iron, which results in induction of ferritin synthesis (Siow *et al.*, 1999).

Our studies in human cultured vascular cells have shown that oxidized LDL, but not native or mildly oxidized LDL, induces time-, concentration- and protein synthesis-dependent increases in GSH and transport of L-cystine, a rate-limiting precursor for GSH synthesis. Pretreatment of cells with vitamin E (100 μM) attenuated oxidized LDL-mediated increases in GSH, whereas pretreatment with vitamin C (20-100 μM) depressed basal and abolished oxidized LDL-induced increases in GSH and L-cystine transport via the Na^+-independent system x_c^-. We have also reported that induction of HO-1 and the macrophage stress protein MSP23 in human and porcine vascular smooth muscle cells exposed to glucose oxidase, $CdCl_2$, diethylmaleate or oxidized LDL is markedly attenuated following pretreatment of cells with vitamin C.

The potential involvement of stress proteins in protecting vascular cells against oxidant injury in atherogenesis is further highlighted by the reports that HO-1 gene transfer confers vascular protection, induction of HO-1 in LDL receptor knockout mice inhibits the formation of atherosclerotic lesions and mice lacking heme oxygenase-2 are more susceptible to oxygen toxicity (reviewed in Siow et al, 1999). Thus, the cystine-GSH pathway and stress response proteins HO-1 and MSP23 may well provide important adaptive antioxidant defences against oxidative stress in atherosclerosis .

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316P ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS IN MAN *IN VIVO*

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Atherosclerosis is initiated by injury to the endothelium. One aspect of this is manifest by impaired endothelium-dependent relaxation to acetylcholine (and other endothelium-dependent agonists). Such endothelial dysfunction can be demonstrated *in vivo* in conditions such as hypercholesterolaemia and type 2 diabetes before the development of overt structural disease. In the case of hypercholesterolaemia, endothelial function can be restored immediately by apheresis of low density lipoproteins (LDL); short term reduction of LDL-cholesterol is associated with improved myocardial perfusion and a reduction in ischaemic events.

Current evidence suggests that endothelial dysfunction is caused, at least in part, through the inactivation of endothelium-derived nitric oxide (NO) by oxygen derived free radicals such as superoxide anion (O_2^-). In animal models hypercholesterolaemia is associated with increased generation of O_2^- from the endothelium; acute exposure of isolated vessels to LDL inhibits endothelium-dependent relaxation and this may be reversed by cell permeable superoxide mimetics. Acute administration of vitamin C can restore endothelial function *in vivo* in both hypercholesterolaemia and type 2 diabetes. Increased oxidative stress may occur through a variety of mechanisms.

In hypercholesterolaemia LDL may activate a membrane bound NAD(P)H oxidase which generates O_2^-; accumulation of an endogenous inhibitor of NO synthase (NOS) such as asymmetric dimethyl arginine or other mechanisms may lead to a relative deficiency of L-arginine, the substrate for NOS, decoupling of NOS with subsequent generation of O_2^- rather than NO; oxidation of the cofactor tetrahydrobiopterin may also lead to uncoupling of NOS resulting in a vicious circle of increasing oxidative stress. In diabetes increased oxidative stress may result from numerous additional mechanisms: glucose induced activation of cyclooxygenase, autoxidation of glucose, increased levels of angiotensin II, alteration in transition metal metabolism or depletion of NADPH required for the glutathione-redox cycle.

Assessment of oxidative stress has hitherto been problematic due to the nonspecific nature of most markers. Plasma concentrations of the F_2-isoprostane 8-epi-$PGF_{2\alpha}$, a non-enzymatic oxidation product of arachidonic acid now appear to provide a reliable measure of oxidative stress *in vivo* and may be used to explore the relationship between oxidative stress and endothelial dysfunction. 8-epi-$PGF_{2\alpha}$ is elevated in patients with hypercholesterolaemia and type 2 diabetes. Therapies, other than lipid lowering treatment, that appear promising in decreasing oxidative stress and restoring endothelial function include L-arginine and antioxidants. In type 2 diabetes oral treatment with a novel water soluble antioxidant appears effective at reducing plasma concentrations of 8-epi-$PGF_{2\alpha}$ and restoring endothelial function.

317P CONVERTING ENZYME, THE SYMPATHETIC NERVOUS SYSTEM AND ENDOTHELIAL FUNCTION IN RELATION TO AGEING

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Almost all of the key homeostatic systems involved in the regulation of vascular tone (e.g. arginine-nitric oxide system, sympathetic nervous system, renin-angiotensin system) suffer a diminution in their effectiveness with ageing. These systems interact at many levels, so that a selective defect in the renin angiotensin system may also result in a secondary loss of sympathetically mediated vasoconstriction. The arginine-nitric oxide system is the principal vasodilatory "system" and the basal release of endothelial nitric oxide decreases with age itself and in a variety of conditions more commonly seen in the elderly, e.g. hypertension, diabetes, hypercholesterolaemia, hypohomocystinaemia and smoking.

The potential consequences of age-related "endotheliopathy" are obvious for vascular risk but there may be more subtle, yet equally important, consequences in other age related diseases, e.g. muscle weakness and osteoporosis. The sympathetic nervous system has been described as a counter regulatory system to the arginine-nitric oxide system, though its regulatory role in orthostasis is greater. Sympathetically-mediated (especially $\alpha_1\beta_1$) vasoconstriction decreases with advancing age. The consequences of this may be considerable not only for the way in which older patients respond to drugs acting on this system but to our understanding of age-related orthostatic problems such as carotid sinus syndrome and orthostatic symptoms. Age-related adrenergic dysfunction can potentially alter the response to drugs acting on the renin-angiotensin system, as both of these systems interact at the level of the α_1 adrenergic receptor.

Changes in the renin angiotensin system, the arginine-nitric oxide system and sympathetic nervous systems with ageing will be discussed, and their consequences for disease in old age.

318P INTERACTIONS BETWEEN THE EFFECTS OF AGEING AND OF CARDIOVASCULAR RISK FACTORS ON ENDOTHELIAL FUNCTION

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Endothelial cells play a key role in modulating vascular tone and structure, mainly through production of endothelium-derived nitric oxide (NO), which is not only a potent vasodilator but also inhibits platelet aggregation, smooth muscle cell proliferation, monocyte adhesion and adhesion molecule expression, thus protecting the vessel wall from the development of atherosclerosis. Almost the totality of cardiovascular risk factors, with the exception of race, are associated with impaired endothelium-dependent vasodilation. However, the main condition characterized by impaired endothelium-dependent vasodilation is ageing. Thus peripheral vasodilation to intrabrachial infusion of endothelial agonists, such as acetylcholine or methacholine, is strongly and inversely related to increased age, while the relationship with an endothelial independent vasodilator, such as sodium nitroprusside, is much weaker.

The negative effect of ageing on endothelial function is so strong that it can be detected even in the presence of other cardiovascular risk factors, such as essential hypertension, *per se* characterized by impaired endothelium-dependent vasodilation. It is worth noting that while in men endothelial dysfunction associated with ageing is a continuous phenomenon starting in young age, in women the presence of endothelial dysfunction appears only after menopause, suggesting that endogenous estrogen can protect the female population against the age-related decline in endothelium-dependent vasodilation. Even in hypertensive patients gender has an effect on age-related endothelial dysfunction, since in females up to menopause, age-related endothelial dysfunction, although present, is attenuated as compared to males of the same age, while after menopause endothelial function shows a steep decline.

In healthy controls endothelial dysfunction is caused by an alteration of the L-Arginine-NO pathway which can be reversed until around the age of 60 years and improved after this age by local infusion of L-Arginine. NO availability, evaluated as the degree of L-NMMA-induced inhibition of vasodilation to acetylcholine, is maintained until the age of 60 years, after which it is no longer detectable. The alteration in NO availability is linked to the appearance of oxidative stress, evaluated by testing the facilitating effect of vitamin C on the response to acetylcholine. Interestingly, in essential hypertensive patients age-related endothelial dysfunction is caused by the same mechanisms, but has earlier onset. Thus the alteration in NO availability and the parallel production of oxidative stress appear at a younger age in essential hypertensive patients as compared to healthy controls. It is conceivable that endothelial dysfunction associated with essential hyper tension is not a specific vascular alteration, but rather an anticipation of a physiopathological phenomenon which is characteristic of advancing age.