Editors' view

Endothelial progenitor cells & translational research

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Robert F Furchgott

Readers will be aware of the death of Robert F Furchgott (1911–2009) on 19th May in Seattle. His brilliant exploitation of a chance finding in 1978 led him to the discovery of endothelium-dependent relaxation and, eventually, to the discovery of nitric oxide (NO) – one of the most important discoveries in the history of cardiovascular medicine. He was a modest man, who continued to do bench-work in the laboratory for many years. He started his research career before the second world war, working on the physical biochemistry of red cell membranes. He made major contributions to receptor theory and mechanisms using organ bath methods (which he described as 'old fashion' pharmacological research), before initiating the present era of vascular biology and medicine with his seminal paper with Zawadzki in 1980 [1]. He marvelled at the numerous directions his research led to and would have been fascinated by the new field of endothelial progenitor cells (EPCs) and its current directions.

Endothelial progenitor cells (EPCs)

EPCs were first isolated in 1997 [2], and their discovery challenged at a stroke the previous orthodoxy that endothelial repair occurred through local migration of neighboring cells from the margin of a focus of endothelial injury and their proliferation to form a neointima. The discovery of EPCs offered an alternative paradigm, in which *circulating* progenitor cells, of bone marrow origin, home in on areas of endothelial injury and are responsible for postnatal formation of blood vessels in health and disease [3, 4]. The therapeutic possibilities opened up by this new way of looking at things are obvious but amazing. Such possibilities are, as yet, in their infancy, and whether EPCs ultimately come to be seen as the biological equivalent of a new planet (Uranus rather than Neptune, since they were

observed rather than mathematically predicted) will depend on whether translational medicine [5] delivers on its early promises in this regard. Future Trekkies may yet come to see 1997 as the birth date of the 'new' medicine of the future, one that may have real-life similarities with what Bones deployed so nonchalantly in the fictional Star Trek series.

Does the emerging field of EPC-based therapy form part of the clinical pharmacological Milky Way or does it belong to another galaxy altogether? Drug regulators would, I believe, take the view that such developments should be under their critical purview with a tweak in nomenclature ('device' instead of 'new molecular entity' perhaps?). Our editorial instinct is similar: while cells are obviously not drugs, understanding how to use them therapeutically depends critically on the principles of clinical pharmacology, and BJCP is delighted to publish work on cell-based therapies and how best to introduce them safely and effectively into clinical practice. Accordingly, in this issue of the Journal Tilling *et al.* review what is known about the mechanism of mobilisation of EPCs from the

bone marrow to the circulation [6]. They consider the complex connections between circulating EPC numbers, risk factors for atherosclerosis, and *in vivo* measures of endothelial function, and then address mechanisms of mobilisation of EPCs from the 'stem cell niche', a microenvironment in the bone marrow where they are tethered to stromal cells. Proliferation and release from this environment, together with acquisition of full function, involves a complex interplay between cytokines, chemokines, proteinases, and cell adhesion molecules. Stromal-derived factor 1 (SDF1), a key chemokine in this regard, is released by hypoxia from platelets and endothelial cells as well as from other cell types, and is a potent chemoattractant of endothelial cells via binding to CXCR4 (C-X-C motif chemokine) receptors ('fusin') and activation of matrix metalloproteinase 9 (MMP9). Release of SDF1 is potentiated by hypoxia-inducible factor 1 (HIF1), and MMP9 activation depends on NO, which plays an important part in EPC mobilisation. Several compounds influence these processes (eg fucoidan, which displaces SDF1 from bone marrow endothelium and extracellular matrix, and AMD3100, a reversible antagonist of SDF1 binding to CXCR4). Vascular endothelial growth factor (VEGF) facilitates EPC mobilisation, as does IL8 and other cytokines. Erythropoietin is stimulated by hypoxia and, distinct from its well-known role in red cell maturation, can also increase circulating EPC numbers. If administered before experimentally induced ischemia, erythropoietin protects against ischemia/reperfusion injury [see 6 for the original references].

Other drugs acting on the EPC cascade include: PPAR- γ agonists (glitazones), which promote NO availability and can prolong EPC survival as well as stimulating EPC mobilisation; TNF- α antagonists, which can both improve endothelial function and increase circulating EPC numbers in patients with rheumatoid arthritis; and angiotensin converting enzyme inhibitors (ACEI) and angiotensin AT1 receptor antagonists (ARBs), which increase the EPC response to hypoxia, despite inhibiting EPO secretion. Signalling pathways that guide EPC to damaged endothelium involve both PI3K/Akt and ERK MAP [kinase] cascades, and these offer further opportunities for pharmacological intervention. EPC mobilisation may thus be a ripe therapeutic target for clinical investigators interested in repairing and maintaining the integrity of the vascular endothelium.

Translation

Elsewhere in this issue we publish a number of papers relevant to translation of basic science to bedside application, some but not all of it directly relevant to EPC applications. Gordon and colleagues describe an investigation of endothelial function by pulse contour analysis [7] building on previous work on endothelium-dependent β_2 adrener-

gic vasodilation [8]. Clinical pharmacology is very much at the heart of developing methods to study pharmacodynamic effects in humans *in vivo*. Two important papers on human neuropharmacology illustrate this. One compares the suitability of two formulations of capsaicin as models of neuropathic pain in healthy volunteers [9], while the other reports pharmacodynamic outcomes from a study using ethanol clamping [10]. The Journal previously published PK data validating this approach [11], which was developed from a method originally designed by O'Connor *et al.* [12]. In another important paper, Ternant and colleagues describe the development of a drug-disease simulation model for rituximab in follicular non-Hodgkin's lymphoma [13]. This usefully predicts progression-free survival and will be used to simulate new dosing regimens in populations of patients to improve the design of future clinical trials.

Pre-translational research

Elsewhere in this issue we publish two essentially negative but important studies related to the cyclic vasoconstrictor undecapeptide urotensin II (U-II). This is a tantalising and incomplete story, which may yet lead to human therapies, but has so far proved a blind alley. U-II was originally identified in the brains of bony fish. In some vascular beds it is many times more potent as a vasoconstrictor than endothelin I (ET1), the previous record holder in this regard. Messenger RNA encoding receptors for U-II (UT) has been found in human brain, endothelium, and vascular smooth muscle cells, and U-II constricts various systemic arteries, including human radial artery *in vitro*, while relaxing pulmonary vessels. Bohm and Pernow [14] reported that intra-brachial artery infusion of U-II reduces forearm blood flow, whereas Wilkinson *et al.* [15] found no such effect. The present study [16] investigated the possibility that the previous contradictions related to differences in age or cardiovascular health. The authors investigated healthy men and men with symptomatic vascular disease using a dose ramp and a separate prolonged (60 min) infusion protocol using both U-II and a potent antagonist peptide (urantide) which displaces U-II from specific binding to recombinant human U-II receptors [17]. They confirmed their earlier negative findings. The difference from the positive study of Bohm and Pernow [14] remains a mystery – one that will intrigue a future Furchgott (whose original finding of endothelium-dependent relaxation resolved the long-standing paradox as to why acetylcholine acted as a potent vasodilator *in vivo* but as a vasoconstrictor *in vitro*). What a challenge to young investigators and trainees in our discipline! Another paper [18] picks up on observations of increased U-II in patients with diabetes and on previously reported beneficial effects of a different U-II antagonist, palosuran (a potent selective competitive antagonist at human UT receptors) in rat models of diabetes. Disappointingly, their

four-week study of palosuran versus placebo in diet-treated patients with type 2 diabetes showed convincingly that antagonism of the U-II system does not influence insulin secretion or insulin sensitivity in the human disease. Species differences bedevil animal models of diseases, and such divergences, disappointing as they are, emphasise the crucial importance of timely, rigorous translational studies in humans.

Robert Furchgott would surely have loved all of this!

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