Cyclosporin A toxicity, and more: vascular endothelial growth factor (VEGF) steps forward

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Toxic effects, which involve some organs with particular intensity, are the main disadvantage in the therapeutical use of cyclosporin A (CsA). Nephrotoxicity, renal vascular damage and hypertension are most relevant among the undesirable effects. Vascular injury has generally been considered a common-ground factor of all types of CsA-induced organ damage [1–3].

Albeit that vascular smooth muscle cells initially appeared to be the main CsA target [2], the finding of multiple endothelial effects [1,3,4–6] indicates that endothelial cell (EC) toxicity is an emerging feature of CsA-induced vascular injury.

Cyclosporin A, endothelium and VEGF

A main issue related to the mechanisms of the effects of CsA is the putative involvement of vascular endothelial growth factor (VEGF) as a relevant cellular response factor. A reasonable possibility is that VEGF has a key role in the cytoprotective mechanisms against CsA-related cell damage and induction of cell resistance against injury. VEGF exerts its effect after binding to membrane tyrosine kinase receptors (VEGFR1, VEGFR2 and VEGFR3, and a complementary receptor, neuropilin-1). Each of these receptors has different signal transduction properties and functions, but VEGFR2 is more important in functional terms [7].

Evidence has shown that circumstances as varied as hypoxia, exposure to reactive oxygen species (ROS), fibroblast growth factor-2 or rupture of interendothelial VE cadherin junctions can all stimulate ECs to produce VEGF [8]. As mentioned above, this autocrine VEGF expression appears to be related to a protective mechanism.

In vitro, blockade of autocrine VEGF using a specific VEGF blocking antibody (α-VEGF) significantly
more, Hernandez et al. [4] have shown that CsA may act in a rather distal mechanism, by influencing VEGF-induced migration of ECs and angiogenesis. However, this is by no means the whole story.

**Exogenous VEGF, endogenous VEGF**

Recent investigations have shown that exogenously administered VEGF is protective against CsA renal toxicity [10]. This finding is in agreement with studies in models other than CsA toxicity, e.g. reduced renal mass, in which VEGF appears to protect the peritubular capillaries in the vasa recta network [11]. Not enough data are available, however, about the role of endogenous VEGF.

In the kidney, VEGF is constitutively expressed in podocytes and tubular cells [7,12–15]. An increase, decrease or redistribution of renal VEGF has been described in several pathological entities and disease models, including ischaemia/reperfusion, chronic tubulointerstitial injury, membranous nephropathy, pre-eclampsia, collapsing glomerulopathy, diabetes mellitus or obstructive nephropathy [7,12–18]. It has been hypothesized that the growth factors modulating embryonic kidney EC survival and capillary morphogenesis may be implicated in the capillary loss that occurs in glomerulonephritis [19]. It is of interest that the mechanisms involved in the change in VEGF expression in the mentioned entities have not yet been clarified; in fact, the hypothesis that renal hypoxia is involved has been raised but not accurately addressed, and a direct role for toxic or inflammatory pathways can also be considered.

Production of VEGF by proximal tubular epithelial cells in culture has been related to increased DNA binding of HIF-1 to hypoxia-responsive elements in the VEGF gene promoter [20]. However, VEGF expression may be turned on by different types of cell stress [8] other than hypoxia. Induction of VEGF gene expression by hypoxia or stress is tissue and agent specific, and changes under different conditions [8,14].

The evidence that VEGF is a main protective agent for ECs has led to the hypothesis that it may be critical in the protection of peritubular vessels. This has been addressed in diverse models, including obstructive nephropathy, remnant kidneys or ageing [11,18,21]. Of outstanding interest is the fact that blockade of endogenous VEGF by several means, from pharmacological inhibition to conditional knock-outs, favours renal injury, including glomerular endotheliosis and glomerular obliteration with renal insufficiency [22]. This aspect would be particularly important in the context of the use of anti-VEGF agents for the treatment of neoplasias or proliferative retinopathy.

The majority of the data available support the notion that vasa recta are protected by VEGF. Vasa recta are rather simple structures, consisting of an endothelial layer partially covered by pericytes. However, the observation that CsA increases tubular VEGF [23,24] favours the possibility that VEGF is endowed with tubular effects. This poses the relevant question of whether the main renal effect of VEGF in CsA toxicity is related to vascular or tubular effects, or both.

The role of endogenous VEGF in CsA toxicity was addressed recently in an *in vivo* study [23], which showed that an α-VEGF antibody markedly enhances renal CsA toxicity, inducing a more severe tubular damage. Even with the limitations of the model, which used quite high doses of CsA (50–150 mg/kg/day, 18 days), CsA-induced tubular VEGF [23] and increased tubular VEGF and Bcl-xL proteins. It is of additional interest that *in vitro* autocrine production of VEGF by a renal tubular cell line, MCT, was identified by western blot; furthermore, CsA toxicity on MCT increased significantly in the presence of α-VEGF [23].

In the same regard, Kanellis et al. [25] have found that VEGF induces a proliferative and anti-apoptotic response in the rat renal tubular epithelial cell line, NRK52-E. Moreover, these cells express VEGFR1 and VEGFR2 mRNA and VEGF protein, leading to the hypothesis that VEGF may act as a survival factor for renal tubular epithelium *in vivo*. Collectively, all these results suggest that endogenous VEGF has direct protective effects on renal tubular cells, at least under CsA challenge. The occurrence of both *in vivo* and *in vitro* effects of VEGF blockade provides a source of interpretation for the actual role of VEGF in potentially injuring circumstances.

It is of mechanistic interest that VEGF expression in chronic CsA nephrotoxicity is increased by nitric oxide (NO) blockade and decreased by increasing NO [24]. In addition, the increased VEGF expression in chronic CsA nephrotoxicity seems to be related, at least in part, to up-regulation of angiotensin II [26]. VEGF mRNA and protein expression is reduced significantly with angiotensin II blockade [26].

The increase in VEGF by CsA therapy is not restricted to the kidney, e.g. an increase in VEGF and matrix metalloproteinase 2 associated with CsA treatment has been described in the myocardium and other organs, and interpreted as the result of the induction of endogenous, emergency cell repair mechanisms by stress conditions [27].

**Intracellular mechanisms of endothelial CsA toxicity**

Intracellular targets of CsA include mitochondrial respiration, cellular calcium signalling, protein kinase C, protein synthesis and peptidyl–prolyl isomerasers (PPIases). However, the significance of
these intracellular effects for CsA nephrotoxicity remains to be demonstrated [28]. The mechanisms of CsA toxicity appear to be fully understood in the lymphocyte, in which CsA binds cyclophilin (CyP), a cytosolic PPIase, and inhibits its activity. The resulting CsA–CyP complex binds to and inhibits the calcium/calcmodulin-dependent protein phosphatase, calcineurin (Cn) [29]. Cn regulates the processes of dephosphorylation and nuclear import of the transcription factor, nuclear factor of activated T cells (NFAT). In the T-cell signal transduction cascade, NFAT is involved in the nuclear stimulation of the inducible expression of a number of genes, such as interleukin (IL)-2 or IL-4. Nonetheless, although Cn inhibition is the cornerstone of CsA-mediated lymphocyte effects, the issue of whether CyP or Cn is the principal mediator of CsA effects in other organs, e.g. the endothelium, has not been thoroughly addressed.

A recent study in ECs in culture, using non-Cn-binding analogues of CsA, i.e. MeVal-4-CsA or Melle-4-CsA, supports the possibility that CyP has a leading role in conveying CsA-related signalling [9]. Of potential practical application, pre-treating the ECs with low concentrations (10 nmol/l) of CsA or MeVal-4-CsA, before adding higher, cytotoxic concentrations of CsA, resulted in a significant increase in EC resistance against further exposure to toxic concentrations. These results suggest that the role of CyP in the endothelium is more relevant than a mere relay step in the pathway to Cn blockade. CyP could be involved in signal transduction pathways through conformational alteration of transcription factors, ion channels or protein kinases, which could stimulate protective mechanisms at low concentrations; these mechanisms may become overwhelmed by larger amounts of the drugs. As putative candidates for the protective mechanisms on ECs, we can cite the production of heat shock proteins, ROS, NO and Bel-2 and, more recently, survivin [7].

In summary, the effects of CsA on cytoprotection and cytotoxicity in ECs may be conveyed through CyP-mediated rather than by Cn-mediated mechanisms, and VEGF has a major role in the cytoprotective effects of CsA at low concentrations, via VEGFR2. Tubular protective effects of VEGF against CsA-induced injury have now been demonstrated in vivo, therefore opening up new possibilities of pathophysiological interpretation, and therapeutics.

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Erythropoiesis-stimulating agents and antibody-mediated pure red-cell aplasia: where are we now and where do we go from here?

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Introduction

Erythropoietin molecules produced by means of gene technology (erythropoiesis-stimulating agents, ESA) have been the agents of choice to correct the anaemia of chronic kidney disease (CKD) since the first of these drugs was licensed in the late 1980s. The side effects seen in the early days may have been due to a too-rapid rise in haemoglobin concentration, along with possible direct effects on non-haematopoietic tissues, including the vascular endothelium. Recently, antibody-mediated pure red-cell aplasia (PRCA) associated with the administration of ESAs has been identified as a serious problem. The number of cases reported has risen from four in the period from 1988, when human recombinant erythropoietin was first introduced to the market, through 1997, to over 100 cases in the last 3 years. To date, cases of PRCA have been predominantly, though not exclusively, associated with a single brand of ESA, Eprex®. Currently, PRCA is a rare but serious complication and thorough investigation and recommendations on how to deal with the problem are needed.

PRCA

PRCA is a severe, non-regenerative form of anaemia, with selective erythroid aplasia of the bone marrow.