Macrophage migration inhibitory factor (MIF) — potential perspectives for immune-intervention in renal disease

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Introduction

Macrophage and T-cell infiltration occurs in virtually all forms of human glomerulonephritis [1]. The degree of renal impairment correlates with macrophage and T-cell accumulation, arguing that immune cell-mediated injury is a common mechanism in the progression of glomerulonephritis. This hypothesis is supported by animal studies in which various depletion strategies have demonstrated a pathological role for macrophages and T cells in experimental models of glomerulonephritis. We know little of the specific antigens involved in the cellular immune response within the kidney, therefore we reasoned that the best strategy for immune-intervention in glomerulonephritis is to target the molecules which operate at the pinnacle of the immune and inflammatory cascades. This led us to study the role of MIF in kidney disease.

What is macrophage migration inhibitory factor?

Macrophage migration inhibitory factor (MIF) was first described as a product of activated lymphocytes over 30 years ago, but the functions of this molecule are only now being delineated [2]. The importance of MIF in the induction of the skin delayed-type hypersensitivity (DTH) response was recently confirmed in mice by administration of a neutralizing anti-MIF monoclonal antibody (mAb) [3]. In addition, MIF plays a central role in endotoxaemia and MIF collaborates in primary T-cell activation [4,5]. Furthermore, a novel and potentially very important finding is the ability of MIF to counter-regulate the action of glucocorticoids [6]. Indeed, MIF is the only molecule known to over-ride the anti-inflammatory action of glucocorticoids, putting MIF at a key point in the regulation of the inflammatory and immune responses.

Macrophage migration inhibitory factor in glomerulonephritis

MIF mRNA and protein are expressed constitutively in many tissues [7]. The 12 kDa MIF protein is encoded by a single gene and the protein product is stored within the cytoplasm, being released when cells are stimulated by factors such as lipopolysaccharide. In normal kidney, MIF is expressed constitutively by some glomerular and tubular epithelial cells. During the development of rat crescentic anti-glomerular basement membrane glomerulonephritis, there is marked up-regulation of MIF expression by intrinsic kidney cells, including endothelium and glomerular and tubular epithelial cells [8]. Importantly, macrophage accumulation is localized exclusively in areas of strong MIF expression, contributing to focal glomerular and tubulointerstitial lesion formation. In preliminary studies, we have also shown that MIF expression is up-regulated in human glomerulonephritis, with strong MIF expression in areas of macrophage infiltration.

The effect of anti-MIF antibody treatment on experimental glomerulonephritis

To explore the therapeutic potential of blocking MIF, we administered a neutralizing anti-MIF mAb in a rat model of crescentic anti-GBM glomerulonephritis. Compared with administration of the control mAb, treatment with the anti-MIF mAb over days 0–14 of
the disease substantially reduced proteinuria, prevented the loss of renal function, significantly reduced histological damage including glomerular crescent formation, and substantially inhibited renal leukocytic infiltration and activation [9]. Further examination showed that anti-MIF mAb treatment prevented the marked up-regulation of IL-1β, ICAM-1, VCAM-1 and iNOS expression seen in the control antibody-treated animals. Anti-MIF mAb treatment suppressed a skin DTH response to the immunizing antigen, but failed to inhibit the secondary humoral response.

Having established the pathological importance of MIF in the induction and development of experimental crescentic glomerulonephritis, we examined whether anti-MIF mAb treatment could halt or even reverse established crescentic disease [10]. To do this, anti-MIF mAb treatment was delayed until day 7, at which time renal impairment and crescent formation were evident. Compared with animals examined on day 7, treatment with the irrelevant control mAb over days 7–21 led to a rapidly progressive glomerulonephritis with severe renal injury (proteinuria), loss of renal function (creatinine clearance), and a marked increase in histologic damage (including glomerular crescent formation). In contrast, anti-MIF mAb treatment over days 7–21 partially reversed the disease by restoring normal renal function and reducing histological damage compared with untreated animals examined on day 7. Reversal of disease was associated with a significant reduction in leucocyte infiltration and activation and renal interleukin-1 production. Importantly, anti-MIF mAb treatment caused a significant increase in endogenous serum corticosterone levels, which correlated with the reversal of disease parameters. This study concluded that blocking MIF activity can partially reverse established crescentic glomerulonephritis and suggests that MIF operates by both enhancing the cellular immune response and suppressing the endogenous anti-inflammatory glucocorticoid response.

The potential role of anti-MIF treatment relative to other immune modulatory interventions

These two studies clearly demonstrate the potential for anti-MIF treatment as an immune-intervention therapy in kidney disease. Furthermore, the counter-regulatory role of MIF in terms of glucocorticoid action and production holds much promise for MIF as a therapeutic target. One reason for the very slow clinical development of specific immunosuppressive drugs is that while they may have substantially fewer side-effects compared with glucocorticoids, they do not have any increased efficacy. It is in this area where anti-MIF treatment could prove to have significant advantages over other anti-inflammatory drugs. MIF can overcome glucocorticoid-mediated inhibition of cytokine production [5], therefore high levels of MIF may be a crucial factor limiting the immunosuppressive effects of even high-dose glucocorticoid treatment. The next key step in developing anti-MIF therapy is to determine whether the combination of anti-MIF mAb plus glucocorticoid treatment can provide a greater degree of disease suppression compared with either agent alone. If this proves to be the case, then a combination of anti-MIF treatment plus low-dose glucocorticoids could become the treatment of choice for many types of kidney disease, and indeed immunological diseases generally.

References

EDHE: Update on an alternative vasodilator with potential renal significance

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Introduction

Local vascular tone is generally determined by extrinsic and intrinsic mechanisms such as autonomic nerve activity, circulating vasoactive compounds, tissue metabolites, the myogenic response and endothelium-derived autacoids. The best characterized autacoids are the potent vasodilators nitric oxide (NO) and prostacyclin (PGI₂) and the vasoconstrictor peptide endothelin. Several studies have, however, convincingly demonstrated the existence of an NO/PGI₂-independent component of endothelium-dependent relaxation in mesenteric, carotid, coronary and renal arteries [1]. Since this NO/PGI₂-independent vasodilatation was coincident with vascular smooth-muscle hyperpolarization, the term ‘endothelium-derived hyperpolarizing factor’ was coined. This term has proved somewhat misleading, as hyperpolarization was not initially monitored under conditions of combined NO synthase/cyclo-oxygenase blockade; thus, the effects originally observed probably represent the combined actions of NO and PGI₂ as well as EDHF. Indeed, assigning specific effects and second messenger pathways to each of these autacoids is hampered by the fact that they can all be produced at one time in response to a single stimulus.

Is EDHF a transferable humoral factor?

The existence of a humoral hyperpolarizing factor produced by native endothelial cells was originally proposed on the basis of an endothelium-dependent hyperpolarization of vascular smooth-muscle cells in an arterial segment. However, it proved difficult to substantiate the release of a diffusible hyperpolarizing factor using classical bioassay techniques. Evidence that EDHF is indeed a transferable factor has recently been obtained by monitoring the membrane potential of detector vascular smooth muscle cells situated downstream from, and electrically isolated from, donor endothelial cells [2].

Physiological stimuli for the release of EDHF

Receptor-dependent agonists have mainly been used to elicit the synthesis and characterize endothelium-derived vasodilator and constrictor substances, although mechanical forces such as fluid shear stress and pulsatile stretch are more physiologically important stimuli for the continuous generation of the endothelium-derived autacoids. Recently the release of EDHF, as assessed by the magnitude of the hyperpolarization of detector smooth muscle cells, was reported to be greater in response to pulsatile stretch than in response to supramaximal concentrations of bradykinin [3]. Thus mechanical stimulation of arteries is likely to ensure the continuous release of this endothelium-derived vasodilator.

Putative nature of EDHF

Is NO an EDHF?

High concentrations of NO have been reported to directly activate Ca²⁺-activated K⁺ (K⁺Ca) channels and to induce smooth muscle hyperpolarization under certain experimental conditions. However, whether physiological concentrations of NO are able to induce the hyperpolarization of vascular smooth muscle cells, which is generally attributed to the actions of EDHF, was initially controversial [4]. While an NO/PGI₂-mediated hyperpolarization can be evidenced in patch clamp experiments, more than 60% of the hyperpolarization elicited by the intraluminal solution from bradykinin-stimulated porcine coronary segments is insensitive to combined NO synthase/cyclo-oxygenase blockade. This residual hyperpolarization is unaffected by either oxyhaemoglobin or selective soluble guanylyl cyclase inhibitors which abrogate NO-mediated dilatation [5]. Moreover, the production of EDHF is actually inhibited by basally produced NO and only under conditions of impaired NO synthesis can a distinct EDHF-like response be demonstrated [5]. These considerations, taken together with the pharmacological characterization discussed below, suggest that the ‘endothelium-dependent’ hyperpolarization observed in response to agonists and mechanical stimulation is
elicited by a hyperpolarizing factor which is distinct from NO and PGJ₂.

**Pharmacological characteristics of EDHF**

Although the exact chemical nature of EDHF is unknown, the hyperpolarizing factor produced by carotid, coronary, and renal arteries displays characteristics similar to those of a cytochrome P450-derived metabolite of arachidonic acid [6,7]. Inhibitors of cytochrome P450 enzymes, such as micodazole and 17-ODYA, markedly attenuate EDHF-mediated relaxation in a number of vessels. Moreover, induction of cytochrome P450 enzymes using β-naphthoflavone enhances the release of EDHF from cultured porcine and human endothelial cells [2]. Although concern has been expressed regarding the non-selective effects of some P450 inhibitors (i.e. clotrimazole and econazole) on K⁺Ca channel activity and endothelial Ca²⁺ signaling, it should be stressed that 17-ODYA exhibits neither of these effects and has been shown to selectively inhibit the production of EDHF rather than interfere with its action on smooth muscle cells [2]. These observations along with others led to the proposal that the EDHF released from the coronary and renal artery endothelium is most probably an epoxyeicosatrienoic acid (EET) synthesized by a cytochrome P450 epoxygenase. Indeed, EETs are generated by endothelial cells and mediate part of the dilator effect of arachidonic acid [8]. Moreover, 11,12-EET and 5,6-EET induce K⁺Ca channel inhibitor-sensitive relaxations of endothelium-denuded arteries and activate large conductance K⁺Ca channels in native and cultured smooth muscle cells. It is, however, likely that more than one EDHF exists since the hyperpolarizing factors released from the human gastroepiploic artery, guinea-pig carotid, and rat mesenteric and hepatic arteries or the rat portal vein exhibit very different pharmacological characteristics from that of the ‘coronary EDHF’ [1]. There is, however, evidence to suggest that EETs may be incorporated into and stored in endothelial membrane lipids, to be released upon cell stimulation [9]. Such a mechanism may account for the apparent insensitivity of EDHF-mediated relaxations to the acute application of P450 inhibitors. The endogenous cannabinoid anandamide was proposed as an EDHF in rat mesenteric vessels, since a selective CB1 cannabinoid receptor antagonist attenuated relaxations mediated by ‘authentic EDHF’ [10]. However, although anandamide and the CB1 agonist HU 210 induce the relaxation of a range of vessels by a cyclo-oxygenase-dependent mechanism, these compounds did not induce an EDHF-like (N⁶-nitro-L-arginine/diclofenac-insensitive, K⁺Ca channel-mediated) dilatation.

**Actions of EDHF**

EDHF-induced hyperpolarization is brought about by an increase in the K⁺ conductance of vascular smooth muscle cells and is abolished in the presence of depolarizing concentrations of KCl. Inhibitors of K⁺Ca channels, such as tetrabutylammonium, apamin, charybdotoxin, or iberiotoxin, virtually abolish the EDHF-mediated relaxation, whereas glibenclamide, an inhibitor of ATP-sensitive K⁺ channels (K⁺ATP) usually has no inhibitory effect. Patch clamp experiments have shown that EDHF activates K⁺Ca channels in a cell-attached mode, i.e. when the channel under investigation is isolated from the extracellular medium by the pipette. Thus it would appear that this factor activates K⁺ channels in an indirect manner, possibly involving membrane-associated second messengers [2,12]. In addition to K⁺Ca and K⁺ATP channels, involvement of the Na⁺/K⁺-ATPase in EDHF-mediated relaxation has been inferred from the inhibitory effect of ouabain. However, the effect of ouabain on EDHF-mediated relaxations is the result of direct depolarization of the smooth muscle cell membrane which offsets any endothelium-dependent hyperpolarization.

20-Hydroxyicosatetraenoic acid (20-HETE), an ω-hydroxylation product of arachidonic acid catalysed by cytochrome CYP 4A, has been proposed to be involved in the development of myogenic tone [13]. 20-HETE is endogenously produced by smooth muscle cells from small renal and cerebral vessels, and once formed it increases smooth muscle tone by inhibiting large-conductance K⁺Ca channels, inducing depolarization and increasing [Ca²⁺], probably by activating L-type Ca²⁺ channels [13]. NO may also modulate

**Interactions between NO and EDHF**

Since a pure EDHF response can only be observed in the presence of N⁶-nitro-L-arginine and diclofenac, the interference of one autacoid with the synthesis of another has been difficult to study. However, under conditions of combined NOS/COX blockade, physiological concentrations of NO—whether generated by the endothelial and the inducible NO synthases or exogenously applied NO donors—are able to attenuate EDHF-mediated relaxations. Using a patch clamp bioassay for EDHF, it has been demonstrated that NO attenuates the synthesis of EDHF rather than interfering with its ability to activate K⁺Ca channels in detector vascular smooth muscle cells [5]. Similarly, cytochrome P450 activity in endothelial cells can be attenuated by NO by two mechanisms; an acute effect involving either a decrease in [Ca²⁺], and/or interaction with the prosthetic haem group of the enzyme, and a chronic effect which results in the decreased expression of P450 protein [5]. Pro-inflammatory cytokines, which induce the expression of the inducible NO synthase in porcine aortic endothelial cells, and the prolonged incubation of endothelial cells with NO donors decrease the expression of cytochrome P450 (CYP) 2C and the release of EDHF [11]. It is therefore likely that the production of EDHF is attenuated by NO under physiological conditions, but serves as a back-up system when NO synthesis is impaired.
the formation of 20-HETE by binding and inactivating the cytochrome P450 haem moiety in much the same way that it inhibits the ‘EDHF synthase’. It is tempting to speculate that the open probability of large-conductance $K_{Ca}$ channels in renal arteries is determined by the balance in the vascular production of 20-HETE and EDHF/NO and that EDHF and NO affect vascular tone by counteracting the 20-HETE-induced inhibition of $K_{Ca}$ channels. Intrarenal infusion of 17-ODYA in rats inhibits the formation of 20-HETE, EETs, and dihydroxyEETs and produces diuresis and natriuresis, which are associated with an increase in renal papillary blood flow [14]. These observations suggest that endogenous cytochrome P450 metabolites of arachidonic acid influence renal medullary haemodynamics and the excretion of water and electrolytes.

The role of EDHF in the vasculature may well extend beyond its function as a vasodilator, as preliminary data suggest that this factor is able to activate tyrosine kinases and stimulate the mitogen-activated protein kinases (Erk1/2) in endothelial and smooth muscle cells, and thus may regulate the expression of a number of genes in the vascular wall.

References


Proteinuria and damage to tubular cells—is complement a culprit?

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Proteinuria is an important risk factor for renal prognosis

A recent report from GISEN (Gruppo Italiano di Studi Epidemiologici in Nefrologia) clearly demonstrated that the decline of glomerular filtration rate (GFR) was correlated with the degree of proteinuria, and that this phenomenon was independent of the nature of any underlying diseases or blood pressure [1]. Patients with a low baseline urinary protein excretion rate (<1.9 g/24 h) had the lowest rate of GFR decline and progression to the end-stage renal failure (ESRF) (4.3%) during ~3 years of follow-up. In contrast, patients with nephrotic-range proteinuria (>3.9 g/24 h) lost kidney function rapidly and 32.5% of patients reached ESRF. Thus, the authors concluded...
that urinary protein excretion was the best single predictor of renal disease progression. However, there is general agreement that the degree of tubulointerstitial injury correlates well, not only with renal function at the time of biopsy [2] but also with functional outcome of the kidney [3]. The possible link between proteinuria and tubulointerstitial injury is the presence of filtered plasma proteins in the tubular lumen. Until now, numerous plasma proteins have been demonstrated to affect the tubular cells and promote tubulointerstitial injuries. These include albumin [4], transferrin [5] and lipoproteins [6]. Complement is one candidate for the promotion of proteinuria-associated tubular injury [7]. The evidence supporting the role of filtered complement has been shown in both in vivo and in vitro studies.

**Selectivity of proteinuria and glomerular filtration of complement**

The composition of plasma proteins filtered through the glomerulus into the tubular lumen in proteinuric patients is dependent on the perm-selectivity of the glomerular barrier. Among complement components of the activation sequence (C1–C9, factor B and factor D), the largest is C4 at 206 kDa and the second largest are C1r, C3 and C5 at 180 kDa. C8 has a molecular mass of 163 kDa. As far as the molecular size is concerned, the key molecules in the alternative and late pathways such as C3, C5 and C8 seem hardly to be filtered through the glomerular barrier in patients with selective proteinuria. In contrast non-selective proteinuria may contain various plasma-derived macromolecules, but the precise composition of the urinary protein components according to the degree of selectivity has not been studied fully. The pioneering work of Ogrodowski showed that urinary excretion of membrane attack complex (MAC), the final product of the complement activation pathway, is increased significantly in patients with complement-independent glomerular diseases such as focal glomerular sclerosis and diabetic nephropathy [8]. MAC is a complex molecule (C5b-9) with a large molecular size, and intravenously injected MAC is hardly filtered through the glomerular barrier even in the nephrotic condition [9]. Thus, the presence of MAC in the urine is thought to indicate not only the presence of leaked complement in the tubular lumen but also the intratubular activation of complement. The only exceptions are the human membranous nephropathy [10] and rat Heymann nephritis [11]. In these conditions, it is hypothesized that MAC is initially formed on the podocytes, endocytosed into the cytoplasm, and finally shed into the urinary space.

From the therapeutic point of view, one of the renoprotective effects of angiotensin converting enzyme (ACE) inhibitor is to reduce the abnormal glomerular traffic of macromolecules through improvement of glomerular size selectivity [12]. Further investigation is needed to see whether ACE inhibitors may reduce urinary excretion of macromolecular complement component such as C3.

**Complement is activated on the apical surface of proximal tubular cells and alters the functions of tubular cells to induce tubulointerstitial injury**

The classical work of Sato and Ullrich [13] showed that isotonic reabsorption by rat kidney proximal tubule was inhibited drastically after short perfusion with fresh sera from rats or other species. They further extended their work to show that complement is involved in this phenomenon although the precise mechanism of complement activation was unknown [14]. Camussi et al. [15] demonstrated, using fresh frozen sections of normal human kidneys, that the brush borders of proximal tubules activate an alternative pathway of complement. Biancone et al. [16] further promoted an in vitro study to confirm Camussi’s observation using normal human proximal tubular cells in culture. Production of reactive oxygen species was increased when human proximal tubular cells were exposed to 25% fresh human serum. Heat-inactivated or C6-deficient serum failed to induce this phenomenon. In addition, human umbilical vein endothelial cells (HUVEC) did not produce reactive oxygen species under the same situation suggesting that the proximal tubular brush border has an as yet unknown mechanism of complement activation and MAC formation on their surface.

In order to see the in vivo role of complement in the proteinuria-associated tubulointerstitial injury, Nomura et al. [17] performed an experiment using aminonucleoside nephrosis rats. Depletion of serum complement by cobra venom factor or regulation of complement by intraperitoneally administered soluble complement receptor type 1 (sCR1) decreased significantly the deposition of C3 and MAC on the apical membrane of the proximal tubular cells and tubulointerstitial injury without affecting the level of proteinuria. Since the injected sCR1 was detected in the urine and was also observed on the brush border of the proximal tubule where C3 was deposited, intraperitoneally administered sCR1 was filtered into the tubular lumen and inhibited locally the further activation of complement on the tubular brush border where C3 activation was taking place. Morita et al. [18] obtained similar results using a different model of proteinuria associated tubulointerstitial injury. Thus, activation of complement in the tubular lumen leads to the tubulointerstitial injuries in proteinuric conditions.

**Mechanism of complement activation and its regulation in the tubulointerstitium**

In our bodies, complement activation via an alternative pathway takes place spontaneously by the interaction
of the C3 molecule with water and ammonia. The internal thiolester bond of C3 is cleaved and exposed by this interaction, and C3 becomes an active form which has the ability to bind to cell membranes. Activated C3 (C3i) can also bind factor B, and factor B in the C3i-B complex is susceptible to factor D, resulting in the formation of C3i-Bb complex. This complex acquires the ability of C3 convertase which cleaves an intact C3 molecule into C3a and C3b. C3b behaves like C3i and generates a new C3 convertase, i.e. C3bBb, and forms an enhancing cycle of C3 activation [19]. If there is no regulatory mechanism at this step, complement activation continues until all the C3 molecules are consumed. Complement regulators in both cell-membrane-bound and serum-soluble forms play crucial role in the regulation of complement activation.

Complement activation proceeds when enhancing factors for complement activation overcome inhibiting factors. In the kidney, there are number of enhancing factors for complement activation. First, ammonia production per unit nephron is increased when there is proteinuria, metabolic acidosis and significant loss of functioning nephrons [20]. However, ammonia excretion into the urine is decreased when there is tubulointerstitial injuries including polycystic kidney diseases [21]. As a consequence, the local concentration of ammonia might be increased to activate C3. Secondly, it has been reported that the functioning factor D is increased markedly in the urine of patients with chronic renal failure [22]. This may also promote acceleration of intratubular C3 activation in proteinuric patients with renal failure. All enzymes promoting C3 activation belong to a serine protease entity. Although many substances with serine protease activity have been reported to exist in the apical membrane of renal tubules, no information is available to date concerning their ability to promote C3 activation.

As for the protection against complement activation in the tubules, there are two mechanisms to be discussed. The first is the high concentration of urea. It inhibits activation of complement at the C3 level [20]. It is probable that urea concentration is disturbed in tubulointerstitial injury and this may lead to the further activation of complement. The second is the membrane regulator of complement. Importance of membrane bound complement regulator was demonstrated by the work of Nomura et al. [23]. The function of C3 level complement regulator (rat Crry) was first inhibited by Fab fragment of a specific monoclonal antibody by kidney perfusion. When the kidney was connected to the systemic circulation again, significant complement-dependent tubulointerstitial injury was induced. Thus, rat Crry was considered to play an important role in protecting the kidney from the autologous complement attack and maintaining normal integrity of the kidney. Precise immunohistochemical localization of major complement regulatory proteins, MCP (membrane cofactor protein, CD46), DAF (decay accelerating factor, CD55) and HRF20 (20 kDa homologous restriction factor, CD59), was studied by Ichida et al. [24] in the normal human kidney. Complement regulators at C3 level (DAF and MCP) were hardly detectable in the apical membranes of proximal tubular cells. This finding partially explains why the alternative pathway is activated on the apical surface of proximal tubular cells when there is non-selective proteinuria, or when proximal tubular cells in culture are exposed to fresh serum.

These in vitro and in vivo studies provide convincing evidence for the role of complement in the proteinuria associated tubulointerstitial injury.

Local (extra hepatic) production of complement components in the kidney

Although the main source of most serum complement components is liver, many studies have demonstrated that various complement components are also produced in extra-hepatic organs or cells. A number of in vitro studies demonstrated that several complement components including C2, C3, C4 and factor B are generated by renal cells under normal conditions, and their production is up-regulated by inflammatory cytokines [25]. In an experimental model of murine lupus nephritis, the increase in renal production of complement components C2, C3, C4 and factor B correlated with the severity of nephritis [26]. Internal C3 synthesis has been shown to increase in immune complex glomerulonephritis [27], tubulointerstitial nephritis [28] and allograft rejection [29]. In human renal allografts, donor-specific C3 was expressed and localized in glomerulus and tubule [30]. These findings strongly suggest that locally produced complement components contribute to the progression of renal inflammation.

Timmerman, however, has attempted to elucidate the role of locally synthesized C6 in the kidney by administering anti-rat Thy1.1 antibody to a C6-deficient rat (PVG/c− strain) transplanted with a kidney from a wild-type C6-sufficient rat (PVG/c+ strain) [31]. Transplanted kidney expressed C6 mRNA in peritubular sites, but there was no C6 or MAC deposition in the glomerulus. Since anti-Thy1.1 nephritis is dependent on MAC, the authors concluded that the renal synthesis of C6 was not sufficient to produce glomerular injury. Thus, the role of complement components produced locally in the kidney is not conclusive. Further efforts are needed to elucidate the role of locally produced complement components in the development of tubulointerstitial injury.

Conclusions

Experimental findings and clinical data strongly suggest that the complement components filtered through
the glomerular barrier are activated on the luminal surface of proximal tubular cells and cause tubulointerstitial damage. Although proteinuria-associated tubulointerstitial injury cannot be attributed solely to intratubular complement activation, it is now clear that complement is one of the important causative factors. However, the exact mechanisms of complement activation on the tubular cells are not elucidated fully. It is completely unknown whether there is any serine protease mediating complement activation in the tubular cells, whether there is increased expression of acceptor molecules which binds activated C3 molecule on the surface of tubular cells, or whether proximal tubular cells can increase production and release of inflammatory cytokines and growth factors in response to the autologous complement attack. Clinically, it is still unknown whether the degree of urinary complement excretion can predict the functional outcome of the kidney, and whether ACE inhibitor can reduce urinary complement excretion. Further studies are needed to understand the proper roles of complement in the proteinuria associated renal injuries.

References

Does the parathyroid ‘see’ phosphate?

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Introduction

Parathyroid hormone (PTH) regulates the serum phosphate (P_i) concentration through its effect on the kidney to decrease P_i clearance and increase serum P_i, which becomes an important problem in severe renal failure. Clinical studies have demonstrated that P_i restriction in patients with chronic renal insufficiency is effective in preventing the increase in serum PTH levels. A number of careful clinical and experimental studies have suggested that the effect of P_i on serum PTH levels is independent of changes in the levels of both serum calcium and 1,25-dihydroxyvitamin D_3 (1,25(OH)_2D_3) [1–4].

In addition, Almaden et al. [5] showed that high P_i directly stimulated both PTH secretion from whole rat parathyroid glands in culture and PTH mRNA levels in human parathyroid tissue in culture. Slatopolsky et al. [6] showed similar results for PTH secretion from rat parathyroid glands maintained in primary culture. These results indicate that P_i regulates the parathyroid directly.

This effect of P_i may be mediated by specific molecules in the parathyroid cell membrane or by metabolic signals associated with increased cellular P_i concentrations. One such mediator may be a Na^+-dependent P_i cotransporter in the plasma membrane of parathyroid cells.

Mammalian Na^+-P_i cotransporters

At least three types of Na^+-P_i cotransporter have been isolated from mammalian cells [7,8]. Type I and type II transporters have been isolated from kidney cortex; they mediate Na^+-dependent P_i cotransport in the apical membrane of proximal tubular cells. Type III transporters were isolated as receptors for gibbon ape leukemia virus (GLVR1 or PiT-1) in mice and humans and amphotropic murine retrovirus (Ram-1 or PiT-2) in rats, and were shown to serve normal cellular functions as Na^+-dependent P_i cotransporters in several tissues [8]. PiT-1 and PiT-2 are approximately 60% identical in amino acid sequence, and exhibit no significant overall sequence homology with the type I or type II transporters. We have isolated and characterized a Na^+-P_i cotransporter cDNA from rat parathyroid glands.

Cloning of parathyroid Na^+-P_i cotransporter

In a previous study, we isolated a Na^+-P_i cotransporter cDNA from rat parathyroid glands [9]. The protein encoded by this cDNA clone, rat PiT-1, showed Na^+-dependent P_i cotransport activity when expressed in Xenopus oocytes and appears to be the homolog of human and mouse PiT-1 (Glvr-1), a Na^+-P_i cotransporter from mice and humans that also functions as a virus receptor (Figure 1). The rat PiT-1 sequence shares weak homology with pho-4^+, a gene implicated in phosphate uptake in Neurospora crassa and also distantly related to partially characterized genes from Escherica coli, Streptomyces hyasledii and Methanobacterium thermoautotrophicum. PiT-1 and pho-4^+ display similar hydropathy plots, contain similar internal repeats, and are homologous in their primary structures. This family of high-affinity P_i transporters allows cells to grow under restrictive conditions requiring a high-affinity uptake of P_i. Thus, PiT-1 is a member of an ancient P_i transporter gene family.

Characterization of a parathyroid Na^+-P_i cotransporter

To characterize the Na^+-dependent P_i cotransport system in rat parathyroid glands, we measured the initial rate P_i fluxes over a wide range of P_i concentra-

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![Fig. 1. The predicted structure of the parathyroid Na^+-P_i cotransporter PiT-1. The 10 predicted transmembrane regions (M1–M10).](image-url)
tions [9]. In the absence of Na\(^+\) in the incubation medium, the transport of P\(_i\) was significantly reduced, amounting to <55% of the total uptake. The kinetic values for the Na\(^+\)-dependent P\(_i\) transport system in rat parathyroid glands were similar to those for PiT-1, suggesting that PiT-1 is a functional Na\(^+\)-dependent P\(_i\) transporter in rat parathyroid glands. The significant difference in \(K_m\) values (89 ± 13 and 140 ± 20 μM, \(P<0.05\)) observed between parathyroid glands and Xenopus oocytes could be the result of uncontrolled variables, such as membrane potential or post-translational modifications [9].

**Regulation of parathyroid Na\(^+\)--P\(_i\) cotransporters**

The amount of PiT-1 mRNA in the thyroparathyroid tissue was reduced in vitamin-D-deficient animals compared with that in normal animals. When expressed relative to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, the administration of 1,25(OH)\(_2\)D\(_3\) to vitamin-D-deficient rats induced ~2.3- and 3.3-fold increases in the amount of PiT-1 mRNA in these glands after 24 and 48 h, respectively [9].

Rats fed a low-P\(_i\) diet for 14 days showed a markedly lower plasma concentration of P\(_i\) than animals fed a high-P\(_i\) diet for the same period [9]. When expressed relative to the amount of GAPDH mRNA, the amount of PiT-1 mRNA in the thyroparathyroid tissue of rats fed the low- and control-P\(_i\) diet was 3.2 and 2.5 times that of the animals fed the high-P\(_i\) diet, respectively [9]. The level of PiT-1 mRNA in the parathyroid glands was also decreased in chronic renal failure (CRF) rats (Table 1).

**How cells sense P\(_i\)**

**Renal proximal tubular cells**

Changes in the extracellular concentration of P\(_i\) modulate cellular function in a physiologically relevant manner. High extracellular P\(_i\) concentrations inhibit and low concentrations stimulate 1,25(OH)\(_2\)D\(_3\) synthesis in the renal proximal tubules. Extracellular P\(_i\) also modulates the renal tubular reabsorption of P\(_i\), with reduced P\(_i\) enhancing and increased P\(_i\) diminishing the tubular reabsorption of P\(_i\) [7]. Extracellular P\(_i\) directly controls the apical Na\(^+\)--P\(_i\) cotransporter in the proximal tubular cells.

In opossum kidney proximal tubular cells (OK cells), a lowering of the medium P\(_i\) results in an increase in apical Na\(^+\)--P\(_i\) cotransport activity, and increasing the P\(_i\) concentration leads to lowered P\(_i\) uptake [10,11]. It was found that the adaptive response to P\(_i\) deprivation can be elicited only when the apical side of the cells is in contact with a low P\(_i\) medium [11]. The up-regulation of the apical P\(_i\) transport is apparent within 2 h of replacement of the 'normal' medium with a low P\(_i\) medium. Replacing the basolateral medium with a low-P\(_i\) medium has no effect on the apical adaptation. In addition, the basolateral Na\(^+\)-independent P\(_i\) transport does not undergo an adaptive response, whether the low P\(_i\) medium is on the apical side or the basal side. Based on these data, it can be speculated that changes in the intratubular P\(_i\) concentration are the determining factors for the adaptive response [11]. This signal may stabilize the Na\(^+\)--P\(_i\) cotransporter mRNA [7]. These observations suggest that apical Na\(^+\)--P\(_i\) cotransporters may act as the P\(_i\)-sensing protein in renal epithelial cells.

**Parathyroid cells**

We suspect that a Na\(^+\)--P\(_i\) cotransporter may mediate the effects of extracellular P\(_i\) on the regulation of PTH synthesis. This transporter was regulated by 1,25(OH)\(_2\)D\(_3\) and by dietary P\(_i\), but there is no evidence that the extracellular P\(_i\) needs to be taken up to exert its effect on the regulation of PTH synthesis. Further studies are needed to clarify whether PiT-1 functions as a cell-surface P\(_i\)-sensing protein in parathyroid cells.

Nuclear transcript run-on assays showed that the effect of low P\(_i\) was post-transcriptional, unlike the predominantly transcriptional effect of 1,25(OH)\(_2\)D\(_3\) on the PTH gene. Changes in the extracellular P\(_i\) concentration may modulate the stability of PTH mRNA (Figure 2). In addition, the effect of calcium on PTH secretion from dispersed bovine parathyroid cells occurs within seconds. However, the effect of P\(_i\),

![Fig. 2. Roles of cell-surface Na\(^+\)--P\(_i\) cotransporter in parathyroid cells. Dietary P\(_i\) content (or plasma P\(_i\)), 1,25(OH)\(_2\)D\(_3\) and PTH each affect the expression of the parathyroid Na\(^+\)--P\(_i\) cotransporter gene. The changes in the Na\(^+\)--P\(_i\) cotransporter activity may modulate the interaction between cellular inorganic phosphate and its binding protein. The P\(_i\) binding protein may bind the 3'-untranslated region of PTH mRNA and modulate its stability.](image)
in vitro in parathyroid glands in culture requires about 4 h before any change in PTH secretion occurs. Naveh-Many et al. [12] suggested that the effect on PTH gene expression correlates with a decrease in PTH mRNA protein binding. This is in contrast to the effect of hypocalcemia, which increases this binding. These results indicate that the final pathway of the effects of low P and low calcium on PTH mRNA share a common mechanism [12].

References

Epidemiology of hantavirus infections in Europe

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Key words: acute renal failure; hantavirus; haemorrhagic fever with renal syndrome; nephropathia epidemica; zoonosis

Introduction

Hantaviruses are found almost world wide and in many areas they are associated with significant mortality and morbidity among humans. They are zoonotic viruses, transmitted to humans from infected rodent carriers. Each hantavirus has its own carrier species with which it has evolved during thousands or millions of years. Transmission to humans is a dead-end for the virus. Hantaviruses are enveloped RNA viruses belonging to the Bunyaviridae family, which otherwise consists of arthropod-borne viruses including some other human pathogens. During the last few years the global interest in hantavirus disease has increased and to date more than 20 distinct hantaviruses have been found. The number is likely to increase because about 2100 rodent species are known and only a small portion of them has been studied. Hantavirus infections are probably underdiagnosed in Europe [1].

In humans, hantaviruses cause two main types of diseases. Haemorrhagic fever with renal syndrome (HFRS) is caused by Puumala (PUU), Dobrava (DOB), Seoul (SEO) and Hantaan (HTN) viruses, while Sin Nombre (SN) and several other hantaviruses found in North and South America cause hantavirus pulmonary syndrome (HPS). Hantaviruses circulating with certainty in Europe include PUU, DOB and Tula (TUL) viruses, of which PUU and DOB viruses are clinically significant.

Nephropathia epidemica (NE) caused by PUU virus is clinically characterized by high fever, headache, abdominal and back pains, haemorrhages and hypotension. The renal involvement best described as an acute tubulo-interstitial nephritis results in transient
Hantaviruses and carrier rodents

Most hantavirus infections in Europe are caused by PUU virus, carried by the bank vole (*Clethrionomys glareolus*), which is found almost everywhere in Europe except southern Spain and Italy and northernmost Norway. The incidence of NE in humans depends strongly on the pattern of population dynamics of the bank vole, which differs greatly in various parts of Europe.

In northern Europe, the bank vole exhibits pronounced 3–4 year population cycles, which are thought to be caused by rodent predators. Most human NE cases occur in the increase and peak phases of the cycle. These cycles are not, however, synchronous over the whole country like Finland and Sweden, and epidemics are encountered in different parts of the country during different years.

In temperate Europe, on the other hand, the bank vole populations are much more stable, basically seasonal, increasing in summer and declining in winter. In these normal years NE is quite rare. However, occasional mast years, heavy seed crops of oak and beech, are followed by improved survival and breeding of seed-eating rodents like bank voles. The mast years are induced by higher than normal summer temperatures and because of this, they can be synchronous over large areas in Europe, consequently giving rise to human epidemics of NE.

DOB virus infections were originally reported only in the Balkans, but recently also in central Europe, Estonia and Russia [4]. DOB virus is carried by the striped field mouse (*Apodemus agrarius*) and yellow-necked forest mouse (*A. flavicollis*). *Apodemus agrarius* is not found in westernmost Europe and DOB virus carried by it seems to dominate in central, eastern and northern Europe up to Estonia while DOB virus in *Apodemus flavicollis* seems to dominate in southern Europe, especially in the Balkans.

In Europe SEO virus has so far been proved to cause only infections transmitted by laboratory rats. TUL virus, found in European common voles (*Microtus arvalis*), can rarely infect man, but has not so far been associated with human disease. Neither is another newcomer, Topografov (TOP) virus carried by lemmings (*Lemmus lemmus*). However, epidemiological data suggest that TOP or a related virus could have caused the lemming-borne military outbreak in Finnish Lapland during World War II.

Hantavirus infections in European countries

NE was first described in 1934 in northern Sweden. A major probable hantavirus outbreak was reported in 1942 among Finnish and German troops mentioned above when hundreds of cases were seen. In Scandinavia most NE cases are reported in Finland (1000/year, incidence 19 per 100 000), while in Sweden 100–300 and in Norway 50–200 clinical cases have occurred annually.

In France, Belgium, the Netherlands, and Germany HFRS cases were first reported in 1982 to 1986. In an endemic forested area across the border between France and Belgium epidemics occurred in 1990, 1993 and 1996. Over the past 10–12 years, the endemic areas in these countries have not been expanding. The recent increase in diagnosed cases of NE in the Netherlands is possibly due to increased awareness of the disease amongst the medical profession [5]. The seasonal distribution in western Europe includes a minor winter/spring peak and a major summer/autumn peak. Most infections in these countries are caused by PUU virus but the first clinical case caused by DOB virus was recently reported in Germany. The number of diagnosed annual infections in Germany is over 200 but there are probably a large number of unrecognized clinical cases [1].

HFRS has been known in the Balkans since 1954. Hundreds of cases have been reported during the last few years. In Bosnia more than 300 patients were admitted to Tuzla hospital in 1995. Several factors such as presence of military camps with large amounts of food stored under primitive conditions, inadequate garbage disposal, or the general breakdown of hygiene caused by the war may have resulted in a higher density of rodents. Most infections in the Balkans are caused by PUU virus. DOB virus has, however, caused severe HFRS in Slovenia, Albania, Greece, and Bosnia [6,7]. According to a recent survey of sera from patients with HFRS in Bosnia and Herzegovina, 75% of the infections were caused by PUU and 25% by DOB virus, whereas no signs of HTN or SEO virus infections were found [7].

In Russia, HFRS is a common disease, although with varying incidence in various parts of the country. PUU virus exists in western Russia and the highest incidences of NE are found in Ural, Volga and Viatka territories with highest morbidity in Bashkiria (43 per 100 000). That area experienced last year an outbreak with 4377 NE cases and 0.6% mortality. In the Far East, more severe HFRS is recognized, probably caused by HNT virus. Moreover, an outbreak of DOB virus-caused disease was recently reported from Russia.
Antibody prevalence and risk groups

In an endemic area in northern Sweden 9% of the population has antibodies to PUU virus [8]. Prevalence rates of up to 30–40% are found in older age whereas they are very low in children. There is no difference between males and females. This is remarkable since most clinical materials include more males than females. It is well known that subclinical or non-typical infections may occur. Whether they are more common among females than males, is not known. In Finland the seroprevalence is 5% on average but up to 20% in endemic areas, and 50% among Finnish mammalogists.

Seroprevalence studies from Germany and the Netherlands have shown that 1.7% and 0.9% of the subjects respectively, have antibodies to PUU virus [1,5]. An increased seroprevalence is found in animal trappers, forestry workers, and employees of horse-farms, but not in farmers. In Sweden and Finland farming has recently been documented to be a risk factor for PUU virus infection [8,9]. A decline in seroprevalence from north to south has been found in Germany [1]. In north-eastern Italy the antibody prevalence ranges from 3 to 9% in occupationally exposed subjects. Hantavirus antibodies are present in 2.5% of the people living in Bosnia and Herzegovina and in 4% in Greece. In Samara in European Russia 7% of the people have antibodies against PUU virus and 1.5% against DOB virus. In Estonia, antibodies to PUU virus are present in 2% and to DOB virus in 1% among sera of healthy persons. The appearance of hantavirus infections in regions where this disease had so far not been observed, e.g. North and South America reveals that the distribution of hantaviruses and particularly of serotypes with high virulence, is geographically more widespread than thought in the past. The high genetic diversity of hantaviruses implies that genomic changes may lead to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 1996; 49: 217–221

References


Hantavirus infection—new threats by an old virus

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Hantaviruses are the aetiologic agents of systemic diseases with renal involvement ranging from the haemorrhagic fever with renal syndrome (HFRS) to the more benign nephropathia epidemica (NE). Recent observations indicate that hantaviruses do not only cause renal disease but also acute respiratory failure, i.e. adult respiratory distress syndrome (ARDS) [1]. The appearance of hantavirus infections in regions where this disease had so far not been observed, e.g. North and South America reveals that the distribution of hantaviruses and particularly of serotypes with high virulence, is geographically more widespread than thought in the past. The high genetic diversity of hantaviruses implies that genomic changes may lead to changes in the severity of the disease. Therefore, it is well justified that the hantaviruses have been grouped as ‘emerging viruses’ [2].
The magnitude of the problem

It has been estimated that hantaviruses cause over 200,000 cases [3] of clinically manifest disease worldwide. This is presumably an underestimate, because the available data from some countries are limited and/or insufficient. China and Korea contribute more than 100,000 cases, Russia more than 10,000 and Western and Northern Europe more than 1000 cases each year. In 1993, a peak of diagnosed cases was noted in Germany, i.e. 200 cases, but subsequently the number has definitely decreased. The lethality of hantavirus infection is known to be 0.1–1%, 3–7%, and >50% for Puumala-, Hantaan-, and HPS (hantavirus pulmonary syndrome)-causing hantaviruses, respectively.

Transmission of the disease—new aspects

Hantavirus serotypes are characteristic for specific rodent host e.g. Hantaan virus in Apodemus agrarius, Seoul virus in Rattus norvegicus, Puumala virus in Clethrionomys glareolus, and Sin Nombre virus in Peromyscus maniculatus. Humans can be infected by inhalation of contaminated rodent excretions e.g. urine, faeces and saliva, in which the virus remains stable for prolonged periods. In rodents, the virus is transmitted horizontally and vertically. Recently, Hantaan virus-like sequences were isolated from lung tissue of bats in Korea [4], pointing to an ever expanding host reservoir.

It is known that rodent population densities expand extensively during warm periods. Global warming is a definite risk of increased frequency of hantavirus. Global warming will generally cause a shift in the ecology of vectors leading to a shift in the epidemiology of vector-born disease, for example malaria tropica.

It is also a chilling prospect that viruses of the haemorrhagic fevers are considered likely candidates for use in bioterrorism [5].

From a clinical point of view it is even more important that, in contrast to past opinion, molecular evidence shows that the virus is transmitted from person to person [6]. These observations in Argentina concerned the highly pathogenic Andes virus causing HPS, but this possibility of virus transmission should also be considered when caring for patients who are infected with other types of hantaviruses.

Virus subtypes and clinical course

Until the beginning of this decade, three clinically relevant distinct hantavirus serotypes were recognized, i.e. Hantaan, Seoul, and Puumala virus. Puumala virus is transmitted via C. glareolus and causes nephropathia epidemica, i.e. self-limited systemic disease with acute renal failure. In contrast, Seoul and Hantaan viruses cause moderate to severe systemic diseases with haemorrhagic fever and multiple organ involvement in addition to acute renal failure. Another hantavirus serotype, Dobrava virus [7] which is genetically related to Hantaan virus causes severe renal disease. This hantavirus serotype is endemic in south-east Europe, in particular in the Balkan. In view of world-wide increasing tourism, knowledge of the pathogenicity and genetic variation of hantaviruses and their geographic distribution is of particular importance. In recent years new hantavirus strains have been identified in those geographic regions previously supposed to be free of virulent hantaviruses. In 1993, an epidemic of highly lethal HPS (with a mortality of over 50%) was recognized in the Four Corner Region of the USA (Utah, Arizona, Colorado and New Mexico). The disease was caused by Sin Nombre virus [8], a novel serotype of hantaviruses that persists in and is carried by Peromyscus maniculatus. In the meantime a variety of hantaviruses causing HPS have been identified in the USA e.g. Black Creek Canal virus, Bayou virus and New York virus: the specific rodent hosts are Sigmodon hispidus, Oryzomys palustris and Peromyscus leucopus, respectively. It is of interest that specific Sin Nombre virus like RNA-genomic sequences have been identified in rodent tissue dating back to the year 1983. This finding and retrospective diagnosis of Sin Nombre virus infection in patient material from 1959 [9] is strong evidence that the virus has been endemic for a long time on the American continent. Since 1995, cases of HPS have been reported from South America, further hantaviruses have been isolated as Rio Marmore virus in Bolivia, Laguna Negra virus in Paraquay, Andes virus, Oran virus, Lechiquanas virus and Pergamino virus in Argentina and Andes virus in Chile, and there are several reports of deaths from hantavirus infection in Brazil as well. The mechanisms of pathogenicity of different hantaviruses with renal and pulmonary tissue tropism are still not understood. One can assume that different cellular and viral factors are involved in those molecular mechanism determining the virulence, pathogenicity and tropism of hantaviruses. Although Black Creek Canal virus and Bayou virus belong to the New World hantaviruses (Sin Nombre virus group) renal failure is frequently observed. Furthermore it is known that the Old World hantaviruses can cause pulmonary symptoms, too [10]. So there is more overlap of pulmonary and renal symptomatology than previously thought.

Sin Nombre virus strains are genetically more related to Puumala virus than to Hantaan virus. As far as the genetic diversity of hantaviruses is concerned, genetic drift and shift and intramolecular recombination and rearrangement seem to play an important role in this process. Reassortment was recently reported for closely related strains of Sin Nombre virus [11]. The distribution of HPS infection throughout the world became a matter of concern since person-to-person transmission is apparently possible. This fact raises the question whether or not HPS may occur in other parts of the world, e.g. in Europe. In north-west Germany, two cases of hantavirus infection with severe pulmonary insufficiency and no renal involvement were reported
[12]. Viral sequences were identified that were genetically more closely related to Puumala strains Haellnaes B1 and Berkel. The authors concluded that hantavirus variants in Germany cause moderate to severe HPS-like disease. In general, a pulmonary involvement of different hantavirus serotypes causing HFRS is plausible from the pathogenetic point of view. This is due to the fact that hantaviruses are acquired by inhalation, therefore lung cells (alveolar epithelium, macrophages and endothelial cells) are the first target cells exposed to the virus. Consequently the initial organ for virus replication seems to be the lung. This is in agreement with the detection of hantaviral antigens in the lung tissue of persistently infected rodents.

Renal manifestations

Viral antigens can be detected in kidneys, but are also expressed in liver, spleen, heart, brain and pituitary of infected humans. Hantaviruses typically cause interstitial nephritis. The pathology correlates with severity and clinical stage of the disease. Histopathological changes in the kidney are interstitial oedema, congested subcortical medullary vessels and medullary haemorrhage, infiltration and desquamation of tubular epithelial cells with loss of the brush border and epithelial cell vacuolization. Viral proteins can be detected in tubular epithelial cells. Patients mostly have substantial proteinuria presumably due to glomerular involvement, indicated by mesangial expansion and mesangial cell proliferation. Concerning the long-term renal prognosis of HFRS and nephropathia epidemicæ, some authors reported persisting hypertension or persisting renal dysfunction, i.e. decreased GFR and elevated serum creatinine in the range of 2 mg/dl in such patients. In our own study, 4 of 42 patients who had had hantavirus infection suffered subsequently from persistingly elevated serum creatinine concentration, hypertension or proteinuria.

Diagnosis—recent progress

The diagnosis of hantavirus infection was based originally on immunofluorescence assay. Concerning cross-reactivity of different hantaviruses it should be underlined that cross reactivity exists between genetically closely related hantaviruses such as Hantaan, Seoul and Dobrava virus and between Puumala, Tula, and Sin Nombre virus. The molecular cloning of the hantavirus genome (cDNA of viral RNA segments) and expression of hantiviral protein allowed the development of a new generation of diagnostic systems based on ELISA [13] and RT–PCR technology. Different IgG and IgM ELISA kits were established using recombinant antigens (PROGEN, Biotechnik GmbH Heidelberg, Germany). These systems allow early and specific diagnosis of infections by various hantavirus subgroups. It is rational to test patients with renal failure for a possible hantavirus infection.

Treatment and prophylaxis

Ribavirin, a RNA inhibitory drug (chemotherapy) has been studied in a variety of interventional trials conducted in different countries. When administered in the initial stage of infection, it will abbreviate the duration of infection and mitigate its course. Although the need for a hantavirus vaccine is urgent an efficient prophylactic system against hantavirus infection is still missing. The strategies for development of a vaccine should be based on the expression of immunogenic epitopes in pro- and eucaryotic expression systems, generation of attenuated viruses, production of antigen-presenting, but replication-deficient, viruses or expression of viral fusion proteins using viral vectors for example as chimaeric hepatitis B virus core particles [14].

References

Novel strategies to retard renal disease progression: combining ACE inhibition with endothelin receptor blocking?

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Background

The glomerular and tubular functions of the kidney are regulated by an intricate network of circulating and locally produced hormones. These substances comprise proteins, peptides, lipids and amino acid-derived molecules, such as arginine vasopressin, angiotensin II (AII), atrial natriuretic peptides, endothelin (ET), bradykinin, arachidonate metabolites and nitric oxide. Recent advances in molecular biology have further elucidated the role of these substances in renal pathophysiology. In particular, genetically manipulated animals in which a hormone is either overexpressed (transgenic) or eliminated (gene knock-out) have served as powerful tools to dissect the often complex and overlapping actions of these substances in the kidney. In parallel, they have also contributed to more insights into the role of vasoactive mediators, such as AII and ET, in the process of progressive tissue destruction in chronic renal diseases. Given the limited therapeutic options available for patients with renal disease who are losing kidney function, these advances have led to interest in defining strategies to better prevent renal-disease progression.

AII and ET-1 as major renal vasoconstrictors

The renal vasculature and tubule epithelium synthesize, and are sensitive to, a variety of circulating and locally derived vasoactive mediators, allowing the kidney to modulate glomerular filtration rate (GFR) as well as Na+ and water excretion. AII, the effector peptide of the renin–angiotensin axis, shares many of the characteristics associated with known endogenous modulators of renal function, being a potent vasoconstrictor of the renal vascular bed. In vivo, AII contracts both afferent and efferent glomerular arterioles, modulating intraglomerular capillary pressure and ultimately glomerular filtration [1]. In addition to its effects on the glomerular microcirculation, AII also has a relevant role in modulating the sieving of macromolecules by the glomerulus. While initial studies attributed the AII’s effect of enhancing glomerular permeability to macromolecules solely to its ability to raise glomerular capillary hydraulic pressure, more recent evidence suggests that AII regulates the glomerular sieving function independently of its hemodynamic effects, by modulating the cytoskeleton assembly of the podocytes and/or the matrix network of the glomerular basement membrane [2]. Increasing evidence suggests that AII controls renal sodium excretion not only by interfering with renal hemodynamics and activating aldosterone biosynthesis, but also by directly regulating tubular epithelial sodium transport. Indeed, AII acts predominantly in the S1 segment of the proximal convoluted tubule to reduce intracellular cAMP, increase the Na+/H+ antiport activity and ultimately enhance Na+ reabsorption [3].

Whereas AII has been recognized for years as the major renal vasoactive hormone it is increasingly recognized that this is just one mediator of renal vasocostriction in normal and disease states. Of crucial relevance are other hormonal systems, one of which, critically dependent on the synthesis and release of vasoactive molecules, belongs to the ET family and comprises three different peptide isoforms: ET-1, 2 and 3, encoded by three different genes [4]. Mature peptides derived by cleavage via an endothelin converting enzyme (ECE), a phosphoramidon-sensitive, membrane-bound metalloprotease, exert their effects by activating specific receptors. Of these ETα mostly mediates vasoconstriction and proliferation, while ETβ receptor activation is coupled to both vasoconstriction and vasodilation. Renal vessels are peculiarly sensitive to the vasoconstrictive effect of ETs [5]. Thus injection of ET-1 into rats raises blood pressure and renal vascular resistance and consequently reduces RBF and GFR [6]. The effect of ET-1 of enhancing systemic blood pressure is fully abolished by a selective blockade of the ETα receptor [7]. A non-selective ETα- and ETβ-receptor antagonist is needed instead to prevent ET-1-induced renal vasoconstriction suggesting a relevant role for the ETβ receptor in the renal response to systemically administered ET-1, at least in the rat. Healthy human volunteers infused with pharmacological doses of ET-1 showed a modest increase in systemic blood pressure but a substantial decrease in...
renal function and excretion of water and sodium [8]. The receptor subtypes which mediate renal vasoconstriction in response to ET-1 infusion in humans are unknown at present. ET-1 has a direct effect on sodium homeostasis and water balance which varies among species. In rats a low dose of ET-1 has a natriuretic effect, while in dogs ET-1 reduces sodium excretion because of a decrease in filtered load and/or renin–angiotensin stimulation. In humans, ET-1 at subpressor doses has no effect on the Na⁺ excretion rate while higher pharmacologic doses induce dose-dependent, long-lasting pressor responses which reduce GFR and alter renal Na⁺ handling through a pressor natriuresis which may or may not be associated with a reduction in Na⁺ filtered load.

Both AII and ET-1 induce fibroblast proliferation and extracellular matrix synthesis

Importantly, AII modulates renal cell growth [9] and evidence is available that intrarenal renin–angiotensin system (RAS) activation, as it occurs in most renal disease conditions, is almost invariably associated with renal growth. This phenomenon, apparently linked to AT₁ receptor activation, depends upon a process of secondary up-regulation of TGF-β and collagen type IV [10]. Injection of AII into the renal artery led to a significant increase in the renal expression of c-fos and Egr-1, the immediate early genes whose activation precedes cell proliferation. These findings may account for the observation that in vivo in rats chronic infusion of AII promotes type IV collagen deposition into renal interstitium and precedes fibrosis. Of interest, angiotensin converting enzyme (ACE) inhibitors limit the up-regulation of genes which favor renal cell growth and the deposition of extracellular matrix proteins and block compensatory renal hypertrophy in most animal models.

ET-1 is also a strong mitogen for mesangial cells [11] and fibroblasts, and by favoring cell proliferation also promotes mesangial matrix protein gene transcription. This is documented by findings in rat cultured mesangial cells and fibroblasts where challenge with increasing concentrations of ET-1 enhances the rates of synthesis of mRNA for collagen type I, III and IV. ET-1, by activating mesangial cell phospholipase A₂, promotes the release of arachidonic acid from membrane phospholipids, which in turn causes excessive formation of thromboxane A₂, one of the factors involved in the synthesis of extracellular matrix proteins. ET-1 is also chemotactic for blood monocytes, a property which in vivo could contribute to the tubulointerstitial damage that accompanies most renal diseases characterized by a progressive course [12].

Cross-talking between AII and ET-1

It is well known that AII stimulates the expression of the ET-1 gene in endothelial and renal cells. Infusion of AII enhances functional ECE activity in vascular smooth muscle and kidney via the ET₄ receptor, whose expression is upregulated by AII in vascular smooth muscle cells, cardiomyocytes and mesangial cells [13]. Part of the mitogenic effect of AII is mediated by ET-1 as suggested by studies with monoclonal antibodies against ET-1 or drugs that block the ET₄ receptor. A reciprocal regulation of AII and ET-1 on matrix protein synthesis and cell proliferation via ET₄ and AT₁ receptors has been suggested in rat mesangial cells [14], AII inhibitors or receptor blockers diminishing the ET-1-mediated effects and vice versa. Consistent data are available indicating that ET-1 in part mediates the increase in systemic blood pressure induced by AII [15–17], a phenomenon dependent on the activation of ET₄ receptors. The observation that in normotensive rats a dose of ET-1 devoid of the pressor effect enhanced arterial pressure when combined with a non-pressor dose of AII, would indicate that vascular ET-1 acts in synergy with AII in inducing vasoconstriction and regulating systemic blood pressure in health and disease. Whether ET-1 also contributes to the effect of AII in impairing glomerular permeability to macromolecules in animal and human proteinuric nephropathies is, however, controversial.

Evidence for AII activation and the effect of ACE inhibitors in experimental progressive nephropathies

AII generated in the kidney constricts the efferent arteriole much more than the afferent arteriole, contributing to the elevated glomerular pressure implicated in the pathogenesis of most experimental and human renal disease. In experimental diabetes early in the course of the disease glomerular hyperfiltration develops depending on afferent arteriolar dilation, possibly due to chronic hyperglycemia or genetic abnormality. Rats with subtotal renal ablation also develop proteinuria and progressive deterioration of renal function owing to high glomerular capillary pressure. A high-protein diet, by promoting an excessive formation of renin mRNA, further enhances glomerular pressure and accelerates the decline in renal function. Thus abundant evidence exists that local stimulation of RAS is uniformly deleterious to the kidney in animal models.

ACE inhibitors, which reduce glomerular pressure by dilating the efferent arteriole, limit the progression of renal disease in experimental diabetes as well as in animals with renal mass reduction. More recent evidence has been provided that ACE inhibitors decrease blood pressure, limit proteinuria and slow the decline in renal function in other models including, male MWF/Ztm rats, puromycin nephrosis, passive Heymann nephritis (PHN) and immune-mediated nephropathies [18].

The protective effect of AII antagonists in diabetic and non-diabetic human renal diseases

ACE inhibitors for the same level of blood pressure control are more renoprotective than other antihypertensives also used in human nephropathies and this appears to be linked to their property of lowering urinary proteins to a greater extent than conventional drugs [18]. Lewis et al. [19] found that patients with insulin-dependent diabetes mellitus given captopril had a lower incidence of doubling of serum creatinine than those on conventional therapy over 4 years of follow-up. A similar effect of ACE inhibitors was shown in patients with immune-mediated chronic nephropathies [18]. That the superior renoprotection achieved by ACE inhibitors compared with other drugs depended on their effect of limiting the urinary protein excretion rate, even at comparable levels of blood-pressure control, has been documented formally by the Ramipril Efficacy in Nephropathy (REIN) study. In this trial the mean rate of GFR decline was three times lower in patients with baseline proteinuria of 1–3 g/24 h as compared with levels of $\geq$ 3 g [20]. Among this latter group the rate of GFR decline was significantly limited by ramipril as compared with conventional treatment. In the ACE inhibitor group the GFR decline was associated with an early reduction in the urinary protein excretion rate which remained significantly lower than in the control group throughout the whole study. In addition, the reduction in the risk reaching an end-point was significantly predicted by the percentage reduction in urinary protein excretion during the treatment period as compared with baseline. In both treatment groups systolic and diastolic blood pressure were comparable at baseline, fell similarly after randomization and remained comparable during the whole follow-up. Chronic treatment with ACE inhibitors, however, does not suppress the formation of AII uniformly, the plasma levels of which, after an initial fall, tend to increase with time toward to baseline levels [21]. A possible explanation for the antihypertensive and renoprotective effects of this class of compounds rests on data which show that AII degradation products, in particular angiotensin [1–7], are formed in excessive amounts in the circulating blood of patients given ACE inhibitors. These compounds act to oppose the pressor action of AII by stimulating vasodilatory prostaglandins and nitric oxide. However, AII is known to stimulate the generation of the vasoconstrictor peptide ET-1 and data are available which show that in animals with progressive nephropathies ACE inhibitors also inhibit the exaggerated synthesis of ET-1. Thus ACE inhibitors given chronically to MWF/Ztm rats genetically targeted to glomerulosclerosis and progressive proteinuria [22], as well as to animals with immune mediated glomerulonephritis [23], reduced renal ET-1 mRNA expression and the synthesis of the parent peptide significantly.

Evidence for ET-1 up-regulation in progressive nephropathies of diabetic and non-diabetic type

Gene expression and the renal synthesis of ET-1 are up-regulated in animal models of disease progression [24]. Thus the remnant kidney model is characterized by a time-dependent increase in renal ET-1 gene expression, as well as an increase in urinary excretion of the peptide, both correlating with progressive renal damage. Similar results were obtained in rats with passive Heymann nephritis, an immune model of glomerular disease resembling human membranous nephropathy. Other studies have found an up-regulation of renal ET-1 and endothelin type B (ET$_B$) receptor gene expression in NZB/W F$_1$ mice that have an immunological disease reminiscent of human lupus. Glomerular ET-1 and ET$_B$ receptor mRNAs were elevated (compared with normal) within days of puromycin amino-nucleoside injection and decreased to control levels by day 20, when the animals were no longer nephrotic. Data are also available in experimental diabetes where the mRNA for ET-1 is overexpressed in the face of unchanged ET$_A$ and ET$_B$ receptors. More direct evidence for ET-1 as a fosterer of renal damage derives from studies using transgenic animals. Mice overexpressing the human ET-1 promoter form more ET-1 in their kidneys and develop renal lesions despite not developing hypertension [18]. Furthermore, rats transgenic for the human ET-2 gene are normotensive and are characterized phenotypically by renal lesions reminiscent of those seen in rats with remnant kidney [18]. Evidence for increased renal ET-1 is also available for patients with chronic progressive nephropathies and for unilateral nephrectomized patients.

ET-1 antagonists improve, at least partially, diabetic and non-diabetic progressive nephropathies

In the renal ablation model both ET$_A$ and ET$_A$ and ET$_B$ receptor antagonists partially reduced proteinuria, ameliorated renal function and prolonged survival despite poor blood pressure control [24]. The renoprotective effect of ET$_A$ antagonists was also evident in mice affected by a lymphoproliferative disease which mimics lupus, and in rats with experimental diabetes treated at the moment of induction of the disease. More recently, an unselective endothelin-receptor antagonist, chronically administered to diabetic animals with proteinuria, was shown to be effective as an ACE inhibitor in lowering blood pressure and controlling total protein and albumin excretion [25]. Unselective inhibition of ET$_A$ and ET$_B$ receptors by bosentan had a beneficial effect on the evolution of nephritis in experimental immune complex nephritis induced by repeated injections of ovoalbumin [18].
Should we combine AII and ET-1 antagonists to maximize renoprotection?

Although inhibiting ACE is the best therapy available to date for animal and human proteinuric progressive nephropathies, such an approach alone is not always sufficient to normalize urinary proteins or to fully prevent renal damage and GFR decline. Possible explanations are that the AII antagonist only partially blocks locally formed AII or, perhaps more relevant, that in chronic nephropathies an exaggerated synthesis of other mediators of vasoconstriction, such as ET-1, takes place in the kidney whose generation can not be limited to an appreciable extent by ACE inhibitors alone. Thus there is a robust rationale for combining different treatments if one intends to maximize renoprotection. In a very recent study performed in rats with passive Heymann nephritis and heavy proteinuria we found that contemporary blocking of AII and ET-1 by an ACE inhibitor associated with a ET_A receptor antagonist was more renoprotective than each drug alone [26]. Specifically, while single therapy reduced proteinuria by 23–25%, the combination resulted in a 45% lowering of urinary proteins. The rationale for combining an ACE inhibitor with an ET-1 antagonist in the above setting rests on data that the kidneys of PHN rats synthesize more ET-1 than normal kidneys [18]. Excessive ET-1 mRNA and protein were localized in proximal tubules where extensive reabsorption of protein took place and preceded the recruitment of inflammatory cells into the interstitium [18]. Findings that contemporary blocking of AII and ET-1 was more renoprotective than each drug alone would suggest that renal disease progresses through an interaction of different mechanism(s) at least two of which have been identified as being suitable for pharmacological modulation. While ACE inhibitors antagonize, at least partially, excessive protein trafficking through the glomerular filter other drugs would instead prevent the consequences of enhanced protein traffic and reabsorption on locally formed vasoactive and inflammatory mediators of renal injury. ET-1 is certainly one of the vasoactive substances formed by proximal tubuli as a consequence of abnormal protein overload, as found in many studies and extensively reviewed recently [18,27]. A combination of drugs to more effectively limit renal injury therefore has a theoretical justification. Since ET_B receptors, but not ET_A, are more frequently up-regulated in progressive nephropathies, the possibility of combining an AII blocker with an ET_A/ET_B receptor antagonist may represent a greater therapeutic advantage and thus should be explored. In the near future results of experimental studies will identify many more of the factors involved in tubulo-interstitial and glomerular damage which are eventually induced by glomerular hypertension and protein traffic. A multidrug approach that targets these molecules will probably be the way to limit, and hopefully fully avoid, progression of proteinuric renal disease towards the need for renal replacement therapy. Even now, patients whose disease responds only partially to ACE inhibitors are candidates for an additional ET-1 antagonist, within the context of controlled clinical designs.

References

Combined morphologic and functional assessment of renal artery stenosis using gadolinium enhanced magnetic resonance imaging

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Introduction

Cost effective treatment options for renal artery stenosis, including percutaneous transluminal angioplasty and surgical revascularization, offer the possibility to cure renovascular hypertension and renal insufficiency. Early diagnosis could prevent dialysis for the majority of patients and save enormous costs [1].

Due to its non-invasiveness, lack of ionizing radiation as well as use of non-nephrotoxic contrast media, magnetic resonance angiography (MRA) is a highly promising screening technique. With the recent introduction of contrast-enhanced ultrafast techniques, MR angiograms with high spatial resolution can be acquired within a single breath-hold of less than 25 s. Typical limitations of unenhanced MRA such as motion artifacts or signal loss in small vessels can be avoided. Therefore, MRA has now gained wide acceptance for replacing more invasive procedures such as conventional X-ray angiography.

The detection of renal artery stenosis with any imaging modality, however, represents only a first step in the diagnostic work-up of renovascular disease. The haemodynamic and functional significance of a detected lesion has to be assessed as well. The capability of combining multiple imaging techniques makes magnetic resonance (MR) imaging particularly suited to address these questions. These various MR techniques allow a comprehensive analysis of renal artery blood flow, renal perfusion and excretory function.

Concept of a combined morphological and functional evaluation of renal artery stenosis

The diagnostic work-up of a patient with suspected renal artery stenosis should always be directed towards clarifying the issue if the surgical or interventional revascularization is indicated. Thus, a single imaging technique should ideally comprise a detailed evaluation of the morphology of the renovascular tree as well as an assessment of the degree of functional impairment of renal perfusion prior to intervention. In addition, the agreement between morphological, functional and haemodynamic abnormalities should be evaluated. Probably the most important question is to predict whether benefit will be derived from revascularization, i.e. whether intervention is indicated. After surgery/ intervention the degree of improvement has to be assessed.

Standard flow sensitive MRA techniques, such as time-of-flight and phase-contrast angiography produce angiograms by detecting the motion of flowing blood. These techniques are limited by patient movement during long acquisition times and by methodological artefacts. The achievable image quality was not comparable to current X-ray angiography. Especially the visualization of smaller details in distal renal artery branches or in accessory vessels was limited. Newly developed contrast-enhanced techniques overcome...
these limitations by acquiring a complete three-dimensional angiogram with high spatial resolution within a single breath-hold period [2]. This method, known as 3D gadolinium MR angiography, requires the administration of gadolinium-chelates as contrast media and therefore allows much faster image acquisition. However, with the current contrast agents, sufficient vessel contrast is only available for a very short time during their first pass requiring optimum timing of the start of the measurement and infusion of the contrast agent. The technique provides true anatomic images with greatly improved image quality similar to conventional X-ray angiography. It also provides adequate spatial resolution as high as 1.5 mm or less in all three dimensions. The gadolinium-chelates used for this procedure are not nephrotoxic and can be administered even in patients with severely compromised renal function [3]. In addition, the method provides functional information of the renovascular system and can be combined with quantitative MR techniques. Since ionizing radiation is not used in MR imaging, there is no radiation hazard and a 3D gadolinium MRA scan can be repeated numerous times for the arterial, venous and excretory phases of the gadolinium passage. This allows temporal assessment of (i) cortical and medullary enhancement, (ii) corticomedullary differentiation [4], and (iii) the rate of excretion of contrast media [5]. With the development of high performance MR systems, acquisition of multiple 3D angiography data sets within a single breath-hold period has become feasible (Figure 1). This so-called ‘multiphasic’ approach helps to assess abnormalities in renal perfusion.

Further characterization of renal artery flow dynamics can be performed by detection of turbulent flow. In unenhanced 3D phase-contrast-angiography techniques, disturbance of the laminar flow pattern causes an artifactual signal dropout in the vessel [4,6] (Figure 2). These haemodynamic abnormalities can be further quantified with 2D MR phase-contrast flow measurements, which can be directly combined with 3D gadolinium MR angiography. The different blood flow velocities within the vessel cross section can be mapped. This allows calculation of absolute blood flow values perpendicular to the vessel axis displayed on the MR angiogram [7] (Table 1). The measurements can be synchronized with the cardiac cycle (cardiac gating) and the pulsatility of blood flow can be recorded and plotted in velocity– or flow–time curves (Figure 3). If these flow curves are recorded with high temporal resolution, characteristic flow profile changes are seen in stenotic vessels (Figure 4). The accuracy of this technique for quantification of mean blood flow as well as for detection of haemodynamic abnormalities has been proven in animal studies when the measurements could be directly correlated to invasive real time blood flow recordings [7]. In the past these techniques had acquisition times of several minutes. In contrast, today recently developed ultra fast MR techniques allows performance of these measurements within a
Fig. 2. Combination of contrast-enhanced 3D gadolinium MR angiography and flow-sensitive 3D phase-contrast angiography for morphologic and haemodynamic assessment of renal artery stenosis. (a) Coronal gadolinium-enhanced MR angiogram reveals 70% stenosis of the proximal right renal artery (arrow). (b) The corresponding 3D phase-contrast angiography shows a mild signal loss in the proximal renal artery indicating disturbance of laminar flow (arrow).

Table 1. Blood flow parameters obtained in 2D phase-contrast flow measurements of 23 patients with different degrees of renal artery stenosis

<table>
<thead>
<tr>
<th>Blood flow parameters</th>
<th>Morphologic degree of stenosis (%)</th>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td>Mean flow (ml/min)</td>
<td>532 ± 146</td>
</tr>
<tr>
<td>Maximum velocity (cm/s)</td>
<td>58 ± 25</td>
</tr>
<tr>
<td>Time to systolic maximum (ms)</td>
<td>184 ± 48</td>
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</table>

single breath-hold period [8] (Figure 3). The morphological and haemodynamic parameters obtained can be correlated to data gained from standard imaging such as kidney size and cortical thickness.

For further quantification of renoparenchymal damage, quantitative assessment of the perfusion of renal tissue is necessary. Certain MR techniques which can be used to determine the amount of blood inflow into the parenchyma have been already successfully applied to the kidneys and the results are promising [9]. Diffusion imaging [10] might also help to further assess parenchymal damage. Both techniques have to be performed prior to administration of gadolinium-chelates.

They can be easily included in the same MR session, before the 3D gadolinium MR angiography and the phase-contrast flow measurements are started, thereby completing the concept of one comprehensive morphological, haemodynamic and functional examination.

Role of comprehensive MR-imaging protocol for detection and characterization of renal artery stenosis

3D gadolinium angiography allows reliable detection of a renal artery stenosis/aneurysm with sensitivities and specificities >90% [11,12], if optimum timing of the measurement and the administration of contrast media is achieved. With common spatial resolutions ≤1.5 mm even accessory renal arteries can be assessed. Further developments in contrast agents might even improve this technique for detection of lesions in very small distal/intrarenal or accessory vessels.

If a renal artery stenosis has been detected, a number of studies must be performed to further characterize the haemodynamic and functional significance of the stenosis. The standard of reference for haemodynamic significance has been retrospectively defined as post-operative improvement, i.e. decrease of serum creatinine, decrease of blood pressure/reduction of antihypertensive medication [4] or prospectively as demonstration of a trans-stenotic pressure gradient >15 mmHg [6] or marked reduction of mean flow [13]. In a comprehensive MR examination, different functional parameters can be identified which correlate with the severity of stenosis. In the 3D gadolinium MRA, significant differences in parenchymal enhancement and cortical thickness were found between normal kidneys and those with haemodynamically significant stenoses [4]. Intravascular signal loss in 3D phase-contrast angiography at the site of stenosis is also an
accurate indicator of a haemodynamically significant stenosis [4,6]. In MR phase-contrast flow measurements, (i) reduction of systolic blood flow velocities in the early systolic peak, and (ii) temporal delay of the systolic flow maximum are objective and accurate criteria that reflect haemodynamic abnormalities in significant renal artery stenoses. The sensitivity and specificity of these indices are comparable with that of DSA [13]. These results are superior to the ultrasound (US) technique [14], which is often compromised by poor visualization of renal anatomy due to overlay of bowel gas or fat. In addition, US assessment of the early systolic flow acceleration in the target vessels, i.e. the smaller intrarenal segmental arteries, is more influenced by downstream microangiopathic lesions.

The different MR imaging techniques analyze only selected aspects of renovascular disease and consequently none of these techniques has perfect sensitivity and specificity [6]. As far as detection of changes in renal artery morphology is concerned, both DSA and 3D gadolinium MRA fail to detect small eccentric arteriosclerotic plaques or the webs and wall irregularities in fibromuscular dysplasia. In fact, abnormalities in renal haemodynamics may still cause renovascular hypertension. In addition, MR techniques to assess flow dynamics may fail, if renal insufficiency is present, because one can no longer reliably differentiate whether parenchymal damage or a significant stenosis is the primary cause of the flow abnormality. Parenchymal damage can only be assessed by quantification of renal...
### Table 2. Modified grading scheme of renal artery stenosis based on comprehensive assessment of morphological and haemodynamic changes

<table>
<thead>
<tr>
<th>Morphologic degree of stenosis</th>
<th>3D Gadolinium MR angiography</th>
<th>2D MR phase-contrast flow measurements</th>
<th>3D Phase-contrast MR angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>normal</td>
<td>normal flow profile</td>
<td>normal</td>
</tr>
<tr>
<td>Mild</td>
<td>mild stenosis &lt;50%</td>
<td>loss of early systolic velocity peak</td>
<td>normal or mildly stenotic, no intravascular signal loss</td>
</tr>
<tr>
<td>Moderate</td>
<td>stenotic ≥50%</td>
<td>decrease of mid systolic velocity components</td>
<td>stenotic, with or without mild intravascular signal loss</td>
</tr>
<tr>
<td>Severe</td>
<td>stenosis &gt;75%</td>
<td>flattened flow profile with no distinct systolic velocity maximum</td>
<td>severe intravascular signal loss simulating occlusion</td>
</tr>
</tbody>
</table>

Parenchymal perfusion/diffusion or disturbance of intrarenal gadolinium-chelate transport and excretion. Although weak differences of some parameters have been found between patients with successful or unsuccessful intervention of renal artery stenosis [4], so far no global MR parameter has been identified which predicts therapy outcome as does renal scintigraphy [15].

Currently, we recommend a combination of the different MR techniques. The techniques are complementary rather than competitive. An approach that can be routinely performed in one MR examination uses a modified stenosis grading scheme. It combines assessment of renal artery morphology by 3D gadolinium MRA and haemodynamic abnormalities in the flow sensitive phase-contrast techniques [4,16] (Table 2). In principle, if one detects an abnormality in either of these techniques, one should suspect that renal artery stenosis is present. Further support of this assumption should then be sought from the results of other MR measurements. A severe stenosis, which is functionally relevant not only shows the morphology of stenosis, but also major changes in flow dynamics. In addition, reduced kidney size and decreased parenchymal contrast enhancement may be present.

Despite the expenses for contrast agents and amortization of MR equipment, the combination of different morphologic and functional techniques in one single MR examination permits a cost effective evaluation. It is a promising substitute for more invasive, nephrotoxic or ionizing techniques with similar cost for contrast agents. It is even promising relative to modalities such as ultrasound.

### References

Nephropathy in type II diabetes—epidemiological issues as viewed from Japan

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Introduction

Diabetic nephropathy is one of the most devastating kidney diseases, ultimately leading to end-stage renal failure in many diabetic patients. According to the statistics of the Japanese Society for Dialysis Therapy, 31,080 of 152,373 (20.4%) chronic dialysis patients suffered from diabetic nephropathy at the end of 1995 in Japan [1]. The annual figures of patients newly admitted to dialysis clearly show that the proportion due to diabetic nephropathy has been increasing year by year. In 1995 the total number of the patients newly admitted to dialysis therapy was 25,858, and 8236 of them (31.9%) had diabetic nephropathy. The figures 10 years before, i.e. in 1986, were 12,565 and 2677 (21.3%), respectively. If one can extrapolate, diabetic nephropathy will have become the leading cause of end-stage renal failure in Japan by the end of this century (Figure 1).

Although the majority of diabetic patients with end-stage renal failure who are admitted to dialysis are supposed to suffer from non-insulin-dependent (type II) diabetes mellitus, the precise proportion has not been well documented. In a previous study we reported that in 1991 there was only one type I diabetic patient among 90 patients with diabetic nephropathy admitted to dialysis in four hospitals affiliated to Shiga University of Medical Science [2]. In these hospitals, three of 101 patients with diabetic nephropathy currently on maintenance dialysis therapy have type I diabetes (personal communication). So it is reasonable to assume that in Japan only few type I diabetic patients with diabetic nephropathy are currently on renal replacement therapy.

Nephropathy in patients with type II diabetes in Japan

Patients with type II diabetes who are registered in our hospitals (total 1229) were categorized as having normoalbuminuria, microalbuminuria or macroalbuminuria according to their albumin/creatinine ratio in the urine samples obtained in the out-patient clinic. The number of patients in each group is shown in Table 1. The prevalence of nephropathy is 14.6% if...
Table 1. Clinical characteristics of type II diabetic patients

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria (U-Alb &lt; 30 mg/g Cr)</th>
<th>Microalbuminuria (U-Alb ≥ 30 mg/g Cr)</th>
<th>Overt proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Gender (M/F)</td>
<td>Number</td>
</tr>
<tr>
<td></td>
<td>758</td>
<td>448/310</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>58 ± 12</td>
<td>166/125</td>
<td>114</td>
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<tr>
<td></td>
<td>6 ± 7</td>
<td>9 ± 12**</td>
<td>74/40</td>
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<tr>
<td></td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>62 ± 12**</td>
</tr>
<tr>
<td></td>
<td>12 ± 6</td>
<td>86 ± 60**</td>
<td>12 ± 8**</td>
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<tr>
<td></td>
<td>522/31</td>
<td>128/37**</td>
<td>1.2 ± 0.3**</td>
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<tr>
<td></td>
<td>474/284</td>
<td>115/176**</td>
<td>1458 ± 1665**</td>
</tr>
<tr>
<td></td>
<td>342/239/177</td>
<td>95/80/116**</td>
<td>17/91**</td>
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<tr>
<td></td>
<td>(Diet/oral agents/insulin)</td>
<td></td>
<td>25/89**</td>
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<td></td>
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<td></td>
<td>12/39/63**</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30/9/27**</td>
</tr>
<tr>
<td></td>
<td>Dialysis (number)</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7**</td>
</tr>
<tr>
<td></td>
<td>Deceased (number)</td>
<td>30</td>
<td>22*</td>
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<td></td>
<td></td>
<td></td>
<td>17**</td>
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<td>32**†</td>
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*P < 0.05; **P < 0.01 vs normoalbuminuria; †P < 0.01 vs microalbuminuria (ANOVA followed by Scheffe’s test or χ² test).

macroalbuminuric patients and 38.3% if both macroalbuminuric and microalbuminuric patients are considered. Approximately two million diabetic patients (one-third of an estimated six million diabetics) are taken care of by hospitals or by general practitioners according to the latest statistics provided by the Ministry of Health and Welfare in Japan. Consequently one can extrapolate that in Japan there are at least 300,000 diabetics with microalbuminuria and 800,000 with either microalbuminuria or macroalbuminuria.

Some of the clinical characteristics of those type II diabetics with nephropathy are listed in Table 1. They are older and the duration of diabetes is longer compared to type II diabetics without nephropathy. The prevalence of retinopathy and hypertension is higher, as expected from previous studies [3]. Interestingly, nearly 40% of type II diabetics without nephropathy, i.e. normoalbuminuric patients, are hypertensive. The close association between hypertension and diabetes has been previously reported. The observation suggests that the two conditions share common pathogenetic factors such as insulin resistance and hyperinsulinaemia [4]. In Japanese normoalbuminuric type II diabetics are not necessarily obese, and their average age is about 58 years (see Table 1). Hypertension is so common in elderly Japanese that we cannot exclude the possibility that the coexistence of diabetes and hypertension occurs by chance [5].

Prognosis of patients with type II diabetes and nephropathy

Fifty-four of the 180 macroalbuminuric patients in Table 1 had progressed to end-stage renal failure during a 4-year observation period, but none of micro- and normoalbuminuric patients. Not unexpectedly, progression to end-stage renal failure was more frequent in patients with serum creatinine concentrations > 2 mg/dl (47/66, 71.2%). In our hospitals the prevalence of chronic renal failure is 5.4% among registered diabetics (66 of 1229). One can extrapolate that in Japan there are approximately 100,000 diabetic patients with chronic renal failure. A recent survey performed by the study group on chronic renal failure organized by the Ministry of Health and Welfare in Japan has estimated that the number of diabetic patients with chronic renal failure is approximately 50,000. Since in recent years nearly 10,000 diabetics are annually admitted to dialysis therapy [1], 10–20% of diabetics with chronic renal failure must have progressed to end-stage renal failure.

In Caucasian patients with type II diabetes several studies indicated that microalbuminuria predicted not only the development of overt proteinuria but also a high rate of cardiovascular death. As shown in Table 1, all-cause mortality was significantly higher in patients with microalbuminuria than in patients with normoalbuminuria. We recently completed a follow-up study to clarify the relationship between microalbuminuria and cardiovascular death in Japanese subjects with type II diabetes [6]. We followed 201 patients with normoalbuminuria and 96 with microalbuminuria for an average of 6.4 years. During the follow-up period, 28 deaths were confirmed (14 in normoalbuminuric and 14 in microalbuminuric patients). Only 10 deaths were attributed to cardiovascular disease (6 in normoalbuminuric and 4 in microalbuminuric patients). Although the age- and sex-adjusted all-cause mortality rate in microalbuminuric patients was significantly higher than in normoalbuminuric patients (13.5 vs 8.2 per 1000 person years, P < 0.05), the mortality rate from cardiovascular disease was not significantly different between the two groups (3.4 vs 3.3 per 1000 person years). On age-adjusted Cox proportional hazards analysis, HbA1c and triglyceride were independent risk factors for the mortality from cardiovascular disease, while microalbuminuria was not associated with cardiovascular death [6]. Thus in Japanese patients with type II diabetes, in contrast to Caucasian patients, the existence of microalbuminuria predicts the development of overt proteinuria [7], but not cardiovascular death.
Concluding remarks

In Japan the total number of the patients newly admitted to dialysis therapy in 1995 was 25,858, of whom 8,236 patients (31.9%) suffered from diabetes. Although in Japan classification of the type of diabetes in patients admitted for renal replacement therapy has not been very precise, only few patients have type I diabetes, at least in our institution. In a recent survey performed by the study group on chronic renal failure, organized by the Ministry of Health and Welfare in Japan, the number of diabetic patients with chronic renal failure was estimated to be approximately 50,000, the majority of whom were classified as suffering from type II diabetes. In the stage of incipient diabetic nephropathy the existence of microalbuminuria predicts development of overt proteinuria. In contrast to Caucasians, microalbuminuria does not predict cardiovascular mortality in Japanese patients with type II diabetes.

References


Intradialytic hypotension: new insights into the mechanism of vasovagal syncope

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Introduction

Sudden hypotension remains to be a major complication of haemodialysis, and occurs in ~25% of dialysis sessions. Two to four litres of fluid need to be removed during a regular session, equivalent to 40–80% of the blood volume. It is therefore not surprising that hypotension occurs so often. Although many factors, patient or treatment related, play a role, a reduction of blood volume is crucial in its pathogenesis. In this comment, we will briefly discuss the haemodynamic responses to progressive volume withdrawal and rationalize on optional ways to withdraw fluid from the dialysis patient.

Haemodynamic response to volume withdrawal

The initial response to progressive hypovolaemia is sympathoexcitation, vasoconstriction, and tachycardia. This results in maintained mean arterial pressure, whereas pulse pressure decreases. Sympathoexcitation follows cardiopulmonary and arterial baroreceptor deactivation, and has been demonstrated by direct measurement of muscle sympathetic nerve activity (MSNA) in the peroneal nerve during lower body negative pressure [1] or haemodialysis [2]. The reflex causes immediate venous and arteriolar constriction. Venocostriction promotes venous return by mobilization of haemodynamically inactive blood. Arterioconstriction helps to maintain blood pressure directly, and also opposes cardiac emptying by an increase in afterload. In addition, it lowers capillary pressure, which facilitates plasma refilling. In aggregate, the available vascular volume is used more efficiently, cardiac emptying prevented, and vascular refilling enhanced.

Once a critical reduction in blood volume has been reached, sudden withdrawal of sympathetic activity occurs [1,2]. This starts vasodilation, bradycardia, and hypotension. This 'second' phase of the response to hypovolaemia is known as the Bezold–Jarisch reflex, cardiac depressor reflex, or vasovagal collapse. Converse et al. [2] were the first to show in a few

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patients that dialysis-related hypotension is indeed characterized by sudden cessation of central sympathetic neural outflow. The reflex is thought to be evoked by vigorous contractions of a progressively empty left ventricle, which activate cardiac mechanoreceptors. This inhibits cardiovascular centres through vagal afferents, and overrules the stimulation by baroreceptor deactivation. Alternative mechanisms must exist as well, since heart transplant patients can experience a typical vasodepressor syncope although the ventricle is denervated [3,4].

Sympathovagal balance

Although very useful in an experimental setting in healthy subjects, recording of MSNA during dialysis hypotension is difficult due to patient-related problems (restless legs), unpredictability of hypotension, and intrinsic difficulties of the technique. Spectral analysis of heart rate variability is a more feasible method to monitor sympathetic and parasympathetic function in this setting [5]. The low frequency component of this analysis (LF, 0.04–0.15 Hz) marks sympathetic activity, and the high frequency (HF, 0.15–0.40 Hz) marks parasympathetic activity. Their ratio (LF/HF ratio) is considered to reflect sympathovagal balance. We used this technique in a large group of patients to study dialysis-related hypotension [6]. During dialysis there first is a consistent steady increase in heart rate, coupled with increasing LF power and LF/HF ratio. However, seconds before and during dialysis hypotension LF/HF ratio drops. This was a consistent finding during the sudden hypotension. The biphasic pattern of events during dialysis is indistinguishable from that seen during progressive volume withdrawal in animals [7] or healthy volunteers [1]. Apparently, dialysis-related hypotension is a physiological response to hypovolaemia.

Plasma refilling

Ultrafiltration rate and tissue hydration state are major determinants of the plasma refilling rate during volume withdrawal. During gross overhydration, plasma refilling can keep up with ultrafiltration to a rate of 2 l/h [8]. However, at this stage the blood volume hardly decreases, and the factors that draw fluid to intravascular, i.e. a decreased capillary hydrostatic pressure due to peripheral vasoconstriction and an increased plasma oncotic pressure, are not operative. It therefore seems a logical approach to start dialysis with a high ultrafiltration rate, to bring the blood volume down to an individually determined safe minimum, and keep it there throughout the procedure by adjusting the ultrafiltration rate. This creates conditions for the highest possible mobilization of fluid over the longest possible period. In addition to ultrafiltration, the dialysate sodium concentration can also be varied. A hypertonic sodium dialysate concentration leads to better preservation of plasma volume [9], but carries the risk of excessive interdialytic weight gain. More studies are needed to determine if sodium and ultrafiltration ‘modeling’ will lead to improvement of intra-and interdialytic blood pressure control.

Prevention of cardiac emptying

As mentioned, venous constriction will help to maintain venous return and cardiac output. It has been suggested that factors associated with dialysis impair venous constriction, since venous return was better preserved during isolated ultrafiltration [10]. However, the physiological role of venoconstriction in humans has not been substantiated. By contrast, the effects of arterioconstriction are clear: not only mean arterial pressure, but also afterload is maintained, which opposes cardiac emptying and impairs cardiac output. We have shown that a brief fluctuation in vasoconstriction imposed during haemodialysis induces a sudden sympathoinhibitory reflex [1]. The underlying mechanism is that relaxation which allows cardiac emptying, starts a vasodilatory reflex that cannot be controlled. Such fluctuations may be caused by any vasoconstrictive (pain, postural changes) or vasodilatory stimulus (a meal, ischaemia-induced adenosine release). Conceivably, fluctuations of arteriolar tone also occur physiologically, and perhaps even more when there is strong sympathetic activation. We submit that under conditions of reduced intravascular volume, and a critically low ventricular volume, the circulation is more vulnerable to fluctuations of vascular tone. Decreased efficiency of the phase 1 vasoconstriction, such as will occur during acetate dialysis, strong osmolar changes, body heating, or use of vasodilatory drugs, will lead to even sooner cardiac emptying and triggering of the Bezold–Jarisch reflex.

Cardiac function

Conceivably, any cardiac condition that impairs ventricular filling will lower the margin to develop a Bezold–Jarisch reflex. On the right heart side, a pathological elevation in atrial pressure such as occurs during tricuspid regurgitation or pulmonary hypertension, will impair maintenance of venous return during volume withdrawal. Left ventricular hypertrophy and stiffness will also oppose ventricular filling. These cardiac conditions will predispose to an early fall in end-diastolic volume and stroke volume during dialysis, and thus promote sudden hypotension [11]. An alternative explanation may be that left ventricular hypertrophy (or myocardial fibrosis) increases excitability of mechanoreceptors. Remarkably, the question whether left ventricular hypertrophy predisposes to syncope in general has received little attention in the literature.
The situation in patients with heart failure is different, in that volume withdrawal may improve myocardial contractility and lead to a paradoxical increase in cardiac output. As recently suggested [12], mechanical impairment of left ventricular function by the distended right heart may also be a factor that is favourably influenced by volume withdrawal. Indeed, these patients may respond with a paradoxical decrease in MSNA and vascular tone during volume unloading [13,14], while cardiac output and pulse pressure increase instead of decrease [14]. The clinical observation that blood pressure may improve during dialysis in severely overfilled patients is also based on this concept. Conceivably, progressive volume withdrawal in these patients will evoke a three-phasic response, i.e. increased cardiac output and vasodilatation, followed by decreased cardiac output and vasoconstriction, and eventually a sudden vasodilatory reflex and hypotension. How fast the final vasodilatory response occurs in these patients depends on the efficiency of the vasoconstrictive (second) phase, and cardiac compliance of the (fibrosed) ventricle, but this has not been studied in detail.

### Influence of the central nervous system

A mismatch between oxygen supply and demand in the brain stem vasomotor centers can also trigger a vasodepressor reflex [15]. This may contribute to the genesis of dialysis hypotension, in particular in patients with an impaired circulation to the brain. Experiments in animals, rabbits in particular, have shown that opioid receptor blockers, serotonin receptor blockers and nitric oxide synthesis blockers not only enhance the (phase 1) vasoconstrictive response to progressive hypovolaemia (haemorrhage), but also can postpone the (phase 2) vasodilatory reflex [7,16]. We found no protective effect of high dose naloxone to lower-body-negative-pressure hypotension in humans [17]. One may doubt, however, whether postponement of a vasodepressor reflex by deceiving the brain vasomotor centre is a preferable or even feasible approach to prevent dialysis hypotension. After all, postponement achieved in that way will only allow more severe tissue ischaemia.

### Conclusions

Central in the pathogenesis of dialysis-related hypotension is a critical decrease of central blood volume. When volume withdrawal and blood volume decrease progress, the occurrence of sudden vasodilatation by the Bezold–Jarisch reflex is only a matter of time. During volume withdrawal, cardiac emptying is prevented by a brittle balance between stimulation of venous return, cardiac function, and vasoconstriction. Any disturbance of this balance, whether happening before, in, or after the heart, may advance the Bezold–Jarisch reflex. Prevention of dialysis-associated hypotension should be aimed at optimization of plasma refilling, cardiac function and venous and arterial vasoconstriction. Interesting subjects for future research are blood volume controlled non-linear ultrafiltration, use of haemodynamic predictors of hypotension such as plasma refilling rate, pulse pressure changes, heart rate, sympathetic activity monitored by spectral analysis, and measures to improve impaired arterioconstriction and to prevent unwanted vasotonic fluctuations.

### References

Folate supplementation in the dialysis patient—fragmentary evidence and tentative recommendations

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Introduction

Anaemia is a disabling and common extra-renal manifestation of chronic renal failure. While inadequate production of erythropoietin is the dominant factor, deficiencies of iron and folate may contribute to patient morbidity. Supplementation of the diet with folate has been proposed as a measure to ensure adequate folate supply for erythropoiesis, but this is probably not necessary in patients with a satisfactory level of nutrition [1–5]. However, more recent evidence indirectly linking folate status with atherosclerosis suggests that a re-appraisal of recommendations on folate supplementation may be opportune, and may be in the best long-term interest of the patients. This is based on the observation that folate nutrition affects more than haematological status (anaemia). Folate is also important in the metabolism of homocysteine, a newly recognized risk factor for atherosclerosis.

Folate and haemopoiesis

Dietary folate enters the circulation as 5-methyltetrahydrofolate (Me-THF). Further metabolism of folate in man requires the demethylation of Me-THF, which is primarily achieved in a reaction requiring vitamin B12 (methylcobalamin) as cofactor and homocysteine as methyl acceptor. In the process, tetrahydrofolate (THF) and methionine are formed (Figure 1). The reaction is catalysed by the enzyme methionine synthetase (5-methyltetrahydrofolate:homocysteine methyltransferase), which, in addition to methylcobalamin, also requires S-adenosylmethionine and reduced flavin adeninedinucleotide as cofactors. Impairment of the reaction, for example in vitamin B12 or folate deficiency, leads to a lack of THF for one-carbon transfer reactions such as required for DNA synthesis, and this is manifest in megaloblastic erythropoiesis and macrocytic anaemia.

While adequate folate intakes are undoubtedly essential for health and well-being, studies in dialysis patients in first world countries have generally concluded that folate supplementation is not necessary to maintain normal folate status and haemopoiesis [4,5]. This is explained by the observation, that while dialysate folate losses are greater than urinary losses, requirements are easily met by a mixed diet containing 60 g protein/day [6,7]. Perhaps surprisingly, alleviation of uraemic anaemia by erythropoietin has not unveiled latent folate deficiency in haemodialysis patients with an adequate level of nutrition [5]. However, given the relative lack of toxicity of folate, and the possible benefit of supplementation, many renal units have continued blanket supplementation of dialysis patients with 1–5 mg folate per day.

Folate, homocysteine and atherosclerotic risk

In recent years, another aspect of folate metabolism has emerged as important in dialysis patients, and this concerns the recycling of the putatively atherogenic amino acid, homocysteine. Homocysteine is formed as a result of methylation reactions involving the active

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form of methionine, S-adenosylmethionine (Figure 1). The product, homocysteine, accumulates unless removed by two diverging pathways: in the transsulphuration pathway, homocysteine reacts with serine to form cystathionine, catalysed by the pyridoxal phosphate–requiring enzyme cystathionine β-synthase. The other (transmethylation) pathway involves methionine synthetase, and in the liver, a secondary reaction catalysed by the enzyme betaine:homocysteine methyltransferase. Thus, accumulation of homocysteine can result from decreased activity of cystathionine β-synthase or impairment of the transmethylation pathways. Deficiency of pyridoxal phosphate (vitamin B₆) limits activity of the trans sulphuration pathway, while vitamin B₁₂ or folate deficiency impairs the methionine synthetase reaction. Supplementation of dialysis subjects with vitamin B₆ has little effect on raised homocysteine levels [8], suggesting that the vitamin is not a limiting factor in the metabolism of homocysteine. In these reactions, vitamin B₁₂ functions as a cofactor (not a substrate), and therefore is required in trace amounts only. In the methionine synthetase reaction on the other hand, folate serves as a substrate (in the form of Me-THF), and methylcobalamin is the cofactor. Thus, folate is required in higher amounts.

It is increasingly being recognized that raised plasma homocysteine levels are an independent risk factor for atherosclerotic vascular disease in the general population [9–11]. Data from the Framingham Heart Study for example showed that plasma homocysteine concentrations in the upper quartile were associated with a 2-fold increase in risk for carotid arteriosclerosis, even after adjusting for age, smoking, hypertension and dyslipidaemia [12]. For reasons which are not clear, homocysteine accumulates in the blood of dialysis patients, and this accumulation is inversely related to folate status [13–15]. Indeed, folate status has been shown to be the primary determinant of homocysteinaemia in end-stage renal failure, with other factors such as disease aetiology, length of time on dialysis and dialysis mode playing a minor role [14]. As suggested by the strong, inverse relationship between folate and homocysteine, concentrations of the latter can be significantly reduced by dietary folate supplementation [8,13,16]. Although reductions of about 25–50% can be achieved, homocysteine levels are not necessarily normalized, and hyperhomocysteinaemia may persist despite supranormal folic acid levels [17].

Very few studies of homocysteine and atherosclerotic risk have been undertaken in subjects with chronic renal failure. Chauveau et al. (1993) found significantly higher concentrations of plasma homocysteine in uraemic patients (non-dialysed) with occlusive arterial disease than in those without vascular disease [18]. In dialysis patients, significant correlations have been observed between total homocysteine levels and an atherosclerotic score in CAPD patients, but not in haemodialysis patients [19]. Recently, a comprehensive study of 176 dialysis patients found that a homocysteine concentration in the upper two quintiles (>27.8 μmol/l) was associated with an odds ratio of 2.9 for any thromboembolic or atherosclerotic vascular event, after adjusting for age, sex, hypertension, diabetes, hypercholesterolaemia, smoking and time on dialysis (95% confidence interval, 1.4–5.8, P < 0.01) [15]. Thus, hyperhomocysteinaemia may well be a risk factor for atherosclerosis in chronic renal failure as well as in subjects with normal renal function. Further studies are needed to confirm these initial observations.

The association of folate with a risk factor for atherosclerosis places a new importance on folate status in dialysis patients. Atherosclerotic disease is a major cause of morbidity and mortality in dialysis patients, and hence reduction of atherosclerotic risk is highly desirable. Not surprisingly, folate supplementation has been widely recommended as a strategy to reduce homocysteine levels [8,13,15,16], even though clinical studies demonstrating benefits are still outstanding.

**Risks associated with folate therapy**

Current evidence indicates that daily oral doses of 5–15 mg folate for long periods are well-tolerated and without toxicity in normal, non-pregnant subjects [20]. The major concern with folate therapy lies in the exposure of vitamin B₁₂-deficient subjects to folic acid, since the inappropriate treatment of vitamin B₁₂ deficiency with long-term folic acid may precipitate severe neurological changes, including subacute combined degeneration of the spinal cord [21]. This complication can easily be avoided by first determining the patient’s vitamin B₁₂ status, and in the event of deficiency, by treating the underlying condition (e.g. malabsorption syndromes), and replenishing vitamin B₁₂ stores.

The issue of side effects of high dose folate supplementation is less clear. Although folic acid has been considered to be remarkably lacking in toxicity in both man and animals, a few cases of sensitivity reactions to folic acid preparations have been noted [21]. Nervous and gastrointestinal side effects have been described in healthy volunteers receiving 5 mg folic acid three times a day for 1 month [22], but these findings were not supported in a subsequent placebo-controlled, double-blind trial [20]. There is evidence of interactions between folate and anticonvulsant drugs (phenytoin, primidone, phenobarbitone), and long-term folate therapy may increase fit frequency in some treated epileptics [21]. Caution should also be exercised when folic acid is given to patients with diseases treated with drugs known to interfere with folate metabolism [20].

**Recommendations**

Most commentators agree that folate supplementation is probably not required for optimizing haemopoiesis [1–7]. However, since additional folate significantly reduces plasma homocysteine levels, folate supplementation should be considered for all uraemic
patients with raised homocysteine levels. The doses of folate which have been suggested [8,13,15–17] are more than 10–30 times the recommended dietary intake (≈ 400 µg/day), and hence it may be more appropriate to consider this ‘folate therapy’ rather than dietary supplementation. Unfortunately, the optimum protocol has not yet been determined, but on present evidence the following can be suggested: before folate is given, vitamin B₁₂ status should be checked, and deficiency corrected. Vitamin B₁₂ status should also be determined (e.g. by measuring plasma pyridoxal 5'-phosphate or erythrocyte aspartate transaminase, a vitamin B₆-requiring enzyme), as deficiency in vitamin B₁₂ cofactors may raise homocysteine levels and blunt the response to folate therapy. Total homocysteine should be measured in all uraemic patients. If plasma homocysteine levels are above the normal reference range (generally held to be 5–15 µmol/l), folate therapy (5 mg/day) should be initiated. Reductions of 25–30% can be expected with this dose [8,13,16], and this should be evident within 4–6 weeks. If homocysteine levels are still elevated after 6–8 weeks of treatment, the dose could be increased stepwise by 5 mg/day, to a maximum of 15 mg/day. The benefit of higher doses is unknown, and therefore clinicians may prefer to wait for the results of safety and efficacy trials before considering higher doses in renal failure patients.

The strategy described here should significantly reduce plasma homocysteine levels, and by inference, minimize the effect of a putative cardiovascular risk factor in this high-risk group. Whether this relatively inexpensive and low-risk intervention will be of long-term benefit to patients is unknown. We await the outcome of controlled clinical trials with interest.

References

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