Editorial Comments

Urinary excretion of aquaporin-2 in disorders of water metabolism

San-e Ishikawa1, Sei Sasaki2, Takako Saito1 and Toshikazu Saito1

1Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical School, Tochigi and
2Second Department of Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Water metabolism plays an essential role in the homeostasis of body fluids in animals and humans. It is regulated by arginine vasopressin (AVP), renal function and water drinking. The disorders of water metabolism result in an increase or decrease in body water and/or fluid, which manifest as hyponatraemia, hypernatraemia, polyuria, dehydration or oedema. In the pathogenesis of such pathological conditions AVP is involved either directly or indirectly. One is often faced with the dilemma to elucidate the exact mechanism of action. A radioimmunoassay for AVP has been established for reliable measurements of plasma AVP levels, but even under pathophysiological conditions plasma AVP does not vary widely. Furthermore, physiological stimulants of plasma osmolality (Posm) and circulatory blood volume are tightly interrelated with one another. Therefore AVP release from the posterior pituitary is unexpectedly complicated in pathological states of water metabolism [1,2]. Accordingly, in the analysis of disorders of water metabolism one has to resort to analysis of tolerance tests monitoring urine volume and urinary osmolality (Uosm) e.g. during water deprivation, hypertonic saline infusion, oral water load and so on.

The water channel molecule AQP-2

Sasaki et al. [3,4] cloned a cDNA of apical collecting duct water channel, aquaporin-2 (AQP-2), of rat and human kidney. In collecting duct cells AQP-2 is translocated from cytoplasmic vesicles to the apical plasma membrane by shuttle trafficking when the cells are stimulated by AVP [5,6]. It is again redistributed into cytoplasmic vesicles after removal of AVP stimulation [7]. The expression of AQP-2 mRNA is increased by exogenous and endogenous AVP in various pathological states such as dehydration, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), liver cirrhosis with ascites and congestive heart failure [3,8,9]. The 5′ flanking region of the AQP-2 gene contains an adenosine 3′,5′-cyclic monophosphate (cAMP)-responsive element and stimulation of gene transcription of AQP-2 by cAMP is observed in experimental promoter assay [10]. All these data indicate that AQP-2 is the AVP-regulated water channel in renal collecting duct cells.

AQP-2 is excreted in the urine

AQP-2 is, in part, excreted into the urine, which accounts for approximately 3% of AQP-2 of renal collecting ducts in the rats [11,12]. Urinary AQP-2 was detectable in both soluble and membrane-bound forms by Western blot. Immunoelectron microscopy showed that AQP-2 was present in membrane structures probably derived from membrane vesicles. Urinary AQP-2 was quantitatively determined by radioimmunoassay, using the antibody against the C-terminal of AQP-2. Exogenous administration of AVP elicited a prompt increase in urinary excretion of AQP-2 in normal subjects and patients with central diabetes insipidus, but not in patients with nephrogenic diabetes insipidus [11].

AQP-2 excretion correlates with plasma AVP

A positive correlation is found between urinary excretion of AQP-2 and plasma AVP levels (Figure 1) [12,13]. Urinary AQP-2 excretion varies within a wide range under ad libitum water drinking. It was 8-fold greater in the normal subjects than in the patients with central diabetes insipidus [13]. A hypertonic saline (5%
NaCl)-infusion test was performed after drinking 20 ml/kg water [13]. After the infusion of 5% NaCl, urinary excretion of AQP-2 elevated by 12-fold, mediated via an increase in plasma AVP, in the normal subjects. When compared with the changes in urine volume and Uosm, the increase in urinary excretion of AQP-2 occurred 60 min later. In contrast, in the patients with central diabetes insipidus urinary excretion did not respond to the 5% NaCl infusion and remained low. These studies indicate that urinary excretion of AQP-2 can be induced to change within a wide range, and is dependent on the endogenous secretion of AVP.

Monitoring urinary AQP-2—a tool for clinical evaluation in disorders of water metabolism

Urinary excretion of AQP-2 reflects the changes in plasma AVP levels, and the magnitude of the changes is larger than that of plasma AVP levels. Therefore, urinary AQP-2 is expected to be a useful tool for evaluating various disorders of water metabolism. Further study will be necessary to evaluate its role in the evaluation of disorders of water retention and hyponatraemia, including SIADH, liver cirrhosis and congestive heart failure, and polyuric states including central and nephrogenic diabetes insipidus or psychogenic polydipsia. Such analyses may lead to the use of urinary excretion of AQP-2 as a bedside test in the near future.

References


Fig. 1. The relationship between plasma AVP levels and urinary excretion of AQP-2 (UAQP-2) in normal subjects. (Cited from ref. no. 13).
Gitelman syndrome comes of age

Leo Monnens, René Bindels and Jean Pierre Grünfeld

Departments of Pediatric Nephrology and Cell Physiology, University of Nijmegen, The Netherlands, and Department de Néphrologie, Hopital Necker, Paris, France

Introduction

Gitelman syndrome is an autosomal recessive inherited disorder first described by Gitelman et al. in 1966 [1]. They characterized this syndrome by hypomagnesaemia, hypokalaemia, and impaired renal conservation of potassium and magnesium. The clinical symptoms consisted of transient episodes of muscle weakness and chronic non-specific dermatitis in two of the three patients. Treatment by aldactone or triamterene decreased the urinary potassium loss but did not influence the magnesium excretion. Further clinical studies allowed a better definition of Gitelman syndrome, separating the syndrome from Bartter syndrome [2,3]. The distinction between the two syndromes is still not perfect (Table 1). Genotype-phenotype studies will ultimately indicate the borders between the syndromes [4].

Molecular basis of Gitelman syndrome

The demonstration of linkage of Gitelman syndrome to the locus encoding the renal thiazide-sensitive NaCl cotransporter (TSC) and the identification of mutations, provided the molecular basis of Gitelman syndrome, although functional studies of the mutant genes are still lacking [5]. The cotransporter consists of 12 transmembrane domains and an intracellular amino- and carboxyterminal region (Figure 1). Most of the mutations observed in the European consortium are localized in the carboxyterminal region. In rat and human the NaCl cotransporter was localized using the in situ hybridization technique. In the human as well as in the rat the cotransporter was present in the distal nephron beginning in the initial distal convoluted tubule. In humans, in contrast to the rat, expression extended into the connecting tubule [6].

In addition Barry et al. [7] showed that the thiazide-sensitive NaCl cotransporter was expressed in osteoblast-like cells, suggesting a direct effect of thiazide on bone-synthesizing cells.

Pathophysiology

The distal tubule consists of several nephron segments. The first segment is the distal convoluted tubule starting at the macula densa and ending with the connecting tubule. The latter segment connects the distal convoluted tubule to the collecting duct. Both segments contain the NaCl cotransporter in humans. A schematic

Table 1. Tentative distinction between Bartter syndrome and Gitelman syndrome

<table>
<thead>
<tr>
<th></th>
<th>Bartter syndrome</th>
<th>Gitelman syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization of defect</td>
<td>Ascending limb of Henle</td>
<td>Distal tubule</td>
</tr>
<tr>
<td>Age of presentation</td>
<td>Prenatal, during infancy, early childhood</td>
<td>Mostly late childhood, or at adult age</td>
</tr>
<tr>
<td>Biochemical differences</td>
<td>Serum magnesium may be decreased</td>
<td>Serum magnesium decreased</td>
</tr>
<tr>
<td>Molecular defects</td>
<td>Urinary excretion of calcium increased or normal</td>
<td>Urinary excretion of calcium decreased</td>
</tr>
<tr>
<td></td>
<td>Na-K-2Cl cotransporter (NKCC2) or apical K channel (ROMK), or basolateral Cl channel (CICNKB) in ascending limb of Henle</td>
<td>Na-Cl cotransporter in the distal tubule</td>
</tr>
<tr>
<td>Functional studies</td>
<td>Concentrating capacity severely disturbed</td>
<td>Concentrating capacity normal or slightly disturbed</td>
</tr>
<tr>
<td></td>
<td>GFR may be normal, decreased or declining</td>
<td>GFR is normal</td>
</tr>
</tbody>
</table>

Correspondence and offprint requests to: Prof. Dr L. A. H. Monnens, Department of Pediatric Nephrology, University Hospital Nijmegen, POB 9101, 6500 HB Nijmegen, The Netherlands.
view of the NaCl and Ca transport in the distal convoluted tubule is given in Figure 2 [8]. Na/K ATPase situated at the basolateral membrane is responsible for the low sodium and high potassium concentration in the cell. An apical NaCl cotransporter is present which is inhibited by thiazide diuretics and which is defective in Gitelman syndrome.

Two other modes of apical entry of sodium have been described in this segment. The first is an apical Na\(^+\) conductance, which is sensitive to amiloride. The second mode consists of parallel Na/H antiport and Cl/base exchange. The inhibition of NaCl absorption in the distal tubule has consequences for the calcium and magnesium reabsorption. Inhibition of the NaCl absorption results in enhanced calcium reabsorption. Two explanations are offered. By inhibition of the sodium entrance into the cell, voltage-gated Ca\(^{2+}\) channels are activated by membrane hyperpolarization. Another possibility is that the decrease in intracellular Na\(^+\) augments the calcium efflux through the basolateral Na\(^+\)/Ca\(^{2+}\) exchanger. Although chronic thiazide treatment may lower the serum magnesium concentration it is rarely as low as in Gitelman syndrome [9].

The magnesium loss observed in Gitelman syndrome is still unexplained. Inhibition of Na\(^+\) transport in the distal tubule will result in a stimulation of Mg\(^{2+}\) uptake as shown also for calcium [10,11]. In a recent review Quamme [12] suggests that the increased magnesium excretion may be due to associated hypokalaemia. Most of the patients with Bartter syndrome, however, with a similar degree of hypokalaemia have a normal serum magnesium. The increased renal potassium excretion is due to the increased distal delivery of Na\(^+\) and water to the cortical collecting duct.

**Clinical symptoms**

Often the syndrome is first discovered in adults during routine investigation. Most of the patients report recurrent periods of carpopedal spasms when the intestinal absorption of magnesium is decreased (vomiting, diarrhoea) or during fever.

Fatigue, hardly noted by some patients, can be so severe that daily activities cannot be performed. The severity of these symptoms is only to a certain degree reflected by the lowering of the serum potassium concentration. The patients are growing normally and are not complaining of polyuria or polydipsia.

In some families chondrocalcinosis is reported, causing swelling, local heat, and tenderness over the affected joints. In experimental studies similar lesions are induced by magnesium deficiency [13]. It is not clear why not more patients are affected by chondrocalcinosis. Chondrocalcinosis may occur in patients with isolated magnesium loss.

**Treatment**

Many patients with Gitelman syndrome remain untreated. When we accept that chondrocalcinosis is due to magnesium deficiency, it is an argument for supplementation of magnesium. Normalization of serum magnesium is, however, difficult to obtain, as high doses of magnesium cause diarrhoea.

Hypokalaemia can be treated by drugs that antagonize the activity of aldosterone or block the sodium channel in the distal nephron.
We prefer the treatment by amiloride, if necessary with additional KCl. Prostaglandin synthetase inhibitors in our experience can also increase serum potassium levels.

References

11. Dai LJ, Friedman PA, Quamme GA. Cellular mechanism of chlorothiazide and cellular potassium depletion on Mg2+ uptake as mouse distal convoluted cells. Kidney Int 1997; 51: 1008–1017
Magnetic resonance angiography—the procedure of choice to diagnose renal artery stenosis?

Christoph J. Olbricht and Ingolf P. Arlart

Departments of Nephrology and Hypertension and 1Diagnostic Radiology, Katharinenhospital, Stuttgart, Germany

Introduction

Arteriography is still the gold standard for the diagnosis of renal artery stenosis (RAS). It is invasive, potentially dangerous and associated with discomfort. Other methods for the diagnosis of RAS are susceptible to a variety of problems such as operator skill, contrast material nephrotoxicity, poor reproducibility between institutions and low values of sensitivity and specificity. Hence, there is still a need for a non-invasive, accurate, reliable and acceptable test for the screening and diagnosis of patients with suspected RAS, to reduce the number of negative angiograms that are performed. Due to its very rapid evolution during recent years, the diagnostic accuracy of magnetic resonance angiography (MRA) has improved substantially and it is a candidate to achieve this diagnostic goal.

Technique of magnetic resonance angiography

The physics of MRA are complex, and a comprehensive grasp of their fundamentals is not really necessary for clinical practice. However, the user should understand the concepts in order to facilitate diagnostic interpretation. Conceptually, MRA is based on the phenomenon that nuclei of certain atoms including hydrogen-1 have a magnetic moment and that they interact with magnetic fields. A strong electromagnetic pulse destabilizes the protons, and their return to stable baseline (relaxation) emits energy (magnetic resonance) that can be measured. MR imaging gives resonance signals limited to body water and some lipids. The distribution and intensities of the magnetic resonance are converted to grey-scale values. Time-of-flight (TOF) and phase-contrast sequences are the two main techniques for imaging flowing blood in vessels by MRA, and they rely on magnetic resonance of the hydrogen of water. Each of these sequences may be acquired as either two-dimensional (exciting sequentially one slice at a time) or as a three-dimensional (when a whole volume is excited at the same time) formulation. TOF techniques are based on the signal produced by flowing blood when it enters the section being imaged. The flowing blood produces a bright signal only if it is fully unmagnetized and unsaturated in terms of electromagnetic pulses compared with surrounding tissue. A low blood flow velocity, turbulent flow condition and a long vessel distance within the excited volume decrease signal intensity. Hence, the TOF technique rarely images more than the proximal 2–3 cm of the renal arteries.

Phase-contrast MRA provides an image of vessels by a different technique. As moving spins (e.g. flowing blood) pass through the imaging volume, they are bombarded by radiofrequency pulses within the magnetic field. This results in the spins undergoing a change in phase relative to stationary tissues, which undergo no phase alteration. Also, the phase shift is broadly proportional to the velocity of the moving spins. This phase difference is used to image moving blood.

Diagnosis of renal artery stenosis by MRA

Studies applying TOF-MRA showed a comparatively low diagnostic accuracy with reliable visualization of only the first 3 cm of the renal arteries. The sensitivity and the specificity for the detection of RAS had a wide scatter [1–7]. Studies using phase-contrast MRA consistently showed a better overall diagnostic accuracy. The sensitivity for RAS > 50% was between 80 and 100%, and the specificity ranged from 93 to 99% [2,6–13]. MRA reliably visualized all main renal arteries, but there was a strong tendency to overlook small accessory renal arteries. Further drawbacks were the inability to visualize intrarenal arteries, to depict fibromuscular dysplasias reliably and to graduate the stenosis correctly.

A major diagnostic improvement was achieved by application of paramagnetic ‘contrast-material’ (i.e. gadopentate dimeglumine), that can shorten the relaxation time of blood. The consequence is a high intravascular signal and an ultrashort breath-hold acquisition of magnetic resonance data within 30 s, avoiding motion artefacts and improving image quality significantly. Studies utilizing the breath-hold gadopentate dimeglumine-enhanced technique gave values of sensitivity and specificity that consistently exceeded 90%. The number of missed accessory renal arteries...
decreased to values <10% [6,7,9,11–16]. In addition to vascular imaging, the blood flow in the renal arteries can be measured reliably by phase-contrast MRA by the breath-hold technique [17–19]. Using a pressure gradient >15 mm across the RAS as the reference standard, digital subtraction angiography (DSA) and MRA had the same accuracy in predicting this haemodynamic significance [20]. A further diagnostic possibility of contrast-enhanced MRA is the renographic analysis of individual kidneys, quite similar to renal scintigraphy with radioactively labelled markers [21].

Role of MRA in screening and diagnosis of renal artery stenosis

Of patients with a high index of clinical suspicion, 30–40% may have clinical significant RAS [22]. It is common practice to proceed directly to arteriography without prior non-invasive testing [23,24]. In our opinion, one of the advanced techniques of MRA should be the diagnostic procedure of choice in these patients. The current published results support the view that a normal MRA is strongly indicative of normal renal arteries and that no further investigation for RAS needs to be carried out under these circumstances. Whenever the renal function is normal, spiral computed tomography angiography (CTA) with intravenous contrast material may still be a good alternative to MRA, since it will detect clinically significant RAS reliably even in small accessory arteries [25,26]. The major risk of CTA is the nephrotoxicity related to the comparatively high contrast volume. Therefore, we do not recommend CTA in patients with impaired renal function. The results of either CTA or MRA will allow careful planning of necessary interventions, either by angioplasty or by surgery. Arteriography could be reserved for endovascular interventions only, sparing 60–70% of patients the discomfort and the complications of arteriography. Currently, the competitive diagnostic modalities including colour Doppler sonography (CDS), intravenous DSA and renal scintigraphy with angiotensin-converting enzyme inhibitors are less well suited to achieve this goal [27–30].

At present, MRA is still too expensive as a general screening method for hypertensive patients with a moderate likelihood of RAS due to the high costs of the ‘contrast-material’. The recent evolution of MR angiography already has decreased the examination time and increased the diagnostic accuracy. This rapid progress will continue and presumably decrease the costs. In a single diagnostic procedure, MRA provides a structural evaluation of renal arteries and it measures single kidney blood flow and filtration rate. With regard to this enormous diagnostic potential, it is very likely that MRA will replace all other diagnostic procedures for detection of renal artery stenosis.

References


---

Cytokines and bioincompatibility

Loreto Gesualdo, Giovanni Pertosa, Giuseppe Grandaliano and Francesco P. Schena

Institute of Nephrology, University of Bari, Bari, Italy

**Introduction**

Bioincompatibility, as it relates to haemodialysis, is a concept based on both scientific and marketing considerations. It can be partly defined, as reported by the 1st Consensus Conference on biocompatibility, held in Koenigswinter on March 1993, as ‘different reactions induced by different materials, procedures, and devices that have not all as yet been fully investigated’ [1]. In the last two decades, there has been growing knowledge on cellular and humoral immunological events occurring during haemodialysis procedures. Such increased understanding of the immune mechanisms underlying the bioincompatibility phenomena has been obtained by the application of several sophisticated immunological procedures and, more recently, of molecular biological techniques [2]. Thus, while in the early days of haemodialysis major emphasis has been given to thrombogenicity and toxicity of the materials used, today it is appreciated that such material may influence several homeostatic systems including the cytokine network.

This editorial comment aims, necessarily in an incomplete fashion, to summarize some of the most important studies on cytokines and bioincompatibility.

**Cytokines involved in ‘bioincompatibility’ reactions**

Cytokines are a family of polypeptides with a molecular weight ranging from 10 to 25 kDa that, produced by different cells following inflammatory stimuli as part of an immunological response, may mediate the cell-to-cell interaction and modulate the cellular level of activation. Moreover, each cytokine can modulate the synthesis or the actions of the others in a complex network of interactions. They are active at picomolar and femtomolar concentrations and may affect cellular responses both proximal and distal to the site of secretion [3].

It has been clearly shown that the contact of the bloodstream with extracorporeal circulation devices can unbalance several homeostatic systems, including the cytokine network [4]. Each of these systems is under a tight feedback control with several checkpoints and balances whose main goal is to maintain the organism functions in a ‘normal state’. Haemodialysis, therefore, modify this ‘normal state’ inducing a state of ‘bioincompatibility’.

The monocyte seems to play a key role in the bioincompatibility reactions as one of the main sources of cytokine secretion [5]. Indeed, upon contact between blood and the extracorporeal system, monocytes are activated directly or indirectly, with the subsequent release of several proinflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, tumour necrosis factor (TNF)-α, and monocyte chemotactic factor (MCP)-1.

Three structurally related polypeptides belong to the IL-1 family: IL-1α, IL-1β and IL-1 receptor antagonist (RA) [6,7]. The first two polypeptides, binding specific receptors (IL-1RI and IL-1RII), exert a wide range of immune and inflammatory effects, whilst IL-1RA blocks IL-1 activity in vitro and in vivo by occupying its receptors. Both animals and humans receiving IL-1 develop fever and hypotension. Similar to IL-1, IL-6...
is a polypeptide that is produced by macrophages and may induce fever. Moreover, it stimulates the proliferation and differentiation of B cells, the release of acute-phase proteins from hepatocytes, and the proliferation of mesenchymal cells [8,9]. IL-8 is a member of a large family of chemotactic peptides synthesized by macrophages, endothelial cells and fibroblasts known as chemokines. It is a potent chemotactic and activating factor for neutrophils [10]. Regarding TNF-α, two different isoforms are known: α and β. TNF-α is a pleiotrophic cytokine with biological properties similar to IL-1. When injected in animals and humans, it may cause hypotension, fever, leukopenia, as well as several metabolic dysfunctions that may evolve in a shock-like syndrome [11]. It has been demonstrated that the effect of TNF-α can be blocked by naturally occurring inhibitors, the TNF-α soluble receptors corresponding to the p55 and p75 cell surface TNF-α receptors [7]. IL-1 and TNF-α effects are mainly due to their ability to induce cyclo-oxygenase and phospholipase A₂ gene expression and, consequently, production of prostaglandins and platelet activating factor [12]. Finally, MCP-1, a chemokine produced by several cells including monocytes, is a potent and specific chemotactic and activating factor for monocytes. It has been shown that both TNF-α and IL-1 together with LPS are the most powerful inducers of this chemokine peptide in a variety of cells [13].

The specific action of any of these monocyte-derived cytokines may be relevant in the pathogenesis of bioincompatibility clinical phenomena. This observation led several investigators to study the plasmatic levels of these pro-inflammatory cytokines. As expected, most of the studies showed a striking increase in IL-1, IL-6, IL-8, TNF-α, and MCP-1 plasma levels [7,8,10,13]. However, some studies reported also a clear increase in the various cytokine-binding proteins, suggesting some, although incomplete, preservation of autoregulatory mechanism [7].

Mechanisms leading to cytokine induction during haemodialysis

Figure 1 depicts three of the most important mechanisms that have been shown to induce both in vitro as well as in vivo cytokine transcription and/or production during haemodialysis:

1. Direct contact of peripheral blood mononuclear cells (PBMC) with dialysis membrane.
2. Complement fractions generated during haemodialysis.
3. LPS contaminated dialysate.

The intrinsic nature of the dialysis material seems to play an important role in cytokine induction during haemodialysis both directly and indirectly, through complement activation. It is well known that, in the absence of any other stimulus, adherence to dialysis membrane induces selective mRNA expression of monocyte mediators and proto-oncogenes. Indeed, Betz et al. have demonstrated that cuprophan membranes stimulate IL-1 expression in monocytes in the absence of complement [14]. On the other hand, cellulosic membranes are known to activate the complement cascade and it has been shown that complement fractions generated during haemodialysis, such as C3a and C5a, stimulate IL-1 gene expression by monocytes [15]. The third possible pathogenetic factor to consider in the increased cytokine production during haemodialysis is the LPS fragments contaminating the dialysate and able to cross the membrane. Recently, it has been demonstrated that the basal release of TNF-α and IL-6 during haemodialysis is considerably influenced by the endotoxin content in dialysate, besides the type of dialysis membrane used [16]. Indeed, it has been shown that monocytes, isolated from uraemic patients treated with non-sterile bicarbonate tanks, spontaneously released a significantly greater amount of TNF-α and IL-6 compared to healthy controls and non-dialysed uraemic patients. This higher production was significantly reduced by the use of sterile-bicarbonate tanks [14].

The contact with the dialysis membrane as well as the interactions with complement fractions, although can induce a selective cytokine gene transcription in monocytes, do not always stimulate automatically the translation of the specific proteins. Indeed, we have previously shown that IL-6 gene expression is strikingly increased during haemodialysis with cuprophan membrane, but its protein secretion is clearly downregulated [17]. Interestingly, Schindler et al. have demonstrated that recombinant C5a in vitro stimulates transcription rather than translation of IL-1 and that LPS or IL-1 itself is required as a translational signal [15]. In support of this hypothesis, we and others have demonstrated that patients treated with cuprophan have higher mRNA levels for different cytokines, but in
order to obtain a higher translation, a second stimulus, such as LPS, is required [15,16].

Clinical relevance of cytokine production in haemodialysis: acute and chronic effects

The production of cytokines during haemodialysis procedures, particularly when using bioincompatible membranes, seems to be responsible for acute complications such as fever, headache and hypotension, as well as for long-term consequences such as dialysis-related amyloidosis, increased susceptibility to infections, and increased muscle protein catabolism [18,19] (Table 1).

The incidence of amyloid bone disease is much greater in patients who have been dialysed with unsubstituted cellulosic membranes than with a biocompatible membrane [20]. Cellulosic membranes may induce increased synthesis and release of β₂-microglobulin (β₂m) by mononuclear cells and endothelial cell via the activation of complement system and cytokines. Particularly, IL-1, TNF-α, and IL-6 have been demonstrated to have a potential role in stimulating the synthesis of β₂m [20,21]. In addition, cellulosic membranes are not able to clear or adsorb β₂m. Finally, proteases and reactive oxygen species (ROS) released by activated neutrophils, could further enhance the polymerization of β₂m into amyloid fibrils [22] and favour its intra-articular deposition. Because of these factors, patients treated with low-flux cellulosic membranes are chronically exposed to a higher concentration of β₂m.

There is increasing evidence that bioincompatible membranes play an important role in enhancing susceptibility to infections in uraemic patients. The use of cellulosic membranes is associated with dysfunction of phagocytic cells, natural killer cells, and other immunological alterations, including altered cytokine production and complement system activation [23]. Growing interest has been recently focused on inflammatory cytokine production during haemodialysis. Contact of mononuclear cells with a complement activating membrane results in accumulation of some cytokine mRNAs, such as IL-1, TNF-α, and IL-6 without a correspondent increased translation into protein [15,17]. Monocytes are at a state of chronic activation when patients are on maintenance haemodialysis with cellulosic membranes. As a result, monocyte became refractory to further stimulation and are unable to mount a cytokine response to antigenic challenge. By contrast, the use of biocompatible membranes normalizes the ability of mononuclear cells to synthesize cytokines [24].

Malnutrition is a common feature in haemodialysed patients and several reports have documented the adverse effect of malnutrition on morbidity and mortality of these patients [25]. Patients on haemodialysis show evidence of an accelerated catabolism, which is particularly evident on haemodialysis days. The altered protein metabolism may be referred to the loss of amino acids induced by the dialytic procedure. However, several studies have underlined the possible role of unsubstituted cellulosic membranes in enhancing protein catabolism [26]. Moreover, experimental evidence [27] supports the hypothesis that the enhanced proteolysis induced by cellulosic membranes is mediated by monocyte activation and the subsequent release of cytokines, such as IL-1 and TNF-α, which may synergistically induce muscle protein catabolism. Thus, it can be speculated that the bioincompatibility of the membrane may contribute to worsening the nutritional status in dialysis patients [28].

Recent reports stress the role of cytokines and their inhibitors in the process of bone remodeling and it has been speculated that bioincompatible membranes play a role in the pathogenesis of renal osteodystrophy [29]. This hypothesis is supported by findings of Ferreira et al. [30] who found that an imbalance between cytokines and their specific inhibitors may have deleterious effects on uraemic bone. Particularly, high plasma IL-1RA concentrations makes the skeleton relatively insensitive to the stimulatory effect of IL-1 on osteoblast; on the other hand, high soluble IL-6 receptor concentrations may balance the stimulatory effect of IL-6 on osteoclast activity. These results strongly support the hypothesis that cytokines may have a pathogenetic role in the development of renal osteodystrophy.

Finally, the biocompatibility of the dialysis membranes may play an important role in the resolution of acute renal failure [31] and may, at least partially, account for the different morbidity and mortality rate between patients dialysed with cellulosic membranes and those dialysed with synthetic membranes [32].

Role of molecular biology techniques in the understanding of cytokine induction during haemodialysis

Several studies, performed both in vitro as well as in vivo, have investigated the production of cytokines by PBMC from dialysis patients compared to normals and uraemic patients not yet haemodialysed [4,7,9,10]. Collectively, these studies can easily confuse the reader. Indeed, due to the enormous variability of the experimental design and techniques used, conflicting results have been reported by different authors [33,34]. Moreover, in the last few years the discovery of cyto-

Table 1. Clinical consequences of cytokine production in haemodialysed patients

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Dialysis-related amyloidosis</td>
</tr>
<tr>
<td>Hypotension</td>
<td>Malnutrition (muscle protein catabolism)</td>
</tr>
<tr>
<td>Headache</td>
<td>Impaired resistance to infections</td>
</tr>
<tr>
<td></td>
<td>Renal osteodystrophy</td>
</tr>
</tbody>
</table>
kine-specific inhibitory proteins, such as IL-1RA and soluble TNF receptors, has further complicated this cloudy issue [7]. The quality of the antibodies, the ELISA or RIA kits used were not always highly reliable. Studies using bioassays (considered useful in the past), in the lights of the discovery of cytokine-specific inhibitors, are now questionable.

The sum of these different pitfalls (interactions of cytokines with specific inhibitors, reliability of the antibodies and techniques used) make the study of cytokine production difficult to evaluate. Recently, the introduction of molecular biological techniques to the study of 'cytokines and bioincompatibility' has overcome some of these difficulties [2]. Unlike bioassays, these techniques are highly specific and are not influenced by cytokine binding proteins and inhibitors. Indeed, using appropriate probes, these techniques allow the researcher to detect specific cytokine mRNAs and their cellular source. Recently, for example, applying a RT-PCR technique, an increased expression of MCP-1 and C3 mRNA between normal and haemodialysed patients has been demonstrated [35,36].

Moreover, using *in situ* hybridization technique, the monocytes were identified as the main cell type expressing MCP-1 mRNA in cuprophan-treated patients (Figure 2) [35].

Although molecular biology techniques cannot demonstrate the clinical impact of an increased cytokine mRNA concentration, at least they can help to identify the mechanisms leading to cytokine transcription during haemodialysis. Overall, the application of these techniques may shed light on the study of bioincompatibility of different dialysers and dialysis procedures. Of course, prospective controlled clinical studies are now warranted to determine the effective clinical consequences of cytokine transcription.

**Perspective**

In summary, biocompatibility of membranes is an important concern in haemodialysis treatment. Bioincompatible treatment may induce an inappropriate monocyte activation and cytokine production.

---

Fig. 2. MCP-1 gene expression studied by *in situ* hybridization in peripheral blood mononuclear cells (PBMC). Bright (A, C) and dark (B, D) field photomicrographs of PBMC isolated from healthy subjects (A, B) and from uraemic patients dialyzed with cuprophan membrane (C, D). Cuprophan treatment induces a clear upregulation of MCP-1 mRNA (black grains in bright field and white grains in dark field).
which, in turn, mediate some of the immune and metabolic dysfunction associated with haemodialysis. It is clear that due to different factors, such as membrane, fluid composition and laboratory assay, the issue of cytokine and bioincompatibility remains a difficult task to address. Thus, as for the general concept on bioincompatibility, the topic on cytokine and bioincompatibility, as Dr Klinkmann said, "is still a puzzle partly solved and partly enigmatic" [35]. In conclusion, to affirm that one membrane is more biocompatible than another, we need prospective clinical studies demonstrating that an up- or down-regulation of cytokine transcription induced by a specific membrane is able to minimize the long-term adverse effects of haemodialysis and to favourably impact on the overall morbidity and mortality of chronic haemodialysed patients.

References

Higher haematocrit levels: do they improve patient outcomes, and are they cost effective?

Allan J. Collins and William F. Keane

Department of Medicine, Hennepin County Medical Center, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Since the introduction of human recombinant erythropoietin (Epo) into clinical practice, numerous studies have evaluated the relationship between correction of the anaemia of chronic renal failure and the associated changes in physiological and functional parameters [1]. Early work showed marked increased exercise tolerance and improved oxygen consumption [2–5]. Similarly, enhancement of brain function, as measured by visual evoked response time and cognitive function, were noted [6,7]. A number of investigators have shown improved cardiac function when haematocrit levels were increased from 25–30% [8–14]. Normalization of haematocrit levels has been studied in only a small number of patients, but improved exercise tolerance and reduced hospitalization rates were demonstrated [15,16]. Although many studies have shown evidence of functional improvement, no studies have had adequate power to determine whether there are beneficial or adverse effects of higher haematocrit concentrations on patient survival or hospitalization.

Haematocrit/haemoglobin levels (haemoglobin concentrations) have only recently been evaluated as a risk factor for mortality in ESRD patients in a mortality model. Madore et al. recently published data on 18,000 National Medical Care patients with haemoglobin levels determined over the last three months of 1992, with follow-up into 1993 [17]. This study showed increased mortality associated with haemoglobins less than 100 g/l, compared to 100 to 110 g/l. Risk factors included in the analysis were age, gender, race, renal diagnosis (as diabetic or non-diabetic), case mix adjustments by biochemical data and serum albumin, and dialysis therapy by urea reduction ratio.

The strength of the study included a large sample size with adjustments for patient, clinical, and biochemical factors, nutritional status, and dialysis therapy. The weaknesses in the study included a short period of haemoglobin assessment, an ill-defined follow-up period in 1993, a study period that included a time during which i.v. iron was not available in the United States, and a low number of patients in the higher haemoglobin group (>110 g/l; 2000 patients). The study also included patients in the over 110 g/l range, which includes Medicare patients receiving Epo and requiring medical justification for haemoglobin levels over 120 g/l. This latter factor places high risk patients in the over 110 g/l group along with the lower risk patients (haemoglobin levels ranging from 110 g/l to 120 g/l), requiring no justification for Epo and possibly biasing the results in the higher haemoglobin group with the sicker patients. Lastly, there were no evaluations of comorbidity and disease severity in the National Medical Care data set. Given the strengths and weaknesses, the study clearly showed the consistent association of haemoglobins less than 100 g/l with significantly higher mortality. Unfortunately, no definite conclusion can be made about the group with haemoglobins over 110 g/l, secondary to the comorbidity and insufficient power.

We have recently reported, in preliminary form, the relationship between haematocrit levels and associated mortality and hospitalization in a large cohort of Medicare patients [18–21]. These studies evaluated prevalent patients at later periods of time than in Madore’s report, with six- and twelve-month intervals of haematocrit determinations and with a longer follow-up of one year. In our cohort, increased hospitalization rates and higher mortality rates were seen in patients with haematocrits below 30%, results which are similar to those reported by Madore et al. Fewer hospitalizations and lower mortality were noted in patients with haematocrits between 33 and 36%. Patients achieving these haematocrit levels had a 7–15% lower risk (P value: 0.001), compared to patients with levels of 30–33%. Our studies were adjusted for comorbidity and disease severity but did not account for dialysis therapy or nutritional status, which were not reported in the Medicare claim data.

The issue that is still not clearly defined is that of which haematocrit level is best for patient survival and reduced morbidity. More recent studies of large patient cohorts indicate that higher haematocrits in the range of 33–36%, or haemoglobins of 110–120 g/l, may be associated with improved outcomes. These more recent studies assessed medical interventions on a national basis and may give insight into large population health care guidelines that provide for improved national outcomes and more cost effective care. The severity of
disease adjustments are critical, since they confound low haematocrit/haemoglobin levels and the associated mortality. These findings would suggest that low haematocrit levels, even after disease severity is taken into consideration, are associated with increased mortality and suggest more aggressive treatment of anaemia would be beneficial.

Recently, the National Kidney Foundation (NKF) developed practice guidelines for anaemia management [22]. The current recommendations indicate achievement of target haematocrits of 33–36%. There were also recommendations for iron replacement. Current practices of i.v. iron replacement in haemodialysis patients consists of ten 100 mg i.v. doses to complete a 1000 mg total dose. Alternative 25–100 mg doses of i.v. iron, administered weekly, has been proposed to improve Epo responsiveness and sustain haematocrit levels [23,24]. Although i.v. iron may be associated with nausea, vomiting, wheezing, hypotension, and rarely anaphylaxia, the rate of these complications is low at approximately 0.6%. Unfortunately, no data were available at the time of publication of the NKF recommendations to assess the long-term mortality associations with these different i.v. iron practices.

We recently reported increased mortality in patients with the high frequency, low dose i.v. iron administration patterns [25]. After risk stratification, the study suggested that there was a 59% higher risk of infectious death in the high frequency (4–6 months of i.v. iron), low dose (5 or less vials per month; mean 3.4) group, compared to standard practice patterns of low frequency (1 to 3 months), high dose (8 vials per month). These results have important implications for clinical use and need further confirmatory studies.

Appropriate iron dosing appears to enhance haematocrit levels and Epo responsiveness [23,26,27]. The haematocrit/haemoglobin morbidity and mortality results along with the i.v. iron pattern outcomes may be viewed differently, depending on the national financing and reimbursement practices for dialysis therapy. A fee-for-service payment system, such as in Japan, the United States, and Germany, would not financially restrict Epo or i.v. iron utilization. However, capitated or fixed regional health care budgeting would place significant financial disincentives to increase Epo and i.v. iron administration without clear evidence of the clinical benefit. The more recent mortality and morbidity studies now place new clinical pressures on nephrologists to address anaemia correction. In the United States, these studies may further improve the relatively lower survival of dialysis patients. In Europe, correction of anaemia may further improve the already excellent outcomes previously noted. We look forward to detailed studies from other countries in which medical practices differ from the United States to determine if a consistent picture will emerge for targets of anaemia correction and the associated outcomes.

References


18. Collins A, Ma J. Early hematocrit correction improves patient survival. Nephrology 1997; 3 [S1]: S187 (Abstract)


Haemolytic uraemic syndrome (HUS) during treatment with cyclosporin A after renal transplantation—is tacrolimus the answer?

Clemens Grupp1, Florian Schmidt1,2, Felix Braun2, Thomas Lorf2, Burckhardt Ringe2, and Gerhard A. Müller1

1Abteilung Nephrologie und Rheumatologie and 2Klinik für Transplantationschirurgie, Georg-August-Universität Göttingen, Göttingen, Germany

Key words: thrombotic microangiopathy, FK 506, cyclosporin A, transplantation, kidney

Post-transplant haemolytic uraemic syndrome (pHUS) is a severe complication after renal transplantation resulting to a high degree in the loss of the affected graft [1]. An incidence of 1–5% in kidney transplant recipients has been observed in the early cyclosporin A (CsA)-era [2–5]. Both recurrence and de-novo disease occur in renal transplants recipients. The major risk factors for HUS in renal transplantation include recurrence of the disease, vascular rejection favoured for example by high levels of HLA antibodies or high HLA-DR mismatch, shock damaged kidneys, in particular an immunosuppressive therapy with CsA [2,6,7]. CsA was recognized as an independent risk factor for the development of HUS unrelated to other factors associated with kidney transplantation when cases of HUS were observed after the use of CsA in bone marrow transplantation [8], liver transplantation [3] or Behcet’s disease [9]. Despite this and other side effects (Table 1), CsA is currently the cornerstone of immunosuppression in kidney transplantation. With the introduction of tacrolimus (FK 506), a further immunosuppressant has become available that has a similar immunosuppressive efficiency. CsA and tacrolimus share many properties both with respect to their mode of action and their side effects (Table 1). However, initial reports suggested that one advantage of tacrolimus in comparison to CsA was a lower incidence of HUS with the former drug [10].

Pathogenesis of HUS

Although in general the pathogenesis of HUS of any cause is still incompletely understood, several factors are thought to play a major role. Endothelial dysfunction, induced for example by toxins or drugs, is an early step in the sequence of events leading to intravascular platelet aggregation [1], caused—amongst others—by release of von-Willebrand-multimers and platelet activating factor (PAF) from damaged endothelial cells [1]. Furthermore endothelial injury leads to an alteration of the prostacyclin/thromboxane balance with a decrease of the vasodilatatory and platelet aggregation inhibitor prostacyclin (PGI2) and an increase of thromboxane, causing vasoconstriction and platelet aggregation. All of these factors favour platelet aggregation and local microthrombosis.

Actions of cyclosporin A favouring the genesis of HUS

CsA is thought to promote the induction of pHUS by several mechanisms. It causes vasoconstriction of the afferent and efferent glomerular arterioles and thereby reduces renal blood flow and glomerular filtration rate. The exact mechanism of this vasoconstriction is still unclear, but there appears to be substantial impairment of endothelial cell function, leading to reduced synthesis of vasodilators (prostacyclin and nitric oxide) and enhanced release of vasoconstrictors (thromboxane and endothelin) [2,8,11]. Endothelial damage is one characteristic of renal biopsies demonstrating CsA toxicity [12].

Are there differences between tacrolimus and cyclosporin A in this respect?

Although tacrolimus and CsA bind to different immunophilins, they exhibit a similar mode of immunosuppressive action, involving inhibition of T lymphocyte activation, primarily via the suppression of the production of interleukin 2 (Table 1). The toxicologic effects of tacrolimus on the vascular system...
Table 1. Comparison between CsA and tacrolimus. Both substances exhibit a similar mode of immunosuppressive action. No major differences exist with respect to their side effects, although some of these may be more pronounced in the one substance than in the other.

<table>
<thead>
<tr>
<th></th>
<th>Cyclosporin A</th>
<th>Tacrolimus (FK506)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>undecapeptide</td>
<td>23-member-β-macrolide-lactone</td>
</tr>
<tr>
<td>Intracellular binding</td>
<td>cyclophilin</td>
<td>FKBP 12</td>
</tr>
<tr>
<td>Mode of immunosuppressive action</td>
<td>Drug-immunophilin complexes (CsA-cyclophilin-FK 506-FKBP 12) block the enzymatic activity of the phosphatase calcineurin →dephosphorylation of NF-ATc →translocation of NF-ATc inhibited →rate limiting for cytokine production (IL-2, IL-3, IL-4, TNFα, INFγ, and GM-CSF)</td>
<td></td>
</tr>
<tr>
<td>Vasoconstriction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nephrotoxicity</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertension</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Gingival hyperplasia</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

NF-ATc, nuclear factor of activated T cells, cytoplasmic component.

also resemble those of CsA. Like the latter drug, tacrolimus is considered to be vasoconstrictive. A more renal but less systemic vasoconstriction than under CsA treatment has been reported [13]. Tacrolimus, in contrast to CsA, does not lower prostacyclin levels at comparable immunosuppressive doses, at least in vitro [14]. However, like CsA, tacrolimus increases the production of the vasoconstrictors thromboxane A₂ and endothelin [15] and inhibits nitric oxide synthase activation [16].

Anecdotal observations of HUS after transplant on tacrolimus

Although no exact data exist as yet, the number of reports of HUS under tacrolimus suggest that there is no difference compared to CsA with regard to this disease [3,17]. As for CsA, cases of HUS under tacrolimus medication unrelated to kidney transplantation have also been reported after liver [3,18] or bone marrow transplantation [8,19,20]. We recently observed two cases of recurrence of HUS under immunosuppressive therapy with tacrolimus. A 31-year-old kidney recipient had been switched from CsA to tacrolimus due to severe, steroid-resistant rejection. Several biopsies taken under CsA immunosuppression showed no signs of microangiopathic purpura but signs of rejection. After conversion to tacrolimus kidney function initially improved but deteriorated again after several weeks. The graft now exhibited the typical histological characteristics of thrombotic microangiopathy and was subsequently lost due to this disease [21]. In a 39-year-old patient, who received primary immunosuppression with tacrolimus, histologically proved recurrence of HUS occurred within 14 days after transplantation. Just by the omission of tacrolimus—the patient refused plasmapheresis—and switch to an immunosuppressive regimen consisting of a dual therapy with mycophenolate mofetil and prednisone, renal function and histology, as evaluated by repeated biopsies, improved dramatically [22].

Practical considerations

On the basis of our own experience and a review of the available data in the literature, we suggest that in patients suspected of CsA- or tacrolimus-induced HUS both of these substances be omitted. In such cases immunosuppressive therapy with mycophenolate mofetil and steroids had been proposed [23]. It is questionable whether such patients should receive antilymphocyte preparations such as OKT 3 or ATG [2,3,24,25]. In immunological high risk patients, especially if a loss of the transplant due to rejection has to be suspected after exploring alternatives of antirejection therapy, continuation of CsA or tacrolimus medication at a reduced dose is conceivable. A switch from CsA to tacrolimus or vice versa might be also considered as repeatedly suggested [3,10,26,27], although the benefit of this manoeuvre is potentially caused by an at least temporary, diminished dose of these immuno-suppressives. Furthermore the other therapeutic options in HUS like plasma therapy and methylprednisolone pulses should be taken into account, despite the effectiveness of these measurements especially in the post-transplant HUS is questionable [1]. Renal insufficiency due to HUS predisposes to a recurrence of this disease after transplantation. However, the genesis of the HUS should be taken into consideration. Low recurrence rates have been reported in HUS with prodromal diarrhoea (D + HUS), usually triggered by verotoxin-producing Escherichia coli (VTEC) and predominantly occurring in children [28], whereas recurrence rates of up to 50% were observed in adults [1,2,7]. This may be due to the fact that HUS in adults often presents as ‘atypical’ sporadic, chronic relapsing or familial D—forms, which are not associated with VTEC. In these latter cases either the omission or a
low dose of both CsA and tacrolimus in the immunosuppressive regimen seems to be advisable. Furthermore, the above mentioned risk factors for pHUS such as high HLA-DR mismatches or the transplantation of shock-damaged kidneys should be avoided [2]. These measurements may help to improve the present poor prognosis of pHUS.

References