Microalbuminuria in essential hypertension. A marker of systemic vascular damage?

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Introduction

Increased urinary albumin excretion (UAE) in the microalbuminuric range (i.e. in amounts not detectable by the usual dipstick methods for urine protein) characterizes a sub-sample of non-diabetic essential hypertensives subjects, probably ranging between 10–20% of the overall hypertensive population [1]. Since abnormality is associated with an augmented frequency of atherosclerotic cardiovascular complications independent of the association with classical risk factors, microalbuminuria may represent an autonomous marker of vascular damage [1]. The hypothesis postulates that an increased UAE reflects the abnormal systemic trans-vascular macromolecular leakage in atherogenesis, where permeability changes occur since the earliest stage [2] as a consequence of damaged vascular endothelium, a structure intimately involved in vascular permeability as well as haemostasis, fibrinolysis and vasomotion [3]. This paper will briefly review the still limited available evidence, either direct, inferential or circumstantial, which supports this possibility with particular regard to recent work in hypertension, taking advantage also of data obtained in apparently unrelated clinical conditions which may help to make the point. One should be aware, however, that several other biological factors, primarily blood pressure levels, may per se influence the levels of albuminuria in hypertensive patients [1]. Due to the constraint of the editorial format, quotations will be generally limited to reviews in which the reader may find the specific references.

Acute phase conditions and vasculitides

Acute increments in UAE occurring within minutes and proportionally to the severity of the injuring stimulus, and receding within few hours from it, were reported in a series of ‘acute phase’ conditions such as trauma, burn injury, extensive skin disease, acute pancreatitis and surgery [4]. Similar short-lived increases in UAE were triggered by acute ischemic cardiovascular syndromes such as myocardial infarction and limb ischemia in which urinary albumin changes paralleled neutrophil activation at the time of exercise-induced pain in arteriopathics or exaggerated vascular albumin permeability after rat hindlimb ligation [4]. In these ‘acute phase’ conditions, inflammatory mediators are likely released in blood and may increase microvascular permeability by damaging vascular endothelium, a prime target for any substance reaching the circulation. Altered glomerular permeability as a part of a systemic endothelial dysfunction and/or injury could, therefore, explain the appearance of microalbuminuria in absence of any evidence of functional or structural renal impairment. This possibility is also suggested by the behaviour in both the early phases of myocardial infarction and surgery of von Willebrand Factor (vWF) levels, an endothelial-derived hemostatic factor produced in greater quantity by a dysfunctional endothelium, and elevated in frank atherosclerotic vascular disease, in presence of all major risk factors of atherosclerosis including diabetes and hypertension, and in most of the inflammatory vasculitides [5]. Similar interpretation may be given to the report of microalbuminuria in patients with systemic sclerosis, a syndrome characterized by excessive collagen synthesis, increased vascular permeability and endothelial damage caused by widespread vascular damage [1]. Whether UAE behaves similarly in other vasculitides is, however, still unsettled.

Diabetes

The concept of microalbuminuria as a marker of vascular damage is corroborated by the consistent correlation with the transcapillary albumin escape rate, an index of transvascular macromolecular leakage across the endothelial layer not only of capillaries but of arteries as well [6]. This evidence, first produced in type I and recently extended to type II diabetics [7], has general relevance, since diabetes overlaps in several respects with essential hypertension, although data obtained in one may not necessarily extend to the other category. Abnormal circulating vWF levels, a
marker of endothelial dysfunction, are frequently found in microalbuminuric diabetics [8] and blunted vasoconstriction to regional nitric oxide synthesis inhibition was shown in microalbuminuric type I diabetes compared with both control subjects and patients without incipient nephropathy [1]. Therefore, one might deduce that endothelial dysfunction may, at least in part, contribute to microvascular permeability not only in acute inflammatory conditions but even in a chronic disease state such as diabetes. This assumption, however, is speculative because a direct link between the degree of endothelial dysfunction and of transvascular molecular leakage has never been shown. Besides, the relative importance of endothelial vs postendothelial mechanisms as determinants of the transcapillary escape rate is still unclear [6]. Whatever the case, the association between UAE and systemic microvascular permeability is not restricted to diabetes since similar data were reported in apparently healthy subjects, and smokers in whom both microalbuminuria and endothelial damage are frequent [1].

**Hypertension**

Beside the epidemiological data of an independent association of microalbuminuria with vascular events [1], other lines of evidence suggest a link between microalbuminuria and systemic vascular status in hypertension. One of them is based upon the evaluation of biochemical markers of endothelial dysfunction and/or damage, mainly vWF, a hemostatic endothelial-derived factor [5] whose levels were found elevated only in apparently uncomplicated [9] and elderly microalbuminuric hypertensives [10] as compared with non albuminuric patients, confirming that—as it is now becoming evident in spite of contrary statements, endothelial dysfunction is not universal in hypertensive patients [3]. Endothelial dysfunction in microalbuminuric essential hypertensives is also suggested by the missing renal vasodilatation in response to L-arginine, the physiological precursor of endogenous nitric oxide (A. Mimran, personal communication), a finding in contrast with the preserved response to acetylcholine, a drug which stimulates release of that substance, in the forearm of patients with elevated UAE [11]. The data may reflect a heterogeneous degree of endothelial dysfunction among regional beds, but also the inherent limits of acetylcholine as a biological test to evaluate endogenous endothelial function. First of all, the response to muscarinic agonists does not necessarily reflect the underlying tonic activity of this biological system. For example, comparable vasodilator responses to muscarinic stimulation were obtained in insulin dependent microalbuminuric patients in whom the response to L-NMMA, an antagonist of the endogenous nitric oxide system, was defective [1]. Further limitation of the technique is the only partial abolition of muscarinic agents by antagonists, so that any assumption that forearm vascular responses to these agents exclusively reflect nitric oxide release is unjustified, especially in pathological states [1]. Unpredictable dependence upon endogenous muscarinic receptor reserve, wide interindividual variability in acetylcholine esterase activity, difficulties in transferring results obtained in a single regional bed to the whole vascular system, discordance with the biological actions of other endothelial active vasodilators and lack of knowledge about the long-term prognostic cardiovascular implications constitute additional problems of the technique. A next not fully addressed question is also whether results obtained through the acetylcholine infusion can be extrapolated to those obtained by vWF determination. As a matter of fact, within-subject comparison of forearm acetylcholine responsiveness and von Willebrand Factor suggests that the two different endothelial markers do not reflect the same underlying alteration [12].

A second line of evidence suggesting a link between microalbuminuria and systemic vascular status in hypertension is based upon the finding of elevated UAE in a sample of well characterized essential hypertensives with atherosclerotic peripheral vascular disease as compared with either uncomplicated hypertensives or normal controls [13]. Thus, urine albumin levels may reflect widespread atherosclerosis, a possibility supported also by the positive correlation with ultrasonographic carotid thickness, a surrogate quantitative measure of the systemic atherosclerotic process [13]. The hypothesis is strengthened by the correlation between UAE and circulating vascular cell adhesion molecule-1 (sVCAM-1), a protein expressed on the surface of activated endothelial cells, and expressed in early atherosclerosis [14]. Since part of the protein is shed in the circulation and can be detected in peripheral plasma, sVCAM-1 may be a circulating marker of the presence and severity of atherosclerosis in humans. Even in this sample of patients, systolic BP—as frequently documented—was a strong predictor of UAE [13], a result that may not only represent the influence of purely hydraulic factors. In fact, systolic hypertension emerges more and more as a marker of atherosclerotic risk, possibly because reduced aortic compliance due to vessel wall stiffening is often seen in association with aortic atherosclerosis which, in turn, reflects general atherosclerosis and predicts symptomatic cardiovascular disease [15].

In conclusion, microalbuminuria might be seen as an integrated marker of risk because of its relationships with the major predictors of cardiovascular mortality and morbidity in hypertensive patients. However, UAE as such, may also mirror a diffusely altered vascular permeability and endothelial damage; whether this is the reason which contributed independently to the greater morbidity and mortality in microalbuminuric subjects is still unsettled.

**References**

ACE inhibitors, AT1 receptor blockers, and the kidney

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One approach employed by historians to highlight the unanticipated involves asking a rhetorical question: What might have been expected, reasonably, on a certain date? In 1972, when ACE inhibition was first emerging, surely no one would have guessed that 25 years later the ACE inhibition story would involve as many therapeutic triumphs as it has. Difficult hypertension requiring three or more drugs for hypertension control was identified early as a reasonable target, followed by scleroderma renal crisis, congestive heart failure, myocardial remodelling after myocardial infarction, diabetic nephropathy, and in all likelihood, other forms of progressive renal injury. Current interest in the specific underlying pharmacological mechanisms by which ACE inhibition achieved its goal reflects not only the pleasure that we all share in understanding a process, but also a more specific and more practical issue. With the birth of alternatives to ACE inhibition for blocking the renin system, we have a new choice and an unambiguous need to understand the mechanisms. The fact that we call the responsible enzyme ‘ACE’ reflects our narrow, provisional view. The enzyme is a peptidyl dipeptide hydrolase and has a wide range of substrates, among them bradykinin, and thus engages prostaglandin release and the nitric oxide pathway. Much of the energy invested to this point on mechanisms has reflected this fact, and a strong suspicion held by many that the kinin pathway makes a very substantial contribution to the therapeutic efficacy of ACE inhibition. Should that be the case, alternatives for blocking the renin system via renin inhibition or competitive Ang II antagonists would have limited therapeutic application.

Another view is possible. No pharmacologist examining the renin cascade would have chosen to block the ACE step. The first step—the renin:angiotensinogen interaction—is rate limiting, and remarkably specific, which would have made it a much more attractive alternative. The AT1 receptor provides a second attractive target. Because Ang II formation can be catalyzed by a number of serine poteases, and they are ubiquitous, pharmacological interruption at the Ang II receptor level has the distinct potential advantage of blocking the action of Ang II, whatever the pathway of its formation. The fact that ACE inhibition came along before the two alternatives was not a product of a planned program, but rather was an unanticipated byproduct of snake toxin pharmacology. This was a happy accident, but an accident nonetheless. With its specificity and efficacy, renin inhibition would have been a very attractive approach to pharma-

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ecological interruption of the renin system. However, because of limited bioavailability of the agents developed, costs, and the rapid development of a pharmacological alternative—the Ang II antagonist class—attempts to develop renin inhibitors for clinical application appear to have ceased. The Ang II antagonists, on the other hand, have gained substantial momentum. Several dozen AT1 receptor antagonists were developed by several dozen pharmaceutical firms and, at the moment these words are being written, two are marketed in Western Europe or the USA, and at least another 11 agents are under active clinical investigation [1]. During these agents’ development, there have been several focuses of interest beyond blood pressure reduction.

The first involved directly the possibility that the lack of specificity of ACE, and thus of ACE inhibition contributed to one of its more annoying and disruptive side effects, cough. One widely held concept was that cough reflected bradykinin accumulation or accumulation of other cough-promoting factors such as substance P [2]. A study designed to address this issue found that the incidence of cough in losartan-treated patients was essentially identical to cough in thiazide-treated patients: As the patient population was selected for study not only because they were believed to have suffered from ACE-inhibitor induced cough, but also showed cough on rechallenge with an ACE inhibitor, the finding was especially persuasive [3]. The AT1 receptor antagonists had passed their first important test beyond blood pressure efficacy, with this successful study.

There has been far more interest in the kidney, and the possibility that AT1 receptor antagonists will prevent nephropathy, than at the same stage in the evolution of ACE inhibitor therapy [4]. There are a number of reasons. Although end-stage renal disease (ESRD) is far less common than acute myocardial infarction and other cardiovascular endpoints, it is enormously costly. Identification of stages, especially the earliest stages, is relatively easy, as is assessment of progression. All make renal injury an attractive therapeutic target with implications that may go well beyond the specifics of ESRD prevention. For these reasons many of our colleagues have urged the pharmaceutical firms involved in AT1 receptor antagonist development to pursue studies on the kidney, and many of these companies have shown interest. All have had to address an issue raised in the title of a recent review by Ichikawa, which has made substantial contribution to this field [5]. ‘Will Ang II receptor-antagonist be renoprotective in humans?’ His essay reflects a substantial body of current thinking.

Ichikawa based his analysis on a range of considerations, the majority of which seem to favor ACE inhibition as these considerations suggest that angiotensin is not involved [5]. ACE inhibitors can influence alternative pathways that might, in turn, influence extracellular matrix protein degradation and the rate of development of glomerulosclerosis. Macrophage infiltration is also ACE inhibitor responsive. Blocking the AT1 receptor opens the short feedback loop, thereby leading to renin release and increased Ang II formation. With the AT1 receptor blocked, this sequence could lead to unopposed activation of the AT2 receptor—with unknown but potentially negative consequences. All of these considerations favor ACE inhibition over Ang II AT1 antagonist action—but it is crucial to remember that each is a construct, an idea based on a slim database, generally obtained in vitro. Perhaps the most important consideration in Ichikawa’s analysis involved glomerular hemodynamics, especially glomerular capillary pressure, which for many goes beyond the construct level. In brief, this analysis suggests that much of the ACE inhibitor dependent improvement in natural history reflects the salutary effect of ACE inhibition on glomerular capillary pressure via bradykinin-mediated efferent arteriolar dilatation. Thus, the kininase action of ACE inhibitors is crucial, and the reduction in Ang II formation is less important—or even irrelevant.

Only a solid therapeutic trial in humans can resolve this issue. Should this apparently compelling argument lead us to dissuade the decision makers from committing to such studies? There are two reasons for going ahead. First, one can make an equally compelling argument for potential efficacy of Ang II antagonists, based on more effective blockade of the renin system. Second, much of the most important data reviewed above were obtained in vitro or in small animal models. If studies in rats never predicted responses in humans, we would probably ever do studies in rats—or at least would not read them. If studies in rats always predicted what would happen in humans, we could not justify studies in humans. Is this an area in which there might be important species differences?

I addressed that issue specifically in a recent editorial on ACE inhibition and the kidney [6]. To isolate species differences one has to apply an essentially identical protocol to multiple species. Bradykinin antagonists blunted the renal vasodilator response to ACE inhibitors in the dog and in the rat, but not in the rabbit. In accord, an Ang II antagonist blunted what ACE inhibitor induced renal vasodilation in the rat and dog, but blocked it completely in the rabbit. ACE inhibition increased prostaglandin release in rat and canine kidneys, but not that of the rabbit. In an elegant study Roman, et al. showed that in the rat it was medullary perfusion that was primarily kinin dependent [7]. Thus, apparent species differences may be primarily anatomical, reflecting the relative contribution of medullary perfusion to total renal blood flow: In this feature humans resemble the rabbit far more than they do the rat or dog [6]. Whatever the explanation one clear message emerges: We cannot extrapolate from studies in a limited range of species, especially the rat, to control mechanisms in humans, even in health and much less so when disease is superimposed.

What of information on the control of the renal circulation in humans, and the mechanisms by which ACE inhibition might influence the renal circulation?
Although there are powerful limitations in the approach that can be employed in humans, several lines of evidence provide an answer. The striking influence of salt intake on the renal vasodilator response to ACE inhibition was recognized early, and supports a dominant role for the Ang II mechanism. This observation is necessary but is not sufficient. More recently, comparative pharmacology has strengthened that conclusion substantially. If the renal vasodilatation induced by ACE inhibitors in humans included a substantial component due to bradykinin, prostaglandins, or nitric oxide, one would anticipate that the renovasodilator response to a renin inhibitor would be substantially less. To our surprise the renal vasodilator response to a renin inhibitor, enalapril, exceeded expectations from early experience with ACE inhibitors [6]. To address this issue, we performed a range of follow-up studies. To ascertain whether the observation represented an idiosyncracy of one renin inhibitor, we studied a second—with an identical result. Because of the notorious risk of employing historic controls, we performed a study in which patients received an ACE inhibitor, a renin inhibitor, or vehicle during the same week. This study was coded and double blind. To avoid an idiosyncrasy of one ACE inhibitor, we employed three, each at the top of the dose response curve. The findings all provided support for a surprising but unambiguous conclusion. Despite the fact that our original premise was reasonable and supported by a wealth of information in animal studies, the renovasodilator response to renin inhibition is in the neighborhood of 140 ml/min/1.73 m$^2$, substantially larger than the response to ACE inhibition, typically in the 90–100 ml/min/1.73 m$^2$.

Although the fundamentals of pharmacology would favor as the explanation more effective pharmacological interruption of the renin system at the rate-limiting step, and thus would favor our drawing that conclusion from these data, there is an alternative interpretation. The two renin inhibitors were structurally related, as most drugs in a class are, and it is possible that they share a renovasodilator action through a mechanism unrelated to a reduction in Ang II formation. In the case of the renin cascade, we have the potential for a ‘tie breaker’. If, indeed, the renin inhibitors operated via this cascade, one would anticipate a similar or larger renovasodilator response to Ang II antagonists, if the studies were performed in the same way. This is precisely what we found in studies performed with an identical model, protocol and technique. Two Ang II antagonists induced a renovasodilator response that matched, or exceeded slightly the response to renin inhibition in healthy humans on a low salt diet [8,9]. From this observation we would draw several conclusions. The renal haemodynamic response to ACE inhibition has underestimated, systematically, the contribution of Ang II to renovascular tone. The effectiveness of renin inhibition suggests that this response represents interruption of primarily renin-dependent, additional non-ACE-dependent pathways. In healthy humans, there might be a small contribution from proteolytic pathways that bypass both renin and ACE. In disease, on the other hand, the latter pathway may provide a more substantial contribution [9].

The final conclusion is that therapeutic trials with Ang II antagonists offer far more promise than did ACE inhibitors, despite the gloomy predictions. They are more effective blockers.

References
Ouabain and hypertension

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The presence of a volume dependent circulating Na-K-ATPase inhibitor was first demonstrated in animals [1]. Subsequently the finding that ouabain sensitive sodium transport of leucocytes from normotensive subjects is depressed by incubation in the plasma of hypertensive patients suggested that the circulating substance responsible might be involved in essential hypertension. It was proposed that a renal defect in sodium excretion was the cause of the increase in the plasma concentration of the Na-K-ATPase inhibitor and that the resultant increase in intracellular sodium and calcium enhanced vascular reactivity and elevated the blood pressure [2]. Initially it was also thought that the inhibitor might inhibit tubular sodium reabsorption and be natriuretic. Later there was a belated recognition that there was little evidence to support the widely held view that Na-K-ATPase inhibitors are natriuretic for the natriuretic effect of ouabain or digoxin, in a normal animal, is negligible unless injected directly into the renal artery in toxic amounts.

There was then a search for a circulating Na-K-ATPase inhibitor which might be involved in hypertension whether or not it also had natriuretic properties. The search was encouraged by the fact that evolution has endowed all animals with highly specific ouabain binding sites and a circulating ouabain-like substance seemed to mirror the relatively recent finding of endorphins as ligands for the opiate receptors.

Plasma ouabain

Hamlyn and Blaustein [3] found a significant correlation between the mean arterial pressure and the capacity of deproteinized boiled plasma to inhibit dog kidney Na-K-ATPase. Goto and colleagues measured the capacity of plant ouabain to bind to cultured canine renal cells before and after they were incubated in plasma [4]. The plasma of hypertensive patients depressed ouabain binding by ~30% and there was a correlation between systolic pressure and the extent of ouabain binding. Though endogenous digoxin-like immunoreactivity (EDLI) was also used to detect endogenous sodium transport inhibitors, there is much evidence that digoxin-like immunoreactivity is often dissociated from digoxin-like biological activity. Indeed in a recent review Goto et al. [4] have deliberately omitted all reference to data obtained using this assay.

In 1991 Hamlyn and colleagues [5] obtained 31 mg of a pure Na-K-ATPase inhibitor from 300 l of human plasma. It inhibited 86Rb uptake of human red cells and isolated Na-K-ATPase and competed with 3H ouabain for binding onto Na-K-ATPase. Mass spectrometry showed that this human substance had spectra identical to those of plant ouabain. Using an antibody raised against plant ouabain it was found that normal human plasma has a mean concentration of 138 ±43 pmol/l, and it was claimed that endogenous ouabain originates from the adrenal.

There was an initial uncertainty that perhaps plant ouabain was an exogenous contaminant. A number of dietary compounds including tea and rat chow contain sodium pump inhibitors. Another difficulty was that while ouabain has a cis junction (CD cis) a biosynthetic pathway for CD cis steroids in humans has not yet been described. It was also pointed out that the glycoside of plant ouabain L-rhamnose is not normally associated with mammals. The principal problem in accepting the discovery of plant ouabain in human plasma was the failure by some workers to reproduce the earlier results with antibodies raised against plant ouabain, particularly if they used a HPLC step in the preparation of the plasma extract for this made it possible to compare the elution fraction of commercial plant ouabain with that of the immunoreactive fractions in the extract [6]. Using this step plant ouabain has either not been found in the plasma or it has been present in much smaller quantities than of other substances which bind onto plant ouabain antibodies. Similarly two groups reported that the plasma level of immunoreactivity to antibodies raised against plant ouabain is unchanged by adrenalectomy. The most divergent finding was that though the plasma level of immunoreactivity to plant ouabain antibodies in the spontaneously hypertensive rat (SHR) was greater than in its normotensive rat the WKY rat the amount of true plant ouabain as authenticated by HPLC was less in the SHR than in the WKY. It was the level of immunoreactive substances to plant ouabain antibodies other than plant ouabain which was raised in the SHR. It seemed that the various antibodies raised against plant ouabain identified several substances, some of which might be isomers of plant ouabain [6].

Several groups have induced hypertension in the rat by long term administration of plant ouabain and in rats immunized against plant ouabain the increase in arterial pressure in the reduced renal mass salt loaded rat and in the Dahl salt sensitive rat is diminished. There is also a recent report by Goto of an ectopic corticotropin syndrome in a man with carcinoma of the lung which gave rise to a mineralocorticoid type of hypertension [7]. Plasma and urinary levels of a
Hypothalamic ouabain

It is very likely that if an endogenous ouabain-like substance has an effect on the blood pressure it usually does so via the hypothalamus [6]. Catecholaminergic denervation of the hypothalamic by an injection into the third ventricle or an incision at the cephalic end of the third ventricle (the AV3V lesion) in the reduced renal mass salt loaded rat and the DOCA salt loaded rat prevent the expected increase in both the arterial pressure and the plasma’s capacity to inhibit Na-K-ATPase. The central administration of a dopamine receptor agonist, pergolide, atrial natriuretic peptide and saline trigger the release of a circulating Na-K-ATPase inhibitor. Plant ouabain instilled centrally increases the blood pressure and renal sympathetic nerve activity. And the intracerebroventricular pre-injection of digoxin specific antibody Fab fragments prevents the increase in arterial pressure induced by the central administration of hypertonic saline. There is also Takahashi and Sano’s demonstration of immunoreactivity to an antibody raised against plant ouabain in the paraventricular and supraoptic nuclei in the hypothalamus and in the organum vasculosum and lamina terminalis of the third ventricle [6].

Haupert has obtained a Na-K-ATPase inhibitor from bovine hypothalamic, up to 750 pmol of material from 1 kg net weight of tissue [8]. Chromatographic and spectroscopic evidence showed that this substance is also plant ouabain but further studies in which the hydroxyl groups were naphthoylated followed by HPLC separation revealed that the retention times of the naphthoylated products of the hypothalamic substance were different from those of commercially available plant ouabain.

Bianchi’s group using the same procedure as Haupert have extracted a Na-K-ATPase inhibitor from the hypothalamic of the Milan hypertensive (MHS) and normotensive rats (MNS) [9]. The yield from the MHS hypothalamic was ~50 times greater than from the MNS, the first demonstration of a quantitative relationship between hypertension and a brain derived ouabain-like Na-K-ATPase inhibitor. Another finding was a striking difference in this substance’s affinity for Na-K-ATPase in contrast to plant ouabain. Rat hypothalamic material inhibits renal and synaptosomal Na-K-ATPase with a potency ~1000 times greater than plant ouabain. In addition, this increased potency appears to change with age for renal Na-K-ATPase obtained from an adult rat is more sensitive to the hypothalamic substance than is renal Na-K-ATPase from a young rat. In contrast the effect of plant ouabain is unaffected by the age of the rat from which the renal Na-K-ATPase is obtained.

Haupert’s group have studied human plasma ouabain, bovine hypothalamic ouabain and plant ouabain after derivatization of all three by naphthoylation [10]. Surprisingly, the products of human plasma naphthoylated ouabain and bovine hypothalamic naphthoylated ouabain have the same retention time but the retention time of the products of naphthoylated plant ouabain are longer. Accordingly the two animal substances are not plant ouabain, but they appear to be the same isomer of plant ouabain, in other words mammalian ouabain. The possibility that mammalian ouabain is an artifact, a product of isomerization of plant ouabain occurring during the isolation process was excluded by submitting plant ouabain to the isolation, purification, naphthoylation protocol employed for the plasma and hypothalamic ouabain. HPLC and CD spectroscopy showed that no structural changes had taken place thus eliminating mammalian ouabain from being an artifact of plant ouabain. These findings dispose of the fear that the endogenous ouabain-like material is a contaminant of exogenous plant ouabain and perhaps explain some of the difficulties encountered when using antibodies raised against plant ouabain.

References
Nonsteroidal anti-inflammatory drugs (NSAIDs) and the kidney

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) comprise a wide group of compounds that, despite differences in their chemical structure, share a similar spectrum of pharmacological activities (anti-inflammatory, analgesic, antipyretic, anti-platelet), side-effects (ulcerogenic and nephrotoxic), and mechanism(s) of action. The latter is dependent, at least in part, upon inhibition of the cyclooxygenase activity of two isoenzymes, the prostaglandin (PG) endoperoxide H synthase-1 (PGHS-1, also referred to as COX-1), constitutively expressed in most cells and tissues, and the prostaglandin (PG) endoperoxide H synthase-2 (PGHS-2, also referred to as COX-2), expressed only in activated cells [1,2]. The kidney is a rich source of these two isozymes, which catalyze the conversion of arachidonic acid to PG endoperoxide H₂ (PGH₂), ultimately leading to the production of PGs [3].

Over the last 10–20 years, a number of experimental as well as clinical studies has univocally attributed to PGs a crucial role in the regulation of many aspects of renal function. This information has alerted clinical nephrologists for the potential nephrotoxicity of NSAIDs, thus contributing to the growing awareness of renal complications by the use of this class of drugs.

The aim of this article is to provide an overview of the present understanding on NSAID-related renal effects. Additionally, proper use of anti-inflammatory agents sparing renal PG synthesis will be addressed. Finally, we shall discuss the potential safety of newly developed NSAIDs selective for PGHS-2, which catalyzes the production of PGs involved in inflammation, and devoid of inhibitory effect on PGHS-1 prostanoid synthesis, implicated in the regulation of renal hemodynamics and water and sodium reabsorption.

NSAIDs and functional renal alterations

An acute reduction of GFR and RBF ranging from 20–50% to acute renal failure can occur within 24–48 h of treatment with virtually all NSAIDs used at full anti-inflammatory dosage, and is usually reversible upon drug withdrawal. The risk for these adverse renal effects is largely dose-dependent, and is negligible for healthy individuals, whose renal hemodynamics is minimally dependent upon intact PG synthesis. In contrast, in subjects with compromised baseline GFR and RBF, renal PGs become critical for the maintenance of renal function, and their inhibition is followed by impaired renal hemodynamics [4–6]. In this situation, a significant decline in renal function is almost inevitable, whereas overt acute renal failure associated with NSAIDs is believed to account for ~16% of cases of drug-induced acute renal failure [7,8]. The populations at greatest risk for renal functional alterations from NSAID therapy include patients with volume depletion from any cause, chronic glomerulonephritis, age-related reduction of GFR, and atherosclerotic cardiovascular disease. Such conditions share either exaggerated vasoconstrictory stimuli to the kidney, or a local reduction in the synthesis of vasodilator PGs. In this setting NSAIDs may remove the modulatory action of PGs on vasoconstrictor agents, thereby inducing renal functional alterations [4].

NSAID administration may also be associated with salt retention, particularly during long-term treatment. The mechanism underlying this effect is likely to be related to decreased GFR and enhanced sodium reabsorption. The risk for clinically evident peripheral oedema is very low. It occurs in only 3–5% of patients chronically taking NSAIDs. Hyperkalaemia has been reported in some cases, mainly associated with potassium-sparing diuretics and ACE inhibitors. NSAID-induced sodium and potassium disturbances are readily reversible after drug discontinuation [9].

NSAIDs and structural renal alterations

In addition to functional renal changes, NSAIDs may cause nephrototoxic effects with mechanisms that are not clearly related to the inhibition of PG synthesis. Acute interstitial nephritis, with and without minimal lesions in the glomeruli and heavy proteinuria, has been reported to occur with a variable incidence, ranging between 0.01% and 2% of patients hospitalized for drug-induced acute renal failure. This complication may develop anytime from days to months after initiation of treatment with NSAIDs, and, in contrast with the haemodynamic variety of acute renal failure, there are not well documented risk factors. However, women and aged individuals seem to be more frequently involved. Spontaneous recovery follows discontinuation of the drug, resolution time varying from days or weeks up to one year. Yet, a full renal recovery is not always the rule, and a permanent renal damage may also occur [9,10].

Membranous nephropathy (MN) with nephrotic syndrome may occur as idiosyncratic reaction to vari-
ous classes of NSAIDs. Approximately 10% of MN is believed to be associated with NSAID use. The temporal association with the intake of modest doses of NSAIDs, the prompt and complete recovery after drug discontinuation, and the absence of recurrent disease may help to clinically distinguish NSAID-associated MN from the idiopathic form [11].

Chronic interstitial nephritis and papillary necrosis (analgesic nephropathy) may develop as a consequence of long-term abuse of a combination of analgesics, mostly those mixture containing phenacetin. Analgesic abuse is a major cause of end-stage renal failure in Western countries. In Europe 2.5% of patients on dialysis had analgesic nephropathy, 1–2% in United States and 12% in Australia. A partial recovery from this nephropathy and/or stabilization of renal lesions may be obtained by withdrawal of the offending drug, depending on the extent of renal damage [12].

NSAIDs and proteinuria

Both short- and long-term NSAIDs treatment, particularly indomethacin, has been reported to reduce proteinuria in patients with the nephrotic syndrome. The antiproteinuric effect of these drugs seems to be enhanced by sodium depletion. Experimental as well as clinical studies have shown that NSAIDs decrease proteinuria by lessening GFR and RPF, and by directly ameliorating glomerular perm-selectivity to macromolecules. Thus, due to their effect on proteinuria as well as on renal haemodynamics, NSAIDs should theoretically delay the progression of glomerular damage. However, contrasting data have been reported in this regard, and at the present time it is difficult to draw conclusions on possible long-term benefits [13]. Besides, one should keep in mind the risk of acute and permanent renal impairment, and the availability of safer antiproteinuric agents such as ACE inhibitors, with the same effect as NSAIDs on glomerular perm-selectivity, but with opposite effects on renal haemodynamics.

Renal sparing NSAIDs

Despite the clinical usefulness of NSAIDs, the growing awareness of their nephrotoxic potential is presently limiting their use. Thus, considerable efforts are being made to identify NSAIDs devoid of renal adverse side-effects.

Low-dose aspirin selectively inhibits platelet prostanoid production without affecting renal PG synthesis. The mechanism is likely to be related to the different rate of recovery of PGH-synthase in the kidney and in platelets, following irreversible acetylation of the enzyme, and possibly to a lesser sensitivity to aspirin of renal PGH-synthase. Due to its selectivity, to date low-dose aspirin represents a drug of choice for the prevention of thrombotic events, even for those patients with underlying renal disease, without the risk of jeopardizing PG-dependent renal function [14,15].

Sulindac is believed to be a NSAID with effective anti-inflammatory activity associated with low nephrotoxicity. Its renal-sparing properties rely upon the presence in the kidney of oxidative enzymes able to rapidly convert the active metabolite of the drug, sulindac sulfide, to the inactive parent sulfoxide compound, as well as to the inactive sulindac sulfone. Due to its unique metabolism, sulindac administration has been reported to be safe, at least in short-term treatment, in situations at risk, such as chronic glomerulonephritis, chronic renal failure, congestive heart failure, and cirrhosis with ascites [16]. On the other hand, the long-term consequences of sulindac treatment in such patients still need to be defined, and caution should be taken when prescribing sulindac for prolonged treatment.

Acetaminophen has been reported to locally inhibit PG synthesis in the hypothalamus, where it exerts its antipyretic and analgesic effect. It is apparently devoid of inhibitory action on renal PG synthesis, and could therefore be the antipyretic/analgesic of choice in subjects with underlying renal disease. Nevertheless, due to its potential for inducing analgesic nephropathy, its habitual consumption should be discouraged [9].

Selective inhibitors of PGHS-2

The vast majority of NSAIDs inhibits the activity of both PGHS-1 and 2. In the kidney, PGHS-1 is constitutively expressed in almost all cell lines, and likely catalyzes the synthesis of prostanoids involved in the maintenance of renal function. In contrast, PGHS-2 is induced in renal native as well as in renal infiltrating cells by inflammatory stimuli. PGs produced by this enzyme are very likely involved in the cellular response to injury. Recently, attention has been focused on the development of NSAIDs selective toward PGHS-2 and, thereby, effective in reducing renal inflammation without altering renal haemodynamics [2,3]. Presently, there is only experimental evidence supporting such hypothesis [17]. Thus, clinical studies are clearly needed to define the therapeutic potential of this new class of NSAIDs.

Summary

In patients with PG-dependent renal function, NSAID administration constantly reduces GFR and RBF in a dose-dependent fashion. In this situation, the risk of overt acute renal failure is high and should be taken into proper account. In contrast, the incidence of NSAID-related renal structural alterations appears to be very low, yet the absolute number of patients may be significant considering the wide use of such drugs. Concerning the antiproteinuric effect of NSAIDs, the unfavourable ratio risk/benefit does not seem to support their indication in proteinuric nephropathies.
The development of PGHS-2 selective inhibitors is promising, and may open new therapeutic strategies in the treatment of the progression of renal disease.

References


Starvation in the midst of plenty—the problem of volaemia in pregnancy and pre-eclampsia

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Pre-eclampsia is characterized by a triad of hypertension, proteinuria, and oedema which appears late in the second trimester or in the third trimester of pregnancy. This complication is seen in 5–10% of pregnancies [1,2]. Pre-eclampsia is the leading cause of maternal mortality and of perinatal mortality. Although this condition has been known since antiquity, there are still gaps in our understanding of the pathophysiology of pre-eclampsia. Hypertension is a foremost sign of pre-eclampsia, while blood pressure is determined by effective blood volume and vascular resistance. Consequently the question arises to what extent abnormalities in blood volume regulation are involved in the pathogenesis of hypertension in pregnant women with pre-eclampsia.

During normal pregnancy an increase of kidney volume, GFR (by 50% after 20 weeks of pregnancy) and RBF (by 60% after 20 weeks of pregnancy) is regularly observed [3, 4]. Changes in renal haemodynamics occur before significant expansion of maternal plasma volume has taken place, but in close temporal relation with a decrease of systemic vascular resistance [4]. Several hormones (prostacyclin, atrial natriuretic peptide, nitric oxide) seem to be involved in the pathomechanisms of these changes [5]. They may be involved in the genesis of glomerular hyperfiltration with subsequent increased urinary excretion of creatinine (thus in pregnancy plasma creatinine is always lower than 88 μmol/l) and proteins (up to 200 mg/d) [1].

Pregnancy is characterized by plasma hypoosmolality (a decrease of plasma osmolality by approximately 10 mOsm/kg H2O), and a slight reduction in colloid osmotic pressure, plasma sodium, and potassium (as compared with preconception values). Plasma hypoosmolality seems to be due to a resetting of the osmostat and thirst center. In pregnancy vasopressin is secreted normally at osmolalities above the lowered threshold and thirst is perceived at lower effective plasma osmolalities than in nonpregnant women.

During a normal pregnancy total body water
increases by 8.5 l, of which 6–6.5 l are located extracellularly and only 2 l intracellularly. From this 6.5 l located in the ECFV, 2 l is accounted for by foetus, factors other than renin are involved in the regulation of sodium excretion by the kidney in pregnancy [9–12]. Plasma aldosterone concentration is normal [11] during pregnancy [9,11], although no clear-out relation between plasma aldosterone levels and plasma renin activity [9–12]. Plasma aldosterone concentrations increase earlier and proportionally more than plasma renin activity. This constellation suggests that factors other than renin are involved in the regulation of aldosterone secretion. As the RAA system responds inadequately to stimulation (upright position) and inhibition (salt load, water immersion), it seems likely that increased activity of this system in pregnancy is a secondary response to primary vasodilation or salt loss factors [8]. The reaction of the RAA system to stimulation suggests that stimulation of this system is submaximal, and that the circulation is underfilled during pregnancy. In the genesis of vasodilation nitric oxide [13], ANP [14] and prostacyclin [15] may play a role for the elevated activity of the RAA system in pregnancy, but other factors, particularly hormones produced by the placenta e.g. progesterone may also be involved.

Contrary to what may be expected from physiological experiments [16] plasma ANP concentrations (in the third trimester) are similar [14] to preconception values [11,17] but elevated in early pregnancy [14]. When related to the increased intravascular volume plasma ANP levels are slightly elevated even in the third trimester of pregnancy. In addition ANP secretion in late pregnancy is responsive to physiologic stimuli such as central hypervolaemia evoked by water immersion [11]. As ANP antagonizes vasoconstriction, it may contribute to the systemic vasodilation during pregnancy [14]. ANP inhibits aldosterone synthesis in the adrenal glands, shifts fluid from the vascular to the interstitial compartment and increases sodium excretion by the kidney [16]. Therefore its role in blood pressure and volume control during pregnancy, a state of sodium retention and hypervolaemia, remains unclear.

In which way does pre-eclampsia differ from normal pregnancy? Pre-eclampsia is characterized by reduction of renal blood flow and glomerular filtration rate, absolute reduction of plasma volume by ~600 ml, and reduced cardiac output [12,17]. Women with severe pre-eclampsia retain more sodium after a saline load, probably because of volume contraction and decreased glomerular filtered load of sodium [17]. In normal pregnancy one observes refractoriness to the pressor action of angiotensin II, such refractoriness is lost [18,19] in pre-eclampsia. Although total sodium and water retention in pre-eclampsia is of the same magnitude or even greater than in normal pregnancy, plasma volume is contracted. This finding suggests a proportionally greater increase of the interstitial fluid space than of the vascular compartment. Redistribution seems to be due to increased capillary permeability [20].

In view of diminished renal excretion of sodium after a saline load, one would expect alterations in secretion of volume related hormones. In fact lower, normal or only moderately elevated levels of plasma renin, and aldosterone are present in pre-eclampsia which are still reactive both to stimulatory (upright position [17]) and inhibitory stimuli (e.g. central hypervolaemia [9–11]). In addition in pre-eclampsia the
dissociation of the physiological relationship between renin and aldosterone secretion is more pronounced than in normal pregnancy (in pre-eclampsia the plasma aldosterone: renin ratio is twice that of normal pregnant women [6]).

The cause of lower plasma renin and aldosterone concentrations in pre-eclamptic women is not clear. Prostacyclin synthesis is reduced in pre-eclampsia [12,15] and prostacyclin is a stimulator of renin secretion. It is likely that prostacyclin is involved in the reduction of renin secretion in pre-eclampsia.

ANP plasma levels in pre-eclamptic women do not differ from those found in normal pregnancy [10,11] although elevated concentrations were also reported [21]. Increase of central volemia induced by water immersion [11] or prolonged lateral recumbency [21] is followed by an increase of plasma ANP level which is less marked than in normal pregnancy [11]. From these studies it follows, that factors other than changes of volemia and atrial stretch are involved in ANP secretion both in normal pregnancy and pre-eclampsia. Thus results on the role of ANP in the pathomechanism of pre-eclampsia are inconclusive.

To summarize in normal pregnancy ECFV is expanded with proportionally greater increase of the intravascular than interstitial fluid space, and GFR and RBF are increased. In pre-eclampsia a similar or even greater amount of sodium and water is retained as in normal pregnancy, but it is located predominantly in the interstitial fluid space. This is due to an increased capillary permeability causing a shift of intravascular fluid into the interstitial fluid space. As a consequence plasma volume is contracted and GFR and RBF are decreased. Although blood volume related hormones (renin, angiotensin II, aldosterone, AVP) are altered both in normal pregnancy and pre-eclampsia, their role in the pathomechanism of water-electrolyte changes taking place during pregnancy is inconclusive.

Recent studies provided evidence of endothelial injury in pre-eclampsia. Presence of increased plasma levels of von Willebrand factor, fibrinectin and thrombomodulin (these factors are markers of endothelial function), decreased synthesis of prostacyclin, but increased production of the vasoconstrictor thromboxane [15] by trophoblastic cells, reduced plasma concentrations of NO and its metabolites [13,22,23] and increased synthesis of vasoconstrictor endothelins (for review see [5]) are consistent with abnormal autocrine and paracrine function of the vascular endothelium in pre-eclamptic women. Imbalance between vasodilatory and vasoconstrictory hormones in favour of vasoconstrictors could influence both vascular permeability and blood pressure and indirectly fluid distribution and balance.

The question arises, which is the primum movens of the above mentioned fluid volumes both in normal pregnancy and pre-eclampsia?

All signs and symptoms of pre-eclampsia disappear after delivery. Thus undoubtedly the utero-fetal unit is instrumental in initiating and maintaining all water-electrolyte alterations noticed in pre-eclamptic women.

Ischaemia of the uteroplacental unit seems to be the central event.

Although recently knowledge of the pathophysiology of pre-eclampsia progressed markedly, better insight into pathomechanisms did have no impact on prevention or therapy of this syndrome. Treatment of endothelial dysfunction may well be required. Administration of endothelin antagonists, inhibitors of the metalloendopeptidase E3,4,11,24 (this enzyme is inactivating ANP), blockers of ANP clearance receptors, promoters of NO, adrenomedullin or prostacyclin synthesis are on the horizon, but whether they will improve the management of pre-eclampsia remains to be elucidated.

References

Is the type of protein in the diet more important than its quantity for slowing progression of chronic renal insufficiency?

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Introduction

The effects of dietary protein on renal function were recognized as early as half a century ago. The issue has recently resurfaced. The past years have seen a debate raging between those nephrologists who claim that the effect of low protein diets on slowing progression of chronic renal insufficiency (CRI) is limited [1–6] according to the results of large-scale intention-to-treat trials [1–4], and those who claim that the effect is limited only because compliance is less than optimal [7–10]. This debate is not purely academic. Its impact goes beyond the patient with CRI, since a lot of money may be saved to the community by postponing the need for dialysis.

If one accepts the hypothesis that reduction of protein intake slows progression of CRI in experimental animals and humans, the logical question arises whether and to what extent protein intake can be safely reduced without adversely affecting the nutritional status or patient compliance.

Is dietary protein restriction effective and safe?

Almost all clinical trials have shown that it is not easy to lower protein intake to less than 0.8 g/kg of ideal body weight over extended periods of time. One should remember that the duration of the pre-dialytic period of CRI may be as long as 10–20 years in some patients. Today nobody knows the level of compliance nor the long-term consequences of strict protein restriction. The published trials are reassuring in this respect, but not sufficient, because they involved only a 2–4 year follow-up. In this context I would like to stress that the dieticians in the clinical centers involved in the MDRD study [4] were all well trained to adjust and monitor protein intake of the patients (whose nutritional education in terms of discriminating the protein content of food items was started in the run in period!). The non-compliant patients were ‘monitored, tracked, and counselled by means of telephone calls and additional visits’ [10]. However, as low protein diets are becoming increasingly used in every day clinical practice, the picture of compliance may be less rosy [5], and this will limit the possibility of slowing progression of CRI safely in such patients [10]. Therefore, the two key questions remain as to how compliance can be improved (i) without adversely affecting the nutritional status, and (ii) while preserving the potential to slow progression of CRI and postponing the need for dialysis.

Are all proteins created equal?

In attempting to solve these problems, research efforts must focus not only on the clinical effects of reducing the quantity of protein intake, but also of modifying its quality. This area has not yet been sufficiently investigated, but a considerable body of evidence suggests that not only the quantity, but also the type of protein ingested may influence their effects on renal haemodynamics. It is well known that vegetarians have a lower glomerular filtration rate than omnivores [11]. This finding is not easy to interpret, however. Not only is the average protein intake lower in vegetarians, but the diet is also different in other important respects. Moreover, it is important to remember that the amount of amino acids present in different diets may not reflect the quantity or pattern absorbed, i.e. their bioavailability. Williams et al. [12] compared the effects of two different dietary proteins on the renal function of normal rats and rats with reduced renal mass. They

showed that subtotally nephrectomized animals which were kept on soya diets had lower glomerular filtration rate and effective renal plasma flow, higher renal vascular resistance, less proteinuria, renal hypertrophy and histological damage, and longer survival than those on casein diets. No differences between the diet were found with respect to growth rates despite the fact that the renal weight of the animals kept on soya diets was significantly lower than that of the animals on casein diets. Differences in the amino acid composition of the diets may have had an effect on the deterioration of renal function: cationic amino acids may block the anionic sites of the glomerular basal membrane and cause proteinuria by disrupting the electrostatic barrier. When a vegetable protein diet is given the reduction in the fractional clearance of plasma proteins suggests that vegetable proteins do not only affect glomerular haemodynamics, but also renal handling of proteins [13].

What are the renal effects of vegetable proteins in humans?

It is important from a clinical point of view that ingestion of vegetable proteins (regardless of their quantity) lowers glomerular filtration rate, renal plasma flow and fractional clearances of albumin and IgG in humans compared with the ingestion of animal proteins. The effect is seen even after short periods of observation [13]. Thus not all proteins are equal in terms of their renal effects. These differences may be partially related to differences in secretion of glucagon and renal vasodilatory prostaglandins [14]. The response of glucagon and renal vasodilatory prostaglandins after a soya meal is blunted compared to the ingestion of a meat meal.

Protein intake versus ketoacid/aminoacid supplements

A very recent secondary analysis of the MDRD study suggests that the trend towards a benefit from the very low diet observed in the intention-to-treat analysis was due more to the lower protein intake (from food and supplement) than the prescription of the very low diet with the ketoacid-aminoacid supplement [10]. These authors also examined the effect of the supplement regardless of achieved protein intake and found that— for a fixed level of protein intake from food only—the assignment to the very low protein intake group was associated with an 86% increase in the risk of renal failure or death at any given point in time. This observation is consistent with a detrimental effect of the amino acid component of the keto acid/amino acid supplement.

The results of this study thus suggest that lower intake of protein but not of supplement may play a role in slowing progression of CRI. This observation points to the importance of looking for alternatives to the simple reduction in protein intake. This idea is also supported by the fact that uraemic patients tend to spontaneously reduce their protein intake because of anorexia.

What we don’t know, but should know

However, many points deserve further clarification. First of all, how much time can be gained in postponing dialysis by restricting, or even better, modifying protein intake? From a clinical point of view, the time gained is unlikely to be very significant, because on the basis of intention-to-treat analysis no major effect was detected in four randomized clinical trials involving hundreds of patients [1–4]. Nevertheless, according to a recent meta-analysis [15], restricted intake of dietary proteins apparently slows progression of diabetic and non-diabetic nephropathy. Furthermore, secondary analysis of the result of the MDRD study [10] suggests that a reduction in total protein intake (including food and supplement) by 0.2 g/kg/day was associated with a slowing of the mean decline in GFR by 1.15 ml/min/year, i.e. by 29%. This would translate into a 41% prolongation of the time to reach end-stage renal failure.

Of course, no data are yet available concerning the long-term effects of a vegetable protein diet. The renoprotective effect of ACE-inhibition has very recently been demonstrated in patients with CRI of various etiologies. A 53% reduction in the risk of progressive renal insufficiency was noted [16]. Obviously it is easier to obtain and maintain lower blood pressure with ACE-inhibitors than to impose demanding dietary restrictions.

Another point that needs to be clarified is the interaction between ACE-inhibition and the total amount of protein intake. On the basis of available data [16] the effects are not additive in terms of slowing progression of CRI, although an additive effect on reduction of proteinuria has been documented [17].

No data are yet available concerning the effects of vegetable protein on progression of CRI at various blood pressure levels. Further, unresolved questions include the following: Are all vegetable proteins equal in their renal effects? And what is their long-term nutritional effect in patients with CRI?

I emphasize that all patients with CRI must arrive at the stage of renal replacement therapy (including renal transplantation) in as healthy a condition as possible. It is ethically unacceptable to delay the start of renal replacement therapy if the patient risks malnutrition [18]. It is estimated that up to 10% of patients with CRI are malnourished at the start of dialysis. Malnutrition is related to shorter survival in dialyzed patients [19].

Conclusion

I conclude that the therapeutic approach of prescribing a vegetable protein diet is fascinating and promising
Preserving the peritoneum in CAPD

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Technique survival in CAPD

It is well established that a significant number of CAPD patients gradually lose their peritoneal membrane function, classically manifest as loss of ultrafiltration and poor solute clearances. After 1 year of CAPD the majority of patients have partial or total disappearance of mesothelium, and the submesothelial tissue becomes fibrous and thickened [1]. This is reflected in the five year technique survival of less than 50% in most published series [2]. Clearly this loss is multifactorial, and infection has been shown to be an important aetiological factor, either as overt clinical peritonitis or as low grade subacute infection. Other factors including chemical agents such as chlorhexidine and acetate dialysate have contributed to peritoneal sclerosis and ultrafiltration failure, but they are no longer used. There remain, however, a cohort of patients in whom no other aetiological factor has been identified except the nature of the technique itself. This has been demonstrated histologically where peritoneal fibrosis can be observed in patients with no previous history of peritonitis, or chemical exposure [3].

The non-physiological nature of dialysate

Many authors have demonstrated how the non-physiological nature of glucose dialysate results in...
alterations in cytokine release in vitro, and hence impairment of peritoneal immunocompetency in vivo when exposed to an infective agent [4,5]. This has been shown to be influenced both by the acidic pH, high glucose, and high osmolality of the solutions. When considering peritoneal fibrosis in the absence of infection there is strong evidence that the same non-physiological features of dialysate influences mesothelial cell injury and fibrosis directly. In addition evidence suggests that the high glucose concentration is a major aetiological factor. High concentrations of glucose are known to suppress mesothelial proliferation and protein synthesis [6], possibly through increased TGF-β gene expression [7], which has independently been shown to suppress mesothelial cell growth and regeneration [8].

Human mesothelial cells produce the extracellular matrix proteins fibronectin, laminin and collagen types I and III [9]. In cultured human mesothelial cells an increasing glucose concentration provokes an increase in both the amount of fibronectin and its messenger RNA [10]. This effect appears to be directly related to glucose concentration and unrelated to increasing osmolality [6]. The production of extracellular matrix protein by mesothelial cells is stimulated by interleukin-1 (IL-1) [11], as is the production of TGF-β [12]. IL-1 was previously shown to be produced by stimulated macrophages and by mesothelial cells themselves [13].

Certain aspects of dialysis with concentrated glucose solutions may produce cell damage by other mechanisms. Peritoneal dialysis solutions are heat sterilized and heat sterilization of commercial glucose-based peritoneal dialysis solutions has been shown to inhibit growth of an in vitro cell culture even when corrected for low pH [14], and has been the subject of a previous editorial [15]. In diabetic patients glucose is known to form abnormal complexes with tissue proteins [16]. Similarly the high glucose concentration in the peritoneal cavity during CAPD results in the complexing of glucose with soluble and structural proteins within the peritoneum. Glucose-containing peritoneal dialysis fluid at the end of a standard dwell and depleted of patient albumin, has been shown to be capable of complexing with supplemented human albumin to form both glycated albumin and advanced glycation end-products (AGE) [17]. In diabetes these AGE are implicated in vascular basement membrane thickening, and could therefore be implicated in peritoneal basement membrane thickening, and progressive peritoneal membrane failure. In addition, exposure to glycated albumin in vitro has also been shown to be directly inhibitory to mesothelial growth and regeneration [18].

Initial studies that exposed confluent peritoneal mesothelial cells to unaltered dialysate for prolonged periods demonstrated considerable cell cytotoxicity [19]. Subsequent studies using lactate dehydrogenase (LDH) release as a marker of cell viability have shown that it is the combination of low pH and high lactate concentration that appears to be toxic to the cultured mesothelial cell [20].

Potential approaches to improve compatibility of the dialysate

Clinical trials comparing a bicarbonate-buffered ‘twin-bag’ dialysate with lactate-buffered dialysate are currently taking place. As the bicarbonate dialysate has a normal or slightly alkaline pH and contains no lactate, the expectation is that biocompatibility will improve. Amino-acid-containing dialysate is now available commercially, and advocated for its potentially beneficial effects on nutrition. In vitro studies, however, are conflicting as to whether they have beneficial effects on mesothelial cell proliferation [21] or have no different an effect from glucose-based dialysate [22]. Amino-acid dialysate may induce less mesothelial cell collagen synthesis than glucose-based dialysate [21], but it may render the cells more susceptible to damage by free radicals [23]. Theoretical concerns over worsening metabolic acidosis from this dialysate formulation appear unfounded in clinical trials, although serum urea does rise [24]. Icodextrin has been recently launched, and has been shown to be safe and effective if used to replace high concentration (3.86%) glucose dialysate for an overnight dwell [25]. In vitro it appears that Icodextrin is less inhibitory to granulocyte and monocyte function, but is little different in its effect on mesothelial cell viability compared with glucose dialysate [26]. However, as with glucose dialysate it less inhibitory if corrected to a physiological pH [4].

Conclusions

In summary, failure of the peritoneal semipermeable membrane cannot be attributed to a single aetiological factor. However, the high glucose concentration and the combination of low pH and high lactate concentration of standard peritoneal dialysis solution are important factors in progressive peritoneal membrane dysfunction. High glucose concentration has been shown to impair cytokine release and cellular immunity, increase production of extracellular matrix proteins, and inhibit cellular proliferation through a variety of different mechanisms. Early studies of non-glucose-containing peritoneal dialysis solutions suggest that they may cause less disruption of cellular mediators [4], and longer-term clinical trials are awaited with increasing interest.

Having used glucose-based peritoneal dialysis solutions for 20 years, is it now time to choose a better alternative?

References

Cyclosporin A-associated hypertension—pathomechanisms and clinical consequences

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Introduction

Cyclosporin A (CsA) has become an integral part of the standard immunosuppressive therapy after organ transplantation although several side-effects seem to limit its use. An increase in systemic blood pressure is the most frequently observed adverse event, and patients after renal and cardiac transplantation [1–4] are at an especially increased risk of postoperative hypertension; this is seen even in liver [5] and lung transplant recipients [6]. However, CsA-associated hypertension is not limited to the transplant popul-
the balance between vasoconstrictor and vasodilator forces might increase blood pressure by sodium retention. Systemic effects of CsA that affect regulation of blood pressure

In patients with myasthenia gravis, CsA treatment increases the sympathetic nerve action potential of the N. peroneus. Furthermore, serum noradrenaline levels and mean arterial blood pressure are reported to be elevated [9], although the altered hormone levels were not found by Kaye et al. [31] in heart transplant recipients. The exact mechanism by which CsA might influence sympathetic nervous tone remains unclear. The suppression of calcineurin-related expressions of immunophilins in specific brain regions might be of importance [32].

Activation of the sympathetic nervous system not only leads directly to vasoconstriction, but also might increase blood pressure by sodium retention. Accordingly, CsA-enhanced proximal renal tubular reabsorptive activity can be reversed by the administration of prazosin, an alpha 1 receptor blocker, which increases sodium excretion in CsA-treated rats [33]. In humans sodium restriction reduces blood pressure in transplant recipients treated with CsA but not in hypertensive patients treated with conventional immunosuppression [34]. However, sodium restriction in these patients cannot be advocated without a note of caution, since it has been shown that a negative sodium balance promotes the development of CsA-related interstitial fibrosis in the kidney. This effect might be mediated by the renin-angiotensin system [35], as Sturrock and co-workers [36] were able to demonstrate that either sodium depletion induced by frusamide or infusion of angiotensin II receptor blockade have been proved beneficial [37,38]. The importance of sodium retention in the pathogenesis of CsA-associated hypertension, however, remains unclear, as administration of CsA for 9 days elevates blood pressure without any effect on sodium balance [19].

In isolated juxtaglomerular cells CsA enhances renin production and secretion [39]; nonetheless, measurement of plasma renin levels in humans, in contrast to...
results obtained in animal studies [40–42] have provided conflicting results [43,44]. Despite these limitations, patients under prolonged CsA treatment exhibit a hyperplasia of the juxtaglomerular apparatus which is reversible once the immunosuppressive regimen has been switched to azathioprine [45]. Furthermore CsA might influence the activity of the renin–angiotensin system independently from the hormonal activity, as it increases the angiotensin II receptor density [46]. Based on our current understanding of the pathophysiological mechanisms of CsA-associated hypertension, sodium retention and the use of diuretics have been advocated [34], although close monitoring of renal function is necessary [34]. Calcium-channel blockers seem to be an attractive alternative because of the primary vasoconstriction induced by CsA [47]. It has been argued that ACE inhibitors will reduce the glomerular filtration rate especially when used in combination with CsA [48] and that these agents will not reduce blood pressure sufficiently. However, this view is dubious because the renin-angiotensin system has been shown to be activated [39,49] not only directly, but also by interaction with for example the sympathetic nervous system, and might exert deleterious effects. In summary, however, one must say that unburdened by potential considerations of pathomechanisms, the single most important issue in a patient with CsA-associated hypertension is to normalize systemic blood pressure.

References


