Protein–RNA interactions in the regulation of PTH gene expression by calcium and phosphate

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Introduction

Parathyroid hormone (PTH) gene expression is regulated transcriptionally by 1,25(OH)2D3, which is able to decrease PTH transcription dramatically [1], and post-transcriptionally by calcium and phosphate [2,3]. The major effect of calcium is exerted by hypocalcaemia which regulates the parathyroid cell at a number of levels and after different time periods of stimulation. Hypocalcaemia increases PTH secretion in the short term, PTH mRNA levels in hours and days, and parathyroid cell number after a more prolonged stimulus [3–5]. The work of Brown et al. has demonstrated elegantly that the parathyroid cell recognizes changes in extracellular calcium by means of a membrane-bound seven transmembrane (7TM) calcium-sensing receptor, leading to activation of inositol triphosphate and mobilization of intracellular stores of calcium [6,7]. How calcium then regulates changes in PTH secretion and synthesis has been an enigma.

Phosphate regulates the parathyroid independently of calcium and 1,25(OH)2D3

We have studied the mechanism of calcium’s effect on PTH gene expression and have shown that in vivo it is post-transcriptional, and we have made observations on the mechanism of this effect. Phosphate also regulates the parathyroid. The contribution of hyperphosphataemia to the pathogenesis of the secondary hyperparathyroidism of chronic renal failure has been documented for many years [8], but it was never possible to separate the effect of hyperphosphataemia from the secondary decreases in serum calcium and 1,25(OH)2D3 [9]. This was established unequivocally first by the work of Kilav et al. in Jerusalem who succeeded in demonstrating that the effect of serum phosphate on PTH gene expression and serum PTH levels was independent of any changes in serum calcium or 1,25(OH)2D3 [2]. In a telling experiment, they bred second generation vitamin D-deficient rats and then placed the weanling vitamin D-deficient rats on a diet with no vitamin D, a low calcium and a low phosphate. After one night of this diet, the serum phosphate had markedly decreased with no changes in serum calcium or 1,25(OH)2D3. These rats with isolated hypophosphataemia had marked decreases in PTH mRNA levels and serum PTH.

Phosphate directly regulates PTH secretion in vitro

To establish that the effect of serum phosphate on the parathyroid was indeed a direct effect, in vitro confirmation was needed and this was provided by three groups. Rodriguez in Cordoba was the first to show that for the in vitro effect of increasing phosphate concentrations to increase PTH secretion it was imperative to maintain tissue architecture [10]. There was an effect in whole glands or tissue slices but not in isolated cells. This effect was soon confirmed by the pioneer and stalwart of research of phosphate on the parathyroid, Slatopolsky and his colleagues in St. Louis [11]. Olgaard’s laboratory in Copenhagen provided elegant further evidence of the importance of cell—cell communication in mediating the effect of phosphate on PTH secretion [12]. In the short period since these studies, there have been a number of studies with parathyroid tissue of different sources and all showing the same result; phosphate regulates the parathyroid directly [13]. In addition, phosphate also leads to marked changes in parathyroid cell proliferation, with hyperphosphataemia increasing and hypophosphataemia decreasing the proliferation [5,14].

How does the parathyroid detect the changes in extracellular phosphate? Miyamoto et al. asked that question in a recent editorial in this journal [15], which was prompted by the recent discovery from their work in Takeda’s laboratory in Tokushima that there is a specific Na–phosphate co-transporter in the parathyroid [16]. Therefore, this flurry of activity in parathyroid laboratories in the last 5 years has shown that
the parathyroid responds to changes in serum phosphate at the level of secretion, gene expression and cell proliferation, but by what mechanism? We can now provide some of the answers for the effect of phosphate and calcium on PTH gene expression.

**Protein–RNA binding to the PTH mRNA 3'-untranslated region and its regulation by calcium and phosphate**

The clearest rat in vivo models for an effect of calcium and phosphate on PTH gene expression are hypocalcaemia with a large increase in PTH mRNA levels and hypophosphataemia with a large decrease in PTH mRNA levels. The first question that Tally Naveh-Man and the students in our laboratory tackled was whether these effects were transcriptional or post-transcriptional. In both instances, they were post-transcriptional, as shown by nuclear transcript run-on experiments. This provided the groundwork for studies to be made on the mechanism of the effect, because post-transcriptional effects often involve protein–RNA interactions. Parathyroid cytosolic proteins were found to bind in vitro transcribed PTH mRNA with three bands at ~50, 60 and 110 kDa. What was particularly interesting was that this binding was increased with parathyroid proteins from hypocalcaemic rats where PTH mRNA levels are increased, and decreased with parathyroid proteins from hypophosphataemic rats, where PTH mRNA levels are decreased. There was protein binding to PTH mRNA with proteins from many tissues, but only with parathyroid proteins was this binding regulated by calcium and phosphate. Intriguingly, the binding was dependent upon the terminal 60 nucleotides of the PTH transcript being present.

**PTH mRNA is degraded in vitro by parathyroid cytosolic proteins and this is regulated by calcium and phosphate**

There is no authentic parathyroid cell line to demonstrate the effect of this protein–RNA interaction on the half-life of PTH mRNA. However, what Naveh-Man and colleagues did was to utilize an in vitro degradation assay to study the effects of hypocalcaemic and hypophosphataemic parathyroid proteins on PTH mRNA stability [3]. In this assay, parathyroid cytosolic proteins of control rats led to the degradation of a radiolabelled PTH transcript at ~40–60 min. Hypocalcaemic parathyroid proteins degraded the transcript only at 180 min, whilst hypophosphataemic parathyroid proteins degraded the transcript already at 5 min. Thus, the in vivo phenomenon is reproduced by the cytosolic proteins in an in vitro assay. Moreover, the rapid degradation of PTH mRNA by hypophosphataemic proteins was totally dependent upon an intact 3'-untranslated region (UTR) and in particular the terminal 60 nucleotides (Figure 1). Proteins from other tissues in these rats were not regulated by calcium or phosphate. Therefore, calcium and phosphate exert their effect on the parathyroid cell to change the properties of cytosolic proteins which bind specifically to the PTH mRNA 3'-UTR and determine its stability. What are these proteins?

**Identification of parathyroid cytosolic protein(s) binding the PTH mRNA 3'-UTR**

Sela-Brown in our laboratory has now utilized affinity chromatography to isolate these RNA-binding proteins. She recently has identified one of the PTH mRNA-binding proteins (50 kDa protein on an SDS–PAGE gel) and demonstrated its functionality [17]. The protein regulates PTH mRNA stability in in vitro degradation assays, and the amount of the protein in the parathyroid correlates with PTH mRNA levels. This preliminary observation should now be supplemented by the identification of the other protein(s) (60
and 110 kDa) which form the protein complex on the PTH mRNA 3'-UTR. It might then be possible to understand how the different messages of changes in serum calcium and phosphate are transduced to the parathyroid cytosol. These proteins then bind to the PTH mRNA 3'-UTR, and in particular the terminal 60 nucleotides, and determine its stability. A stable transcript, for instance after hypocalcaemia, would then be translated into the hormone and available for rapid secretion. An unstable transcript, such as after hypophosphataemia, would be degraded rapidly and less PTH would be translated and secreted.

**Clinical implications for the patient with chronic renal failure**

The clinical complication of secondary hyperparathyroidism in renal failure patients is a vexing problem. We have long known that meticulous control of serum phosphate is the essential first step in the management of these patients, allowing us then to prescribe a vitamin D metabolite which would increase the serum calcium to normal and also have a direct effect on the parathyroid to decrease PTH gene transcription. However, so many patients fail in the arduous and chronic task to take their phosphate binders as prescribed. The net result is hyperphosphataemia and subsequent secondary and tertiary hyperparathyroidism. The studies reported here provide some light on the mechanisms involved and hopefully will lead to therapy designed to target the effects of phosphate on the parathyroid cell.

**References**

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**References**

Endotoxin and renal function: perspectives to the understanding of septic acute renal failure and toxic shock

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Key words: acute renal failure; endotoxin; renal function; sepsis

Introduction

Gram-negative sepsis is commonly associated with acute renal failure (ARF). There is strong evidence that the septic state is due to a combination of direct and indirect effects of endotoxin. Impaired renal function due to endotoxin has been widely reported in both human [1,2] and experimental models of endotoxaemia, sepsis and septic shock.

Endotoxin administration is associated with an array of local and systemic responses with production of different biologically active mediators, many of which are responsible for the impairment of renal blood flow (RBF), glomerular filtration rate (GFR) and tubular dysfunction seen in patients with sepsis. In this review, by discussing these different responses and mediators, we shall try to understand the effects of endotoxin on renal function.

Endotoxin and renal function

Renal blood flow

The altered renal function in endotoxaemia has been attributed predominantly to renal hypoperfusion. However, in experimental models of endotoxaemia, as well as in septic patients, a wide range of values for RBF has been reported (see Table 1) [3]. Differences in models and techniques, nature and amount of endotoxin administered, the severity of shock (hypovolaemia vs hyperdynamic), the compensatory reactions evoked and the extent of concomitant fluid resuscitation may all contribute to discrepancies in RBF between studies [4–20].

The increase in RBF and a decrease in renal vascular resistance may be seen in early stages of sepsis [6] and may reflect the systemic decrease in peripheral vascular resistance or may be primarily intrarenal, an early response to renal vasoconstrictors. This renal vasoconstriction may be mediated by prostaglandins, PGE2 and PG12 [10,15], and/or nitric oxide [23,24], with increased intrarenal activity of the vasodilating kallikrein system [25]. However, even when RBF seems adequate, intrarenal redistribution of flow favours juxtaglomerular areas at the expense of outer cortical flow, especially in the presence of an inadequate effective circulatory volume [8], may have adverse effects on renal function.

Glomerular filtration

Even with decreased RBF, the depression of GFR is greater than any depression of RBF, leading to a reduction in filtration fraction [15,21]. The effects of endotoxin on GFR are probably mediated by extrarenal factors, as infusion of endotoxin into the isolated perfused kidney fails to reduce the GFR [28]. A reduction in filtration fraction is best explained by a reduction in the ultrafiltration coefficient (Kf) and/or a disproportionate decrease in transcapillary hydraulic pressure (Pgc), in relation to blood flow. The reduction in Pgc is related to an increase in the ratio of afferent to efferent arteriolar resistance.

Tubular function

In sepsis, sodium reabsorption is affected in a biphasic manner. Sepsis is among the conditions associated with a low fractional excretion of sodium (FeNa) during ARF. In animal models of endotoxaemia, early retention of sodium and a low FeNa are often reported [21,29]. With the clinical progression of ARF, late sodium wasting with high FeNa is observed [21]. This most likely reflects the onset of tubular damage. This effect of endotoxin was not observed in isolated per-
Table 1. Renal blood flow in sepsis and septic shock.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Model</th>
<th>Technique</th>
<th>Renal Perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermreck et al. (1969)</td>
<td>dogs-septic limb</td>
<td>electromagnetic flow transducer</td>
<td>RBF ↑ ↑ ↓</td>
</tr>
<tr>
<td>Tristani et al. (1970)</td>
<td>septic patients</td>
<td>indicator dilution</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Lucas et al. (1973)</td>
<td>septic patients</td>
<td>PAH</td>
<td>ERPF =↑ ↓</td>
</tr>
<tr>
<td>Ravikant et al. (1977)</td>
<td>piglets-septic limb</td>
<td>microspheres</td>
<td>RBF ↑ =↓</td>
</tr>
<tr>
<td>Cronenwett et al. (1978)</td>
<td>dogs-live pseudomonas</td>
<td>microspheres</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Carroll et al. (1982)</td>
<td>primates-live E. coli</td>
<td>microspheres</td>
<td>PAH =↑ ↓</td>
</tr>
<tr>
<td>Henrich et al. (1982)</td>
<td>dogs-E. coli endotoxin</td>
<td>PAH</td>
<td>ERPF =↑ ↓</td>
</tr>
<tr>
<td>Van Lambalgen et al. (1984)</td>
<td>dogs-E. coli endotoxin</td>
<td>microspheres</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Van Lambalgen et al. (1987)</td>
<td>rats-E. coli endotoxin</td>
<td>microspheres</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Fink et al. (1984)</td>
<td>dogs-E. coli peritonitis</td>
<td>PAH</td>
<td>ERPF =↑ ↓</td>
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<td>Hussain et al. (1985)</td>
<td>dogs-E. coli endotoxin</td>
<td>microspheres</td>
<td>RBF =↑ ↓</td>
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<tr>
<td>Bader et al. (1986)</td>
<td>rats-E. coli endotoxin</td>
<td>electromagnetic flow probe</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Brennet et al. (1986)</td>
<td>septic patients</td>
<td>PAH corrected</td>
<td>RBF =↑ ↓</td>
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<tr>
<td>Breslow et al. (1987)</td>
<td>pigs-E. coli endotoxin</td>
<td>microspheres</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Cumming et al. (1988)</td>
<td>sheep-peritonitis</td>
<td>PAH</td>
<td>ERPF =↑ ↓</td>
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<tr>
<td>Gullichsen et al. (1989)</td>
<td>dogs-E. coli endotoxin</td>
<td>electromagnetic flow meter</td>
<td>RBF =↑ ↓</td>
</tr>
<tr>
<td>Schaer et al. (1990)</td>
<td>primates-E. coli</td>
<td>PAH</td>
<td>ERPF =↑ ↓</td>
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RBF, renal blood flow; ERPF, effective renal plasma flow; PAH, PAH clearance technique; CO, cardiac output; MAP, mean arterial pressure.

fused kidney, suggesting a systemic effect rather than a direct tubular effect of endotoxin [28]. These effects may be mediated by cytokines, hormones or autacoids. Interestingly, interleukin-1, a cytokine released during endotoxaemia, appears to depress sodium reabsorption directly [30].

Mediators in sepsis and ARF

Sepsis is characterized by the release of a vast array of inflammatory cytokines, arachidonic metabolites, vasoactive substances, thrombogenic agents and other biologically active mediators. A large body of experimental data suggests that these various mediators and neuro-endocrine mechanisms are involved in the pathogenesis of renal dysfunction in sepsis. These mediators and mechanisms have important interactions. The inappropriate and uncontrolled release of these mediators and their interactions may play a major role in mediating the systemic and renal haemodynamic responses discussed earlier. Also, as mentioned earlier, since endotoxin has little [31] or no direct [28] effect on isolated perfused kidney, it may be the release of these biologically active mediators that causes much of the renal dysfunction seen during endotoxaemia.

Also, it is noteworthy that in many experimental studies, in addition to the evidence for a crucial role of these mediators in sepsis and renal dysfunction, is the fact that upon antagonism of many of these mediators, significant improvements have been noted in renal function. However, given this impressive spectrum of mediators of renal dysfunction, it is not surprising that few studies have clearly implicated any one agent as being of primarily pathogenic importance in endotoxaemic acute renal failure. The following section, with respect to some important mediators, will touch briefly on areas of relevance in the pathogenesis of renal dysfunction.

Tumour necrosis factor

Tumour necrosis factor (TNF) is a polypeptide, with human TNF containing 157 amino acids with a mol wt of ~17000 Da. TNF has been demonstrated to play a major role in the pathogenesis of Gram-negative shock, mediating a broad spectrum of host responses to endotoxaemia [32]. TNF release is caused by endotoxin [33], and TNF levels are elevated in patients with septic shock, compared with normal, with levels of TNF correlating with the severity of sepsis and mortality rate [34]. In the kidney, endotoxin stimulates release of TNF from glomerular mesangial cells [35]. In another study, passive immunization to TNF was markedly protective against renal cortical damage resulting from intravenous administration of endotoxin to rhesus monkeys [36]. Also, TNF stimulates production of a cascade of mediators, vasoactive and inflammatory, e.g. prostanooids, leukotrienes, nitric oxide (NO) and endothelin-1 (ET-1) [38–43].

On the other hand, in a different study, intravenous infusion of low dose TNF had no effect on systemic haemodynamics and RBF. A higher dose also failed to reduce RBF, but did increase cardiac output and decreased systemic vascular resistance and reduced mean arterial pressure, to some degree [37].

Thus, it seems that TNF may be an important mediator of endotoxin effects, with renal effects of TNF seen in severe sepsis.

Endothelin

Endothelin-1 (ET-1), a 21 amino acid peptide, is an extremely potent vasoconstrictor which has been implicated in the reduction of GFR and RBF that
characterizes septic ARF. Intravenous administration of endotoxin to experimental animals was observed to greatly increase plasma ET-1 concentration [44]. In humans, marked elevations in plasma ET-1 concentration are seen in the setting of sepsis [45]. The most convincing evidence for a role of renal ET-1 in the pathogenesis of septic ARF is the observation, that endotoxin-induced renal hypofiltration is prevented by intrarenal infusion of anti-ET-1 antibodies [46]. In this study, infusion of anti-ET-1 antiserum into the left renal artery prevented systemic endotoxin-induced hypofiltration on that side, but did not affect the reduction in GFR in the contralateral control kidney.

**Thromboxane A2**

Thromboxane A2 (TXA2) is the major vasoconstrictor product of the cyclooxygenase pathway. It is a potent platelet activator and promotes intravascular platelet aggregation and thrombosis. Administration of a TXA2-like stable prostaglandin H2 (PGH2) analogue, U-44069, to the isolated perfused rat kidney results in renal vasoconstriction accompanied by a severe reduction in filtration fraction, suggesting a predominant pre-glomerular action [47]. In addition to its own intrinsic renal vasoconstrictor actions, TXA2 appears to mediate renal vasoconstrictor responses to platelet-activating factor (PAF), an important pro-inflammatory lipid [48].

Endotoxin is a potent stimulator of TXA2 synthesis in renal cortex, as well as in extrarenal tissues [15,49–51]. Numerous reports have provided evidence for a role for TXA2-induced renal vasoconstriction in models of experimental sepsis [15,49–51]. In these studies, selective antagonism of the actions of TXA2 was shown to afford greater protection against renal ischaemia than generalized, cyclooxygenase inhibition (such as with aspirin), which concomitantly inhibits the formation of intrarenal vasodilator prostaglandins [15].

**Leukotrienes**

In activated leukocytes, arachidonic acid may undergo oxygenation to form a family of highly potent vasoconstrictor eicosanoids through the action of the 5-lipoxygenase enzyme and subsequent enzymatic addition of glutathione. The resulting products (sulfidopeptide leukotrienes (LTs)) were collectively known as the ‘slow-reacting substance of anaphylaxis’ and are composed of LTC4, LTD4 and LTE4. Systemic administration of LTC4 and LTD4 in rat leads to reduction in RBF and GFR [52,53]. During endotoxic injury, production of these leukotrienes is enhanced [54,55]. Also, in studies using a relatively non-specific LT antagonist [15], and subsequently using a specific antagonist of the LTD4 receptor [56,57], salutary effects on the reduction in RBF induced by systemic administration of endotoxin, were noted.

**Other mediators**

Adenosine, PAF, NO, interleukin-1, the renin–angiotensin system, the kallikrein–kinin system and quite a few other mediators and neuro-endocrine systems have been implicated in the pathogenesis of sepsis and renal dysfunction associated with sepsis. These mediators directly or indirectly, by their effects on other mediators, may be responsible for the different effects of endotoxin.

**Non-haemodynamic responses to endotoxin**

Endotoxin, together with the above noted mediators, is believed to activate the complement systems (again releasing vasoactive and chemotactic factors), the coagulation and fibrinolysis cascades [58]. Moreover, circulating leukocytes are activated and the function and structure of the vascular endothelium changes [58] with changes in the production of mediators such as NO, ET-1 and others mentioned above [45,59]. There is evidence that in patients with ARF and sepsis, leukocyte activation is greater than in patients with ARF from other causes [60]. In the isolated rat kidney, perfusion with endotoxin-activated leukocytes resulted in a reduction in glomerular filtration and sodium excretion [61].

Under certain circumstances, endotoxin given at intervals or even during continuous infusion in rodents may induce overwhelming and widespread intravascular coagulation in both kidneys and extrarenal organs, the so-called generalized Shwartzman reaction, during which cytokine release, activation of complement and coagulation systems play an important role [25,62,63]. This induces intrarenal microembolization of fibrin and platelets, and renal cortical necrosis [63]. The Shwartzman reaction may mimic cortical necrosis in patients, a rare complication of sepsis and diffuse intravascular coagulation in man, except when caused by meningococci [25,62,63].

**Conclusion**

The association between sepsis, endotoxaemia and renal failure has been suspected for several decades. During this period, numerous direct, secondary and tertiary effects of endotoxin have been identified. Endotoxin seems to release a vast array of mediators, stimulate several compensatory systems, e.g. the adrenergic system and the renin–angiotensin system (not discussed here), all of which, in more than one capacity, have major affects on RBF, the determinants of GFR and tubular function (see Figure 1). Also, as noted, there is increasing evidence that similar mechanisms are involved in human septic shock. Experimental studies are beginning to unravel the complexities of the systems involved. It seems that the balance between appropriate production and overproduction of these mediators, the balance between vasoconstriction and vasodilating systems and other important balances that
Fig. 1. Potential mechanisms and their interactions in endotoxin-mediated renal dysfunction.

characterize these complexities will ultimately determine the host’s eventual response and its effect on renal function.

References


Editor’s note
Please see also the Molecular Basis of Renal Disease article by Haeffner-Cavaillon (pp. 853–860 in this issue).
Importance of vitamin D repletion in uraemia

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Introduction

In an editorial in Nephrol Dial Transplantation, Cunningham and Makin [1] discussed some basic issues regarding vitamin D deficiency in uraemia. Based on some recent findings, we address this issue again and try to answer the following questions: (i) Which is the most appropriate definition of hypovitaminosis D?; (ii) What is the prevalence of hypovitaminosis D in uraemia?; (iii) Is calcitriol the sole active vitamin D metabolite?; and (iv) Does the plasma concentration of calcitriol [1,25(OH)2D3] depend upon the plasma concentration of 25(OH)vitamin D3?

25(OH)D3 has a much longer half-life than native vitamin D, i.e. 2–5 weeks vs several hours. Its plasma concentration is, therefore, much better suited to provide an integrated assessment of the vitamin D status. The problem is to define its ‘normal’ values. In principle, two approaches are conceivable: the statistical approach and the pathophysiological one.

The statistical approach consists of measuring plasma 25(OH)D3 concentration in a large sample of ‘normal’, i.e. healthy, people. The ‘normal range’ is defined as mean ± 2 SD for values with a Gaussian distribution or median and 10th–90th percentile for values with a non-Gaussian distribution.

This approach will define a lower limit, that will vary with the vitamin D status of the reference population, which in turn depends on its sun exposure and dietary intake of vitamin D (i.e. the amount of dairy products, eggs, fatty fishes and meat) [2–4]. That in the studies of post-menopausal women, renal 25(OH)D3 concentration of 37.5 nmol/l [3] defined as mean ± 2 SD for values with a Gaussian distribution or median and 10th–90th percentile for values with a non-Gaussian distribution.

Several authors have used this approach and produced different definitions of what represents different degrees of severity of hypovitaminosis D. In decreasing severity, these comprise (see also Figure 1): (i) severe hypovitaminosis D (or vitamin D deficiency): plasma 25(OH)D3 concentrations < 8 ng/ml, i.e. 20 nmol/l [5]; or 10 ng/ml, i.e. 25 nmol/l. Lower values are usually associated with histological evidence of osteomalacia [6]; (ii) moderate hypovitaminosis D (vitamin D insufficiency): plasma 25(OH)D3 concentration < 12 ng/ml, i.e. 30 nmol/l, because these levels in neonates [7] or in post-menopausal women [8] are associated with increased PTH levels. This threshold is close to that proposed by the Harvard group [9] [15 ng (or 37.5 nmol/l)] on the basis that it corresponded to the mean – 2 SD of the September values in the US reference population and was associated in their population of medical in-patients with high intact PTH concentrations; (iii) mild hypovitaminosis D (vitamin D insufficiency) could be defined by plasma 25(OH)D3 concentrations < 20 ng/ml, i.e. 50 nmol/l, the threshold below which the calcitriol levels become dependent upon those of 25(OH)D3 in a normal population, explaining the seasonal variation of calcitriol [10].

Apart from considerations on the methodology of the 25(OH)D3 measurement, we draw attention to the fact that the definition of hypovitaminosis D described above strictly applies only to individuals without renal insufficiency. In the latter condition, the appearance of osteomalacia is favoured by multiple factors other than low plasma 25(OH)D3, such as aluminium load, hypocalcaemia and metabolic acidosis, whereas hyperparathyroidism is favoured also by hypocalcaemia, metabolic acidosis, hyperphosphataemia and low levels of 1,25(OH)2D3, but on the contrary is suppressed by hyperaluminaemia. It should be pointed out, however, that in the studies of post-menopausal women, renal function was considered normal relatively to age, i.e. actually lower than in younger adults [8].

It is also likely that dietary intake of calcium is a confounding factor which can influence the threshold of plasma 25(OH)D3 taken for the definition. At a lower intake of dietary calcium, the plasma concentration of 25(OH)D3 should be higher. For example, to suppress the seasonal variation of hip bone density in post-menopausal Bostonian women [11], whose calcium intake was only 750 mg/day, a plasma 25(OH)D3 of 100 ± 10 nmol (40 ng/ml) was necessary, whereas in the Netherlands, where post-menopausal women had a calcium intake > 1100 mg, a plasma 25(OH)D3 of > 30 nmol was sufficient [8].

Considering that taking the threshold of 100 nmol/l to define mild vitamin D insufficiency would lead to considering the majority of our Western population as having hypovitaminosis D throughout the year with the exception of late summer [12], we do not recom-

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Fig. 1. Plasma 25 OH vitamin D range useful for definitions of vitamin D status. Epidemiological range of P 25 OH vitamin D concentration in late summer and range of P 25 OH vitamin D for (1) possible occurrence of radiological osteomalacia, (2) abnormal increase of intact PTH in ‘healthy’ controls (often post-menopausal women with physiological reduction of GFR), (3) dependency of calcitriol and (4) of mineral density upon P 25 OH vitamin D. In healthy controls these ranges are used to define vitamin D deficiency [in relation to (1)] and vitamin D insufficiency (or hypovitaminosis D) of decreasing severity (in relation to criteria 2, 3, 4) (a) higher Ca diet decreases this range; (b) with aluminium intoxication osteomalacia occurs even with vitamin D repletion in uraemic patients (c) in individuals with physiological kidney function.

Evolution of vitamin D status in individuals with physiological kidney function:
- deficiency
- insufficiency

Epidemiological range in late summer:
- USA
- Southern Germany
- Paris

Range of P 25 OH vit D for occurrence of:
(1) [a,b] radiological osteomalacia
(2) [a] P "intact" PTH > above upper limit of normal
(3) [b] calcitriol dependence upon P 25 OH vit D
(4) [a] Seasonal variation of hip density of post-menopausal women

Prevalence of hypovitaminosis D in renal insufficiency

For the purpose of this discussion, we shall adopt the somewhat arbitrary threshold of <15 ng/ml, i.e. 37.5 nmol/l [9].

A cross-sectional study demonstrated a high prevalence of hypovitaminosis D (57%) in a medical in-patient population in Boston; dialysis and nephrotic syndrome were the two variables most strongly associated with hypovitaminosis D [9]. A high frequency of low 25(OH)D3 concentrations had also been found in pre-dialysis and dialysis patients in the past by Eastwood [13] in London and, not unexpectedly, hypovitaminosis D was also common in healthy controls studied during winter. Two studies from France, one in Toulouse [14] and one in Picardy [15], confirmed this observation. Even in sunny Algeria, we found that the median plasma 25(OH)D3 concentration during September was relatively low, i.e. 47.5 nmol/l (range 2.5–170) and the prevalence of hypovitaminosis D was 42% [16].

These studies document that hypovitaminosis D is common in the general population and even more common in elderly renal patients. Renal disease may affect vitamin D status by several mechanisms, i.e. altered life style with reduced sun exposure, loss of vitamin D metabolites together with the vitamin D-binding protein in nephrotic proteinuria or peritoneal dialysis fluid, decreased intrinsic skin capacity to synthesize cholecalciferol [17,18]. Calcium deficiency, common in renal failure, aggravates vitamin D insufficiency by accelerating hepatic catabolism of 25(OH)D3 through secondary hyperparathyroidism and increased synthesis of calcitriol [2,16].

Is calcitriol the only physiologically active metabolite of vitamin D?

When 25(OH)D3 and calcitriol are compared in terms of capacity of binding to the vitamin D receptor (VDR), the molar ratio is 1:2400. Since the plasma concentration ratio of 25(OH)D3 to calcitriol is 1000:1, a physiological role for 25(OH)D3 has long been regarded as unlikely. There are functional systems, however, such as the vitamin D-responsive element-dependent transcription of the growth hormone gene in cultured cells, where the efficiency ratio is 1:500, suggesting that circulating 25(OH)D3 could actually contribute to the overall vitamin D effect on intestinal calcium absorption or PTH secretion [1]. Furthermore, not all effects of vitamin D metabolites are mediated via the VDR and 25(OH)D3 may also...
act through VDR-independent pathways [19,20]. Heaney et al. [21] recently showed that the molar potency ratio comparing 25(OH)D₃ and calcitriol was 1:100 when the effective dose to produce a 25% increase of calcium absorption in healthy vitamin D-replete humans was assessed. It was calculated that plasma 25(OH)D₃ could account for one-eighth of the vitamin D effect.

These results raise the possibility that physiological plasma concentrations of 25(OH)D₃ act to suppress PTH synthesis and secretion. This is suggested by an inverse correlation between plasma concentrations of 25(OH)D₃ and PTH, independent of calcitriol, in patients with hip fractures [22] and in Algerian dialysis patients [16]. In this latter group, low plasma 25(OH)D₃ concentrations were also the only biochemical difference distinguishing patients with and without Looser zones. This possibility deserves serious consideration, although such cross-sectional data do not directly prove a direct effect on PTH secretion and could conceivably be explained by other mechanisms, such as displacement of bound calcitriol from the vitamin D-binding protein [23] or specific action of metabolites derived from 25(OH)D₃ other than calcitriol, such as 24,25(OH)₂D₃ [24–29].

It is also of interest that low doses of 25(OH)D₃, i.e. 10–25 μg/day, cannot only cure osteomalacia in pre-dialysis patients with vitamin D deficiency (i.e. plasma 25(OH)D₃ concentrations <25 nmol/l [28]), but can also suppress PTH concentrations and improve histological osteitis fibrosa [15].

**Are the plasma concentrations of 1,25(OH)₂D₃, (the product) dependent upon the concentration of 25(OH)D₃, (the precursor)?**

In healthy controls without renal failure, we have seen that plasma 1,25(OH)₂D₃ concentrations are dependent upon plasma 25(OH)D₃ only when these latter are <50 nmol/l [10]. When these controls are replete so as to achieve plasma 25(OH)D₃ concentrations between 50 and 150 nmol/l, the interconversion of the precursor 25(OH)D₃ into the product 1,25(OH)₂D₃ by renal 1-α-hydroxylase is tightly regulated and plasma 1,25(OH)₂D₃ does not increase any more.

In patients with renal failure, a correlation between plasma 25(OH)D₃ and plasma 1,25(OH)₂D₃ concentration was noted only by Lucas et al. [33,34] only when the range of plasma 25(OH)D₃ included values <50 nmol/l [33,34]. In an Algerian haemodialysis population [16] in which plasma 25(OH)D₃ concentration ranged spontaneously from 2.5 to 170 nmol/l, a good correlation was found between plasma 25(OH)D₃ and plasma 1,25(OH)₂D₃ concentrations. When plasma 25(OH)D₃ concentrations in haemodialysis patients are increased pharmacologically up to 300 nmol/l, plasma 1,25(OH)₂D₃ further increases proportionally [30]. Surprisingly, an increase of plasma 1,25(OH)₂D₃ concentration upon such administration of 25(OH)D₃ (or vitamin D₃) is seen even in anephric patients [31]. This observation suggests that 1,25(OH)₂D₃ is produced by extrarenal tissues. The most likely candidate is the monocyte–macrophage system which, when activated by contact with bioincompatible dialysis membranes, expresses an 1-α-hydroxylase activity [32].

**What is the optimal range of plasma 25(OH)D₃ in renal failure to prevent renal osteodystrophy?**

As proposed by Cunningham and Makin [1] and by Fournier et al. [15], respectively administration to uraemic patients of 1000–2000 U per day of the parent vitamin D (D₂ or D₃) or of 20–30 μg of 25(OH)D₃ will maintain their plasma 25(OH)D₃ concentrations close to the upper limit of the reference population, with few if any side effects. These replacement doses are far from the pharmacological doses which have been used in the past: 1 mg of vitamin D₂ (40 000 IU/day) or 100–500 μg/day of calcidiol to increase calcium absorption [35] or 50–100 μg/day to improve osteitis fibrosa [29]. These therapeutic strategies are obsolete today because of the risk of prolonged hypercalcaemia resulting from the long half-life of 25(OH)D₃.

In healthy elderly patients, a supplement of 800 IU cholecalciferol (20 μg of vitamin D₃) increases the plasma 25(OH)D₃ concentration from 40 to 100 nmol/l. Plasma calcium increases only minimally (0.01 nmol/l), but plasma 1,84 PTH decreases significantly, i.e. from 54 to 30 pg/ml, and a significant reduction in the risk of hip fractures is noted [36]. In view of these findings, the above daily dose of cholecalciferol recommended by Cunningham in renal failure makes sense, more so because this dose does not cause an increase in calciuria in pre-dialysis patients nor hypercalcaemia, since these latter complications are seen only when daily doses are in excess of 2400 IU or 60 μg/day [37], inducing plasma 25(OH)D₃ concentrations >200 nmol/l. Based on the work of Chapuy et al. [38], one can expect that a supplement of 1600 IU of cholecalciferol increases the plasma 25(OH)D₃ concentration from 40 to 160 nmol/l.

Cross-sectional studies in dialysis patients in Algeria [16], France [39] and Romania [40] suggest that a higher plasma 25(OH)D₃ concentration, renal osteodystrophy is prevented. In the Algerian study, no Looser zones nor subperiosteal resorptions were seen when plasma 25(OH)D₃ concentrations were >40 nmol/l and 100 nmol/l, respectively. This observation is all the more impressive because hypocalcaemia, hyperphosphataemia and metabolic acidosis persisted, and that the PTH-suppressive effect of aluminium overload, although present, was mild since the plasma levels of aluminium were <1.5 μmol/l. Indeed with a plasma 25(OH)D₃ ≥ 100 nmol/l (but <170 nmol/l), the plasma level of intact PTH was <25 pmol/l (the upper limit of the optimal range for a population exposed to aluminium). In the population of Amiens (never exposed to aluminium for 16 years and for whom the PTH upper limit for prevention of increased bone
formation rate is therefore lower (11 pmol/l), plasma 25(OH)D$_3$ concentrations >120 pmol/l were required to maintain the PTH values below this optimal limit. In this population, 69% of the patients had plasma PTH <25 pmol/l without any administration of 1α-hydroxylated vitamin D compounds.

In pre-dialysis patients, an average daily dose of 20 μg of 25(OH)D$_3$ increases the plasma 25(OH)D$_3$ concentration from an average of 34 to 75 pmol/l. At least for an observation period of 2 years, this measure was sufficient (in association with 3 g of CaCO$_3$ taken as phosphate binder) to prevent hyperparathyroidism and to improve osteitis fibrosa, whereas hypercalcaemia was not seen but plasma phosphate increased from 1.13 to 1.45 mmol/l in parallel with an increase in plasma creatinine from 300 to 440 μmol/l [15]. Such a beneficial effect contrasts with persistently elevated plasma 1,25PTH values, despite correction of hypocalcaemia, in patients who received 0.25–1 μg/day of 1α-calcidol (but no calcium) for 2 years.

**Conclusion**

Undoubtedly low concentrations of plasma 25(OH)D$_3$ are pathophysiologically relevant in uraemic patients, which justifies plasma 25(OH)D$_3$ monitoring. Observational studies show a link between low plasma 25(OH)D$_3$ concentration and hyperparathyroidism and Looser zones respectively, independently of plasma calcitriol levels. We acknowledge that such correlations do not prove causality. Nevertheless, we feel that repletion of vitamin D to achieve optimal plasma 25(OH)D$_3$ concentrations is a legitimate therapeutic strategy. This can be achieved by systematic daily administration of 1000–2000 IU of cholecalciferol or 20–30 μg of 25(OH)D$_3$. This recommendation probably will be even more justified when the use of the novel calcium–aluminum-free phosphate binders such as sevelamer hydrochloride (Renagel®) spreads, because they bind biliary salts and decrease plasma cholesterol, and therefore possibly interfere with the vitamin D enterohepatic cycle.

**References**


**Nephrol Dial Transplant (1999) 14: Editorial Comments**
Standard precautions in haemodialysis—the gap between theory and practice

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Del dicho al hecho hay gran trecho (prevention—easier said than done) Introduction

There is evidence that haemodialysis entails a high risk of exposure to blood-borne viruses. Until the mid 1970s, the most important hygienic problem in haemodialysis units was hepatitis B virus (HBV) [1]. After it had come under control, hepatitis C virus (HCV) became the most prevalent viral infection causing the well-known associated clinical sequelae [2,3]. The nosocomial mode of transmission has apparently become the most frequent mode of transmission on dialysis [4], since transmission of HCV via transfusion has been practically eliminated thanks to serological monitoring of blood donors and diminution of transfusion requirements after introduction of erythropoietin. The magnitude of the risk of transmission by health care personnel in dialysis units is unknown, but should be small if standard precautions recommended by the Centre for Disease Control (CDC) are adhered to [5,6]. Hand washing and use of gloves are the most simple, but also the most important, methods—assuming that they are implemented. This raises the following question: are such measures actually adhered to in haemodialysis units? On which occasions are such precautionary measures necessary? Which measures are most frequently adhered to in clinical practice? Which measures are most frequently neglected?

Nosocomial transmission of HCV in haemodialysis units

Taking HCV as an example, several observations argue that nosocomial transmission is the principal mode of infection. On the one hand, some patients with HCV...
had never received a blood transfusion, and this is true for up to 40% of seropositive patients in some units [7]. The prevalence of HCV-positive patients on haemodialysis is higher than for patients maintained on other treatment modalities, e.g. home haemodialysis and peritoneal dialysis [8,9]. Sampietro et al. [10] and other authors [11] studied HCV-positive patients dialysed in one haemodialysis unit and analysed the 5′-region of the viral genome which is highly conserved. In this population, one type of virus which was rare in the general population, was most frequent, suggesting nosocomial transmission. Isolation of HCV-positive patients effectively reduced the incidence of HCV infection in haemodialysis units [12,13]. This observation is compatible with the notion that the haemodialysis technique itself provides a mode of viral transmission.

Transmission of HCV by haemodialysis is controversial, and the data on this point are contradictory. The dimensions of the virus particles and the dialysis membrane characteristics make this mode of transmission impossible on theoretical grounds, at least in the absence of membrane rupture. In a study performed in our unit, we found an association between certain dialysis machines and infection by HCV [3], but attempts to isolate HCV in the ultrafiltrate remained unsuccessful (unpublished data) as was also found in most other studies [14,15]. Only some authors noted passage of viral particles into the ultrafiltrate when very high transmembrane pressures were applied and microleaks possibly occurred [16]. Probably the most important mode of transmission which, unfortunately, is the most difficult to document and control, is patient–patient transmission either directly or indirectly by contaminated surfaces.

**Standard precautions**

The recommendations concerning prevention and control of HCV and human immunodeficiency virus (HIV) infection have been laid down in specific protocols, which have been disseminated widely [5]. These recommendations are based on the systematic implementation of ‘universal precautions’ which currently are part and parcel of the concept of ‘standard precautions’ [6]. They have two goals, to avoid transmission of the microorganisms first from patient to health care personnel and second from health care personnel to patients.

The ‘standard precautions’ basically comprise four points. (i) washing of hands and cutaneous surfaces after contact with blood and other body fluids and after removal of gloves, (ii) utilization of gloves during contact with blood, body fluids, secretions, excretions and contaminated material, (iii) utilization of other barrier methods (masks, protective glasses and clothes, etc.) during patient care which can easily lead to splashes and aerosol formation; and (iv) adequate cleaning, disinfection and sterilization of all reutilized material.

**Activities in haemodialysis which necessitate precautions (hand washing and gloves)**

In all activities which lead to direct contact with blood products, it is necessary to wear gloves and to wash hands before and after said activities. Which recommendation should be made for the different dialysis-related activities? Hand washing is necessary when dialysis material is made ready for use; hand washing is also necessary whenever the patient is connected to and disconnected from the machine, particularly for dressing of the puncture site and, furthermore, whenever the patient requires attention in the course of the dialysis session and the blood line is manipulated. It is not necessary to wash hands before used material is disposed of and before the room is cleaned, but gloves must be worn for these activities and hands must be washed after the activities are completed.

**Are standard precautions adhered to in haemodialysis units?**

It is necessary to stress that it is important that the staff handles adequately all material which is contaminated with blood of HCV-positive patients in order to avoid dissemination of the virus in dialysis units. It is obligatory to wear gloves and to wash hands before and after handling blood lines or potentially infectious material. Furthermore, it is necessary to clean bloodstains carefully and not to use leftover material that has been in contact with the patient. Although these rules are mandatory, unfortunately they are not always adhered to.

These proposals are apparently simple but when one monitors whether they are adhered to or not, a high degree of non-compliance by health care personnel is noted. This corresponds to what has been found in intensive care units by others too [17,18].

**Isolation or meticulous application of standard precautions?**

Isolation of infectious patients in separate units interrupts potential chains of transmission and eliminates nosocomial infection, as documented in several studies [12,13]. However, the issue remains whether such costly measures are obligatory or whether strict application of standard precautions is sufficient. Some authors, e.g. Gilli et al. [19], advocate no separation of infectious patients, but strict application of universal precautions, because a similar reduction in the incidence of hepatitis C is seen with and without isolation. Jadoul et al. [29] also advocate strict application of standard precautions as a way to control infection in the haemodialysis unit. Isolation is of proven efficacy for controlling infection. In certain circumstances, it may still be necessary to adopt this measure (units with very high prevalence or control of outbreaks of hepatitis C). This issue raises economic as well as
logistic problems. It is difficult to know on which criteria the separation of patients should be based: with the available methods, it is difficult to identify the highly infectious patient. Because of their immuno-suppressed state, some dialyzed patients do not have increased transaminases; such patients have no antibodies but are PCR-positive; in some patients, PCR becomes negative after treatment with interferon, but one cannot exclude the possibility that they turn positive again some time after cessation of treatment.

**Perspectives**

It is necessary to consider why adherence to standard precautions by the health care personnel in dialysis units is so low. Although we do not have quantitative data, generally health care personnel are apparently more concerned about the possibility of infection by the patient. In contrast, they tend to disregard their role in transmitting HCV to the patient [21]. We believe that this explains why the use of gloves was the measure which has been adhered to most frequently, followed by hand washing after (and less frequently before) manipulations [22]. Another factor which contributed to non-compliance is the time factor: staff have to work in a hurry because the shifts closely follow each other and because the timetable has to be strictly adhered to. The poor compliance of staff with standard precautions obliges us to give instructions on the mechanism of transmission of HCV in dialysis units and its potential prevention repeatedly on different occasions. Staff must be made conscious of the importance of systematic adherence to preventive measures. This simple measure could diminish the incidence of hepatitis C and would also have the advantage of being cheap. The impact of instructions given to health care personnel must be evaluated, i.e. adherence to recommendations before and after instruction and the impact of instruction on the incidence of HCV infection.

**Acknowledgement.** We thank Professor E. Ritz for translating the text.

**References**

20. Jadoul M. Transmission routes of HCV infection in dialysis units. We thank Professor E. Ritz for translating the text.

**Editor’s note**

Please see also the Clinical Observation article by Arenas Jiménez (pp. 1001–1003 in this issue).