and transcription factors, and altered levels would not affect the vitamin D system exclusively. A recent report demonstrated reduced RXR levels in hyperplastic parathyroid glands [12] of uremic rats which could reduce the VDR-mediated inhibition of PTH gene expression and contribute to PTH overproduction in this disorder.

Summary

The control of gene transcription by vitamin D compounds is initiated by binding to the VDR, which enhances the receptor’s ability to heterodimerize to RXR, interact with response elements in target genes and attract components of the transcriptional initiation complex. A number of factors are capable of influencing this process, including (i) the rate of uptake and catabolism of the ligand, (ii) the nature of the conformational change induced by a specific ligand, (iii) the cellular content of the VDR, (iv) post-translational modifications of the VDR and (v) the availability of other transcriptional components. Vitamin D analogues may affect these factors differently to 1,25(OH)2D3 to produce unique biological profiles that can be exploited for therapeutic use.

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Molecular mechanisms of vitamin D hyporesponsiveness in renal failure

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Key words: calcitriol, uraemia, vitamin D

Introduction

Calcium homeostasis depends on two major hormone systems. Parathyroid hormone (PTH) and calcitriol (1, 25(OH)2D3), the active form of vitamin D, interact to balance calcemia; they influence intestinal calcium uptake, renal calcium losses, and calcium exchange at the bone. Calcemia itself influences PTH secretion.

In the classical model[1] both a rise in blood calcium and calcitriol cause a negative feed-back on PTH release, whereas both PTH and calcitriol cause a rise in blood calcium. PTH also increases an inducer of calcitriol.

In renal failure this subtle balance is disturbed, when active kidney mass is lost; the capacity of the kidney to produce calcitriol from precursors by the activity of the enzyme 1α-hydroxylase is reduced in parallel with renal function. Consequently, serum calcium and calcitriol decrease, parathyroid hormone secretion is enhanced, and a new equilibrium is found, however,
at the expense of a loss of the calcium content in the bone. The end-point is hyperparathyroidism; the degree of this PTH hypersecretion may, however, largely differ from patient to patient, and within the same patient, from time-point to time-point in his/her evolution.

This imbalance is further affected by phosphate; renal failure causes phosphate retention, which in turn causes serum calcium to decrease, with the above-mentioned consequences on calcitriol and PTH. In addition, phosphate itself also decreases calcitriol and increases PTH directly or indirectly [2].

Curiously enough, several studies point to the fact that at the initiation stage of chronic renal failure, without supplementation of vitamin D analogues, calcitriol levels remain normal; in spite of this, PTH is elevated [3]. This data suggests the development of a certain degree of vitamin D resistance, as both the metabolic degradation of calcitriol and the inhibition of PTH secretion are regulated by calcitriol. These findings have, however, not consistently been confirmed [2,4], but this might be attributed to differences in patient selection, e.g. with regard to kidney function.

Several additional arguments point in the direction of calcitriol resistance in renal failure. Already in association with moderate defects of renal function, intestinal absorption of Ca$^{2+}$ is decreased and whole-body retention of Ca$^{2+}$ is increased [5]. In response to increasing supplements of calcitriol, urinary calcium remains lower in early renal failure, compared to healthy subjects [3]. Also in rats with moderate renal failure (reduction of GRF <50%), PTH increases compared to sham-treated rats with normal renal function, in spite of identical serum calcitriol levels [6].

All these data suggests that the response to calcitriol is depressed, even if the fall in calcitriol levels normally occurring with renal failure can be prevented. Calcitriol exerts its biological actions through binding with the calcitriol receptor, which has two functional domains, one hormone binding and one DNA-binding. The DNA-binding domain has similarities to that of other steroid hormone receptors (e.g. glucocorticoid, oestrogen, thyroid, and retinoic acid receptors). After complexation of calcitriol with the vitamin D receptor (VDR), binding occurs with the DNA of vitamin-D-responsive elements (VDREs—e.g. osteocalcin, osteopontin, 9K calcium binding protein gene), which are present in vitamin D responsive genes.

Impaired response to vitamin D at the receptor level could be the consequence of: (i) decreased binding of calcitriol to the VDR; (ii) decreased expression of the VDR; (iii) decreased binding affinity of the vitamin D–VDR complex to the VDREs (Figure 1).

What is the evidence that one or more mechanism play a role in uraemia? If this were the case, hyperparathyroidism would not be prevented, even if adequate calcitriol levels can be obtained.

**Decreased binding of calcitriol to the VDR**

This issue has been studied repeatedly. In none of these studies could a decrease of binding affinity be demonstrated.

**Decreased calcitriol receptor content**

It is of note that under normal conditions calcitriol upregulates its own receptor, i.e. increasing serum calcitriol induces an increase of VDR. VDR content in uraemia has most frequently been studied in parathyroid glands. A decrease in VDR of parathyroid glands has been demonstrated in the rat [7], the dog [8], and in men [9]. Similar data were also reported for human peripheral blood mononuclear cells [10] and rat intestine [11].

Szabo et al. could not confirm this decrease in VDR expression in parathyroid glands of uraemic patients [12]. The reason for this inconsistency remains unclear. One of the reasons could be that in the studies by Szabo et al., protease inhibitors were used in the experimental set-up, preventing proteolytic degradation of the receptor during its preparation from the uraemic sample. This approach had not been followed in earlier studies. However, later studies, where proteolysis was prevented in a similar way as in the study by Szabo et al., nevertheless also demonstrated a decrease of VDR [7].

Another possible explanation for these divergences, is that the expression of VDR depends on the type of parathyroid hyperplasia, which may be nodular or diffuse. Hyperplastic parathyroids of the nodular type contain substantially less VDR than glands of the diffuse type [13].

Even if the parathyroid glands from the experiments by Szabo et al. contain a similar number of VDR, with or without renal failure, the normal upregulation in response to calcitriol is inverted in the uraemic
condition [12]. Parallel data were reported by Koyama et al. in duodenum of rats with chronic renal failure [14]. Several authors have studied the levels of VDR mRNA in renal failure, but a decrease could not be demonstrated [12,14,15]. Also the changes in VDR mRNA in response to calcitriol administration were similar in the uremic and in the normal condition. Infusion of uremic ultrafiltrate to rats resulted in a rise of VDR mRNA in spite of a fall in VDR [15]. These data suggest that uremic toxins inhibit VDR synthesis at the post-transcriptional level.

Decreased binding affinity of VDR with VDRE

In earlier studies based on DNA-cellulose chromatography it has been suggested that interaction of VDR with DNA was inhibited in chronic renal failure [11]. Binding affinity of the hormone receptor for DNA was also reduced when the receptor was preincubated with uremic ultrafiltrate [16]. Again, these findings were not uniformly confirmed [17].

Hsu et al. used the electrophoretic mobility shift assay to compare the ability of VDRs from normal and renal failure rats to bind to the osteocalcin gene VDRE [18]. DNA binding capacity was reduced by 50% in renal failure rats. In transfected JEG-3 cells, calcitriol-induced reporter gene expression was blocked by uremic ultrafiltrate [18].

Using the same electrophoretic assay, Sawaya et al. observed a significant reduction in kidney calcitriol-receptor complex binding to mouse osteopontin VDRE [6]. This defect occurred already with moderate renal failure despite normal calcium, phosphorus, calcitriol and VDR concentrations.

If uremic compounds inhibit VDR binding to its target genes, it could be expected that 24-hydroxylase activity would be decreased in renal failure. This enzyme is responsible for the transformation of active calcitriol to 1,24,25(OH)3D3; its synthesis is a VDR-receptor-mediated process [19]. Uremic ultrafiltrate indeed reduced production rate of 1,24,25(OH)3D3 in kidney homogenates by ± 50%, in the presence of both 1 μM and 25 nM calcitriol [16].

VDR functions primarily as a heterodimer with retinoid X receptor (RXR), at least in the case of the stimulation of gene transcription. Since both VDR and RXR can bind to direct repeats of the sequence AGGTCA [20], the question can be raised whether uremic toxins will chemically modify VDR alone, RXR alone, or the two of them together. Patel et al. demonstrated that the inhibitory effect of uremic toxins on the formation of the VDR–RXR–VDRE complex is due to a modification on the VDR alone, and not of the RXRα [21].

VDR and the immune system

An often neglected functional aspect is that calcitriol also shows immunomodulatory effects. Anti-proliferative, prodifferentiating, and immunosuppressive actions have been demonstrated.

Macrophages are potential targets for immunomodulatory action of 1,25(OH)2D3. Following treatment with 1,25(OH)2D3, macrophages exhibit enhanced antimicrobial action. 1,25(OH)2D3 regulates cytotoxic cells and biosynthesis of immunologically active humoral substances [22].

The question arises whether the uremic milieu also blunts this aspect of response to vitamin D. Recently we could demonstrate that uremic ultrafiltrate obliterated the calcitriol-induced CD14-expression on the surface of isolated monocytes in culture [23]. Preliminary data suggest that a similar inhibitory action is observed with respect to the differentiation of the promyelocytic leukemia cell line, HL-60, to differentiate (unpublished results).

Conclusions

End-stage renal disease is characterized by a state of relative calcitriol resistance. The following mechanisms play a possible role: (i) lower expression of VDR, at baseline and in response to calcitriol; (ii) diminished VDR binding to VDRE. One of the consequences is decreased breakdown of calcitriol. VDR and VDRE play an important role in the hormonal regulation of Ca/P metabolism and homeostasis of the bone, but may also play a role in other systems, e.g. the immune system.

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Glomerular lesions of diabetes mellitus: preventable and reversible

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Glycaemia and the development of diabetic nephropathy

Although preceded by a series of observations documenting advanced lesions of the glomerulus in the diabetic patient [1,2], Ruth Østerby of Aarhus University first emphasized in man the early development of glomerular basement membrane (GBM) widening and mesangial expansion [3,4]. She and colleagues measured these structures in successive renal biopsies, demonstrating convincingly the progressive nature of diabetic renal disease, even early in its course. Furthermore, she worked towards unifying the underlying pathophysiologic mechanisms of diabetic nephropathy stressing the accumulation of basement-membrane-like material. Although widening of the GBM and expansion of the mesangium may not reflect identical biochemical or biological processes, they do evolve similarly in the diabetic environment—as reflected by studies in identical twins in which GBM width and mesangial expansion were increased uniformly in the diabetic twin [5]. Importantly, these morphometric measurements of glomerular lesions enabled quantitative comparisons among normal and non-diabetic subjects, and those subjects treated with insulin or islet/pancreas transplantation both in rats [6–8] and in man [9–12].

Euglycaemia and the prevention or reversal of diabetic nephropathy

With animal models, islet transplantation or optimum insulin therapy altered the course of the structural and functional lesions of diabetic renal disease [6–8]. Applied to rodent models, the time for reversal encompassed 2–6 months—a duration much shorter than the time for efficacy demonstrated in people; however, studies in rats must account for the short time span available over the life of the rodent. This problem is especially complicated in diabetic rats because of the minimum of 6 months of diabetes necessary to establish most glomerular lesions [8]. Thus these time demands within an animal of relatively short life span reduce the questions that may be answered. Nevertheless, much important work has been accomplished in rodents demonstrating the feasibility of subsequent studies in man. Of importance are those issues raised above concerning the resiliency of glomerular lesions, once established, to reversal with implementation of