1,25-Dihydroxycholecalciferol: endocrinology meets the immune system

Evelyne van Etten, Brigitte Decallonne and Chantal Mathieu*

Laboratory of Experimental Medicine and Endocrinology, Catholic University of Leuven, Herestraat 49, 3000 Leuven, Belgium

Abbreviations: DC, dendritic cells; 1,25(OH)2D3, 1,25-dihydroxycholecalciferol; NOD, non-obese diabetic; Th, T-helper.

Corresponding author: C. Mathieu, fax +32 16 345934, email chantal.mathieu@med.kuleuven.ac.be

The activated form of vitamin D, 1,25-dihydroxycholecalciferol (1,25(OH)2D3) is well known for its effects on Ca and bone metabolism, but also non-classical effects of the molecule have been described. In non-obese diabetic (NOD) mice primary (before disease onset), secondary (after insulin but before diabetes onset) as well as tertiary (after transplantation of syngeneic islets) prevention of diabetes was demonstrated with 1,25(OH)2D3 and its chemically-manufactured non-hypercalcaemic analogues. 1,25(OH)2D3 exerts its immune effects both at the level of the T lymphocyte (shift in cytokine profile from T-helper (Th)1 to Th2, enhanced sensitivity to apoptosis-inducing signals) as well as at the level of the antigen-presenting cell (reduced antigen presentation, reduced production of Th1-promoting cytokines, reduced expression of co-stimulatory molecules). Also, physiologically, 1,25(OH)2D3 is believed to have a role in the immune system by serving as a negative feedback signal, limiting the mounted immune reaction. To test the clinical applicability of 1,25(OH)2D3 as treatment for type 1 diabetes in genetically-at-risk young children, we tested whether short-term early-life intervention with cholecalciferol or non-hypercalcaemic analogues of 1,25(OH)2D3 could prevent diabetes in NOD mice. Significant protection of pancreatic β cells against autoimmune destruction was observed in analogue-treated and especially in cholecalciferol-treated NOD mice as compared with controls (P<0·005). This short-term early-life intervention was, however, not able to protect the mice from developing diabetes during their lifetime. Possible solutions are longer or combined treatments with other immunomodulators that have synergistic effects with 1,25(OH)2D3 and its analogues.

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Primary prevention of type I diabetes by 1,25-dihydroxycholecalciferol and its analogues

It has been demonstrated that type I diabetes can be prevented in NOD mice by 1,25(OH)₂D₃ (Mathieu et al. 1994b). Treatment of NOD mice from weaning until old age not only prevented clinical diabetes, but also the histological lesion, insulitis, became less prevalent (Mathieu et al. 1992). As mentioned earlier, a major problem when using 1,25(OH)₂D₃ in vivo is its effects on Ca and bone metabolism; protection against diabetes was achieved using doses near to the toxic range. Structural analogues of 1,25(OH)₂D₃ with a dissociation between calcemic and immune effects, obtained in cooperation with the Department of Organische Chemie (University of Ghent, Ghent, Belgium; Professor M. Vandewalle and Professor P. De Clercq), and also analogues developed by pharmaceutical companies (Leo Pharmaceutical Products, Ballerup, Denmark; Hoffman-La Roche Inc., Nutley, NJ, USA; Schering AG, Berlin, Germany), have been used in the diabetes model in the NOD mouse (Mathieu et al. 1995). Some of these substances were able to reduce the incidence of insulitis and diabetes in the NOD mouse, without any effects on Ca and bone metabolism.

Also, in human subjects prevention of type I diabetes would be optimally achieved by primary prevention, i.e. treatment of genetically-at-risk young children. No side effects can be tolerated in early-life treatment of these children and, therefore, attention has been mainly focused on dietary interventions (e.g. avoidance of cow’s milk in the first months of life; Kimimaki et al. 2001). In the EURODIAB study (Dahlquist et al. 1999) a positive correlation between cholecalciferol supplements in early life and protection against diabetes has been demonstrated.

Based on this clinical study and on the positive results obtained with long-term treatment with 1,25(OH)₂D₃ or its analogues in NOD mice, we investigated whether short-term early-life intervention with supplements of cholecalciferol or with non-hypercalcemic analogues of 1,25(OH)₂D₃ could prevent type I diabetes in the NOD mouse. Thus, NOD mice were treated intraperitoneally with cholecalciferol (25 μg/d) or with one of the non-hypercalcemic analogues of 1,25(OH)₂D₃, TX22 (30 μg/kg per d) or TX527 (2.5 μg/kg per d); both analogues were originally synthesised by M. Vandewalle and P. De Clercq and later provided by Théramex SA, Monaco, from 3 to 70 d of age, corresponding to neonatal life and childhood in human subjects, and followed up for diabetes incidence until 30 weeks of age. Vehicle-treated mice served as controls. At diabetes onset or at 30 weeks of age, mice were killed and pancreatic insulin content was measured (Gysemans et al. 2002). To evaluate drug toxicity, serum and bone Ca levels and serum osteocalcin content (as an index of bone turnover) were measured (Mathieu et al. 1995). None of the treatments resulted in long-lasting effects on Ca or bone metabolism (data not shown). Although none of the treatments protected NOD mice from diabetes development (Table 1), a significant (P<0.005) conservation of β-cell mass (expressed as higher pancreatic insulin levels) was observed for all treatments (Fig. 1). When analysing diabetic and normoglycaemic mice separately this difference remained evident. This preserved pancreatic

Table 1. Effects of short-term early-life interventions with cholecalciferol and its analogues TX522 and TX527 in NOD mice on diabetes incidence

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<td>Control</td>
<td>21/34</td>
<td>53</td>
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<tr>
<td>Cholecalciferol (25 μg/d)</td>
<td>5/13</td>
<td>8/19</td>
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<tr>
<td>TX522 (30 μg/kg per d)</td>
<td>9/20</td>
<td>11/32</td>
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<td>TX527 (2.5 μg/kg per d)</td>
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*Originally synthesised by M. Vandewalle and P. De Clercq, University of Ghent, Ghent, Belgium and later provided by Théramex SA, Monaco.

Fig. 1. Pancreas insulin content of (A) all mice, (B) diabetic mice and (C) normoglycaemic mice of the different treatment groups. Mice were treated intraperitoneally with vehicle, cholecalciferol (25 μg/d), TX522 (30 μg/kg per d) or TX527 (2.5 μg/kg per d) from 3 to 70 d of age. At diabetes onset, or at 30 weeks of age, mice were killed and the pancreatic insulin content was measured. Values are expressed as means with their standard errors represented by vertical bars. Mean values were significantly different from those for the control group: *P<0.05, **P<0.01, ***P<0.005.
insulin content confirms that as well as effects on the immune system, cholecalciferol and its analogues also have direct protective effects on the β-cells (Hahn et al. 1997; Riachy et al. 2001), making these target cells more resistant to autoimmune-mediated destruction. Unexpectedly, β-cell protection was most pronounced in mice treated with regular cholecalciferol supplements. This finding could be explained by a defect in the immune stimuli-mediated up-regulation of 25-hydroxycholecalciferol-1α-hydroxylase (the enzyme responsible for the final and rate-limiting step in the synthesis of the active form of cholecalciferol, 1,25(OH)2D₃) in the macrophages of NOD mice (Overbergh et al. 2000a). This defect might be responsible for inappropriately low levels of 1,25(OH)2D₃ locally at inflammatory sites, and might be corrected by cholecalciferol supplements. Presently, we are investigating whether defects in macrophage 25-hydroxycholecalciferol-1α-hydroxylase levels are also present in human subjects with type 1 diabetes.

This study indicates that, in contrast to previous results for NOD mice treated for a long period of time with 1,25(OH)2D₃ or its analogues, treating NOD mice for a short period of time early in life with an analogue of 1,25(OH)2D₃ is not sufficient to prevent diabetes. Longer or combined treatments with other immunomodulators remain necessary for primary prevention in NOD mice.

Mechanisms of protection from type 1 diabetes by 1,25-dihydroxycholecalciferol and its analogues

Investigating the mechanisms of protection, we discovered that the well-known defect in suppressor cell function in NOD mice was normalised by treatment with 1,25(OH)2D₃ or its analogues (Mathieu et al. 1995). In subsequent studies involving cyclophosphamide-induced diabetes we were able to demonstrate again that these suppressor cells were clearly present after 70 d of therapy in 1,25(OH)2D₃- or analogue-treated NOD mice (Casteels et al. 1998b). Nevertheless, in the latter studies it was shown that these suppressor cells were not indispensable for the protective effect of 1,25(OH)2D₃ and its analogues. Analysing the cytokine profile locally in these pancreases, we found a clear shift from T-helper (Th)1 to Th2 expression patterns in 1,25(OH)2D₃-treated mice. These observations and the fact that protection continued after withdrawal of the treatment, suggested that 1,25(OH)2D₃ treatment had resulted in the induction of tolerance for β-cells, possibly by elimination of autoimmune effector (Th1) cells that otherwise would have caused diabetes. In the following studies we were able to demonstrate that treatment with 1,25(OH)2D₃ caused a restoration of the impaired apoptosis sensitivity in NOD thymocytes and splenocytes, both in female and male mice (Casteels et al. 1998c). At present we are investigating the molecular signals involved in the apoptosis resistance in NOD lymphocytes and the way in which 1,25(OH)2D₃ influences these signals.

Although it is well known that 1,25(OH)2D₃ directly affects T lymphocytes in vitro as well as in vivo (as shown in NOD mice by the generation of suppressor cells and improved elimination of autoreactive effector cells), it is believed that the effects of 1,25(OH)2D₃ are primarily mediated through its action on antigen-presenting cells, of which dendritic cells (DC) are the most potent. In a study using in vitro-generated human DC, we showed that treatment with the non-hypercalcaemic 1,25(OH)2D₃ analogue TX 527 has a major impact on the differentiation and maturation, as well as function, of these monocyte-derived DC (Van Halteren et al. 2002). With TX 527 treatment during DC maturation, a shift was induced from a Th1-promoting phenotype towards a Th2-promoting phenotype. These results confirm the in vitro effects of 1,25(OH)2D₃ itself on human DC (Penna & Adorini, 2000). Subsequent stimulation of an autoreactive T-cell clone resulted in a profound redirection of its cytokine profile (lower levels of Th1 cytokines), suggesting a mechanism of action by which the secondary and tertiary prevention of diabetes in the NOD mouse by 1,25(OH)2D₃ could be explained. In this context, we are now investigating the effects of 1,25(OH)2D₃ treatment on in vitro and in vivo DC development of NOD mice.

On the other hand, when treatment was initiated during monocyte differentiation, a totally different cell type was generated, lacking the DC-specific surface markers and morphology. It is known that monocytes may be considered as relatively immature precursors with multiple differentiation potential, depending on the micro-environment. Cytokines and inflammatory substances play an important role in the final decision, determining whether monocytes will acquire DC, macrophage, osteoclastic or other characteristics and functions (Chomarat et al. 2000). Currently, we are analysing further the phenotype and function of the cell type generated by continuous treatment with 1,25(OH)2D₃ or its analogues.

Secondary prevention of type 1 diabetes by analogues of 1,25-dihydroxycholecalciferol

We have shown that treatment with analogues of 1,25(OH)2D₃ can lead to secondary prevention, i.e. prevention of the progression of an initiated β-cell attack (reflected by the presence of insulitis) to clinical disease, diabetes (Casteels et al. 1998a). Some analogues of 1,25(OH)2D₃ were very effective in preventing this progression from insulitis (demonstrated in each individual mouse in pancreatic biopsies taken at the age of 70 d, before starting therapy) to overt diabetes, even when given alone. In combination with a short induction treatment of 3 weeks with the classical immunosuppressant, cyclosporin A, the efficacy of the analogues in blocking disease progression was further increased. Again, because of the very short period of treatment with cyclosporin A, no long-term immunosuppression was detected. Interestingly, in this model of secondary prevention no suppressor cells could be demonstrated in co-transfer experiments. Within the pancreases of protected mice, we again noted a shift from Th1-type cytokines towards Th2-type cytokines by measuring cytokine mRNA production locally in the islets. In further experiments we have been able to demonstrate that this shift from a Th1 cytokine profile to a Th2 cytokine profile mediated by 1,25(OH)2D₃ or its analogues only happens for autoantigens, but not ‘diabetes-irrelevant’ antigens (Overbergh et al. 2000b).
Tertiary prevention of type I diabetes

Islet transplantation is a potential cure for patients with type 1 diabetes. Compared with patients that have become insulin dependent due to pancreatic surgery, patients with type 1 diabetes destroy the newly-transplanted islets more vigorously, thus creating the need for heavy immunosuppressive regimens (Mathieu, 2000). A cause for this more vigorous reaction is most probably the recurrence of the autoimmune disease itself. In recent years we have focused specifically on this phenomenon of autoimmune recurrence.

We have tested analogues of 1,25(OH)2D3 in a model of pure recurrence of autoimmunity, after syngeneic (NOD to NOD) islet transplantation. When neonatal NOD islets were transplanted into spontaneously-diabetic NOD mice, a rapid and specific destruction of the transplanted β-cells was always found. In this model again therapy with analogues of 1,25(OH)2D3 alone was as efficient as cyclosporin A in prolonging graft survival. Moreover, a combination of analogues of 1,25(OH)2D3 and low doses of cyclosporin A not only synergistically prolonged graft survival, but also allowed the withdrawal of all drugs after 2 months without subsequent recurrence of the disease (Mathieu et al. 1997; Casteels et al. 1998d). This observation suggests that even in the case of tertiary prevention, autoimmune recurrence can be overcome, and thus re-induction of self-tolerance can be achieved. Analysis of the cytokine profile in the grafts themselves again demonstrated the induction of an immune shift from a Th1 profile towards a Th2 profile in mice receiving the combination treatment. In the meantime, we have screened other immunosuppressants for their capacity to act synergistically with analogues of 1,25(OH)2D3 and found several good candidates (Mathieu et al. 1994a; Branisteanu et al. 1997; van Etten et al. 2000; Gysemans et al. 2002).

In view of the potential of the xenotransplantation technique as a solution for the human organ shortage which will be encountered should islet replacement become used as a widespread therapy for type 1 diabetes, we studied xenogeneic islet transplantation (rat islets) in NOD mice. We have discovered that after transplantation of xenogeneic islets, an important proportion of these grafts never functions, even under technically controlled circumstances. This phenomenon is known as primary non-function or early graft failure. We demonstrated that this graft failure is far more frequent in autoimmune NOD mice than in non-autoimmune NOD mice or other non-autoimmune-prone mouse strains, and is characterised by high interleukin 1 and low transforming growth factor β levels locally in the grafts, as measured by reverse transcription–polymerase chain reaction (Gysemans et al. 2000). Furthermore, we found that analogues of 1,25(OH)2D3 or of the classical immunosuppressant leflunomide, both in combination with cyclosporin A, could prevent this primary non-function (Gysemans et al. 2001a,b).

1,25-Dihydroxycholecalciferol as a physiological immunoregulator

A final part of our work focused on the analysis of the physiological role of 1,25(OH)2D3 in the immune system. Receptors for 1,25(OH)2D3 have been described in many cells of the immune system (Veldman et al. 2000), and indirect evidence has existed for many years to suggest that cells of the immune system can produce 1,25(OH)2D3. We were the first to demonstrate at the molecular level that the enzyme responsible for the final hydroxylation and activation of vitamin D, 25-hydroxycholecalciferol-1α-hydroxylase, is present in macrophages and originates from the same gene as the enzyme in the kidney, typically involved in Ca homeostasis (Overbergh et al. 2000a). However, the macrophage gene is regulated in a completely different manner, with immune signals in particular influencing the transcription, while the typical Ca signals have no effect. On the basis of these findings and the data available on the effects of 1,25(OH)2D3 on different cells in the immune system and their cytokine production, we have proposed a physiological role for 1,25(OH)2D3 as a negative feedback signal in the activated immune system (Fig. 2). Moreover, we found that NOD mice have a defective up-regulation of their macrophage 25-hydroxycholecalciferol-1α-hydroxylase in response to immune stimuli, another possible factor contributing to their autoimmunity-prone
immune system. With analysis of the 25-hydroxycholecalciferol-1α-hydroxylase promoter using transfection experiments we are currently further addressing this issue.

Conclusions

Our work has demonstrated a clear pharmacological potential for 1,25(OH)2D3, particularly its structural analogues with less calcemic effects, as tools in the prevention and treatment of autoimmune diseases. This effect is based on shifts in cytokine profile (from Th1 to Th2), that occur specifically for the autoantigens, and on enhanced sensitivity of the autoreactive T lymphocytes to apoptotic signals, thus leading to improved elimination of these potentially-dangerous cells. These effects of 1,25(OH)2D3 are primarily mediated via its actions at the T-cell level of the antigen-presenting cell (with altered phenotype and cytokine production), although direct effects at the T-cell level are also present. 1,25(OH)2D3 or its analogues can therefore be considered in interventions aimed at the prevention or treatment (islet transplantation) of human type 1 diabetes.

References


Penna G & Adorini L (2000) 1α,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation and survival of dendritic cells leading to impaired alloreactive T cell activation. Journal of Immunology 164, 2405–2411.


