Preliminary report

Dipeptidyl peptidase-4 inhibitors (DPP-4i) combined with vitamin D3: An exploration to treat new-onset type 1 diabetes mellitus and latent autoimmune diabetes in adults in the future

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\textbf{A R T I C L E  I N F O}

Keywords:
Type 1 diabetes mellitus (T1DM)
LADA
DPP-4 inhibitor
Vitamin D3

\textbf{A B S T R A C T}

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by destruction of pancreatic beta cells through cell injury caused primarily by cytotoxic T lymphocytes (CD8\textsuperscript{+}). The pathophysiologcal basis of T1DM seems to be an imbalance between a reduced function of T regulatory lymphocytes and an increased inflammatory activity of Th17 lymphocytes caused by increased production of inflammatory cytokines, as IL-1β, IL-6, IL-17 and IFN-gamma due to environmental factors and genetic predisposition. The preservation of the reserve of beta cells in new-onset T1DM and latent autoimmune diabetes in adults (LADA) by immunomodulation in addition to the incretin effect seems to be possible with an association of DPP-4 inhibitors and vitamin D3. In this review, we discuss the effects of both drugs on the immune system and on beta cell function and their eventual additive effects in preserving the residual function of beta cells in new-onset T1DM and LADA.

\section{1. Introduction}

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by injury to and destruction of pancreatic beta cells secondary to activation of dendritic cells and macrophages, CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes, and B lymphocytes. These cells interact to generate an inflammatory response, leading to insulitis with a predominance of CD8\textsuperscript{+} T lymphocytes [1]. The exact trigger of the autoimmune process is not fully understood but may involve genetic predisposition, viral infections, changes in the intestinal flora, and low vitamin D levels [2–5].

The continuous destruction of beta cells promotes loss of secretory reserve of insulin, leading to clinical diabetes when the mass of beta cells reduces to less than 20% of the total amount present before the disease [6]. Beta cells are known to be able to replicate after birth, and even patients with long-standing T1DM have residual beta cells producing insulin [7]. Preservation of residual pancreatic insulin function in patients with T1DM is fundamental to reduce blood glucose fluctuations, decrease insulin requirement and improve metabolic control, thereby reducing the occurrence of complications related to hyperglycemia [8,9]. One of the goals of treatment in T1DM is to preserve/regenerate the mass of pancreatic beta cells through intensive insulin therapy [10], reducing glucotoxicity on these cells, or through therapies that regulate the immune system, reducing the inflammatory response and cell apoptosis and improving the function of beta cells [11–14]. In T1DM, there is an imbalance between regulatory T cells (Treg), which have reduced suppressive function [15,16], and increased activity of inflammatory Th17 cells, which are responsible for the autoimmune process against beta cells [17,18]. IL-17, CD8\textsuperscript{+} T cells (also known as Tc17 cells) [19] and IL-17 CD4\textsuperscript{+} are increased in children with new-onset type I diabetes compared to age-matched healthy controls [20]. This imbalance seems to be generated by increased serum IL-6 levels [21] and other cytokines as IL-1β, IL-17 and INF-γ [22,23]. However, it is important to emphasize the importance of IL-6 levels to control the Treg/Th17 cells differentiation and programamtion [24]. Thus, therapies correcting this imbalance between Treg and Th17 cells could assist in preserving the secretory function of beta cells in T1DM.

In fact, other authors and we have reported cases of prolonged clinical remission and preservation of secretory beta cell reserves with dipeptidyl peptidase-4 (DPP-4) inhibitors and/or vitamin D3 in patients with T1DM and latent autoimmune diabetes in adults (LADA) [25–31].

Based on the above, the aim of this review is to discuss the
complementary mechanisms between DPP-4 inhibitors and vitamin D3 in regulating the imbalance between the functions of Treg and Th17 cells, as well as their possible beneficial effects on beta cell function in T1DM, showing that this association may be a future line of research in the treatment of new-onset T1DM and possibly, also, for other autoimmune diseases.

2. Dipeptidyl peptidase-4 inhibitors in type 1 diabetes mellitus and LADA

DPP-4, a serine peptidase also known as CD26, is a cell surface antigen (DPP-4/CD26) expressed in T and B lymphocytes, macrophages and natural killer, acinar, endothelial and epithelial cells [32,33]. The difference between the soluble DPP-4 form, present in the plasma and seminal fluid, and the form attached to the cell membrane (CD26), is that the soluble form the intracellular and transmembrane portions of the molecule is absent. The CD26 molecule has three main functions: (A) binding of adenosine deaminase (ADA), (B) peptidase activities, and (C) binding of the extracellular matrix. All these functions can influence the proliferation and chemotaxis of T lymphocytes [33]. According to Bengsch et al. (2012) [34], T lymphocytes expressing CD26 are subdivided into Th17(26bright), Th1(26++), Th2(26++), and Treg (26low/−). The null/profile of the CD26 molecule in T cells (CD4−CD26bright) appears to have immunosuppressive functions in these cells, since the CD26–caveolin-1 interaction would promote a positive regulation of CD86 in antigen presenting cells (APCs) with subsequent binding to CD28 on T cells, leading to the activation of antigen-specific T cells [35]. Thus, the absence of CD26 in T cells could maintain low CD86 levels in the APCs and prevent T cell activation [36]. CD26, as an ADA binding protein, prevents the immunosuppressive effects of adenosine through its intracellular receptor A2A in effector T cells. Since Tregs do not express CD26 and, therefore, are unable to have ADA binding to their membranes, the pericellular concentration of adenosine would be high. In this way, Tregs would use this excess of surrounding adenosine as a soluble suppressive factor in effector T cells, in addition to using other mechanisms dependent on cell-cell contact and independent of contact, such as secretion of IL-10 and TGF-β [37–39].

As a serum serine peptidase, DPP-4 degrades two intestinal incretin hormones involved in increasing insulin release by beta cells after a meal, glucagon-like peptide-1 (GLP-1) produced by intestinal L cells, and the gastric inhibitory polypeptide (GIP), produced by intestinal K cells. DPP-4 also inactivates several other serum peptides such as proline or alanine in the second aminoterminal (NH2) position, cleaving the dipeptides in the molecule with their consequent biological inactivation and activation [32]. DPP-4 inhibitors (sitagliptin, vildagliptin, linagliptin, saxagliptin and alogliptin) have been used for > 10 years in the treatment of type 2 diabetes mellitus (T2DM), since they promote glucose-dependent increased insulin secretion by leading to increased serum GLP-1 and GIP levels, as well as inhibiting the release of glucagon and reducing the hepatic production of glucose [40,41]. In addition, these molecules have demonstrated anti-inflammatory and immunomodulating effects, both in vitro and in vivo [42–46].

Due to their anti-inflammatory and immunomodulating effects and due to the fact that they increase GLP-1, which would have protective effects on beta cells through the GLP-1 receptor [47,48], DPP-4 inhibitors have already been tested in T1DM and LADA with conflicting results. In T1DM, treatment with DPP-4 inhibitors improved HbA1c levels and reduced daily requirements of insulin without causing hypoglycemia, but they had no benefits in preserving pancreatic reserves [49–52]. In contrast, benefits on beta cells have been observed in patients with LADA [53,54]. Of note, the selection in TIDM studies of adult patients with long-time disease and without pancreatic reserve may have generated a biased result. In the REPAIR-TID study [50], which included patients with new-onset T1DM treated with sitagliptin and lansoprazole, the inclusion criteria of up to 6 months since diagnosis seemed for us to be too long, since during this period, much of the pancreatic insulin reserve may have already been lost [55]. In addition, the use of a reduced dose of sitagliptin (50 mg/day) in patients younger than 18 years did not seem adequate, since the DPP-4 activity is higher in T1DM [56–58], even when compared with patients with T2DM [59]. Additionally, patients with T1DM have lower postprandial GLP-1 levels [60], which may explain the fact that several participants failed to achieve adequate GLP-1 levels in this study. These results differ from the excellent response observed with DPP-4 inhibitors in the remission of T1DM in animal models [61–63]. However, the doses used in these animal studies were higher than those tested in humans. Our group has shown that the inhibition of the proliferation of human peripheral blood mononuclear cells (PBMC) with sitagliptin was dose-dependent, and that the concentration of 50 μg/mL of sitagliptin was able to modulate the differentiation of Th17 cells/Th1 in regulatory cells producing TGF-β1, reducing the production of IL-6, IFN-gamma, and IL-17 [64]. This same effect has been observed in animal models [65,66]. Thus, future studies with DPP-4 inhibitors in new-onset T1DM should take into account the used dose and the duration of the diagnosis of diabetes, in addition to assessing the serum DPP-4 activity, as well as the expression of CD26 on lymphocytes, in an attempt to define a minimum dose able of inhibiting DPP-4, increasing GLP-1 and modulating the immune cellular and humoral responses.

3. Vitamin D in type 1 diabetes mellitus and LADA

In humans, vitamin D is synthesized through the conversion of 7-dehydrocholesterol in the skin into pre-vitamin D3 in response to ultraviolet B radiation from sunlight and is quickly converted into vitamin D3. Vitamin D can also be obtained from some perishable, in natura foods or supplemented as vitamin D2 or D3. In the liver, vitamin D3 is metabolized into 25-OH vitamin D3 through the action of 25-hydroxylase and, subsequently, converted into its active form 1,25-dihydroxyvitamin D3 by 1-α-hydroxylase in the kidney and in some cells, including immune system cells [67]. Monocytes, macrophages, dendritic cells (DCs), T and B lymphocytes express a receptor for vitamin D (VDR) in addition to enzymes that activate 25-OH-vitamin D3 into 1,25-dihydroxyvitamin D3, indicating its importance in maintaining the homeostasis of the immune system [68].

The main function of vitamin D in the immune system appears to be immunoregulation. In DCs 1,25(OH)2D3 is able to change the morphology and function of DCs to tolerogenic DCs (tolDCs), impairing the differentiation of B cells into different functions, both in vitro and in vivo [42–46].

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has shown a significant increase in the suppression function of Treg cells in these patients, without an increase in these cell number, while in the placebo group, there was a reduction in the suppressive role of Treg cells after 12 months [78]. On the other hand, in 61 patients with T1DM with ages between 8 and 15 years and disease duration < 8 weeks, the use of alfacalcidol at a dose of 0.25 μg twice a day showed benefits in preserving fasting C peptide levels, with requirement of lower daily insulin doses, an effect that has been shown to be more important in men [79]. The use of calcitriol 0.25 μg/day administered to children at high risk for T1DM, aged between 1.5 and 13 years and with positive antibodies against T1DM (anti-GAD and anti-insulin) but without clinical T1DM, was able to induce disappearance of antibodies in all patients after 1–3 years of treatment [80]. Gabbay et al. (2012) treated 17 patients with T1DM with cholecalciferol 2000 IU/day and showed a significantly increased percentage of Treg cells, with a more slowly decline in the levels of stimulated C peptide when compared with placebo [81]. In patients with LADA, treatment with 1-alpha(OH) D3 0.5 μg/day was able to preserve at 1 year the levels of C peptide at fasting and 2 h after a 75-g glucose overload, but the effect was only beneficial in those patients with a diagnosis duration below 1 year [30]. In healthy volunteers treated for 3 months with cholecalciferol 140,000 IU/week, there was a significant increase in the number of Treg cells in peripheral blood, while in the placebo group, there was no change in the number of these cells, or changes in C peptide levels during fasting or after stimulation with OGTT [82]. However, in other studies with treatment of patients with new-onset T1DM with calcitriol 0.25 μg/day, no benefit was observed [83,84].

4. DPP-4 inhibitor combined with vitamin D3 - potential therapeutic on immune system and beta cell function

The immunomodulating effects of DPP-4 inhibitors, as well as those of vitamin D3, seem unquestionable. However, the benefits of these drugs in the treatment of new-onset T1DM and LADA still require confirmation, mainly those of DPP-4 inhibitors, which despite a similar efficacy in the treatment of T2DM [85], may have different immunological effects due to peculiarities of each molecule in their connection with DPP-4/CD26, in addition to the interference on the dimerization of CD26 [86,87]. Yazbeck et al. [88] proposed a model of the potential anti-inflammatory mechanism of DPP inhibitors. After binding of the inhibitor to the active site of membrane-bound DPP4/CD26, they propose a conformational change in the intracellular domain of the enzyme. Alternatively, the inhibitor could pass across the cell membrane and bind to DPP8 or DPP9. This might then lead to initiation of an intracellular pathway, resulting in a reduction in pro-inflammatory cytokines secretion, suppressed T-lymphocyte proliferation and increased production of the cytokine TGF-β [88]. To date, no clinical randomized, double-blind, placebo-controlled study has been carried out with this therapeutic combination in new-onset T1DM and LADA. Probably no immunomodulating therapy alone can promote prolonged T1DM remission, and combined therapy is already accepted as the most suitable due to the involvement of several immunological pathways, in which the blockade of one pathway can lead to increased activation of other pathways [89]. Ideally, such therapy should be safe, inexpensive and have effects both on the immune system and on beta cells [90]. The association of DPP-4 inhibitors and vitamin D3 appears to satisfy these requirements, as both drugs have effects on the immune system and on beta cells [91,92], have demonstrated to be safe and are relatively inexpensive. The best doses of each drug have yet to be defined since the DPP-4 activity seems to be greater in T1DM and LADA [56-58,93], and polymorphisms in the vitamin D receptor and the various enzymes involved in the metabolism of vitamin D may influence its serum concentration and effectiveness [94]. In children with type 1 diabetes, BsmIBB, BsmIBb and TaqIt polymorphisms of the VDR were associated with an increased risk of T1DM, whereas BmIBb and TaqITT had protective effect for T1DM [95]. In addition, it is necessary to define whether treatment with higher doses of 25-OH vitamin D3 or 1,25(OH)2D3 would be more effective. The use of high doses of 25-OH vitamin D3 seems safer against the occurrence of hypercalcemia [96] and perhaps more effective by allowing higher concentrations of free 25-OH vitamin D3 as substrate to 1-a-hydroxylase in immune system cells in the inflamed areas [97]. In a recent review about vitamin D in autoimmunity, Dankers et al. (2017) share the same opinion as our group: ‘This lack of effect could be due to the low level of remaining β cell function at the start of the study, suggesting that the therapeutic window for vitamin D supplementation is in the earliest phases of the disease’ [98]. In other words, time to start intervention is crucial. We believe should starting at diagnosis of the T1DM and LADA.

Our group has shown that the use of sitagliptin 100 mg/day associated with vitamin D3 5.000 IU/day enabled prolonged clinical remission (mean 27.1 ± 18.9 months) in patients with T1DM, showing a reduction in serum CD8+/CD26+ T cells when compared to T1DM treated with insulin alone (Fig. 1) [99]. Of note, human CD26+/CD26− T cells appear be memory cells with profound cytotoxicity capacity, multi-functional and enzymatically active, producing more Granzime B, CD107A, IL-2, INF-gamma, IL-17A, IL-22 and TNF-alpha than CD26−.
and CD26 int T cells [100,101]. Moreover, the highest concentrations of VDR are found in CD8+ lymphocytes [102], which could explain the reduction of CD8+/CD26+ cells in our study, as well as good clinical results at noted by levels of HbA1c (mean: 6.33% ± 1.12%) and C-peptide (1.19 ± 0.72 ng/mL) even after 27 months of evolution. However, this was not a randomized, double blind study, which limits its interpretation.

5. Conclusion

The additive effects of DPP-4 inhibitors and vitamin D3 on the immune abnormalities that occur in T1DM, in addition to their beneficial effects on beta cells, have a rational theoretical (Fig. 2) that still needs to be proven in new clinical studies.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

The authors declare that they have no competing interests.

References


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O.E. Johannsen, B.O. Boehm, V. Grill, P.A. Torjesen, S. Bhattacharya, S. Patel, K. Wetzl, H.J. Woelle, C-peptide levels in latent autoimmune diabetes in adults treated with lansoprazol versus glipizide: exploratory results from a 2-year double-blind, randomized controlled study, Diabetes Care 37 (1) (2014) e1–2, http://dx.doi.org/10.2337/dc13-1523 (727/1/e1 [pii]).


