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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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ABSTRACT

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 ($CD8^+$). The pathophysiological 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.

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, $CD4^+$ and $CD8^+$ T lymphocytes, and B lymphocytes. These cells interact to generate an inflammatory response, leading to insulitis with a predominance of $CD8^+$ 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 to 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^+ T cells (also known as Tc17 cells) [19] and IL-17^+CD4^+ 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- < gamma > [22,23]. However, it is important to emphasize the importance of IL-6 levels to control the Treg/Th17 cells differentiation and programmation [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

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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($CD26^{Bright}$), Th1($CD26^{++}$), Th2($CD26^{+}$), and Treg ($CD26^{Low/-}$). The null/low profile of the CD26 molecule in Treg cells ($CD4^{+}CD25^{high}$) 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]. Then, the absence of CD26 in Treg 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 A_{2A} 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 (NH_2) position, cleaving these dipeptides in the molecule with their consequent biological inactivation or 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 T1DM studies of adult patients with long-time disease and without pancreatic reserve may have generated a biased result. In the REPAIR-T1D 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 [$1,25(OH)_2D_3$], 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 $1,25(OH)_2D_3$ is able to change the morphology and function of DCs to tolerogenic DCs (tolDCs), impairing the turnover of DCs and maintaining them in an immature state, reducing the expression of the major histocompatibility complex (MHC class II) and costimulating molecules such as CD40, CD80, and CD86 [69]. In addition, $1,25(OH)_2D3$ can upregulate the secretion of the chemokines CCL2, CCL18, and CCL22, which are involved in the recruitment and induction of Treg cells, polarization for Th2 and maintenance of DCs in an immature state, in addition to reducing the recruitment of Th1 cells by decreasing the production of the chemokine CXCL-10 [68–71]. $1,25(OH)_2D3$ and its analog TX527 directly change the function of T cells by inhibiting the production of Th1 cytokines (IL-2 and IFN-gamma), Th2 (IL-4), and Th17 (IL-17 and IL-21) to the extent that they induce Treg cells with increased expression of IL-10 and CTLA-4 [72–74]. $1,25(OH)_2D3$ also inhibits the differentiation of B cells into plasma cells, as well as their proliferation, and IgG and IgM secretion, inhibiting the generation of B memory cells and inducing their apoptosis [75]. Considering all that, vitamin D3 has a potential in the treatment of autoimmune diseases like T1DM.

In studies of primary prevention of T1DM in humans, vitamin D supplementation has been shown to significantly reduce the incidence of new cases of T1DM when administered early in childhood [76,77]. In contrast, intervention studies in patients with new-onset T1DM have shown conflicting results, which may have occurred due to differences in intervention type. Some studies have used cholecalciferol, while others used calcitriol, or even alfacalcidol, a vitamin D analog; all at different doses, variable T1DM diagnosis duration and different age