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Review

Dipeptidyl peptidase inhibitor therapy in type 2 diabetes: Control of the incretin axis and regulation of postprandial glucose and lipid metabolism

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ABSTRACT

Dipeptidyl peptidase 4 (DPP4) is a widely expressed, serine protease which regulates the bioactivity of many peptides through cleavage and inactivation including the incretin hormones, glucagon like peptide −1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP). Inhibitors of DPP4 are used therapeutically to treat patients with Type 2 Diabetes Mellitus (T2DM) as they potentiate incretin action to regulate islet hormone secretion and improve glycemia and post-prandial lipid excursions. The widespread clinical use of DPP4 inhibitors has increased interest in the molecular mechanisms by which these drugs mediate their beneficial effects. Traditionally, focus has remained on inhibiting the catalytic activity of DPP4 within the plasma compartment, however evidence is emerging on the importance of inactivation of membrane-bound DPP4 in selective tissue beds to potentiate local hormone gradients. Here we review the recent advances in identifying the cellular sources of both circulating and membrane-bound DPP4 important for cleavage of the incretin hormones and regulation of glucose and lipoprotein metabolism.

1. Introduction

The molecular underpinnings of Type 2 diabetes mellitus (T2DM) include insufficient insulin action, aberrant glucagon secretion and insulin resistance in peripheral tissues. Collectively, these disrupt the tight coordination of nutrient uptake and disposal contributing to hyperglycemia and dyslipidemia [1,2]. Cleavage of target peptides, chiefly the incretin hormones, glucagon like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) by the serine protease dipeptidyl peptidase 4 (DPP4) is an important regulator of metabolic homeostasis, particularly linking nutrient intake with uptake and utilization. The incretins are secreted in response to nutrients and rapidly stimulate glucose-dependent insulin secretion from β-cells. GLP-1 and GIP exert their metabolic effects through activation of their respective G protein coupled receptors, the GLP-1 receptor (GLP-1R) and the GIP receptor (GIPR) and their biological activity is significantly reduced upon degradation by DPP4 [3–5].

DPP4 is an exopeptidase which exists in both a membrane anchored and a circulating soluble form [6]. Both forms exhibit enzymatic activity as the catalytic active site which contains a serine triad is located at the C-terminal and it is a short sequence at the N-terminus which extends into the cytoplasm. DPP4 is widely expressed in many tissue compartments (intestine, kidney, liver, islets), endothelial and immune cells and soluble DPP4 is present in many bodily fluids (blood, urine, bile, seminal fluid, cerebral spinal fluid) [7]. DPP4 is a member of a large family proline specific peptidases including DPP2, DPP6 (catalytically inactive), DPP8, DPP9, fibroblast activation protein (FAP) and prolyl endopeptidase (PEP) and prefers a position 2 proline or alanine, however many peptides with glycine, valine, serine, threonine or leucine at the second position are also cleaved [8]. It is known that the circulating isoform of DPP4 increases in patients in response to metabolic disease including obesity [9–12], increased HbA1c levels and T2DM [13,14] and non-alcoholic fatty liver disease (NAFLD) [15]. However the biological significance of elevated, circulating DPP4 levels remains unknown. Here we assess the literature relevant to identifying the cellular sources of DPP4 important for incretin degradation and regulation of glucose and lipoprotein metabolism. We also discuss the current state of knowledge regarding sources of circulating DPP4 and summarize the mechanisms underlying DPP4 release.

2. Mechanism of action of DPP4 inhibitors

2.1. Dipeptidyl peptidase 4 and cleavage of the incretin hormones

GLP-1 and GIP are peptide hormones which in response to nutrient ingestion are traditionally defined to be secreted by the gut and act as elegant messengers to regulate hormone secretion from pancreatic α-cells and β-cells to properly direct nutrient disposal [16]. It was the collective efforts of a number of groups, using complimentary models and approaches which defined the relationship between DPP4 cleavage

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and regulation of the bioactivity of GLP-1 and GIP. These important preclinical experiments laid the critical foundation for the successful development of DPP4 inhibitors to treat T2DM [7].

In healthy individuals GIP and GLP-1 account for 50–70% of the postprandial rise in insulin and in patients with T2DM, the incretin effect is impaired and only accounts for approximately 20% of the postprandial rise in insulin [17,18]. Traditionally, DPP4 inhibitors have been administered to inhibit DPP4 truncation of GLP1 and GIP and potentiate their actions on islet hormone release. Studies in mice with whole body deletion of both incretin receptors (GLP-1R and GIPR) known as double incretin receptor knock out (DIRKO) mice have demonstrated that systemic concentrations of DPP4 inhibitors require intact signaling through both incretin receptors to lower glucose as mice with single receptor elimination still respond to acute, systemic treatment with DPP4i (Val-Pyr, SYR106124) [19] or chronic treatment with vildagliptin [20]. In support of contributions from both GLP-1 and GIP, genetic elimination of the β-cell *Glp1r* does not diminish glycemic regulation by the DPP4 inhibitor vildagliptin (150 μg) [21] and chronic anagliptin (0.625 mg/mL in drinking water) treatment of diabetic mice lacking proglucagon expression ($Gcg^{-/-}$) results in a reduced glucose excursion in response to glucose challenge [22]. Studies in human subjects treated with the GLP-1R antagonist exendin 9–39 have also revealed that GLP-1 signaling accounts for approximately 50% of the improvement in glycemia observed with vildagliptin treatment [23]. Interestingly, administration of low concentrations of sitagliptin which do not impact plasma DPP4 activity (40 μg/mouse) [24] to healthy mice null for the β-cell *Gipr* resulted in no improvement in glucose tolerance [25]. Also, in healthy participants 3 day treatment with sitagliptin stimulated levels of active GIP in response to oral glucose to a much greater degree (20-fold) compared with GLP-1 (1.4 fold) [26] suggesting under healthy, physiological conditions a small potentiation in GIP may be glucoregulatory. However, in contrast with control participants, patients with T2DM infused with active GIP exhibit a weak insulinotropic response while the insulin response to active GLP-1 infusion remains intact [27]. The exact mechanism underlying the lack of response to GIP in β-cells may be linked to the hyperglycemia induced desensitization of signaling through the GIPR or down regulation of receptor expression which develops in T2DM [28–30]. An improvement in glycemic control mediated by twelve week treatment of T2DM patients with sitagliptin or by 4 weeks of insulin treatment has been shown to improve the β-cell response to GIP infusion [31,32].

2.2. Molecular mechanisms underlying DPP4 shedding

Significant DPP4 activity has been reported in many body fluids as it is proposed to be shed from cells to a circulating soluble form lacking the transmembrane domain and cytoplasmic tail [8]. Consistent with shedding and not secretion, treatment of primary human myotubes, smooth muscle cells (SMC) or adipocytes with brefeldin A had no effect on DPP4 release into the media [10,33]. Matrix metalloprotease (MMP)-2 and MMP9 were identified through a protease profiling screen to be significantly differentially expressed in adipocytes and consistent with this observation, incubation of both SMC and adipocytes with the broad spectrum MMP inhibitor, Batimastat, reduced soluble DPP4 in the media by 25–40% [10]. Recent experiments in peripheral blood mononuclear cells isolated from patients with T2DM determined treatment with the kallikrein related peptidase (KLK) inhibitor aprotinin decreased levels of soluble DPP4 by ~60%. Additionally, KLK5 was identified to increase DPP4 shedding from transfected HepG2 cells and recombinant KLK5 treatment increased shedding from T2DM patient derived CD4+ T cells [34]. DPP4 has also been demonstrated to be released by human differentiated myotubes, however acute exercise did not change serum DPP4 levels [33].

2.3. Direct signaling effects of soluble DPP4

The direct signaling effects of increased levels of soluble DPP4 have been evaluated in a number of models. Treatment of human vascular SMC with soluble DPP4 increased activation of the MAPK ^{erk} pathway and phosphorylation of NF-ƙb p65 subunit and increased secretion of the inflammatory cytokines: interleukin 6 (IL-6), IL-8 and monocyte chemoattractant peptide −1 (MCP) [35]. In addition, treatment of lipopolysaccharide-stimulated RAW 264.7 cells with soluble DPP4 increased protein levels of inducible nitric oxide synthase (iNOS) and phosphorylated JNK. Associated with these increases, media concentrations of nitrate, tumour necrosis factor (TNFα), IL-6 and IL-1β were also significantly elevated. Extension of these studies in which C57BL/6J mice were intravenously injected with soluble DPP4 (1.2 μg/mouse) demonstrated significant increases in mRNA levels of the Toll like receptors 2 and 4 in both kidney and white adipose tissue [36]. Treatment with a DPP4 inhibitor prevented these inflammatory effects suggesting catalytic activity is required [35,36]. Studies characterizing the adipocyte-enriched yellow marrow and its role in bone healing have demonstrated that adipogenic progenitor cells but not osteochondrogenic progenitor cells shed DPP4 into the medium after differentiation. In addition, treatment with soluble DPP4 impaired osteogenic differentiation but did not alter adipogenic differentiation suggesting signaling by soluble DPP4 may mediate detrimental effects on bone homeostasis [37]. Clearly, the factors regulating DPP4 shedding are complex and the impact of elevated levels of circulating DPP4 to elicit a paracrine or endocrine effect directly on metabolic tissues requires further investigation.

3. Cellular sources of DPP4: regulation of incretin cleavage and glycemia

3.1. Intestine

Understanding the molecular and cellular sites of action responsible for the clinical effectiveness and potentiation of active GLP-1 and GIP observed with DPP4 inhibition has been of significant interest. Given its traditional characterization as a gut-derived peptide hormone and the significant degradation of active GLP-1 that occurs prior to exit of the portal system, the importance of a local gut GLP-1R axis for postprandial glucose has been investigated. In fact, DPP4 expression has been specifically localized to the gut within endothelial cells (EC) immediately adjacent to the enteroendocrine L cells which secrete GLP-1 [38]. Consistent with these data, local enteral DPP4 inhibition (plasma DPP4 activity is unaffected) with orally administered sitagliptin under both chow and high fat diet conditions increases active GLP-1 and GIP levels within the portal circulation and improves glucose tolerance [24,39] (Fig. 1). Experiments in which mice were treated with the peripheral, muscarinic antagonist, atropine ± the DPP4 inhibitor NVPDPP728 decreased insulin secretion supporting DPP4 potentiation of GLP-1 action may occur through vagal signaling [40]. Consistent with this, treatment of mice with low concentrations of sitagliptin also increased vagus nerve activity which was inhibited by treatment with exendin 9–39 [24]. In order to identify the cellular source of DPP4 which is targeted to mediate these beneficial effects on postprandial glycemia, three experimental lines of mice were generated: 1) $Dpp4^{gut-/-}$ in which DPP4 within cells from the absorptive epithelium was targeted for deletion by Villin-Cre, 2) $Dpp4^{EC-/-}$ in which DPP4 in the vascular endothelium and in hematopoietic derived cells was genetically eliminated with Tie2–controlled CRE expression and 3) $Dpp4^{EC-/-(BMT)}$ in which $Dpp4^{EC-/-}$ received a bone marrow transplant from congenic donors to confine DPP4 deletion to Tie2+ cells of non-hematopoietic origin [39]. $Dpp4^{gut-/-}$ were extensively characterized compared to all control groups including CRE expressing controls [41] and no differences were observed in glucose tolerance in response to chow or HFD feeding [39]. Also, glucose stimulated, active