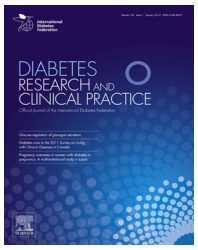
Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation



The dipeptidyl peptidase-4 inhibitor vildagliptin does not affect ex vivo cytokine response and lymphocyte function in patients with type 2 diabetes mellitus☆

P.C.M. van Poppel[a,1,*], M.S. Gresnigt[a,b,1], P. Smits[a,c], M.G. Netea[a,b], C.J. Tack[a]

[a] Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[b] Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen, The Netherlands
[c] Department of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 21 May 2013
Received in revised form
30 October 2013
Accepted 19 December 2013
Available online 3 January 2014

Keywords:
DPP-4 inhibitor
Immune function
Side effects
Type 2 diabetes

ABSTRACT

Aims: The enzyme dipeptidyl peptidase-4 (DPP-4) is a key player in the degradation of incretin hormones that are involved in glucose metabolism. DPP-4 is also expressed on immune cells and is associated with several immunological functions. Some studies have reported increased rates of infections in patients treated with DPP-4 inhibitors. We therefore assessed whether treatment with the DPP-4 inhibitor vildagliptin affected cytokine production and T-cell differentiation.

Methods: Patients with type 2 diabetes were treated with vildagliptin or an active comparator, acarbose, for four weeks, in a randomized cross-over trial. Blood was sampled at the end of each treatment period and peripheral blood mononuclear cells were isolated and stimulated with a broad spectrum of pattern recognition receptor agonists.

Results: Serum cytokine concentrations and ex vivo cytokine production (both monocyte and T-cell derived) did not differ during treatment with vildagliptin compared to acarbose. Similarly, ex vivo relative upregulation of mRNA transcription of T-cell lineage specific transcription factors was unaffected by vildagliptin treatment.

Conclusions: These data show that a four-week treatment with vildagliptin in patients with type 2 diabetes mellitus does not result in a significant modulation of cytokine responses. This observation suggests that inhibition of DDP-4 does not lead to an increased risk of infection by diminishing cytokine production.

☆ Clinical trial registration number: clinicaltrials.gov NCT01000688.

* *Corresponding author at*: 463 Department of Internal Medicine, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 243618819; fax: +31 243541734.
E-mail address: p.vanpoppel@aig.umcn.nl (P.C.M. van Poppel).
[1] These authors contributed equally to this work.

0168-8227/$ – see front matter
http://dx.doi.org/10.1016/j.diabres.2013.12.039

1. Introduction

Dipeptidyl peptidase-4 (DPP-4) inhibitors are approved for the treatment of type 2 diabetes mellitus. Inhibition of DPP-4 prevents degradation of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which results in stimulation of insulin secretion and inhibition of glucagon release, both in a glucose-dependent manner. Together, these effects improve glycaemic control in type 2 diabetes [1]. The enzyme DPP-4 is expressed on epithelial and endothelial cells in liver, lung, kidney, intestinal brush-border membranes and exists as a soluble form [2].

DPP-4 is also expressed on circulating T-, B-lymphocytes and natural killer (NK) cells, and is synonymous with the cell-surface antigen CD26 [3]. DPP-4 functions as a peptidase, acts as adenosine deaminase binding site and interacts with the extracellular matrix. DPP-4/CD26 has been suggested to be a co-stimulator of T-cell activation [2,3]. DPP-4 cleaves N-terminal dipeptides from oligopeptides. Besides incretin hormones, also chemokines and cytokines might serve as substrates [4]. Theoretically, DPP-4 inhibition may thus affect immune responses, and this could translate into an altered susceptibility to infections. Some meta-analyses focusing on side effects of DPP-4 inhibitors, have reported a slight increase in upper respiratory infections [5,6], although these reports are not consistent [7].

Recently, we completed a randomized, double-blind, active treatment controlled cross-over trial in which endothelial function after treatment with the DPP-4 inhibitor vildagliptin was assessed [8]. Here we present the results of experiments assessing the effect of DPP-4 inhibitors on the plasma concentrations of inflammatory markers, and on the ex vivo peripheral blood mononuclear cells (PBMC) responses to various pattern recognition receptors (PRR) stimuli.

2. Materials and methods

2.1. Study population

This study was combined with a trial assessing endothelial function [8]. Included were subjects with type 2 diabetes mellitus treated with metformin with or without sulphonylurea or thiazolidinediones, aged 35–75 years and a HbA1c $< 8.0\%$ (<64 mmol/mol). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The local ethics committee (Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands) approved the study and all subjects gave written informed consent before participation.

2.2. Protocol

Details of the study are described elsewhere [8]. In short: participants were randomized to receive either vildagliptin 50 mg bid for 28 days or acarbose 50 mg tid for 7 days followed by 100 mg tid for the remaining 21 days on top of their medication in a cross-over double dummy design. There was a wash-out period of 1 week. At the end of each treatment period, arterial blood was collected for PBMC isolation and stimulation.

2.3. PBMC isolation and stimulation

Blood was diluted with PBS (1:1) and fractions were separated by Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) centrifugation. PBMCs were washed twice in PBS, resuspended in RPMI (ICN Biomedicals, Costa Mesa, CA) (supplemented with 10 μg/ml gentamycin, 10 mM L-glutamine and 10 mM pyruvate), counted using a particle counter (Beckmann Coulter) and plated in 96-well round bottom plates at a concentration of $2.5 * 10^6$/ml. Cells were stimulated for 24 h with: lipopolysaccharide (LPS) (10 ng/ml) (*Escherichia coli* serotype O55:B5) (Sigma Chemical Co., St Louis, MO), Pam_3Cys (10 μg/ml) (EMC microcollections, Tübingen, Germany), β-glucan (10 μg/ml, kindly provided by Prof. David Williams, Tennessee University), a combination of Pam_3Cys (10 μg/ml) + β-glucan (10 μg/ml), heat killed (HK) *Staphylococcus aureus* ($1*10^7$/ml) and HK *Candida albicans* yeasts (ATCC MYA-3573 (UC820) strain, $1 * 10^5$/ml). A four day stimulation assay in the presence of 10% human serum was performed with RPMI or HK *C. albicans* ($1 * 10^5$/ml), and a seven day stimulation assay was performed with the stimuli: RPMI, LPS (10 ng/ml), HK *S. aureus* ($1 * 10^7$/ml), HK *C. albicans* ($1 * 10^5$/ml) and beads coated with anti-CD3/anti-CD28 antibodies ($1.25 * 10^6$/ml) (Miltenyi Biotec, Germany), for the stimulation of T-cells. After the 24 h and 7 days incubation at 37 °C and 5% CO_2, the supernatants were collected and stored at −20 °C until cytokines were measured. After four days incubation at 37 °C at 5% CO_2, cells were resuspended in 200 μl TRIzol (Sigma Chemical Co., St Louis, MO) and stored at −20 °C until RNA isolation was performed.

2.4. Quantitative RT-PCR (qPCR)

RNA was isolated according to the protocol supplied with the TRIzol reagent, mRNA (1 μg) was reverse transcribed into cDNA using iScript cDNA synthesis kit (BIORAD, Hercules, CA). qPCR was performed using Power SYBR-Green master mix (Applied Biosystems, Foster City, CA) and primers: 5′-ATG-AGT-ATG-CCT-GCC-GTG-TG-3′ (Beta2M FW) and 5′-CCA-AAT-GCG-GCA-TCT-TCA-AAC-3′ (Beta2M REV); 5′-CAA-GGG-GGC-GTC-CAA-CAA-T-3′ (T-BET FW) and 5′-TCT-GGC-TCT-CCG-TCG-TTC-A-3′ (T-BET REV); 5′-TCA-CAA-AAT-GAA-CGG-ACA-GAA-CC-3′ (GATA3 FW) and 5′-GGT-GGT-CTG-ACA-GTT-CGC-AC-3′ (GATA3 REV); and 5′-CTG-CCC-CTA-GTC-ATG-GTG-G-3′ (FOXP3 FW) and 5′-CTG-GAG-GAG-TGC-CTG-TAA-GTG-3′ (FOXP3 REV). All primers were synthesized by Biolegio (Malden, The Netherlands). PCR was performed using Applied Biosystems 7300 real-time PCR system with PCR conditions 2 min 50 °C, 10 min 95 °C followed by 40 cycles at 96 °C for 15 s and 60 °C for 1 min. The RNA levels of the T-cell transcription factors T-bet, GATA-3 and FoxP3 were corrected using the signal of housekeeping protein β2-microglobulin.

2.5. Cytokine measurements

Cytokines were measured using ELISA-kits (Sanquin, Amsterdam, the Netherlands and R&D Systems, Minneapolis, MN),