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Is there an impact of treatment with DPP-4 inhibitors on lymphocyte subpopulations in type 2 diabetic patients?

Czy leczenie inhibitorami DPP-4 ma wpływ na subpopulacje limfocytów u chorych na cukrzycę typu 2?

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Abstract

Introduction: Dipeptidil peptidase 4 inhibitors (DPP-4) are a group of antihyperglycemic agents. DPP-4 is an enzyme expressed on lymphocyte surface as co-stimulatory molecule in activation processes. The aim was to assess lymphocyte subpopulations initially and after 14 days of treatment with DPP-4 inhibitors sitagliptin, saxagliptin and vildagliptin.

Material and methods: The study was conducted in three groups 10 subjects each, of type 2 diabetic patients. In subjects studied an initial tests followed by repeated ones after 14 days of treatment with sitagliptin, saxagliptin, and vildagliptin in therapeutic doses were performed. Baseline test as well as lymphocyte subpopulations (total T cells, and T-cell subsets CD4+, CD8+, CD26+, CD45RA+, CD45RO+, CD4+/CD25+) using 7-colour flow cytometry method were performed.

Results: In patients receiving sitagliptin no significant increase in lymphocyte subpopulations were observed. In patients who received vildagliptin significant increase of total T-cells ($p < 0.05$); in patients treated with saxagliptin significant ($p < 0.05$) though mild increased percentage of total T-cells and CD4+, CD26+, CD45RO+ subsets were found.

Conclusions: The study showed mild but significant increase of several T-cell subsets after treatment with saxagliptin and vildagliptin with non significant elevation after treatment with sitagliptin. It seems that changes are not expressed enough to have a clinical impact.
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Key words: *diabetes type 2; DPP-4 inhibitors; lymphocyte subpopulations*

Streszczenie

Wstęp: Inhibitory dipeptydylo peptydazy 4 (DPP-4) są nową grupą leków hipoglikemizujących. DPP-4 jest enzymem występującym między innymi na powierzchni limfocytów, molekułą ko-stymulującą w procesach aktywacji. Celem niniejszej pracy była ocena subpopulacji limfocytów przed i po 14-dniowym leczeniu inhibitorami DPP-4 sitagliptyną, saxagliptyną i vildagliptyną.

Materiał i metody: Badanie przeprowadzono w trzech 10-osobowych grupach pacjentów z cukrzycą typu 2. U badanych wykonano badania wstępne, a następnie badania powtórzono po 14 dniach pobierania sita-, saxa- i vildagliptyny w dawkach terapeutycznych. U badanych wykonano badania podstawowe, a także oznaczono subpopulacje limfocytów (całkowite limfocyty T oraz subpopulacje limfocytów T CD4+, CD8+, CD26+, CD45RA+, CD45RO+, CD4+/CD25+; całkowite limfocyty B i subpopulacja CD26+) metodą 7-kolorowej cytometrii przepływowej.

Wyniki: U badanych otrzymujących sitagliptynę nie obserwowano znamiennego wzrostu w zakresie badanych subpopulacji limfocytów. U chorych otrzymujących vildagliptynę obserwowano istotny ($p < 0,05$), choć niewielki wzrost całkowitej puli limfocytów. Pacjenci otrzymujący saxagliptynę wykazywali istotny ($p < 0.05$), choć niewielki wzrost odsetka limfocytów T całkowitych, TCD4, CD26+, CD45RO+.

Wnioski: Badanie wskazuje na niewielki wzrost puli limfocytów T po zastosowaniu saxagliptyny i vildagliptyny, bez wpływu sitagliptyny. Wydaje się, ze stwierdzane zmiany, mimo że znamienne, są na tyle niewielkie, że nie powinny mieć znaczenia klinicznego.
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Słowa kluczowe: *cukrzyca typu 2; inhibitory DPP-4; subpopulacje limfocytów*

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Introduction

Diabetes is a serious medical and social problem [1]. The number of patients is increasing, and thus so is the number of patients affected by late complications [2]. There is therefore a need to seek new forms of therapy, which could reduce the risk of developing complications by improving metabolic control. Dipeptidyl peptidase-4 inhibitors (DPP-4) are a new group of anti-hyperglycaemic drugs whose mechanism of

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action consists of the inhibition of degradation of glucagon-like peptide 1 (GLP1) and thus increasing its concentration, resulting in increased glycaemic stimulus-induced insulin release [3, 4]. Numerous clinical studies have documented the hypoglycaemic efficacy of these drugs, both as monotherapy and combination therapy [5, 6]. The use of drugs from this group allows the reduction of HbA_{1c} by 0.6–0.8% [7]. DPP-4, also known as CD-26, is an enzyme present on the surface of most cell types, as well as on those circulating in the blood. It decomposes peptides containing proline or alanine; in addition to GLP-1, these are various growth factors, chemokines, cytokines, neuropeptides [8]. It has been reported that patients with type 2 diabetes have a reduced expression of CD26 on lymphocytes compared to healthy subjects; moreover, the expression of surface CD26 has been shown to be negatively correlated with DPP-IV activity in the lymphocytes [9].

CD26/DPP-4 has many physiological effects, including immune regulation, as a molecule transmitting co-stimulatory signals in T-cell activation processes [10, 11]. *In vitro* studies have shown to affect the immune system, associated with shifts in the lymphocyte pool [12]. Clinical observations have shown that the use of DPP4 inhibitors may increase the risk of infections, especially nasal-pharyngeal [5, 13].

Therefore, the question arises as to what extent the use of DPP-4 inhibitors in routine clinical practice may modify the immune system?

Such analysis has not been performed in an *in vivo* study so far. Since CD26/DPP-4 is expressed on a subset of normal lymphocytes, we hypothesised that the application of DPP-4 inhibitors might influence the composition of lymphocyte compartment and their function. To support our assumption, there is evidence showing clinical improvement of patients with autoimmune diseases after treatment with DPP-4 inhibitors. [14]. In contrast, in diabetic patients treated with sitagliptin, there was no effect on CD4+ T-cell activation [15].

The aim of this study was to evaluate selected lymphocyte subpopulations before and after 14 days of treatment in three groups of patients receiving therapeutic doses of saxagliptin, sitagliptin and vildagliptin.

Material and methods

This study was conducted on a group of 30 patients selected according to the following criteria: type 2 diabetes treated with insulin in monotherapy without other hypoglycaemic agents, no clinical signs of infection on clinical examination, normal levels of inflammatory markers (CRP and IL-6), no signs of liver damage (ALT < 2 x ULN), and no signs of nephropathy (GFR > 90 mL/min/1.73 m^2). Patients with known contraindications for DPP-4 inhibitors, as well as immunocompromised or allergic subjects, were excluded.

Table I. *Study group characteristics. Data presented as means ± SD*

Tabela I. *Charakterystyka badanych grup. Dane przedstawiono jako średnie ± SD*

	Saxagliptin (n = 10)	Sitagliptin (n = 10)	Vildagliptin (n = 10)
Age (years)	61 ± 17	60 ± 11	57 ± 9
Sex M/F	3/7	3/7	6/4
Duration (years)	15 ± 9	15 ± 13	9 ± 6
BMI [kg/m^2]	33.9 ± 4.9	30.8 ± 11.9	31.9 ± 13.8
HbA_{1c} (%)	9.5 ± 1.1	8.7 ± 1.2	9.5 ± 1.7
Insulin dose [IU/kg]	0.97 ± 0.31	0.69 ± 0.47	0.70 ± 0.25

BMI — body mass index; NS

The patients were randomly divided into three groups, consisting of ten persons each. Each group received saxagliptin, sitagliptin or vildagliptin respectively in therapeutic doses as indicated in diabetes management, in addition to the current insulin therapy. During the study, the insulin dose was titrated according to blood glucose levels. The characteristics of the study groups are shown in Table I. No significant differences between the groups were observed. The study was approved by the Bioethics Committee at the Medical University of Silesia.

The study was conducted in an inpatient setting. All subjects were screened and blood was collected for baseline analyses, then the study drug was added to the current antidiabetic therapy — saxagliptin, sitagliptin or vildagliptin respectively. After 14 days of treatment, blood was collected again for tests. The following assessments were performed:

— anthropometric measurements (body weight, height);
— HbA_{1c} — using the HPLC method;
— fasting blood glucose — using the immunoenzymatic method.

Immunophenotyping of each sample was performed using the 7-colour flow cytometry on a BD FACSCantoII flow cytometer. Each time, 2 mL of blood was collected on anticoagulant (EDTA) by peripheral vein puncture, then the sample was analysed within two hours of collection.

Samples were analysed using a computer program DIVA (Becton Dickinson Biosciences, San Jose, CA, USA). The method of whole blood typing with subsequent RBC lysis was used. In order to determine individual lymphocyte subpopulations, a panel of nine

Table II. *Monoclonal antibody panel for lymphocyte subpopulation assessment*
Tabela II. *Panel przeciwciał monoklonalnych do oceny subpopulacji limfocytów*

No.	FITC	PE	PerCP	PE-Cy7	APC	APC-Cy7	Pacific Blue
1.	CD45RO Dako	CD26 BD	CD3 BD	CD45RA BD	CD25 BD	CD8 BD	CD4 Biolegend
2.		CD26 BD	CD3 BD	CD19 BC			

Monoclonal antibodies used in the study: BD — Becton Dickinson, San Jose, CA, USA; BC — Beckman Coulter; Dako — Dakopatts, Glostrup, Denmark; FITC — fluorescein isothiocyanate; PE — phycoerythrin; PerCP — peridinin-chlorophyll; PE-Cy7 — phycoerythrin-cyanine 7; APC — allophycocyanin; APC-Cy7 — allophycocyanin-cyanine 7

Table III. *Subpopulations of studied T-lymphocytes in groups of patients receiving saxagliptin, sitagliptin and vildagliptin before and after 14 days of treatment. Data presented as mean percentages ± SD of total lymphocyte population*
Tabela III. *Subpopulacje badanych limfocytów T w grupach chorych pobierających saxagliptynę, sitagliptynę, i vildagliptynę przed i po 14 dniach leczenia. Dane przedstawiono jako średnie ± SD odsetków populacji limfocytów*

		Total T-lymphocytes	T-lymphocytes CD4+	T-lymphocytes CD8+	T-lymphocytes CD26+	T-lymphocytes CD45RA+	T-lymphocytes CD45RO+	T-lymphocytes CD4+/CD25+
Sitagliptin	Before	69.2 ± 5.5	45.0 ± 7.3	19.9 ± 8.1	54.0 ± 7.5	25.3 ± 8.7	38.1 ± 7.7	23.5 ± 4.7
	After	69.6 ± 6.9	45.9 ± 7.8	19.7 ± 7.1	55 ± 12.3	26.0 ± 7.6	39.2 ± 10.3	22.6 ± 8.3
Saxagliptin	Before	69.2 ± 9.4	42.7 ± 6.8	22.6 ± 6.1	51.1 ± 9.1	30.1 ± 10.6	35.7 ± 6.5	19.9 ± 5.1
	After	72.8 ± 7.8*	45.4 ± 6.9*	23.1 ± 5.3	54.6 ± 10.1*	30.5 ± 10.6	38.3 ± 6.5*	17.8 ± 4.4
Vildagliptin	Before	66.9 ± 5.1	42.2 ± 7.8	21.1 ± 6.2	45.5 ± 7.2	26.1 ± 7.7	35.7 ± 4.7	17.3 ± 8.1
	After	70.1 ± 3.9*	44.2 ± 9.5	19.7 ± 8.3	48.2 ± 8.4	25.2 ± 7.9	38.6 ± 8.5	17.8 ± 10.7

*$p < 0.05$ v. baseline value

monoclonal antibodies was used, in accordance with the standard staining protocol [16, 17]. The combinations of antibodies used in the diagnostic panel are shown in Table II.

Results

The results for T-lymphocyte subpopulations are shown in Table III, and Figure 1 illustrates an example distribution of lymphocyte subpopulations.

A small, significant increase in T-lymphocyte percentage was shown after 14 days of using saxagliptin and vildagliptin ($p < 0.05$), with no significant change with sitagliptin. In patients treated with saxagliptin, a significant increase of helper T-cells (CD4+) with memory cell phenotype (CD45RO+; $p < 0.05$) was found. In addition, the use of saxagliptin was associated with an increase in T-cells showing CD26 expression ($p < 0.05$). There were no changes in the pool of suppressor-cytotoxic T-cells (CD8+) and the population of T-cells containing regulatory cells (CD4+/CD25+).

There were no changes in the percentage of B-cells including the small subpopulation of B-cells expressing CD26.

Discussion

This study demonstrated that 14-day use of saxagliptin and vildagliptin resulted in a significant, albeit slim, increase in the entire T-cell population. A non significant increase was observed in patients receiving sitagliptin. Additionally, in patients receiving saxagliptin helper T-cells expressing the phenotype of memory cells were found. At the same time, there was an increase in the subpopulation of T-lymphocytes expressing CD26 antigen. One would expect that the inhibition of DPP-4 decreases the proportion of CD26-expressing lymphocytes in peripheral blood. What we observed was the complete opposite. Thus, inhibition of DPP-4 stimulates increase in CD26+ lymphocytes most probably to compensate in this way the inhibitory effect of the drug.

The study was conducted in type 2 diabetics treated initially with insulin only. We selected such a group of subjects because the aim was to analyse the lymphocyte subpopulation but not the antihyperglycaemic effect of DPP-4 inhibitors. Thus we decided not to include patients treated with oral drugs to avoid potential interactions.

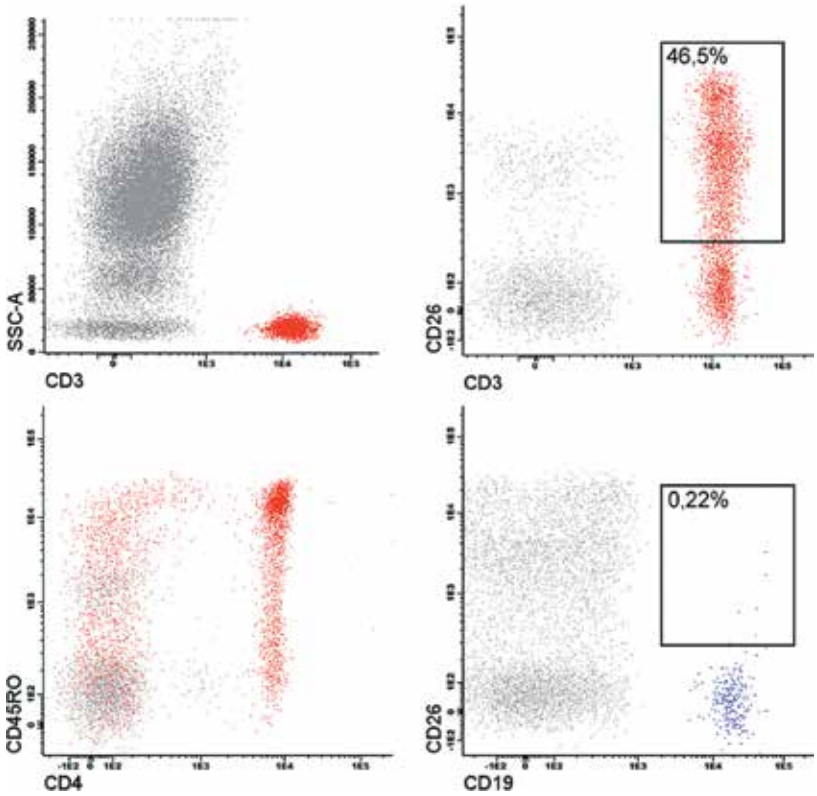

Figure 1. *Example of cytometric analysis of lymphocyte subpopulations. More than 46% of T-cells express CD26, whereas the antigen is expressed only by a small fraction of B-cells*

Rycina 1. *Przykład analizy cytometrycznej subpopulacji limfocytów. Ponad 46% limfocytów T wykazuje ekspresję CD26, podczas gdy ten antygen eksponowany jest przez niewielką frakcję limfocytów B*

In humans, CD26/DPP4 appears late in the differentiation of T-cells in the thymus, and is preferentially restricted to a subpopulation of helper/memory cells [16]. CD26/DPP4 plays a role of co-stimulator in the processes of activation. CD26 expression is the highest in CD4+ T-cells, which produce, among other substances, IL-22, IL-17, GM-CSF or TNF (Th17 cells), playing a key role in inflammatory reactions in the body [19]. The presented results indicate that DPP4 inhibition causes a discrete increase in the percentage of these populations.

In the literature, we found a single report on the effect of DPP4 inhibitors on T-cell subpopulations. White et al. evaluated CD4+ lymphocyte subpopulations in two groups of 20 patients receiving sitagliptin for half a year, or treated with other groups of hypoglycaemic drugs. These authors found no differences between the study groups [15]. Our study adds new elements of knowledge. Lymphocyte subpopulations were analysed before and after 14 days of treatment with drugs studied in the same group of patients. In addition, the study was extended to additional subpopulations of lymphocytes. Our results confirm the data obtained by the above-cited authors for sitagliptin. However, they suggest a significant, albeit small, effect of saxagliptin and vildagliptin, and non significant influence of sitagliptin. A similarly increasing trend in lymphocytes subpopulations of each drug suggests that it is a group effect rather than the influence of a particular drug.

Conclusion

The present study showed a minor increase in the lymphocyte content after treatment with DPP-4 inhibitors. It seems that the observed changes are so discrete that they might not have clinical relevance.

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