Original paper

Ustekinumab therapy changes the transcriptional activity pattern of TGF-β1–3 genes

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Abstract

Introduction: One of the examples of genes whose expression can be altered by the action of ustekinumab is TGF-β. It is a pleiotropic cytokine whose activity affects psoriatic changes and the state of homeostasis of the whole organism.

Aim: To evaluate the effect of ustekinumab on the transcriptional activity of TGF-β family genes in patients with psoriatic arthritis and to check whether the results obtained can be helpful in monitoring the progress of treatment.

Material and methods: From total PBMCs obtained from peripheral blood of 14 patients with psoriatic arthritis, total RNA was isolated. The expression level of the *TGF-β1*, *TGF-β2* and *TGF-β3* genes was determined by RT-qPCR in real time.

Results: In all the analysed samples, the presence of mRNA of three TGF-β isoforms was quantitated in each week of therapy. *TGF-β3* and the smallest *TGF-β2* showed the highest expression. Statistically significant correlations were observed in the amount of *TGF-β1* and *TGF-β3*/μg mRNA RNA, *TGF-β2* and *TGF-β2*/μg RNA and *TGF-β3* and *TGF-β3*/μg RNA.

Conclusions: Ustekinumab influences the transcriptional activity of TGF-β genes, and the changes caused have a bearing on the patient's health.

Key words: ustekinumab, *TGFβ1-3,* psoriasis arthritis, molecular marker.

Introduction

The use of biological drugs involves interference in the processes taking place in the body, which may result in the change not only of the target sites of the drug, but also the entire signal pathways. One of the examples of genes whose expression may change due to the action of ustekinumab is transforming growth factor β (TGF-β) [1, 2].

Its three isoforms (TGF-β1, TGF-β2, TGF-β3) have pleiotropic effects and their main function is the broad understanding of homeostasis maintenance [3, 4]. In the pathogenesis of psoriasis, TGF-β is responsible for releasing the cells of the Th17 pathway, which is the main pathway for inflammation [1, 2, 5]. Changing the expression of the gene encoding TGF-β under the influence of ustekinumab may contribute to disturbing homeostasis of the body, resulting in a decrease in the effectiveness of therapy or the occurrence of side effects. In mammals, 3 TGF-β isoforms are distinguished: TGF-β1, TGF-β2 and TGF-β3, which are homodimers and their quaternary structure is composed of 2 identical polypeptides [6, 7].

With respect to the skin, each isoform exhibits the highest concentration in the various layers of the skin. For TGF-β1, it is stratum corneum and granular for TGF-β2 [8, 9].

Qualification of patients for the treatment with biological drugs is not accidental. Only patients with a disease classified as moderately severe or severe are

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included in this form of therapy, in whom no improvement was observed after using at least two different methods of classical therapy at maximum doses or patients having contraindications to undertake such treatment [10, 11]. The decision about the selection of a specific biological drug is made by the doctor individually for each patient [11]. The advantage of therapy with the use of biological drugs is the fact that it is highly effective and brings a long-lasting remission of changes. It can be used in severe cases and it is also possible to re-implement it during relapse. Biologic agents also do not cause toxic effects on the liver and kidneys [12]. It should be noted that ineffective therapy with a specific biological medicine does not mean failure with another [13]. Ustekinumab has a significantly lower incidence of opportunistic infections compared to patients treated with anti-TNF therapy. In addition, its use does not affect the probability of cancer, cardiovascular disease, severe infection or tuberculosis, unless their previous absence was confirmed in a history [14–16]. Only about 5% of patients receiving ustekinumab detected the appearance of antibodies against the substance [17].

Aim

The aim of the study was to evaluate the effect of ustekinumab on the transcriptional activity of genes encoding *TGF-β1, TGF-β2* and *TGF-β3* in patients with psoriatic arthritis.

Material and methods

The approval of the Bioethical Commission of the Medical University of Silesia in Katowice (KNW/0022/KB1/59/13) was obtained for the tests in accordance with the Declaration of Helsinki on medical research with the participation of people. Patients included in the study consciously decided to participate in the project by signing the informed consent form.

Blood was collected both before treatment (week 0) and at week 16, 28 and 40. Blood samples were collected into sterile tubes containing disodium edetate, preventing blood from clotting by binding calcium ions, and then peripheral blood mononuclear cells (PBMC) were obtained.

The study included a group of 14 patients suffering from psoriasis vulgaris of both sexes (10 men and 4 women) aged 48 ±10 years who had not been biologically treated before and in whom conventional psoriasis therapy was ineffective.

Inclusion criteria:

- Patients with changes in the course of psoriatic psoriasis eligible for biological therapy.
- Patients with severe forms of psoriasis who have stopped responding to treatment, have contraindications or do not tolerate other general treatments.
- Patients who have not improved after treatment using at least two different methods of classical general therapy.
- Male and female patients.
- Patients who are 18 or over who can decide for themselves during the research.
- Patients who are able to understand the purpose and risks associated with the study, after signing the Patient Consent Form for participation in the study.
- Informed consent of the patient to use his/her biological material for scientific purposes.

Exclusion criteria:

- Persons under 18 years of age and persons over 70 years of age.
- Pregnant and nursing women.
- Hypersensitivity to the active or auxiliary drug.
- Active bacterial, viral, parasitic and fungal infection, especially tuberculosis, HIV infection, hepatitis B (HBV; hepatitis B virus).
- People with known systemic lupus erythematosus, demyelinating diseases, active cancer and severe heart failure (III and IV NYHA; New York Heart Association).
- Lack of informed consent and cooperation of the patients.
- Abuse of the drug or alcohol stated by the patient in the interview.
- Patients with a diagnosed mental illness or other disorder that may impair cooperation with the treating physician.

The biological drug ustekinumab was administered subcutaneously, at a dose of 45 mg, initially at 4-week intervals and in the subsequent stages of the study, the interval between administrations was increased. Evaluation of the expression of the *TGF-β1*, *TGF-β2* and *TGF-β3* genes was performed by Real-time Quantitative Polymerase Chain Reaction (RT-qPCR). This is the quantitative reaction of the deoxyribonucleic acid polymerase (DNA; Deoxyribonucleic Acid) with the prior rewriting of the RNA strand for complementary DNA (cDNA; Complementary DNA) using reverse transcriptase. The RT-qPCR reaction allows simultaneous monitoring of the amount of the product being created as well as the reproduction of selected fragments. After each cycle, fluorescence is measured, which increases in direct proportion to the amount of DNA in the cycle. The cycle in which the limit value is exceeded makes it possible to detect the signal. The number of cycles needed to determine the threshold cycle (CT) is inversely proportional to the amount of matrix. A set of Sensi-Fast™ reagents (Bioline, London, Great Britain) and a set of primers (Forward and Reverse) with sequences complementary to the tested genes were used to carry out the RT-qPCR reaction. For each RT-qPCR reaction, a standard curve was constructed from which the Opticon™ DNA Engine Sequence Detector sequence detector (MJ Research Inc., Watertown, MA, USA) calculated the copy number of the test mRNA in

the reaction mixture. The standard curve was determined from the RT-qPCR results of the quantitative template – a fragment of the β-actin gene (TaqMan® DNA Template Reagents Kit and β-actin Control Reagent Kit – Applied Biosystems, catalogue No. P/N 401970) in five different concentrations (from 1 to 10,000 copies of cDNA/µl). On the basis of the fluorescence curve recorded after each amplification cycle, the number of messenger RNA copies (messenger RNA) of each gene tested in the conversion to 1 µg of RNA was determined. Obtained values of the copy number of *TGF-β1*, *TGF-β2* and *TGF-β3* RNA were introduced to the Excel spreadsheet (Microsoft Office Professional Plus 2016). Next, a database was created from the results, which was implemented in the Statistica program (StatSoft, Tulsa, OK, USA, version 13.1) and a statistical analysis was made.

Statistical analysis

The normality of the data distribution was assessed on the basis of the Shapiro-Wilk test. In order to verify the differences in the studied groups, a one-way analysis of ANOVA variance with the conservative post-hoc test of the least significant difference (LSD) was used. For each conducted analysis, the most important elements of descriptive statistics were also determined: mean, standard deviation, minimum and maximum values. The statistical analysis used the general statistical significance assumed in studies of $p < 0.05$ for each of the tests.

Results

The transcriptional activity of the genes of all three types of TGF-β was found in peripheral blood mononuclear cells, in all test samples taken from patients, both before treatment (week 0) and at week 16, 28 and 40 (Table 1). Comparing the average number of copies of the TGF-β/1 µg total RNA mRNA for individual isoforms, it was demonstrated that before the start of treatment (week 0) and at week 16, 28 and 40, the highest number of copies was observed for *TGF-β3* and the lowest for *TGF-β2*. At individual points of the study, the following relationships were recorded for the number of mRNA/µg copies *TGF-β* (*TGF-β3 > TGF-β1> TGF-β2)*.

By analysing the expression profile of the *TGF-β1* gene during ustekinumab therapy, an increase in its transcriptional activity can be observed in the period from the beginning of treatment to the 28th week of therapy, and then the number of copies of the *TGF-β1* mRNA is reduced. The same relationships can be observed in the case of the *TGF-β2* gene, where we also find an increase in its transcriptional activity up to the 28th week of therapy. The amount of *TGF-β3* mRNA, as opposed to other isoforms, decreases after the initiation of ustekinumab therapy by week 16 and then shows an upward trend. Regarding *TGF-β1*, there were statistically significant differences in the expression of this gene during ustekinumab therapy using a one-way analysis of ANOVA variance ($p = 0.0085$), and their character was specified by the post-hoc Least Significant Difference test. There were sta-

Table 1. Values of descriptive statistics of mRNA/µg copy number of TGF-β1, TGF-β2, TGF-β3 in PBMCs in patients with psoriatic arthritis before treatment (week 0) and at week 16, 28 and 40 of therapy

mRNA	Week of therapy	Copy number of mRNA/1 µg total RNA.			
		Average	Standard deviation	Minimum	Maximum
TGF-β1	0	439	364	94	1115
	16	805	907	107	2792
	28	1073	517	520	2092
	40	639	410	197	1358
TGF-β2	0	28	16	7	57
	16	42	35	3	99
	28	50	28	10	97
	40	32	21	5	84
TGF-β3	0	2549	3567	266	13011
	16	2366	2764	264	9251
	28	3834	3639	13	9135
	40	5383	3476	779	12780
ACTB	0	7599	5734	1969	17075
	16	9031	8613	420	18427
	28	4898	4214	125	12402
	40	27328	14995	8696	54020

tistically significant differences in the expression of *TGF-β1* between the time before the start of treatment (week 0) and 28 weeks after drug administration (post-hoc test, $p = 0.016561$). In turn, in relation to *TGF-β2*, a one-way analysis of the ANOVA variance ($p = 0.10310$) determined changes in the expression level of this isoform as statistically insignificant. For *TGF-β3*, there were also statistically significant differences in expression (one-way analysis of ANOVA variance, $p = 0.01618$), and the post-hoc test clarified their occurrence between time before treatment (week 0) and 40 weeks of therapy ($p = 0.035379$) and between week 16 and 40 of therapy ($p = 0.031357$).

Discussion

The TGF-β family responsible for the maintenance of the broadly understood homeostasis of the body belongs to those genes whose change of expression is extremely important in psoriasis and psoriatic arthritis [18–20]. Among the various pathways that trigger the activity of TGF-β in psoriasis, the induction of Th17 pathway cells is particularly important [19]. This pathway is the main axis for the emergence of proinflammatory cytokines and, as a result, for psoriatic lesions. It is worth noting that changes in the gene expression profile of TGF-β signalling pathways and subsequent modifications in the body are still a research problem for many scientific papers [20]. However, due to the multifactorial background and complexity of processes that occur in psoriasis, scientists are constantly looking for new therapeutic options and diagnostic techniques that will enable early detection and complete cure of the disease.

According to the research conducted by Michalska-Bańkowska *et al.*, which evaluated the changes in TGF-β1, TGF-β2 and TGF-β3 expression under the influence of cyclosporine A, although the method of handling the test samples was analogous to that described in our own study, other relationships in the transcriptional activity occurring after administration of the drug may be noticed. In the study on cyclosporine A, the expression of both *TGF-β1* and *TGF-β2* after 42 days (6 weeks) decreases, so that in the next stage, it will slightly increase [21]. On the other hand, in the case of *TGF-β3*, an increase in expression was initially observed, which decreased after 42 days. In our studies in the case of ustekinumab, the number of copies of *TGF-β1* and *TGF-β2* mRNA increased in the initial treatment period (up to week 28), and in the case of *TGF-β3* up to week 16, the expression decreased gradually after 40 weeks of therapy. Despite the fact that both drugs are used in the treatment of psoriasis, the expression of TGF-β family genes showed a completely different nature of changes. This can be explained by another mechanism of action of both drugs and another test group. Gedebjerg *et al.* quantitatively compared mRNAs, including TNF-α, INF-α, IL-17 and IL-23, and the results of the PASI index in patients with psoriasis during ustekinumab therapy, with pre-therapy results and the results of patients not affected by the disease. Expression of the mRNA of the given genes was determined by the RT-qPCR technique, similarly to our own studies. With the exception of TNF-α, an increased level of expression of the examined genes was observed in the skin of the patients in relation to the healthy skin. After 4 days of treatment, the level of expression did not change, indicating that ustekinumab does not have an immediate effect on the expression of these genes, however, after 28 days, the expression was reduced in relation to the skin before therapy. After about 112 days of therapy, the expression determined in the diseased skin was comparable to that in the healthy skin [18]. The study confirms the choosing our test method was correct, however, we cannot compare the results obtained due to the different test material and the lack of information about the TGF-β genes.

Kavanaugh *et al.* conducted two-year studies on 615 patients with psoriatic arthritis during which they observed the impact and safety of ustekinumab on the course of the disease. Throughout the study, group receiving ustekinumab showed a greater reduction in the clinical symptoms of the disease, both joints and skin, and improved living standards relative to the placebo group. They also compared their findings with anti-TNF drugs and concluded that ustekinumab shows faster efficacy, measured on the PASI scale, than etanercept. This confirms that ustekinumab therapy for psoriatic arthritis is effective [4]. Papp *et al.* also compared the effects of two biological medicines: risankizumab and ustekinumab in people suffering from psoriasis and psoriasis of the joints. The comparative indicator was the PASI value and the quality of life of patients. According to research by Papp *et al.*, risankizumab is the most effective drug, which only affects the p19 subunit [22]. The dose described in the study was the same as the dose used in studies by Tsai *et al.* [23], and the PASI value has also been decreasing since ustekinumab administration [22]. Steglich *et al.* described the case of a black man with very advanced psoriatic skin and positive HBV serology. The patient was severely affected by psoriasis because his BSA score was 81% and the DLQI value was very high. Despite the positive HBV serology, Steglich *et al.* [24] decided to use ustekinumab, supporting the patient's immunity with antiviral drugs. Ustekinumab therapy not only failed to reactivate HBV, but also caused a sudden decrease in BSA to 47% after just 3 weeks of treatment [24].

Summarizing the review of the available literature and the results of our own research on the effect of ustekinumab and its effect on the expression of TGF-β genes and dependent genes, we can conclude that the results obtained in this study are consistent with the results of studies on similar subjects [25].

Conclusions

The use of ustekinumab in the treatment of psoriatic arthritis results in a change in the transcriptional activity of the TGF-β pathway genes. Analysis of TGF-β gene expression patterns in patients with psoriatic arthritis may be helpful in monitoring and determining the effectiveness of therapy.

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Conflict of interest

The authors declare no conflict of interest.

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