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Glucocorticoid-induced Changes in the Transcriptional Activity of Genes of the Innate and Adaptive Immune System in the Blood of Patients with Acute Urticaria

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Abstract

BACKGROUND: A number of the main effects of glucocorticoids (GCs) are their direct action on T cells, mainly through the transcriptional regulation: Elevated expression of immune regulatory proteins, inhibitory receptors, and reduced expression of pro-inflammatory cytokines, costimulatory molecules, and cell cycle mediators. However, controversies arise due to the clinical effectiveness of GCs in the treatment of acute urticaria (AU).

AIM: Our work aimed to study the effect of the administered GCs on the transcriptional activity of the genes of the innate and adaptive immune system in the peripheral blood in patients with AU.

MATERIALS AND METHODS: In our research, we applied a pathway-specific polymerase chain reaction (PCR) array (Human Innate and Adaptive Immune Responses RT2 Profiler PCR Array, QIAGEN, Germany) to detect and verify innate and adaptive immune responses pathway-focused genes expression in the blood of patients with AU who received treatment with GCs in addition to standard therapy.

RESULTS: Adding GCs to standard therapy did not notably affect the nature of the clinical presentation of AU, which was assessed according to the urticaria activity score scale. Analysis of the transcriptional profile of peripheral blood mononuclear cells in patients with AU against the background of GC therapy showed the induction expression of the FOXP3 and interleukin (IL)-10 genes against the background of repression of the transcriptional activity of the genes for chemokines and cytokines CCL5, CXCL8, interferon gamma, IL2, IL5, IL17A, IL1B, and tumor necrosis factor. GC-induced changes in the transcriptome also manifested by pronounced repression in genes of CD40 and CD80 (B7-1) costimulatory molecules, transcriptional regulators of Th1 cells differentiation – TBX21 and STAT1, Th17 cells – RORC, NLRP3 – inflammasome genes, and the transcription factor NFKB1 compared with the control group.

CONCLUSIONS: Adding GCs to the standard therapy of AU has a pronounced immunosuppressive potential at the transcriptome level of immune response genes in the blood; however, it does not have any noticeable clinical effect.

Introduction

Urticaria is considered a common dermatologic disorder typically manifesting in intensely itchy, well-circumscribed, raised wheals that vary in size from some millimeters to several centimeters or even larger [1]. Urticaria that resolves within a period of fewer than 6 weeks is considered acute. Second-generation H1 antihistamines are chosen as first-line medications for the treatment of acute urticarial [2]. In some cases, they may be titrated up to two or even 4 times the usual dose to control symptoms. If symptoms are not sufficiently under control with second-generation H1 antihistamines, H2 antihistamines may be put in [3]. In severe cases, the following glucocorticoids (GCs) as prednisone or prednisolone (0.5 to 1 mg/kg/day) may be considered to add for 3-10 days to control symptoms [4]. The current EAACI/GA^{2}LEN/EDF/WAO health-care recommendations in 2018 proposed to regard the use of a short course of oral GCs for up to 10 days to reduce the continuity of symptoms in acute urticarial [5]. The theoretical mechanism of GCs does not inhibit mast cell degranulation but is probably activated by suppressing a range of stimulating inflammatory processes and T-cell activations [6]. The clinical effectiveness of GCs in the treatment of acute urticaria (AU) produces controversy [7]. In truth, short courses of oral GCs may cause significant adverse events [8].

Determination of the level of gene transcriptional activity is an essential index of the functional activity of cells [9], [10], [11], [12], [13]. Our previous studies show significant changes in the transcriptional profile and genetic variations in different pathological conditions [14], [15], [16], [17], [18], [19]. Changes in some genes expression of the innate and adaptive immune system play a vital role in the mechanisms of AU development. On the other hand, the noted effect of GCs is their high ability to suppress the immune response. GCs can suppress the initiation of T cell responses by decreasing the antigen presentation, costimulation, and cytokine

production functions of innate immune cells [20]. Many of the ultimate critical effects of GCs, however, are their direct actions on T cells, primarily through transcription regulation, namely, elevated expression of immunoregulatory proteins, inhibitory receptors and apoptotic genes, and reduced expression of pro-inflammatory cytokines, costimulatory molecules, and cell cycle mediators [21]. Therefore, our work aimed to study the effect of the administered GCs on the transcriptional activity of the genes of the innate and adaptive immune system in the peripheral blood in patients with AU.

Materials and Methods

Participants and study design

For our study, we isolated peripheral blood mononuclear cells from 12 patients with AU who were administered standard treatment H1- and H2-antihistamines (control group) and 12 patients with AU who received short-course therapy of GCs injected intramuscularly (dexamethasone, 4 mg/ml, once a day, for 3 days). The diagnosis AU is based on the appearance of skin lesions. No laboratory reference standard is available. Diagnosis starts with a routine patient evaluation, which comprises a thorough history and physical examination, and the ruling out of severe systemic disease by basic laboratory tests. Physical examination of the patient includes a test for dermographism. Disease activity was determined using urticaria activity score (UAS). This simple scoring system is based on the assessment of key urticaria symptoms (wheals and pruritus). The 2018 EAACI/GA2LEN/EDF/WAO diagnosis guideline recommends using the UAS proposed in the previous version of the guideline to measure disease severity and monitor treatment results in daily practice [5]. The UAS assigns a score from 0 (no disease activity) to 3 (intense activity) for each of the two key urticaria symptoms, wheals and pruritus. The sum of the scores represents disease severity on a scale from 0 (minimum) to 6 (maximum). We excluded patients meeting the following criteria: Angioedema; anaphylaxis; the use of antihistamines or GCs within 7 days before the ED visit; and chronic urticaria.

We used a pathway-specific polymerase chain reaction (PCR) array (Human Innate and Adaptive Immune Responses RT2 Profiler PCR Array, QIAGEN, Germany) to identify and verify immune pathway-focused genes expression in randomly selected 12 individuals from each group using real-time PCR due to the procedure described below.

Experimental procedures

RNA isolation

Total RNA was isolated from white blood cells using NucleoZOL (Macherey-Nagel, Germany),

according to the manufacturer's instructions. NucleoZOL is designed for the isolation of total RNA (small and large RNA) in a single or separate fraction from a variety of sample materials, such as cells, tissue, and liquids of human or animal origin. White blood cells were lysed and homogenized in NucleoZOL reagent based on guanidinium thiocyanate and phenol.

cDNA synthesis

The RNA quality was determined and it was reverse transcribed. The concentration and quality of the isolated total RNA were determined on a NanoDrop spectrophotometer (Thermo Scientific™, USA). For the reverse transcription procedure with a cDNA conversion RT² First Strand Kit (QIAGEN, Germany, Cat. no. 330401), RNA samples with the following parameters were selected: Ratio A260/A280 within the range of 1.8–2.2. The RT2 HT First Strand Kit procedure comprises two steps: Elimination of genomic DNA contamination and reverse transcription, which enable fast and easy handling of 96 RNA samples simultaneously. After genomic DNA elimination, the RNA sample undergoes reverse transcription with an RT master mix, as well as random hexamers and oligo-dT prime reverse transcription to capture more difficult-to-detect genes.

PCR array

The cDNA was then used with RT2 Profiler PCR Array (QIAGEN, Cat. no. PAHS- 052ZA) in combination with RTI SYBR® Green qPCR Mastermix (QIAGEN, Cat. no. 330504), following the complete RT2 Profiler PCR Array procedure (www.qiagen.com). Samples were assigned to control and study groups. Cycle threshold (CT) values were normalized based on an automatic selection from the full panel of reference genes. Any Ct value >35 was considered to be a negative call. The RT2 Profiler PCR Array data analysis software calculates the fold change based on the widely used and agreed upon ΔΔCt method. The data analysis web portal calculates fold change/regulation using the delta-delta CT method, in which delta CT is calculated between the gene of interest and an average of reference genes housekeeping genes, followed by delta-delta CT calculations (delta CT [Test Group]-delta CT [Control Group]). Fold change is then calculated using a 2^ (-delta-delta CT) formula. This data analysis report was exported from the QIAGEN web portal at GeneGlobe. The software allows defining the best reference genes for normalization.

Statistical analysis of PCR array data

The RT2 Profiler PCR Array Data Analysis software does not perform any statistical analysis beyond the calculation of p-values using a Student's t-test (two-tailed distribution and equal variances between the two

samples) based on the triplicate 2^(-ΔCT) values for each gene in the experimental group compared to the control group. The Microarray Quality Control published results indicating that a ranked list of genes based on a fold change and such a p-value calculation was sufficient to demonstrate reproducible results across multiple microarrays and PCR arrays including the RT2 Profiler PCR Arrays.

Ethical approval

The ethical principles contained in the Declaration of Human Rights adopted in Helsinki, in 1975, and revised in 2008, were fully respected in our study. The subjects enrolled, voluntarily participated in this study, and completed and signed written informed consent. The protocol of the study was approved by the local ethics committees of I. Horbachevsky Ternopil National Medical University.

Results

Applying the RT2 Profiler PCR Array Human Innate and Adaptive Immune Responses, we studied the expression of 84 genes involved in the immune response. Given the active involvement of both congenital and adaptive components of the immune system in the pathogenesis of AU, we initially used an array that includes genes from several functional groups such as (1) innate immunity genes, including pattern recognition receptors, (2) adaptive immunity genes, including Th1, Th2, Th17, and Treg cells markers, (3) T-cell activation, costimulation molecules, and transcriptional factors, (4) T-cells and B-cells dependent cytokines, (5) humoral immunity genes, and (6) inflammatory response genes and other.

The use of oral GCs as an adjunct to standard therapy with H1- and H2-antihistamines did not significantly affect the nature of the clinical manifestations of AU, assessed by the UAS scale.

We had found that in patients with AU during therapy with GCs, the expression in the blood of 17 genes out of the studied 84 genes of interest significantly changed (Table 1, Figures 1 and 2). Thus, adding GCs to standard therapy led to the transcriptional induction of the FOXP3 gene, which regulates the formation of Treg cells – 13.68-fold (p = 0.007291) and the main Treg-mediated suppressor cytokine interleukin (IL)-10 – 13.81-fold (p = 0.018205) compared to the control group. The administration of GCs led to a decrease in the transcriptional activity of the genes CCL5 (3.81-fold, p = 0.00006) and CXCL8 (9.06-fold, p = 0.013104) and a number of cytokines: Th1-mediated interferon gamma (IFNG) – by 24.69-fold (p = 0.000112), Th2-mediated IL2 and IL5 (3.79-fold, p = 0.013243 and 18.89-fold, p = 0.027027, respectively), Th17-mediated IL17A – 10.87-fold (p = 0.039417), and systemic pro-inflammatory cytokines IL1B – 9.78-fold (p = 0.005831) and, more pronounced, tumor necrosis factor (TNF) – 33.66-fold (p = 0.000071). GC-induced changes in the transcriptome also manifested with pronounced repression of the genes of costimulatory molecules CD40 – by 33.04-fold (p = 0.002482), CD80 (B7-1) – by 48.93-fold (p = 0.021489); transcriptional regulators of Th1-cells differentiation – TBX21 (12.04-fold, p = 0.007368) and STAT1 (3.15-fold, p = 0.025298), regulator of differentiation of Th17 cells – RORC (14.45-fold, p = 0.000205), as well as the genes of NLRP3 – inflammasome (17.30-fold, p = 0.005008) and the transcription factor NFKB1 (6.30-fold, p = 0.000154) compared to the control group.

Fold change (2^[-Delta Delta CT]) is the normalized gene expression (2^[-Delta CT]) in the test sample (AU + GCs, AU + GCs) divided the normalized gene expression (2^[-Delta CT]) in the control sample (AU). Fold regulation represents fold change results in a biologically meaningful way.

Discussion

Several previous studies have demonstrated that GCs combined with antihistamines may influence

Table 1: Changes in the transcriptional activity of immune response genes in patients with AU on the background of GCs therapy compared with patients with AU

Gene symbol	Description	Fold change	t-test	Fold regulation
		AU + GCs/control group (AU)	p value	AU + GCs/control group (AU)
CCL5	Chemokine (C-C motif) ligand 5	0.26	0.000060	−3.81
CD40	CD40 molecule, TNF receptor superfamily member 5	0.03	0.002482	−33.04
CD80	CD80 molecule	0.02	0.021489	−48.93
FOXP3	Forkhead box P3	13.68	0.007291	13.68
IFNG	Interferon, gamma	0.04	0.000112	−24.69
IL10	Interleukin 10	13.81	0.018205	13.81
IL17A	Interleukin 17A	0.09	0.039417	−10.87
IL1B	Interleukin 1, beta	0.10	0.005831	−9.78
IL2	Interleukin 2	0.26	0.013243	−3.79
IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	0.05	0.027027	−18.89
CXCL8	Interleukin 8	0.11	0.013104	−9.06
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	0.16	0.000154	−6.30
NLRP3	NLR family, pyrin domain containing 3	0.06	0.005008	−17.30
RORC	RAR-related orphan receptor C	0.07	0.000205	−14.45
STAT1	Signal transducer and activator of transcription 1	0.32	0.025298	−3.15
TBX21	T-box 21	0.08	0.007368	−12.04
TNF	Tumor necrosis factor	0.03	0.000071	−33.66

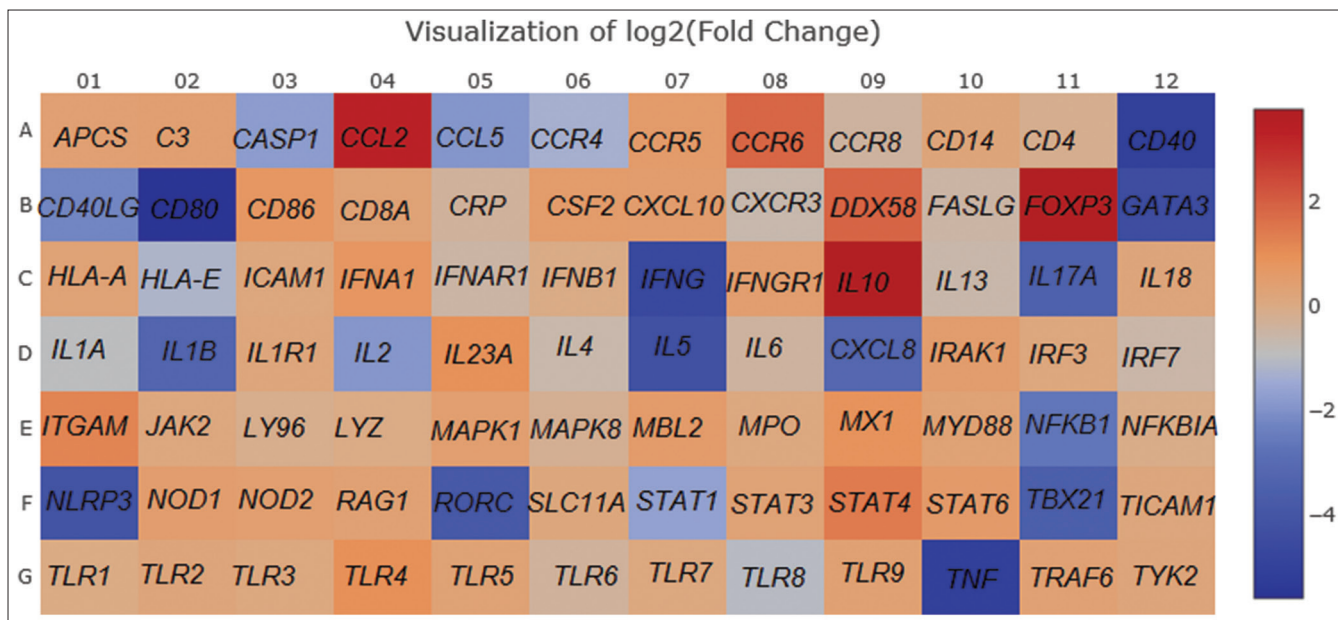GCs: Glucocorticoids, AU: Acute urticaria.

Figure 1: Heat map plot. Visualization of log2 (fold change) gene expression. Acute urticaria + glucocorticoids versus acute urticaria (control)

faster remission in AU patients [22]. Adding oral GCs are related to notable symptom improvement and lessened attack duration. Therefore, GCs are predominantly administered to emergency department (ED) patients with AU [23]. However, there are some inconsistent data regarding the effectiveness of GCs in the treatment of AU. A previous study by Barniol *et al*. (2018) of a 4-day course of prednisolone with antihistamines has not shown advancement in clinical response 2 days after attending ED [7]. A retrospective study of 2011 described 93% of 459 Italian patients visiting an ED for this condition who received GCs [23]. Another recent observational study
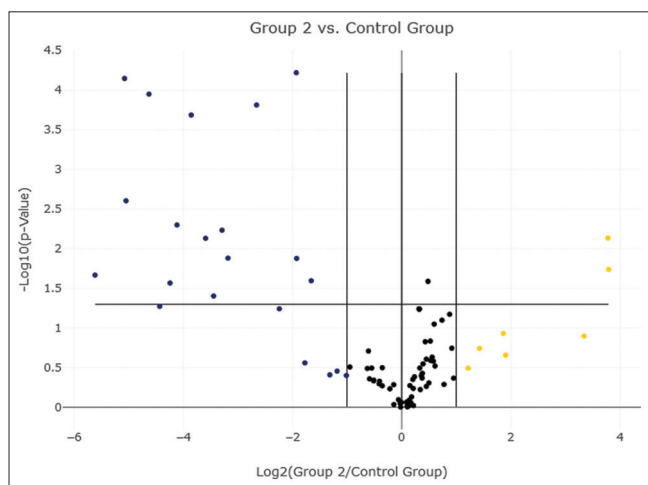

Figure 2: Volcano plot identifies significant gene expression changes by plotting the log2 of the fold changes in gene expression on the X-axis versus their statistical significance on the Y-axis. The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold. The horizontal line indicates the selected p-value threshold. Genes with data points in the far upper left (downregulated, blue) and far upper right (upregulated, yellow) sections meet the selected fold regulation and p-value thresholds. Group 2 – acute urticaria+ glucocorticoids. Control group – acute urticaria

in Canada reported frequent GCs use (48% of 2701 ED visits) to treat allergic reactions or anaphylaxis, which had no significant benefit [24]. These data coincide with our outcomes on the absence of clinically significant changes in the course of AU when GCs are added to standard therapy. Furthermore, a recent study by Arga *et al*. (2021) did not favor adding GCs to the antihistamine in treatment for severe pruritus in patients with AU [25]. The authors summarize that using GCs as an adjunctive treatment is discouraged due to the lack of clinical benefits and possible side effects. A randomized study by Javaud *et al*. (2019) is still ongoing [26].

Given the uncertain clinical benefit of GCs, we focused on elucidating their effects on the transcriptional activity of immune response genes, which could provide a rationale in searching for more targeted therapies for such conditions. GCs can suppress the initiation of T-cell responses by decreasing the antigen presentation, costimulation, and cytokine production functions of innate immune cells [6]. However, the utmost vital effects of GCs are their immediate impact on T cells, mainly through regulation of transcription, namely, elevated expression of immune regulatory proteins, inhibitory receptors and apoptotic genes, and reduced expression of pro-inflammatory cytokines, costimulatory molecules, and cell cycle mediators [27]. The GCs receptors (GRs) directly bind with nuclear factor kappa B (NF-κB) and AP-1 family proteins to inhibit their transcriptional activity [28]. The outcome is the following: GCs suppress T-cell expression of costimulatory molecules (such as CD28), cytokines (IL-2, IL-4, IL-5, IL-6, IL-8, TNF, and IFNγ), and chemokines (CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, and CCL13) [29]. Furthermore, GCs upregulate coinhibitory molecules such as PD1, CTLA4, LAG3, and TIM3 [30]. The final result demonstrates the potent suppression of T-cell effector programs.

All T-cells express GR, but different cells have different GC sensitivity. Thus, although the total impact of GC signaling is suppression of T-cell activation, differential suppression of subsets sufficiently suggests that GCs stimulate particular T-helper cell responses over others, namely, they force inhibition of Th1 cells, moderate inhibition of Th2 cells, and contribute permission for Th17 cell responses [6]. These investigation patterns are partly confirmed by our results. We observed transcriptional repression of Th1-cell differentiation regulators – TBX21 and STAT1, Th17 cells – RORC, as well as genes Th1-, Th2-, and Th17-dependent cytokines – IFNG, IL2, IL5, and IL17A.

GCs strongly inhibit Th1 cell responses. Inhibition of IL-12-induced STAT4 phosphorylation prevents its activation promoting transcriptional activity, and inhibiting STAT1 gene expression prevents IFNγ signaling, with both effects controlling Th1 cell differentiation [31]. Furthermore, GCs inhibit the expression of T-bet (Tbx21) and IFNG genes, and the GR directly binds with T-bet protein, which prevents the expression of a TH1 cell transcriptional program [32]. GCs also suppress Th2 cell differentiation, but relatively less than Th1 cell differentiation. GCs have a bare effect on IL-4-induced STAT6 phosphorylation, but GC inhibits induction of GATA3, therefore may prevent expression of IL-4, IL-5, and IL-13 [33]. GCs upregulate T-cell expression of the IL-6 and transforming growth factor beta (TGFβ) receptors and stimulate Th17 cell differentiation. Th17 cells are often refractory to GCs, which emerge partly due to increased expression of the GC exporting membrane channel multidrug resistance 1 [34]. Moreover, GCs increase RORγt expression and IL-17 production. Most commonly, GCs allow and even promote Th17 responses. The impacts of GCs on Th9, Th22, and T follicular helper cells are less marked and often conflicting [35].

Treg cell differentiation is certainly primed by GC signaling. Upregulation of TGFβ receptors, FOXP3 and IL-10, is consistent with elevated Treg cell differentiation and function [36]. GCs improvement of Treg cell function is a crucial mechanism by which endogenous GCs effect immunosuppression [37]. Our results also report the activation of the expression of the FOXP3 gene, which regulates the formation of Treg cells and the main Treg-mediated suppressor cytokine IL10, that, against the background of inhibition of the genes of costimulatory molecules CD40 and CD80, the transcription factor NFKB1, and systemic pro-inflammatory cytokines IL1B and TNF contributes to the prevalence of suppressor signals.

Conclusions

Thus, our study has shown that adding GCs to the standard treatment for AU have a pronounced immunosuppressive potential at the transcriptome level of the immune response genes in the blood; still, it does not have any noticeable clinical effect.

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