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Selected cytokines and acute phase proteins in psoriatic patients treated with cyclosporin A or Re-PUVA methods

Wybrane cytokiny i białka ostrej fazy u chorych na łuszczycę leczonych cyklosporyną A lub metodą Re-PUVA

Systemic treatment options for severe psoriasis include cyclosporin A and Re-PUVA method, both very effective immunosuppressive and antiinflammatory regimens. Cyclosporin A (CyA) by blocking the IL-2 gene transcription impairs IL-2 driven proliferation of activated Th and cytotoxic T lymphocytes (11, 19, 20). Although the primary target for CyA is CD4 T cell, functions of a lot of various types of cells are influenced secondarily, including B cells, keratinocytes, Langerhans cells, endothelial cells, macrophages, neutrophils, mast cells, basophils, fibroblasts (7, 11, 15, 19). A number of proinflammatory cytokines are directly or indirectly inhibited as a result of preventing T cell activation by CyA, among them : IL-2 and its receptor, Il-3, Il-4,IL-6, IL-8, TNF-α, IFN-γ, GM--CSF (1, 19). One of the most important effects of CyA relevant to the pathogenesis of psoriasis is suppressing the antigen presentation by Langerhans cells, macrophages and adhesion molecules' and HLA-DR' expression on the surface of papillary endothelial cells (1, 11, 15, 19). Chemotaxis within the psoriatic skin is also strongly affected by CyA. CyA can inhibit TNF-α and GM-CSF production by peripheral blood monocytes and these two cytokines are believed to be responsible for the chemotactic activity of polymorphonuclear cells (7, 19). Another mechanism of CyA in psoriasis may be preventing vasodilatation and inflammation as a result of inhibition of mast cell and basophil degranulation (15, 19).

Thus CyA prevents the egress of various inflammatory cells from vascular system by reducing adhesion molecules on the vessel surface. *In vitro* CyA inhibits keratinocyte proliferation and specifically prevents keratinocyte cell cycle progression in the G1 phase (18, 19) without significantly decreasing expression of TGF-α/EGF receptors (11). So the benefit of CyA in reducing the hyperproliferation of psoriatic epidermis may also include a direct effect on keratinocytes (19, 20).

Re-PUVA is believed to be one of the most powerful methods for very severe psoriasis, including psoriatic erythrodermia and pustular psoriasis (12, 17, 10, 12, 17). Re-PUVA can increase the efficacy

of treatment by combining the advantages of two methods and diminishing the long-term risks, such as actinic degeneration and malignant tumors (17).

Both retinoids and photochemotherapy can reduce the DNA synthesis (4, 12, 14), which leads to normalization of epidermal cell proliferation (3). Retinoids are especially effective in controlling keratinocyte differentiation and aging (5, 6, 13). Re-PUVA acts by modulating cutaneous immune responses (8). It may produce temporary alterations in number, morphology and function of Langerhans cells in involved and uninvolved tissue (4, 6, 8, 9) and cause the HLA-DR down-regulation in psoriatic skin (6, 9).

Temporary decrease in circulating T cells (especially CD4 T cells), as well as a dose-dependent inhibition of NK cell activity, were also reported following various UV regimens (4, 8, 12), but these alterations are believed to be only transient. Re-PUVA can also diminish the intensity of inflammation through its influence (mostly by retinoids) upon the granulocyte chemotaxis. It leads to limiting the neutrophils and eosinophils migration into inflammatory areas of psoriatic skin (12, 13). Beneficial effect of Re-PUVA in psoriasis may be also explaned by the influence of both photochemotherapy and retinoids on skin microvasculation through decreasing of the nitric oxide production in psoriatic skin (3, 16).

Apart from many possible mechanisms involved, the antiinflammatory and immunological efects of Re-PUVA therapy are believed to be due to inhibition of cytokines which are likely to be important in the pathogenetic events in psoriasis (2, 4, 6, 8, 9, 14).

Our study was undertaken to investigate the influence of the treatment with two potent antipsoriatic therapeutic managements: low-dose cyclosporin A and Re-PUVA method on the plasma levels of selected proinflammatory cytokines and acute phase proteins.

MATERIAL AND METHODS

Two groups of male patients were included in the study: 1) 13 patients with severe plaque type psoriasis (including 1 erythrodermic patient), mean age was 40 years, range 24-60. They suffered from psoriasis from 2 to 44 years, mean duration of disease was 17.6 years. 2) 10 patients with severe psoriasis, mean age was 39 years, range 23 – 50. They suffered from psoriasis from 4 to 30 years, mean duration of disease was 15.7 years.

The activity of psoriasis was evaluated by the same investigator according to the PASI score. In CyA group it ranged from 18.2 to 70.8; mean PASI value was 31. In Re-PUVA group it ranged from 30.2 to 65.4; mean PASI value was 39.9. Control group consisted of 20 healthy male volunteers, mean age was 39.5 years.

The following proteins were measured in plasma: a) cytokines : IL-2, sIL-2R, IL-6, TNF-α, IFN-γ, GM-CSF, b) acute phase proteins: C-reactive protein (CRP), α-2 macroglobulin (α-2 MG).

The blood samples were taken from all the patients: a) during the active phase of the disease, before the treatment was administered; and b) after clearing of psoriatic lesions following the treatment.

Measurement of the protein concentrations: An enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify the presence of the cytokines and acute phase proteins in plasma. The kits for ELISA were provided by Genzyme,USA (IL-6, TNF-α, IFN-γ, GM-CSF), Endogen, USA (IL-2), Immunotech, France (sIL-2R), Immunodiagnostik, Germany (α-2 MG), Eucardio Laboratory, USA (CRP). The measurements were done in duplicates according to the instructions included in

the assays. The absorbance was read at 405 nm (sIL–2R), 450 nm (TNF–α, IL–2, IL–6, GM–CSF, CRP), 490 nm (α–2 MG), 492 nm (IFN–γ) and the values were taken from the standard curve. Metertech 960 Microplate ELISA Reader was used for the assay.

Statistical methods: The obtained data were put to statistical analysis. Average (M), median (Me), standard deviation (SD), the mean error of the average (SE) and variation coeficient (V%) were evaluated. Significance of differences between the averages was tested by the Student's t–test or Cochran's and Cox's test.

RESULTS AND DISCUSSION

In both groups of patients : mean plasma levels of the cytokines and acute phase proteins examined before treatment were highly significantly elevated ($p < 0.001$) in psoriatic patients when compared with healthy controls (Tab. 1 and 2; Fig. 1 and 2). The after treatment concentrations of all proteins were significantly lower ($p < 0.001$) than before treatment values. The CRP and IFN–γ plasma levels despite their deep decrease following both methods of treatment, were still significantly higher in comparison with the control values (Fig. 1, 2).

Comparing these two treatment options, there were no differences in the mean levels of the investigated cytokines before treatment with cyclosporin A or Re–PUVA method. But there were significant differences in the acute phase proteins measured in both groups of patients: CRP plasma concentrations were higher and α–2 MG were lower in the Re–PUVA group. After treatment the concentrations of the proteins (except α–2 MG) were lower in the CyA group, although the level of significance was achieved only in relation to CRP and α–2 MG. It is worth to stress that the GM–CSF level was even unmeasurable due to the treatment with cyclosporin A. Therefore, it means that, although both antipsoriatic methods were very effective in reducing the plasma levels of evaluated proinflammatory proteins, cyclosporin A proved to be much more potent agent.

Beneficial effect of CyA and Re–PUVA method in the systemic treatment of psoriasis have been clearly documented (1, 7, 10, 12, 17, 20). Both therapeutical methods have the advantages but also their specific risk factors. Cyclosporin A and Re–PUVA exert immunosupressive effects and can also inhibit cell proliferation (4, 18). Both these methods are capable to affect the skin circulation and also to influence production of the acute phase proteins, possibly through the cytokine suppression. However, in contrast to the most other immunosupressive agents, CyA does not produce marrow suppression, does not inhibit phagocytosis and is not known to be mutagenic or teratogenic (1). Most of the effects of CyA on the immune system, such as inhibition of T cell proliferation, influence on generation of cytotoxic cells and down–regulation of T cell–dependent antibody production are the direct consequences of cytokine inhibition. But it appeared that specific cellular targets that mediate the therapeutic effects of CyA in psoriasis may include not only lymphocyte but also keratinocyte, Langerhans cell, mast cell, basophil and endothelial cell (7, 11, 15). The culminative effects may result in disruption of the self propagating mechanisms of inflammation in psoriasis.

Therefore, efficacy of cyclosporin A and Re–PUVA method as immunosupressive agents in decreasing the cytokine secretion in psoriatic patients confirms the pivotal role of the immune activation in pathogenesis of psoriasis.

Tab. 1. Plasma concentrations of selected cytokines and acute phase proteins in patients treated with Cyclosporin A

Parameter	Group	M	SD	p patients vs control
Age	P C	40.92 37.07	9.01 11.45	> 0.25
PASI	P	31.00	13.98	–
Duration of psoriasis (years)	P	17.69	11.16	–
IL – 2 (pg/mL)	P_1	348.09	115.10	< 0.001
	P_2	114.50	25.64	> 0.04
	C	123.34	33.04	
sIL–2R (pM)	P_1	104.32	27.35	< 0.001
	P_2	21.37	6.47	< 0.15
	C	24.42	6.95	
IFN–γ (pg/mL)	P_1	196.20	26.60	< 0.001
	P_2	132.80	8.90	< 0.001
	C	12.50	4.70	
TNF–α (pg/mL)	P_1	45.50	16.00	< 0.001
	P_2	8.00	5.40	< 0.05
	C	13.10	9.30	
IL–6 (pg/mL)	P_1	39.70	17.90	< 0.001
	P_2	2.50	4.30	< 0.05
	C	6.30	5.40	
GM–CSF (pg/mL)	P_1	29.84	11.47	< 0.001
	P_2	0.00	0.00	< 0.001
	C	4.33	4.82	
CRP (mg/L)	P_1	8.40	5.09	< 0.001
	P_2	0.60	0.41	< 0.001
	C	0.29	0.03	
α–2 MG (mg%)	P_1	659.20	407.10	< 0.001
	P_2	104.20	53.90	< 0.01
	C	129.50	42.90	

P – patients, P1 – patients before treatment, P2 – patients after treatment,
C – control, M – average, SD – Standard Deviation,
p – level of significance

Tab. 2. Plasma concentrations of selected cytokines and acute phase proteins in patients treated with Re–PUVA method

Parameter	Group	M	SD	p patients vs control
Age	P C	39.00 37.07	8.91 11.45	> 0.60
PASI	P	39.92	11.04	-
Duration of psoriasis (years)	P	15.70	9.42	-
IL - 2 (pg/mL)	P_1	365.9	70.1	< 0.001
	P_2	134.2	31.1	> 0.30
	C	123.3	33.04	
sIL-2R (pM)	P_1	124.5	19.76	< 0.001
	P_2	26.59	4.70	> 0.30
	C	24.42	6.95	
IFN-γ (pg/mL)	P_1	106.8	43.7	< 0.001
	P_2	141.2	12.6	< 0.001
	C	12.5	4.7	
TNF-α (pg/mL)	P_1	47.2	15.78	< 0.001
	P_2	12.0	5.81	> 0.70
	C	13.1	9.3	
IL-6 (pg/mL)	P_1	34.2	17.5	< 0.001
	P_2	3.2	5.18	> 0.010
	C	6.3	5.4	
GM-CSF (pg/mL)	P_1	36.8	23.72	< 0.01
	P_2	4.2	5.69	> 0.90
	C	4.33	4.82	
CRP (mg/L)	P_1	13.484	8.056	< 0.001
	P_2	1.296	0.705	< 0.01
	C	0.29	0.30	
α-2 MG (mg%)	P_1	339.5	325.8	< 0.03
	P_2	85.8	65.2	0.07
	C	129.5	42.9	

P – patients, P1 – patients before treatment, P2 – patients after treatment,
C – control, M – average, SD – Standard Deviation,
p – level of significance

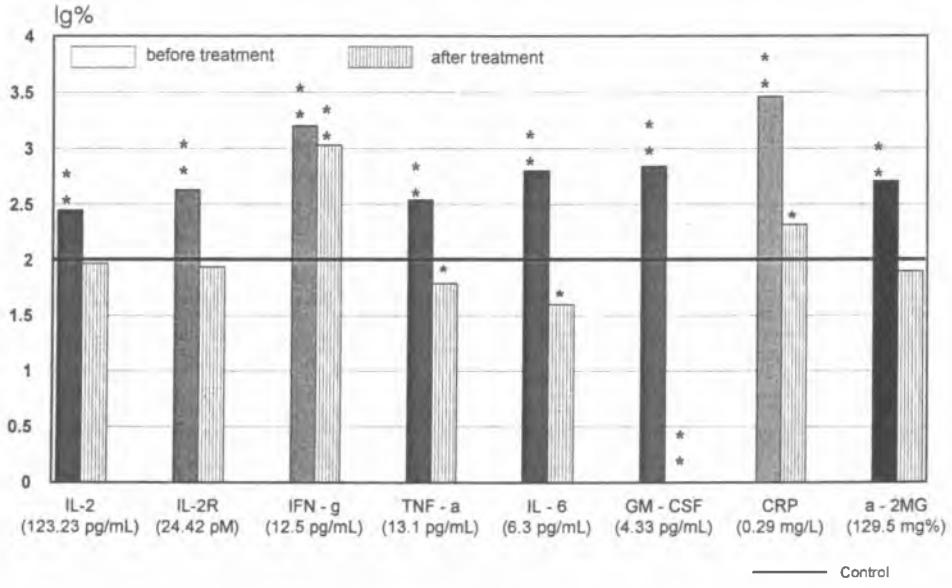

Fig. 1. Plasma concentrations of selected cytokines and acute phase proteins before and after treatment with Cyclosporin A expressed as lg% of the control values
1) control values are expressed below the respective bar, 2) significance of differences in comparison with control expressed as: * * ($p < 0.001$), * ($p < 0.01$)

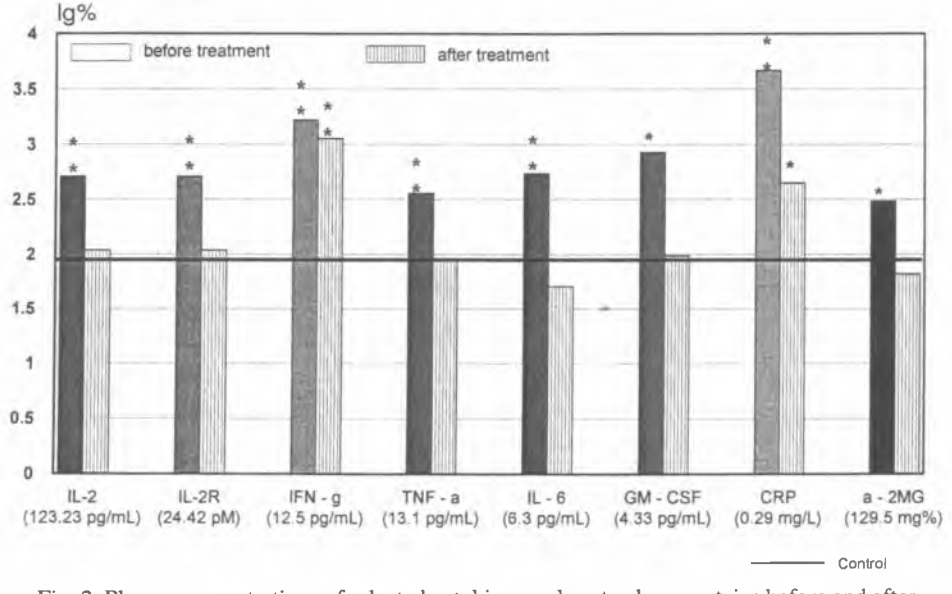

Fig. 2. Plasma concentrations of selected cytokines and acute phase proteins before and after treatment with Re–PUVA expressed as lg% of the control values
1) control values are expressed below the respective bar, 2) significance of differences in comparison with control expressed as: * * ($p < 0.001$), * ($p < 0.01$)

CONCLUSIONS

1. Clinical clearing of psoriatic lesions is associated with the significant decrease of proinflammatory proteins in plasma.

2. Cyclosporin A proved to be more potent antipsoriatic agent then Re–PUVA in reducing the plasma concentrations of measured proinflammatory proteins.

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STRESZCZENIE

Dwie grupy pacjentów z ciężką plackowatą łuszczycą leczono stosując jedną z metod ogólnych: 1) 13 chorym podawano cyklosporynę A w dawce dziennej 2,5 mg/kg. Średni czas leczenia wynosił 11 tygodni, średni wskaźnik PASI – 31; 2) 10 chorych leczono metodą Re-PUVA przez okres średnio 7 tygodni, średni wskaźnik PASI – 39,9. Badano stężenia osoczowe IL-2, sIL-2R, IFN-γ, TNF-α, IL-6,GM-CSF, białka c-reaktywnego i α-2 makroglobuliny przed i po leczeniu, posługując się metodą immunoenzymatyczną ELISA. Średnie poziomy badanych cytokin i białek ostrej fazy były znacząco wyższe przed leczeniem u pacjentów chorych na łuszczycę w porównaniu z grupą kontrolną ($p < 0,001$). Po leczeniu wartości mierzonych białek były znacznie obniżone w obu grupach pacjentów, lecz cyklosporyna A okazała się bardziej skuteczna w obniżaniu poziomu badanych prozapalnych cytokin i białek ostrej fazy. Stężenia osoczowe białka c-reaktywnego i IFN-γ, pomimo obniżenia w następstwie leczenia obiema metodami, były nadal wysoce istotnie wyższe w porównaniu z grupą kontrolną. Uzyskane wyniki wskazują na to, że chociaż osiągnięto stan klinicznej remisji, to nadal utrzymują się cechy aktywacji immunologicznej poprzedzające kolejny nawrót zmian łuszczycowych.