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Plasma activity of Interleukin-6 and some acute phase proteins in severe drug-induced skin adverse reactions

Contemporary pharmacological treatment is connected with an increasing number of undesired drug-induced reactions affecting approximately 20% of all hospitalized patients. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are regarded as the most severe cutaneous adverse drug reactions, because they are still life-threatening despite the achievements of immunosuppressive therapy (3, 6, 8, 10). Although not all pathogenetic mechanisms involved are sufficiently explained, literature data indicate the participation of immunological phenomena of cell-mediated type in the development of these two dangerous drug-induced skin diseases. Especially, the presence of activated CD8 and CD4 T-cells in inflammatory skin infiltrate taken together with clinical and histological similarity of bullous drug-induced reactions to acute graft-versus-host disease (GVHD) (3, 8, 10) are the observations of the utmost importance. Moreover, efficacy of SJS/TEN treatment with cyclosporine A additionally proves the involvement of activated T lymphocytes in pathogenic events in severe cutaneous drug-induced diseases (8, 10). Immunological reactions of all types are mediated by cytokines released from the activated cells in lesional skin. Many cell types, including T lymphocytes, macrophages, endothelial cells and keratinocytes, can be regarded as the potential source of cytokines and other immune and inflammatory mediators in the skin. A study of these proteins' activity in the drug-induced skin diseases may be useful in understanding the complex pathogenic mechanisms partaking in their origin and development. Among the cytokines, Interleukin-6 (IL-6) produced by majority of skin cells should be considered to be possibly involved in the development of SJS/TEN (1, 4, 5, 9). This cytokine, being the growth factor for T and B lymphocytes can indirectly affect the immune reactions mediated by these cells (4, 5, 9). Moreover, despite its influence upon the maturation of lymphocytes as the effector cells, IL-6 is the main activator of the acute phase protein synthesis (1, 5, 9). For, systemic symptoms observed frequently in patients, such as elevated temperature

and leukocytosis, indicate the possibility of the acute phase response being initiated in the course of severe drug-induced reactions. Among a large group of the acute phase proteins, C-reactive protein (CRP) and α-2 macroglobulin (α-2 MG) may be of special interest in SJS/TEN because of their relations with cytokines engaged in skin inflammation. The aim of this study was to evaluate the activity of IL-6 and induced by this cytokine C-reactive protein and α-2 macroglobulin in plasma of patients suffering from Stevens-Johnson syndrome and toxic epidermal necrolysis.

MATERIAL AND METHODS

9 patients, including 3 with toxic epidermal necrolysis and 6 with Stevens-Johnson syndrome were included into the study. Among them were 5 women and 4 men. Mean age of the group was 47.9 years, range 18-70. The blood samples were taken from all the patients: a) during the acute stage of the disease before the treatment was applied; b) after clearing of skin lesions following the effective treatment. Duration of treatment ranged from 17 to 29 days. Control group consisted of 30 healthy volunteers in appropriate age.

Measurement of protein concentrations was as follows: An enzyme-linked assay (ELISA) was used to detect and quantify the presence of selected proteins in plasma. The kits for ELISA were provided by Endogen Inc. USA (IL-6); Eucardio Laboratory Inc. USA (CRP); Immunodiagnostik GmbH, Germany (α-2MG). The measurements were done in duplicates according to the instructions included in the assays. Microplate ELISA Reader 960 Metertech Inc. Austria was used for the assay. The obtained data were put to statistical analysis. Average (M), median (Me), standard deviation (SD), the mean error of the average (SE) and variation coefficient (V%) were evaluated. Significance of differences between the averages was tested by the Student's t-test and Mann-Whitney's test.

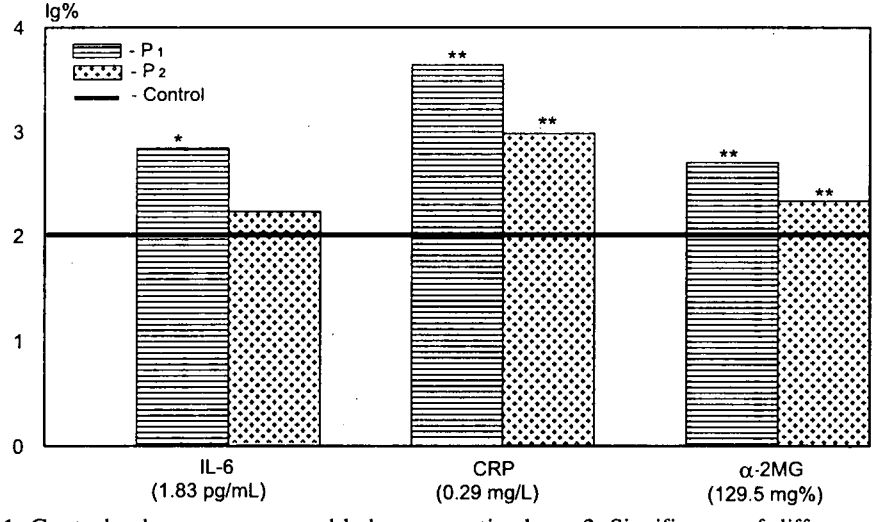
RESULTS

In the acute stage of the investigated diseases before treatment was introduced a considerable increase of plasma levels of IL6 ($p<0.05$), CRP and α-2 MG ($p<0.001$) was observed in comparison with control group (Table 1, Fig. 1). Efficient treatment caused changes of these proteins' activity. After clearing of the skin lesions the deep decrease of protein concentrations in peripheral blood was observed. IL-6 plasma concentrations were lowered towards the control values, but both CRP and α-2 MG mean plasma concentrations, despite their deep decrease after treatment, were still highly significantly elevated ($p<0,001$) when compared with control values (Fig. 2). It is worth to stress, that

Tab. 1. Plasma concentrations of IL-6, CRP and α–2 MG in 9 patients with severe drug-induced cutaneous reactions (SJS, TEN) before and after treatment

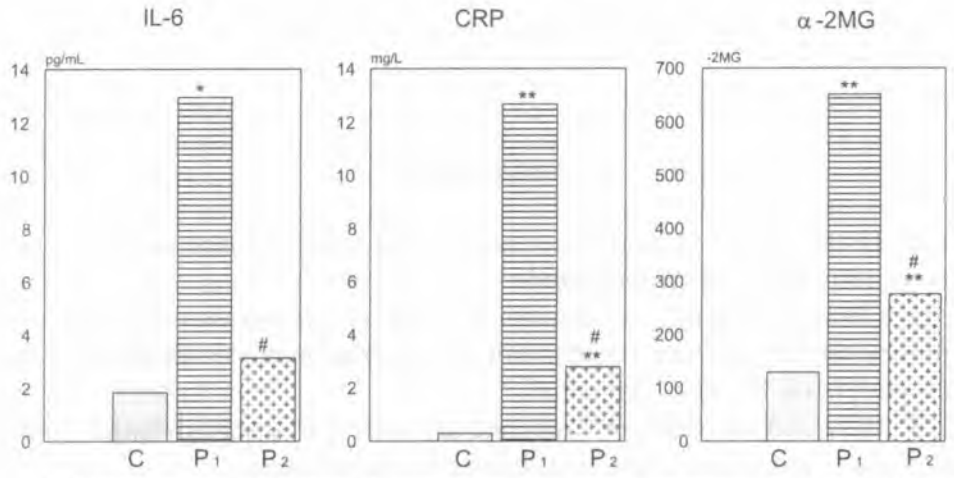
Protein	Group	Statistical characteristics						Comparison with control	
		min	max	M	SD	SE	V%	p	lg%
IL-6 (pg/ml)	C	0	5.60	1.83	1.34	0.25	73.21		2.00
	P_1	0.80	44.60	12.95	13.94	4.41	107.66	< 0.05	2.84
	P_2	0.20	12.80	3.13	3.85	1.21	123.07	> 0.05	2.23
CRP (mg/L)	C	0	0.86	0.29	0.30	0.05	102.46		2.00
	P_1	0.31	19	12.67	5.74	1.81	45.28	< 0.001	3.64
	P_2	0.32	10.47	2.79	3.07	0.97	110.17	< 0.001	2.98
α-2MG (mg%)	C	30	190	129.53	42.87	7.83	33.10		2.00
	P_1	466	740	651.60	83.95	26.55	12.88	< 0.001	2.70
	P_2	104	620	276.80	162.97	51.54	59.00	< 0.001	2.33

P1 - patients before treatment, P2 - patients after treatment, C - control



1. Control values are expressed below respective bars. 2. Significance of differences in comparison with control expressed as * $p < 0.05$, ** $p < 0.001$

Fig. 1. Plasma concentrations of IL-6, CRP and α-2 MG in 9 patients with severe drug-induced cutaneous reactions (SJS, TEN) expressed as lg% of the control values



1. Significance of differences in comparison with control expressed as * $p < 0.05$, ** $p < 0.001$. 2. Significance of differences in comparison P1 vs P2 expressed as # $p < 0.05$

Fig. 2. Plasma concentrations of IL-6, CRP and α-2 MG in 9 patients with severe drug-induced cutaneous reactions (SJS,TEN) before and after treatment

IL-6, CRP and α-2 MG plasma concentrations measured when clearing of disease was achieved were significantly lowered in comparison with acute stage, before the treatment was administered ($p<0.05$) (Figs 1, 2). It means that the activity of investigated proteins can change together with changes in the clinical stage of the patients suffering from the severe bullous drug-induced skin reactions.

CRP is regarded as the most sensitive indicator of inflammation, whose concentration may increase even more than 100 times during the first day of illness and may lower to almost normal level within only few days after clearing of inflammation (7). In the examined patients, in the acute stage of their disease 44-fold increase of CRP concentration was observed in comparison with mean control value. The possibility of the acute phase being initiated in the course of SJS and TEN seems to be especially interesting, because the cytokines involved in this reaction affect the activity of T lymphocytes both autocrinally and paracrinally. Among these cytokines, IL-6 as the main inductor of the acute phase proteins activates the synthesis of α-2 MG, which is a carrier-protein for a lot of immune mediators, among them for IL-6 (2).It creates a unique relationship between IL-6 and α-2 MG, because both these proteins may modulate each other's activity this way. It seems that α–2 MG, through its influence on distribution and activity of many cytokines may be regarded as one of the paracrinal regulators of growth and differentiation of many cells, including B and T lymphocytes (2). Increased levels of measured proteins observed in patients with drug-induced cutaneous reactions mean that they may be engaged in pathological process and moreover, changes in their concentrations following

the changes in the disease activity suggest the possibility of using these parameters in clinical practice.

CONCLUSIONS

1. In the course of severe cutaneous drug-induced reactions the acute phase response may be mobilized.
2. Clinical clearing is connected with a significant decrease of Interleukin-6, C-reactive protein and α-2 macroglobulin concentrations in the peripheral blood of patients.
3. Increased activities of examined proteins are observed longer than clinical symptoms of severe drug-induced skin diseases.

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SUMMARY

Plasma concentrations of Interleukin-6 (IL-6), C-reactive protein (CRP) and α-2 macroglobulin (α-2 MG) were examined in 9 patients with severe drug-induced cutaneous reactions (3 patients with toxic epidermal necrolysis and 6 patients with Stevens-Johnson syndrome). The activity of selected protein was measured using the immunoenzymatic ELISA method: a) in the acute stage of disease, before the treatment was applied and b) after clearing of skin lesions due to effective treatment. In the acute stage of the disease considerably increased plasma levels of IL-6 ($p<0.05$) and both acute phase proteins ($p<0.001$) were found. After clearing of clinical symptoms the concentrations of the examined proteins lowered towards control. But despite deep decrease, CRP and α-2 MG levels were still highly significantly increased ($p<0.001$) in comparison with the control values. The results of this study indicate that in severe drug-induced skin reactions the acute phase response can be initiated and that the increased activity of examined proteins is longer observed than clinical symptoms of the disease.

Aktywność osoczowa Interleukiny-6 i niektórych białek ostrej fazy w ciężkich relacjach polekowych

Badano stężenia osoczowe Interleukiny-6(IL-6), białka C-reaktywnego (CRP) i α-makroglobuliny (α-2 MG) u 9 chorych z ciężkimi reakcjami polekowymi (3 chorych z toksyczną nekrolizą naskórka i 6 chorych z zespołem Stevensa-Johnsona). Aktywność wybranych białek oznaczano w osoczu przy pomocy metody immunoenzymatycznej ELISA: a) w ostrym okresie choroby przed rozpoczęciem leczenia oraz b) po ustąpieniu zmian klinicznych skóry w następstwie skutecznego leczenia. Stwierdzono znaczące podwyższenie stężeń IL-6 ($p<0,05$) i wybranych białek ostrej fazy ($p<0,001$) w nasilonym okresie choroby oraz obniżenie poziomu badanych białek w kierunku wartości kontrolnych wraz z poprawą stanu klinicznego. Jednak pomimo znacznego obniżenia stężenia osoczowe CRP i α-2 MG po leczeniu były nadal wysoce istotnie podwyższone w porównaniu z kontrolą ($p<0,001$). Uzyskane wyniki wskazują na to, że w przebiegu ciężkich reakcji polekowych skóry dochodzi do uruchomienia odpowiedzi ostrej fazy i że podwyższona aktywność badanych białek utrzymuje się dłużej niż objawy kliniczne choroby.