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Drug-induced hyperergic vasculitis - activity of selected cytokines and acute phase proteins in plasma

Drug-induced hyperergic vasculitis belongs to the clinically differentiated group of adverse skin reactions. Although its pathogenic mechanism is complex and not entirely elucidated yet, it is believed to be basically the result of type III immunological reaction (2,3,6,8). Immune complexes interacting with vessel wall stimulate intense inflammatory response involving activated endothelial cells, cytokines and adhesive molecules (2,3,7). Damage of the vessel walls in skin and/or in internal organs is the main pathological feature of various intensity.

Skin lesions in hyperergic vasculitis are of polymorphic character mostly because different types of lesions can develop according to the vessel involved. Usually the symmetrical skin eruptions appear on the extremities, especially the lower legs. Numerous purpuric macules, bullae and tiny vesicles with hemorrhagic content developing into ulcers covered with necrotic crusts are always visible (2,3,4,6). Drug-induced hyperergic vasculitis reactions are frequently caused by sulfonamides, non-steroidal antiinflammatory drugs, psychotropic agents (1,2,3, 6,7,8). They may also be the result of the combined effects of infection/inflammation and drug (2). It is worth to consider that participation of immune complexes in pathogenic events is often connected with clinically dramatic course, which is observed for example in systemic hyperergic vasculitis, urticarial vasculitis or serum-sickness urticaria.

The aim of the present study was to evaluate the intensity of inflammatory and immune response in hyperergic vasculitis expressed as activity of some cytokines, their receptors and activated by them acute phase proteins in drug-induced hyperergic vasculitis.

MATERIAL AND METHODS

Drug-induced hyperergic vasculitis was diagnosed in 14 adult patients included into the study. Among them were 6 women and 8 men. The mean age of the group was 50.5 years, the range 18-73 years. The patients took the culprit drugs 7 to 14 days before their vasculitis appeared. All the patients received more then one offending drug, and 8 persons among them reported at least 3 various agents. In the examined group the most frequent causative drugs were nonsteroidal antiinflammatory drugs, analgetics/antipyretics and various antibiotics.

Blood samples were taken from all the patients: a) during the acute stage of disease, before the treatment was administered; b) after clearing of skin lesions following the effective treatment. Duration of treatment was 14–26 days. The control group consisted of 30 healthy volunteers of approproate age.

Plasma concentrations of the following proteins were examined: interleukin-2 (IL-2), soluble interleukin-2 receptor (sIL-2R), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), p55 soluble TNF receptor (p-55 sTNF-R), C-reactive protein (CRP), α-2 macroglobulin (α-2 MG).

Measurements of protein concentrations. An enzyme-linked immunosorbent 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 (cytokines and receptors); Eucardio Laboratory Inc.USA (CRP); Immunodiagnostik GmbH Germany (α-2 MG). The measurement were done in duplicates according to the instructions included in the assay. The obtained data were put to statistical analysis. Average (M), median (Me), standard deviation (SD), the mean error of the averrage (SE) and variation coefficient (V%) were evaluated. Significance of differences between the average values was tested by Student's t-test, Cochran's-Cox's test and Mann-Whitney's test.

RESULTS

Acute symptoms of drug-induced hyperergic vasculitis in a group of 14 patients were followed by high increase of plasma levels of the examined proteins ($p<0.001$) in comparison to the controls (Tab.1, Fig.1). Clearing of the disease lesions was connected with distinct decrease of

Table 1. Plasma concentrations of examined proteins in patients with hyperergic vasculitis and in the control group

Protein	Group	Statistical characteristics				Comparison with control			
		n	M	SD	SE	Min	Max	p	lg%
IL – 2 pg/mL	C	30	2.08	1.95	0.36	0	8.00		
	P_1	14	25.67	6.65	1.77	12,40	36.00	$p < 0.001$	3.09
	P_2	14	6.26	4.21	1.12	0.80	16.00	$p < 0.001$	2.47
sIL - 2R U/mL	C	30	253.33	106.35	19.42	96.00	42.00		
	P_1	14	2963.86	979.11	261.68	885.00	4426.00	$p < 0.001$	3.07
	P_2	14	801.64	237.91	63.58	509.00	1297.00	$p < 0.001$	2.50
IL – 6 pg/mL	C	30	1.83	1.34	0.25	0	5.6		
	P_1	14	11.48	9.36	2.50	2.50	36.40	$p < 0.001$	2.98
	P_2	14	2.39	1.87	0.50	0	5.60	$p > 0.05$	2.12
IL – 10 pg/mL	C	30	3.14	1.80	0.33	0.60	7,60		
	P_1	14	16.96	8.28	2.21	6.20	35.40	$p < 0.001$	2.73
	P_2	14	8.37	2.96	0.79	2.70	11.60	$p < 0.001$	2.43
TNF – α pg/mL	C	30	2.57	3.03	0.55	0	10.20		
	P_1	14	28.47	12.50	3.34	13.90	64.60	$p < 0.001$	3.04
	P_2	14	11.66	5.54	1.48	0.90	18.50	$p < 0.01$	2.65
p55 TNF – R pg/mL	C	30	210.50	73.97	13.50	52.00	352.00		
	P_1	14	646.71	470.12	125.65	218.00	1730.00	$p < 0.001$	2.48
	P_2	14	349.14	205.60	54.95	104.00	840.00	$p < 0.01$	2.22
CRP mg/L	C	30	0.29	0.30	0.05	0	0.86		
	P_1	14	14.15	5.13	1.37	3.65	18.86	$p < 0.001$	3.68
	P_2	14	3.72	4.20	1.12	0.78	17.56	$p < 0.001$	3.10
α - 2MG mg%	C	30	129.53	42.87	7.82	30.00	190.00		
	P_1	14	599.86	115.48	30.86	412.00	740.00	$p < 0.001$	2.66
	P_2	14	145.57	49.42	13.21	90.00	242,00	$p > 0.05$	2.05

C – control, P_1 – patients before treatment, P_2 – patients after treatment

the proteins concentrations in comparison with the active stage ($p<0.001$). T he activity of IL-6 and α-2 MG measured after treatment was not different from the control values ($p>0.05$). On the contrary, mean plasma levels of 6 other proteins, despite their deep decrease, were still significantly ($p<0.001$) or significantly ($p<0.01$) higher than those observed in the healthy control.

We believe that there are some striking findings of our study. First of all, high concentrations of measured proteins (with an exception of IL-6 and α-2 MG) remain after total clinical recovery. It suggests that biochemical parameters of inflammation and homeostasis disturbance are much more long-lasting than clinical symptoms of the disease. What is more, similar changes in activity of IL-6 and induced by this cytokine α-2 MG are also worthy to stress. Both these proteins were highly elevated in peripheral blood during the acute stage and both returned towards control values after clearing of hyperergic vasculitis. It may be especially interesting because these two proteins create a functional unit exerting influence upon each other's activity. Interleukin-6 is acknowledged as the main stimulator of α-2 macroglobulin synthesis and α-2 MG being protein carrier is a regulator of IL-6 activity decreasing or increasing its concentration in biological fluids. Moreover, increase of CRP as well as α-2 MG plasma levels observed in this study indicate that in drug-induced vasculitis the acute phase response can be activated. Some of the examined cytokines take part in the control of this dynamic process as its positive (IL-6, TNF-α) or negative (IL-10, p55 TNF-R) regulator.

Another point deserving attention is the parallel character of changes in the activity of two cytokines: IL-2, TNF-α and their soluble receptors. It may be in accordance with belief that cytokine activation is usually connected with activation of membrane receptors as well as with increase of soluble receptors for cytokines (5, 7). Soluble receptors released from cellular membrane create an essential element of immune homeostasis because they may modulate both positively and negatively the response of appropriate cytokines (5). After being released from cell,

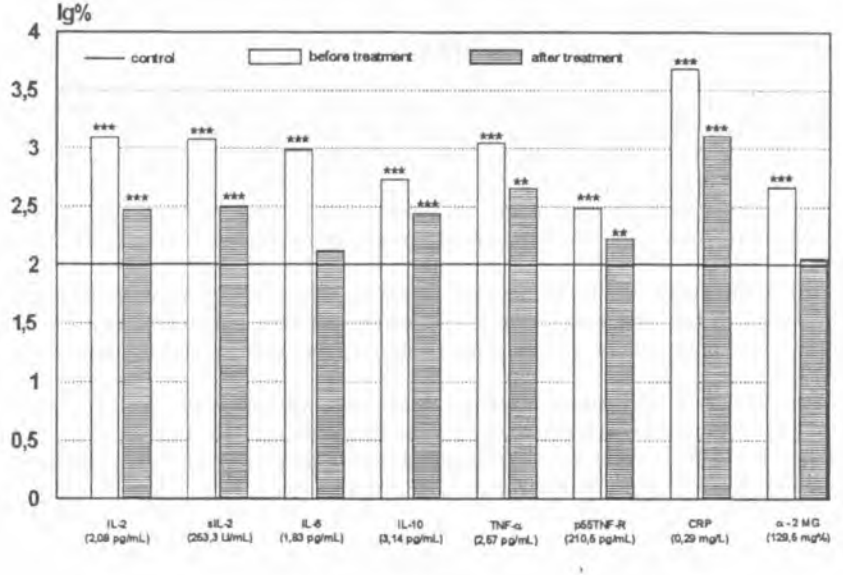

Fig. 1. Plasma concentrations of examined proteins in patients with hyperergic vasculitis before and after treatment expressed as lg% of the control values; 1) control values are expressed below the respective bars, 2) significance of differences in comparison with control expressed as *** $p < 0.001$, ** $p< 0.01$

cytokine interacts with either specific cellular receptor or soluble receptor, which can play role of both cytokine carrier/transporter or inhibitor blocking activity of appropriate cytokines (5). Presence of soluble receptors in peripheral blood and other biological fluids may exert activity of negative feedback by creating complexes with cytokines and changing their ability to bind with membrane receptors. Soluble receptors may also act as stabilizers on cytokine biochemical structure prolonging their activity. So, the results of this study support the belief that soluble receptors, unlike membrane receptors, remain usually longer in circulation and their elevated concentrations can be a more durable trace of immune activity.

The results may also strongly suggest that vessel wall damage by immune complexes, which is the basic pathogenic event in hyperergic vasculitis, is connected with the deep disturbance of immune balance and causes the engagement of systemic regulatory mechanisms.

CONCLUSIONS

1. In drug-induced hyperergic vasculitis plasma concentrations of the examined cytokines, receptors and acute phase proteins are highly elevated and they can change with the disease activity.

2. Biochemical parameters of inflammation and homeostasis disturbance are much more long-lasting than clinical symptoms of hyperergic vasculitis.

3. Prolonged increase of the examined proteins (with an exception of IL-6 and α-2 MG) in peripheral blood shows that drug-induced hyperergic vasculitis is connected with deep disturbance in immune homeostasis.

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SUMMARY

Plasma concentrations of 8 proteins, including cytokines: interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), receptors: soluble IL-2 receptor (sIL-2R), p55 soluble TNF receptor (p55 sTNF-R) and acute phase proteins: α-2 macrogloulin (α-2 MG) , C-reactive protein (CRP) were examined in 14 patients with drug-

induced hyperergic vasculitis. The activity of selected proteins were measured using the immunoenzymatic ELISA method: a) in the acute stage of disease before treatment was administered, and b) after clearing of skin lesions, after treatment. In the acute stage of disease highly elevated concentrations of the examined proteins ($p<0.001$) in comparison to the control were found. After clearing of clinical symptoms the concentrations of IL-6 and α-2 MG were not significantly different from the control values. But despite deep decrease, plasma levels of remaining six proteins were still highly significant ($p<0.001$) or significant ($p<0.01$) when compared to the control. Results of this study indicate that in the course of drug-induced hyperergic vasculitis the systemic inflammatory and immune response is activated and elevated concentrations of the examined proteins are present in peripheral blood despite clearing of the clinical symptoms of the disease.

Polekowe hyperergiczne zapalenie naczyń – osoczowa aktywność wybranych cytokin i białek ostrej fazy

Badano stężenia osoczowe 8 białek, w tym cytokin: interleukiny-2 (IL-2), interleukiny-6 (IL-6), interleukiny-10 (IL-10), czynnika martwicy nowotworów-α (TNF-α); receptorów: rozpuszczalnego receptora IL-2 (sIL-2R), rozpuszczalnego receptora p55 TNF (p55TNF-R) oraz białek ostrej fazy: α-2 makroglobuliny (α-2 MG) i białka C-reaktywnego (CRP) u 14 chorych z polekowym hyperergicznym zapaleniem naczyń. Aktywność wybranych białek oznaczano w osoczu przy pomocy metody immunoenzymatycznej ELISA: a) w ostrym okresie choroby przed rozpoczęciem leczenia oraz b) po ustąpieniu zmian chorobowych i zakończeniu leczenia. Stwierdzono znaczne podwyższenie stężeń badanych białek przed leczeniem ($p<0.001$) w porównaniu z grupą kontrolną. Po ustąpieniu objawów klinicznych stężenia IL-6 i α-2 MG nie różniły się od wartości obserwowanych w grupie kontrolnej, natomiast poziomy osoczowe pozostałych sześciu białek, pomimo znacznego obniżenia, pozostały nadal wysoce istotnie ($p<0.001$) lub istotnie ($p<0.01$) podwyższone w porównaniu z grupą kontrolną. Uzyskane wyniki wskazują na to, że w przebiegu polekowego hyperergicznego zapalenia naczyń dochodzi do uruchomienia ogólnoustrojowej odpowiedzi zapalnej i immunologicznej, a podwyższone stężenia badanych białek utrzymują się we krwi obwodowej pomimo ustąpienia klinicznych objawów choroby.