BEHAVIOR OF SERUM ADENOSINE DEAMINASE AND ITS ISOENZYMES IN PATIENTS WITH URTICARIA

ILINCA NICOLAE*, LUCIA DINU**, GABRIELA COMAN *, MARILENA CIORTEA**, LUCREȚIA DULGHERU*, ALINA MUSETESCU*, SIMONA ROXANA GEORGESCU*,***

Summary

Urticaria is a complex inflammatory disease, with often unidentified causes and incompletely elucidated etiopathogenic mechanisms. The purpose of the paper is to determine the serum profile of adenosine deaminases (total ADA, ADA-1, ADA-2), endogenous mediators possibly involved in the persistence and resolution of spontaneous urticaria. Based on the enzymatic activities determined in 84 patients with spontaneous acute urticaria, 60 patients with spontaneous chronic urticaria and 64 healthy volunteers, it was found that: two ADA-1 and ADA-2 isoenzymes were detected in the human serum, based on which the two types of urticaria can be differentiated. The authors acknowledge that modulation of adenosine deaminase activity could influence urticaria resolution.

Key words: *urticaria, adenosine deaminase and isoenzymes, histamine, adenosine, purine metabolites.*

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Introduction

Urticaria is a condition characterized by various clinical manifestations, which consist of changes in the skin and sometimes in the mucous membranes. The disease evolves with periods of exacerbations and spontaneous remissions. Urticaria should be seen as a clinical reactive pattern with multiple aetiologies and complex symptomatology. Paraclinical studies dedicated to identifying the etiopathogenic mechanisms involved in the development of urticaria are ample and include interference between immunity, inflammation, oxidative stress, neuroendocrine status. Regardless of causative agent in urticaria, there is a tissue injury that induces cell membrane permeability alteration. Mast cells, basophils and keratinocytes synthesize and secrete significant levels of histamine in the extracellular space, the key mediator in urticaria [1]. The release of histamine into the interstitial space is modulated by numerous stimuli. Adenosine is one of the endogenous mediators involved in regulating histamine release from basophils and enterochromaffin cells, in cell signalling, in immunological and inflammatory connections in injured tissues [1, 2, 3, 4, 5, 6]. The extracellular bioavailability of adenosine is limited by adenosine deaminase, the enzyme catalysing the hydrolytic deamidation of adenosine [6]. Considering the interaction between adenosine and histamine in urticaria, we can assume that adenosine release in extracellular spaces promotes a negative feedback, indicating ADA induction. ADA synthesis is stimulated in tissues subjected to acute insults, metabolic stress, hypoxia, or an inflammatory process[6]. ADA activity is accelerated by Zn2+, Co2+, estradiol, IGF-1 (insulin-like growth factor 1), simvastatin.

* Dermatovenerology Research Laboratory, Clinical Hospital for Infectious and Tropical Diseases "V Babes".
** MedLife Clinic, Bucharest.
*** University of Medicine and Pharmacy "Carol Davila", Bucharest.

In addition, ADA is inhibited by Hg 2+, Cd2+, gp120, progesterone, IL-13, curcumin, nettle, coformycin, DPP4 inhibitors [7,8,9].

Adenosine deaminase (ADA, E.C.3.5.4.4) is a Zn-dependent amino-hydrolase largely distributed in the body that has specificity for different nucleoside analogues. ADA catalyses the irreversible conversion of adenosine and deoxyadenosine to inosine and deoxyadenosine, respectively, according to the following scheme: Adenosine / Deoxyadenosine ---------ADA------→ Inozine / Deoxyiniosin--------- Phosphorylase ----→ Hypoxanthine---- Xanthine oxidase -----→ Xanthine --------- Xanthine oxidase-→ Uric acid.

ADA plays an important role in initiating a variety of cellular responses, including purine metabolism, immune system development and maintenance, epithelial cell differentiation, neurotransmission, gestation maintenance, aging, cell adhesion, immune response and metabolic stress regulation, cell signalling. ADA is a negative regulator of adenosine receptors, circadian cycle, inflammatory response, leucocyte migration, apoptotic process of mature B cells and thymocytes. ADA is an indirect modulator of histamine release from basophils, of IL-6, IL-1beta, IL-2 synthesis. ADA is a positive regulator of T cell differentiation, B cell proliferation, calcium mediated signalling, cardiac rhythm, smooth muscle contraction, regulation of ligand affinity for T cell receptors [6,7,8,9]. Two isoenzymes, designated ADA-1 and ADA-2, which differ in the nature of genetic information, kinetic properties, substrate specificity, molecular weight, tissue distribution [2,4,6,10-19], have been identified. ADA-1 is the major isomorph, the 20q11.3 gene locus, which reduces the intracellular adenosine level and prevents the lymphotoxicity of this nucleoside. It is widely spread in different tissues, with the highest concentration being recorded in the cecum and the smallest in the spleen. ADA-1 has been identified in several molecular variants (monomeric form and homodimer complex with membrane glycoproteins). The ADA-1 monomeric variant is composed of a polypeptide chain of 363 amino acid residues and a molecular weight of 38KDa, preferentially located in the cytoplasm and nucleus. The complex variant of the enzyme is composed of the ADA-1 homodimer coupled with two 300 kDa CD26/DPP4 molecules, located in the plasma membrane and oriented with the active catalytic centre towards the outside. ADA-2 is the minor isoenzyme of 110KDa, the 22q 11.2 gene locus; it has been detected in the spleen, monocytes and macrophages. ADA-2 is the dominant form of plasma and serum. ADA-1 shows increased thermal stability compared to ADA-2. ADA-2 has a lower affinity for adenosine and lower catalytic activity to deoxyadenosine compared to ADA-1 [7, 10-20].

Under physiological conditions, ADA measured in the serum is predominantly represented by ADA-2 and a small portion is represented by ADA-1. Elevated ADA levels have been reported in some dermatological conditions, such as psoriasis, lupus erythematosus, alopecia areata [9-15,19, 20].

The objective of this study is to investigate the activity of ADA isoenzymes, endogenous factors possibly involved in the maintenance and resolution of urticaria.

Material and method

Patients were selected from amongst those who had been consulted following a skin rash, an eritemato-squamous rash and a pruritic rash or a rash accompanied by a burning sensation/pain and/or subcutaneous or submucosal cellular tissue oedema [1]. Of these, there were selected 82 patients with acute spontaneous urticaria, 60 with chronic spontaneous urticaria for paraclinical determinations intended for a possible involvement of purinergic signalling in urticaria, study initiated in the Dermatology Research Laboratory of the Victor Babes Hospital for Infectious and Tropical Diseases, Bucharest.

Inclusion criteria for participation in the study:

- adults, aged over 20;
- patients diagnosed with acute spontaneous urticaria, chronic spontaneous urticaria;
- adequate nutritional status;
- no treatment for the underlying disease prior to inclusion;
- persons from whom informed consent was obtained.

Exclusion criteria for participation in the study

- chronic alcohol, drug abuse;
- treatment with corticosteroids, immunosuppressants, nutritional supplements;
- pregnancy and breastfeeding.

Laboratory tests. In addition to the microbiological, coproparasitological, allergo-logical, serological, haematological and bio-chemical analyses, there was analysed the variation of ADA, ADA-1, ADA-2 activities in the pre-therapeutic phase in patients with urticaria versus controls. ADA activity was determined by colorimetric technique [17]. The differentiation between ADA-1 and ADA-2 was achieved using a specific inhibitor (erythro-9-2-hydroxy-nonyl-adenine, EHNA) that inactivates ADA-1. Enzymatic activity was expressed in U/L serum [9.17].

Statistical analysis. The comparison of experimental results between batches for quantitative variables was performed using the t or Annova tests. Correlations between variables were established by linear regression. The presentation of the relationship between two parameters was assessed by the Pearson correlation coefficient (r). We chose a significance threshold (p) of 0.05 (5%), the 95% confidence level indicating that the decision was right.

Results

ADA activity was determined in 64 healthy volunteers, grouped by age and gender (Table 1), to determine the enzyme reference range. The ADA value determined at controls is 13.1 ± 3.2U/L serum, with no significant deviations in relation to the age groups or the sex of the examined subjects.

At controls there were no differences in ADA-1 activity based on age or sex (Table 2). The set reference range is 3.2 ± 1.2U/L serum.

The ADA-2 level measured in the control serum is invariably based on sex and age in the examined adults. The optimal reference range is 10.1 ± 2.8 U/L serum (Table 3).

Following evaluation of ADA activity in patients with urticaria, the following values were obtained: 12.6 ± 4.5 U/L serum for patients with acute spontaneous urticaria and 22.7 ± 4.6 U/L serum for patients with chronic spontaneous urticaria. The ADA level was significantly higher in patients with chronic spontaneous urticaria versus controls (p=0.009). ADA-1 showed statistically significant variations between patients with acute spontaneous urticaria and patients with chronic spontaneous urticaria (5.3 ± 1.6 U/L vs. 4.1 ± 1.8 U/L, p=0.043). The ADA-2 level was invariable in patients with chronic spontaneous urticaria. In contrast, ADA-2 was significantly higher in patients with chronic spontaneous urticaria versus controls (p=0.018). The Total ADA/ADA-1 ratio showed significant variations in patients with chronic spontaneous urticaria versus controls (p=0.007) and versus patients with acute spontaneous urticaria (p=0.028). The Total ADA/ADA-2 ratio had no statistically significant oscillations between the analysed groups (Table 4).

The analysis of the correlations between total ADA, ADA-1, ADA-2 activities in each investigated group showed a positive association between total ADA and ADA-1 ($r = 0.87$, $p<0.05$) and a negative association between ADA-1 and ADA-2 ($r = - 0.62$, $p<0.05$) only in patients with acute spontaneous urticaria (Table 5).

Discussions

On the basis of the determinations made in the present study, interesting variations of ADA activities were noted, as follows:

- ADA shows genetic polymorphism, two molecular forms being detected in the human serum, which differentiate by behaviour towards EHNA;
- Total ADA, ADA-1 and ADA-2 do not show variations depending on the sex and age of the investigated adults;
- at the pre-therapeutic time, the increases in total ADA and ADA-2 are specific to chronic spontaneous urticaria, and the increase in ADA-1 is specific to acute spontaneous urticaria;
- the ratio between total ADA and ADA-1 could be a differentiating factor between acute spontaneous urticaria and chronic spontaneous urticaria.

Based on these results, the authors conclude that by regulating the level and activity of ADA,

Table 1. Distribution of ADA activity (U/L serum) depending on age and sex at controls

Sex	Age	n	Average	Standard deviation	Minimum	Maximum
Men	20-29	6	11.9	2.0	9.9	13.4
	30-39	4	11.4	1.2	10.9	13.0
	40-49	9	12.9	1.9	11.4	15.1
	50-59	5	14.3	1.2	12.7	15.4
	60-69	2	13.7	1.0	12.6	14.8
	>70	4	13.3	1.7	11.6	14.9
		30	13.0	2.1	10.9	15.3
Women	20-29	5	12.1	1.3	10.8	13.2
	30-39	4	11.6	0.9	10.7	12.4
	40-49	10	11.8	1.2	10.7	13.1
	50-59	8	13.6	2.4	11.7	16.2
	60-69	4	14.7	1.0	13.1	15.7
	>70	3	13.9	0.8	13.0	14.8
		34	13.3	2.3	10.6	15.7
Total		64	13.1	3.2	9.9	16.2

Table 2. Distribution of ADA-1 activity (U/L serum) depending on age and sex at controls

Sex	Age	n	Average	Standard deviation	Minimum	Maximum
Men	20-29	6	2.9	0.5	2.3	3.4
	30-39	4	3.4	0.3	3.2	3.7
	40-49	9	3.2	1.0	2.1	4.1
	50-59	5	3.3	0.2	3.1	3.6
	60-69	2	2.8	0.1	2.8	2.9
	>70	4	2.9	0.5	2.4	3.5
		30	3.1	1.0	2.1	4.1
Women	20-29	5	2.9	0.3	2.6	3.2
	30-39	4	3.6	0.6	3.1	4.2
	40-49	10	3.3	0.4	2.9	3.8
	50-59	8	3.3	1.0	2.3	4.0
	60-69	4	3.1	0.6	2.5	3.8
	>70	3	2.9	0.2	2.7	3.1
		34	3.3	1.1	2.2	4.2
Total		64	3.2	1.2	2.1	4.2

Table 3. Distribution of ADA-2 activity (U/L serum) depending on age and sex at controls

Sex	Age	n	Average	Standard deviation	Minimum	Maximum
Men	20-29	6	9.0	1.2	7.6	10.0
	30-39	4	8.0	1.3	7.3	9.3
	40-49	9	9.8	0.8	9.3	11.0
	50-59	5	11.0	1.6	8.6	12.8
	60-69	2	10.9	1.1	9.8	11.6
	>70	4	10.4	1.2	9.2	11.4
		30	9.9	2.2	7.6	12.8
Women	20-29	5	9.2	1.0	8.2	10.0
	30-39	4	8.0	0.6	7.4	8.3
	40-49	10	8.2	1.3	7.0	9.3
	50-59	8	10.2	1.2	9.4	12.2
	60-69	4	11.6	1.2	10.4	12.6
	>70	3	11.0	0.7	10.3	11.7
		34	10.2	1.7	7.4	12.2
Total		64	10.1	2.8	7.4	12.8

Table 4. ADA activity (U/L serum) in patients with acute spontaneous urticaria, patients with chronic spontaneous urticaria and controls

ADA (U/L serum)	Acute urticaria (82 cases)	Chronic urticaria (60 cases)	Controls (64 cases)
Total ADA	12.6 ± 4.5	22.7 ± 4.6*	13.1 ± 3.2
ADA-1	5.3±1..6 **	4.1 ± 1.8	4.7 ± 1.2
ADA-2	7.3 ± 3.9	18.6 ± 4.3 *	8.4 ± 2.9
Total ADA/ADA-1	2.38 ± 0.22	5.54 ± 0.57 */**	2.79 ± 0.31
Total ADA/ADA-2	1.73 ± 0.20	1.22 ± 0.14	1.56 ± 0.18

* $p < 0.05$ acute or chronic urticaria versus controls, ** $p < 0.05$ acute urticaria versus chronic urticaria.

Table 5. Statistical relations between total ADA, ADA-1, ADA-2 in the studied batches

Pair variables	Acute urticaria		Chronic urticaria		Controls	
	R	p	r	p	r	p
Total ADA/ADA-1	0.870	0.005	0.103	0.637	0.011	1.00
Total ADA/ADA-2	0.068	0.977	0.09	0.798	-0.094	0.998
ADA-1/ADA-2	-0.620	0.006	-0.033	0.899	0.042	0.966

adenosine modulates the immune and inflammatory response in the urticarial process. The level of extracellular adenosine is very low in tissues, and its release from cells is accelerated in acute urticaria in response to various stimuli. The acute increase of adenosine is required to limit excessive inflammation, but high levels of adenosine become toxic [6, 9, 20]. Adenosine modulates cellular responses via specific receptors expressed on T cells, mast cells and macrophages, on endothelial cells, on neutrophils, on dendritic cells. Blocking of endogenous adenosine signalling exacerbates immune activation and aggravates tissue dysfunction as a result of harmful acute stimulation [3,4,6,9,20]. The induction of ADA in urticarial lesions could be a mechanism for eliminating toxic substances to suppress unwanted effects on tissues.

In addition, adenosine turnover is extremely rapid. Adenosine is formed both in the extracellular and in the intracellular space. Tissue hypoxia and ischemia stimulate 5-nucleotidase, suppress adenosine kinase and accelerate intracellular synthesis of adenosine. After accumulation of increased amounts of adenosine in the intracellular space, adenosine is transferred outside the cells with the help of nucleoside transporters. In the extracellular space, adenosine synthesis increases by stimulation of CD39 (DNTP) and CD73 (5-nucleotidase), enzymes that degrade nucleoside precursors released from cells [2,6,18]. The extracellular bioavailability of adenosine is limited by adenosine deaminase. In the human skin, ADA has been identified in the dermis and epidermis, having a higher activity in the superficial layer. It is believed that ADA participates in the skin immune function [18]. In alopecia areata lesions, ADA activity and oxidative stress are significantly elevated compared to normal scalp. The increase in ADA in these lesions may be due to the infiltration of T lymphocytes, hyperproliferation and cell differentiation. In alopecia areata, ADA is therefore involved in cell-mediated immunity [19]. Other reports have highlighted the increase in ADA in patients with psoriasis prior to treatment and the reduction in enzyme activity after treatment with PUVA and cyclosporin. These results show that ADA is related to disease activity [20].

Conclusions

Elevated ADA levels have been reported in patients with spontaneous urticaria. The ADA isoenzymes detected in the patients' serum behave differently depending on the type of urticaria investigated. The increases in total ADA and ADA-2 are specific to chronic spontaneous urticaria, and the increase in ADA-1 is specific to acute spontaneous urticaria. Finally, the authors state that the therapeutic modulation of adenosine deaminases could influence the resolution of urticaria by: regulating histamine secretion, mitigating inflammation, regulating immune response, removing purine and deoxypurine metabolites accumulated in the skin.

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Conflict of interest
NONE DECLARED

Correspondance address: Gabriela Coman
email: noime85yahoo.com