Plastic Materials as Potential Triggers in Chronic Diseases

ILINCA NICOLAE[1], LUCIA DINU[2], CORINA-DANIELA ENE (NICOLAE)[3]*, CLARA MATEI[3], MIRCEA TAMPA[1,3], SIMONA ROXANA GEORGESCU[1,3]

[1] Victor Babes Hospital of Clinical and Infectious Diseases, Research Department of Dermatology, 281 Mihai Bravu, Bucharest, Romania

[2] Medlife Clinic, Bucharest, 1 Hans Christian Andersen, 040289, Bucuresti, Romania

[3] University of Medicine and Pharmacy Carol Davila, Bucharest, 8 Eroii Sanitari Blv., 060474, Bucharest, Romania

A number of epidemiological, clinical and experimental studies show that cholinergic stimulation induced by the contact between human body and several plastic materials plays an important role in the pathogenesis of diseases. Extraneuronal acetylcholine mediates the reduction of cytokines synthesis, the increased synthesis and release of histamine from dermal mast cells or basophils, and the suppression of the inflammation. Based on these considerations, the authors proposed to verify the importance of acetylcholine in the mechanism of generation of the inflammatory response, using Helicobacter pylori infection as an experimental model for systemic inflammation. We also studied the impact of antihistamines/anti-Helicobacter pyori treatment on acetylcholine levels in chronic idiopathic diseases. Determination of the inflammation tests and the cholinesterase activity, as the level of non-neuronal acetylcholine, showed a significant association between cholinesterase activity, inflammatory response and therapeutic efficacy. In conclusion, evaluation of the inflammation tests and the cholinesterase activity could be an adjuvant factor for therapeutic management in chronic idiopathic diseases subjects.

Keywords: plastic materials, inflammatory response, non-neuronal acetylcholine, cholinesterase activity

The use of plastics has become a global scourge. Plastics are some of the most widely used materials worldwide and a significant number of workers are employed in the plastic industry [1]. Plastic materials can be successfully used in the packaging, leather, footwear and textiles industry and also in consumer goods, pharmaceuticals, medicine, agriculture. Plastic material composition is varied. The plastic industry uses a number of chemicals which range from the monomers used to manufacture the plastic resins themselves, to additives that are necessary to impart certain characteristics to the final plastic product. Some of these additives include:

- antioxidants (alkylated phenols, amines, organic phosphites and phosphates, and esters);
- lubricants (stearic acid, waxes, fatty acid esters and fatty acid amines);
- antistatics (quaternary ammonium compounds, anionics and amines);
- blowing/ foaming agents (azodicarbonamide, modified azos and 4,4'-oxybis (benzenesulfonyl hydrazide));
- colourants (titanium dioxide, iron oxides, anthraquinones, and carbon black); heat stabilizers (lead, barium-cadmium, tin and calcium-zinc);
- organic peroxides (methyl ethyl ketone peroxide, benzoyl peroxide, alkyl peroxide and peresters);
- flame retardants (antimony trioxide, chlorinated paraffins and bromophenols); plasticisers (adipates, azelates, trimellitates and phthalates)
- ultraviolet stabilisers (benzophenones, benzotriazole and salicylates) [2].

Currently, the most challenging properties of plastic materials used in the medical field are: biocompatibility and their toxic and antigenic potential.

Workers can be exposed to these chemicals at one or more of the several stages of plastic production [1].

From our experience, contact dermatitis is the most common occupational skin disease, but contact diseases, skin cancer and infectious may also be caused by occupational exposure.

Contact diseases is common in occupational settings and its prevalence should be expected to increase because of workers increasing exposure to a variety of industrial materials [3].

While occupational contact dermatitis is an eczematous eruption caused by irritation or a type 4 response (delayed type hypersensitivity) to a workplace agent, contact diseases are an immediate reaction that occurs within minutes after exposure to an allergen. The majority of cases of occupational contact dermatitis are irritant, only 20-25% being allergic [4].

The mechanisms underlying immediate reactions in contact diseases are divided into two main types: immunologic and nonimmunologic. However, there are substances that cause immediate contact reactions, whose mechanisms are not kown.

Immunologic contact diseases is a type 1 hypersensitivity immunologic reaction in individuals who have previously contacted the causative agent and synthesized specific immunoglobulin E against that agent. IgE molecules react with IgE receptors on the mast cells, basophils, eosinophils, Langerhans' cells and other cells. Within minutes, histamine, exoglycosidases, neutral proteases and proteoglicans are released from mast cells, resulting in an immediate skin response. Massive amounts of these mediators lead to anaphilaxis [3]. Most cases of contact diseases are type 1, Ig E-mediated type. Atopics are more prone to develop this type of contact diseases. An important cause is rubber latex. Unlike allergic contact dermatitis to rubber, due almost exclusively to the antioxidant chemicals and to vulcanizing agents used in the manufacturing process, immunologic contact diseases is caused by the rubber latex itself [5].

Other frequent causes of immunologic contact diseases include phthalic anhydride, methylhexahydrophthalic

email: koranik85@yahoo.com

anhydride and methyltetrahydrophthalic anhydride which are used as curing and hardener agents for epoxy resins.

Nonimmunologic contact diseases occurs in individuals non sensitized to the contactant (almost any normal subject). The mechanism of action consists in a direct release of vasoactive substances which cause a localized reaction [3].

The pathogenesis of nonimmunologic contact diseases is not completely clear, being probably multifactorial. It is belived that this reaction is mediated by prostaglandins. Substance P from the axons of unmyelinated C fibers of sensory nerves may also contribute to the pathogenesis of nonimmunologic contact diseases. Low molecular weight chemicals like aldehydes and weak acids and their salts can cause nonimmunologic contact diseases [5].

The appearance of the clinical signs depends mainly on the concentration of the contactant, the duration of exposure and rubbing or scratching [3].

In the context of immediate response to chemicals, dermal vessels dilate, skin become red and nerve endings are excited.

Simultaneously appears the inflammation, which includes a number of local (metabolic) and systemic (endocrine, hematologic, hepatic, immunologic) changes. To this process take part cellular components that accumulate at the site of inflammation, molecular factors derived from complement system, lipid-derived chemotactic factors, chemokines, cytokines.

Inflammatory response and *hypothalamic-pituitary-adrenal axis (HPAA) impairment* plays a crucial role in the pathogenesis of diseases skin lesions (hives) [6-23], this statement being supported by numerous clinical and experimental observations. Chronic diseases is common in subjects with bacterial [6] or viral [7] infections associated with a systemic inflammatory status, without being detected a causal relationship between this infection and the onset of diseases. Sometimes, hives are triggered [8,9] by overloading the nerves and do not respond to antihistamines, being relieved by anxiolytic medications.

Hives [6-10] are produced by dermal edema induced by histamine, heparin, serotonin (released from mast cells or basophils) and acetylcholine, kinin, prostaglandins, chemotactic factors for neutrophils, eosinophils, lymphocytes (cells that accumulate at the site of the wheal). Atropine competitively blocks histamine secretion mediated by acetylcholine, from mast cells and basophils, this statement being explained by the presence of the cholinergic receptors on these cells [9]. Acute stress and intradermal administration of CRH (corticotropin-realising hormone) stimulates skin mast cells, increases vascular permeability and regulates the activity of the histidine decarboxylase, the enzyme that mediates the production of histamine [8,10]. CRH and ACTH (adrenocorticotropic hormone) activates basophils degranulation, cells which release vasoactive substances, including histamine [11].

CRH, urocortin derivatives of POMC (proopiomelanocortin) and the corresponding receptors are expressed in different cells of the skin (keratinocytes, hair follicles, sebocytes, monocytes, mast cells, melanocytes, fibroblasts). These factors modulate proliferation, differentiation, cell apoptosis and exert pro- and anti-inflammatory activity in the skin [12].

Chronic diseases can be triggered by emotional stress by exacerbating dendritic cell function [13] and also cholinergic agonists (methacholine) cause hives.

Mice exposed to bacterial lipopolysaccharide shows a reduction in the number of circulating lymphocytes and an increase in cholinesterase activity in early inflammation, followed by a decrease in this enzyme activity [10-12,14]. Acetylcholine (fig. 1a,1b) has an important role in reducing the secretion of cytokines (IL-1, IL-2, IL-6, IL-18, TNF, IFN, NF-kB macrophage migration inhibitor factor, the high mobility group box-1 protein, without affecting the production of IL-10) and releasing vasoactive substances.

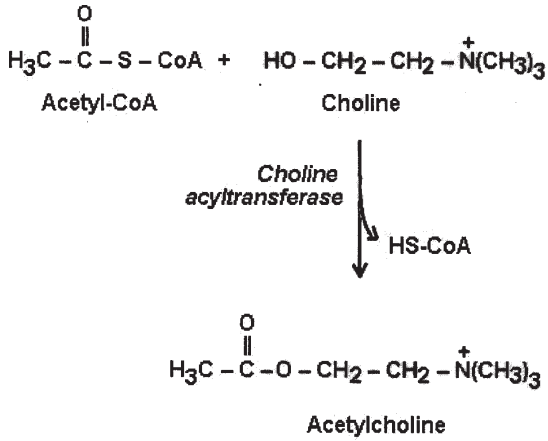

Fig. 1a. Biosynthesis of acetylcholine. Acetylcholine is the ester formed by the esterification reaction of acetyl coenzyme A and choline, catalyzed by choline acyltransferase. (EC 2.3.1.6.)

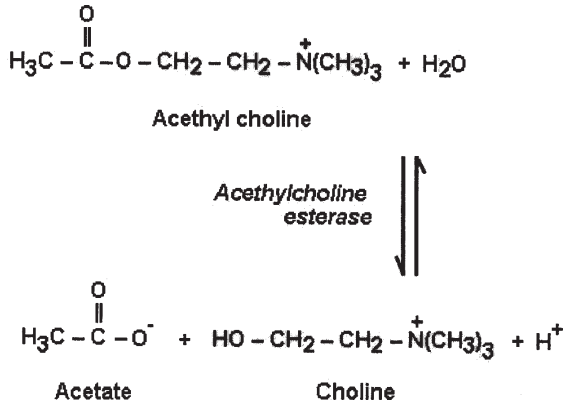

Fig. 1b. Degradation of acetylcholine is accomplished by hydrolysis, catalyzed by acetylcholinesterase (EC 3.1.1.7) and pseudocholinesterase (EC 3.1.1.8). The end-products of the reaction are choline and acetic acid

Suppression of skin inflammation, mediated by acetylcholine, is due to its interaction with cholinergic receptors located on macrophages, fibroblasts, epithelial cells, mast cells, lymphocytes [15-17]. Sensory nerve fibers, located at the *dermoepidermal junction,* form a functional unit with mast cells, contributing to the regulation of neurogenic inflammation. Mast cells can function as sensors between emotional stress and environment [10-12,18,19]. These data show a direct involvement of acetylcholine in the pathogenic mechanism of diseases, by enhancing the release of histamine from dermal mast cells and regulating the inflammation.

There are few research and major studies are lacking to evaluate cholinergic disturbances in chronic idiopathic diseases (CIU). In this study we discuss the changes in butyrylcholinesterase (BChE) level, as a measure of extraneuronal acetylcholine level, depending on the disease severity score (UAS) and the magnitude of the inflammatory process, and also the correlation between these changes and the effect of anhistamines/anti-Helicobacter pylori treatment in subjects with chronic idiopathic diseases complicated/not with systemic inflammation (associated with an infection caused by Helicobacter pylori).

Experimental part

Material and Methods

We conducted a prospective study, which included 67 subjects aged 18 and over, with chronic idiopathic diseases (CIU). The study was made between December 2008 and December 2012 using Helicobacter pylori infection (HP) as an experimental model for systemic inflammation.

Subjects were divided into 3 groups depending on the presence/absence of anti-HP antibodies and the treatment protocol:

Group A included 23 HP-negative subjects, UAS=5.17±0.63, who received H1-antihistamines treatment;

Group B included 24 HP-positive subjects, UAS=5.26±0.73, who received H1-antihistamines treatment;

Group C included 20 HP-positive subjects, UAS=5.47±0.36, who received H1-antihistamines treatment and anti-HP therapy.

H1-antihistamine therapy consisted of levocetirizine 5mg in association with desloratadine 5mg, given at 12 h. For Helicobacter pylori eradication, subjects received twice-daily doses of omeprazole 20 mg, amoxicillin 1g and clarithromycin 500 mg for 14 days.

Inclusion criteria. Untreated subjects with chronic idiopathic diseases, with *negative autologous serum skin test* (ASST (-)) and adequate nutritional status with/without Helicobacter pylori infection.

Exclusion criteria. We excluded from the study subjects with diseases vasculitis, subjects with ASST (+), chronic diseases subjects with known etiology: physical urticaria, cholinergic urticaria, hives caused by food allergy, medications, connective tissue, thyroid diseases and malignancies, subjects who were receving corticosteroids and immunosuppressive therapy. We also excluded pregnant and lactating women.

Investigations. At study entry, all subjects were evaluated clinically and paraclinically (complete blood count, biochemical, serological, immunological, parasitological, bacteriological and allergy tests). Blood tests were performed using ABX Pentra 60 automatic analyzer (ABX Diagnostics France) and biochemical determinations were performed using HumaStar Analyzer (Human GmbH, Wiesbaden, Germany).

Subjects were monitored by butyrylcholinesterase activity (BChE, determined by spectrophotometric method), protein C reaction (CRP measured by immunoturbidimetric method) albumin (determined by photometric method) and malondialdehyde (MDA, determined quantitatively using thiobarbituric acid) and Helicobacter pylori antibodies (determined by immunochromatographic/ELISA method).

The assessment of disease activity in CIU subjects was performed using UAS score (Urticaria Activity Score), which is the sum of lesions score (0-3) and pruritus score (0-3).

All the subjects were evaluated at baseline, at 1, 3 and 6 months after therapy initiation, to assess Urticaria Activity Score (UAS) and anti-HP antibodies. The favorable therapeutic response was assessed by reduction of more than 50% in baseline UAS. (UAS_0).

Statistical analysis of data was performed using SPSS software, version 11.5.

The study was approved by the Hospital Committee of Ethics. All subjects consented for the use of their biological samples in research and for teaching, without prejudice of the diagnosis or their personal image.

Results and discussions

In *table 1* we registered the main clinical and demographic characteristics of CIU subjects included in the study. The analyse of presented data shows that the groups formed were similar in terms of clinical characteristics and biological profile. Groups A, B, C were differentiated by the presence or absence of anti-HP antibodies.

UAS determination before the treatment (H1-antihistamines for groups A and B, respectively H1-antihistamines and anti-Helicobacter pylori for group C),

Variables	Group A (n=23)	Group B (n=24)	Group C (n=20)
Age (years)	52±9	48±4	50±7
Sex (M/F)	8/15	7/17	9/11
Area (rural:urban)	14/9	15/9	12/8
Smokers/nonsmokers	5/18	7/17	8/12
BMI (Kg/m^2)	22.4±3.1	23.2±3.4	22.9±0.9
ASST	Negative	Negative	Negative
Anti-Hp antibodies	Negative	Pozitive	Pozitive
UAS	5.17±0.63	5.26±0.73	5.47±0.36

BMI: body mass index, ASST: intradermal skin testing to autologous serum, UAS: Urticaria Activity Score.

Table 1
BASAL CHARACTERISTICS OF SUBJECTS INCLUDED IN THE STUDY

Time of assessment (months after treatment initiation)	Variable	Group A	Group B	Group C
0	UAS_0	5.17±0.63	5.26±0.73	5.47±0.36
1	UAS_1	3.12±0.98[a]	4.62±1.42	4.32±1.65
	Responders/Non-responders	9/14	5/19	8/12
3	UAS_3	2.01±1.66[a]	3.76±1.37	3.18±1.21
	Responders/Non-responders	19/4	14/10	17/3
6	UAS_6	0.16±0.42[a]	1.18±1.10[a]	0.62±0.78[a]
	Responders/Non-responders	23/0	19/5	20/0

a = statistical significant variation of UAS from baseline (UASo)

Table 2
UAS EVALUATION AND THE THERAPEUTIC RESPONSE IN CIU SUBJECTS DURING THE TREATMENT WITH H1-ANTIHISTAMINES (GROUPS A, B), RESPECTIVELY H1-ANTIHISTAMINES AND ANTI-HP ANTIBODIES (GROUP C)

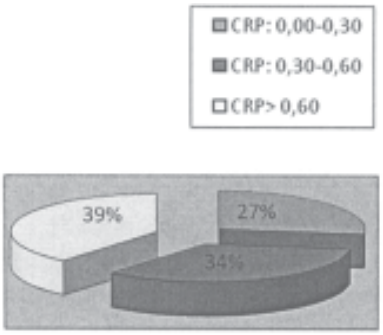

Fig. 2a. The distribution of CRP values in CIU subjects before treatment

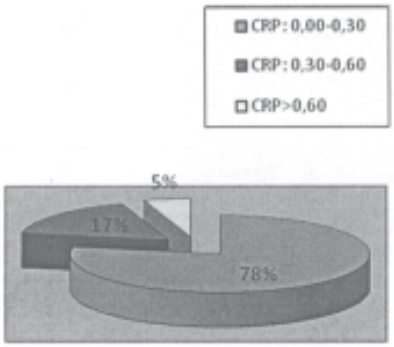

Fig. 2b. The distribution of CRP values in CIU subjects after 6 months of surveillance.

during and after the treatment, was expressed by the mean value and mean standard deviation for every group and moment of the monitoring (table 2).

We obtained a substantially reduction of UAS after 1, 3 and 6 months of surveillance with H1-antihistamines compared to baseline in group A. UAS reduction was much slower in CIU subjects associating HP infection (groups B and C).

The therapeutic success, consisting in the reduction with more than 50% in baseline UAS, is presented in table 2. If it untreated, the HP infection complicates both clinical manifestations and therapeutic response in CIU subjects. We observed a therapeutic failure in 26% of CIU subjects (group B) who were not treated for HP infection.

Responders/Non-responders = number of subjects who had the reduction/non-reduction of more than 50% in UAS.

CRP, acute phase reactant, registered elevated values in subjects with CIU monitored in this study, perhaps in response to hives, inflammation and infection (table 3). UAS reduction and decrease of HP infectious activity, were associated in a large number of cases with normalization of CRP (fig. 2a si2b).

Malondialdehyde (MDA) synthesis is accelerated under oxidative stress conditions. The reduction of MDA serum level after treatment, in subjects with CIU, was associated with dermatological symptom (table 4). In treatment refractory cases, there was no strict concordance between UAS and serum levels of MDA.

At study entry, in CIU subjects, the reduction of butyrylcholinesterase activity was correlated with the activity of the inflammatory response. Butyryl-cholinesterase (fig. 3a and 3b) changed its activity during H1-antihistamines and anti-HP treatment (table 5).

Albumin synthesis was suppressed in CIU subjects associating HP infection (table 6) before treatment. With the disappearance of hives, we observed the increase in albumin serum level.To calculate the relationship between CRP levels (fig. 2a and 2b) and cholinesterase activity (fig. 3a and 3b) in CIU subjects, we divided the subjects for these serum levels intervals of CRP: 0-0.30mg/dL, 0,30-0.60 mg/dL, and> 0.60mg/dL for each time of the assessment (table 7). In subjects with values between 0 and 0.30 mg / CRP/dL we did not obtained a conclusive relationship between CRP and BChE. For the range 0.30 - 0.60 mg /CRP/dL, we obtain a moderate negative

Time of assessment (months after treatment initiation)	Group A	Group B	Group C
0	0.48±0.36	0.88±0.79	0.96±1.15
1	0.32±0.28[a]	0.74±0.71	0.75±0.77
3	0.29±0.17[a]	0.50±0.46	0.39±0.25[a]
6	0.18±0.18[a]	0.38±0.26[a]	0.30±0.26[a]

a = statistically significant variation of CRP from baseline

Table 3
CRP (mg/dL) VARIATION WITHIN 6 MONTHS IN THE STUDIED GROUPS

Time of assessment (months after treatment initiation)	Group A	Group B	Group C
0	292±107	336±93	319±126
1	251±69[a]	299±101[a]	278±102
3	234±57[a]	273±88	244±65[a]
6	216±43[a]	237±49[a]	221±53[a]

a = statistically significant variation of MDA from baseline

Table 4
MDA (μmol/L) VARIATION WITHIN 6 MONTHS IN THE STUDIED GROUPS.

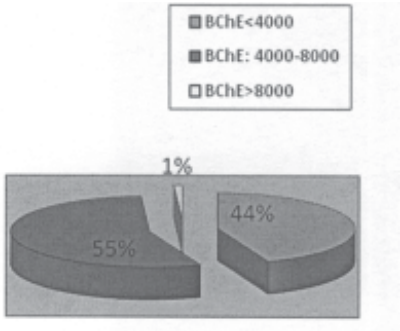

Fig. 3a. The distribution of cholinesterase activity in CIU subjects before treatment.

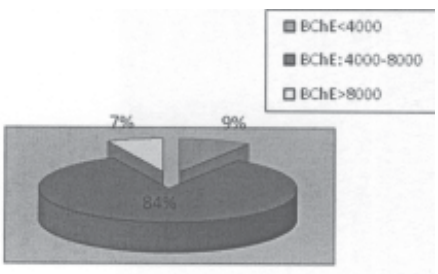

Fig. 3b. The distribution of cholinesterase activity in CIU subjects after 6 months of surveillance.

Time of assessment (months after treatment initiation)	Group A	Group B	Group C
0	4266±1204	4006±835	3922±1411
1	4301±966	4111±1308	3989±1107
3	5535±1160[a]	4966±966	4705±925[a]
6	5802±2007[a]	5409±780[a]	5906±706[a]

a = statistical significant variation of BChE from baseline

Table 5
BChE (U/L) VARIATION WITHIN 6 MONTHS IN THE STUDIED GROUPS

Time of assessment (months after treatment initiation)	Group A	Group B	Group C
0	3.84±0.53	3.77±0.62	3.68±0.79
1	3.92±0.47	3.79±0.66	3.77±0.54[a]
3	3.93±0.36[a]	3.88±0.74	3.90±0.57
6	4.01±0.29[a]	3.96±0.51[a]	4.11±0.48[a]

a = statistical significant variation of albumine from baseline

Table 6
ALBUMIN (g/dl) VARIATION WITHIN 6 MONTHS IN THE STUDIED GROUPS

Time of assessment (months after treatment initiation)	Variable	CRP (mg/dL)		
		0-0,30	0,30-0,60	□0,60
0	Number of subjects	18	26	23
	BChE (U/L)	4973±1204	4017±981	3125±774
	r	0.092	- 0.396	- 0823
	CI 95%	0.027-0.285	0.268-0.576	0.537-0.975
	p	0.729	<0.001	<0.001
1	Number of subjects	29	24	14
	BChE (U/L)	5762 948	4428 675	3088 542
	r	0.106	- 0.415	- 0.901
	CI 95%	0.021-0.140	0.117- 0.721	0.678-0.984
	p	0.420	< 0.002	< 0.001
3	Number of subjects	36	22	9
	BChE (U/L)	6289 1103	4878 325	3511 243
	r	0.083	- 0.389	- 0.672
	CI 95%	0.015-0.421	0.193-0.835	0.512-0.801
	p	0.091	<0.001	<0.0001
6	Number of subjects	52	11	4
	BChE (U/L)	6989 940	5437 622	4122 103
	r	-0.013	- 0.518	- 0.738
	CI 95%	0.006-0.311	0.221-0.734	0.405-0.927
	p	0.089	< 0.0001	<0.0001

CI= confidence interval
r = correlation index
p = statistical significance

Table 7
THE RELATIONSHIP BETWEEN CRP AND BChE IN CIU SUBJECTS (n=67)

relationship, and for the CRP > 0.60 mg CRP/dL we obtained a very close relationship.

Hives and associated inflammation are a reactive pattern that requires active measures for prevention and control. The inflammatory response varies from person to person depending on factors related to patient (general condition, level of nutrition, immune status), the intensity and duration of action of the trigger agent and the variability of mechanisms involved in generating the inflammatory response. In addition, dermal mast cells can release different amounts of mediators in response to the same stimulus. Although there are clear evidences regarding the role of inflammation and cholinergic imbalances in diseases, it was not established the precise mechanism of the relationship between the changes in the level of acetylcholine, clinical severity of disease and the response to antihistamines/anti-Helicobacter pylori treatment in subjects with urticaria . There are also a number of clinical and experimental observations that assign to acetylcholine a major role in the modulation of inflammation, pruritus and histamine release from dermal mast cells [11,14,15,19,20-25].

The results presented in the previous section demonstrate the complexity of factors that participate in the occurrence, progression and resolution of urticaria flare.

Increased levels of cholinesterase activity were recorded in acute disease compared with the low level of enzyme activity in chronic idiopathic disease. Butyrylcholinesterase activity could be an important criterion to distinguish between the acute and chronic urticaria phase (unpublished results). The increased cholinesterase activity could lead to low concentrations of acetylcholine, which could trigger a systemic inflammation.

Chronic disease, uncomplicated with infectious foci (group A) responds relatively well to H1-antihistamines. In these conditions, the body can neutralize excess histamine.

Chronic disease associated with Helicobacter pylori infection is progressively improved. After 6 months of close supervision, 48% of CIU cases were clinically healed, 40% were improved and 12% had the same condition as at the study entry.

In CIU cases beginning with generalized flare we found considerable changes in CRP, malondialdehyde (table 4), albumin (table 5) and acetylcholine (table 6). These phenomena that occurred immediately after the initiation of the inflammatory response, could be explained by the

regulation of gene expression of proteins in the liver, under the influence of cytokines.

When CIU did not debute with acute generalized flare, inflammation changes tests were lower than expected for the severity of the condition and this can be explained by down-regulation of the synthesis.

It should be noted that normalization of inflammation tests progressed slowly, after several months of close supervision and vigorous treatment. The resolution of the inflammatory process not always leads to CIU healing. Overall concordance between inflammation and treatment exists in 28% of CIU cases, partial concordance exists in 38% of cases and discordance exists in 34% of CIU cases. Besides removing the causative factors and the infectious foci, our results suggest the need for suppression of the inflammation in CIU subjects.

There is a total match between reduced BChE and increased CRP in 35% of CIU cases, a partial concordance in 38% of cases and lack of any association in 27% of CIU subjects.

The simultaneous determination of inflammation tests and BChE could be an adjuvant factor that influences the therapeutic decisions in CIU subjects.

Conclusions

These results provide additional evidence that supports the observation that cholinergic disturbances play a crucial role in pathogenesis of urticaria. Non-neuronal acetylcholine mediates the histamine release from dermal mast cells and the reducing of inflammation.

The authors recommend a careful clinical and biological monitoring of CIU subjects and interdisciplinary collaboration to ensure a correct therapeutic management. It is very important the communication with the patient to detect the causal agent. In this way, the doctor can narrow the list of possible causal factors in order to perform allergy testing. Identification of the causal agent imposes to avoid it, for faster resolution of the condition. When a plastic material is incriminated in the pathogenesis of disease, we must know as much as possible about the production, the composition, the properties and its action on the environment.

References

1. MWANGA H. Contact dermatitis in the plastic industry production-a case series. Current Allergy & Clinical Immunology. 2011;**24**, 1: 44-6

2. U.S. ENVIRONMENTAL PROTECTION AGENCY. Office of Compliance Sector Notebook Project: Profile of Rubber and Plastic Industry 2005. Washington, DC: EPA, 2005.

3. IRIS ALE S, MAIBACH HI. Occupational contact urticaria In: Kanerva L, Wahlberg JE, Elsner P, Maibach HI. Handbook of Occupational Dermatology. Heidelberg: Springer Verlag Berlin Heildelberg New York, 2000: 200-1

4. HOLNESS DL, ARRANDALE VH, MATHIAS CGT. Occupational urticaria and allergic contact dermatitis. In: Malo JL, Chan-Yeung M, Berstein DI. Asthma in the Workplace. Boca Raton: CRC Press, Taylor & Francis Group, 2013: 418

5. LEOW YH. Contact urticaria. In: Ket NS, Leok GC. The Principles and Practice of Contact and Occupational Dermatology in the Asia-Pacific Region. Singapore: World Scientific Publishing, 2001: 33.

6. DINU L, NICOLAE I, CEAUSU E, DIACONU DJ. Influenta infectiei cu Helicobacter pylori asupra raspunsului la tratamentul cu antihistaminice la pacientii cu urticarie cronica idiopatica. Rev Rom Boli Infectioase. 2013; **6,** 3

7. DINU L, NICOLAE I, ENE NICOLAE C, IONESCU P, CALOMFIRESCU C, GEORGESCU SR. Inflamatia si seroprevalenta infectiei cu virusul hepatitei C la pacientii cu urticarie cronica idiopatica. Rev Rom Boli Infectioase. 2013; **6**, 3

8. SLOMINSKI A, ZBYTEK B, ZMIJEWSKI M, SLOMINSKI RM, KAUSER S, WORTSMAN J, TOBIN DJ. Corticotropin releasing hormone and the skin. Front Biosci. 2006; **11**: 2230-48.

9. SLOMINSKI A,ZMIJEWSKI M, SKOBOWIAT C, ZBYTEK B, SLOMINSKI R, STEKETEE J. Sensing the Environment: Regulation of local and global homeostasis by the skin neuroendocrine system. Adv. Anat. Embryol. Cell Biol. 2012; **212**: 1-115.

10. PAVLOVSKY L. FRIEDMAN A. Pathogenesis of Stress-Associated Skin Disorders:Exploring the Brain -Skin Axis, Skin and the Nervous System:Stress,Itch,and More., Tur E (ed): Environmental Factors in Skin Diseases. Curr Probl Dermatol. Basel: Karger, 2007; **35**: 136-45.

11. DYKE SM, CAREY BS, KAMINSKI ER. Effect of stress on basophil function on chronic idiopathic urticaria. Clin Exp Allergy. 2008; **38**, 1: 86-92.

12. WALLENGREN J. Neuroanatomy and neurophysiology of itch. Dermatol Ther. 2005; **18**, 4: 292-303.

13. SAINT-MEZARD P, CHAVAGNAC C, BOSSET S, IONESCU M, PEYRON E, KAISERLIAN D, NICOLAS JF, BÉRARD F. Psychological stress exerts an adjuvant effect on skin dendritic cell functions in vivo. J Immunol, 2003; **171**, 8: 4073-80.

14. DA SILVA CB, WOLKMER P, DA SILVA AS, PAIM FC, TONIN AA, CASTRO VS, FELIN DV, SCHMATZ R, GONÇALVES JF, BADKE MR, MORSCH VM, MAZZANTI CM, LOPES ST. Cholinesterases as markers of the inflammatory process in rats infected with Leptospira interrogans serovar Icterohaemorrhagiae. Journ Med.Microbiology. 2012; **61**, 2: 278-284.

15. VALENTIN A PAVLOV, HONG WANG, CHRISTOPHER J CZURA, STEVEN G FRIEDMAN, AND KEVIN J TRACEY. The cholinergic anti-inflammatory pathway. Mol Med. 2003; **9**, 5-8: 125–134.

16. COSTA MM, SILVA AS, PAIM FC, FRANÇA R, DORNELLES GL, THOMÉ GR, SERRES JD, SCHMATZ R, SPANEVELLO RM, GONÇALVES JF, SCHETINGER MR, MAZZANTI CM, LOPES ST, MONTEIRO SG. Cholinesterase as inflammarory markers in a experimental infection by Trypanosoma evansi in rabbits, An Acad Bras.C. 2012; **4**: 1105-13.

17. ULLOA L. The vague nerve and the nicotinic anti-inflammatory pathway. Nat. Rev.Drug. Discov. 2005; **4**, 8: 673-84.

18. WESSLER I, REINHEIMER T, KILBINGER H, BITTINGER F, KIRKPATRICK CJ, SALOGA J, KNOP J. Increased acetylcholine levels in skin biopsie of patients with atopic dermatitis. Life Sci. 2003; **72, 18-19:** 2169-72.

19. KURZEN H, WESSLER I, KIRKPATRICK CJ, KAWASHIMA K, GRANDO SA. The non neuronal cholinergic system of human skin. Horm Metab. Res.2007; **39**, 2: 125-35.

20. ENE NICOLAE C, NICOLAE I, TAMPA M, MATEI C, GEORGESCU SR. Rev. Chim. (Bucharest), **64**, no. 6, 2013, p. 654

21. JUDIT M, JORDI C., NAT LIA F., RAUL B., ANNA R., BHARTI M., MICHAEL M., JORGE J. Paraoxonase-1 is related to inflammation, fibrosis and PPAR delta in experimental liver disease. BMC Gastroenterology. 2009; **9:**3 doi:10.1186/1471-230X-9-3.

22. WESSLER IK, KIRKPATRICK CJ. The non-neuronal cholinergic system: an emerging drug target in the airways. Pulm Pharmacol Ther. 2001; 14: 423-34.

23. RACKE K, MATTHIESEN S. The airway cholinergic system: physiology and pharmacology. Pulm Pharmacol Ther. 2004; 17: 181-198.

24. REINOUD G., JOHAN Z., HERMAN M. ANDREW J. H. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. Respiratory Research, 2006; **7**, 73: doi:10.1186/1465-9921-**7**-73.

25. NICOLAE I, ENE(NICOLAE) C.-D, SCHIPOR S, TAMPA M, GEORGESCU SR. Rev Chim.(Bucharest), **64,** no.10, 2013, p.1201

Manuscript received: 12.09.2013