Relationship Between Gamma-glutamyl Transpeptidase Activity and Inflammatory Response in Lichen Planus

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Chemicals used in the manufacture of synthetic fibers have been associated with undesirable side effects such as itching or skin lesions and it seems that they are involved in the induction of pathological processes such as oxidative stress and inflammation. Lichen planus (LP) can be regarded as an inflammatory disorder, chemical and physical factors playing an important role in the perpetuation of the inflammatory process. Gamma-glutamyl transpeptidase (GGT) plays an important role in the preservation of skin architecture and modulation of skin inflammation. In this study, we found that GGT activity is increased in LP patients with mild inflammation, whilst GGT is inactivated under conditions of severe inflammation. Therefore, GGT is involved in the inflammatory process, but there is no a positive correlation between its activity and the intensity of the inflammatory response. This functional adaptation of the enzyme may be due to down-regulation of its synthesis under free radical overload conditions. Understanding the molecular mechanisms involved in the modulation of intracellular redox homeostasis is an important step in the pharmacological management of patients with LP.

Keywords: inflammation, lichen planus, gamma-glutamyl transpeptidase, C-reactive protein, sulfhydryl groups

Chemicals used in the manufacture of synthetic fibers have been associated with serious health problems such as immune system dysfunctions, behavioural disorders and hormonal imbalances [1-3]. Clothes that come into contact with the skin can cause itching, allergies or skin lesions. Synthetic materials favour perspiration, which may increase the risk of developing fungal or bacterial infections and eczema. In addition, dyes used in the clothing industry can dissolve due to perspiration resulting in skin lesions. These external factors could enhance the itching and inflammation associated with lichen planus (LP) lesions [1,2].

LP can be regarded as an inflammatory disorder, chemical and physical factors playing an important role in the maintenance of the inflammation [2,3]. Regardless of the agent which may be involved in the onset of the disease, there is an increase in the activity of proteolytic enzymes and a release of active substances and chemokines in the intercellular spaces [4-15]. An excessive amount of oxygen and nitrogen free radicals are produced and that promotes chronic inflammation through the oxidative degradation of certain biomolecules [7, 8, 16, 17]. It can be said that inflammation and oxidative stress are inseparable processes, which influence each other [3, 4-9]. In patients with inflammatory skin diseases, endogenous antioxidants become deficient being consumed in the tissues and that could contribute to the perpetuation of oxidative stress (fig. 1) [3, 4-13].

The gamma-glutamyl cycle plays a central role in the preservation of skin architecture and protection against oxidative stress. This cycle is initiated by gamma glutamyl transpeptidase (GGT) [18-26]. This enzyme is expressed in most eukaryotic cells and is predominantly found on the luminal surface of cells with secretory and absorptive functions (kidneys, gall bladder, pancreas, liver, brain, seminal vesicles, endothelium), as well as in malignant tissues, immune and inflammatory cells [2-4]. GGT is

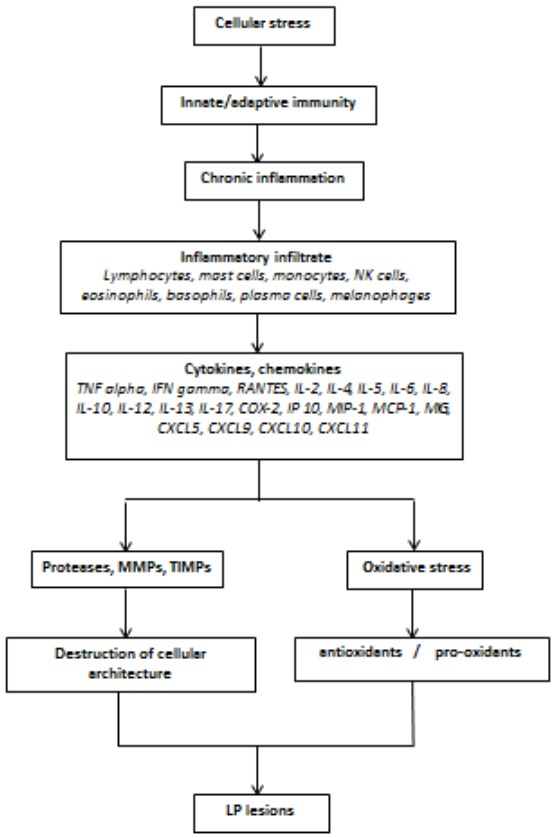

Fig. 1. The role of inflammation in LP
TNF – tumour necrosis factor, IFN- interferon, RANTES - Regulated on Activation, Normal Cell Expressed and Secreted, IL – interleukin, COX - Cyclooxygenase, IP - Interferon gamma-induced protein 1, MIP - macrophage Inflammatory Proteins, MIG - monokine induced by IFN-gamma, MCP – Monocyte Chemoattractant Protein, CXCL - chemokine ligand , MMPs-metalloproteinases, TIMPs - tissue inhibitor of metalloproteinases

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involved in the maintenance of intracellular homeostasis of redox status, the transfer of amino acids through the cell membrane, the metabolism of inflammatory mediators and turnover of glutathione [1, 18-26]. GGT is a rate-limiting enzyme in the catabolism of glutathione (1,2) and in the conversion of leukotriene LTC4 to LTD4 (3). Cysteinyl-glycine reduces Fe (3+) to Fe (2+) and generates reactive species with pro-oxidant effect (reactions 4-7).

$$GSH + \text{amino acid} \xrightarrow{GGT} \text{glu-amino acid} + \text{cys-gly} \quad (1)$$

$$\text{Cys} - \text{gly} + H_2O \xrightarrow{\text{Dipeptidase}} \text{cys} + \text{gly} \quad (2)$$

LTC4(5S-Hydroxy-6R-(S-glutathionyl)-7E,9E,11Z,14Z-eicosatetraenoic acid) GGT→ LTD4 (5S-Hydroxy-6R-(S-cysteinylglycinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid)+ glu (3)

GC-SH (cysteinil-glycine)→ GC-S(-) (thiolate anion, *p*H>7) + H(+) (4)

GC-S(-) + Fe (3+) → GC-S• (thiyl radical) + Fe(2+) (5)

$$Fe(2+) + O_2 \rightarrow Fe(3+) + O_2(-) \text{ (superoxide anion)} \quad (6)$$

$$O_2(-) + 2H_2O \rightarrow 1/2O_2 + 2H(+) + H_2O_2 \text{ (hydrogen peroxide)} \quad (7)$$

A change in GGT activity, an increase in C-reactive protein (CRP) level, or a reduction in total-sulfhydryl (SH) groups level may be considered early signs of an acute phase response in LP (25). The objective of this study is focused on the investigation of the relationship between GGT activity and inflammatory response in LP patients. We enrolled 60 patients with LP and 60 healthy subjects (control group) (fig. 2).

Experimental part

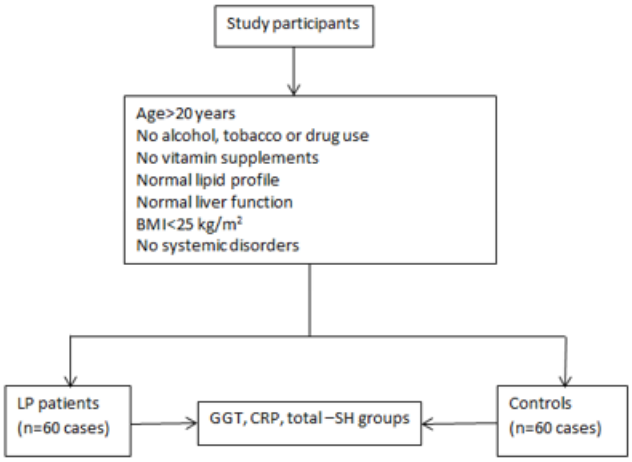

Fig. 2. Study design

The processing of biological samples: blood samples were collected using a holder-vacutainer system under basal conditions, before any diagnostic or therapeutic procedures. Venous blood samples collected in tubes containing an anticoagulant (K3EDTA) were used for hematological determinations; serum obtained from venous blood collected in vacutainer without an anticoagulant was used for biochemical, serological and immunological determinations. Samples were immediately processed or frozen at -60 degrees Celsius; hemolyzed or lactescent samples were rejected.

Laboratory determinations

The GGT Activity Colorimetric Assay kit provides a simple and direct procedure for measuring GGT activity in a variety of samples. GGT activity is determined by a coupled enzyme assay, in which the GGT transfers the λ-glutamyl group from the substrate L-λ-Glutamyl-p-nitroanilide, liberating the chromogen p-nitroanilide (pNA, 418 nm) proportional to the GGT present. One unit of GGT is the amount of enzyme that will generate 1.0 μmole of pNA per minute at 37°C. CRP level was determined by immunoturbidimetric method (340 nm). The CRP-Turbilatex is a quantitative turbidimetric test for the measurement of CRP in human serum or plasma. Latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination cause an absorbance change, dependent upon the CRP content of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

Thiol groups in biological samples (serum, liver homogenate) react with DTNB (5,5-dithiobis-2-nitrobenzoic acid). The N-mercaptobenzoic acid anion produced is yellow colored and has a maximum absorbance at 412 nm. The results are expressed in μm -SH/L in serum, or μm -SH/g protein [28].

Statistical analysis. The comparison of obtained results between the groups for quantitative variables was performed using the t-test or ANOVA. The correlations between variables were determined by linear regression. The relationship between pairs of two parameters was assessed by Pearson's correlation coefficient (r). We chose a significance level (p) of 0.05 (5%) and a confidence interval of 95% for hypothesis testing.

Results and discussions

After a rigorous selection of the study participants, two groups with similar demographic and clinical characteristics were made: 60 patients with LP and 60 healthy subjects (fig. 2). In this section we present:

- baseline data on GGT, CRP and total -SH groups in LP patients and controls;
- correlations between GGT and CRP level and between total -SH groups and CRP level in LP patients before the initiation of the treatment.

In patients with LP and controls we analyzed the serum level variations of GGT, CRP, total -SH groups, as well as the variations of those parameters in correlation with the severity of inflammation. Table 1 shows the mean values of GGT, CRP, total -SH groups, standard deviations, and the statistical significance for each studied variable in LP patients and controls. Thus, in LP patients the GGT level (U/L) was increased compared to controls (29.8 ± 14.3 vs.21.2 ± 14.1, p> 0.05). The variations of CRP (mg/dL) and total -SH groups (ìmol/L) level did not show statistically significant differences between the two groups, LP patients and controls (0.41 ± 0.58 vs. 0.19 ± 0.09, p> 0.05, respectively 351± 62 vs.399 ± 54, p> 0.05).

Table 1
LEVELS OF GGT, CRP AND TOTAL –SH GROUPS IN LP PATIENTS AND CONTROLS

Variable	LP patients	Controls	P
GGT (U/L)	29.8±14.3	21.2±14.1	0.193
CRP (mg/dL)	0.41±0.58	0.19±0.09	0.062
Total –SH groups (μmol/L)	351±62	399±54	0.177

Severity of inflammation	CRP (mg/dL)	LP (n=cases)	Control (n=cases)
Absent	0.00	16	42
Mild	≤0.30	12	18
Moderate	0.31-0.60	12	0
Severe	0.61-1.00	13	0
Very severe	>1.00	7	0

Table 2
DISTRIBUTION OF LP PATIENTS AND CONTROLS ACCORDING TO THE SEVERITY OF INFLAMMATION

CRP (mg/dL)	GGT (U/L)	P
0	16.1±12.7	-
≤0.30	18.3±7.3	0.836
0.31-0.60	33.3±17.3	0.064
0.61-1.00	47.3±23.5	0.007
>1.00	38.7±4.2	0.049

Table 3
GGT ACTIVITY ACCORDING TO THE SEVERITY OF INFLAMMATION IN LP PATIENTS

The severity of inflammation was assessed according to serum CRP levels (table 2). In 26.7% of LP cases no systemic inflammatory process was identified, 20.0% had mild inflammation, 20% moderate inflammation, 21.7% severe inflammation and very severe inflammation was present in 11.6% of them. In the control group, the inflammation was absent in 70.0% of cases and a mild inflammation was noticed in 30.0% of them.

The serum profile of GGT activity varies in LP patients according to the severity of inflammation (table 3). Compared to GGT levels (U/L) detected in LP patients with no signs of inflammation (16.1 ± 12.7), higher GGT levels were obtained in LP patients with signs of inflammation, as follows: 18.3 + -7.3, p> 0.05 for CRP < 0.30 mg/dL; 33.3 ± 17.3, p> 0.05 for CRP + 0.31-0.60 mg/dL; 47.3 ± 23.5, p> 0.05 for CRP = 0.61-1.00 mg/dL; 38.7 ± 2.4, p < 0.05 for CRP> 1.00 mg/dL (fig. 3).

The level of total -SH groups varies in LP patients according to the severity of inflammation (table 4). Compared to the total -SH level (µmol/L) detected in LP

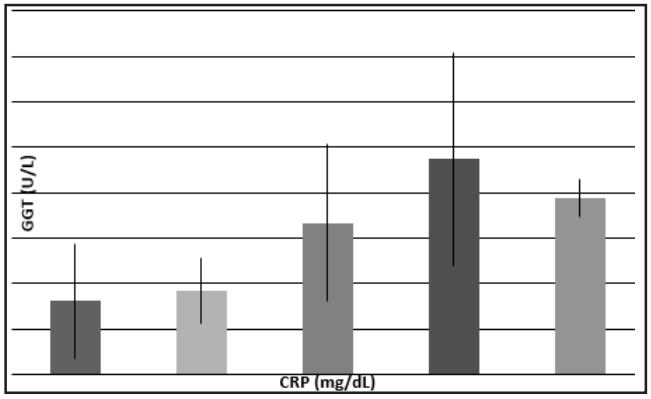

Fig 3. Relationship between GGT activity and inflammation in LP patients GGT- (gamma-glutamyl transpeptidase), CRP- (C-reactive protein)

patients with no signs of inflammation (401 + 98), there were reductions in the total-SH level in LP patients with signs of inflammation as follows: 389 + 677, p> 0.05 for CRP <0.30 mg/dL; 331 + - 85, p> 0.05 for CRP + 0.31-0.60mg/dL; 300 + 94, p <0.05 for CRP = 0.61-1.00mg/dL; 286 + 52, p <0.05 with CRP> 1.00mg/dL (fig. 4).

Table 4
TOTAL -SH GROUPS LEVEL ACCORDING TO SEVERITY OF INFLAMMATION IN LP PATIENTS

CRP (mg/dL)	Total -SH groups (µmol/L)	p
0	401±98	-
≤0.30	389±67	0.947
0.31-0.60	331±85	0.164
0.61-1.00	300±94	0.043
>1.00	286±52	0.031

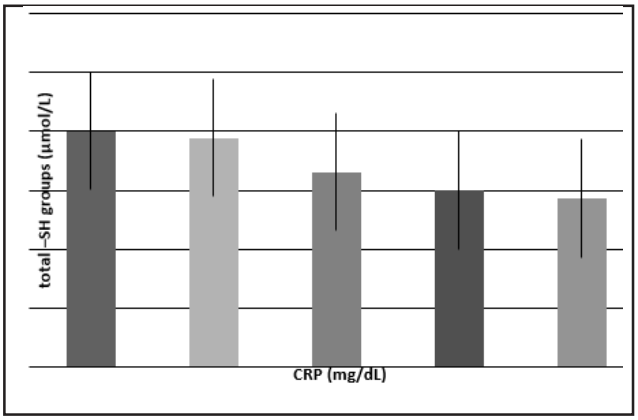

Fig. 4. Relationship between total-SH groups level and inflammation in LP patients Total- SH groups- (total-sulfhydryl groups), CRP -(C-reactive protein)

In this study, we determined the relationship between GGT and CRP as well as between total –SH groups and CRP in LP patients according to the severity of inflammation (table 5). No correlation was obtained between the studied parameters when CRP levels were below 0.60 mg/dl. There was a weak statistically significant positive correlation between GGT and CRP for CRP levels between 0.60-1.0 mg/dL (r = 0.118, p <0.05) and a strong statistically significant negative correlation between GGT and CRP for CRP levels > 1.00 mg/dL (r = - 0.510, p <0.05). There was a negative association, with no statistical significance between total -SH groups and CRP, for CRP levels higher than 0.60 mg/dL

Oxidative stress related-factors represent key components in the induction of inflammation in the human body, being responsible for the production of pro and anti-inflammatory molecules as well as pro and anti-cancerous agents in epithelial tissues. The endogenous anti-inflammatory system acts throughout the body as a natural defense system against all aggressive factors, contributing to the maintenance of a vital biological balance [3-7, 14, 15, 27-32].

In this study, we found that the serum alterations of GGT, a rate-limiting enzyme in the glutathione conversion and modulation of total-SH groups level, could be an early event

Severity of inflammation	GGT/CRP		-SH/CRP	
	r	p	r	p
Absent	0.011	1.00	0.111	0.899
Mild	0.094	0.901	0.102	0.887
Moderate	0.122	0.572	-0. 163	0.156
Severe	0.118	0.045	-0.198	0.056
Very severe	- 0.510	0.003	- 0.132	0.087

Table 5
STATISTICAL CORRELATIONS BETWEEN GGT AND CRP, TOTAL-SH GROUPS AND CRP

in the inflammatory signaling pathway in LP patients. A statistically significant positive association was observed between GGT and CRP in the early stages of inflammation. In the LP cases associated with very severe inflammation, GGT activity was lower than expected. The relationship between GGT activity and the parameters of inflammation in LP patients could be explained by the over-loading with free radicals with effect on the enzyme gene expression at the level of its synthesis. GGT could be regarded as a molecular switcher that provides the turnover of glutathione and maintains the intracellular redox status in epithelial disorders [25-28].

The pathogenesis of LP is complex and involves the interplay of immunological, endocrine and biochemical factors [3, 4, 25]. Oxidative stress (overproduction of free radicals and antioxidant deficiency), plays a crucial role in LP pathogenesis. In LP lesions, it was revealed an increased activity of certain proteolytic enzymes, the release of active substances in intercellular spaces and synthesis of proinflammatory chemokines [3-15]. GGT activity is influenced by toxic substances, redox reactive metals, ischemia, inflammatory processes, infections, apoptosis, excessive levels of free radicals and cell membrane damage [1, 18, 23].

Inflammation is a process characterized by an excessive production of oxygen and nitrogen free radicals. These reactive species maintain the chronic inflammation through the oxidative degradation of lipids, proteins, carbohydrates and nucleic acids [7, 10, 16, 17, 19, 23, 29]. Inflammation and oxidative stress are inseparable processes, which influence each other [4-9, 25, 27, 28, 32]. The presence of inflammation in LP patients can play an important role in the choice of treatment strategy, and the patient's quality of life can be improved by adopting individualized regimens.

Conclusions

In patients with LP, GGT is involved in the elimination of toxic metabolites produced under oxidative stress conditions. GGT activity is influenced by the intensity of the inflammatory response. In an inflammatory process the enzyme is early stimulated but if a severe inflammation is present its activity is suppressed. This functional adaptation of the enzyme might be due to down-regulation of its synthesis under free radical overload conditions. Understanding the molecular mechanisms involved in the modulation of intracellular redox homeostasis is an important step in the pharmacological management of patients with LP.

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