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EVALUATION OF AMINO ACID L-CANAVANINE IN THE DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS. A CLUE TO THE PATHOGENESIS OF DISEASE

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ABSTRACT

Objective.- The aim of this study was to observe if there is any relation between serum canavanine levels and antinuclear antibody (ANA) titers in samples from patients with systemic lupus erythematosus (SLE).

Material and methods.- We analyzed 128 blood samples from patients with SLE and 128 samples from healthy subjects for ANA by indirect immunofluorescence and serum canavanine with the Rosenthal technic.

Results.- The levels of serum canavanine in healthy subjects were 1.8 to 3.8 mg/dl with a mean of 3.1 and the ANA titers were 0 to 1:1280 and in patients with SLE values of canavanine were 3.8 to 16 mg/dl with a mean of 7.1 and $p<0.01$ with ANA titers from 1:128 to 1:280. The sensitivity of the canavanine test is 96% and 93% specificity. It was observed that as the values of canavanine increase, the ANA titers increase as well.

Conclusions.- With the obtained values it is concluded that evaluation of serum canavanine can be useful as a support for the diagnosis of SLE and can be used as a screening test

KEYWORDS : L-canavanine, lupus erythematosus, diagnostic

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is the prototype of systemic rheumatic disease, is an autoimmune disease that is not organ specific, where tissue damage is primarily mediated by DNA anti-DNA immune complexes. The typical case of SLE presents an average of 3 different circulating antibodies present simultaneously. The prevalence of antibodies is very variable and more than 25 different types of autoantibodies have been identified in SLE (1, 2).

The first laboratory study employed for the detection of autoimmune diseases was conducted by Waaler in 1940 with sheep red blood cells sensitized with rabbit anti-sheep red blood cells which are agglutinated in sera of patients with rheumatoid arthritis. He named the factor responsible for the agglutination "rheumatoid factor". The next discovery was made by Hargraves in 1948 he observed an atypical cell and he named LE cell. The cell was a polymorphonuclear leukocyte (PMN) that contained engulfed amorphous nuclear material. The amorphous material was from a damages lymphocyte nucleus. Subsequently it was observed that the purified DNA from different species reacted with the serum of patients with SLE and the reactivity was due to an immunoglobulin (3). Then in 1954 the technique of immunofluorescence was applied for the detection of specific antigens. By 1957 applied this technique to patients with SLE demonstrating that there were antibodies in patients sera that would bind to nuclear antigens. A follow-up study showed that immune complexes of DNA and anti-DNA could be eluted from the kidney of some patients with lupus nephritis. The assay for detection of antibodies to DNA that is still considered the gold standard today was developed in 1968 (4).

SLE is characterized by the presence of multiple autoantibodies directed to nuclear and cytoplasmic antigens; other antibodies to phospholipids, B2-glucoprotein 1 and also to cell surface, platelets, erythrocytes, lymphocytes and neuronal cells, are also observed. The antibodies to DNA may be to either the double stranded native DNA (nDNA) or a denatured single strand DNA (ssDNA). Antibodies to nDNA occur in 70% of patients with SLE. The detection of these antibodies in high titers is of clinical importance. Historically, antibodies to nDNA have been considered as the main antibodies implicated in the pathogenesis of SLE. Immune complexes containing both DNA and chromatin are deposited in the kidney and may initiate an inflammatory response causing severe glomerular damage leading to renal failure. The chromatin is constituted by 40% DNA, 40% histones and 20% non-histone proteins. The histones are organized along DNA as repeating units named nucleosomes. Antinucleosome or antichromatin antibodies react with DNA. In a mouse model of SLE it was demonstrated that the antichromatin antibodies are the first to appear and that specific antibodies to DNA and histone develop later (5). It is thought, that this may also occur in human SLE. Chromatin antibodies are detected in up to 80% of patients with SLE.

Making a diagnosis of SLE is difficult due to its several initial manifestations without presenting a well defined clinical picture. Usually when the diagnosis of the disease is made, it is in advanced stage and some of the injuries caused by the disease are almost always irreversible. Therefore have been searched new methodologies that are specific to make an early diagnosis of the disease.

In mice immunized with polylysine peptides they developed antibodies to DNA, anti-dsDNA and also other antibodies characteristic of lupus including anti-histone, anticardiolipin and antiribo-nucleoprotein; at 3 months the presence of IgM and IgG deposits in the renal glomeruli of the immunized mouse was demonstrated (6). On the other hand in monkeys fed with alfalfa stems these developed a disease with the characteristic of SLE; in the blood serum, anti-DNA antibodies were found, as well as low levels of complement and complement deposits were found in the kidneys and in the skin (7). Alfalfa and some leguminous contain a non-protein amino acid named L-canavanine which is an analogue of arginine. In a previous study the concentration of canavanine in blood samples from patients with SLE was determined, finding high values in 92% of the patients (8). With this information and knowing that canavanine is incorporated in the helix of DNA and RNA in formation instead of arginine, resulting in the synthesis of aberrant proteins; we think that canavanine can induce the formation of ANA in man (9). Therefore we decided to determine the levels of canavanine in serum of patients with SLE along with the titers of ANA and evaluate their usefulness as support to the diagnosis of the disease.

Material and methods

We analyzed 128 blood samples from clinically healthy people and 128 samples from patients with SLE, both between 12 and 40 years old and of both sexes.. The diagnosis of the disease was made with the clinic, laboratory and cabinet studies, The evaluation of the amino acid L-canavanine was done with the Rosenthal technic (10) and the ANA were evaluated by indirect immunofluorescense with Tan technic (11). The data are presented as mean ± SD, $p< 0.05$ is considered statistically significant; the diagnostic sensitivity and specificity were analyzed by the characteristic graphs.

Results

The values obtained for L-canavanine in clinically healthy people were 1.8 to 3.8 mg/dl, with a mean of 3.1 and standard deviation ± 0.49 and the ANA titers were from 0 to 1:128. In patients with SLE values of 3.8 to 18 mg/dl were found with a mean of 7.1 and standard deviation ± 2.6 and the value of $p<0.01$ which is statistically significant, see table 1.

ANA values for patients with SLE were 1:128 to 1:1280. The canavanine test has a sensitivity of 96% with a specificity of 93%.

DISCUSSION

The SLE was first described in the year of 1822 and since that time laboratory studies have been sought to help the diagnosis of the disease in a quick and safe way; and several studies have been done to know how the ANA are generated which to date is unknown. The SLE is so frequent in the relatives of the patients who live in the same house in comparison with the population in general. This frequency of illness in relatives is explained because every family consumes the same foods and among these are leguminous. The development of the disease is the result of the interaction of genetic, environmental and hormonal factors. Among the genetic factors are some phenotypes of slow acetylation; of the environmental factors is sunlight that triggers the onset of the disease and on the other hand most of the patients are women this indicates the role of hormones. The slow acetylation of the drugs is of special interest because it has been observed that ANA and even the typical cellular phenomenon of the disease are produced and also the clinical symptoms characteristic of the disease as it happens with the hydralazine (12). The collateral effects of the drugs can be directly attributed to differences in the acetylator phenotype. Slow acetylators tend to easily produce ANA when they are administered isoniazid or hydralazine and a high incidence of lupus erythematosus is also observed in them. In an investigation done by Drayer found that of 134 patients with SLE, 104 of them were slow acetylators (13). In an study done in 1010 patients with SLE, a sensitivity of 93% was found for the ANA with a specificity of 86% (14,15). In this study for canavanine test a sensitivity of 96% was found and specificity of 93% which leads us to think that the evaluation of serum canavanine in patients with SLE can be very useful as it supports the diagnosis of the disease.

At the moment it is still difficult to make a diagnosis of SLE because there are several clinical manifestations without presenting a well defined clinical picture. Some of the injuries caused by the disease are almost always irreversible, so it is important to make an early diagnosis of the disease. We present here the determination of serum canavanine as a laboratory test that can be useful as a support to make an early diagnosis of the disease. This amino acid of vegetable origin is found in some leguminous that are eaten daily as food, including lentils, common beans, great beans, sprouts soybeans and alfalfa. These leguminous are consumed since childhood and it has observed that canavanine accumulates in the blood and in the different organs causing disease when consumed for a long time. In previous studies it has been observed that the metabolism of canavanine is closely related to the levels of the enzyme acetyltransferase present in the liver. Slow acetylators tend to produce ANA after consuming canavanine for 2 to 3 months and rapid acetylators produce ANA after 2 years or more when consumed in large quantities in the form of food complements or in the form of tablets containing alfalfa and/or soy protein (16). There are several reports of SLE caused by the consumption of alfalfa. We mention the case of a 40 years old female patient with inactive SLE controlled for 4 years taking prednisone at a dose of 15 mg/day, suddenly appeared high titers of ANA, elevated cryoglobulins, low complement levels and proteinuria. The patient reported that she had been taking 15 alfalfa tablets daily for the previous 9 months (17,18). This suggests that possibly a good number of patients with autoimmune diseases have consumed alfalfa. When analyzing the alfalfa tablets by high pressure liquid chromatography and colorimetric analysis, high levels of canavanine were found.

The amino acid L-canavanine is an analogue of arginine was discovered in seeds of **Canavalia ensiformis** and later found in other plants. In the plants it works as a chemical defense against insects and other herbivores. In rats fed with canavanine, it was found that it stimulates the synthesis of immunoglobulins, as well as the production of double stranded antiDNA antibodies and antibodies that produce glomerular damage. In studies carried out with radioisotopes it was observed that canavanine alters protein synthesis, inhibits the transport of arginine through the membranes, is incorporated into the new chains of polypeptides in the place of arginine by the action of the enzyme arginil-ribosintetase. This substitution results in the production of aberrant proteins that alter metabolic reactions, DNA synthesis, RNA synthesis and synthesis of several proteins (19). It has been observed that canavanine is incorporated into the proteins in an equal amount of arginine; in addition to the activation of the production of immunoglobulins which function as ANA it is probable that the existing arginine in tissues is consumed, decreasing its levels. For this reason, in a previous study, serum levels of arginine were reduced in 77% of patients with SLE in which it was observed that as the ANA titers increase arginine values decrease (20). This may be because arginine is consumed in the production of ANA.

Renal arginase transforms arginine into ornithine and this compound through the enzyme ornithine-decarboxilase is transformed into polyamines which have a very important role in the processes of cell growth, multiplication and differentiation. As a result of their multiple positive charges, the polyamines are easily linked to DNA and RNA, they also stimulate RNA biosynthesis and therefore the synthesis of proteins. The canavanine inhibits the metabolism of ornithine therefore decreases the synthesis of polyamines and consequently also decrease the growth and cell division and as a result of the above there is erytropenia, leukopenia and thrombocytopenia (21). All this is observed in patients with lupus erythematosus which may be due to the action of canavanine. On the other hand, during the metabolism of canavanine, the enzymes ornithine-aminotransferase and ornithine-decarboxylase and other enzymes involved in the synthesis of the collagen are inhibited and therefore the collagen is synthesized in a deficient form and consequently the peptides of lysine, proline and hydroxyproline do not bind to form the protein peptides excreted in the urine in amounts 4 times more than is normally excreted. Even some authors have tried to evaluate these peptides in urine to make a diagnosis of SLE; but like the ANA they appear when the disease is already very advanced (22). In summary it has been accepted that for the development of lupus erythematosus, genetic factors are involved due to the acetylating phenotype; with slow acetylating individuals being affected by chemical compounds used in agriculture and industry that have ingested or been in contact with these agents (23). Exposure to sunlight is a well known environmental factor in the induction and exacerbation of disease (24). Viral infections have not been demonstrated in the tissues of patients with SLE therefore there is little evidence to support that an infections agent causes SLE. The ingestion of alfalfa which contains L_canavanine has been linked to the development of lupus erythematosus in several reports (17,18,25,26). In SLE produced by drugs, dermatological and joint manifestations are frequent and renal and neurological manifestations are rare; on the other hand when L-canavanine is administered, renal damage and neurological alterations are produced. This indicates that canavanine may be the triggering factor of the disease and according to the results obtained in this study, it can be considered that patients with serum canavanine values greater than 3.8 mg/dl are diagnosed with SLE. In addition, as shown in table No 1, the canavanine vaues increase at the same together with ANA titers which suggest that the canavanine triggers the formation of ANA. For the observed in this study, the evaluation of canavanine should be done before starting the steroid treatment because the canavanine values decrease due to the activation of several enzymes that participate in the metabolism of arginine. Finally, it is concluded that the evaluation of serum canavanine can be useful as a support for the diagnosis of SLE and could be used as a screening test.

This is the first study done on the evaluation of serum canavanine and the ANA titers in blood patients with SLE; therefore can not be compared with previous studies. It is necessary to make an evaluation of serum canavanine in a larger number of patients with SLE and also in other autoimmune diseases to confirm the usefulness of this finding.

TABLE 1: Values of serum canavanine and ANA titers found in healthy subjects and patients with SLE

healthy subjects			patients with SLE		
n	canavanine mg/dl	ANA	n	canavanine mg/dl	ANA
3	1.8	0	5	3.8	1:128
3	2.0	0	3	3.9	1: 160
2	2.3	0	18	4.1	1:160
4	2.4	0	9	4.5	1:512
6	2.5	0	2	5.0	1:512
8	2.7	0	8	6.0	1:512
19	3.	1:40	10	7.2	1:1024
8	3.1	1:40	10	7.6	1:512
13	3.2	1:40	8	7.8	1:512
12	3.3	1:64	7	8.2	1:512
21	3.4	1:64	17	8.5	1:1280
7	3.5	1:80	9	9.0	1:1024
12	3.6	1:80	11	10.2	1:1024

4	3.7	1:80	3	11.5	1:640
6	3.8	1:128	6	13.0	1:640
			2	16.0	1:640

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