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ROLE OF NON-PROTEIN AMINO ACIDS IN AUTOIMMUNE DISEASES

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ABSTRACT Many nonprotein amino acids (NPAAs) are plant secondary metabolites and have a similar chemical structure , size, shape and charge to protein amino acids and can be mistakenly used in protein synthesis, interfere in biochemical pathways, overstimulate receptors or chelate metal ions. The Consumption of a number of plants that contain NPAAs has been shown to have acutely toxic effects in humans. The consumption of some seeds or vegetables that contain NPAAs influences some people as a trigger for some diseases or as an exacerbating factor. The effects of NPAAs on human health are not well understood. It has been observed that chimpanzees fed alfalfa and also a volunteer subject who have intake alfalfa tablets have both developed a disease with all the characteristics of lupus erythematosus (an autoimmune disease) and by suppressing the consumption of alfalfa the disease disappears.

Most of NPAAs are of plant origin; these do not form part of the primary structure of proteins, they act as antimetabolites and most are found in higher plants; they have the same basic structure as amino acids of animal origin. The substitution of a protein amino acid in protein synthesis produces abnormal proteins which generate antibodies and modification of autoantigens as mechanism to break immunological tolerance and trigger autoimmunity. Auto-antibodies formation is a characteristic of autoimmune disease. The toxic potential of NPAAs depend the amount ingested and the consumption time. Chronic exposure to NPAAs could contribute to autoimmune disease development in genetically susceptible individuals.

KEYWORDS : Non protein amino acids , plant toxin, autoimmune diseases

INTRODUCTION

Plants synthesize a great number of secondary compounds that are toxic to other forms of life; non-protein amino acids (NPAAs) may be toxic to microorganisms, plants, insects or higher animals. Most NPAAs have low toxicity, but their prolonged administration can be toxic in some mammalian tissues. Some of these compounds interfere with the metabolism of proteins amino acids and the inclusion in specific tissues such as the skin and some organs. Thus we have mimosine that affects the thyroid and its metabolite (indospicin) affects the liver, skin and brain. NPAAs alter protein synthesis by competition with protein amino acids in messenger RNA and some have anti-nutritional effects (1,2).

The discovery in plants of amino acids that are structurally related to components of mammalian central nervous system glutamate receptors suggests that many of the analogues of glutamic acid are synthesized by plants; may have a signaling function "in vivo" in addition to any protective role that they might play (3) . The most striking example of amino acids toxicity is neurolathyrism (lathyrism) one of the oldest neurotoxic diseases known; described by Hippocrates on 400 BC; *lathyrus sativus* is an insect-resistant crop that can grow in poor soils and in drought conditions and is often eaten in times of famine when there is a dietary shortage of protein amino acids; this plant contain the NPAA β-N-oxalil-L-αβ-diaminopropionic acid (B-ODAP) (4).

There are data on leguminous crops from the V to IV centuries BC; wheat, barley, peas, *lathyrus*, lentils, beans and almortas were grown. *Lathyrus sativus* is still cultivated in some regions of Spain, in North Africa and Asia. Leguminous are rich in protein in such a way that they are used as a substitute for meat. The content of toxic factors in beans, peas, common beans, soybeans is important (trypsin inhibitors, tannins, lectins and NPAAs).

Nonprotein amino acids have been reported from a wide variety lot of plants including leguminosae, liliaceae, sapindaceae, cycadaceae, compositae, rubiaceae and lecythidaceae. However, NPAAs are most often found in leguminous and are mainly abundant in seeds (5). The chemical and physiological properties of some NPAAs found in plants which have been shown to be toxic to man, livestock and domestic animals are reviewed.

The toxicity of (NPAAs) was first examined systematically in the early 1960s and many were found to have growth-inhibitory properties toward microorganisms. This is because they are similar with some of the 20 protein amino acids. Protein synthesis is a fundamental process in all life forms, therefore by replacing an amino acid in protein synthesis, abnormal proteins are produced, which generate antibodies and when it has an important role in the active site of an enzyme this result in the loss of the activity of the enzyme. These NPAAs are called analogues amino acids and may be incorporated into proteins (6).

Diseases associated with protein misfolding or abnormal protein synthesis such as Parkinson´s disease and amyotrophic lateral sclerosis are predominantly sporadic (less than 10% genetic), but the factors that cause the disease have not been identified. No causes have been identified for many chronic diseases in humans.

Alfalfa induces autoimmunity in humans

L-canavanine is the most studied amino acid. This amino acid was isolated of Jack beans and wilds potatoes (*hedysarum alpinum*); it is also abundant in Albizzia julibrissin; and compete with L-arginine in protein synthesis. Canavanine a structural analog of arginine, is a NPAA naturally occurring in Leguminosae (7). In some species the canavanine contents ranges from 10% to 13% of the seed dry weight and constitutes more than 95% of the free amino acid nitrogen. Also is present in various beans, clover, onions, seeds and sprouts of alfalfa and other higher plants. An ordinary dish may contain around 25g beans and thus is assumed the intake of canavanine would be 200mg (5).

Association of Systemic Lupus Erythematosus (SLE) and alfalfa was first reported in a volunteer who developed lupus-like autoimmunity while ingesting alfalfa seed for a hypercholesterolemia study. This also was observed in chimpanzees fed with alfalfa sprouts that developed SLE. By suppressing the consumption of alfalfa disease disappeared both in the monkeys and in the voluntary subject (8). L-canavanine, present in alfalfa, was suspected as a cause of this phenomenon. During the study, the subject was ingesting crushed alfalfa seeds for six weeks. He developed moderate splenomegaly, pancytopenia, coombs positive, autoimmune hemolytic anemia and antinuclear antibodies (ANA) (9). When alfalfa seeds ingestion was discontinued the spleen size and the laboratory abnormalities returned to normal. Following these observations, six cases of subjects who developed SLE disease were reported from the consumption of alfalfa tablets for 3 weeks to 7 months (10). All the patients presented ANA and anti-

dsDNA antibodies. After stopping the consumption of tablets, all the patients were asymptomatic.

L-canavanine works as a potent antagonist that exhibits antimetabolic activity in many living fungi as well as in animals by being incorporated in the cell nucleus and other proteins and interfering with DNA and RNA synthesis (11, 12). Animal experiments have shown a number of deleterious effects following consumption of legumes containing canavanine; so has been observed a significant reduction in plasma concentration of plasma arginine, lysine and histidine (13). Prolonged consumption of alfalfa seeds by humans has been associated with pancytopenia, anemia, leukopenia and the development of antinuclear antibodies, attributed to canavanine in the seeds (9).

Mechanisms of toxicity of NPAAs

The first mechanism of toxicity identified for NPAAs was their ability to replace a protein amino acid in protein synthesis resulting in the synthesis of abnormal or nonnative protein. It has been shown that there are a linear correlation between the concentration of the NPAA and the level of incorporation into protein ((14). The replacement of a protein amino acid by an NPAA resembles a mutation (missence), in which the replacement of a single base in DNA encodes another protein amino acid in the polypeptide chain.

The typical toxic mechanism of NPAAs is that they function as mimic of 20 protein amino acids and are mistakenly incorporated in proteins in the place of the corresponding protein amino acids similar in structure, thereby leading to the production of unnatural proteins that cannot function properly. It is the case with azetidine-2-carboxilic acid found in *Canavalia majalis* (Liliaceae) which is a mimic of proline and a legume toxin canavanine that occurs in the seeds of jack bean, *canavalia ensiformis* and *Dioclea megacarpa* in very high concentrations (up to 6 and 10% respectively), which is a mimic of arginine (15).

Interference in metabolic pathways

The L-arginine analogue, L-canavanine is synthesized by over 350 species of Papilionoideae including jack beans (*canavalia ensiformis*), vine (Dioclea megacarpa) and wild potato *(Hedysarum)alpinum*). Concentrations can reach up to 13% of the dry weight of seeds; It is a very close structural analogue of L-arginine and serves as a substrate in virtually every enzyme-mediated reaction that employs L-arginine. In the rat L-canavanine is converted by arginase into urea and L-canaline toxin. Arginine participates in the urea cycle, in the formation of connective tissue and the synthesis of polyamines by the action of arginase producing ornithine and this with ornithine-descarboxilase (ODC) becomes putrescine (16,17).

Canavanine is an arginine antagonist, able to manifest antimetabolic effects in viruses, bacteria and fungi as well as in animals by being incorporated in the cell nucleus and proteins interfering with DNA and RNA synthesis (11,18). In macrophages and polymorphonuclear leucocytes, canavanine is able of prevent L-arginine derived synthesis of nitric oxide (12). On the other hand has been shown than canavanine cause a marked reduction in RNA synthesis in Escherichia coli and also to decrease DNA synthesis in herpes virus. Canavanine has an isoelectric point of 8.1 which decreases the basicity of histones which are rich in arginine. This alters the function of DNA and decreases the synthesis of histones, which has been demonstrated in mice, hamsters, also in monkey kidney cells. L-canavanine supplied in high concentration is lethal in rats and caused the death of an subject who feeds on wild potatoes with a high content of canavanine (7). When was administered in rats canavanine marked with radio isotopes; it was observed that the amount of canavanine that is incorporated in the proteins is equal to the amount of arginine (19).

Amino acids of Lathyrus

Several species of genus *lathyrus* are of economic importance*; L odoratus* (sweet pea) and *L. latifolius* (everlasting pea) are cultivated as ornamentals, while others such as *L sativus, L. cicero* and *L. clymenum* are sources of food for humans and domestic animals; these last three species have been implicated as causes of neurological disorders in both humans and animals. In addition to the genus Lathyrus , the genus *Vicia* also has 15 species that are lathyrogens, among them *Vicia faba*. The disease known as lathyrism, really is about two different diseases also produced by two different substances; one that affects the central nervous system causing neurolathyrism which is an irreversible paralytic disease and the other is osteolathyrism in which bone and connective tissue problems appear. A study of the free amino acids and related compounds in the seeds of 49 species of lathyrus were found two NPAAs. While working on an experimentally induced connective tissue disease, has been observed that β-aminopropio-nitrile (BAPN) an NPAA found in leguminous plant *lathyrus*; produced disastrous results in the test animals; this compound it inhibited the cross-linking of collagen; therefore was discovered that BAPN increased the solubility of collagen such as bone collagen which had been hitherto unavailable for study without prior denaturation. A lathyrogenic compound was defined as any compound which induced an increase in the connective tissue fragility and in collagen solubility in the 14-days-old-chick embryo. L-2-4-Diaminobutanoico acid (2-4-DABA) is a NPAA present in seeds of many species of lathyrus when injected into the peritoneum of rats, caused liver damage and neurotoxicity.

Lathyrus sativus (grass pea) is found in Europe, North America, Asia, East Africa, Ethiopia and South America; it is used as food in Spain in the form of porridge , contains various NPAAs; it is known as almorta, alverjón and pea; its toxic effects appear when consumed over a period of several weeks or months due to the presence of the compound β-N-Oxalil-L-αβ-diaminopropionic acid (β-ODAP). ***L sativus*** is the most widely cultivated species of *Lathyrus* and a valuable source of food and fodder in some of the poorest regions of the world. The best documented plant derived NPAA is B-ODAP produced by the legume *lathyrus sativus*. Prolonged consumption results in degenerative changes in the major central nervous pathway responsible for regulation of skeletal muscle function. The initial effects of B-ODAP include cramping and weakness in the muscles of the legs and can be reversible; however prolonged exposure leads to irreversible damage and permanent central motor system deficits.

B-ODAP may be regarded as a structural analogue of glutamic acid, acts as a neuroexciting to central nervous system neurons and is selective for glutamate receptors. B-ODAP inhibits tyrosine aminotransferase, which catalyzes the reaction between tyrosine and 2-oxoglutarate to yield glutamate and the ketoacid corresponding to tyrosine; B-ODAPs stimulates neural protein kinase C and causes mitochondrial dysfunction (20). 4-glutamil-3-aminopropio-nitrile is the toxin responsible for osteolathyrism since it preventing the formation of crosslinks in collagen and elastin which results in weak muscles and collagen disorders (collagen diseases), also produces fragility of the blood capillary walls.

L-2-4-diaminobutiric acid, L-2,4-diaminobutanoic acid (DABA). DABA was isolated from *L. latifolius* and shown to be acutely neurotoxic to rats. This amino acid is a homologue of ornithine. Induces ammonia toxicity (and hence neurotoxicity) in rats by inhibiting the liver enzyme ornithine transcarbamylase. DABA may cross the blood-brain barrier, in rats and other animals, using the cationic amino acid transporter system and once introduced into the brain, DABA acts as an anticonvulsant.

Sweet pea (peas or lodges) these seeds containing β-oxalylamino-L-alanine (BOAA) that alters the synthesis of collagen; inhibits the formation of collagen fibrils by the enzyme lysyl-oxidase, preventing the formation of hydroxylysine. In rats fed a diet of 50% sweet pea developed enlarged adrenals relative to control animals. The main effect is on the formation of collagen fibrils. Symptoms are similar to those of scurvy and copper deficiency, which share the common feature of inhibiting proper formation of collagen fibrils.

B-N-(ϒ-Glutamyl)-aminopropionitrile(ϒ-glutamil-BAPN) was isolated originally from seeds of *L odoratus and L pusillus*. The physiologically active part of the molecule is the BAPN moiety.

Both BAPN and ϒ-glutamyl-BAPN exercise their effects by inhibiting the formation of cross-linkages between the polypeptide chains during the synthesis of collagen. These seeds in animals produce aneurysm of the aorta; this is similar a condition that occurs in humans (aneurysm of the aorta).

β-L-Cyanoalanine (BCNA). Seeds of L *sativus* and *Vicia sativa* their seeds contain significant concentrations of BCNA and even higher amounts of the ϒ-glutamil derivative. Administration of BCNA by stomach tube to weanling male rats at a concentration of 15 mg per 100g body weight produced reversible hyperactivity, tremors,

convulsions and rigidity. BCNA inhibits cystathionase in the pyridoxal-5-phosphate-requiring conversion of cistathionine to cysteine and also the activity of aspartate decarboxylase. In mammalian liver this is the major pathway by which cysteine is synthesized from methionine, which is an essential amino acid.

Mimosine

Mimosine was isolated from *Mimosa púdica* but its present in high concentrations in the leaves and seeds of *leucaena leucocephala* containing as much as 8-10% dry weight of mimosine, while even higher concentrations can occur in the seeds. It is an analogue of tyrosine. Mimosine (hydroxyphenylalanine) present in seeds, stem and leaves is an inhibitor of DNA replication; inhibits various metal-containing enzymes involved in the synthesis of DNA, purines and thymidines; it also induces apoptosis of leukemic cells due to damage to the mitochondria (21).

The ingestion of leaves and seeds of *leucaena* has been held responsible for the loss of hair in both animals and humans. It is also found in guajes that in Mexico are used for human consumption due to their high protein content; it is a powerful chelating agent it kidnapping copper, iron, zinc and magnesium therefore it inhibits the enzymes responsible for the synthesis of DNA (ribonucleotide reductase). It is also a substrate for phenyl-alanyl-RNA synthetase and can replace tyrosine as a substrate for the enzyme tyrosinase. Mimosine inhibits a number of enzymes, such as dopamine β-hydroxylase and tyrosinase. Dopamine-β-hydroxylase was inhibited because of the removal of Cu by the strong chelating properties of the amino acid. Mimosine also reduces the synthesis of dopamine and serotonine, furthermore it is an inhibitor of pyridoxal phosphate, transaminases, tyrosine-decarboxylase, cystathionine synthetase and cystathionase. In addition to its potent metal-chelating ability (including Cu, Zn and Fe) (22-23)).

Indospicine an analogue of arginine, which is found in several members of the genera *indigofera* a leguminous native of Africa and Asia and which is a potent antimetabolite of arginine. The *indigofera* plant has a high protein content and is considered very useful because it tolerates droughts , floods and salinity; has 1.5% of the amino acid. Indospicine inhibits, but is not hydrolyzed by arginase, also inhibits the incorporation of arginine and consequently other amino acids, into liver protein. Indospicine is a competitive inhibitor of arginase and causes both liver degeneration and abortion. As an arginine analogue indospicine has the potential to interfere with a range of mammalian arginine metabolic pathways, as arginine is a precursor for the synthesis not only of protein but also of nitric oxide, urea, polyamines, proline, glutamate, creatine and agmatine(24). Many species of *Indigofera* contain a second toxic amino acid, canavanine is also produced, plus a third toxin β-nitropropionic acid, which is a "suicide inhibitor" of succinate dehydrogenase. Camel or horse meat contaminate with indospicine readily killed dogs but spared camels and horses that had fed on indospicine-containing plants.

Mucuna pruriens (velvet bean). Other NPAA is 3-4-dihydroxy-phenylalanine (L-DOPA) which is found in high concentrations (6-9%) in *mucuma* seeds; which is able to replace the protein amino acid tyrosine in protein synthesis, the catechol group on the L-DOPA molecule allows it to interact strongly with divalent metals affecting tyrosinase activity (25).

L-homoarginine. which is present in *Lathyrus cicero and Lathyrus sativus* differs from L-arginine only in that it contains an additional backbone methylene group (CH3) and can replace L-arginine in mammals in most physiological processes. Homoarginine is recognized as a normal metabolite in humans. L-arginine and L-homoarginine compete as a substrate of nitric oxide synthase. This amino acid was a substrate for rat liver arginase (26). This enzyme catalyze the hydrolysis of homoarginine to yield urea and lysine, the latter being an essential diet requirement for animal species. Also can be formed homocitrulline in tissue in several types of reactions. Antibodies directed against proteins that contain homocitrulline have been found in rheumatoid arthritis patients sera. More recently, it has been shown that homocitrulline-containing proteins are present in rheumatoid arthritis (27). Homoarginine induces hypersensitivity and mortality in rats when administered intraperitoneally at 10mmol/Kg body weight. When homoarginine was fed to rats on a lysine-deficient diet food intake and growth rate were both reduced. The seeds of *Lens culinaris* (lentils), also contain homoarginine, have been not observed adverse effects in human. Homoarginine is an uncompetitive inhibitor of human liver and bone alkaline phosphatase, but is not active against the intestinal and placental forms of the enzyme.

l-homoarginine, which is present in *lathyrus cicero*, *lathyrus sativus* and *lens culinaris* (lentils), can replace L-arginine in mammals in most physiological process. L-homoarginine inhibits bacterial growth. Missincorporation into protein in humans since it is an endogenous amino acid synthesized in the kidneys, also can decrease the production of nitric oxide by endothelial cells where it is an important determinant of vascular tone and blood pressure (28).

AZETIDINE-2-CARBOXYLIC ACID (AZE)

Aze is ubiquitously present in low concentrations in vegetation, which kidnapping metals from the soil and transfer then to various parts of the plants. In certain species, aze accumulates to exceedingly high levels notably in the bulbous roots of table beets and sugar beets (*beta vulgaris*) up to 5%. Thus, aze is present in the food chain in small concentrations in vegetables. However it is in high concentrations in certain foods, especially in dairy products derived from livestock fed sugar beet by products (15).The worldwide prevalence of multiple sclerosis (MS) has been linked to beet agriculture (*Beta vulgaris*). Beets contain Aze which can replace proline in proteins leading to neurodegeneration and autoimmune disorders. There is a possibility that aze present in beet a potent cell toxin, implicated in MS, can enter the human feed chain (2).

Multiple sclerosis is considered as an autoimmune disease; attack the central nervous system. Epidemiologists have seen an increased pattern of MS cases in countries located farther from the equator. People of northern European descent are at highest risk of developing MS. Rubenstein (15) proposed that aze replaces L-proline residues in myelin basic protein (MBP) of the myelin sheath. Aze is the lower homolog of proline that is identical to proline except in that it contains four instead of five members in its ring . Aze is highly toxic to many organisms because it is incorrectly incorporated into proteins instead of proline modifying the three dimensional structure of proteins including collagen, keratin and hemoglobin. A link was also established between the geography of beet agriculture and the worldwide prevalence of MS. In addition, MS is a relatively modern disease and correlates with the increase in cultivation of beets for sugar which now accounts for around 30% of the world supply of sucrose.

Aze which is present in a number of plants including *Convallaria majalis* (lily of the valley),some liliaceae and *Beta vulgaris* (sugar beets) readily replaces L-proline in protein synthesis. Aze was shown to inhibit the uptake of 14C-proline by E *coli* and inhibit proline biosynthesis from glutamate through feedback inhibition. This feedback inhibition has also been demonstrated by analogues of the aromatic amino acids: phenylalanine, tyrosine and tryptophan.

Canavanine

L-canavanine is synthesized by over 350 species of leguminous plant including jack beans (*Canavalia ensiformis*), vine (*Dioclea megacarpa*) and wild potato (*Hedysarum alpinum*).

L-*canavanine* serves as a substrate in virtually every enzymes mediated reaction that employs L-arginine. In the rat L-canavanine is converted by arginase into urea and the toxin L-canaline. L-canavanine is also a substrate for inducible nitric oxide synthase; the enzyme that converts L-arginine in nitric oxide; a potent vasodilator and inhibition of platelet activation (7).

Canavanine is found in many species of the Papilionoideae, which is a sub-family of the Leguminosae. Seeds are a particularly rich source of this amino acid and concentrations as high as 13% dry weight has been reported in those of *Dioclea megacarpa*. Canavanine is incorporated into mammalian protein in competition with arginine because the arginyl-tRNA synthetase is unable to differentiate between arginine and canavanine. It is significant that macaques fed on the seeds and sprouts of *Medicago sativa* (alfalfa), which contain canavanine, develop hematological and serological abnormalities (28). These abnormalities are similar those seen in human SLE, an autoimmune disease that adversely affects the kidney and skin. SLE can be induced in apparently normal subjects that are fed leguminous which contain L-canavanine. (29)

Canavanine can affect B-cell function, accelerating the disease in

autoimmune mice and inducing antibody-mediated autoimmune phenomena on normal mice. Canavanine also affects the charged surface membrane properties of autoimmune B-cells.; such alterations may be associated with an abnormal (auto) immune response. L-canaline is formed by the hydrolysis of canavanine (a reaction catalyzed by arginase) which is an importan factor in the toxicity of canavanine (30).

L-canavanine is a substrate for arginyl tRNA synthetase and replace l-arginine during protein synthesis; this substitution can occur in every arginine-containing proteins and results in the production of structurally aberrant canavanyl proteins (31). Arginine constitutes 8% of amino acids in the human collagen. The result is a disruption of enzymatic activity and a rapid degradation of the protein. L-canavanine is a less basic molecule than l-arginine; the pK of the guanidooxi group is 7.04 vs 12.48 of the guanido group in l-arginine and this can impact the function and structure of the protein and the health and function of the organism. Substitution of a protein amino acid for a NPAA has similarities a missense mutation in which substitution of a single base in DNA will encode another protein amino acid in the polypeptide chain.

The toxicity of L-canavanine in animals has been well documented; has been observed cases of poisoning among cattle allowed to forage on jack beans, *Canavalia ensiformis*; the seeds of which contain approximately 2.5% L-canavanine by dry weight. Several studies of feeding trials that showed L-canavanine sulfate induced toxic effects in mice. All mice fed L-canavanine at a concentration of 2.0 g/kg mouse died within 24 hours. Also has been reported hematologic and serologic abnormalities similar to those observed in human (32).

Sword beans (*canavalia gladiata*) are one of many underutilized but exceptionally productive, large-seeded tropical leguminous. This leguminous is consumed in Srilanka, southern India, Indonesia as a substitute for mashed potatoes; In Guatemala as a coffee substitute and other Central American countries. Sword beans are not commonly used as a food; the reason is the presence of antinutritional factors such as haemagglutinins, tannins, phytates and canavanine 4% (7).

The existence of the genetic component in SLE is based mainly on family aggregation and high concordance in twins monozygotes; it is estimated that the prevalence of SLE in relatives is 66 times higher than in general population. This frequency of the disease is due to the fact they live in the same house and eat the same foods. On the other hand leguminous are consumed since childhood and it has been observed that canavanine accumulates in the human organism causing illness in some people when they consume them for a long time (33).

Vicia faba. Broad beans contain vicin and convicin that are hydrolyzed in the digestive tract by the action of β-glucosidase, giving divicin and isouracil. Beans, broad beans and soy produce methane causing intestinal cramps and flatulence. The beans in large quantities produce agglutination of erythrocytes causing small clots that decrease the fluidity in the blood capillaries. Favism is a genetic disease. People with a deficiency of the enzyme glucose-6-dehydrogenase have hemolytic problems due to the ingestion of broad beans (*Vicia fava*) and also decreases the concentration of glutathione due to the action of the compounds known as vicin and convicin; which when hydrolyzed in the digestive tract give rise to divicine and isouracil, which are directly responsible for the disease. The gene that encode for the enzyme is found on the X chromosome; therefore the disease is more frequent in men than in women. The dihydroxyphenilalanine found in broad beans and velved beans is also an agent of favism. Broad beans also contains canavanine 1 g% in dry weight (5).

Lentils. Lentils also contain canavanine 1.0 g% dry weight and homoarginine; furthermore contain several antinutrients such as trypsin inhibitors, an enzyme that metabolizes phytic acid which binds to iron and zinc minerals (5).

Autoimmune Diseases

Autoimmune diseases also called collagen diseases; these diseases have three things In common; their etiologies remains unknown, the prognosis is not hopeless, remain poor and the specificity of available drugs questionable. SLE considered to be the prototype of autoimmune disease in which the immune response is directed against a wide variety of self-antigens resulting in damage to various organs and systems. In chimpanzees feed alfalfa, they developed a disease with all the characteristics of lupus erythematosus and on the other hand a volunteer developed lupus erythematosus while ingesting alfalfa seeds for a study of hypercholesterolemia. By suppressing the consumption of alfalfa the disease disappeared both in the monkeys and in the voluntary subject. Subsequently several cases of induction or exacerbation of the disease due to the intake of alfalfa tablets were reported. (8, 34). When was administered in rats canavanine marked with radio isotopes it was observed that the amount of canavanine that is incorporated in the proteins is equal to the amount of arginine (18). This fact causes the loss of positive charge of arginine and therefore the interactions of the amino acids with its neighbors are modified; therefore immune system can attack the canavanine proteins, leading to autoimmune diseases such as rheumatoid arthritis, MS, psoriatic arthritis, SLE, and Sjogrens syndrome. Anti-DNA antibodies were found in the blood serum of chimpanzees and rats fed alfalfa. Canavanine has an isoelectric point of 7.1 wherever arginine its isoelectric point is 12.48 which decreases basicity in proteins that are rich in arginine. The proline used in the production of collagen is synthesized from arginine and in the first reaction participates the arginase enzyme producing ornithine. Proline is hydroxylated by prolyl hydroxylase enzyme to hydroxyproline which stabilizes the collagen. On the other hand has been observed that when prolyl-hydroxylase enzyme is inhibited, collagen loss its native configuration. The final step of collagen synthesis consists of cross-link formation by the enzyme lysyl-oxidase whose two cofactors are copper and pyridoxal-phosphate; this gives the fibril the strength to perform its major tensile role. This enzyme when is inhibited by canaline , (a metabolite of canavanine), then synthesis of collagen is disturbed and on the other hand antibodies are generated. The lysyl-oxidase enzyme is also inhibited by BAPN a NPAA present in *lathyrus sativus*, this blocks the activity of the enzyme by kidnapping copper. The tensile effects decreased due to this fact that interlinked links are diminished. Collagen is distributed in all the organs of the human body and the skin; due to this fact there are injuries throughout the body during the disease. For this reason the autoimmune diseases also are known as collagen diseases (35).

SLE can be induced in apparently normal subjects that are fed leguminous which contain NPAAs. The toxic potential of these NPAAs depend on the amount ingested and the time of consumption.

Canavanine is the NPAA more studied; when this amino acid is metabolized by the arginase enzyme, canaline is produced which inhibits metabolism of ornithine, decreasing the production of several compound necessary for cell division and growth. On the other hand protein synthesis is disturb and autoantibodies are produced generating immune complexes which inducing the autoimmune disease. Canavanine disturb the collagen synthesis; others NPPAs inhibit the activity of several enzymes and others such as BAPN found in sweet bean (*lathyrus odoratus*), specifically inhibits the formation of cross-linking of collagen and elastin chains. This legume in addition contain canavanine (5); is added to rice and consumed frequently worldwide; furthermore there are other legumes such as alfalfa, lentils, soy bean, common bean, that also contain canavanine. See Table 1. Furthermore, in seeds of various lathyrus species several toxic amino acids have been found such as homoarginine, mimosine, and indospicine in seeds, sprouts and all parts of the leguminous plant.

Table 1. Plant Derived Non-protein Amino Acids, Source(s), Known Protein Amino Acid Analogues And What Their Effects

Source	amino acid or derivates	protein amino acid	action or disease
Alfalfa	L-Canavanine	L-arginine	cytotoxic
Mucuma pruriens	3-4-dihydroxyphenylalanine	L-tyrosine	favism
Canavalia ensiformis	L-canavanine	L-arginine	cytotoxic
Dioclea megacarpa	L-canavanine	L-arginine	cytotoxic
Lathyrus cicero	Homoarginine	L-arginine	cytotoxic
Lathyrus	α-γ- diaminobutyric acid	L-ornithine	latyrogenic
Brassica	glucosinolate	L-tyrosine	goitrogenic
Leucaena glauca	Mimosine	L-tyrosine	goitrogemic
Lathyrus sativus	β- aminopropionitrile	L-glutamic	osteolathyrogen
Lathyrus odoratus	**β**-diamino propionic acid	L- glutamic	osteolathyrogen

Convallaria majalis	azetidine-2-carboxilic acid	L-proline	multiple sclerosis
Indigofera spicata	indospicine	L-arginine	hepatotoxic
Beta vulgaris	azetidine-2-carboxilic acid	**L-**proline	multiple sclerosis

It has been shown that a lower concentrations of NPAAs, there is a linear correlation between the concentration of the NPAA and the level of incorporation into protein which can produce changes in the protein affecting its tertiary structure and its function. The immune system can attack these proteins, leading to autoimmune diseases such as rheumatoid arthritis, MS, psoriatic arthritis, SLE, Sjogrens syndrome, scleroderma, dermatomyositis, also in cells with Alzheimer´s disease (36). This demonstrates the need for additional studies on NPPAAs to support the diagnosis of some diseases.

This information suggests that majority of patients with some autoimmune disease have consumed some leguminous plant that contain canavanine (see table 2), homoarginine or other NPAA que disturb the synthesis of collagen and induce formation of autoantibodies.

Table 2. Legume Plants Containing L-canavanine And Their Family To Which They Belong

PLANT	COMMON NAME	FAMILIA
Albizia julibrissin	acacia	fabáceae
Canavalia ensiformis	Jack bean	fabáceae
Canavalia gladiata	sword beans	fabaceae
Canavalia majalis	sable bean	fabáceae
Cicer arietinum	chickpea	fabaceae
Dioclea megacarpa		fabaceae
Hedysarum alpinum	wild potatoes	fabaceae
Indigofera tintorea	creeping indigo	fabaceae
Lathyrus odoratus	sweet pea	Fabaceae
Lathyrus sativus	grass pea	fabaceae
Lens culinaris	lentils	fabaceae
Leucaena leucocephala	guaje	fabaceae
Medicago sativa	alfalfa	fabaceae
Vicia fava	broad bean	fabaceae

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