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Role of non-protein amino acid L-canavanine in autoimmunity

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Abstract

Association of SLE and alfalfa was first reported in a volunteer who developed lupus-like autoimmunity while ingesting alfalfa seed for a hypercholesterolemia study. This was corroborated with studies in monkeys fed with alfalfa sprout that developed SLE. Re-challenge with L-canavanine relapsed the disease. Arginine homologue L-canavanine, present in alfalfa, was suspected as a cause. L-canavanine can be charged by arginyl tRNA synthetase to replace L-arginine during protein synthesis. Aberrant canavanyl proteins have disrupted structure and functions. Induction or exacerbation of SLE by alfalfa tablets reported in a few cases remains controversial. Epidemiological studies on the relationship between alfalfa and SLE are sparse. In mice, NZB/W F1, NZB, and DBA/2 mice fed with L-canavanine show exacerbation/triggering of the SLE, however, BALB/c studies were negative.

L-canavanine incorporation may be more efficient in the presence of inflammation or other conditions that can cause arginine deficiency. The L-canavanine induced apoptotic cells can be phagocytosed and a source of autoantigens processed by endosomal proteases. Endogenous canavanyl proteins are ubiquitinated and processed via proteasome. Incorporation of L-canavanine into proteasome or endosome can also cause disruption of antigen processing. Alfalfa/L-canavanine-induced lupus will be an interesting model of autoimmunity induced by the modification of self-proteins at the translational level.

Keywords: Lupus; L-canavanine; Arginine; Autoantibodies; Antinuclear antibodies

Contents

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1. Introduction

Development of SLE in monkeys fed with alfalfa sprout was a sensational observation when it was initially reported more than 20 years ago [1,2]. These findings corroborated the occurrence of a lupus-like syndrome in a healthy volunteer ingesting alfalfa seed for a hypercholesterolemia study [3]. L-canavanine [4,5], an abundant amino acid in alfalfa seed and sprout, was suspected as the cause of this phenomenon. L-canavanine is usually not used to synthesize cellular proteins (non-protein amino acid), but is known to replace L-arginine and produce aberrant canavanyl proteins. Case reports on exacerbation/development of SLE after taking alfalfa tablets followed [6,7]. However, only a few studies reported the in vitro immunological effects of L-canavanine [7–10] and a few reports mentioned alfalfa in studies of the environmental factors in SLE [11–13]. Both clinical and basic studies on alfalfa/L-canavanine-induced SLE are very limited despite the impact of the initial reports. Furthermore, alfalfa/L-canavanine-induced lupus has not been evaluated with the recent view of modification of autoantigens as mechanisms to break immunological tolerance and trigger autoimmunity. It will be of interest to reevaluate alfalfa/L-canavanine-induced SLE as a model in which autoantigens are modified at the translational level via replacement of arginine by L-canavanine. This article is aimed to review what was the alfalfa-induced SLE and what is known about the mechanisms.

2. Non-protein amino acid L-canavanine

L-canavanine, a non-protein amino acid present in various beans, clover, onions, seeds and sprouts of alfalfa, and other higher plants, is a natural homologue of L-arginine [4,5]. L-canavanine works as a potent antagonist that exhibits antimetabolic activity in many living systems in vitro and in vivo. L-canavanine can compete with L-arginine when cellular enzymes such as arginyl tRNA synthetase, inducible nitric oxide synthase (iNOS), and arginase target free arginine. More importantly, L-canavanine is a substrate for arginyl tRNA synthetase; L-canavanine can be charged by arginyl tRNA synthetase and replace L-arginine during protein synthesis, creating aberrant canavanyl proteins [4]. Although the pKa of the guanidiooxy group of L-canavanine is far less than the value of arginine, up to 30% of arginine residues can be replaced by L-canavanine based on analysis of proteins synthesized in vitro [4,5].

This substitution can occur in every arginine-containing proteins and results in the production of structurally aberrant, canavanyl proteins [4]. L-canavanine substitutions disrupt the tertiary and/or quaternary structure that is responsible for the three dimensional conformation unique to the protein [14]. The result is a disruption of enzymatic activity [4,5] and a potentially rapid degradation of the proteins [15]. Persistent presence of L-canavanine may result in apoptotic cell death.

3. Alfalfa induced autoimmunity in human

Reduced serum cholesterol levels [16], inhibition of cholesterol absorption [16], and the prevention and regression of atherosclerotic plaques [17], caused by the ingestion of alfalfa seed/meal/saponin, were reported in rats, rabbits, and monkeys. Based on these favorable effects of alfalfa in animals, a human study to examine the effects of alfalfa was conducted [18]. A reduction in cholesterolemia has been observed in man after a short period of alfalfa seed ingestion [18]. However, one volunteer developed a lupus-like autoimmune syndrome during the study [3]. A healthy 59-year-old man ingested 80–160 g of ground alfalfa seeds daily on eight occasions or periods of up to 6 weeks [3]. Although no symptoms were observed, he developed moderate splenomegaly, pancytopenia with a Coombs positive autoimmune hemolytic anemia (AIHA), antinuclear antibodies (ANA), and hypocomplementemia. When alfalfa seeds ingestion was discontinued, the spleen size and laboratory abnormalities returned to normal. This observation lead to the studies in monkeys fed with alfalfa for the presence of lupus-like autoimmunity (next section) [1].

Following these observations [1–3], two lupus patients with clinically and serologically quiescent SLE who had reactivation of their systemic diseases in association with the ingestion of alfalfa tablets (8–15 tablets/day, 9 months and 2 1/2 years) were reported [6]. Analysis of the alfalfa tablets demonstrated the presence of L-canavanine. Another 4 cases of previously healthy patients, who developed lupus-like symptoms including arthralgias, myalgias, mild rash, positive ANA, and anti-dsDNA antibodies in one patient, were also reported. All the patients in the 4 cases had consumed 12–24 alfalfa tablets per day for 3 weeks to 7 months. [19]. All 4 patients became asymptomatic after discontinuing the tablets and the ANA disappeared in 2 patients.

Another report described a family of 3 generations who have been taking supplements including alfalfa tablets for 7 years [20]. The propositus had intermittently elevated ANA and was diagnosed as having SLE. Three family members had arthritis and one had