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Relation of Arginine-Lysine Antagonism to Herpes simplex Growth in Tissue Culture

Richard S. Griffith, Donald C. DeLong and Janet D. Nelson

Lilly Laboratory for Clinical Research and Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind., USA

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Abstract. In the studies conducted, arginine deficiency suppressed herpes simplex virus replication in tissue culture. Lysine, an analog of arginine, as an antimetabolite, antagonized the viral growth-promoting action of arginine.

The in vitro data may be the basis for the observation that patients prone to herpetic lesions and other related viral infections, particularly during periods of stress, should abstain from arginine excess and may also require supplemental lysine in their diet.

Introduction

It has been shown that arginine-deficient tissue culture media inhibit viral replication [5–8, 17, 20, 22–23, 29, 31–32, 34]. In both in vitro and in vivo experiments, removal of arginine by mycoplasma has produced an antiviral action [16, 28]. Lysine has an antiviral action in tissue culture [24, 31]. In contrast to other studies [18], two reports have appeared which suggest that dietary arginine restriction and supplemental lysine used clinically has a suppressive effect on herpes simplex infection [9, 15].

The present in vitro study compares the results of increasing concentrations of arginine on the viral suppressive action of lysine under experimental conditions.

Materials and Methods

Virus and Cells. Herpes simplex type I virus (Mayo 1814) was obtained from the Research Resources Branch of NIAID and passed 28 times in our laboratory prior to these studies. The BS-C-1 line of green monkey kidney cells was used. The stock cell cultures were grown at 37 °C on glass with complete Medium 199 with 5% heat-inactivated fetal calf serum (FCS), 100 U/ml peniciilin, and 100 μg/ml streptomycin.

Virus Growth Condition. Special Eagle's minimum essential medium (EMEM) was prepared without arginine or lysine [31, 33]. Arginine and

lysine were added to the EMEM and mixed prior to addition to infected cells in the concentrations shown in table I. Falcon flasks (25 cm^2) containing confluent monolayers of BS-C-1 cells were inoculated with approximately 500 plaque-forming units (PFU) of herpes simplex virus. After adsorption for 1 h at room temperature, the cell sheet was washed three times with physiological saline to remove the nonadsorbed virus and any arginine-containing residual growth medium. Special EMEM containing various concentrations of arginine and/or lysine (5 ml) was added and the flasks were incubated at 37 °C. Flasks were observed microscopically and when control flasks exhibited 4+ cytopathic effect (CPE), all flasks were frozen, thawed, sonicated, centrifuged, and titrated for the number of PFU/ml (multiple back titration).

Virus Titration. Dilutions of virus suspension from growth studies were added to confluent monolayers of BS-C-1 cells in 25 cm^2 flasks. After adsorption for 1 h at room temperature, the infected cell sheet was overlaid with equal parts of 1% agarose and 2× complete Medium 199 with 2.5% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. All flasks were incubated at 37 °C until control flasks indicated optimum plaque size. A 10% formalin and 2% sodium acetate solution was added to each flask to inactivate the virus and fix the cell sheet to the plastic surface. The plaques were counted after staining the surrounding cell areas with crystal violet.

Results

When arginine was omitted from the medium, herpesvirus did not grow. Lysine did not encourage herpes replication as indicated by lack of effect in concentrations as high as 400 μg/ml (table I). Maximum herpes growth was obtained in the flasks containing 10–25 μg/ml of arginine without lysine: 211,000–290,000 viable viral PFU were found.

The addition of 50 μg/ml of lysine to the flasks containing 2.5 and 5.0 μg/ml of arginine reduced the number of viable PFU per flask from 3,300 and 48,800 to less than 10. A reduction of plaque formation by 0.5 $\log_{10}$ is considered significant [3]. In the flasks containing 7.5 and 10 μg/ml of arginine, the plaque formation was also reduced.

It is obvious from table I that the maximum antagonism of arginine was achieved with the addition of 50 μg/ml of lysine since the addition of higher concentrations of lysine did not produce a proportionately greater effect. The effect of arginine on viral

Table I. Lysine Inhibition of arginine utilization; 500 PFU/flask multicycle back titration

Arginine, μg/ml	Lysine, μg/ml					
	0	50	100	200	300	400
0	< 10	< 10	< 10	< 10	< 10	< 10
2.5	3,300	< 10	< 10	< 10	< 10	< 10
5	48,800	< 10	< 10	< 10	< 10	< 10
7.5	56,000	60	60	33	33	130
10	211,000	160	99	66	66	260
12.5	227.000	5,200	6,900	NR[1]	2,510	1,485
25	290,000	160,000	NS[2]	290,000	230,000	330,000

Values are mean PFU/flask

[1] No reading – poor cells

[2] No sample

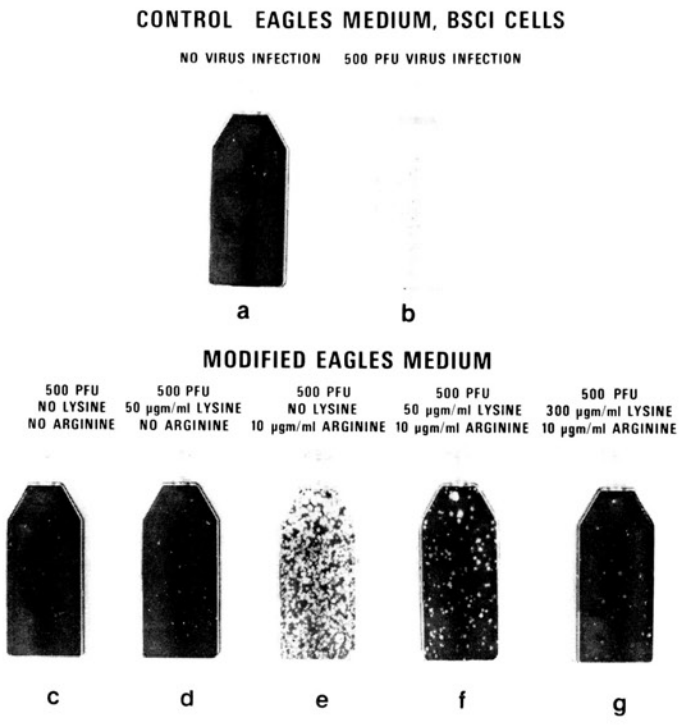

Fig. 1. A composite using representative flasks (a–g) illustrates the antagonism of arginine by lysine on viral replication. **a** The noninfected cell layer stained evenly. **b** When the cell layer was infected with 500 PFU, virtually all cells were killed so staining did not occur. In both figure 1a and b the control media (EMEM) contained 58 µg/ml lysine and 105 µg/ml of arginine. **c** When both arginine and lysine were omitted from the medium, the addition of 500 PFU did not produce any cytopathogeneicity, as evidenced by all cells taking the stain. **d** The addition of lysine in the absence of arginine in the medium did not support viral growth. **e** 10 µg/ml of arginine supported viral growth but not to the degree seen in the infected control (fig. 1b). **f, g** 50 and 300 µg/ml of lysine added to the flasks containing 10 µg of arginine antagonized viral growth: compare figure 1f and g with figure 1e.

plaque formation and its antagonism with lysine is illustrated in figure 1.

Discussion

Our results verify the 'nutritional' requirements for the amino acid arginine to support herpes simplex type I viral growth [17, 23]. It is also evident that lysine antagonizes, or is inhibitory to, the growth-supporting action of arginine for the herpesvirus in tissue culture [31]. Lysine and its precursor, aminoadipic acid, have been shown to be suppressive to the RNA-type mouse encephalitis virus in tissue culture [24]. Adenovirus type 2, a DNA virus also with a capsid coat, requires arginine for growth in tissue culture [26]. Other viruses have been shown to be arginine dependent: adenovirus type 1 [7], SV 40 [8], polyoma [34], cytomegalovirus [20], and measles [25]. A herpesvirus causing Marek's disease in chickens has been reported to be arginine dependent in vivo [16].

Lysine appears to be an antimetabolite acting as an analog of arginine [33]. Lysine competes with arginine for the transport system across the wall of the intestine [12–14, 19, 27] and also with the reabsorption of arginine by the transport system in the renal tubules [4, 19]. The net result with the oral administration of lysine is a reduction of arginine absorption and an increased excretion of arginine in the urine. Lysine competes with arginine for the enzyme argi-

nase and is, therefore, a potent arginase inducer resulting in catabolic degradation of arginine [11, 30]. Lysine entering the transport system of the tissue cells, decreases the intracellular content of arginine [2]. The total result is a depletion of the amino acid pool of arginine with a preponderance of lysine, a detrimental ratio for viral replication. A genetic variation in arginine and lysine utilization may make certain individuals more susceptible to viral infection [21].

On the basis of the observation that a reduction in arginine concentrations in tissue culture suppresses viral replication and lysine as an analog of arginine acts as an arginine antagonist, it seems reasonable in the treatment of infections due to arginine-dependent viruses to curtail the intake of arginine while administering lysine. Increasing the concentrations of arginine in the tissue culture antagonized the inhibitory action of lysine. This suggests that the appearance of viral infection may be related to either a reduced intake of lysine (as seen in the weaning of infants) (Dr. *Benjamin Kagan*, Cedars Sinai Medical Center, Los Angeles, Calif.) or the ingestion of large amounts of arginine (all nuts, including peanuts, peanut butter, and chocolate) [10]. Since it has also been shown in vitro that lysine deficiency may activate certain viruses [1], then the treatment of viral infections should include supplementary lysine accompanied by a reduction of arginine intake in the diet as outlined in our clinical study [9].

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R. S. Griffith, MD, Lilly Laboratory for Clinical Research, Wishard Memorial Hospital, 1001 West 10th Street, Indianapolis, IN 46202 (USA)