Note

Reduction of Tumor Growth and Hypercholesterolemia by Arginine Administration to Rats with Transplanted Hepatoma

Kazumi Yagasaki, Toshihiro Tanabe, and Ryuhei Funabiki

Department of Applied Biological Science, Tokyo Noko University, Fuchu, Tokyo 183, Japan
Received August 31, 1993

The effect of the oral administration of large amounts of arginine or lysine on tumor growth and hypercholesterolemia was studied in rats into which hepatoma AH109A cells had been transplanted. Arginine reduced both hepatoma growth and hypercholesterolemia, and lysine tended to have these effects. The oral administration of large amounts of arginine might be useful to decrease cachexia arising from cancer and to inhibit the tumor growth.

Large amounts of arginine suppress the growth of some tumors when animals-bearing tumors are given the amino acid orally or intravenously. The growth of an ascites hepatoma cell line, AH109A, in rats implanted with the hepatoma is inhibited when rats are given intravenous hyperalimentation with excess arginine imbalance. The proliferation of AH109A cells in culture is suppressed by lysine as well as arginine. When rats receive a subcutaneous implantation of AH109A cells in the back, the hepatoma cells grow rapidly, forming a solid tumor and causing hypercholesterolemia characterized by a striking increase in very-low-density lipoprotein and low-density lipoprotein (VLDL + LDL)-cholesterol (Ch) with a slight but significant decrease in high-density lipoprotein (HDL)-Ch. The basic amino acids arginine and lysine are hypocholesterolemic and histidine is hypercholesterolemic. Our study was done to find whether the oral administration of large amounts of arginine or lysine would lessen hypercholesterolemia and inhibit tumor growth in rats after transplantation of AH109A cells. Arginine reduced hepatoma growth and hypercholesterolemia, and lysine tended to have the same effects.

Male Donryu rats (four weeks old; NRC Haruna, Gunma) were kept on a stock pellet diet (CE-2; CLEA Japan, Tokyo) for 3 days and on a 20% casein diet for the next 9 days in an air-conditioned room with an 8:00 a.m. to 8:00 p.m. light cycle. All of the animals then received a subcutaneous implantation of 5 x 10^6 AH109A cells (provided by SRL, Tokyo), which produced a solid tumor in the back, as previously described. They were thereafter kept on the 20% casein diet and water ad libitum. Eight days after the implantation, rats bearing hepatomas were assigned to one of three groups with other rats with equal tumor size and body weight. l-Arginine or l-lysine (acetic acid salts, Ajinomoto Co., Inc., Tokyo) was suspended in water and given to rats for 7 days by oral intubation twice a day (9:00 a.m. and 5:00 p.m.) at a dose of 3.44 mmol/ml each time, for 6.88 mmol daily. Control rats received water instead. The oral dose (6.88 mmol daily) was approximately ten times as much arginine or four times as much lysine as the control rats ingested daily from the 20% casein feed. The final administration was at 9:00 a.m., at which time rats were deprived of feed but were given free access to water until being killed 4 h later by decapitation. Blood was collected in a glass tube, left to clot at room temperature, and centrifuged to obtain the serum. The serum and solid hepatoma were quickly removed, washed with cold 0.9% NaCl solution, blotted on filter paper, and weighed.

Table Effect of Oral Administration of Arginine or Lysine on Food Intake, Body Weight Gain, Liver Weight, and Lipid Levels in Liver and Hepatoma of Rats with Transplanted AH109A Cells

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>Arginine</th>
<th>Lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/15 days)</td>
<td>229 ± 8^a</td>
<td>187 ± 4^b</td>
<td>197 ± 9^b</td>
</tr>
<tr>
<td>Body weight gain (g/15 days)</td>
<td>83.9 ± 4.1^a</td>
<td>44.8 ± 2.9^b</td>
<td>57.2 ± 4.0^b</td>
</tr>
<tr>
<td>Liver weight (g/100 g body weight)</td>
<td>4.1 ± 0.2^a</td>
<td>3.9 ± 0.1^b</td>
<td>4.7 ± 0.1^b</td>
</tr>
</tbody>
</table>

Abbreviations: Ch, cholesterol; TCh, total cholesterol; TG, triglyceride; PL, phospholipid; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Each value is the mean ± standard error for 7 rats (control group) or 6 rats (other groups). Values not sharing a common letter are significantly different at p < 0.05 by Duncan's multiple-range test.
The serum lipoproteins were separated into HDL and VLDL + LDL fractions by the precipitation method. The total Ch content of the unfractionated serum (TCh) and the Ch in the HDL fraction (HDL-Ch) were assayed by an enzymatic method with a commercial kit (Wako Pure Chemical Industries, Osaka), and the difference between TCh and HDL-Ch was regarded as the (VLDL + LDL)-Ch. The serum triglyceride (TG) and phospholipid (PL) levels and the TCh, TG, and PL contents of the liver and hepatoma were assayed after extraction of total lipids as previously described. Statistical analysis was done with Duncan’s multiple-range test.

As shown in Fig., the weight and hence the growth of the solid hepatoma was suppressed by the oral administration of either arginine or lysine; the effect of lysine was not statistically significant. The administration of either of the two amino acids reduced food intake and retarded the growth of the animals compared with the controls, and the liver was slightly enlarged when lysine was given (Table). Changes in the lipid levels of the serum, liver, and hepatoma also are shown in the Table. Arginine reduced the serum TCh level, because the (VLDL + LDL)-Ch level decreased. Arginine decreased the serum PL level and tended to decrease the TG level. Lysine tended to be hypolipidemic, but none of the effects of lysine were statistically significant. The two amino acids had no effect on the HDL-Ch level, or on liver and hepatoma lipids, except that rats given lysine had increased the liver TG level. The liver enlargement caused by the lysine administration might be partly due to the accumulation of TG; lysine has this effect on liver TG in nephritic rats with hyperlipidemia.

The results showed that the oral administration of large amounts of arginine had both antitumor and hypocholesterolemic effects in rats with transplanted AH109A. Lysine tended to have the same effects. The acetic acid moiety of these amino acids may not be involved in the inhibition of tumor growth, since acid-free arginine and lysine suppress the proliferation of cultured AH109A cells. Littman et al. showed that reduction of the availability of Ch, either by dietary restriction or by administration of hypocholesterolemic drugs, slows the growth of a number of transplantable animal tumors. Schneider et al. have reported that estrone, which inhibits Ch biosynthesis and lowers rat plasma Ch, prolongs the survival of rats bearing hepatoma subcutaneously and suppresses hepatoma growth. They demonstrated that there is correlation between low tumor weight and prolonged survival with low plasma Ch and low Ch in VLDL and LDL fractions. There is correlation between the hepatoma weight and (VLDL + LDL)-Ch concentration and negative correlation between hepatoma and HDL-Ch in rats with transplanted AH109A. At least two interpretations are possible of the suppression of tumor growth and hypercholesterolemia by these two amino acids; the hypocholesterolemic effect of the amino acids could lead to growth suppression, or vice versa. However, each effect may occur independently, since arginine and lysine directly inhibit AH109A growth in vitro and since both amino acids are hypocholesterolemic in tumor-free animals.

References