Suppression of Tumor Growth in Experimental 9L Gliosarcoma Model by Copper Depletion

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Abstract

The effect of copper (Cu) depletion on the growth of tumors was investigated in a rat brain tumor model. 9L gliosarcoma cells were injected subcutaneously in 5-week-old male Fischer-344 rats. The control group (n = 18) received a normal diet throughout the experiment and the depletion group (n = 18) received a Cu-deficient diet starting 3 weeks prior to tumor implantation, and 2 mg of D-penicillamine orally, once daily, on the 3 days before and after implantation. Six animals from each group were killed at 1, 2, and 3 weeks following the implantation to measure the tumor weights and determine the tissue Cu concentration by atomic absorption spectrophotometry. The tumor weights increased much more rapidly in the control than in the depletion group. The Cu concentrations in tumor tissue of the depletion group were significantly lower than in the control group. There was no statistical significance in Cu concentration in the brain tissues of the control and depletion groups. Our study indicated that a Cu-deficient diet and D-penicillamine treatment can inhibit subcutaneous glioma growth in this rat model.

Key words: angiogenesis, copper, gliosarcoma

Introduction

Copper (Cu), a trace element essential for homeostasis and important in cell growth, is accumulated in the serum and tumor tissue of patients with various cancers and is correlated with the tumor malignancy and patient prognosis. We previously reported abnormally elevated levels of Cu in malignant gliomas. Cu is a cofactor of angiogenesis and several molecules that bind Cu are potentially angiogenic. Cu has a high affinity with heparin and fibroblast growth factor which are both capable of stimulating tumor angiogenesis. Angiogenesis is a key factor of tumor growth. The inhibition of tumor angiogenesis may control tumor growth by preventing the exponential vascular growth phase. The mechanism of Cu-bound molecular stimulation of cell growth and the potential inhibition of the bioactivity of tumor cells must involve the affinity and transfer kinetics between Cu and the molecule.

A rat brain glioma model was previously used to show the effect of reduced Cu serum concentration caused by Cu depletion on angiogenesis. This study determined the effects on tissue Cu concentration and inhibition of tumor growth of Cu depletion caused by a Cu-free diet and D-penicillamine treatment in the rat brain glioma model.

Materials and Methods

9L gliosarcoma cells were cultured in incubation medium (RPMI-1640 supplemented with 10 mg/ml of gentacin) and maintained in a humidified incubator at 37°C. The trypsinized and lifted cells were resuspended in phosphate buffered saline at a concentration of 10⁶ cells/ml. Thirty-six male 5-week-old Fischer-344 rats, weighing 240–250 g, were anesthetized with ether. Cell suspension containing 10⁶ cells (100 μl) was slowly injected subcutaneously through a 26-gauge needle. We divided the rats into two groups by the methods of Brem et al. as follows. The control group (n = 18) was fed normal diet through the experiment. The depletion group (n = 18) was fed a Cu-deficient diet starting 3 weeks prior to the tumor cell implantation and also received 2 mg of D-penicillamine per day orally for 6 days, beginning 3 days before implantation.

Six animals from each group were killed with ether 1, 2, and 3 weeks after the implantation. The brain
and tumor tissue were removed. The mass of the tumor tissue was determined. Tissue Cu levels were measured using a Hitachi 170-30 atomic absorption spectrophotometer (Tokyo). The statistical significance of differences in values was established using the Student’s t-test.

### Results

Table 1 summarizes the results. In the control group, the Cu concentration in the gliosarcoma tissue was significantly higher than that in brain tissue (1 wk, $p < 0.001$; 2 wks, $p < 0.001$; 3 wks, $p < 0.001$). The Cu concentration in the tumor tissue of the depletion group was significantly lower than that in the control group at each week of tumor growth (1 wk, $p < 0.05$; 2 wks, $p < 0.05$; 3 wks, $p < 0.001$). The Cu concentration of the brain in the depletion group showed no statistical difference from the control group.

All the subcutaneous tumors were well marginated from the surrounding subcutaneous tissue, and were easily removed. The tumor weight increased rapidly in the control group but much more slowly in the depletion group (Fig. 1). The tumor tissue in the depletion group weighed significantly less than that in the control group at each week (1 wk, $p < 0.05$; 2 wks, $p < 0.01$; 3 wks, $p < 0.001$).

Three weeks after implantation, the animals in both groups had developed normally. The animal weights of both groups showed no significant difference. No symptoms such as behavioral disorders or paresis were observed.

### Discussion

Our study showed that 9L gliosarcoma tissue contains a significantly higher Cu concentration than normal rat brain tissue. Such abnormal elevation of Cu level has been found in several malignant tissues. Brem et al. suggested several roles for Cu ion in neoplastic development. Increased Cu accumulation in the peritumoral zone of brain tumor provides a chemokinetic stimulus to mobile tumor cells similar to the migration of endothelial cells in response to Cu ion gradients. The extracellular matrix of brain tumor is enriched with adhesive proteins, fibronectin, and collagen that stimulate the migration of neoplastic cells and could be linked to peritumoral invasion. Cu binds heparin and angiogenic protein, fibroblast growth factor, which both stimulate cell mobility. Experiments with a 9L gliosarcoma rat model showed that Cu-deprived rat developed tumors characterized histologically by lack of pseudopodial protrusion at the tumor margin, an indicator of spreading neoplastic cells, suggesting a defect in cell migration. Brem et al. 1,2)
mainly focused on the effect of the serum concentration of Cu ion on angiogenesis using Cu depletion and D-penicillamine treatment in experimental animals, showing that such therapy diminished nuclear staining with Cu. Our study in the same rat model found that Cu depletion and D-penicillamine treatment achieved significant inhibition of tumor cell growth. Our results also indicated that the treatment reduced the concentration of Cu ion in glioma tissue.

D-penicillamine is used to treat patients with rheumatoid arthritis, as well as Wilson’s disease or congenital Cu storage disease. D-penicillamine has a superoxide-dismutase-like action. Production of H₂O₂ by D-penicillamine in the presence of Cu ion was observed in a cell-free system and inhibited endothelial cell proliferation. This inhibition was synergistically enhanced by the presence of Cu ion in a dose-dependent fashion. Endothelial cell proliferation and collagenase are closely concerned with angiogenesis. Such superoxide-dismutase-like and angiosuppressive actions may be another factor in the observed inhibition of tumor growth.

Severe Cu deficiency can result in normocytic or megaloblastic anemia, neutropenia, and osteoporosis. No specific neurological abnormalities have been described. We found that the tissue concentrations of Cu were significantly depleted in tumor tissue, but no statistical difference was seen in the brain between control and treated animals. These results suggest that Cu-depletion therapy affected the subcutaneous tumor and might not have an effect on tumor implanted in the brain. Mechanisms such as the blood-brain barrier may prevent central nervous system Cu depletion, but the central nervous system may tolerate Cu deficiency for a limited period allowing clinical usage. Brem et al. found that selective Cu depletion may occur in tumor implanted in the brain, but with increased peritumoral vascular permeability and brain water content. Methods to achieve Cu depletion of tumor implanted in the brain should be thoroughly investigated.

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References


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