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Iron Nutrition and Tumor Growth: Decreased Tumor Growth in Iron-deficient Mice[1]

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ABSTRACT

Groups of 15 mice of three different laboratory strains (BALB/c, C3H/He, DBA/2) were fed on a low iron diet (5 mg iron/kg diet), and three similar groups of 15 mice were maintained on a normal iron diet (312 mg iron/kg diet). When the low iron diet group became iron deficient, tumor cells (5×10^5 cells/mouse) of CA07-A (colon adenocarcinoma), HE129 (hepatoma), and M119 (mammary adenocarcinoma) were inoculated s.c. in BALB/c, C3H/He, and DBA/2 mice, respectively. All mice developed tumors, tumors grew more slowly, and the mean tumor sizes were smaller in the low iron diet group at nearly all weekly observations in all three strains of mice. No apparent differences in the behavior, activity (*e.g.*, movement, climbing, running, grooming, etc.), and appearance were observed between low iron diet and normal iron diet mice. The mean body weight of mice at transplantation was less in the low iron than in the normal iron groups for the BALB/c strain but higher in the low iron groups of C3H/He and DBA/2 mice, indicating that food intake of mice on a low iron diet was not impaired. These results suggest that iron nutrition of the host affects tumor growth; tumor cells grow better in an iron-rich environment. This knowledge should be considered when designing treatment for patients with cancer. Iron oversupply in cancer patients might enhance tumor growth and adversely affect cancer therapy.

INTRODUCTION

Iron is required for the growth of all living cells, including tumor cells. Studies have shown that transferrin, the major serum iron binding protein, is one of the essential substances required for the growth of cells in serum free media (1). Deferoxamine and picolinic acid, which are iron chelating agents, inhibited the growth of tumor cells in tissue culture (2, 3). Blatt and Stiteley (2) showed that the antitumor (neuroblastoma) activity of deferoxamine *in vitro* could be reversed by concomitant addition of ferric citrate. Iatrogenic iron overload has been associated with neoplasia; parenteral administration of iron dextran has induced sarcomas at the site of injection in rodents, rabbits, and humans (4–6). Idiopathic hemochromatosis, a genetic disease characterized by iron overload, is associated with a very high risk of primary liver cancer and other malignancies (7–9). Increased ferritin levels are associated with the development of cancer in humans (10).

We proposed that iron deficiency would retard tumor growth. The effects of dietary iron on the growth of tumors in mice are described in this study.

MATERIALS AND METHODS

Animals. Thirty female BALB/cAnNIcr, 30 C3H/HeNIcr, and 30 DBA/2HaIcr mice were obtained at weaning from the Laboratory Animal Facility of the Fox Chase Cancer Center.

Diet and Bedding. Low iron diet (5 mg iron/kg diet) was purchased from the United States Biochemical Corporation (Cleveland, OH) and contained 56% sucrose, 27% casein, 14% vegetable oil, and 3% salt and vitamin mixture. Iron free synthetic bedding (Alpha-dri) was purchased from Shepherd Specialty Papers, Inc. (Kalamazoo, MI).

Tumor Cells. CA07-A (colon adenocarcinoma of BALB/c mice), HE129 (hepatoma of C3H/He mice), and M119 (mammary adenocarcinoma of DBA/2 mice) were obtained from the Division of Cancer Treatment Tumor Repository at NCI-Frederick Cancer Research Facility (Frederick, MD).

Induction of Iron Deficiency. Fifteen BALB/c, 15 C3H/He, and 15 DBA/2 mice were placed on the low iron diet (5 mg iron/kg diet) at weaning, and the other 15 of the respective groups of mice were placed on a normal iron diet (312 mg iron/kg diet) at weaning. The only element that the low iron diet contained in an abnormal amount was the iron content (5 mg iron/kg diet). The diet contained all of the nutritional elements recommended by the Nutritional Research Council subcommittee on nutritional requirements for normal growing mice. If the iron was supplemented in normal quantity, mice were shown to grow normally. Normal iron diet also contained the same nutritional components, except the amount of iron (312 mg iron/kg diet). With regard to the iron content of the mouse diet, NIH 31 diet, the standard diet for breeding mice, contains 345 mg iron/kg diet, and NIH 7 diet, a maintenance diet, contains 250 mg/kg diet. Several commercial diets for mice, which follow the Nutritional Research Council guidelines, usually contain between 289 and 340 mg iron/kg diet. Mice were placed in plastic cages (5/cage). Mice on the low iron diet were also given double distilled deionized water dispensed with a glass straw from a glass bottle and maintained on the synthetic bedding (Alpha-dri). These iron diets were maintained for the entire length of the experiment. Mice were weighed weekly, and monthly measurements of Hct[3] were made.

Preparation of Tumor Cells and Transplantation. Tumors obtained from the Division of Cancer Treatment Tumor Repository were transplanted via trochar into mice as follows: CA07-A in BALB/c; HE129 in C3H/He; and M119 in DBA/2. Tumors were kept growing in these mice until ready for transplantation into the experimental animals. When the Hcts of mice on the low iron diet became significantly lower than the Hcts of mice on normal iron diet ($P \leq 0.05$ by Mann-Whitney or Student's t test), tumor cells were prepared as follows. Tumor bearing mice were first sacrificed by cervical dislocation. Tumors were surgically removed, minced in RPMI 1640, and passed through mesh (gauge 80; Cellector tissue sieve), under sterile conditions. Cells were centrifuged, resuspended in RPMI, and counted on a hemocytometer. Cell viability was determined by trypan blue dye exclusion.

Each experimental mouse was shaved on the right flank. The shaved area was cleaned with an alcohol sponge and treated by s.c. injection of 5×10^5 tumor cells in 0.5 ml volume. Each BALB/c mouse received 5×10^5 CA07-A cells, each C3H/He mouse the same inoculum size of HE129, and each DBA/2 the same inoculum size of M119 cells. The mice were observed for the frequency of tumor growth, time of tumor appearance, size of tumor, and survival time. At weekly intervals, tumor dimensions were measured with a caliper.

RESULTS

Body Weights and Hematocrit Measurements. Mean body weights and Hcts of low and normal iron diet mice were compared with each other at the beginning of the diet and at the time of transplantation. Hcts of low iron mice were significantly lower than Hcts of normal iron mice at the time of transplantation ($P \leq 0.05$ by Mann-Whitney or Student's t test) in all three groups of mice; mean ± SE of percentage of Hct

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[3] The abbreviation used is: Hct, hematocrit.

for low and normal iron mice were 28.8 ± 1.33 and 51.5 ± 1.22 for BALB/c, 27.9 ± 17.4 and 48.0 ± 0.6 for C3H/He, 36.9 ± 2.15, and 51.2 ± 0.28 for DBA/2 mice. The time interval between the initiation of the low iron diet and transplantation was 4.5 months for BALB/c, 5 months for C3H/He, and 4 months for DBA/2. Mean body weight of mice on low and normal iron diets were similar to each other at the entry of diet in all three groups of mice. At transplantation, mean body weight of the low iron mice (21.7 ± 0.45 g) was lower than body weight of the normal iron group (26.7 ± 0.46 g) in BALB/c mice. On the contrary, mean body weight was higher at transplantation in the low iron than the normal iron group in C3H/He and DBA/2 mice: 33.5 ± 1.09 *versus* 28.8 ± 1.03 g for C3H/He and 27.3 ± 0.26 *versus* 24.3 ± 0.35 g for DBA/2.

Time of Tumor Appearance. All mice developed tumors, and the time of initial tumor appearance was similar between the low and normal iron diet mice in all three strains of mice: 2 weeks for BALB/c; 1 week for C3H/He; and 2 weeks for DBA/2.

Survival after Tumor. End point for survival was the time of death with tumor. There was no difference in survival (between time of transplantation and death) between the low and normal iron in all of the mouse strains. However, survival times differed among three groups of mice which carried different tumors: 11–13 weeks for BALB/c; 13–14 weeks for C3H/He; and 6–7 weeks for DBA/2.

Size of Tumor. At every observation period, the largest tumor was almost always seen in the normal iron group in all three groups of mice. Comparisons of mean tumor sizes between low and normal iron diet mice measured at weekly intervals are shown in Figs. 1–3. Mean tumor size was always numerically smaller in low iron diet than normal iron diet mice at each weekly observation in all three groups of mice. On most occasions, tumor sizes between low and normal iron diet mice were significantly different ($P \leq 0.05$ by Mann-Whitney or Student's *t* tests) in all strains, as indicated by arrows in the figures.

Included in these data are the measurements on the live animals made at the time indicated. Therefore, as mice began to die, the number of tumors evaluated decreased. However, death of mice was not related to size of tumor in any of the groups. The mean tumor sizes of the animals that had died in the previous week varied; the dead mice did not necessarily have the largest tumors. This ruled against the explanation that for the low iron diet, the large tumor animals were eliminated earlier, thereby accounting for the difference in tumor between the two groups.

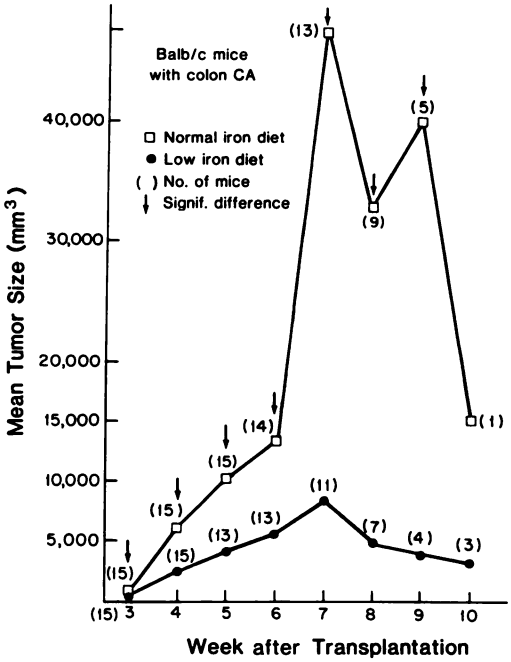

Fig. 1. Comparison of mean tumor sizes between low and normal iron BALB/c mice at weekly observations. Significant differences ($P \leq 0.05$) are indicated by *arrows. CA*, cancer.

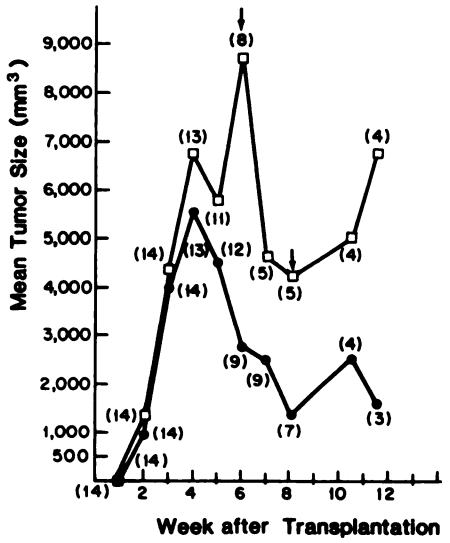

Fig. 2. Comparison of mean tumor sizes between low (●) and normal (□) iron diet C3H/He mice with hepatoma at weekly observations. Significant differences ($P \leq 0.05$) are indicated by *arrows. Numbers in parentheses,* number of mice.

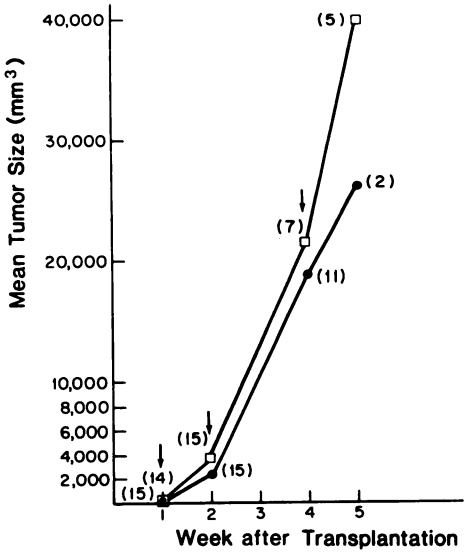

Fig. 3. Comparison of mean tumor sizes between low (●) and normal (□) iron diet DBA/2 mice with mammary cancer at weekly observations. Significant differences ($P \leq 0.05$) are indicated by *arrows. Numbers in parentheses,* number of mice.

Metastasis. Distant tumor growth in places other than inoculation sites was also noted but only in C3H/He mice carrying hepatoma. The inoculation site was the right back (flank) s.c. and metastasis was either to the left flank, shoulder, or upper back. Metastasis was seen in 6 C3H/He mice of the normal iron and only one C3H/He mouse of the low iron diet group.

DISCUSSION

These results show that tumors grew slower and were smaller in mice on the low iron than the normal iron diet. In all three

strains of mice, tumors were generally smaller in the low iron group at every observation period. There were no obvious differences in the animals' behavior and/or activity such as running, climbing, grooming, etc., or appearance between low or normal iron diet mice except in BALB/c mice, in which low iron diet mice had paler eye color (eyes are usually pinkish red) and less glossy hair. There were no differences in the time of tumor appearance and/or survival from the time of transplantation between low and normal iron mice. The tumor inoculum used in the current study is the most commonly used size (number of cells from the primary tumor) in transplantation experiments in mice to assure tumor take. (More cells are needed if they are from the cell line in culture.) Consequently, every animal developed a tumor; therefore, we were able to see the difference in tumor size and growth rate between mice on a low iron diet and those on a normal iron diet. However, because of the successful tumor take in all mice, the effect of iron deficiency on tumor occurrence and survival of the animal could have been minimized or lost. Possibly, with smaller inocula, detectable differences in the survival, incidence of tumor, and tumor appearance time between low iron diet and normal iron diet mice might appear. Nevertheless, if this observation is extrapolated in human situations, patients with small and slow-growing tumors have a great advantage with regard to their prognosis because surgery can easily be done to remove these tumors as opposed to larger tumors.

Iron is known to be an essential element for growth of all cells including tumor cells. Human hepatoma cells (PLC/PRF/5) (11) grow faster in an iron supplemented than un unsupplemented medium.[4] In view of these findings and other known associations of iron overload with increased incidence of cancer, iron supplementation in cancer patients or older people at high risk of cancer might enhance tumor growth (10). Blatt and Stitely (2) have recently shown that deferoxamine has potent antitumor activity *in vitro* as a result of its ability to chelate iron. Our results, combined with other data, warrant continued study of the possible adverse effects of iron supplementation in experimental animals and in humans with cancer or at risk of developing cancer.

[4] H-W. L. Hann *et al.*, unpublished data.

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