Iron Nutrition and Tumor Growth: Decreased Tumor Growth in Iron-deficient Mice

Hie-Won L. Ilann, Mark W. Stahlhut, and Baruch S. Blumberg
Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

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 x 10⁶ 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 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 Stitelye (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/cAnNcr, 30 C3H/HeNcr, and 30 DBA/2Hacr 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). Animals were housed in plastic cages (5/cage). Mice on the low iron diet were also given oxamine and picolinic acid, which are iron chelating agents, required for the growth of cells in serum free media (1). 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).

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 < 0.05 by Mann-Whitney or Student’s t test) in all three groups of mice; mean ± SE of percentage of Hct

4168

Received 9/22/87; revised 12/21/87; accepted 4/4/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by USPHS Grants CA-40737, RR-05895, CA-06927, and CA-39949 from the NIH and by an appropriation from the Commonwealth of Pennsylvania.

2 To whom requests for reprints should be addressed.

1 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 < 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.

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 an unsupplemented medium. 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.

ACKNOWLEDGMENTS

The authors wish to thank the late Dr. Morris Ross at the Fox Chase Cancer Center for his invaluable advice in designing and conducting this study; Dr. Howard Blatt for his help in obtaining animal tumors for the study; Dr. Katherine McGlynn for her statistical help; Drs. W. Thomas London, Darrell Brown, Timothy Poole, and Gary Hudes for review of the paper; and Maureen Walsh for her assistance in preparing the manuscript.

REFERENCES


4170

H-W. L. Hann et al., unpublished data.
Iron Nutrition and Tumor Growth: Decreased Tumor Growth in Iron-deficient Mice

Hie-Won L. Hann, Mark W. Stahlhut and Baruch S. Blumberg