SHORT COMMUNICATION

Hemopoietic function in rats with carcinomatous anemia

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Introduction

The cause of carcinomatous anemia can be considered relative to three major abnormal conditions: reduced hemopoietic response to anemia, red blood cell destruction, and excess loss of blood from blood vessels¹⁻³⁾. In the present study, we investigated the reduction of the erythropoietic function of hemopoietic tissues, together with iron metabolism, in order to clarify the initiating mechanism of carcinomatous anemia in animals bearing tumors in the oral region.

Methods

Eight-week-old male Wistar rats were used. The animals were divided into two groups: the experimental group 27 comprised cancerous rats treated with 0.5 % 9,10-dimethyl-1, 2-benzanthracene (DMBA) solution in acetone on the cheek skin three times a week for 32 weeks, and the control group comprised 19 normal rats treated with acetone alone on the cheek skin three times a week in the same manner as with the experimental group. In both groups, blood samples were collected via the tail vein 32 weeks after initiation of the carcinogenesis experiment. Hematologic parameters (red blood cell count, hemoglobin level and hematocrit value) were determined with the Hematology Analyzer HA/5 (Clay Adams, USA), percent reticulo-

cyte was determined with brilliant cresyl blue stain, and plasma iron level and iron-binding capacity were measured with the Technicon AA-2 (Technicon, USA).

After administration of ⁵⁹Fe-labeled ferric citrate, peripheral blood samples were collected at specified intervals to obtain blood counts and to determine ⁵⁹Fe radioactivity in the plasma^{4,5)}. The parameters examined on the basis of ⁵⁹Fe radioactivity included: (1) the disappearance rate of ⁵⁹Fe in the plasma; (2) the ratio of ⁵⁹Fe radioactivity uptake by red blood cells to total ⁵⁹Fe radioactivity, (i.e., the percent ⁵⁹Fe utilization of red blood cells, % RCU); and (3) the radioactivity per mg in the cheek skin (tumor), spleen and femur following sacrifice and autopsy at 72 hours post-injection of ⁵⁹Fe-labeled Fe citrate. The tumors in the experimental group and the bone marrow in both experimental and control groups were double-stained with hematoxylin-eosin and subjected to an examination of the histopathology.

Results

Black-brown tumors approximately 10 mm diameter, designated histologically as squamous cell carcinoma, appeared in all animals at the DMBA application site within 32 weeks following initiation of the experiment in the experimental group (Figs. 1, 2).

The red blood cell counts, hemoglobin lev-

Fig. 1 Tumors are observed in the cheek skin of rats 32 weeks after initiation of the carcinogenesis experiment

Fig. 2 Histological image of the tumor
Squamous cell carcinoma is found. $\times$ 177, H-E.

els, and hematocrit values in the experimental group were lower than those of the control group. Anemia occurred in the former, together with an increased percent reticulo-

cyte count (Table 1). The plasma iron (PI) decreased in the experimental group compared with the controls, while there was an increase in the unsaturated iron binding capacity (UIBC) (Table 2).

The disappearance rate of ^{59}Fe in the plasma was higher in the experimental group (Fig. 3). The percent ^{59}Fe utilization of red blood cells (% RCU) increased relatively early (Fig. 4). At 72 hours post-injection of ^{59}Fe -labeled Fe citrate, the radioactivity per mg in the cheek skin (tumor) was 16.3 ± 7.0 cpm/mg in the experimental group and 6.4 ± 1.2 cpm/mg in the controls. These values were 74.0 ± 30.5 cpm/mg and 69.0 ± 18.1 cpm/mg for the spleen, and 12.2 ± 8.2 cpm/mg and 16.6 ± 2.6 cpm/mg for the bone (femur). Therefore, although ^{59}Fe uptake in the experimental group increased relative to the controls in the cheek skin (tumor), it decreased in the femurs.

The control rats had yellow (fatty cell) bone marrow composed mainly of adipose tissue, while the experimental group rats had red bone marrow composed mainly of hematopoietic cells (Figs. 5, 6).

Discussion and Conclusion

Attention has been focused on abnormal iron metabolism associated with anemia in organisms with cancer⁶⁾. We, therefore, investigated whether cancer-related anemia in rats was due to a lack of available iron for hemoglobin synthesis or to a reduction of iron-transferring carriers. In the present study, transferrin, the iron carrying factor, was increased in rats with carcinomas. It was proposed that the decreased iron transfer via plasma (PI) in these animals was due to a lack of iron itself⁷⁾. Hyperactivity of hema-

Table 1 Hematological values

Anemia occurred in cancerous rats, while the percent reticulocyte count increased.

	Red blood cells ($10^6/\mu\text{l}$)	Hemoglobin (g/dl)	Hematocrit (%)	Reticulocytes (%)
Control group	6.21 ± 0.69	17.58 ± 0.63	48.67 ± 4.00	3.33 ± 1.26
Experimental group	4.15 ± 0.68	14.05 ± 2.35	32.81 ± 5.94	14.60 ± 9.03

mean $\pm$ S.D.

Table 2 Content of plasma iron(PI) and unsaturated iron binding capacity (UIBC) PI of cancerous rats decreased compared with that of controls, while UIBC increased.

	Plasma iron ($\mu\text{g}/\text{dl}$)	Unsaturated iron binding capacity ($\mu\text{g}/\text{dl}$)
Control group	200.9 ± 9.9	376.6 ± 36.2
Experimental group	165.7 ± 34.7	421.3 ± 63.8

mean $\pm$ S.D.

Fig. 3 Disappearance of ^{59}Fe in plasma
 ^{59}Fe disappearance rate was higher in the experimental group.

Fig. 4 Percent red blood cell utilization of ^{59}Fe (%RCU)

In the experimental group, the percent plateaued 48 hours after injection, and the %RCU was increased.

topoietic tissues of cancerous rats is based on the increased percent reticulocyte count, higher disappearance rate of ^{59}Fe in the plasma, increased utilization of red blood cells, and a decrease in fatty cells in the bone marrow⁸⁾.

Fig. 5 Histological image of the bone marrow in the experimental group

The red marrow contains many parenchymal cells such as erythroblasts. $\times 44$, H-E.

Fig. 6 A histological image of the bone marrow in the control group

Many fatty cells are observed, showing characteristics of yellow marrow. $\times 44$, H-E.

Increased uptake of ^{59}Fe in tumor tissue in cancerous rats as compared with control rats is attributed to iron uptake by the tumor tissue^{9,10)}, tumor ferritin synthesis^{11,12)}, hemorrhage¹⁾ and other factors¹³⁻¹⁵⁾. However, uptake of ^{59}Fe by bone marrow decreased in the tumor-bearing animals. This seems to be because most of the ^{59}Fe in bone marrow are utilized to synthesize hemoglobin for newly produced red blood cells within the 72 hours following ^{59}Fe administration, and they are thereafter soon released into the peripheral blood.

Based on these findings, we conclude that carcinomatous anemia is partly attributable to a lack of plasma iron, a constituent of hemoglobin, but probably does not reflect hypofunction of hematopoietic tissues.

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