Tumor Res. 1(1966) 203-216

STUDIES ON FERROKINETICS IN TUMOR-BEARERS

II. Ferrokinetics by ^{59}Fe-labeled Colloid Iron*

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Introduction

Our previous report of ferrokinetic study on tumor-bearers by the use of ^{59}Fe-globulinate, failed to identify the presence of any inhibitory action of bone marrow erythropoiesis in cancer (1, 2). A number of published reports also agree with our report (3, 4, 5).

Now, it is known that more than 90 per cent of daily iron required by the bone marrow to synthesize hemoglobin must come through the reutilization of iron from senescent red blood cells (6). Thus, studies on the iron metabolism in the reticu-

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* This study was supported by a research grant from the Ministry of Education of Japan, #95677.

loendothelial system of tumor-bearers might be helpful in understanding the mechanism of cancer anemia.

Colloid iron, when injected intravenously, is largely taken up by the reticuloendothelial system and metabolized as storage iron, i.e., hemosiderin and ferritin, and thereafter released into circulation (7, 8).

In this paper the fate of infused ^{59}Fe-labeled colloid iron was pursued in order to evaluate the effect of tumor-bearing upon the activity of the reticuloendothelial system.

Materials and Methods

I. Clinical Experiments

Fourteen patients with gastric and bronchogenic carcinomas, 5 patients with iron deficiency anemia and 5 normal subjects were investigated.

1) Hematological: Erythrocyte count, hematocrit and hemoglobin were determined on all patients. Reticulocyte count and bone marrow examination were performed on most of the patients.

2) Studies of ferrokinetics by ^{59}Fe-labeled chondroitin sulfuric acid iron: Ten patients with carcinomas, 5 patients with iron deficiency anemia and 5 normal controls were subjected

to radioiron studies.

a) Plasma radioiron disappearance half-time

^{59}Fe-labeled chondroitin sulfuric acid iron secured from Dainabbot Radioisotope Laboratory, Tokyo, with a specific activity of 0.55μCi per mg. iron was used. A 5 ml. aliquot of this material was injected intravenously, containing approximately 20 mg of iron with a radioactivity of 8 to 11μCi. The disappearance of ^{59}Fe from the plasma was measured at five minute intervals. Two ml. sample was counted in the liquid state using a well type scintillation counter.

b) Red cell utilization percentage of ^{59}Fe

Following an intravenous injection of ^{59}Fe-labeled chondroitin sulfuric acid iron, blood samples were drawn every 2 days to measure the incorporation of radioiron into the red cells and less frequently over the following two weeks.

3) Serum iron and iron binding capacity: Serum iron concentration was determined by the method of Trinder (9) with Nakao's modification (10). Total iron binding capacity was determined by the method of Peters (11).

II. Animal Experiments

Adult albino rats (Gifu-strain) weighing approximately 170 g were used. The rats were fed with Oriental solid diet and water ad libitum over 10 days before use for experiments. These were subcutaneously inoculated into one hind leg with 2×10^6 freshly harvested cells of Yoshida sarcoma. On each animal, determinations of serum iron concentration and total iron binding capacity were made.

1) Studies of ^{59}Fe-labeled chondroitin sulfuric acid iron: One mg of ^{59}Fe-labeled chondroitin sulfuric acid iron with a specific activity of 0.55 μCi was injected intravenously into normal and Yoshida sarcoma-bearing rats. Two tenth ml. of blood samples were taken three times at 5, 30 and 60 minutes following the injection of radioiron to determine the clearance rate of ^{59}Fe from the circulating plasma.

One mg of ^{59}Fe labeled chondroitin sulfuric acid iron was injected intravenously into another group of normal and tumor-bearing rats. Two tenth ml of blood samples were taken three times at 24, 48 and 72 hours to measure the incorporation of radioiron into red cells.

2) Distribution of ^{59}Fe-labeled chondroitin sulfuric acid iron in various tissues: Twenty four hours after an intravenous

injection of ^{59}Fe-labeled chondroitin sulfuric acid iron, the distribution of radioiron was examined on the plasma, liver, kidney and tumor tissues. Iron contents of the liver were determined following fractionation into hemosiderin and ferritin fractions by the method of Konro and Yoneyama (12).

Results

I. Clinical Experiments

The hematological and radioiron studies of 14 patients with carcinomas, 5 patients with iron deficiency anemia and 5 normals were summarized in Table 1 and 2. The details of the individual hematological data not included in the tables were presented in a previous paper (2).

1) Plasma radioiron disappearance half-time: The half-time of ^{59}Fe-labeled chondroitin sulfuric acid iron in the plasma was shortened definitely in cancer patients as compared to the healthy controls, ranging from 14.0 to 19.5 minutes (Fig. 1). The correlation between hemoglobin value and plasma disappearance half-time could not be demonstrated in cancer patients (Fig. 2).

2) Red cell utilization percentage of ^{59}Fe: As shown in Table 2 and Fig. 3, the utilization curves of red cells in

Table 1. Hematological Data on Patients with Carcinoma

	No.	RBC * $\times 10^4$	Hb * g/dl	Ht * %	Serum Iron * r/dl	TIBC * r/dl	Nucleated Cells ** in Bone Marrow $\times 10^3$
Normal	5	453.4 ± 37.4	14.6 ± 1.4	42.2 ± 3.2	110.4 ± 25.6	302.2 ± 56.6	94.9
Carcinoma	14	346.5 ± 48.2	10.5 ± 4.2	30.1 ± 8.8	53.4 ± 16.6	241.0 ± 62.4	122.8
Iron Deficiency Anemia	5	336.0 ± 66.4	8.4 ± 2.8	28.2 ± 5.4	43.2 ± 17.6	388.0 ± 52.2	213.2

* Mean ± S.D.

Table 2. Radioiron Data on Patients with Carcinoma

	No.	TBV ** (ml)	TPV ** (ml)	PID T/2 * (min.)	% RCU * 2nd	4th	6th	8th	10th	15th day
Normal	5	5015.8	2898.8	21.0 ± 1.7	6.6 ±1.4	19.9 ±3.2	35.9 ±4.4	48.2 ±5.8	56.7 ±8.6	69.9 ±11.6
Carcinoma	14	3807.3	2606.0	16.1 ± 2.7	4.9 (2.3 -6.5)	13.9 (4.6 -29.4)	24.3 (9.5 -50.8)	30.0 (14.7 -61.2)	37.5 (16.1 -67.4)	43.9 (16.8 -79.6)
Iron Deficiency Anemia	5	4739.0	3418.8	12.8 ± 3.2	31.0 ±11.2	51.8 ±17.2	64.1 ±8.8	72.6 ±12.4	78.6 ±11.4	88.4 ±10.2

* Mean ± S.D. ** Mean Value only () Range

cancer patients significantly differed from those of the normal subjects, showing a gradual increase of utilization in accordance with the lapse of time. The maximum utilization of radioiron was observed on the 15th day following the injection. In cancer patients the levels of the curves were lower than those of the normal subjects. On the other hand, in the patients with iron deficiency anemia the curves were remarkably higher than those of the above two groups. The difference among these three groups became evident after the 4th day and was more pronounced on the 15th day. The maximum utilization of radioiron observed on the 15th day in the normal group was 60 % or more, whereas cancer patients showed a wide variation ranging from 17 % to 80 % depending upon the severity of the anemia. The correlation was obtained when the hemoglobin concentration of each patient was plotted against the percentage of utilization of iron (Fig. 4).

II. Animal experiments

When Yoshida ascites tumor was transplanted subcutaneously into albino rats, the tumor usually killed the animal in 12 to 14 days. The experiments were performed on 7th day following tumor inoculation.

1) Plasma radioiron disappearance half-time: Measurement was done on 5 normal and 5 tumor-bearing rats. Plasma radioiron disap-

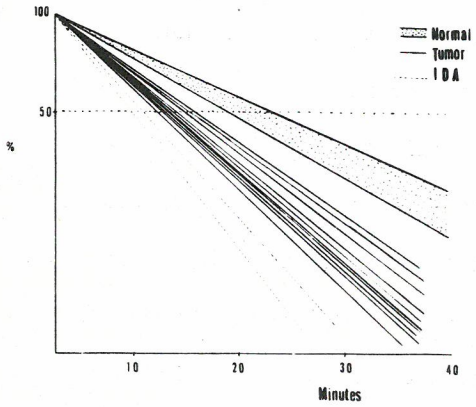

Fig.1. Plasma radioiron disappearance curves in cancer patients and others after intravenous injection of ^{59}Fe labeled chondroitin sulfuric acid iron.

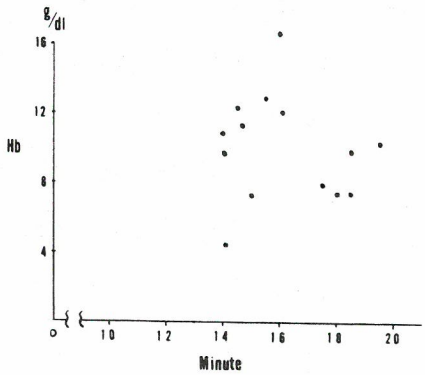

Fig.2. Correlation between Hb and PID half time of ^{59}Fe labeled chondroitin sulfuric acid iron in cancer patients.

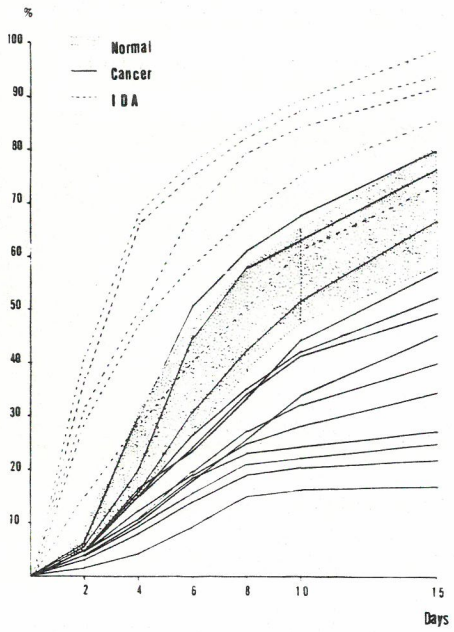

Fig.3. % RCU in cancer patients and others after intravenous injection of ^{59}Fe labeled chondroitin sulfuric acid iron.

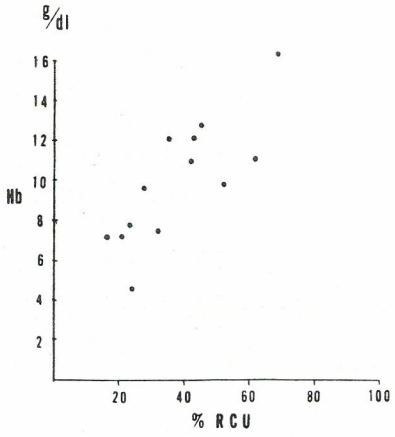

Fig.4. Correlation between Hb and % RCU of ^{59}Fe labeled chondroitin sulfuric acid iron in cancer patients.

pearance half-time in tumor-bearers was summarized in Table 3. It was markedly retarded in tumor-bearers varying from 65.0 to 79.0 minutes.

2) Utilization percentage of red cells: Five rats bearing Yoshida sarcoma and 5 untreated rats were used for the determination of utilization percentage of red cells. The utilization percentage into red cells was markedly lowered in tumor-bearing rats (Table 3).

3) Distribution of radioiron in various tissues: Twenty four hours after an injection of ^{59}Fe-labeled chondroitin sulfuric acid iron, 80 % of the loaded radioiron could be detected in the liver of normal rats, while only 60 % of the infused colloid iron was found in the liver of tumor-bearers. On the other hand, approximately 10 % of radioiron was recovered from the plasma.

Only 1 or 2 % radioiron was detected in the spleen and kidney. The fractional determination of injected ^{59}Fe in the liver disclosed a marked decrease of incorporation of radioactivity into the hepatic ferritin fraction of tumor-bearing rats when compared with that of normal controls (Figs. 5, 6).

Discussion

The pathogenesis of the anemia frequently associated with

Table 3. PID T/2 and % RCU in Rats with Subcutaneously Inoculated Yoshida Sarcoma After Injection of ^{59}Fe-Labeled Chondroitin Sulfuric Acid Iron

Condition	No.	PID T/2 * (min)	No.	% RCU *	
				24 hr.	120 hr.
Normal	7	50.2 ± 4.22	7	9.0 ± 1.28	56.4 ± 4.5
Yoshida Sarcoma Bearing	5	58.2 ± 3.81	5	6.8 ± 1.58	37.9 ± 3.5

*Mean ± S D

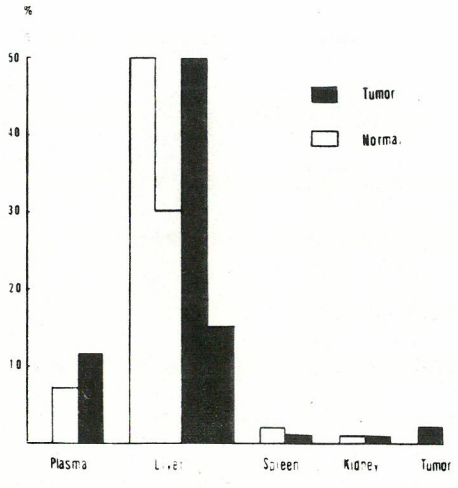

Fig.5. Distribution of ^{59}Fe labeled chondroitin sulfuric acid iron 24 hours after injection in rats with subcutaneously inoculated Yoshida Sarcoma.

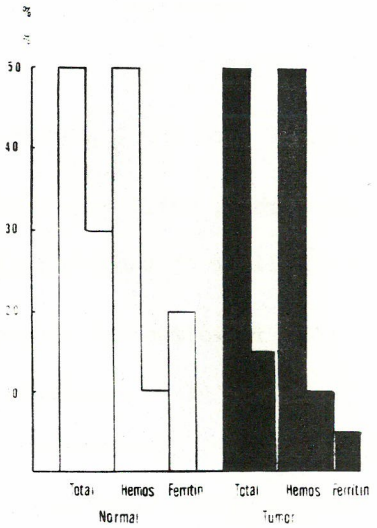

Fig.6. Distribution of ^{59}Fe labeled chondroitin sulfuric acid iron 24 hours after injection in liver of rats with subcutaneously inoculated Yoshida sarcoma.

neoplastic diseases has been poorly understood. Bone marrow examination reveals normal cellularity with adequate erythroid activity (13). Moreover, erythrokinetic studies including radioiron clearance, red cell radioiron utilization, often do not help in clarifying the mechanism of anemia associated with neoplasia (4). On the other hand, the red cell survival studies by Ashby technic in tumor-bearers showed a marked shortness of red cell life span indicating an increased red cell destruction (3). The iron released from senescent red blood cells in the reticuloendothelial system is capable of re-entering the plasma iron compartment. Therefore, a metabolic study of iron in the reticuloendothelial system is quite important to clarify the process of the utilization of iron in tumor-bearers.

A larger part of infused colloid iron is taken up by the reticuloendothelial system as storage iron and metabolized further as hemosiderin and ferritin which is capable of re-entering circulation as plasma iron. Shoden (7, 8) and Urushizaki (14) previously have pointed out that the metabolic fate of intravenously administered iron can be different according to the type of colloid iron used. The chondroitin sulfuric acid iron employed in the present experiments, rapidly disappeared from the blood stream and at first was stored as hemosiderin and later was utilized as ferritin.

The plasma radioiron clearance after an injection of ^{59}Fe-labeled chondroitin sulfuric acid iron was accelerated in cancer patients, however it was retarded in Yoshida sarcoma-bearing rats. Especially, in cancer patients the decrease of utilization of red cells and the delay of the maximal utilization of radioiron were the prominent features when compared with the normal subjects. Moreover, it was quite interesting to note that the decreased hemoglobin levels in cancer patients were in parallel with the decrease of percentage of red cell utilization. As was reported previously, the utilization of radioiron by the bone marrow of the tumor-bearers appeared essentially normal. However, the study of iron utilization by the use of ^{59}Fe-labeled chondroitin sulfuric acid iron as a tracer revealed a marked decrease of the utilization of this type of iron compound in tumor-bearers.

The defective reutilization of iron in tumor-bearers probably accounts primarily for the anemia associated with neoplasia. Although the exact mechanism responsible for this defective utilization of iron is still obscure, it is presumably due to the alteration of the activities of the reticuloendothelial system in the tumor bearing state. It might be pointed out from our results that the defective utilization of iron in tumor bearers seemed to be related to the depressed formation of hepatic ferritin through which iron is transferred from tissues to plasma and reaches the bone

marrow where it is utilized for the hemoglobin synthesis.

Conclusion

In order to clarify the process of iron utilization in the reticuloendothelial system in tumor-bearing hosts, 14 cancer patients, 5 patients with iron deficiency anemia and 5 healthy controls were subjected to metabolic survey of infused ^{59}Fe-labeled chondroitin sulfuric acid iron. Animal experiments were also performed on Yoshida sarcoma-bearing rats. Plasma radioiron clearance was definitely accelerated in cancer patients and retarded in Yoshida sarcoma-bearing rats. However, the decrease of red cell utilization of infused colloid radioiron and delay of the maximal utilization were observed in both cancer patients and tumor-bearing rats. Moreover, it was interesting to note that the decreased hemoglobin levels in cancer patients were in parallel with the decrease of percentage of red cell utilization after the administration of ^{59}Fe-labeled chondroitin sulfuric acid iron. The previous report concerning the ferrokinetics by ^{59}Fe-globulinate in neoplastic disease, showed that the utilization of radioiron by the bone marrow appeared to be normal. Therefore, the defective utilization of iron in the reticuloendothelial system of tumor-bearers probably accounts primarily for the causation of anemia.

(Received March 15, 1966)

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