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STUDIES ON FERROKINETICS IN TUMOR-BEARERS

I. Ferrokinetics by ^{59}Fe-globulinate*

Ichiro Urushizaki, Morimichi Fukuda, Jun Ibayashi,
Mikihito Matsuda, and Koji Shiramatsu

Division of Clinic, Cancer Research Institute
Sapporo Medical College

Introduction

Anemia is a common feature in tumor-bearers. It is often associated with chronic blood loss and occasionally with infection. However, in the majority of instances, the anemia cannot be ascribed to these causes.

Recently, it is well confirmed that tumor bearing, results in a derangement of the iron metabolism of the host. Hypoferremia in cancer in the absence of chronic blood loss has been amply demonstrated by a number of investigators (1, 2, 3). Iron exchange between body tissues is accomplished by a mechanism in which iron is bound to a fraction of plasma protein and transported. The major portion leaving the plasma is normally directed towards the bone marrow where it is used

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for hemoglobin synthesis. With the tracer techniques introduced by Huff and co-workers (4, 5), it is possible to obtain more quantitative data on the amount of iron transported through the plasma.

The purpose of the present series of the investigation is to study the systemic effect of tumor bearing on iron metabolism and to deliniate the mechanisms responsible for the anemia in tumor-bearers. The following study was undertaken to elucidate the erythropoietic activity in tumor-bearers by the use of ^{59}Fe-globulinate.

Materials and Methods

I. Clinica Experiments

26 patients with gastric and bronchogenic carcinomas, 10 patients with iron deficiency anemia, 5 patients with hypoplastic anemia and 10 normal controls were selected for the present investigation.

1) Hematological: Erythrocyte count, hematocrit and hemoglobin were determined on each patient. Reticulocyte count by cresyl-blue method and bone marrow examination by sternal puncture were performed on most of the patients. Sideroblast count was obtained by the method of Kaplan (6).

2) Iron metabolism:

a) Serum iron and total iron binding capacity.

The serum iron concentration was determined by the method of Trinder (7) with Nakao's modification (8). The total iron binding capacity was measured by the method of Peters (9).

b) Radioiron studies.

Ferrokinetic studies were performed on 12 patients with carcinomas, 5 patients with iron deficiency anemia, 3 patients with hypoplastic anemia and 5 healthy controls. 10 to 15 μCi of radioactive iron as ferrous citrate, in which the specific activities were 8.1 to 16.4 μCi per μg, was incubated at 37°C for 30 minutes with 15 to 20 ml of plasma obtained from a normal human subject. Following intravenous injection of a 10 ml aliquot of this tagged plasma, blood samples were drawn 5 times at 5, 15, 30, 60 and 120 minutes from the cubital vein to determine the clearance rate of radioiron from the plasma. Blood samples were taken thereafter every 5 days to measure the incorporation of radioiron into red blood cells. The plasma volume was determined by extrapolating the ^{59}Fe activity of the plasma to 0 time. Total blood volume and cell volume were calculated from the plasma volume, employing a correction of 0.9 to the venous hematocrit. The disappearance of ^{59}Fe from the plasma, the plasma iron turnover, red cell utilization of ^{59}Fe, red cell iron turnover and red cell iron renewal rate were determined by the method of Huff et al. (4, 5). Samples were counted in the liquid state using a well type scintillation counter.

3) Red cell survival studies: The survival of red cells in each case was determined by the ^{51}Cr method (10).

II. Animal Experiments

Adult albino rats (Gifu-strain, Japan) weighing approximately

170 g were used. The rats were fed with Oriental solid diet and water ad libitum over 10 days before the experiments. Each animal received an injection of approximately 2 x 10^7 freshly taken Yoshida sarcoma cells into one hind leg. Since tumor inoculation causes the death of the animal within 14 days, the experiments were carried out on the 7th day after the tumor cell inoculation.

1) Serum iron and hemoglobin concentration: Serum iron concentration was measured by the above microdetermination. Hemoglobin was determined by the cyanmethemoglobin method.

2) Radioiron studies: In each experiment radioactive ferrous citrate from 0.3 to 0.5 μCi with specific activities of 8.1 to 16.4 μCi per μg of iron was used. This was added to 5 ml aliquot of fresh serum obtained from normal rats. After incubation at 37°C for 30 minutes, 0.3 ml of this tagged serum was injected intravenously into normal and tumor-bearing rats. Blood samples each 0.2 ml were taken three times at 5, 30 and 60 minutes after the injection of radioiron to determine the clearance rate of ^{59}Fe from the plasma of normal and tumor-bearing rats.

In another group of tumor-bearers, similar amounts of blood samples were also aspirated three times at 24, 48 and 72 hours after the injection of the tagged serum to measure the incorporation of radioiron into the red cells. The plasma volume was determined by extrapolating the ^{59}Fe plasma activity to 0 time. Microdetermination of hematocrit was also carried out.

3) Distribution of radioiron in various tissues: At 24 hours after an intravenous injection of ^{59}Fe-globulinate, normal and tumor-bearers were sacrificed and the radioactivity of the each organ was determined. In this way the percentage of the recovered radioiron was determined in the blood, liver, spleen and tumor tissues.

4) Red cell survival studies: The survival studies of red cells in Yoshida sarcoma bearing and normal rats were determined by the modified ^{51}Cr method (11).

Results

I. Clinical Experiments

1) Hematological: The hematological data in each group of patients were listed in Table 1. The patients with carcinoma revealed the presence of a moderate anemia. The mean value of the erythrocyte count and hemoglobin were 371.6 x 10^4 and 10.5 ± 2.7 g/dl, respectively.

Bone marrow examination, revealed a increase of the total number of nucleated cells with a normal ratio of E/1,000 M, as compared with those of healthy controls. The nucleated cell count of the marrow in cases with iron deficiency anemia exceeded remarkably the upper limit of the normal. However sideroblast counts of the bone marrow were markedly reduced below normal in all cases of carcinoma as in the case of iron deficiency anemia.

2) Iron metabolism:

a) Serum iron and total iron binding capacity.

Table 1 indicats a marked depression of serum iron concentration

Table 1. Hematological Data on Patients with Carcinoma

	No	RBC $\times 10^4$	Hb g/dl	Ht %	Serumiron γ/dl	TIBC γ/dl	*Nucleated cells in B.M. $\times 10^3$	E/1000 N Ratio	Sidero-blaste %
Normal	10	475.3 ±38.9	15.1 ±1.2	43.5 ±3.0	109.4 ±17.3	287.3 ±17.3	110.6	334 ±47.1	21.1 ±4.7
Carcinoma	26	371.6 ±133.8	10.5 ±2.7	32.2 ±7.0	58.1 ±17.5	249.8 ±33.9	124.5	332.7 ±62.3	5.7 ±4.3
Iron Deficiency Anemia	10	359.2 ±62.7	8.2 ±2.2	27.9 ±5.3	39.8 ±15.5	411.4 ±40.7	185.4	670.3 ±147.3	2.0 ±0.9
Hypoplastic Anemia	5	189.6 ±26.1	5.6 ±0.84	16.6 ±2.5	176.6 ±15.1	279.0 ±14.4	37.3	398.6 ±149.3	67.0 ±12.3

* mean value only

Table 2. Radioiron Data on Patients with Carcinoma

	No	*TBV ml	*TPV ml	PID½ minute	PITR mg/day/kg	%RCU at 10th day	RCIT mg/day/kg	PIP mg	TRCI mg	RCIRR %/day
Normal	5	4520.4	2473.2	90.4 ±22.4	0.53 ±0.23	88.1 ±6.7	0.48 ±0.08	2.9 ±0.65	2481.6 ±333.1	1.17 ±0.2
Carcinoma	12	4057.6	2623.1	71.9 ±24.6	0.46 ±0.11	87.9 ±6.7	0.44 ±0.05	1.43 ±0.82	1736.9 ±375.5	1.3 ±0.3
Iron Deficiency Anemia	5	4976.2	3688.8	27.9 ±7.1	0.80 ±0.08	105.2 ±3.1	0.83 ±0.02	1.0 ±0.65	1258.2 ±200.7	3.7 ±0.6
Hypoplastic Anemia	3	3243.6	2780.0	181.3 ±39.6	0.55 ±0.07	26.1 ±8.8	0.14 ±0.03	5.0 ±0.5	584.3 ±9.4	1.2 ±0.02

* mean value only

in cases with carcinomas in which the mean value was 58.1 ± 17.5 per 100 ml of the blood. On the other hand, the total iron binding capacities of the cancer cases were found to be low, showing a median of 249.8 ± 33.9 per 100 ml.

b) Radioiron studies.

The results of radioiron studies on 12 patients with carcinomas, 5 patients with iron deficiency anemia, 3 patients with hypoplastic anemia and 5 normal subjects were summarized in Table 2.

a. Plasma disappearance half-time of ^{59}Fe (PID T/2).

The mean value for the half-time of radioiron clearance determined in the plasma of the carcinomas was shortened to 71.9 ± 24.6 minutes, as compared with the normal mean of 90.4 ± 22.4 minutes. The rapid clearance of radioiron from the plasma in cases with iron deficiency anemia and delayed clearance in cases with hypoplastic anemia were demonstrated in Fig. 1.

b. Plasma iron turnover (PIT).

The plasma iron turnover was within a normal range, showing a median of 0.48 ± 0.11 mg iron per day per kg in carcinomatous cases. However, this was greatly increased over the normal rate in cases with iron deficiency anemia (Fig. 3).

c. Red cell utilization of ^{59}Fe (% RCU).

Incorporation of radioiron into the circulating red cell mass was measured on the 10th day after the

administration of radioiron, at which time the incorporation had ordinally reached a plateau, as shown in Fig. 2. The mean value for the red cell utilization was 87.9 ± 6.7 % in cases with carcinomas. However, it was markedly increased in cases with iron deficiency anemia and decreased in cases with hypoplastic anemia.

d. Red cell iron turnover (RIT).

The red cell iron turnover is the amount of iron entering into newly formed red cells per unit of time. In cancer cases this was within the normal range showing a mean value of 0.44 ± 0.05 iron per day per kg.

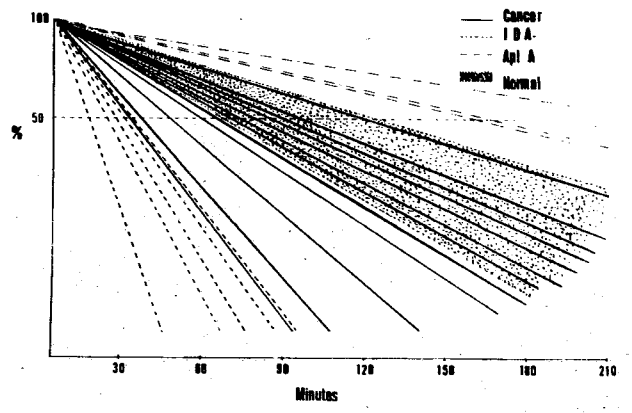

Fig. 1. Plasma iron disappearance curves in cancer patients and others.

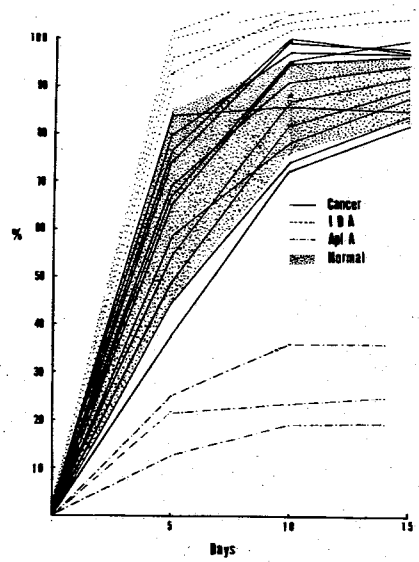

Fig. 2. Red cell utilization in cancer patients and others.

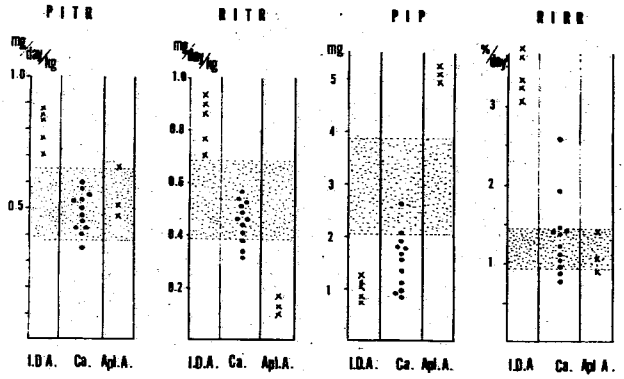

Fig. 3. Ferrokinetic data on cancer patients and others.

e. Red cell renewal rate (RIRR).

The red cell renewal rate is the fraction of red cells renewed daily. The mean value of the red cell renewal rate in cancer cases was 1.3 ± 0.3 % per day (Fig. 3).

f. Plasma iron pool (PIP).

The plasma iron pool is calculated from the plasma iron level and the plasma volume. It was markedly decreased in cases with carcinomas and had a mean value of 1.43 ± 0.82 mg. The plasma iron pool was greatly decreased in cases with iron deficiency anemia and increased in cases with hypoplastic anemia.

3) Red cell survival studies: The survival of ^{51}Cr tagged red cells was significantly shortened in cancer cases. In contrast to a normal half-life of 26 days, these patients had a half-life of 9 to 26 days, as shown in Fig. 4.

II. Animal Experiments

Yoshida ascites tumor was transplanted subcutaneously into albino rats. The survival days of tumor-bearers ranged from 10 to 14 days.

1) Serum iron concentration and hemoglobin value: The serum iron concentration and hemoglobin were measured in tumor-bearing rats. When measurements were repeated during the course of the tumor growth, a progressive decline in either of them could be demonstrated (Table 3).

Fig. 4. Half life of red cells by ^{51}Cr Method.

Rable 3. Serum Iron and Hemoglobin in Rats with Subcutaneously Inoculated Yoshida Sarcoma.

Condition	Fifth day after Inoculation			Tenth day after Inoculation		
	No.	Serumiron γ/dl	Hb g/dl	No.	Serumiron γ/dl	Hb g/dl
Normal	8	210.0 ± 19.2	14.4 ± 0.76	8	210.0 ± 19.2	14.4 ± 0.76
Yoshida Sarcoma bearing	5	186.8 ± 4.6	13.2 ± 0.37	5	110.6 ± 10.5	10.4 ± 0.6

2) Radioiron studies: The plasma radioiron disappearance half-time and percentage utilization of red cells in tumor-bearing rats and in their respective controls were summarized in Table 4. The mean values of PIDT/2 were 72.6 ± 6.27 minutes in the control group and 82.0 ± 3.37 minutes in the tumor group, as may be seen in Table 4. Incorporation of radioiron into the circulating red cell mass was usually measured on the 3 day after an administration of radioiron, at which time the incorporation reached a plateau.

Hematocrit, total plasma volume and total blood volume are markedly decreased in tumor-bearers, as is shown in Table 4. The mean values of the red cell utilization percentage were 78.1 ± 9.5 % in the control group and 73.9 ± 5.32 % in the tumor group on the 3rd day after the injection of ^{59}Fe-globulinate.

Table 4. Radioiron Data on Rats with Subcutaneously Inoculated Yoshida Sarcoma.

Condition	No	Ht %	TPV %/BW	TBV %/BW	PID$\frac{1}{2}$ Minute	No	%RCU 24 h	%RCU 72 h
Normal	8	45.8±1.42	4.35±0.31	8.08±0.35	72.6±6.27	7	66.3±5.14	78.1±9.49
Yoshida Sarcoma bearing	7	35.0±2.26	3.92±0.04	6.0±0.12	82.0±3.37	5	60.7±3.65	73.9±5.32

3) Distribution of radioiron in various tissues: 24 hours after an intravenous injection of ^{59}Fe-globulinate, 70 % of radioiron was detected in the blood of tumor bearing and normal rats. On the other hand, 10 % or less and 4 % of radioiron were demonstrated in the liver of the tumor bearing and the normal, respectively. Only 1 or 2 % of radioiron was detected in the spleen and kidney, as is shown in Fig. 5.

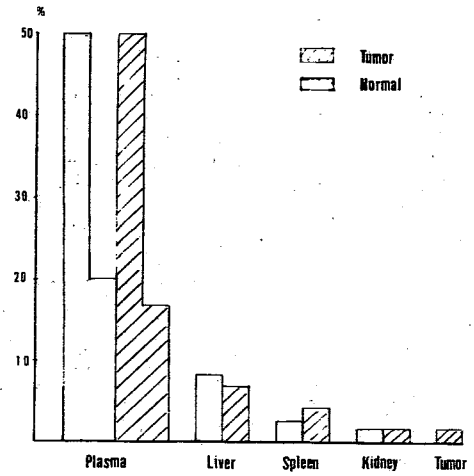

Fig. 5. Distribution of ^{59}Fe-globulinate 24 hours after injection in rats with subcutaneously inoculated Yoshida sarcoma.

4) Red cell survival studies: The half-life of red cells, determined by ^{51}Cr method, was definitely shortened in tumor bearing rats (Fig. 6).

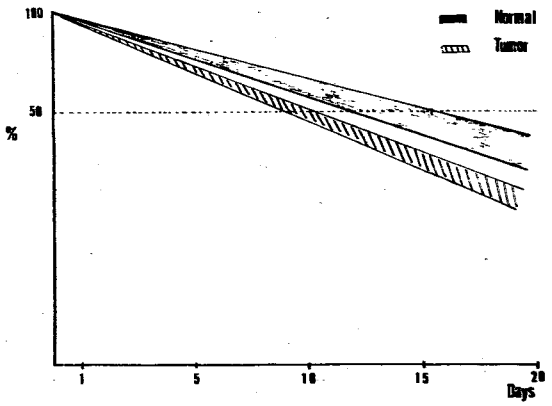

Fig. 6. Red cell survival half time in rats with subcutaneously inoculated Yoshida Sarcoma.

Discussion

In the present study, morphological observations on the bone marrow revealed normal or increased cellularity. However, the sideroblast counts of the bone marrow in cancer patients were markedly decreased. The depression of serum iron concentration tends to be in parallel with the degree of tumor growth (12). The hypoferremia and further the decreased plasma iron pool seemed to suggest that the tumor-bearers were in an iron deficient state.

The determination of the level of serum iron, however, gives no idea concerning the dynamic aspect of iron metabolism, especially there is nothing to indicate the actual amount of iron being transported through the plasma. For this reason a number of attempts have been made to obtain additional quantitative data on iron transport.

A tracer technique using isotope of iron permits an investigation of iron metabolism and erythropoietic function (13, 14, 15). Radioiron binds with transferrin which has an activity of accepting 2 atoms of iron per molecule of protein. Subsequently, transferrin bound iron in plasma is rapidly removed at an exponential rate by the bone marrow where it is utilized in hemoglobin synthesis. The distribution of radioiron 24 hours after an intravenous injection into normal rat showed that less than 10 % of radioiron was detected in the liver and the spleen.

The erythropoiesis could be estimated from the rate of removal of iron from the plasma and the fraction of radioactive iron in circulating erythrocytes.

The present results indicated that the plasma clearance time was slightly shortened, whereas utilization percentage of radioiron into red cells was within a normal range in cancer patients. In Yoshida sarcoma-bearing rats, the clearance time was slightly delayed but the utilization percentage showed normal values. In cases with iron deficiency anemia the depressed serum iron level and the increased erythropoiesis was regularly associated with a shortened T/2 and

increased percentage of red cell utilization. On the other hand T/2 was prolonged in cases with aplastic anemia with high serum iron levels and depressed erythropoiesis.

The plasma iron turnover which is a moderately accurate index of erythropoiesis, was within normal limits in all cancer patients. In addition to this, the red cell turnover rate and red cell renewal rate were normal in almost all of the cancer patients.

Thus the direct and indirect evidences made possible by ferrokinetic studies, as well as that of the marrow morphology, support the view that erythropoiesis is normal in tumor-bearers. The concept of bone marrow depression due to a noxious agent released from tumor could not be substantiated by the present experimental results. It would appear, therefore, that anemia in cancer may be caused by some different pathophysiological mechanisms separate and apart from the depressed erythropoiesis. However, the plasma iron pool in tumor-bearers is greatly reduced and the number of sideroblast in bone marrow is decreased in a similar manner as was seen in cases with iron deficiency anemia. The overall results seemed to indicate that the bone marrow of tumor-bearers is definitely in an iron deficient state.

The red cell survival studies employing ^{51}Cr showed a marked shortness of red cell life span. Recently, it has been proposed by many investigators that the anemia in cancer might be caused by a diminution in the life span of the red cells (16). If the normal

production of red cells can not keep pace with the increased red cell destruction, the hemoglobin concentration declines to a level at which production and destruction rates are in an equilibrium.

Therefore it is postulated that the characteristic abnormality of the tumor-bearers is a failure to compensate fully for an increased rate of red cell destruction. The marked increase in erythropoietic activity displayed by the normal bone marrow in iron deficiency anemia, was not observed in tumor-bearers. It might be pointed out that the tumor-bearers actually showed functional inadequacy in the face of iron supply.

Conclusion

Ferrokinetic studies by infused ^{59}Fe-globulinate in 26 patients with carcinomas and on a group of rats with Yoshida sarcoma showed that the plasma radioiron disappearance half-time was slightly prolonged in Yoshida sarcoma-bearing rats. The utilization percentage of red cells was within normal limits in both cancer patients and Yoshida sarcoma bearing rats. The plasma iron turnover, red cell iron turnover and red cell renewal rate were all within a normal range, whereas the plasma iron pool, serum iron level and sideroblast count in bone marrow were markedly decreased.

These results seem to support the view that the tumor-bearers are in an iron deficient state, however, the erythropoietic activities are maintained within normal limits.

The red cell survival studies showed a marked shortness in both cancer patients and Yoshida sarcoma-bearing rats.

In summary, the characteristic abnormality of the tumor-bearers may be accounted for as a failure to compensate fully for an rate of red cell destruction. It might be pointed out that the tumor-bearers actually showed functional inadequacy in the face of iron supply.

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