The Life Span and Osmotic Fragility of Erythrocytes in Mice Bearing Benzo(a) Pyrene – Induced Fibrosarcoma

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Red cell survival studies in mice with benzo(a) pyrene [B(a)p]-induced fibrosarcoma indicate marked reduction in RBC ^{51}Cr $t^{1/2}$. In contrast, the $t^{1/2}$ of erythrocytes from B(a)p injected but tumor-free mice was not declined. Increasing tumor burden resulted in increment in the severity of this disorder, and preincubation of normal red cells with tumor supernatant caused significant reduction ($p < 0.05$) in ^{51}Cr $t^{1/2}$. It is suggested that the observed alteration in RBC life span may be due to the effect of tumor. Apparently, both intrinsic and extrinsic cellular defects could be implicated for this abnormality. However, the shortened survival cannot be attributed to altered osmotic fragility, since the erythrocytes of the fibrosarcomatous mice were more resistant to hypotonic hemolysis *in vitro*.

Introduction

Malignancy is often associated with a series of paraneoplastic disorders in the apparently non-involved tissues of the host [1]. The most common hematologic manifestation of such alterations is the development of anemia, the etiology of which is not clearly understood [2]. The pathogenesis of this disorder has been studied in experimental animal models involving a variety of transplanted malignant tumors. A consistent finding, and obviously an important factor, is the significant reduction in the life span of the circulatory erythrocytes [3–7]. However, little is known in animals with primary tumors. Obviously this has contributed, among others, to the controversy around the validity of the extrapolation of data from experimental animals to the human situation. Therefore, an evaluation of the red cell survivality in primary tumor bearing animals seems to be of great importance. In this regard, our previous studies on the hematologic [8] and ferrokinetic [9] alterations in a chemically-induced primary fibrosarcoma in Strain A mice have indicated the possibility of a hemolytic episode. In an an attempt to gain additional insight into the problem, red cell survivality, in terms of ^{51}Cr-labeled RBC half-life ($t^{1/2}$), has been studied in the present investigation.

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Materials and Methods

Eight to ten week old closed-colony-bred male Strain A mice weighing approximately 20–22 g were used. Tumors were induced by a single subcutaneous injection of 0.3 ml olive oil containing 0.4 mg of benzo(a) pyrene [B(a)p, Schuchardt, München, GFR] in the left hind leg. Palpable tumors developed usually within 3–5 months and histological examinations revealed it as fibrosarcoma. The tumor volume was calculated according to the formula of Auda *et al.* [10].

Erythrocyte survival studies were done essentially by the method of Brodsky *et al.* [11] using radioactive chromium ($Na_2{}^{51}CrO_4$). Homologous red cells were incubated for one hour at 37 °C with 20 µCi/ml of $Na_2{}^{51}CrO_4$ (sp. activity 1.5 mCi/ml, Bhava Atomic Research Centre, Trombay). After reduction of chromate to Cr^{3+} by addition of 0.3 mg ascorbc acid per ml cell suspension [12], the washed, labeled erythrocytes were transfused into recipient mice in a volume of 0.5 ml. At different time intervals after transfusion ranging from 1 to 10 days, blood samples (0.1 ml) were withdrawn and the radioactivity was counted. Taking the value on 24 h as 100% the radioactivity of the samples on the following days were expressed as percent of 24 h count. The data were plotted against time on a semilogarithmic paper and the ^{51}Cr-labeled red cell half-life ($t^{1/2}$) was estimated by extrapolating from the ^{51}Cr disappearence curve.

Tumor supernatant was prepared by employing the method of Vaillier and Vaillier [13]. The method of Dumont *et al.* [14] was followed for erythrocyte osmotic fragility measurements. Red cells were labeled with ^{59}Fe *in vivo* by intravenous injection of 1.0 µCi $^{59}FeCl_3$ (sp. act. 887 mCi/g Fe, BARC, Trombay). The fragility of the young cells (^{59}Fe-labeled) was compared to that of whole population by measuring ^{59}Fe and hemoglobin release respectively [15]. Total erythrocyte cholesterol was determined by the method emplyoing the $FeCl_3$-acetic acid – H_2SO_4 reaction [16].

The results were subjected to Students's t test, and recorded as significant when $p < 0.05$. Values are reported as means ± S.E.

Results

The mean survival time of erythrocytes from normal and tumor-bearers, measured by ^{51}Cr-labeling technique, is shown in Table I. It is evident that the RBC ^{51}Cr $t^{1/2}$ of the tumor-bearing mice was decreased ($p < 0.05$). On the other hand no significant change in red cell survival was noted in B(a)p injected non-tumorous mice.

Comparison of the RBC $t^{1/2}$ in mice with increasing tumor burden revealed maximum reduction in those with greater tumor volume (Table II). Preincubation of normal red cells with tumor supernatant for 1 h at 37 °C resulted in a reduction of about 18% in the $t^{1/2}$ compared to that of control erythrocytes (Table II).

It is observed that the red cells from the tumor hosts were osmotically more resistant than were the erythrocytes from normal controls. While the mean corpuscular fragility (the saline concentration at which 50% of the red cells hemolyze) in the tumor bearing mice was 0.437% NaCl with a range of 0.44–0.455%, in the controls and tumor-free groups 50% hemolysis occurred in 0.462% (range, 0.440–0.483%) and 0.459% (range 0.442–0.047%) saline respectively. However, there was no significant difference ($p > 0.05$) in osmotic fragility between the newly-formed and whole population of circulating red cells of the tumor hosts.

Estimation of red cell cholesterol envisages a higher mean value in the fibrosarcomatous mice. For example, in contrast to 0.85 mg of cholesterol per ml of packed red cells in the normal mice, it was 1.31 mg/ml packed RBC in the tumor-bearing animals. Thus, an increase of about 54% ($p < 0.05$) was noted.

Table I. Erythrocyte ^{51}Cr $t^{1/2}$ in normal and tumor-bearing mice.

RBC Donor	Recipient	$t^{1/2}$ [days]
1. Normal Strain A mice	normal	17.6 ± 1.5
2. Normal + Vehicle (Olive Oil)	normal	16.9 ± 1.6
3. Tumor-bearing	normal	7.5 ± 0.8
4. Normal	tumor-bearing	12.6 ± 1.2
5. Tumor-bearing	tumor-bearing	6.1 ± 1.3
6. B(a)p treated tumor-free mice	normal	15.8 ± 1.7

p values are as follows: 1 *vs.* 2 and 6, $p > 0.05$: 1 *vs.* 3, < 0.01: 1 *vs.* 4, < 0.05: 3 *vs.* 5, > 0.05.

Table II. Effect of increasing tumor burden and preincubation with tumor supernatant on erythrocyte survival time.

Donor	^{51}Cr $t^{1/2}$ [days] in normal recipient mice
1. Tumor-bearing (tumor volume < 1500 mm^3)	8.0 ± 0.5
2. Tumor-bearing (> 1500 mm^3)	6.2 ± 0.8
3. Normal RBCs preincubated with tumor supernatant	13.4 ± 0.8
4. Normal RBC preincubated in HBSS[a]	16.4 ± 1.2

[a] Hanks' balanced salt solution.

1 *vs.* 2, $p > 0.05$.
3 *vs.* 4, $p < 0.05$.

Discussion

The present study demonstrates that the mean red cell $t^{1/2}$, measured by ^{51}Cr-labeling, of the fibrosarcomatous mice is significantly reduced in comparison to that of their normal counterparts. The normal RBC ^{51}Cr $t^{1/2}$ in the B(a)p injected tumor-free mice, and the close relationship between the increase in tumor burden and the severity in the degree of ^{51}Cr $t^{1/2}$ reduction probably reflect that the tumor growth was responsible for the observed changes in RBC survival. In addition, the decrease in the mean $t^{1/2}$ of red cells from the normal mice preincubated with tumor supernatant strongly suggest that the supernatant contains the property for such alterations. But the factor(s) responsible for such changes is not clearly understood.

The marked reduction in the $t^{1/2}$ of red cells from fibrosarcomatous mice in the circulation of the normal recipient animals probably implicate the defect as intrinsic. In this regard, increased resistance of the RBCs from tumor-bearers to hypotonic hemolysis in the present study perhaps indicate some alteration in the cell membrane. The abnormal osmotic fragility was not merely due to the effect of carcinogen since the B(a)p injected non-tumorous mice have a normal MCF. Furthermore, it cannot be attributed to reticulocytosis observed in the tumor hosts [8] because the fragility of young RBCs and the whole population of red cells was virtually identical. Decreased osmotic fragility has often been

attributed to increased membrane cholesterol [17]. Accordingly, the altered fragility may be related to the increased red cell cholesterol observed in the present study. But in any case decreased osmotic fragility seems unlikely to be an important contributing factor for the reduction in red cell survival time.

A change in the red cell membrane, however, may not be the sole explanation for the shortened red cell survival. The fact that red cells from normal mice have also a reduced ^{51}Cr $t^{1/2}$ when transfused into the circulation of tumor-bearers suggests that the tumor growth is probably associated with both intrinsic and extrinsic RBC defect. The exact nature of the extracorpuscular defects are currently being explored.

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