Evaluation of Recommended Methods for Radioisotope Red Cell Survival Studies

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UCHIDA, T., KARIYONE, S., MIKI, M., SATO, M., FUJIMORI, K. and NAKAJIMA, K. *Evaluation of Recommended Methods for Radioisotope Red Cell Survival Studies.* Tohoku J. exp. Med., 1976, 120 (3), 219-223 — Mean red cell life-span in normal subjects and in patients with various hematological disorders was examined with ^{51}Cr and DF^{32}P . The results with ^{51}Cr were corrected for ^{51}Cr elution using correction factors. The results by the two methods agreed fairly well with each other. Elution rate in various hematological disorders was 2.3% per day or less except for the patients with extracorporeal hemolytic agents such as autoimmune hemolytic anemia or congestive splenomegaly. It is concluded that estimates of mean red cell life-span by corrected ^{51}Cr method are more useful and sufficient than uncorrected ^{51}Cr or DF^{32}P method in general hematological disorders. ——— mean red cell life span; corrected ^{51}Cr method; elution rates; DF^{32}P method

Red cell survival studies with radioactive chromate (^{51}Cr) are widely used in clinical hematology. The elution of ^{51}Cr from intact cells has raised difficulties in the interpretation of survival studies. The International Committee for Standardization in Hematology (ICSH, 1971) recommended the methods for radioisotope red cell survival studies, in which mean red cell life-span (MCL) was estimated. The present study was undertaken to evaluate this method by tagging red cells simultaneously with ^{51}Cr and diisopropylfluorophosphate (DF^{32}P) in various hematological disorders, and comparing the MCL by both methods. The elution rate of ^{51}Cr from red cells in each hematological disorder is also presented here.

METHODS

The red cell life-span was measured with ^{51}Cr and DF^{32}P simultaneously in 54 patients with normal routine hematological laboratory data and various hematological disorders. Labeling was carried out according to the methods recommended by ICSH (1971). Twenty ml of venous blood were obtained in a plastic syringe containing 3.0 ml

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of NIH formula "A" acid-citrate-dextrose (ACD) and transfused into the vial. Radioactive sodium chromate solution (^{51}Cr : $1.5 \mu\text{Ci/kg}$ body weight) was added and the mixture was incubated for 30 min at 37°C . Fifty mg of ascorbic acid were added to the vial and a 20 ml aliquot of the labeled red cell suspension was removed for re-infusion into the subjects. At 30 min after the completion of the infusion, 5 ml of blood specimen were taken. Duplicate 2 ml blood samples were washed with physiologic saline and the supernatant was discarded.

Further samples per week were taken until four weeks after the infusion. The radioactivity of each sample was measured in an automatic well-type scintillation counter (Nuclear Chicago, Chicago, Ill.). $T_{50}\text{Cr}$ was determined from the time for the radioactivity of ^{51}Cr in the circulation to fall to 50% of its initial value. In the calculation of MCL, all ^{51}Cr measurements were corrected for elution according to the tables of correction factors (ICSH, 1971). The data were plotted on linear and semilogarithmic graph paper. If a straight line could be fitted to the data points, the line was obtained according to the method of least squares, MCL was estimated directly from linear disappearance curves by extrapolating to zero activity. If an exponential disappearance curve was obtained, MCL was calculated from the half-time divided by the natural logarithm of 2.

DF ^{32}P (Radiochemical Centre, Amersham, England) in sterile propylene glycol was dissolved in 20 ml of saline and administered intravenously. The amount of DFP injected was not greater than 0.02 mg/kg body weight, and the radioactivity injected did not exceed $0.7 \mu\text{Ci/kg}$ body weight. One ml of blood sample was taken at 24 hr and thereafter at least two specimens per week for the duration of the study. Red cell ^{32}P radioactivity was measured by a gas-flow counter equipped with an automatic sample changer (Model 181A decade scaler: Nuclear Chicago). Presentation of red cell survival data was the same as the calculation of MCL by ^{51}Cr method.

The % chromium elution rate was calculated by Cline's method (Cline and Berlin 1963). If the data points show a straight line, and if the elution of ^{51}Cr from intact cell is exponential, then

$$N = N_0 \left(1 - \frac{t}{T}\right) e^{-K_e t},$$

where N is the ^{51}Cr counts per min/ml of red cell at any time " t " and N_0 is the ^{51}Cr counts per min/ml of red cell at time zero. T is the MCL, T' is the $T_{50}\text{Cr}$ and K_e is the chromium elution rate constant. If MCL and $T_{50}\text{Cr}$ are known from simultaneous

TABLE 1. *Average values of mean red cell life-span*

	Number of cases	Hematological data		
		RBC ($\times 10^4/\mu\text{l}$)	Hb (g/100 ml)	Ht (%)
Hematologically normal subjects	12	486.3 ± 45.8	14.5 ± 1.7	44.5 ± 2.6
Iron deficiency anemia	10	304.4 ± 51.0	6.7 ± 1.2	25.3 ± 4.6
Hereditary spherocytosis	4	300.8 ± 42.8	10.0 ± 0.7	28.8 ± 4.9
Autoimmune hemolytic anemia	4	271.5 ± 10.0	9.6 ± 1.7	28.0 ± 4.5
Aplastic anemia	5	250.6 ± 63.8	7.0 ± 3.0	27.0 ± 7.4
Congestive splenomegaly	6	372.7 ± 40.8	10.5 ± 0.7	32.2 ± 5.1
Acute leukemia & lymphoma	6	246.0 ± 100.6	7.0 ± 3.6	22.2 ± 9.8
Myeloproliferative disorders	5	543.6 ± 161.6	15.6 ± 4.6	45.8 ± 13.2
Idiopathic thrombocytopenic purpura	2	403.0 ± 3.0	12.8 ± 0.6	40.5 ± 1.5

Mean $\pm$ s.d. * Significantly higher values than normal ($p < 0.01$).

measurement of red cell survival with DF^{32}P and ^{51}Cr , then K_e can be determined as follows:

$$K_e = \frac{\ln 2 + \ln \left(1 - \frac{T'}{T}\right)}{T'}$$

If the red cell disappearance curve shows exponential fashion, then

$$N = N_0 e^{-(K_d + K_e)t}$$

where K_d is the rate of random destruction of red cells. In DF^{32}P labeling method, declining equation may be expressed by

$$N' = N_0' e^{-K_d t},$$

where N' and N_0' are the counts per min/ml of red cells with DF^{32}P at any time " t " and extrapolated from the declining portion to time zero, respectively. Then,

$$K_e = \frac{\ln 2}{T'} - \frac{1}{T}$$

RESULTS

The values for MCL and elution rates in 54 patients with various hematological disorders are shown in Table I. The mean value obtained by the corrected ^{51}Cr method in hematologically normal subjects was 102.1 ± 10.8 days (mean $\pm$ s.d.) and that of DF^{32}P method was 111.3 ± 9.1 days. The chromium elution rate calculated from simultaneous DF^{32}P measurements in this group was 1.44 ± 0.18 %. The elution rates in patients with iron deficiency anemia, hereditary spherocytosis, autoimmune hemolytic anemia, aplastic anemia, congestive splenomegaly, lymphoma and leukemia, myeloproliferative disorders and idiopathic thrombocytopenic purpura were 1.13 ± 0.57 , 1.22 ± 0.13 , 5.31 ± 2.62 , 0.66 ± 0.47 ,

by ^{51}Cr and DF^{32}P in various diseases

^{51}Cr method		DF^{32}P method	Elution rate % per day
$T_{50}\text{Cr}$ (days)	MCL (days)	MCL (days)	
28.0 ± 2.5	102.1 ± 10.8	111.3 ± 9.1	1.44 ± 0.18
23.4 ± 2.6	82.1 ± 13.0	66.2 ± 10.1	1.13 ± 0.57
11.4 ± 2.4	41.0 ± 9.2	25.5 ± 4.7	1.22 ± 0.13
6.0 ± 1.1	20.6 ± 4.5	20.7 ± 7.3	$5.31 \pm 2.62^*$
12.9 ± 4.3	49.9 ± 12.1	31.2 ± 8.7	0.66 ± 0.47
20.8 ± 4.3	74.3 ± 19.7	74.8 ± 21.8	$2.14 \pm 0.77^*$
16.5 ± 7.5	63.9 ± 24.1	49.0 ± 30.3	1.21 ± 0.50
26.1 ± 5.0	96.0 ± 17.7	82.6 ± 28.5	1.10 ± 0.24
20.3 ± 1.8	73.0 ± 10.0	74.8 ± 14.8	1.82 ± 0.08

2.14 ± 0.77 , 1.21 ± 0.50 , 1.10 ± 0.24 and 1.82 ± 0.08 , respectively. The range of elution rates in various hematological disorders was 0.13–2.32% per day except for autoimmune hemolytic anemia (0–8.00% per day) and congestive splenomegaly (1.04–3.40% per day), which were significantly higher than normal value. There was a positive correlation between MCL obtained by DF³²P and corrected ⁵¹Cr methods in hematologically normal subjects ($r=0.704$, $p<0.01$). The similar positive correlation was obtained between them in the entire group ($r=0.868$, $p<0.01$).

DISCUSSION

Radioactive sodium chromate has been widely used for red cell survival studies. The standardized method and MCL obtained by correction factors of ICSH (1971) are very convenient for quantitative measurement of red cell destruction. DF³²P is a satisfactory label, because it is not eluted from the red cells at least after the first 24 hr (Hjort et al. 1960) and MCL obtained by DF³²P is thought to express the true life-span of red cells. We compared MCL obtained by corrected ⁵¹Cr with that by DF³²P techniques and evaluated the elution rates in various hematological disorders according to Cline's method (Cline and Berlin 1963). The mean values for MCL by corrected ⁵¹Cr and DF³²P in hematological normal subjects were 102.1 and 111.3 days, respectively. These values agree with the results of Eernisse and Rood (1961) (121,126 and 127.5 days), Shirakawa (1967) (113.4 ± 8.2 days) and Bentley et al. (1974) (DFP, 111.2 ± 20.3 ; corrected Cr, 110.4 ± 8.2 days). The chromium elution rate in this group was 1.44%, which is in accordance with generally accepted value (Garby and Mollison (1971), 1.19%; ICSH (1971), 1.31%). In various hematological disorders, Cline and Berlin (1963) demonstrated that elution rates ranged from 0.67 to 2.27% per day, and Garby and Mollison (1971) obtained the similar results (0.56 to 2.04% per day). Although the elution rate is considered to vary greatly in patients with hematological diseases, that in each disorder has not been determined yet. From the calculation of elution rates in 12 hematologically normal subjects and 42 patients with hematological disorders, we found that elution rate in each disorder is 2.3% per day or less except for autoimmune hemolytic anemia and congestive splenomegaly. These findings suggest that extracorporeal hemolytic agents such as an attachment of autoantibody to red cell membrane or red cell sequestration in marked splenomegaly might relate to elution process from red cells.

MCL obtained by corrected ⁵¹Cr method coincided fairly well with that of DF-³²P method ($r=0.868$, $p<0.01$). Therefore, it is convenient to express the red cell survival as MCL rather than the raw ⁵¹Cr data ($T_{50}\text{Cr}$) in general cases. Other advantages of ⁵¹Cr as the label in comparison with DF³²P are that only small volume of blood need to be labeled, observation period is four weeks or less and sites of cell destruction can be detected by surface counting. However, DF³²P should be used when a precise measurement of MCL is necessary, especially in patients with autoimmune hemolytic anemia or congestive splenomegaly. It would be concluded that ⁵¹Cr method is sufficient in general red cell survival studies.

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