A NEW TECHNIQUE TO DENATURE RED CELLS FOR SPLEEN SCANNING

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The value of spleen scanning is well established in determining the presence and degree of splenomegaly in the differential diagnosis of obscure abdominal masses and in the diagnosis of space-occupying lesions of the spleen. In addition, we have found it useful in locating the best site for external irradiation of the spleen and in assessing the response of the spleen to therapy in blood dyscrasias (*1*).

Commonly used methods of splenic scintiscanning involve modification of the red cells by sensitization (*2*), by heating (*3*) and by chemical means (*4,5*) so that there is a rapid and selective sequestration of the red cells in the spleen. The scope of the sensitized red-cell technique is limited because the procedure is complex and is dependent on the red-cell genotype. The heat-denaturation technique yields excellent results but it tends to be time-consuming and cumbersome. Chemical denaturation with simultaneous labeling of the red cells with ^{203}Hg or ^{197}Hg-labeled bromomercury hydroxypropane (BMHP) or with ^{197}Hg-Cl_2 is the simplest method for spleen scanning. There is, however, a significant uptake by the nontarget organs such as the kidney and liver resulting in undesirable radiation exposure to these organs. Moreover, the possibility of chemical toxicity from these mercury compounds has not been totally excluded although no untoward effects have been reported in the literature (*6*). For visualizing the reticuloendothelial system ^{99m}Tc-sulphur colloid has also been found to be an excellent agent and has been effectively used for spleen scintigraphy (*7,8*). However, simultaneous visualization of liver with the spleen may not be desirable at times.

In view of these limitations of conventional techniques, exploration of alternative means of denaturing the red cells for splenic scintiscanning was considered worthwhile. A possible method of denaturing the red cells was indicated by the work of Mayer and his colleagues (*9*) who reported an impairment of red-cell viability by exposure to an excess acid citrate dextrose mixture. The other pointer to an alternative method of red-cell denaturation is the evidence that the survival of red cells is impaired when red-cell labeling is carried out with low-specific-activity hexavalent ^{51}Cr. Szur has noted that the red-cell half-life is reduced to 21–24 days with 50 μg of carrier chromium and to about 16 days with 100 μg of carrier chromium (*10*).

This paper presents organ distribution studies in animals after administration of ^{51}Cr-labeled cells denatured by concentrated ACD and/or stable sodium chromate. Splenic scintiscans obtained in dogs and patients with the new technique are also presented.

MATERIAL AND METHODS

Animal experiments were carried out on male Wistar rats and mongrel dogs.

Collection of blood samples. About 1 ml of blood was collected from lightly anesthetized rats through a direct cardiac puncture. Ether was used as an anesthetic.

About 5 ml of blood was collected from one of the leg veins of the dog. Nembutal or i.v. sodium pentothal was used as an anesthetic for the dogs.

Labeling of the red cells with ^{51}Cr. The blood was quickly transferred to a glass vial containing ACD solution. The concentration of ACD varied with the techniques for denaturation of red cells. About 15 μCi of high-specific-activity ^{51}Cr-Na_2CrO_4 was then added, and the mixture was incubated at 37°C for 10 min. For dogs, 200 μCi of ^{51}Cr-Na_2CrO_4 was used for labeling the red cells.

Denaturation of red blood cells. One of the following techniques was used to denature the red cells.

1. Blood was collected in a four-times concentrated solution of ACD in the usual proportion of 1:4 (ACD:blood)—1 ml of concentrated ACD for 4 ml of blood. Fifteen microcuries of hexavalent ^{51}Cr in

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rats and 200 μCi in dogs was added to the mixture and incubated for 15 min. The composition of four-times concentrated ACD was as follows:

Citric acid, 3.2 gm
Disodium citrate, 10.00 gm
Dextrose, 4.8 gm
in 100 ml of distilled water.

The composition of eight-times concentrated ACD had double the amount of ingredients present in four-times concentrated ACD.

2. After the labeling of the red cells with ^{51}Cr was completed in normal ACD solution, inactive sodium chromate was added (1 mg of sodium chromate per ml of blood). The mixture was incubated at 37°C for 15 min.

3. After ^{51}Cr-labeling of the red cells was completed in concentrated ACD solution, stable sodium chromate was added and incubated as in Technique 2.

4. Heat denaturation of ^{51}Cr-labeled red cells was carried out with the conventional technique (*3*).

Administration of the ^{51}Cr-labeled red cells after denaturation. A known amount of labeled blood was injected in the penile vein of the rats. Dogs were injected in one of the leg veins. A suitably measured standard diluted to an appropriate known volume was kept to calculate the counts injected. Care was taken to insure a smooth intravenous injection. Whole-body counting of the animals was carried out immediately after the injection of labeled cells and just before sacrifice to estimate the radioactivity eliminated from the body.

Counting of body organs. The animals were sacrificed after 4 or 24 hr of administration of ^{51}Cr-labeled red cells. Spleen, liver, kidneys and heart were removed and washed in running water. The weight of the organs was noted. In the case of rats all the organs except the liver could be put into the counting tubes, and they were counted as a whole. Aliquots of the rat liver and dog organs were weighed and counted. One milliliter of blood was counted, and the total blood activity was calculated assuming the blood volume to be 7% of the body weight in grams. The total activity in the organs and blood is expressed as a percent of the administered dose. Integral counting was done in a well scintillation counter.

Spleen scans in animals. Scanning was carried out at 4 hr and 24 hr in dogs before they were sacrificed. Scanning of the removed spleen was also performed. An isotope scanner with a 3 × 3-in. NaI(Tl) crystal fitted with a 19-hole honeycomb collimator was used. The dogs were scanned in prone position. The scattered radiation from surrounding tissues was selectively eliminated by using a gamma-ray spectrometer adjusted around the photopeak of ^{51}Cr (0.32 MeV) with a window width of 10 volts. The scanning speed was 24–27 cm/min. The dot factor for dot scans and the factors for photoscans were adjusted according to the counting rates over the spleen. Ordinary photographic printing paper was used in most of the cases for photoscans. The use of readily available and inexpensive photographic printing paper in place of single-emulsion photoscanning film has been described previously (*11*).

Spleen scans on patients were carried out in the following way. Five milliliters of the patient's blood was collected and transferred into a sterilized screw-cap vial containing 1.25 ml of concentrated ACD

TABLE 1. EFFICIENCY OF LABELING RED CELLS WITH ^{51}Cr AT DIFFERENT TIME INTERVALS

Time of incubation at room temp. (min)	% efficiency of labeling
2	81.4
4	85
5	85
7	85.3
10	84.5
15	86.2
20	86

TABLE 2. EFFICIENCY OF LABELING RED CELLS WITH ^{51}Cr IN DIFFERENT CONCENTRATIONS AND PROPORTIONS OF ACD AT 25 MIN (ROOM TEMPERATURE)

1. Normal standard labeling (0.5 ml ACD + 2 ml blood : 94.5%
2. 4 ml normal ACD + 2 ml blood : 94%
3. 0.5 ml conc ACD (8 times) + 2 ml blood : 90.04%
4. 0.5 ml conc ACD (4 times) + 2 ml blood : 86%
5. Normal ACD + 2 mg Na_2CrO_4 : 87%

TABLE 3. ORGAN DISTRIBUTION DATA IN RATS 4 HR AFTER ADMINISTRATION OF ^{51}Cr-LABELED RED CELLS*

Organ	Usual proportion of ACD & No. treatment (%)	Conc. ACD (4 times usual concentration) (%)	Conc. ACD (4 times) + Na_2CrO_4 (1 mg/ml blood) (%)
Spleen	3.06	35.6	57
Liver	5.67	5.6	4.95
Kidneys	0.66	0.86	0.46
Heart	0.04	0.43	0.03
Blood	100	75.5	41.5

* % administered dose.

FIG. 1. 24-hr dog spleen scan with conc. ACD and stable sodium chromate.

TABLE 4. ORGAN DISTRIBUTION DATA IN RATS 24 HR AFTER ADMINISTRATION OF ^{51}Cr-LABELED RED CELLS*

Organ	Usual proportion of ACD & No. treatment (%)	Usual proportion of ACD & heat treated (%)	Conc. ACD (4 times) (%)	Usual ACD + Na_2CrO_4 (1 mg/ml blood) (%)	Conc. ACD (4 times) + Na_2CrO_4 (1 mg/ml blood) (%)
Spleen	2.8	24.8	46.0	29.0	86.5
Liver	6.2	27.6	26.8	8.1	8.35
Kidneys	2.65	1.72	0.46	1.28	0.36
Heart	0.5	0.41	—	0.33	—
Blood	100	3.7	4	75.7	1.88

* % administered dose.

TABLE 5. ORGAN DISTRIBUTION DATA IN DOGS 24 HR AFTER ADMINISTRATION OF ^{51}Cr-LABELED RED CELLS*

Organ	Conc. ACD (4 times) (%)	Conc. ACD (4 times) + Na_2CrO_4 (1 mg/ml blood) (%)
Spleen	22.9	56.5
Liver	15.4	3.66
Kidneys	0.22	0.31
Heart	0.05	0.014
Blood	10.2	2.79

* % administered dose.

solution to which 200–250 μCi of high specific activity ^{51}Cr in the form of sodium chromate was added (110 μCi/μg). The mixture was incubated at 37°C for 5–10 min. Five milligrams of stable sodium chromate was added to the labeled blood (1 mg/ml of blood). After incubating for 15 min, the blood was reinjected into the patient. Spleen scans were done after 4 hr and in some patients after 24 hr.

RESULTS

Preliminary studies to determine the time required for maximum incorporation of hexavalent ^{51}Cr by the erythrocytes showed 85% labeling of the cells by 4 min (Table 1).

Table 2 shows a minimal change in the efficiency of ^{51}Cr-labeling of the red cells by varying the quantity and by increasing the concentration of ACD. The efficiency of labeling in four-times concentrated ACD is 86% as compared to 94.5% in usual ACD.

Table 3 gives the organ distribution data in rats sacrificed 4 hr after administration of ^{51}Cr-labeled red cells with and without denaturation. Denaturation of red cells with concentrated ACD alone and with a combination of concentrated ACD and stable sodium chromate resulted in 35.6% and 57% accumulation of activity in the spleen, respectively.

The 24-hr distribution studies in rats (Table 4) show 46% sequestration of red cells denatured with concentrated ACD and 86.5% sequestration when denatured with a combination of concentrated ACD and carrier chromate. Erythrocyte modification with stable sodium chromate alone after ^{51}Cr-labeling in the usual ACD showed much reduced sequestration by the spleen.

Similar studies were carried out in dogs, and the 24-hr organ-distribution data are presented in Table 5. Combination of concentrated ACD and stable chromate promoted more sequestration of ^{51}Cr-tagged red cells (56.5%) as compared to concentrated ACD alone.

Spleen scintiscans were done in the dogs just before sacrifice at 24 hr. Figure 1 shows the spleen scan of the dog injected with concentrated ACD and stable sodium chromate-treated red cells.

Concentrated ACD and stable sodium chromate denaturation of ^{51}Cr-tagged cells to obtain spleen scintigrams was tried in 20 patients. Two representative scans are presented. Four-hour spleen scintiscan of a patient suffering from chronic anemia with splenomegaly is shown in Fig. 2. The spleen is well delineated and shows enlargement. Figure 3 shows the spleen scan of a patient with chronic myeloid leukemia with the disease in remission. The spleen

is normal in shape and size. Scanning was performed 24 hr after injection of the labeled and denatured red cells.

DISCUSSION

Initial studies revealed that good and almost maximum red-cell incorporation of hexavalent ^{51}Cr occurred by 4 min and the use of four- or eight-times concentrated ACD in place of the normal ACD did not significantly affect the efficiency of labeling (Tables 1 and 2). As compared to the efficiency of labeling at different time intervals (Table 1), there appears to be a slight overall increase in the percentage incorporation of hexavalent ^{51}Cr in the labeling studies carried out with different concentrations of ACD (Table 2). This is probably due to the difference in room temperatures at the time of the two experiments. The time of incubation for the latter set of experiments was also 25 min compared with 20 min. However, these experiments showed that incubation of blood with radioactive sodium chromate and concentrated ACD for 10 min was adequate.

Organ distribution studies of ^{51}Cr-labeled red cells in rats and dogs show that maximum splenic sequestration of red cells was achieved with combined concentrated ACD and stable sodium chromate denaturation (Tables 3–5). The radioactivity in the spleen is significantly increased at 24 hr. Studies in the rats show a markedly higher concentration in the spleen with concentrated ACD and stable chromate compared with the concentration after heat modification. Table 4 showing 24-hr organ-distribution data in rats injected with heat-denatured and concentrated ACD-denatured cells does not account for about 42% and 23% of the administered activity. A similar deficit of 51% and 37% is also seen in dogs injected with concentrated ACD denatured red cells and with concentrated ACD and stable chromate denatured red cells (Table 5). Since whole-body counting before sacrifice at 24 hr also showed a decrease in activity compared with initial whole-body counts, it appears that the rest of the activity was excreted from the body through the urinary tract. It is noteworthy that 4-hr organ-distribution data in rats (Table 3) does not show any excretion. On the basis of these experiments, combined concentrated ACD and stable chromate denaturation was adopted for spleen scanning in patients.

FIG. 2. Enlarged spleen in patient suffering from chronic anemia with splenomegaly. Scan done with conc. ACD and stable sodium chromate at 4 hr.

FIG. 3. Spleen scan in patient with myeloid leukemia. Scan done with conc. ACD and stable sodium chromate at 24 hr.

The spleen scans in dogs (Fig. 1) and in patients (Figs. 2 and 3) with concentrated ACD and stable chromate denaturation show a good delineation of the spleen with very little background activity in the circulation and adjacent organs. No significant difference was noted in the quality of the spleen scans done at 4 and 24 hr. Since a 4-hr scan is more convenient for our patients, we have adopted it as a routine. The entire procedure takes about 30 min for ^{51}Cr-labeling and denaturation compared with the 1½ hr needed with the heat-modification tech-

nique. In addition, the technique is simpler and does not require any special apparatus such as a water bath with temperature control.

Although simultaneous labeling and denaturation of red cells with ^{203}Hg-BMHP is the simplest and quickest, its use is questioned because of the unresolved question of chemical toxicity. The other undesirable aspects of the ^{203}Hg-BMHP method are the radiation exposure of nontarget organs and the difficulty in interpreting some of the spleen scans due to partial visualization of the left kidney. Absence of beta emission during ^{51}Cr decay considerably reduces the radiation exposure to the spleen compared with that from ^{203}Hg. Use of ^{51}Cr is therefore preferable to ^{203}Hg from the radiation-safety point of view.

In the combined concentrated ACD and stable sodium chromate technique about 10 mg of sodium chromate has been employed which works out to 0.2 mg/kg body weight in a 50-kg patient. A subcutaneous lethal dose of stable chromate in rabbits is known to be as high as 243 mg/kg (*12*). It appears that the small amounts of stable sodium chromate used in the combined denaturation technique are not likely to cause any undesirable toxic effects in patients. No toxic side effects were noted in any of the animals and patients in our series.

SUMMARY AND CONCLUSION

The use of concentrated ACD and stable sodium chromate to denature the ^{51}Cr-tagged autologous red cells for spleen scintiscanning is described. Organ distribution data in rats and dogs 4 and 24 hr after i.v. injection of labeled erythrocytes with and without modification is presented. Studies show that combination of concentrated ACD and stable chromate yields maximum sequestration of labeled cells in the spleen with very low concentrations in liver, kidneys and circulating blood.

The technique has been successfully used in 20 patients. Representative spleen scans of two patients are shown.

The advantages of combined ACD and stable chromate denaturation over the heat-treatment technique are simplicity and quicker denaturation. The ^{203}Hg-BMHP has the drawback of significant uptakes in nontarget organs and the possibility of chemical toxic effects.

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