In vitro Labeling of Platelets with Stable Rubidium Compounds

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Abstract. It is a common practice in diagnostic hematology to mark blood cells with radioactive tracers, such as $^{51}$Cr, $^{55}$Fe, $^{75}$Fe, etc., to determine their life-span and study their metabolism.

A program has been started to verify the possibility of marking blood cells with stable indicators, assayed by radioisotope-induced X-ray fluorescence analysis. All elements may be considered as indicators aggre-gating to blood cells, and for in vivo measurements, which are not toxic in the quantities injected, calculated on the basis of the limit of detection of the technique.

In this study platelets were marked with a very small quantity of stable compounds of rubidium, a potassium analog. Measurements were carried out in vitro on platelets of normal subjects and in vivo on platelets of rats and rabbits. Several survival curves were deduced and life-span values obtained in accord with previously reported values.

Introduction

Radionuclides and labeled molecules are applied to an increasing extent in hematology because of their adequacy for quantification of dynamic life processes under physiologic and pathologic conditions. The labeling of blood cells with radioactive tracers provides a suitable method for measuring their life-span, studying their metabolism, and determining parameters such as red-cell volume, plasma volume, etc.

Recently the use of activable tracers and stable tracers has also proved advantageous and its present applicability extends to some areas where radioactive tracers have been used previously, as well as to some new applications. In the case of activable tracers activation analysis has been successfully employed. In the case of stable tracers, x-ray fluorescence analysis (XRFA) induced by small sealed-off radioactive sources has been employed (Cesareo et al., 1975, 1976; Kaufman et al., 1975; Price et al., 1976).

The sensitivity of XRFA is generally sufficient for counting the labeled blood cells, without the drawbacks of radiation exposure and radioactive contamination of patients or assisting personnel.

The basic steps involved in the use of stable tracers are:

- introduction of a carefully chosen chemical form of the tracer into the system to be studied;
- XRFA of the system, opportuneely treated, in order to quantify the tracer present.

A research program has thus been started to explore in detail the possibility of employing stable tracers assayed by a 'single element' version of XRFA in hematology.

In a previous paper the labeling of platelets with stable selenocystine (Se-cystine) was studied (Cesareo et al., 1976) in order to verify the program with a well-known radioactive indicator of platelets. The toxicity of the selenium compounds therefore makes Se-cystine not so suitable for the eventual labeling of platelets in vivo.

In this paper, platelets were labeled in vitro with stable compounds of rubidium, a potassium analog. Rubidium salts, such as RbI and RbCl were employed, which are nontoxic and therefore also employable for 'in vivo' labeling of platelets.

Methods

Human blood was collected from healthy subjects by clean venepuncture with disposable plastic syringes; 5 ml aliquots were transferred to siliconized centrifuge tubes containing 1 ml EDTA
at 2%. Platelet-rich plasma was prepared by centrifugation at 250 g for 10 min and platelets successively separated from the plasma by centrifugation at 1000 g for 10 min. One ml of 0.154 M NaCl containing 5 mg Rubidium (in the form of RbI or RbCl) was then added to the platelet concentrate. The suspension was then incubated for 30 min permitting a good uptake of the Rb compounds, without platelet damage. This was proved by carrying out platelet-function tests such as platelet adhesiveness to glass following the method of Wright (Wright, 1941) and platelet aggregation induced by adrenaline, ADP, and collagen studied by a modification of the optical-density technique devised by Born (Born, 1963). Release reaction was studied by determining adenosine nucleotides from the ultraviolet spectrum recorded against a water-blank with a Beckmann spectrophotometer (Müller, 1968), and oxygen uptake measured with a Clark electrode, as reduction in oxygen pressure.

The platelet suspension was successively filtered through millipore filters 0.65 µm in size, thus permitting the retention of platelets (which have diameter of about 1–2 µm) and the passage of free RbI or RbCl molecules. The filters were successively washed several times using a 0.154 M NaCl solution in order to remove free RbI or RbCl molecules.

Measurements on rabbits were also carried out (Cesareo et al., 1977). Female rabbits were employed, and about 8 ml blood withdrawn from a marginal vein of the ear. Platelets were separated as described above, labeled with 0.9 mg RbCl, and reinjected. At successive intervals, about 4 ml blood were withdrawn and the platelets concentrated and filtered through millipore filters with a pore size of 0.22 µm.

The rubidium content of the filters was then XRF analyzed using a single-element version of the technique characterized by a 3 mCi Cd-109 radioactive source coupled to a Xe-gas proportional detector, an amplifier chain, a single-channel analyzer, and a timer-scaler.

**Results**

With respect to the in vitro labeling of human blood, measurements were carried out to establish that the rubidium detected in the filter was bound to the platelets, and was not free ions retained in the filter despite the numerous washings. To this purpose normal platelets and lysate platelets were treated in parallel in the same manner, filtered, and analyzed. The spectrum of the XRF of normal platelets compared to the XRF spectrum of lysate platelets showed a clear difference in the Rb uptake of the platelets. Free ions were in fact trapped in the same manner by the filter through which normal or lysate platelets were filtered.

The rubidium and iodine uptake of the platelets was further compared, when RbI was employed as stable tracer. It was estimated that the Rb uptake was about four times larger than the I uptake.

It was further estimated that the Rb uptake of normal platelets corresponds to about 10 µg Rb/10 ml blood.

Further, several in vitro survival curves were measured in different experimental conditions: Figure 2 shows, for example, the survival curve of platelets treated with EDTA. The survival time of 95 h agrees with the values reported in literature and with the values obtained by labeling platelets with stable Se-cystine (Cesareo, 1976).

In other experiments platelets were incubated at first with antibody against platelets and successively with the above-mentioned RbCl solution. A survival time of about 45 h was deduced.

From the measurements carried out in vivo on rabbits, it was deduced that the total quantity of Rb taken up by the platelets was about 100 µg and that the sensitivity of the apparatus in a measuring time of 500 s was 0.03 µg/cm². No Rb was detected in the filters containing plasma, red cells, and white cells.