TEACHING EDITORIAL

Cell Labeling with Oxine Chelates of Radioactive Metal Ions: Techniques and Clinical Implications

The development of a nonpolar lipophilic chelate of In-111 for labeling purified cell preparations in vitro is one of the most exciting avances in radiopharmaceutical chemistry in the last several years. It is now possible to label the various cellular components of the blood with a gamma-emitting nuclide, and image their distribution in the body. This technique already has important clinical application in clot and abscess localization, and promises to be useful for lymphocyte and monocyte kinetics and distribution as well.

In 1975 McAfee, working at the Hammersmith Hospital, London, with Thakur, made an extensive study of both soluble and particulate agents for labeling of phagocytic leukocytes (*1,2*). The use of particles, however, presented several problems, the principal one involving the difficulty in separating surface adherent as well as free particles from those completely engulfed by the cells. Therefore soluble agents were used, and of this group only nonpolar lipid soluble chelates labeled cells to a significant degree. Some of these lipophilic chelates eluted from the cell, but In-111 (and Tc-99m) oxine did not elute and labeled cells effectively. The authors showed that plasma decreased the labeling yield and that the labeling was nonspecific ("indiscriminate")—i.e., it labeled all types of cells. This necessitates cell purification before labeling, and has proven to be the most difficult technical problem with all oxine labeling methods. It is crucial to preserve cell viability by selection of the proper anticoagulant and medium, and to use gentle centrifugation and pipetting techniques. No special equipment is needed but *practice* is essential for the success of the method, and this may prove to be the biggest obstacle to the widespread use of these methods. A change of scenery seems to have been stimulating to research in this area, as Thakur in 1975, working with Coleman and Welch at the Mallinckrodt Institute of Radiology in St. Louis, developed the In-111 oxine method for leukocyte labeling, and showed it to be superior to Ga-67 citrate for abscess localization in dogs (*3*). The abscess-to-blood ratio of In-leukocytes was 70:1 compared to 4:1 for Ga-67 citrate. Thakur suggested that the mechanism of labeling was passive diffusion of the In-111 oxine through the cell membrane. From these studies it was apparent that the behavior of the labeled cells in vivo, such as their ability to accumulate in abscesses, and the blood half-time clearance curves were extremely sensitive indicators of cell viability, probably more sensitive than any of the simpler in vitro tests such as trypan blue exclusion. The large safety factor ($\sim 10^4$) for oxine toxicity was pointed out. Thakur, Welch, and Joist soon extended the technique to labeling of dog platelets and showed the excellent thrombus localizing properties, as well as accumulation in damaged arterial intima (*4*). These studies laid the groundwork for subsequent developments and clearly showed the potential for human application.

Recent developments in radiolabeling platelets with Tc-99m are described by Wistow and Grossman et al. in this issue of *The Journal of Nuclear Medicine*. Using novel methods for producing venous thrombi in rabbits, they have shown excellent images of Tc-99m-labeled platelets in these clots as well as in acute arterial endothelial lesions. The ability to use Tc-99m with its excellent properties and availability is an important advance. In spite of the shortened biologic half-life of Tc-99m-labeled platelets compared with that of In-111-labeled platelets, the authors point out that completely satisfactory images of the lesions were obtained with the Tc-99m label. Either elution of Tc-99m or damage to the platelets are suggested as possible causes of the shortened half-life. One wonders about the possible toxic effect of the stannous ion complexed with oxine and carried into the cell as the 1:2 chelate (*5*). The saline washed In-111-labeled platelet blood curves described in these papers followed a single exponential disappearance and showed no significant difference from the curves obtained with the

plasma method (*6* (plasma environment retained throughout). This observation is in disagreement with that of Scheffel et al. (*6*), who showed retention of some plasma was necessary for rabbit platelet viability. Our own observations in humans (*7*) showed that retention of plasma was necessary, as we are unable to label human platelets in saline and preserve their viability as determined by recovery, blood disappearance rates, and accumulation in lesions. Further work is needed to determine the factor or factors in plasma that preserve platelet viability. In the meantime, it is preferable to use an agent with a lower labeling yield (~ 20%) and ensure viability. Careful studies using Cr-51-labeled human platelets have shown a linear disappearance with a life span of about 9.5 days (*8*). Random damage or elution of label will convert this to a single exponential type of curve. It is certain that statistical analysis of curve shape, as well as the simple determination of half-life, will be important in determining platelet viability.

Reports of the clinical use of In-111 leukocytes (*9*) for abscess localization are enthusiastic. Our 35 cases collected to date by P. Doherty (unpublished data) confirm these optimistic findings. No false positives were noted in noninflammatory lesions, and intense uptake occured in abscesses. The technique also promises to be of value in acute inflammatory conditions such as osteomyelitis, and the arthritides such as rheumatoid arthritis and ankylosing spondylitis, as well as determining the biologic activity and effectiveness of leukocyte transfusions. The very low background activity in the kidneys, bladder, and bowel facilitates intepretation.

The prospect for the clinical use of In-111-labeled platelets is equally exciting. We have shown in our series of 22 cases (*7*) visualization of deep-vein thrombosis, acute arterial trauma, and acute pulmonary embolism. The method provides a rational approach for studying the role of the platelet in hemostasis and thrombosis in vivo, as well as the effect of the various antiplatelet drugs on these processes. Other workers (personal communication, B. Siegel and M. Welch) have shown similar promising initial results in humans.

A recent study of In-111 lymphocyte kinetics in two normals and two patients with Hodgkin's Disease has shown normal recirculation of lymphocytes (labeled with 250–500 μCi In-111) through lymph nodes back into the blood, with no apparent difference in distribution in Hodgkin's Disease (*10*). This suggests the future possibility of studying subpopulations of cells such as T lymphocytes and activated macrophages (*11*) for use in tumor localization. Methods for harvesting and processing ("activating") these cells to provide sufficient numbers of viable cells for labeling must be developed, however, before such studies can be attempted. From our experience with granulocytes, platelets, and red blood cells, the In-111 oxine method will probably label these other types of cells adequately, providing large enough numbers of cells are available.

Consideration of the small numbers of cells usually available for labeling (approximately 10^9 platelets, 10^8 leukocytes, and 10^6 lymphocytes) relative to the number of atoms per millicurie of In-111 (1.28×10^{13}) leads to the conclusion that each cell must contain large numbers of In atoms—for 1.0 mCi In-111 platelets, 10^4 atoms/cell, leukocytes, 10^5 atoms/cell, and lymphocytes, 10^7 atoms/cell. This produces large self radiation exposure principally from low energy Auger electron emissions (unpublished data, D. J. Sylvester). Estimates of 1.8×10^4 rads for 10^8 leukocytes (1mCi to total decay), 9.4×10^4 rads for 10^9 platelets, and 8.8×10^5 rads for 10^6 lymphocytes (500 μCi In-111 to total decay) are probably reasonable figures for radiation received by these cells (personal communication, S. Smith). The estimation and effect of this deposition of intimate radiation energy in cells is not completely understood but must be considered especially with "radiosensitive" lymphocytes. Even the shorter-lived Tc-99m used to label lymphocytes in 2.2 ml volume, (25 mCi) delivers about 20–50 rads to the cells (*12*). This factor may well put an upper limit on the amount of activity one can use with lymphocytes and possibly other cell types as well.

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