No Difference in Sensitivity for Occult Infection Between Tropolone- and Oxine-Labeled Indium-111 Leukocytes

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There is considerable disagreement as to whether oxine or tropolone is the best labeling agent for indium leukocytes. We have previously looked at the sensitivity of oxine-labeled 111In leukocyte scans for occult infections and now present a similar group of patients imaged with tropolone-labeled 111In leukocytes. Thirty-four patients (38 studies) with possible occult infection were prospectively studied. Patients were imaged 1-4 hr after injection and again at 24 hr postinjection. The early tropolone images had a sensitivity of 53% while the delayed images at 24 hr had a sensitivity of 93%. Based on a previous study, oxine-labeled leukocyte scans have an early sensitivity of 33% and a delayed sensitivity (at 24 hr) of 95%. The differences in sensitivity between oxine and tropolone when imaged early and at 24 hr were not statistically significant. We conclude that there is no significant difference in the ability to detect infection between oxine- and tropolone-labeled leukocytes, both early at 1-4 hr, and on delayed imaging 24 hr after injection.


There is an ongoing debate as to whether oxine or tropolone is the best labeling agent for indium-111 (111In) leukocytes. Although oxine-labeled leukocytes have been used extensively to detect sites of occult infection (1-3), questions have been raised about the viability and biologic behavior of oxine-labeled cells (4-7).

Oxine labeling requires the removal of the leukocytes from plasma because of the high affinity of transferrin for the indium-oxine complex; however, there is evidence that plasma protects the leukocytes and that its removal may damage the cells (8). For that reason, some investigators have recommended switching to tropolone for leukocyte studies since the labeling can be done in plasma (7). However, recent studies have given conflicting results concerning leukocyte phagocytosis, chemotaxis, and the ability to detect abscesses early with the two labeling agents (4-7, 9-11).

We recently reported the sensitivity of oxine-labeled leukocytes for occult infection (11) and now present a similar group of patients imaged with tropolone-labeled 111In leukocytes to evaluate whether one labeling agent is superior to the other.

MATERIALS AND METHODS

Patient selection

Thirty-four consecutive patients (22 males, 12 females, age range 18-74, mean 48) suspected of having sites of infection were prospectively studied after obtaining informed consent. During their hospitalization some patients underwent several exams, making a total of 38 examinations performed.

Leukocyte separation and labeling technique

Autologous white cells were labeled with [111In]tropolone using a slight modification of the technique described by Peters et al. (7). Specifically: to 40 cc of whole blood collected in a syringe containing 5 cc acid citrate dextrose solution, 6% hydroxyethyl starch (10% total volume) was added and the syringe was placed needle...
FIGURE 1
22-yr old man who had undergone decompressive laminectomy for metastatic teratocarcinoma developed fever postoperatively. A: 4-hr leukocyte image over back is normal. Note lung uptake. B: Posterior 24-hr image shows focus of increased activity in spinal area. CT and surgery confirmed epidural abscess

up in an incubator at 37°C for 45 min. The leukocyte rich plasma (LRP) was expressed into another 50-ml centrifuge tube and centrifuged for 5 min at 450 g. The plasma was poured off into another 50-ml tube and centrifuged at 1600 g yielding platelet poor plasma (PPP). The leukocyte cell button was resuspended in 1 ml PPP, 0.1 ml tropolone solution (26 µg/0.1 ml in HEPES buffer) and 700 µCi of [111In]Cl3 was then added and the suspension was incubated at 37°C for 10 min. The cells were "washed" with 6 ml PPP, centrifuged for 5 min at 450 g. The activity in the cell button and supernatant was measured to calculate the labeling efficiency. The radiolabeled cell button was resuspended in 6 ml PPP and 500 µCi of activity associated with the leukocytes were withdrawn into a syringe and reinjected into the patient. An average tagging efficiency of 80% was obtained.

Imaging technique
The patients were imaged 1–4 hr after injection (mean 3.0) and again at 24 hr. Images were obtained on a large-field scintillation camera with a medium-energy collimator, using 20% windows set on the 173- and 247-keV photopeaks of 111In. An initial image was performed over the liver for an information density of 500 ct/cm². The time needed to obtain that image was recorded and the remainder of the images were performed for that length of time.

The patient's chest, abdomen, and pelvis were routinely imaged; the extremities and head were imaged if clinically indicated.

Review of images and confirmation of diagnosis
All images were read by two experienced observers who were unaware of the clinical history and the results of other imaging studies. There were no disagreements between the observers. The clinicians were informed of the results of the early images, but in all cases elected to wait until a definitive answer was given at 24 hr before instituting therapy. The final clinical diagnosis was confirmed by a combination of the clinical course and supplemental radiographs, transmission computed tomography (TCT), ultrasound, surgery, needle biopsy, or necropsy.

Statistical analysis was performed using the chi-square test.

RESULTS
Fifty percent (19/38) of either early or late studies were positive for infection. The 1–4 hr images had a sensitivity of only 53% (10/19) compared with 95% (18/19) for 24-hr imaging. The falsely negative early images included: three abdominal abscesses, two infected aorto-femoral grafts, one paraspinal abscess, one infected pseudocyst, one wound infection, and one case of osteomyelitis. The one false-negative, delayed study (also falsely negative on the early image) was an infected hip prosthesis. These were no false-positive scintigrams with either early or delayed imaging. The diagnoses were confirmed by surgery, biopsy, or autopsy in 21 cases and by clinical course, laboratory data, and supplemental radiographs in 17.
Of the ten studies positive on both early and delayed scans, 60% (6/10) had more intense uptake at 24 hr. There were no cases in which the early image was more intense than the delayed image. None of the studies that were positive early became negative on delayed imaging.

The results of the tropolone study were compared with a similar group of patients imaged with oxine-labeled leukocytes that we recently reported (7/). Thirty-three percent (7/21) of oxine-labeled leukocyte studies were positive when imaged early (1-4 hr after injection, mean 2.8) compared to 53% (10/19) with tropolone. The improvement with tropolone-labeled cells was not statistically significant (p > 0.05). There also was no statistical difference (p > 0.05) between oxine’s and tropolone’s ability to detect infection at 24 hr. Oxine-labeled cells picked up 20/21 patients who had a site of infection compared to tropolone’s 18/19. This gave both labels ~95% sensitivity. The specificity for both oxine- and tropolone-labeled leukocytes was the same, 100%. Representative cases are illustrated in Figs. 1-3.

DISCUSSION

Indium-111 leukocytes labeled with oxine have been shown to be a sensitive technique for detecting abdominal abscesses and other sites of infection (1-3). Recently, however, some investigators have suggested that the oxine labeling technique injures the leukocytes (4-7). They have recommended tropolone for cell labeling, suggesting it is less toxic (7).

To obtain high labeling efficiencies with indium-oxine, the leukocytes must be washed free of plasma since the indium-oxine complex avidly binds to transferrin. Tropolone offers the possibility of less toxicity since it allows cells to remain in their normal physiologic environment, plasma, during the labeling process (12). Leukocytes deprived of plasma show morphologic changes within 10 min (8) and studies of experimental inflammatory lesions have shown greater accumulations of leukocytes labeled in plasma than those deprived of it (7).

However, studies of the behavior of both oxine- and tropolone-labeled leukocytes have given conflicting results. Segal reported that, in vitro, oxine reduced random migration and chemotaxis by 50% (4). On the other hand, Zakhireh found the standard oxine labeling technique had no effect on viability, random migration, chemotaxis, bactericidal capacity, or the ultrastructure of white cells (9). The results of studies directly comparing the in vitro behavior of oxine- and tropolone-labeled cells are just as confusing. Burke found oxine decreased leukocyte chemotaxis and phagocytosis by 30% compared to controls whereas tropolone was significantly less toxic (6). Others have reported similar results (13). However, when Gunter compared the chemotactic responsiveness of tropolone-treated neutrophils to oxine-treated neutrophils, he found the oxine-treated cells’ chemotactic response to be essentially normal while the tropolone cells showed a diminished response (10). Hall et al. also found tropolone to be a more toxic cell label than oxine (14).

Peters et al. feel that in vitro studies may not accurately reflect in vivo behavior (7, 15). They recommend studying the in vivo kinetics of labeled leukocytes to detect subtle damage. Although lung uptake of labeled leukocytes may be due to physiological margination, Saverymuttu believes it is more likely a reflection of cell damage (13). Indeed, when leukocytes are heat dam-

FIGURE 2
28-yr-old woman with fever and RLQ pain following abdominal surgery. Left: 4-hr leukocyte exam. Anterior view over pelvis is normal. Right: 24-hr scan shows increased uptake in RLQ. Abscess was confirmed at surgery.
aged, more lung uptake occurs (16). In a study of the pulmonary uptake of $^{111}$In-labeled leukocytes, leukocytes labeled in plasma with tropolone stayed in the lungs a shorter period of time than cells labeled in saline with acetylaceonate (13). The latter cells showed more liver uptake as well. Confusing the issue, however, cells labeled with either tropolone or acetylaceonate displayed several different patterns of transit, and some cell preparations labeled out of plasma showed no evidence of lung retention. In our previous oxine-labeled $^{111}$In leukocyte study, we looked at lung uptake to see if significant uptake correlated with a decreased sensitivity for infection when imaged early (11). We found no difference in lung uptake between the true-positive and false-negative group.

The present tropolone-labeled leukocyte study is a continuation of our previous oxine-labeled leukocyte study. We wanted to determine which labeling technique was superior in a clinical setting. Our results for tropolone were the same as for oxine-labeled cells; we found no significant advantage of one labeling technique over the other as far as sensitivity for detecting infection. We did find, however, that the tropolone labeling technique was less time-consuming and simpler to perform than the oxine technique.

Our results with tropolone-labeled cells were not as good as reported by Peters et al. when imaged early at 1–4 hr, although our results were identical to theirs at 24 hr (7,11). The reason for the difference in early sensitivity between the two studies is not clear. The first consideration is differences in patient population. Peters imaged patients with a variety of diseases in addition to infection; in fact, one-half of his patients had inflammatory bowel disease. All of our abnormal studies, on the other hand, were due to infection. Noninfectious diseases may react differently or, the uptake may reflect blood pool as has been suggested by some (17). Second, Peters used mixed cell preparations obtained by sedimentation in patients who were neutrophilic (those with a leukocyte count of at least 12,000/mm$^3$ of which more than 80% were neutrophils) and pure cell preparations obtained by Percoll or Metrizamide separation in the remainder. We used mixed cell preparations in all patients. Although Peters did not break down his results by cell separation technique, perhaps differences in the mixed compared with pure cell preparations may account, at least partially, for the differences in results. Finally, in 60% of our tropolone studies, the degree of uptake in the infected sites was significantly more intense on the delayed images. Peters also observed similar findings in his study (7). We conclude that there is no significant difference between oxine- and tropolone-labeled $^{111}$In leukocytes in their ability to detect infection by clinical imaging, both when imaged early at 1–4 hr on delayed imaging, 24 hr after injection.

REFERENCES


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