

Imaging of Human Infection with ^{131}I -Labeled Recombinant Human Interleukin-8

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The chemotactic cytokine interleukin-8 (IL-8) plays an important role in attraction and activation of polymorphonuclear leukocytes in infection and inflammation. A pilot study was conducted to determine if radiolabeled IL-8 would depict infection in humans.

Methods: Human recombinant IL-8 (rhIL-8) labeled with ^{131}I (specific activity, 0.4–0.7 MBq [11–18 μCi] $^{131}\text{I}/\mu\text{g}$ IL-8) was injected intravenously into 8 diabetic patients with active foot infections and evidence of osteomyelitis, 2 patients with successfully treated osteomyelitis, and 1 patient with cellulitis of the thumb. **Results:** Focal accumulation of ^{131}I -rhIL-8 was seen in 8 of 8 patients with active foot infection and diffuse uptake was seen in the thumb of the 1 patient with cellulitis. In the 2 patients with successfully treated bone infection, multiphase $^{99\text{mTc}}$ -hydroxyethylene diphosphate bone scans were negative early, but late-phase (>3 h) uptake depicted degenerative lesions that did not image with ^{131}I -rhIL-8. **Conclusion:** ^{131}I -rhIL-8 accumulates rapidly within infected foci in osteomyelitis and cellulitis but not in successfully treated infections or degenerative joint disease.

Key Words: infection; osteomyelitis; cytokine; interleukin-8; radioiodine

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Interleukin-8 is a chemotactic cytokine that exhibits a high affinity for receptors on the plasma membranes of neutrophils (1,2). Cellular activation occurs after IL-8 binding in the presence of calcium and protein kinase, resulting in neutrophil activation and chemotaxis in infection and inflammation (2). IL-8 thus functions to mediate neutrophil recruitment, accumulation, and activation. We have previously shown that radiolabeled IL-8 rapidly images sterile abscesses in an animal model (3). In this article we describe the use of ^{131}I -labeled human recombinant IL-8 (^{131}I -rhIL-8) for the scintigraphic depiction of infection in humans.

MATERIALS AND METHODS

Patients

Eight diabetic patients with documented, active foot infections and evidence of osteomyelitis and 2 diabetic patients with success-

fully treated osteomyelitis were selected for ^{131}I -rhIL-8 imaging. The diagnosis of osteomyelitis was based on clinical, laboratory, and imaging studies including plain x-rays and multiphase bone scans (Tables 1 and 2). Each patient gave human experimentation committee-approved written, informed consent before administration of supersaturated potassium iodide solution (2 drops, 3 times per day) beginning 24 h before and continuing for 7 d after the intravenous injection of 37 MBq (1 mCi) ^{131}I -rhIL-8. Blood samples were obtained immediately after ^{131}I -rhIL-8 injection and at 0.5, 1, 2, 3, 6, 24, and 48 h later. Urine was collected between 1 and 24 h, 24 and 48 h, and 48 and 72 h after ^{131}I -rhIL-8 administration. Laboratory studies were performed to assess red and white cell counts (including differential cell counts), platelets, urea nitrogen, creatinine, serum electrolytes, calcium, phosphorous, alkaline phosphatase, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and bilirubin levels at multiple selected time points after ^{131}I -rhIL-8 injection.

Image Acquisition and Analysis

Whole-body and spot images were obtained using a gamma camera equipped with a high-energy, parallel-hole collimator and interfaced to a computer (Vertex; ADAC Corp., Milpitas, CA; or ZLC; Siemens Corp., Hoffman Estates, IL). A 20% window centered around the 364-keV photopeak of ^{131}I was used to obtain images of 250,000–300,000 counts. All patients were scanned at 5, 30, 60, 120, 180, and 360 min; at 24 and 48 h, scans were obtained for the first 3 patients studied. Relative activity was determined by computer-assisted region of interest (ROI) analysis of areas of ^{131}I accumulation and the background-corrected counts per pixel were plotted over the 24-h postinjection imaging interval. Multiphase bone scans were performed with 925 MBq (25 mCi) $^{99\text{mTc}}$ -hydroxyethylene diphosphate ($^{99\text{mTc}}$ -HDP) injected intravenously with blood-flow (6 s per frame \times 20 frames), blood-pool (200,000 counts), and delayed images obtained at 2–3 h and, in some instances, at 24 h after injection.

Radiolabeling Procedures

rhIL-8 was obtained from Pepro Tech Inc. (Rocky Hill, NJ). ^{131}I was purchased as NaI (480–630 MBq [13–17 mCi] per μg iodine) from Amersham Corp. (Arlington Heights, IL). Radioiodination of rhIL-8 was performed using previously described methods (3). Specific activity ranged from 0.4 to 0.7 MBq (11–18 μCi) ^{131}I per μg IL-8. The chemotactic activity of the product was assayed using purified human neutrophils as responder cells and nonlabeled IL-8 as a chemotaxis standard (3).

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TABLE 1
Clinical Findings in 11 Patients

Age (y)	Clinical findings	Sedimentation rate (mm/h)	Culture
66	Osteomyelitis, right fifth distal phalanx	109	Mixed*
69	Osteomyelitis, first metatarsal, right foot	120	MRSA
53	Osteomyelitis, left great toe	119	Mixed
77	Osteomyelitis, proximal phalanx, left second toe	89	MRSA
67	Osteomyelitis, left foot	102	Pseudomonas, staph
50	Treated osteomyelitis, right great toe	31	<i>S. aureus</i>
44	Left ankle fusion	28	Enterococcus, pseudomonas
72	Recurrent osteomyelitis	29	MRSA
43	History of tibial fracture, treated osteomyelitis, right tibia	3	Not done
75	Osteomyelitis, right foot	79	MRSA
39	Puncture wound, right thumb	Not done	Not done

*Multiple pathogens in specimen.

MRSA = methicillin-resistant *Staphylococcus aureus*.

RESULTS

Clearance of ¹³¹I-rhIL-8

A biexponential, rapid fall of blood ¹³¹I levels was seen after injection of ¹³¹I-rhIL-8. There was an initial phase within the first 50 min (half-life, 5.3 min) and a second

phase with a half-life of 6.6 h (Fig. 1A). The activity of ¹³¹I-rhIL-8 was equally divided between plasma and red blood cells (RBCs) in blood. Cumulative urinary clearance of ¹³¹I radioactivity (\pm SD) was 59.0 ± 13.7 percentage injected dose at 72 h (Fig. 1B).

TABLE 2
IL-8 in Infection

Age (y)	Diagnosis	^{99m} Tc TPBS	X-ray	¹³¹ I-rhIL-8
66	Osteomyelitis, right fifth distal phalanx, DM	Abnormal uptake in all phases of distal phalanx, fifth digit, right foot	Fracture vs. osteomyelitis of distal phalanx of fifth digit, right foot	Uptake in distal phalanx of fifth digit, right foot
69	Osteomyelitis, first metatarsal right foot, DM	Abnormal uptake in all phases of first tarsometatarsal joint, right foot	Osteomyelitis of first metatarsal, right foot	Uptake in first metatarsal, right foot
53	Osteomyelitis, left great toe, DM	Abnormal uptake in all phases of distal phalanx, left great toe	S/P amputation of second and third digits, left foot	Uptake in distal phalanx, left great toe
77	Osteomyelitis, proximal phalanx, left second toe, DM	Abnormal uptake in all phases of proximal phalanx, left second toe	Erosive changes, second and third metatarsophalangeal joints, left foot	Uptake in proximal phalanx of second toe, left foot
67	Osteomyelitis, left foot, DM	Abnormal uptake in all phases, fifth left tarsometatarsal joint	Soft tissue ulceration/erosion, fifth metatarsal, left foot	Uptake in fifth digit, metatarsal left foot
50	Treated osteomyelitis, right great toe, DM	Uptake in delayed phase, right great toe	Soft tissue swelling in distal right foot, no bony changes	No rhIL-8 localization
44	Left ankle fusion	Abnormal uptake in all phases of distal left fibula	Left tibiotalar/tibiocalcaneal ankle fusion	Uptake in left ankle
72	Treated osteomyelitis recurrence, DM	Not done	Destruction and subluxation of second and third metatarsal heads, left foot	Faint uptake, third metatarsal region, left foot
43	History of tibial fracture, treated osteomyelitis, right tibia	Abnormal uptake in flow/blood pool, only distal right tibia	Previous fracture, right tibia	No rhIL-8 localization
75	Osteomyelitis, right foot, DM	Abnormal uptake in all phases, fifth metatarsal, right foot	Possible erosion fifth metatarsal, right foot	Uptake in fifth metatarsal, right foot
39	Puncture wound, right thumb	Diffuse uptake in flow/blood pool phases, right thumb	Soft tissue swelling, right thumb	Diffuse uptake, right thumb

DM = diabetes mellitus; S/P = status post; TPBS = triple-phase bone scan.

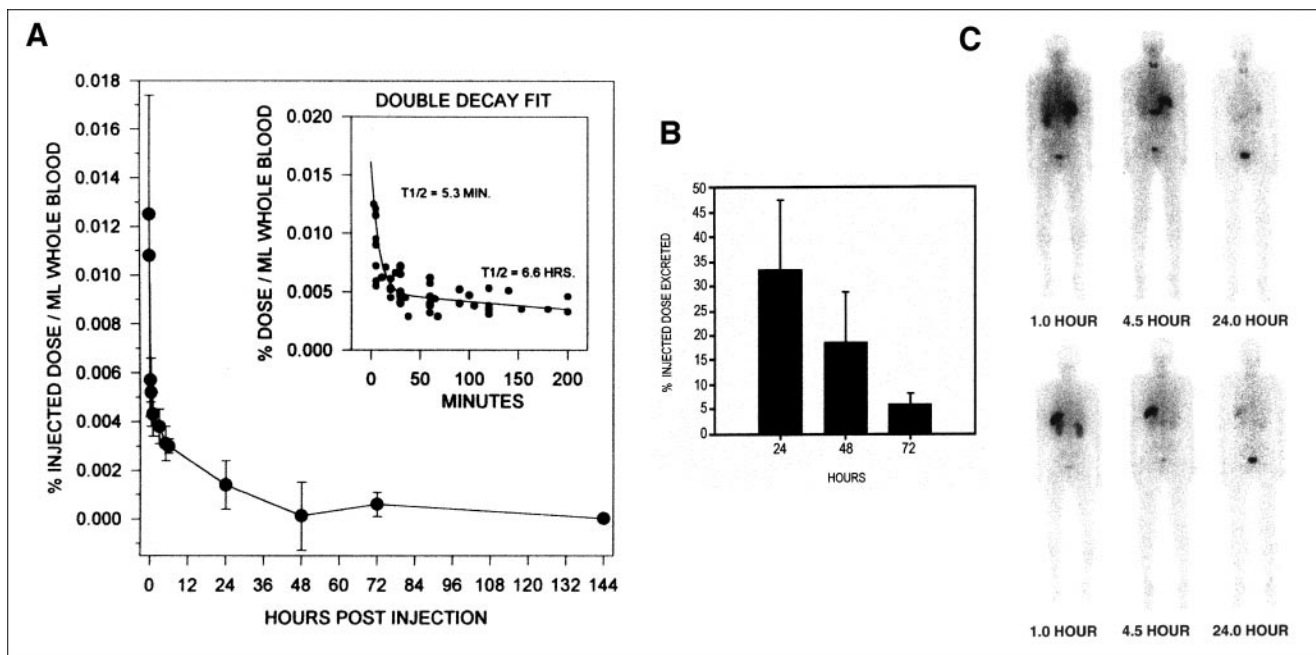


FIGURE 1. Blood clearance of ¹³¹I-rhIL-8 (A), urinary excretion of ¹³¹I radioactivity (B), and anterior (top row) and posterior (bottom row) body images over 24-h interval show faint uptake in lungs and prominent accumulation in spleen with discernable activity in small bowel, kidneys, and bladder (C). T1/2 = half-life.

Effect of ¹³¹I-rhIL-8 on Laboratory Values

White blood cell (WBC) counts fell briefly in 6 of the 11 patients (mean decrease, 600 cells per mm³; decrease range, 200–1,000 cells per mm³) after rhIL-8 injection. In all but 2 patients, WBC levels returned to baseline (patient 1, 7,200 vs. 7,500 [baseline] cells per m³; patient 2, 6,000 vs. 7,000 [baseline] cells per m³) by 60 min. No differences were noted from baseline in levels of RBC, platelets, urea nitrogen, creatinine, serum electrolytes, calcium, phosphorous, alkaline phosphatase, ALT, AST, LDH, or serum bilirubin after ¹³¹I-rhIL-8 injection.

¹³¹I-rhIL-8 Imaging of Human Infection

Accumulation of ¹³¹I radioactivity was seen in nasal and oropharyngeal areas of the head and neck. Uptake was also seen in the stomach, spleen, and bladder. Faint, transient, early lung uptake was also seen after injection. The distribution of ¹³¹I radioactivity changed little over a 24-h imaging interval (Fig. 1C). Focal accumulation was seen in the areas of foot infection in the 8 patients with confirmed osteomyelitis (Fig. 2A). The intensity of uptake appeared to be related to the duration of infection, with the most acute infections showing the most prominent accumulation of ¹³¹I activity (Fig. 2B). ROI analysis of the areas of infection showed a progressive increase of ¹³¹I radioactivity that peaked between 3 and 6 h after ¹³¹I-rhIL-8 injection. In 2 patients with successfully treated osteomyelitis, there was no ¹³¹I accumulation in either of the areas of prior infection. There was also no ¹³¹I accumulation in the 1 patient with degenerative joint disease of the foot identified on the 3-h delayed images of a multiphase bone scan (Figs. 2C and

2D). Acute cellulitis of the thumb after a rose thorn puncture wound was depicted as diffuse uptake of ¹³¹I-rhIL-8.

DISCUSSION

IL-8 is 1 of >40 chemotactic cytokines that act to induce neutrophil and other WBC activation and migration (1,2). Secretion of certain cytokines results in the selective recruitment of neutrophils into infected or inflamed tissues (1,2). In earlier studies, it has been shown that radioiodinated IL-8 will localize sterile and infected abscesses in animal models (3,4). In this study, we have shown that radiolabeled IL-8 successfully depicts infections in 8 of 8 patients with active foot infection and osteomyelitis documented by routine clinical, laboratory, radiographic, and multiphase bone scans. Accumulation of ¹³¹I-rhIL-8 was rapid with discernable depiction of infection as early as 3 h after injection. The physical properties of ¹³¹I limit spatial resolution for small structures such as foot bones, but the intense uptake in these infected feet was encouraging. Clearly, better resolution to separate soft tissue from bony infection might be achieved with a ¹²³I or ^{99m}Tc radio-label. Blood ¹³¹I radioactivity fell rapidly with uptake in the stomach, most likely reflecting deiodination. The fact that blood-pool activity did not appear to hamper imaging and uptake of IL-8 into sites of known infection was indicative of the rapid clearance and mechanism of local IL-8 receptor binding and activation of neutrophils (1,2). Splenic uptake is in keeping with the observation that IL-8 binds to erythrocytes, although in our studies and those of others, blood-pool activity does not appear to interfere with ¹³¹I-rhIL-8 imaging (3,4).

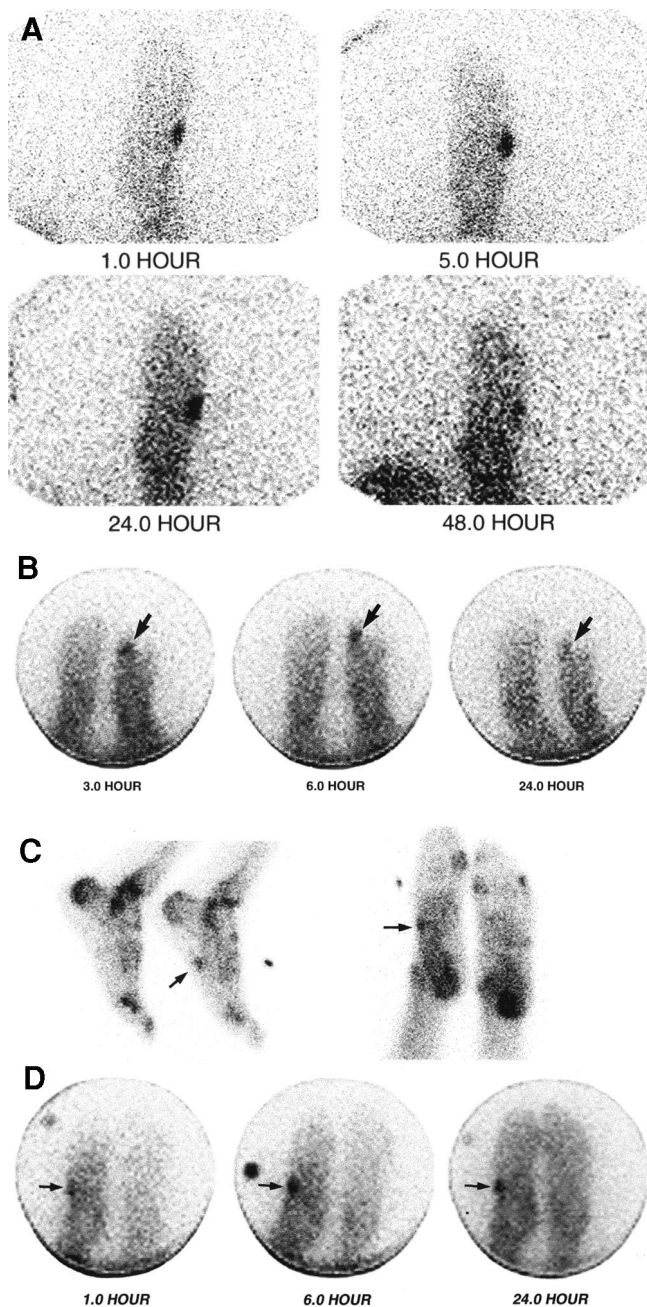


FIGURE 2. Focal uptake of ^{131}I -rhIL-8 in acute infection of left foot (A), less intense but focal ^{131}I -rhIL-8 uptake (arrow) in patient with chronic infection in foot (B), 3-h delayed $^{99\text{m}}\text{Tc}$ -HDP bone scan shows multifocal degenerative changes in ankles and feet in patient with chronic infection (arrow) of right foot (C) and corresponding ^{131}I -rhIL-8 images depict infection (arrow) but do not image extensive degenerative arthritis (D).

Furthermore, the lack of accumulation of IL-8 in areas of degenerative joint disease confirmed previous studies that have shown a specific receptor-mediated mechanism for localization of ^{131}I -rhIL-8 (3).

There were no significant side effects from ^{131}I -rhIL-8. Peripheral WBC counts fell slightly in 6 patients (mean, 4% decrease; range, 3%–14%) after IL-8 injection at doses of $1\mu\text{g}/\text{kg}$. When they occurred, the fall in peripheral WBC counts was small, transient, and unaccompanied by any observable untoward effects. At doses 10-fold greater than those administered in this study, a biphasic neutrophil response has been described with a fall of WBC at 30 s after IL-8 administration to $<10\%$ of baseline followed by a return to baseline levels within 15 min (5). Our findings also confirmed the absence of hemodynamic changes after bolus ($10\mu\text{g}/\text{kg}$) or constant infusion of IL-8 in baboons (5).

These data confirm, in humans, the findings of our initial animal studies (3) and the studies of others (4) that radiolabeled IL-8 can be used to depict foci of infection. van der Laken et al. (4) have shown early scintigraphic localization of radiolabeled IL-8 in rabbits with focal infections induced by *Escherichia coli* and *Staphylococcus aureus*. In these models, early localization of tracer has been shown with high target-to-nontarget radioactivity ratios, especially in studies using infected abscess models (4).

This study was not undertaken to formally evaluate efficacy of ^{131}I -rhIL-8 for the localization of osteomyelitis or in infection detection in humans but rather to provide pilot biodistribution data (under radioactive drug research committee approval) in one example of human infection to determine if such a trial is warranted. On the basis of the data now available, we believe that a trial is warranted, although a smaller peptide fragment of IL-8 or a related cytokine, perhaps labeled with a metal chelate of either indium or technetium, may be preferable to an iodinated cytokine or cytokine analog as an optimal diagnostic imaging agent.

CONCLUSION

In this preliminary study, we showed that ^{131}I -rhIL-8 depicts acute infection in humans, confirming studies in animals. However, the mechanisms of localization and the clinical value of IL-8 in the diagnostic evaluation of either infection or inflammation in humans have yet to be established.

REFERENCES

1. Hoch RC, Schraufstatter IU, Cochrane CG. In vivo, in vitro, and molecular aspects of interleukin-8 and the interleukin-8 receptors. *J Lab Clin Med.* 1996;128:134–145.
2. Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med.* 1998;338:436–445.
3. Hay RV, Skinner RS, Newman DC, et al. Scintigraphy of acute inflammatory lesions in rats with radiolabeled recombinant human interleukin-8. *Nucl Med Commun.* 1997;18:367–378.
4. van der Laken CJ, Boerman OC, Oyen WJG, et al. Radiolabeled interleukin-8: specific scintigraphic detection of infection within a few hours. *J Nucl Med.* 2000;41:463–469.
5. van Zee KJ, Fischer E, Hawks AS, et al. Effects of intravenous IL-8 administration in non-human primates. *J Immunol.* 1991;148:1746–1752.