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EDITORIAL

Chemotactic Peptides: New Locomotion for Imaging of Infection?

Approximately one year ago, Fischman, Khaw, and Strauss expressed their disappointment about the relatively slow progress with radioimmune imaging in neoplasia, while success in non-neoplastic areas had been very encouraging (*1*). Among other molecules, which do not directly involve the immune response, leukocyte-attractant peptides, which were originally derived from bacteria, were proposed as agents for imaging the cells to which they bind. The authors noted three potential advantages of this type of molecule over antibodies: smaller size and hence better diffusibility to the extravascular space; faster blood clearance resulting in low background activity; and the presence of well-defined receptor systems on known populations of tissue cells. Furthermore, analogs of these peptides can be synthesized and, by varying the size, charge, and other properties, a radiopharmaceutical with optimal imaging characteristics can be selected.

In this issue, Fischman et al. (*2*) report their study using such labeled chemotactic peptide analogs to image focal sites of infection. The study was performed in rats with a deep-thigh infection caused by *Escherichia coli*. Four different analogs were synthesized, coupled to diethylenetriaminepentaacetic acid (DTPA) and labeled with indium-111 (^{111}In). The parent compound of the molecules, N-formyl-methionyl-leucyl-phenylalanine (N-formyl-Met-Leu-Phe) has a molecular weight of 437. Five minutes after injection in rats, definite localization of the ^{111}In-labeled DTPA-derivatized chemotactic peptide analogs was present at the site of infection, thus showing rapid diffusion to the extravascular space. To evaluate the effect of increased permeability of infected tissue on peptide accumulation, a group of animals was co-injected with ^{99m}Tc-DTPA and ^{111}In-labeled DTPA-derivatized chemotactic peptide analogs. The authors reported that the images acquired in the ^{99m}Tc-window were of lower intensity and decreased in intensity more rapidly than those acquired in the ^{111}In-window using the upper peak only. One might wonder whether this is a fair comparison since both compounds differ significantly in molecular size, radiolabel, charge, renal handling, and other characteristics that are known to influence local accumulation and wash-out in regions with increased permeability such as infected tissue. To better understand the impact of molecular size and other properties, further experiments are needed.

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The blood clearance of the ^{111}In-DTPA chemotactic peptide analogs was rapid with half-lives varying from 21.5 to 33.1 min. Clearance from non-target organs like muscles and heart was similar. So far, the ^{111}In-DTPA chemotactic peptide analogs fulfilled the expectation raised by the same investigators (*1*). But what about their binding to well-defined receptor systems on granulocytes and mononuclear phagocytes in vivo, and how should the low uptake found in an organ rich in phagocytes, such as the spleen, be explained? To answer these questions, comparison with another recently developed radiopharmaceutical seems appropriate. In 1988, Fischman, Rubin et al. proposed human nonspecific polyclonal immunoglobulin G (IgG) as a new agent for the detection of focal inflammation and infection (*3,4*).

The utility of this radiopharmaceutical for the detection of bone and joint infection was recently confirmed by our group (*5*). Clinical utility, however, does by no means indicate that the mechanism by which ^{111}In-IgG accumulates in focal sites of infection and inflammation has been elucidated. Animal experiments showed that Fab and $F(ab)_2$ fragments of IgG labeled with ^{111}In did not localize at sites of infection, while Fc fragments did (*3, 6*). Thus, a major role was assigned to the Fc portion of the molecule and tentatively to Fc receptor binding. In severe neutropenia, the number of Fc receptors in an infectious focus is low. To investigate the impact of the presence of low levels of Fc receptors, we studied the utility of ^{111}In-IgG in a neutropenic rat infection model and in humans with fever and severe neutropenia. Somewhat to our surprise, ^{111}In-IgG appeared to perform at least as well in patients with neutropenia as in patients with normal or elevated white blood cell counts (*7*). Furthermore, autoradiographic studies in another infection model in the rat revealed that ^{111}In-labeled IgG and human serum albumin (HSA) were not associated with inflammatory cells, but localized primarily within the edematous interstitial spaces of the infection (*8*). Both studies do not support the receptor binding hypothesis. Nevertheless, a significant entrapment of ^{111}In in infectious foci was observed after administration of ^{111}In-IgG or ^{111}In-HSA (*5,9*). Enhanced vascular permeability, followed by macromolecular entrapment in tissues at focal sites of infection, probably offers a better explanation for ^{111}In-IgG accumulation. The role of the indium itself and of the characteristics of the protein (such as molecular weight, charge, and polarity), with respect to diffusion and entrapment at sites of infection, remain unclear.

In their article in this issue, Fischman et al. elegantly demonstrate *in vitro* that the chemotactic peptide analogs maintain biologic activity after coupling to DTPA. They claim that this activity persists *in vivo* as demonstrated by the cell association of the radiolabel and the images of infection in the rat, and they suggest that the mechanism of infection localization may be due to receptor-mediated binding to phagocytes. The question is whether this is what actually happens. In several patients injected with ^{111}In-IgG for suspected bone and joint infection, we have found a net increase of the count rate (after correction for physical decay) in the affected area from 4 to 48 hr, whereas the count rate in the noninfected contralateral area significantly decreased in the same period of time. These observations demonstrate ongoing entrapment of activity over a time span of at least two days. It is remarkable that the animal experiments with ^{111}In-labeled chemotactic peptide analogs do not show such entrapment (*2*). Quantative analysis of the scintigraphic images showed that the target-to-contralateral background ratio increased from approximately 1.2 after 5 min to a little over 3 after 60 min. The activity in the isolated nonaffected muscle specimens after 60 min had decreased, on average, to one-tenth of the activity present at 5 min postinjection. These data lead to the conclusion that the net count rate over the abscess dropped dramatically, being consistent with rapid wash-out; however, that drop in count rate is delayed compared to the noninfected contralateral muscle. The rapid decline in activity in the infected site from 5 to 60 min, makes it hard to believe that the ^{111}In-DTPA peptides localize at the site of infection by virtue of receptor-mediated binding to phagocytes and subsequent internalization by these cells. Therefore, with respect to specific binding, it remains to be shown that ^{111}In-DTPA chemotactic peptide analogs offer a significant advantage over ^{111}In-labeled proteins like IgG and HSA (*9*).

Will further research in the area of chemotactic peptides open new horizons for the scintigraphic detection of focal sites of infection and inflammation? To answer this question, one has to consider which requirements should be met for such radiopharmaceuticals in the 1990s. Briefly, they should provide:

1. Rapid delineation of foci and extent of infectious disease.
2. No significant physiologic accumulation in the blood and organs like liver, spleen, gastrointestinal tract, bone, bone marrow, kidneys, and muscle.
3. Relatively quick wash-out from background and target.
4. Specificity, i.e., discrimination between infection and nonmicrobial inflammation.
5. Low toxicity and absence of immune response.
6. Wide availability of the radionuclide at relatively low cost and easy preparation of the radiopharmaceutical.

With regard to item 1, it is clear that a major disadvantage of all radionuclide techniques currently available for imaging infection is the rather lengthy time required to answer the clinical question. This time span varies from 6 hr to 3 days, whereas techniques such as ultrasound, x-ray computed tomography, and magnetic resonance imaging can provide a diagnostic conclusion within minutes after finishing the imaging procedure. Therefore, radiopharmaceuticals that provide diagnostic results within 1, 2, or 3 hr, at the most, would signify a major step forward. All four DTPA-derivatized chemotactic peptide analogs studied by Fischman et al. yielded good quality images of the infection sites within 1 hr after injection (*2*).

With regard to item 2, the animal experiments of Fischman et al. (*2*) showed no significant accumulation of labeled chemotactic peptides in the spleen, gastrointestinal tract, testicles, and heart with a rapid washout, similar to that from muscle. Activity in the liver and lungs 5 min postinjection was approximately twice as high as in the organs just mentioned. Clearances from the liver and the infected tissue were similar. Accumulation in the kidneys was high. Therefore, the prospects for imaging infection within or near these organs are not promising.

Third, the rapid wash-out of ^{111}In-labeled chemotactic peptide analogs (hours) from both the target and the background tissues, offers a major advantage: the ability to perform repeat studies in a short period of time (days) to monitor therapeutic effects of drugs and invasive procedures. However, if labeling is performed with radionuclides that have a relatively short physical half-life, such as ^{99m}Tc, item 3 is irrelevant.

Item 4 states that the ideal radiopharmaceutical should be able to discriminate between infection and noninfectious inflammation accompanying tumors, fractures, hematomas, etc. At present, no radiopharmaceutical for imaging infection reaches sufficient specificity. From a theoretical point of view, the specificity of the labeled chemotactic peptides is likely to be greater for sterile inflammation since bacterial infections are rich in N-formyl-Met-Leu-Phe residues and sterile inflammations are devoid of these residues. As a consequence, bacterial and non-bacterial inflammation should differ in the uptake of intravenously administered labeled chemotactic peptide analogs.

Item 5 relates to safety. One problem of an anti-granulocyte IgG_1 (mouse) monoclonal antibody, which has been shown to detect focal sites of infection (*10,11*), is the development of human antimouse antibodies (HAMA). Furthermore, the presence of these antibodies in blood can interfere with in vitro radioimmunoassays of hormones and tumor markers (*12*). Although it is perhaps unlikely that chemotactic peptides induce immune responses, this should be investigated.

The development of marked neutropenia after the administration of chemotactic agents, even in minute quantities, is of major concern. A transient fall in white blood cell (WBC) count to 50% was induced in rabbits by intravenous administration of only 0.5 μg of the chemotactic factor N-formyl-Met-Leu-Phe (*13*). Fischman et al. found significantly lower WBC levels at 40 min postinjection than at baseline, 5 min, and 10 min; levels at 20 min were significantly lower than at 5 and 10 min (*2*). The evidence thus far indicates that labeled oligopeptide chemotactic factors and related peptides induce profound metabolic changes in neutrophils (*13*). Measurements of markers for neutrophil activation (e.g., elastase) are indicated. The step from animal to human studies with these peptides will probably be difficult to take. It seems inevitable that the amounts of peptides to be administered must be reduced from pharmacologic to tracer levels.

With regard to item 6, it is clear that ^{111}In is not the ideal radiolabel for imaging agents that clear so rapidly from target and background tissues. The optimal radionuclide for labeling chemotactic peptides should be widely available and not very expensive. It should have a physical half-life of hours rather than days, and the radiation burden should not be particularly high. These requirements imply that positron emitters like ^{11}C or ^{18}F and also ^{123}I are out of the scope for routine diagnostic work-up and therapeutic follow-up. As progress in radiochemistry moves very rapidly, it will hopefully become possible to label these compounds with ^{99m}Tc with high-specific activities, thereby allowing for a reduction in the dose of the biologically active ligand to be administered.

In summary, the synthesis of labeled chemotactic peptides certainly offers a new and interesting approach for the scintigraphic detection of inflammatory processes. It is by far too early to place these compounds in competition or concert with well-established radiopharmaceuticals and non-radionuclide techniques for the imaging of focal sites of infection. The development of small peptides for imaging purposes fits very well in the trend toward smaller molecules like meta-iodobenzylguanidine and somatostatin (*14*), offering much higher uptake in target organs compared to large size compounds such as proteins. However, the potential toxicity of pharmacologic doses of chemotactic peptides poses a very serious problem. Therefore, with these compounds, progression from animal to human studies will probably not be as easy as with the virtually non-toxic and well studied human polyclonal immunoglobulins.

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