The uptake mechanisms of infection and inflammation imaging agents

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Scintigraphic visualization of the localization of infection and inflammation is a challenging problem in clinical practice, because it may have important implications for the management of patients with infectious or inflammatory disorders. In order to enable clinicians to install rapidly the most appropriate treatment, adequate delineation and diagnosis of inflammatory foci in these patients is of critical importance. If the clinical history and physical examination are indecisive, the clinician can choose from several diagnostic modalities to determine the localization, the extent and the severity of the disease. New highly sensitive radiological investigations like magnetic resonance imaging (MRI) and spiral computerized tomography (CT) are able to locate relatively small focal abnormalities. However, these radiological methods rely on morphological changes, and as a result they are less accurate in early stages of infection or inflammation and are unable to discriminate active processes from anatomical changes due to a cured infection or after surgery.

In contrast, radiopharmaceuticals used for imaging infection and inflammation accumulate in the infectious/inflammatory lesion due to the locally changed physiological condition, such as enhanced blood flow, enhanced vascular permeability, or influx of leukocytes. Thus, scintigraphic imaging does not depend on morphological changes, but is based on physiochemical processes in tissues. Therefore, scintigraphic techniques can also visualize infectious foci in their early phases, when morphological changes are not yet apparent. In addition, scintigraphic imaging is an excellent noninvasive method of whole-body scanning that can determine the extent of the infectious or inflammatory disease throughout the body. Here the characteristics of the radiopharmaceuticals that are currently used for infection and inflammation imaging are reviewed.

Gallium-67-citrate

The use of ^{67}Gallium-citrate for infection and inflammation imaging was described for the first time in 1971.[1, 2] Upon intravenous injection ^{67}Ga binds to transferrin, and the ^{67}Ga-transferrin complex extravasates at the site of inflammation due to the locally enhanced vascular permeability. In the inflammatory lesion ^{67}Ga may transchelate to lactoferrin as present in leukocytes.[4] ^{67}Ga is excreted partly via the kidneys, and at later time points also via the gastrointestinal tract. Physiological uptake of ^{67}Ga is observed in the liver, bone, bone marrow and bowel.

Radiolabeled leukocytes

Because it was known that leukocytes actively migrate from the circulation into the inflamed tissue, in the 70s of the past century the in vivo localization of radiolabeled leukocyte preparations was studied. McAfee et al.[5] labeled leukocytes with ^{111}In complexed as ^{111}In-oxinate. After intravenous administration, the labeled cells home in the lungs with subsequent rapid clearance from the lungs. The radiolabeled leukocytes rapidly clear from the blood and part of the cells accumulate in the infected/inflamed tissue, while a substantial portion of the leukocytes (presumably the damaged cells) accumulate in the spleen. Three phases can be distinguished in the migration of the radiolabeled leukocytes from the circulation into inflamed tissue: (1) adherence to the vascular endothelium due to locally enhanced expression of adhesion molecules, (2) diapedesis: active migration through the endothelial lining and the basal membrane and (3) chemotaxis: active migration up the chemotactic gradient into the affected tissue. Ten years later, the labeling of leukocytes with ^{99m}Tc was developed using the lipophilic chelator HMPAO. Due to the more optimal radiation characteristics of ^{99m}Tc for scintigraphic application ^{99m}Tc-labeled leukocytes have replaced ^{111}In-labeled leukocytes for most indications.

Radiolabeled anti-granulocyte antibodies

The main limitation of the use of radiolabeled leukocytes is their cumbersome preparation. Therefore investigators have tried to develop a method that would allow the *in vivo* labeling of leukocytes (as opposed their labeling *ex vivo*). The use of radiolabeled monoclonal antibodies against surface antigens of granulocytes was one of the first approaches tested to accomplish *in vivo* labeling of leukocytes. Two

of these preparations are now available as radiopharmaceutical for infection/inflammation imaging: anti-CD67 IgG (anti-NCA-95, BW250/183)[7], and anti-CD66 Fab' (Leukoscan®)[8, 9, 10] (Fig. 1), and a third preparation, the anti-CD15 IgM (LeuTec®: anti-CD15)[11, 12] has been submitted for registration. With each of these ^{99m}Tc-labeled anti-granulocyte antibody preparations infectious and inflammatory foci can be delineated. The actual mechanism by which these antibody preparations accumulate in infectious or inflammatory lesions has been a matter of debate. Initially it was thought that the antibodies would bind to circulating granulocytes and subsequently migrate to the lesion. Alternatively, the antibodies could also target cells that have already migrated into the interstitial space of an inflammatory lesion. For the ant-CD67 antibody it was shown that only 5-10 % of the ^{99m}Tc activity in the blood was associated with circulating granulocytes.[13] Skehan et al. carefully compared the kinetics of ^{99m}Tc-labeled anti-CD66 Fab' and ^{99m}Tc-HSA in the blood, the inflammatory focus and in the control tissue.[14] This analysis confirmed that the ^{99m}Tc-anti CD66 Fab' fragments did not localize in inflammation as a result of binding to circulating granulocytes.

Apart from antibodies directed against surface antigens expressed on granulocytes, investigators have tried to use antibodies directed against bacteria which theoretically would allow specific detection of the micro-organism causing the infection. However, these studies have shown that IgG molecules show enhanced extravasation in any inflamed tissue, due to the locally enhanced vascular permeability. This finding was exploited by using human nonspecific polyclonal immunoglobulin (HIG) for infection and inflammation imaging. HIG was labeled with ^{111}In and ^{99m}Tc and extensively characterized clinically. Both ^{111}In and ^{99m}Tc-labeled HIG have a long circulatory half-life and physiological uptake in the liver and spleen. A general limitation is the long time span of at least 24 h between injection and diagnosis. In a comparative study, it was shown that ^{99m}Tc-HIG labeled via the chelator hydrazinonicotinamide (HYNIC) has in vivo characteristics highly similar to those of ^{111}In-HIG and in most cases is suitable to replace the ^{111}In-labeled compound. ^{99m}Tc-HIG imaging, however, has more limited sensitivity in chest disease and in chronic inflammatory processes than ^{111}In-HIG scintigraphy.

^{18}F-Fluorodeoxyglucose

The widespread successful use of ^{18}F-labeled fluorodeoxyglucose ([^{18}F]-FDG) for the detection of a wide range of malignancies has revealed that not only neoplastic cells but also leukocytes may show enhanced uptake of [^{18}F]-FDG. In fact, all activated leukocytes (granulocytes, monocytes and lymphocytes) have enhanced uptake of [^{18}F]-FDG, and thus acute and chronic inflammatory processes can be imaged with FDG-PET.[26] There have been many reports of [^{18}F]-FDG accumulation in different infections and inflammatory lesions[27,28], and with the growing availability of PET scanners, it is expected that infection imaging with [^{18}F]-FDG-PET will acquire its place in clinical practice of infection imaging in the near future. Obviously, FDG-PET is not specific for infection as tumor lesions, inflammatory lesions and granuloma will also be detected by FDG-PET.

Interleukin-8 (IL-8)

IL-8 is a small protein (72 amino acids, 8.5 kDa) and belongs to the CXC subfamily of the chemokines. IL-8 binds to both types of CXC receptors (CXCR1 and CXCR2) that are expressed at high levels on freshly isolated human neutrophils.[89] The affinity of IL-8 for both receptor types is relatively high (Kd = 0.3-4 x 10^{-9} M). The potential of radiolabeled IL-8 to image inflammation was reported for the first time by Hay and colleagues. The labeling method appeared to have major effects on the in vivo biodistribution of radioiodinated IL-8. The imaging characteristics of IL-8 labeled via the Bolton-Hunter method were clearly superior to those of IL-8 labeled via the iodogen method. We developed a ^{99m}Tc-labeled IL-8 preparation using HYNIC as a chelator. In rabbits with focal infection this preparation was characterized: uptake in the abscess was extremely high (0.3-0.4 %ID/g). As a result more than 15% of the injected activity accumulated in the abscess and abscess-to-muscle ratios exceeded 100. In neutropenic rabbits, the uptake of ^{99m}Tc-IL-8 in the inflamed muscle was less than 10% of the uptake obtained in rabbits with normal neutrophil counts, indicating that the uptake was dependent on the presence of IL-8 receptor-positive cells.

The high specific activity of the preparation (50 MBq/µg) allows injection of very low doses of IL-8 for imaging (< 0.1 µg/kg), and therefore even temporary cytopenia is not anticipated. Detailed analysis of serial images of rabbits that received ^{99m}Tc-IL-8 revealed that a substantial part of the ^{99m}Tc-IL-8 is trapped in the lungs immediately after i.v. injection. Concomitant with the clearance of the activity from the lungs (between 1 and 6 h p.i.) the accumulation of the activity in the abscess occurs, suggesting that the neutrophil-bound ^{99m}Tc-IL-8 that is initially trapped in the lungs migrates to the inflammatory focus.

Platelet Factor 4 (PF4)

PF4 is like IL-8 a member of the CXC chemokines, but has no relevant affinity for either of the two CXC receptor types. In fact, the PF4-receptor has not been identified yet. PF4 is also called the 'body's heparin neutralizing agent'. A 23 aminoacid peptide analogue of FP4, designated as P483, was synthesized. This peptide contained the heparin-binding region of PF4, a lysine-rich sequence to facilitate renal clearance and CGCG-sequence to allow labeling with ^{99m}Tc. When P483 was complexed with heparin its affinity for leukocytes increased and this complex (P483H) was studied in a rabbit model of infection. ^{99m}Tc-P483H clearly delineated the infectious foci as early as 4 hours after injection. Upon intravenous injection high pulmonary uptake was observed.^{99m}Tc-P483H was studied in 30 patients to test its applicability as imaging agent for scintigraphic detection of infection and inflammation with good results (86% sensitivity, 81% specificity, 83% accuracy). Due to the high physiological uptake in the lungs, the agent is not suited for detection of pulmonary infections. In addition, the agent showed physiologic uptake in the thyroid, the salivary glands, stomach and gastrointestinal tract. To overcome these unfavorable characteristics of ^{99m}Tc-P483H all lysine residues were substituted by arginine residues giving the P1827 peptide. In addition, heparin was replaced by the chemically defined dermatan sulphate (DS). In rabbits with *E.Coli* infection maximum accumulation of the ^{99m}Tc-P1827DS complex in the infection was obtained at a molar peptide:DS ratio of 1:10. ^{99m}Tc-P1827DS like P483H showed high uptake in the lungs, but had less uptake in the thyroid, salivary glands and the GI tract with similar uptake in the infection site. A direct comparison with ^{99m}Tc-IL-8 in the same rabbit model showed that the uptake of ^{99m}Tc-P1827DS in the infection was three times lower than that of ^{99m}Tc-IL-8.

Leukotriene B_4 (LTB_4)

Leukotriene B_4 (LTB_4) is a potent chemoattractant that activates granulocytes and macrophages and it is an important mediator in both acute and chronic inflammatory diseases. Two distinct types of leukotriene receptors have been identified (BLT1 and BLT2). LTB_4 has a high affinity for the BLT1 receptor (Kd = 10^{-9} M) that is mainly expressed on human neutrophils, while the recently characterized BLT2 receptor is a low-affinity receptor (Ka = 23 x 10^{-9} M) expressed more ubiquitously. Binding of LTB_4 to BLT1 and BLT2 promotes chemotaxis and chemokinesis. In search for an effective infection-imaging agent, a series of LTB_4 receptor antagonists were synthesized.

DPC11870-11 consists of two quinolone moieties for receptor binding linked via a cysteic acid-based hydrophilic backbone and a DTPA moiety to allow labeling with ^{111}In. In rabbits with intramuscular *E.coli* infection, ^{111}In-DPC11870-11 rapidly delineated the infection with high abscess-to-background ratios. Blocking experiments with an excess of the nonradiolabeled agent indicated that the localization of ^{111}In-DPC11870-11 was dependent on the specific interaction with LTB_4-receptors expressed in the infected tissue. The mechanism of accumulation of this tracer was studied in rabbits with soft tissue *E.coli* infection. Detailed analysis of serial images and biodistribution data of the rabbits revealed that 40-45% of the tracer localizes in the bone marrow within 1 h after injection, whereas the accumulation in the infected tissue occurred during the next few hours concomitant with clearance of the tracer from the bone marrow, suggesting that the targeted cells in the bone marrow migrate to the infectious focus. This hypothesis was confirmed by demonstrating that the tracer also localized in an abscess that was induced 4 h after injection of ^{111}In-DPC11870-11.

Interleukin-2 (IL-2)

Chronic inflammation is characterized by infiltration of monocytes and lymphocytes. Thus to visualize chronic inflammatory processes, ligands of receptors on these mononuclear cells should be applied. Radiolabeled IL-2 (MW = 15 kDa) can target IL-2 receptors expressed on activated T-lymphocytes. In a mouse model of autoimmune diabetes and in rats with renal allografts, ^{123}I-IL-2 adequately detected areas of lymphocytic infiltration. Studies in patients with autoimmune disorders as Hashimoto thyroiditis, Graves' disease, Crohn's disease and coeliac disease demonstrated localization of ^{123}I- or ^{99m}Tc-labeled IL-2 at the site of lymphocytic infiltration. In patients with active Crohn's disease, the focal uptake of ^{123}I-IL-2 in the intestinal wall decreased after corticosteroid therapy, and potentially this technique can be used to monitor the effect of therapy. Scintigraphic results using ^{123}I-IL-2 in patients with coeliac disease was consistent with the histologically determined number of infiltrating IL-2 receptor-positive cells in the jejunal mucosa. ^{99m}Tc-IL-2 also accumulated in the thyroid glands of patients with Hashimoto's thyroiditis and Graves' disease.

Ciprofloxacin

The agents described above have a specific affinity for receptors expressed on cells that are recruited during the inflammatory response, and these agents target the cells infiltrating the inflamed tissue. As these agents accumulate in the focus due to a common feature of infection and inflammation, they cannot be used to differentiate between infection and inflammation. Agents that specifically target the infectious organism (e.g. bacteria, fungi or viruses) have the potential to distinguish microbial from non-microbial inflammation. The most intensively studied agent in this category is ^{99m}Tc-ciprofloxacin. ^{99m}Tc-ciprofloxacin, also referred to as Infecton™ (Draximage, Kirkland, Canada) is ciprofloxacin labeled with ^{99m}Tc. Ciprofloxacin is a fluoroquinolone antimicrobial agent that binds to prokaryotic topoisomerase IV and DNA gyrase as expressed in proliferating bacteria. It has been hypothesized that ^{99m}Tc-labeled ciprofloxacin could specifically target living bacteria in vivo and thus that ^{99m}Tc-ciprofloxacin is able to specifically visualize bacterial infection. In clinical practice, such an agent would be extremely useful, as it would allow the discrimination between infection and inflammation due to other causes. The first results obtained with ^{99m}Tc-ciprofloxacin in 56 patients with known or suspected sites of infection were published in the Lancet in 1996, and suggested that ^{99m}Tc-ciprofloxacin had a high sensitivity (84%) to visualize infection. The efficacy of ^{99m}Tc-ciprofloxacin for imaging infections was evaluated in 879 patients in a large multicenter study coordinated by the IAEA. The overall sensitivity and specificity for infection were 85.5% and 81.6%, respectively. Despite these impressive figures from this large multinational study, several reports dispute the claim that ^{99m}Tc-ciprofloxacin only visualizes bacterial infection. In rabbits with infected and uninfected knee prosthesis and in patients with osteomyelitis or with septic arthritis, the specificity of ^{99m}Tc-ciprofloxacin for bacterial infection was not found.