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Radiopharmaceuticals for Imaging Chronic Lymphocytic Inflammation

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ABSTRACT

In the last few decades, a number of radiopharmaceuticals for imaging inflammation have been proposed that differ in their specificity and mechanism of uptake in inflamed foci as compared to the traditional inflammation imaging agents. Radiolabelled cytokines represent a reliable tool for the preclinical diagnosis of chronic inflammatory processes, even before anatomical and functional changes occur in affected tissues. Moreover, the introduction of radiolabelled monoclonal antibodies and sophisticated technique like PET/CT now make the field of inflammation imaging highly specific and accurate. In this review, different approaches of the established and experimental radiopharmaceuticals for imaging of chronic inflammation are discussed.

Key words: Radiopharmaceuticals, Inflammation, Molecular Imaging

INTRODUCTION

Diseases with chronic inflammations are always prominent in clinical medicine and characterised by a wide range of histopathological features. Clinicians frequently face problems in diagnosis and therapy dealing with patients of presumed or established inflammatory disorders, radiopharmaceuticals play a major role in providing the best possible solutions. In last few decades, a large number of radiopharmaceuticals are developed for the imaging of infection and inflammation. Radiopharmaceuticals used for scintigraphic imaging locate the site of acute/chronic infection and inflammation in their early phases. Infection specifically refers to the invasion of malignant micro-organisms, whereas inflammation is the response of the immune system against any type of disorder or injury. There may be inflammation without infection or infection without inflammation depending upon the cause of the disease or injury. Chronic lymphatic inflammation are characterised by infiltration of lymphomononuclear cells in the target organ, whereas acute inflammation have a predominant neutrophil infiltration. It is possible to distinguish different cellular infiltrates for chronic inflammation characterised by a prevalence of cellular immunity (Th1 lymphocytes and monocytes) or humoral immunity (Th2 and B lymphocytes) (Signore et al., 2000). The mechanism of localisation of radiopharmaceutical in lymphocytic inflammation imaging varies; non-specific radiopharmaceuticals (such as ^{67}Ga-citrate and ^{99m}Tc-HIG) accumulation depends upon the enhanced transudation, increased vascular

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permeability and increased blood supply processes whereas uptake of specific radiopharmaceuticals (such as ^{99m}Tc-mAbs, ^{99m}Tc-cytokines) depends upon the antigen-antibody interaction or a specific receptor binding process. Non-specific radiopharmaceutical accumulation is due to processes that are common in infection and inflammation and therefore it can not distinguish between infection and inflammation. In this respect, it should be noted that all the radiopharmaceuticals accumulate to some extent in the inflamed area due to increase blood supply and vascular permeability. For the specific detection of infection and inflammation different radiopharmaceuticals demonstrate different levels of accuracy in their sensitivity and specificity according to the pathophysiology and patho-biochemical process of individual disease.

This review will focus mainly on the established and routinely available radiopharmaceuticals for chronic lymphocytic inflammation, although it will also deals with potential radiopharmaceuticals for the future.

No Ideal Radiopharmaceutical
Typical characteristics for an ideal radiopharmaceutical have been defined by different authors (Rennen et al., 2001; Bleeker-Rovers et al., 2004; Bernardo-Filho et al., 2005, Buscombe, 2006). Although, no radiopharmaceutical has been discovered till now that fulfils all the characteristics of an ideal one, each has its own advantages and disadvantages. However, in the present scenario, we especially need more specific and sensitive radiopharmaceuticals for the diagnosis of infection and inflammation, which can specifically detect the level of different activation markers, adhesion molecules and receptors. The information these radiopharmaceuticals can provide could be used for the therapy decision making and to detect the response to the therapy. Conti et al. (2005) have shown the use of radiolabelled anti-TNFα monoclonal antibody as a prognostic tool for arthritis and suggest that the selection of patients who are the candidates for innovative anti-TNFα intra articular therapy should be guided by anti-TNFα scintigraphy.

Some radiopharmaceuticals also have the potential to distinguish between infection and inflammation, as advancement in the molecular imaging field, radiolabelled antibiotics are developed which are highly specific for the microbial infection rather than sterile inflammation (Benitez et al., 2006). In two different studies, Venjamuri et al. (1996) using ^{99m}Tc-Ciproflaxacin and Benitez et al. (2006) using ^{99m}Tc-Certizoxime antibiotics show their diagnostic specificity for the infection foci that could differentiate sterile from septic inflammation.

In this way, the research is now more focused on the development of particular tracers with more accuracy, sensitivity and specificity profile for the investigation of a particular infection or inflammation rather than searching for any ideal agent.

Radiolabelled Antibodies
Radiolabelled antibodies can be of following two kinds:

Radiolabelled Human Polyclonal Immunoglobulin G
Human polyclonal immunoglobulin (HIG) is a non antigen specific IgG antibody that can be labelled with ^{99m}Tc, having a half-life of 6 hours and 140 keV energy. It can also be labelled with ^{111}In for imaging at the time points beyond the 24 hours. The advantages of ^{99m}Tc over ^{111}In are its low radiation exposure, easy availability, short half-life and low price. Since HIG is of human origin, it does not produce any allergic response. The accumulation of radiolabelled HIG at the site of inflammation is non-specific and is related to the increase in vascularization, extracellular fluid volume and endothelial permeability within the inflamed site (Corstens et al., 1993).

A study was performed by Nijhof et al. (1997), in the patients with different infections and inflammation using ^{111}In- labelled non-specific immunoglobulin. In this study, 226 patients underwent the scintigraphy examination for 232 possible infection or inflammation foci. Imaging was performed 4 h, 24 h and 48 h post injection. The conclusion of the study was that ^{111}In- labelled non-specific immunoglobulin scintigraphy is a very sensitive tool for detection of infectious and inflamed bone and joint disorders. Another study reported that the ^{99m}Tc- non-specific immunoglobulin scintigraphy is more specific for detecting synovitis in comparison with ^{99m}Tc-diphosphonate (Bois de et al., 1995). A study was performed by Cindas et al. (2001) to investigate whether ^{99m}Tc labelled polyclonal human immunoglobulin (^{99m}Tc-HIG) scintigraphy detects

synovial inflammation in the patients with rheumatoid arthritis (RA). 29 patients with active RA underwent the scintigraphic examination and a total of 928 joints were evaluated for active inflammation. The authors concluded that the 99mTc-HIG uptake correlates with active inflammation and in RA it reflects the synovial inflammation specifically and distinguishes joints with and without inflammation.

Recently, Ortapamuk et al. (2002) performed a study to clarify whether 99mTc-HIG can detect and determine the severity of orbital involvement in patients with Graves' ophthalmopathy. Planar and SPECT examination were performed 4 hours after the injection of 99mTc-HIG in 26 patients with Graves' ophthalmopathy. In this study, it was concluded that 99mTc-HIG can clearly identify patients with clinically active inflammation, but also subclinicial inflammation can be seen by 99mTc-HIG scintigraphic evaluation.

These studies demonstrate that the 99mTc-HIG could not only be used for the assessment of disease activity but also as an effective prognostics tool for the inflammation mediated disorders.

Radiolabelled monoclonal antibodies

In last decade, radiolabelled monoclonal antibodies (mAbs) and their Fab' fragments were developed as a new class of radiopharmaceuticals for radioimmunoscintigraphy. These radiolabelled-mAbs allow the excellent molecular imaging of inflammation and infection in several immune-mediated disorders by binding with highly specific receptors expressed on particular sub cell population.

Anti-TNFα Antibodies

99mTc-Infliximab

Infliximab (Rimicade®) is a chimeric IgG1κ monoclonal antibody (MoAb), with a murine variable (Fv) domain of mouse anti-human TNF-α antibody and constant (Fc) sequences of human IgG1. Infliximab is produced by recombinant cell culture techniques. It specifically recognizes and binds to both soluble and membrane-bound TNF-α with high avidity and high affinity ($Ka = 10^{10}$ M^{-1}) (Knight et al., 1993).

Infliximab is labelled with 99mTc, using a direct radiolabelling method (Annovazzi et al., 2002). A 99mTc-Infliximab scintigraphic study was successfully performed by Conti at al. (2005) to assess the degree of TNF-α mediated inflammation in the affected knees in patients with active rheumatoid arthritis. Scintigraphy showed the intense accumulation of 99mTc-infliximab in the affected knee which represents the high levels of intralesional TNF-α, interestingly they did not find uptake in the unaffected joints.

Another pilot study was performed by Chianelli et al. (2006) on seven rheumatoid arthritis (RA) patients. In this study, nine inflamed joints were examined with 99mTc-infliximab scintigraphy. Post-treatment scintigraphy demonstrated the different amounts of radiopharmaceutical uptake in the inflamed joints; 3 inflame joints showed significant changes in uptake, whereas in the other 4 joints uptake was slightly reduced and was unchanged in 2 joints. Moreover, these imaging results affected clinical treatment.

Recently, we have performed a study with 99mTc labelled infliximab in the patients with active Crohn's Disease (CD), to investigate *in-vivo* biokinetics of anti-TNF-α antibodies and to predict the clinical response of anti-TNFα therapy (D'Alessandria et al., 2007). Scintigraphic scanning with 99mTc-Infliximab allowed us to visualize *in vivo* the intestinal sites where TNF-α was present. In this study, we found little TNF-α in the affected bowel of patients with active CD. Therefore, we concluded that, the clinical benefit that patients have from anti-TNF-α therapy is unlikely the consequence of a local a reduction of TNF-α. The mechanism of action, in therapeutic doses, therefore deserves further investigation.

These preliminary studies in humans showed the specific targeting of this radiopharmaceutical in the inflamed foci. Moreover, these studies also demonstrate that the selection of the candidates for infliximab therapy and prediction of therapy response could be possible by using 99mTc-infliximab scintigraphy before initiating anti-TNF therapy.

99mTc-Adalimumab

Adalimumab (Humira®) is the first 'fully human' recombinant monoclonal IgG1 antibody against TNF-α. It is engineered through the phase display technology in a Chinese Hamster Ovary (CHO) mammalian expression system. It recognizes both soluble and membrane-bound TNF-α with high specificity and high affinity ($K_D=6x10^{-10}$M) and inhibits its biological activity by blocking interaction with p55 and p75 cell surface TNF

receptors (Rau, 2002). It can also lyse TNF-expressing cells in the presence of complement. Adalimumab was radiolabelled with ^{99m}Tc, using an indirect radiolabelling method as described by Abrams et al. (1990). A scintigraphic imaging study was successfully performed by Barrera et al. (2003) to assess the sensitivity and biodistribution of systemically administered ^{99m}Tc-Adalimumab in ten patients with active RA. All the patients underwent two scintigraphic examinations, first to assess the biodistribution of the radiolabelled antibody and second after two weeks to assess the specificity for TNF targeting and sensitivity to reflect decreased inflammation after the administration of systemic corticosteroids. Each patient received a sub-therapeutic intravenous dose of ^{99m}Tc-anti-TNF mAb, prior to scintigraphy. The results demonstrate that the inflamed joints imaged using ^{99m}Tc-Adalimumab were clearly visualized at 4 h and 24 h after injection and the increase in uptake at 20 h was 20% to 30%. Interestingly, no uptake of the ^{99m}Tc-Adalimumab was seen in the normal joints. Furthermore, this study shows that the radiopharmaceutical specifically targets to TNF in the arthritic joint and it can detect clinical relevant changes in disease activity. Therefore, ^{99m}Tc labelled human anti-TNF mAb allows clear visualization of the inflamed joints in patients with active rheumatoid arthritis (RA). Joint localization and radiopharmaceutical retention is due to specific TNF targeting in synovitis.

Anti-E-selectin
E-selectin is an endothelial-specific, cytokine-inducible adhesion molecule (Bevilacqua, 1993), which is exclusively expressed on the luminal surface of vascular endothelium during the inflammatory response. Its expression has been demonstrated by immunohistochemistry in a variety of acute and chronic inflammatory diseases, including RA (Mason et al., 1994). E-selectin plays a key role in the inflammatory process; it mediates neutrophils, monocytes and eosinophil adhesion to activated vascular endothelium via carbohydrate ligands such as sialyl Lewis X (Bevilacqua et al., 1989; Bhushan et al., 2002).
As it is well known that, the expression of E-selectin on endothelial cells is induced after stimulation by interleukin-1 (IL-1), TNF or lipopolysaccharide, it is not expressed by resting endothelial cells. Moreover, the increased expression of E-selectin has been detected in

several inflammatory disorders (Bevilacqua and Nelson, 1993). A monoclonal antibody against E-selectin labelled with ^{111}In and has successfully been used for imaging of inflammation in an animal model (Jamar et al., 1995), thereafter also in humans (Chapman et al., 1996).
Keelan et al. (1994), performed an *in vivo* study in an animal model with ^{111}In-labelled anti-E-selectin monoclonal antibody (1.2B6), to assess the imaging potential of the antibody. The accumulation of intravenously injected ^{111}In-labelled mAb was compared to that of ^{111}In-control antibody in a model of arthritis in the pig. This study demonstrated higher accumulation of ^{111}In-anti-E-seletin antibody in the synovitis and the authors concluded that radiolabelled anti-E-selectin mAb can be used to image localized inflammatory tissue.
A comparative study was also performed by Jamar et al. (1997), between ^{99m}Tc-HIG, an established tracer for arthritis imaging, and ^{111}In-labelled anti-E-selectin mAb (1.2B6) in 11 patients with active RA. In this study, net ^{111}In counts in the joints increased significantly between 4 h and 24 h with a mean change of $54 \pm 40\%$. Moreover, the images obtained from ^{111}In-labelled anti-E-selectin mAb demonstrates much less vascular activity than with ^{99m}Tc-labelled non-specific immunoglobulin and the study reveals that radioimmunoscintigraphy using ^{111}In-labelled anti-E-selectin is more sensitive, effective and specific than ^{99m}Tc-HIG scintigraphy to identify the active synovitis.

Anti-CD20 antibody (^{99m}Tc-rituximab)
Rituximab (Rituxan$^®$) is a genetically engineered chimeric murine/human monoclonal antibody to CD20. Rituximab is produced by cell suspension culture technique, in a Chinese Hamster Ovary (CHO) mammalian expression system. Rituximab consists of IgG1 kappa immunoglobulin containing murine variable region sequences and human constant region sequences. CD-20 is a B-cell specific antigen expressed on the surface of B lymphocytes throughout the differentiation from pre-B cell to the mature B-cell stages, but it is not found on haematopoietic stem cells, plasma cells or in normal tissues (Stashenko et al., 1980; Tedder et al., 1985). B-cells are involved in the production of auto-antibodies and rheumatoid factor (RF), T cell activation, pro-inflammatory cytokine production and therefore play an important role in the pathogenesis of different autoimmune diseases (Dorner et al., 2003).

Rituximab binds to the CD20 antigen expressed on B lymphocytes with its Fab domain, and the Fc domain recruits immune effector functions to mediate B-cell lysis *in vitro*. *In vivo* rituximab induces depletion of both normal as well as malignant B cells (Reff et al., 1994). Rituximab's cytotoxicity is mediated by 3 different mechanisms, which are complement-mediated lysis (CDC), antibody-dependent cytotoxicity (ADCC) and direct disruption of signalling pathways and triggering of apoptosis. Different mechanisms predominate in the treatment of different diseases (Johnson et al., 2001; Olszewski et al., 2004).

Rituximab was radiolabelled with ^{99m}Tc for imaging inflammation and infection (Stopar et al., 2006) by a photo-activation method developed by Stalteri et al. (1996). Besides labelling with ^{99m}Tc, rituximab was also labelled with ^{90}Y and ^{131}I, but these radiopharmaceuticals are used in the cancer radioimmunotherapy mainly for B-cell lymphoma (Lindén et al., 2005).

Recently, this radiolabelled ritumixmab was used in mice as well as in breast cancer patients for sentinel lymphoscintigraphic examination. In this study, five balb/c mice and ten breast cancer patients underwent dynamic sentinel lymph node (SNL) mapping procedure using ^{99m}Tc- rituximab. Results demonstrated that the ^{99m}Tc-labelled rituximab was found to be very reliable in showing the status of sentinel lymph nodes (SLN) both in mice and in breast cancer patients with a yield of 95% (Wang et al., 2006).

Anti-CD4 antibody

CD4 is a membrane glycoprotein expressed on T lineage cells, including the majority of thymocytes and a subset of peripheral T cells and monocytes. The extracellular domains of CD4 bind to the conserved regions of MHC II molecules on antigen-presenting cells (APCs). CD4$^+$T cells constitute the helper subset which regulate T and B cell function during T cell dependent responses. A number of anti-CD4 monoclonal antibodies including murine and primatized have been available for the management of different autoimmune diseases. These monoclonal antibodies bind with high affinity to human CD4, induce CD4 receptor down regulation, and are potent inhibitor of T cell response.

Several studies have been performed in patients with different autoimmune diseases using anti-CD4 monoclonal antibody which demonstrated its specificity for inflammation. A ^{99m}Tc-labelled CD4 specific antibody scintigraphic study was performed by Becker et al. (1990) in patients with active RA. In this study, the CD4 monoclonal antibody (MAX. 16H5) was labelled with ^{99m}Tc by a direct method using 2-mercaptoethanol. Each patient received a sub-therapeutic dose of ^{99m}Tc-labelled CD4 specific antibody and they were examined at 1.5 h, 4 h and 24 h post-injection. This study demonstrated that the ^{99m}Tc-labelled CD4 specific antibody can specifically detect the diseased joints in the patients with active RA.

In another study to detect the specific binding of anti-CD4 mAb to its target molecule performed by Kinne et al. (1993), a direct comparison between radiolabelled specific anti-CD4 mAb and non-specific human immunoglobulin for imaging inflamed joints in RA patients was performed. Patients with active or severe RA were intravenously injected with a sub-therapeutic dose of ^{99m}Tc- labelled murine anti-human CD4 mAb (MAX.16H5) or ^{99m}Tc- labelled HIG. Whole body and joint specific scintigraphic images were acquired at 1 h, 4 h and 24 h. In this study, the authors concluded that the ^{99m}Tc- anti-CD4 mAb allows more specific detection of inflammatory infiltrates which are rich in CD4-positive cells.

Anti-CD3 antibody (^{99m}Tc-muromonab-CD3)

Muromonab-CD3 (Orthoclone OKT®3) is a murine monoclonal antibody to the CD3 antigen of human T cells. The antibody is a biochemically purified IgG$_{2a}$ immunoglobulin. Muromonab-CD3 reacts with and blocks the function of CD3 in the membrane of human T cells that has been associated *in vitro* with the antigen recognition structure of T cells and is essential for signal transduction. Binding of muromonab-CD3 to T lymphocytes result in early activation of T cells, this leads to cytokine release, followed by blocking of T cell functions. *In vivo*, muromonab-CD3 reacts with most peripheral blood T cells and T cells in body tissues, but has not been found to react with other haematopoietic elements or other tissues of the body.

Recently, in a study performed by Martins et al. (2004), OKT3 was labelled with technetium-99m according to the technique developed by Martins and Gutfilen (2002). The use of ^{99m}Tc-OKT3 scintigraphy in diagnosis of acute rejection in renal transplants was subsequently evaluated. Among 22 patients that underwent renal transplantation, they reported an increased ^{99m}Tc-OKT3 kidney uptake

with time in 3 patients with rejecting allografts, differentiating them from those without this abnormality. Furthermore, these findings agreed with those of biopsies. In this study, the authors concluded that the ^{99m}Tc-OKT3 scans may be used as a diagnostic method to identify kidney allograft rejection, possibly allowing a balance between adequate immunosuppression to prevent rejection of an allograft and excessive immunosuppression. We have recently radiolabelled with 99mTc a different MoAb anti-human CD3, Visilizumab (Nuvion®) and demonstrated its capacity to bind in vitro and in vivo to human T-lymphocytes (unpublished data). Indeed, this antibody is very promising not only for in vivo imaging of lymphocytes but also to provide a rationale for therapy with Nuvion® and to early follow-up the efficacy of therapy.

Anti-DR antibody

The HLA-DR antigens play important roles in the cellular interaction involved in immune response. The HLA-DR is normally expressed on B lymphocytes, activated T lymphocytes, macrophages, monocytes, dendritic cells, activated NK cells and progenitor haemopoietic cells. During the resting state of T lymphocytes DR is not expressed and is therefore very specific for T cell activation. It is well known that this activation antigen is normally expressed on tissue infiltrating lymphocytes in inflamed tissues, in a high percentage of cells and for a longer time span compared to other activation markers, such as CD25 (IL2 receptor). It is therefore, a stable and reliable marker for detection of T cell mediated inflammation. Isobe et al. (1992), described that other tissues, such as vessel endothelium, may express DR following the release of local inflammatory molecules. Using ^{111}In-labelled anti-DR monoclonal antibody, Isobe (1993) found the expression of MHC class II antigens in a rat model of heart rejection and in mouse kidney allograft rejection. Indeed the scintigraphy revealed the presence of DR molecules on both the graft endothelium and the infiltrating mononuclear cells.

We have recently, radiolabelled labelled anti-DR monoclonal antibody (1D09C3) with technetium-99m. Using the 2-ME method, a high labelling efficiency of 90% and specific activity of 110.2 mCi/mg was obtained (unpublished data). 1D09C3 is a fully humanised IgG_4 monoclonal antibody and does not induce Fc-portion-mediated side effects, like chimeric antibodies do (Billing and Chatterjee, 1983; Jonker et al., 1988). Studies demonstrated that 1D09C3 has in vivo as well as in vitro tumoricidal activity and it can act selectively on tumor-transformed and activated cells via a non-apoptotic mechanism (Nagy et al., 2002). The [99m]Tc- labelled Anti-DR monoclonal antibody can provide a useful tool for imaging of inflammation and several cancer types, mainly leukaemia and lymphoma cells.

Furthermore, Rimsza et al. (2007) demonstrated that the HLA-DR can be used as a prognostic marker in the patients with diffuse large B-cell lymphoma (DLBCL). They demonstrated that the HLA-DR protein status predicts the survival in patients with DLBCL treated with the MACOP-B chemotherapy regimen. These findings have suggested the use of anti-DR monoclonal antibodies for diagnostic and therapeutic purposes.

Radiolabelled Cytokines

Cytokines are the soluble proteins produced by different types of activated cells (mainly T cells and macrophages). Cytokines play a prominent role in the regulation of cell function, homing, motility and metastasis through interaction with specific cell surface receptors expressed on a known cell population. Cytokine receptors, usually of high affinity, are normally present at low levels on non-activated cells, but expression is up regulated during the cell activation and therefore these receptors on the affected tissue are suitable targets for the detection of infection/inflammation.

Interleukin 2

Presently, Interleukin 2 (IL-2) is among the best approaches for the imaging of chronic inflammation, although it binds both to Th_1 and Th_2 cells. IL-2 is a single chain glycoprotein of 133 amino acids that is mainly produced by activated T cells. IL-2 interacts with a specific receptor expressed by activated T lymphocytes and plays a prominent role in regulating the immune mediated response through long term T cell proliferation and also promotes the proliferation of macrophages, B cells and NK cells (Smith et al., 1988). In inflammatory conditions, infiltrating cells in the inflamed tissue express the high affinity IL-2 receptors which are targeted by the radiolabelled IL-2. Initially, interleukin 2 was labelled with ^{35}S, ^{125}I and ^{131}I for in-vitro studies and in-vivo biodistribution in animal models

(Koths et al., 1985; Robb et al., 1985; Gennuso et al., 1989). After that IL-2 was radiolabelled with ^{123}I and ^{99m}Tc for the imaging of chronic inflammation, its application in the patients with different disorders including inflammatory bowel diseases, autoimmune thyroid diseases, insulin dependent diabetes and melanoma has been investigated.

In a study in the patients with active Crohn's disease, ^{123}I-IL2 was able to detect the presence of activated T lymphocytes in inflamed tissue. Ex-vivo autoradiographic examinations demonstrated the specific binding of ^{123}I-IL2 to activated lymphocytes in the inflamed gut mucosa (Signore et al., 1999a). In another study in the patients with coeliac disease, a positive correlation was observed between the uptake of ^{123}I-IL-2 measured by gamma camera and the number of histologically determined IL-2 receptor-positive cells in the inflamed jejunal mucosa (Signore et al., 1999b). Similarly, ^{99m}Tc-labelled IL-2 also showed specific accumulation in the inflamed tissue in several diseases such as insulin dependent diabetes (Signore et al., 1996) and melanoma (Barone et al., 1998). These studies suggest that the radiolabelled IL-2 could be used as a potential radiopharmaceutical for scintigraphic imaging of chronic inflammation.

^{67}GA-CITRATE

^{67}Ga-citrate as a radiopharmaceutical for imaging infection and inflammation was discovered in 1971 (Lavender et al., 1971). As an analogue of iron, ^{67}Ga-citrate binds in ionic form to circulating transferrin. Transferrin-bound ^{67}Ga uses transferrin receptors (CD71) to access the cell and then remains stably trapped within the cells (except RBCs) (Chianelli et al., 1997). During acute inflammation condition, ^{67}Ga-citrate is extravasated at the site of inflammation due to the locally enhanced vascular permeability. The extravasated radiopharmaceutical binds with high affinity to lactoferrin excreted by leukocytes or to siderophores, produced by microorganisms growing in a low-iron environment. Approximately, 25% of the total injected dose is excreted through urinary system and rest is retained in the bone, bone marrow, liver and soft tissues (Hoffer, 1980). However, this radiopharmaceutical has long physical half-life (78 hours) and high energy gamma radiations, which are unfavourable characteristics for γ-camera imaging and cause high radiation absorbed dose in the patients.

The use of this radiopharmaceutical is mainly restricted to chronic osteomyelitis, lung infection and fever of unknown origin (FUO), especially in immunocompromised patients.

^{99m}Tc-JOO1X

The non-pyrogenic acylated polygalactoside, J001X, is isolated from the membrane proteoglycans of a non-pathogenic strain of *Klebsiella pneumoniae*. JOO1X selectively binds to CD11b, the complement receptor 3 expressed on monocytes, NK cells and macrophages, as well as to CD14, the lipopolysaccharide receptor expressed on macrophages and monocytes (Chianelli et al., 2006; Hmama et al., 1992). It preferably binds to macrophages, mainly in their activated state (Bois de et al., 1995). As inflammatory foci are characterised by the recruitment of macrophages, they could in principle be imaged with ^{99m}Tc-J001X. Radiolabelled J001X has been used in several experimental animal studies including alveolitis and inflammatory lymph nodes in chronic berilliosis in baboons (Diot et al., 1992), inflammatory lesions in pigs (Perin et al., 1993) and rabbit arthritis model (Goupille et al., 1994; Miot-Noirault et al., 1996).

^{18}F-FDG (^{18}F- fluorodeoxyglucose)

^{18}F is a positron emitter with a physical half-life of 1 hour and 50 min. The FDG (2'-deoxy-2'-fluro-D-glucose) accumulation in activated lymphocytes, monocytes and granulocytes is based on the fact that these cells use glucose as an energy source only after activation during the metabolic burst. Transport of FDG across the cellular membrane is mediated by glucose transporter proteins. Intracellular FDG is subsequently phosphorylated to [^{18}F]FDG-6 phosphate by the hexokinase enzyme and phosphorylated radiopharmaceutical remains trapped inside the cell in contrast to phosphorylated glucose that enters in the glycolysis.

Several PET (positron emission tomography) studies have been performed using ^{18}F-FDG in patients with different inflammatory disorders such as vasculitis, Crohn's disease, sarcoidosis and rheumatoid arthritis, in addition to infection such as osteomyelitis, spondylodicitis and prosthetic joint infection. Recently, Beckers et al. (2006)

performed a study using ^{18}F-FDG PET on 16 patients with active RA. They found PET was positive in 69% of the knees, while magnetic resonance imaging (MRI) and ultrasonography (US) were positive in 69% and 75%, respectively. A study demonstrated that visual identification of RA knee synovitis by ^{18}F-FDG PET is related to its visual identification with MRI and US. The standardised uptake values also correlated with serum CRP (C-reactive protein) and MMP-3 (matrix metalloproteinase-3) levels.

Studies in the patients with spondylodiscitis proved FDG PET to be superior to MRI, ^{67}Ga-citrate scintigraphy and three phase bone scan for visualization (Gratz et al., 2002; Schmitz et al., 2001). Moreover, FDG PET is also able to differentiate between mild infection and degenerative changes (Stumpe et al., 2002). These studies conclude that FDG PET is highly sensitive tool to detect suspected osteomyelitis of the central skeleton, spondylodiscitis or chronic low-grade infections of the peripheral skeleton.

Although FDG PET is a useful tool for detection of inflammation and infection in many disorders, it has some limitations in others. FDG PET does not seem suitable for detection of other types of inflammation such as the insulitis in the pancreas of diabetic patients. Although ex-vivo studies in NOD mice demonstrated enhanced FDG uptake in islets of Langerhans that were affected by insulitis, the relatively small difference between diseased and healthy islets and the small size of the islets combined with the limited resolution of the PET camera will most likely prevent successful application in patients (Kalliokoski et al., 2005). Besides FDG, no validated PET tracers for imaging of peripheral inflammation are available and thus more sensitive tracers for PET imaging of insulitis are urgently awaited.

The brain is another organ, in which application of FDG PET for detection of inflammation or infection is limited. Since the brain uses glucose as its source of energy, basal FDG uptake in healthy brain is high and consequently increased FDG uptake as a result of inflammation can be hard to detect. Moreover, changes in FDG uptake are not specific for inflammation, but can also be induced by other physiological causes, like activation of a specific brain region by an external stimulus or a specific task (Grafton, 2000). In addition, the inflammation-induced increase in FDG uptake can be obscured by neurodegeneration, which leads to a reduction in glucose consumption. Consequently, neurodegenerative diseases that are associated with chronic inflammation, such as Alzheimer's disease and Parkinson's disease, show characteristic patterns of glucose hypometabolism on PET scans due to destruction of brain tissue (Herholz, 2003), whereas acute encephalitic disorders usually exhibit distinct areas of glucose hypermetabolism, sometimes accompanied with hypometabolism in atrophic brain regions (Lee et al., 2004).

Other PET radiopharmaceuticals for neuroinflammation

Because of the aforementioned limitations of detection of neuroinflammation by FDG PET and the limited brain penetration of large proteins like radiolabelled cytokines and antibodies, radiopharmaceuticals targeting specific processes in neuroinflammation, like activation of microglial cells, have been developed. Microglia is the macrophage equivalents of the central nervous system. Activation of microglia by an inflammatory stimulus is accompanied by an increase in density of their mitochondria and a strong upregulation of the expression of peripheral benzodiazepine receptors (PBR) on the outer mitochondrial membrane. Not only microglia cells, but also astrocytes show increased PBR expression in response to brain inflammation. Thus, the PBR is an attractive target for imaging of neuroinflammation.

[^{11}C]PK11195

Currently, [^{11}C]PK11195 is the best validated PET tracer for imaging PBR expression as a measure of inflammation-induced microglia activation. [^{11}C]PK11195 PET has been successfully applied in a variety of neurological disorders. In patients with viral encephalitis, like for example HSV encephalitis (Cagnin et al., 2001a), Rasmussen's encephalitis (Banati et al., 1999) and AIDS (Hammoud et al., 2005), [^{11}C]PK11195 PET could clearly identify brain areas with activated microglia. In fact, increased uptake was also detected in brain areas that appeared normal on MRI at the time of investigation, but which developed marked atrophy several months later. [^{11}C]PK11195 PET could also be applied successfully in dementia. In a PET study on patients with Alzheimer's disease, including patients with mild disease, increased [^{11}C]PK11195 binding was observed in affected brain regions (Cagnin et al., 2001b). Brain regions

with high [11C]PK11195 uptake exhibited signs of atrophy on follow-up MRI and FDG PET scans. [11C]PK11195 PET could also demonstrate microglia activation in other forms of dementia, like frontotemporal dementia (Cagnin et al., 2004). In drug-naive patients with early Parkinson's disease, [11C]PK11195 PET revealed enhanced microglia activation in midbrain, as compared to healthy controls (Ouchi et al., 2005). The uptake of [11C]PK11195 in midbrain positively correlated with the severity of motor deficits. An inverse correlation was found between midbrain [11C]PK11195 uptake and nerve terminal loss in striatum. In patients suffering from multiple sclerosis, enhanced [11C]PK11195 uptake seems to correlate with new lesions, whereas old lesions show little tracer uptake (Banati et al., 2000). Focal [11C]PK11195 binding in structures that appeared normal by MRI and asymmetric increased tracer uptake in thalamus and brain stem were also observed. T2-weighted MRI lesions with elevated [11C]PK11195 binding showed an increase in tracer uptake during relapse (Debruyne et al., 2003). In stroke patients, areas with enhanced [11C]PK11195 uptake overlap areas with abnormalities on MRI, but tend to be bigger. Several months after the stroke, the area of [11C]PK11195 binding has extended from the primary infarction site into connected areas in the same hemisphere and the contralateral thalamus, whereas atrophy has developed in the areas that originally exhibited the highest tracer uptake (Gerhard et al., 2005).

As follows from the above, [11C]PK11195 PET can reveal early inflammatory responses, before abnormalities are visible by MRI. Increased PK11195 binding in areas that appear normal on MRI, were shown to develop atrophy afterwards, suggesting that microglial activation precedes neurodegeneration and thus could be used as an early predictor of disease progression.

Novel tracer for the PBR
Despite the successful applications reported so far, [11C]PK11195 is not an ideal tracer. Signal to noise ratios can be rather low, because of the high lipophilicity, high plasma protein binding and relatively poor brain penetration of the PET tracer. Therefore, various novel PET tracers for the PBR with improved properties are currently being developed. Amongst these novel tracers [11C]DPA713, [11C]DAA1106 and [18F]FEDAA1106 appear to be most promising.

[11C]DPA713 is still in the preclinical phase of development. In rats with unilateral lesions induced by intra-striatal injection of AMPA, [11C]DPA713 displayed a higher lesion-to-background contrast than [11C]PK11195, which was mainly due to lower nonspecific binding of [11C]DPA713 in healthy brain (Boutin et al., 2007). However, further evaluation in humans is still required before the value of this tracer can be assessed. [11C]DAA1106 and its [18F]fluoroethyl derivative [18F]FEDAA1106 have a much higher affinity for the PBR than [11C]PK11195 and consequently exhibit 4-6 fold higher uptake in PBR-rich regions in monkey brain (Maeda et al., 2004; Zhang et al., 2004). Like [11C]DPA713, [11C]DAA1106 demonstrated increased tracer uptake in lesioned brain areas of rats that were unilaterally injected with kainic acid in the hippocampus. [11C]DAA1106, [18F]FEDAA1106 have already been evaluated in healthy human volunteers. For both tracers quantitative analysis of receptor binding is feasible, but long scan times are required, because of the low kinetics of tracer dissociation from the receptor (Fujimura et al., 2006; Ikoma et al., 2007). None of the novel tracers have been used in patients to measure neuroinflammation yet.

RESUMO

Nas últimas décadas, foram propostos vários radiofármacos para obtenção de imagens de sítios de inflamação, diferindo em suas especificidades e mecanismos de captação quando comparados aos tradicionais agentes utilizados para essa finalidade. Citocinas radiomarcadas representam uma ferramenta confiável para o diagnóstico pré-clinico precoce de processos inflamatórios crônicos, anterior às alterações anatômicas e funcionais, em tecidos afetados. Além disso, a introdução de anticorpos monoclonais radiomarcados e técnicas sofisticadas, como PET/CT, tornaram a obtenção de imagens de focos de inflamação altamente específica e apurada. Nesta revisão, diferentes abordagens com radiofármacos já bem estabelecidos e com outros em nível experimental para a obtenção de imagens de sítios de inflamação crônica são discutidas.

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