Design, (radio)synthesis and applications of radiolabelled matrix metalloproteinase inhibitors for PET
Matusiak, Nathalie

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
General discussion
In the present dissertation, the preparation and evaluation of four novel matrix metalloproteinase (MMP)/a disintegrin and metalloproteinase (ADAM) inhibitors radiolabelled with fluorine-18 (\(^{18}\text{F}\)FB-ML5, \(^{18}\text{F}\)-1A, \(^{18}\text{F}\)-2 and \(^{18}\text{F}\)-1B) are described. While most of the developed probes exhibited excellent in vitro results (IC\(_{50}\) ranged in the 10\(^{-9}\) to 10\(^{-7}\) M), they led to rather disappointing in vivo results. For PET, Marimastat-ArB\(^{18}\text{F}\)F\(_3\) [1] and \(^{18}\text{F}\)FB-ML5 (chapters 3 and 4) demonstrated specific signal accumulation in tumor mouse model/mouse model of pulmonary inflammation. However, overall, the signal-to-noise ratios were relatively low. The disappointing results may be caused by several reasons as listed below. These aspects should be taken into consideration in the design of new MMP/ADAM imaging probes for PET.

**Affinity**

As specific tracer retention is associated with the ratio of target density (B\(_{\text{max}}\)) and affinity of the radioligand for its target (K\(_{d}\)), the affinity of the PET probes may have been not adequate in consideration of the B\(_{\text{max}}\) for MMP/ADAM imaging. In addition, expression levels of active MMPs/ADAMs may have been too low for PET-labelled MMP/ADAM inhibitors with affinities in the 10\(^{-9}\) to 10\(^{-7}\) M range to be considered as suitable imaging agents.

**Specific radioactivity**

The specific radioactivities of \(^{18}\text{F}\)FB-ML5, \(^{18}\text{F}\)-1A, \(^{18}\text{F}\)-2 and \(^{18}\text{F}\)-1B may have been insufficient for MMP/ADAM imaging as endogenous tissue inhibitor of matrix metalloproteinases (TIMPs) bind to the same domain as MMPIs with very high affinity in the picomolar range, and in an irreversible manner [2]. Therefore, the competition of TIMPs with radioactive probes for MMP/ADAM imaging is most probably quite severe. After activation from pro-MMPs to MMPs, the fast majority of active MMPs is inhibited by TIMPs. As a result, the concentration of free active MMPs/ADAMs is very low.

**Lipophilicity**

In chapter 5, the effect of the lipophilicity was assessed and it did not result in a substantial difference in probe binding. As two thirds of MMPs are soluble, it is more logical to develop hydrophilic MMPIs. In addition, the radiolabelled probes with
very high log P values reported in chapter 2 resulted in very strong non-specific binding. Consequently, log P values higher than 2.5 should be avoided.

**Alternative to hydroxamic acid**

In an MMPI, the modification of the zinc binding group (ZBG) has more of an effect on binding properties of the inhibitor to its target than change of the substituents in the different pockets [3]. Therefore, further research should be performed to develop and synthesize novel MMPIs with alternative ZBGs. Despite the potency of the hydroxamic acid, it has some disadvantages such as challenging synthesis, metabolic instability and most importantly a too high potency for zinc binding. Indeed, the hydroxamate binds many zinc proteases and can also chelate metals other than zinc such as iron. Other ZBGs, which are more selective than hydroxamic acids, have been designed and developed such as hydantoins, 1,3,4-triazol-2-ones and imidazol-2-ones, however radiolabelled probes with such alternative ZBGs were not yet assessed *in vivo* for PET.

**Antibodies**

The use of radiolabelled monoclonal antibodies could also lead to improved results as they can lead to high selectivity and potency. In addition, radiolabelling of monoclonal antibodies is often straightforward. However, clearance is usually problematic except if low molecular weight fragments, like 15 kDA nanobodies, are used. Temma et al. [4] evaluated a $^{99m}$Tc-anti-MT1-MMP antibody in breast tumor-bearing rodents. They obtained promising results with about 38% of specificity by using a non-specific antibody as a negative control agent. Other groups developed a monoclonal antibody REGA-3G12 as a selective inhibitor of MMP-9 [5]. REGA-3G12 is directed against the catalytic domain but not against the fibronectin or zinc-binding domain. Interestingly, the use of functional blocking antibodies often enables inhibition of specific inhibition of specific functions of the MMP rather than its general proteolytic activity. REGA-3G12 was not radiolabelled for nuclear imaging purposes.

**Substrates**

The use of radiolabelled substrates (contrary to radiolabelled inhibitors) could lead to better results as substrates can be expected to show signal amplification. However, the determinants of substrate specificity of MMPs/ADAMs are not well
understood. Contrary to other proteases, such as caspases, most MMPs/ADAMs have no obvious or strict consensus amino acid recognition sequence, which hampers the production of substrate-based inhibitors.

**Activatable cell penetrating peptides (ACPP)**

Cell penetrating molecules consist of known peptide sequences which signal for the internalization of a molecule, or a positively charged sequence which will allow the agent to stick to the negatively cell membrane to be nonspecifically internalized. In the case of ACPP, the targeted MMP can bind and process the agent to remove an inhibiting moiety (antiCPP), allowing the agent to enter neighbouring cells. After entering the cells, the agent is stuck in the tissue of interest, resulting in a long lasting contrast. Watkins et al. [6] developed a radiolabelled MMP-14 specific ACPP tracer; but *in vitro* results, in MDA-MB-231 cells, showed that further work is required since only 27% of the activated probe entered the cells. Van Duijnhoven et al. [7] tested both a negative (scrambled, non ACPP) and a positive (pre-activated, CPP) control along their novel MMP-2/MMP-9 ACPP probe in a HT1080 fibrosarcoma mouse model 24 h p.i. [8]. A great absolute accumulation of their ACPP probe was obtained (ACPP on non-ACPP ratio > 1), however, this higher retention was present in all organs, including the muscle in which the activity of MMP-2 and MMP-9 was found to be null by zymography. The authors concluded that activation was present in the vasculature, and that tissue accumulation was unspecific. The similitude in the distribution of the ACPP and the CPP positive control supports this conclusion. Besides, van Duijnhoven et al. [8] tested their MMP-2/MMP-9 ACPP 3 h p.i. in mice with MMP-2/9 positive subcutaneous HT1080 tumors and in mice bearing subcutaneous BT-20 tumors with low MMP-2/9 expression. *Ex vivo* biodistribution showed no improved tumoral ACPP activation in HT1080 fibrosarcoma mice at 3 h p.i. compared to 24 h p.i. In addition, tumoral uptake and relative tumoral activation for ACPP were similar in both BT20 and HT1080 tumor bearing mice. The authors suggest that tumoral ACPP uptake in these tumor models originates from probe activation in the vasculature instead of tumor-specific activation. Finally, van Duijnhoven et al. [9] tested their MMP-2/MMP-9 ACPP and Alb-ACPP, which is an ACPP modified with an albumin binding ligand that prolongs blood clearance, in a Swiss mouse model of myocardial infarction. Both peptides probes showed a significantly higher uptake in infarcted myocardium compared to remote myocardium. The biodistribution for dual-isotope radiolabeled probes showed increased
retention of activated ACPP and activated Alb-ACPP in infarcted myocardium compared to remote myocardium. The enhanced retention correlated to gelatinase levels determined by gelatin zymography, whereas no correlation was found for the negative control (scrambled, non ACPP). In conclusion, radiolabelled MMP sensitive ACPP probes enabled to assess MMP activity in the course of remodelling after myocardial infarction in vivo.

**Optical imaging of MMPs/ADAMs**

Radiolabelled inhibitors or antibodies are used as 1:1 agents. As a result, they require a very large target concentration. The advantage of using activatable fluorescence probe is that they have the potential to be fully and directly quantitative, since the activation rate, a measurable quantity, can be related to the enzymatic activity of interest. However, even if near infrared fluorescence imaging has a limited spatial resolution, a low signal to background ratio and is restricted to animals and ex vivo studies [10], it can be worth to explore this imaging modality for the visualization/quantification of the proteolytic activity of MMPs and ADAMs.

**Applications of radiolabelled MMPIs**

Although MMPs/ADAMs are a difficult target for imaging, with many challenges as listed above, they remain an attractive target since suitable probes could be applied in the study of many diseases. Indeed, MMPs/ADAMs are involved in cardiology [11], oncology [12] and further other disorders such as sepsis [13], intestinal inflammation [14] and brain inflammation [15-18].

**General conclusion**

To conclude, this dissertation exhibits novel lead structures in order to further optimize suitable PET tracers for MMP/ADAM imaging. Additional studies using MMPIs with alternative ZBGs or the use of antibodies should be explored to obtain an appropriate radioligand to target the proteolytic activity of MMPs and ADAMs.
References