III. 1. Development of $^{18}$F-Labeled Matrix Metalloproteinase Inhibitors for Tumor Imaging by PET

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Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases involved in the extracellular matrix degradation. The mammalian MMP family is now known to include at least 20 enzymes and is categorized into several classes based on substrate specificity and domain structure. Among the sub-families of MMPs, gelatinases (MMP-2 and MMP-9) have become attractive targets for research on cancer and development of anticancer drugs. MMPs have been closely involved in the invasion, metastasis, and angiogenesis that are crucial for the progression of malignant tumors. Moreover, increased expressions and activities of gelatinases, especially MMP-2, have been observed in a variety of human cancer. Thus, estimating MMP activities in vivo is thought to contribute to diagnosis of the tumor invasiveness and to clinical evaluation of the efficacy of MMP inhibitors. For these reasons, we believe that MMP inhibitors labeled with a positron emitter should become a unique type of tracer that might be clinically beneficial for predicting cancer invasion and cancer therapy with anticancer drugs targeting MMPs through the use of positron emission tomography (PET).

Considerable effort has been devoted to the development of potent MMP inhibitors and numerous inhibitors are reported in the literature. Current focus in the field of MMP inhibitor development is directed towards the synthesis of selective inhibitors. For examples, Warner-Lambert, Shionogi, and Bayer have reported gelatinase selective inhibitors which contain a carboxylic acid group and a linear side chain (Fig. 1). Based on these structural features of the selective inhibitors, we began our research to develop a novel PET tracer targeting MMP-2. The design and radiosynthesis of the new MMP inhibitors selective for gelatinases are reported here.
Results and Discussion

Design strategy for $^{18}$F-labeled MMP-2 inhibitor

Compounds A-C were used as references for the design of $^{18}$F-labeled MMP inhibitors. The essential structural features of the reference inhibitors are the carboxylic acid group (zinc binding group; ZBG) and the linear side chain ($R_1$). Thus, carboxylic acid-based $^{18}$F-labeled MMP-2 inhibitors with a linear side chain was designed (Fig. 2).

Precursor synthesis

The precursors (5a-c) were synthesized from the commercially available D-form of methionine, tryptophan, and valine, respectively, as shown in Scheme 1. Protection of the carboxyl group was carried out in methanol under reflux with $p$-toluenesulfonic acid and $p$-toluenesulfonyl chloride. Then, the obtained methyl esters (2a-c) were coupled with 4-iodobenzenesulfonyl chlorides. Utilizing the reaction conditions developed by Sonogashira, the iodophenylsulfonamides (3a-c) were coupled with 5-hexyn-1-ol to yield the desired alkynyl phenylsulfonamides (4a-c) in good yield. Conversion of the hydroxyl group to the tosylate completed the precursor 5a-c synthesis.

Radiosynthesis of $[^{18}F]$MMP inhibitors

The gelatinase inhibitors labeled with fluorine-18 (1a-c) were easily prepared via a one-pot synthesis outlined in Scheme 2. The one-pot synthesis, radiofluorination (12 min) and deprotection (6-12 min), was done in the same vial with heating at 110°C using an oil bath. The initial fluorination of the precursor was performed by nucleophilic displacement with $[^{18}F]$fluoride in the presence of potassium carbonate and Kryptofix 2.2.2. After fluorination, basic hydrolysis of the methyl ester group of 6a-c was carried out by adding 2N NaOH to the reaction solution. Under these conditions the total radiosynthesis times were about 60-70 min including the preparative HPLC separation. The average of the overall radiosynthesis yields of 1a-c were 43%, 13%, and 33%, respectively (n=5-7, decay corrected).

After purification of 1a-c their radiochemical purities were evaluated by analytical reverse phase HPLC. The purities of 1b and 1c stored in preparative HPLC mobile phase remained 99% or more for at least 2 hours. On analysis of 1a, however, a radiochemical impurity which increased gradually with time was obseve, suggesting chemical decomposition of 1a. The chemical instability of 1a would make it unsuitable as a PET tracer even though it was synthesized in the best radiochemical yield among the three types.
of tracer.

In conclusion, we have designed and synthesized a new type of PET tracer for cancer imaging, carboxylic acid-based MMP inhibitors labeled with fluorine-18. Fluorine-18 labeled compounds (1a-c) were synthesized from the precursors by a simple one-pot preparation and purified by HPLC. 1a was prepared in the best total radiochemical yield, but turned out to be unstable chemically and thus unsuitable for use to biological studies. Among three compounds, 1c is undergoing biological evaluation as a prospective candidate for cancer tumor imaging agent by PET.

References

Fig. 1. Selective MMP inhibitors especially for gelatinases. ZBG: Zinc binding group.

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<tr>
<th>Inhibitors</th>
<th>IC_{50} (μM)</th>
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<tr>
<td>A</td>
<td>0.005</td>
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<tr>
<td>B</td>
<td>0.034</td>
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<tr>
<td>C</td>
<td>0.042</td>
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Fig. 2. New radiopharmaceuticals designed for gelatinase inhibition.
Scheme 1. Precursor synthesis of the newly designed MMP inhibitors from the D-form of various amino acids. (i) TosCl, TosOH, MeOH, reflux; (ii) 4-Iodobenzenesulfonyl chloride, NMM, CHCl₃; (iii) 5-hexyne-1-ol, Pd(PPh₃)₂Cl₂, CuI, TEA, DMF; (iv) TosCl, NMM, CHCl₃.

Scheme 2. Radiosynthesis of 1a-c by a one-pot procedure. (i) [¹⁸F]KF, K₂CO₃, Kryptofix 2.2.2, CH₃CN, reflux; (ii) aq. NaOH, then aq. HCl.