Automated radiosynthesis of 1-(2-[18F]fluoroethyl)-tryptophan, a potential substrate for indoleamine 2,3-dioxygenase PET imaging

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Introduction

Indoleamine 2,3-dioxygenase (IDO) is an enzyme catalyzing the initial and rate-limiting step in the catabolism of tryptophan along the kynurenine pathway (Figures 1 & 2). This enzyme is located in brain and has been detected in high concentration in human tumor cells. Furthermore this enzyme could be responsible for the suppression of immune responses by blocking locally T-lymphocyte proliferation.

Figure 1 | The two first steps in the catabolism of L-tryptophan along the kynurenine pathway

Therefore, a radiotracer based on tryptophan structure seems to be well adapted to bring out the presence of h-IDO, and thus, of tumors.

Figure 2 | Structure of h-IDO

Results

The automated radiosynthesis of 18FETrp 5 needs the prior synthesis of a precursor owing a tosyl moiety which allows the easy introduction of the radioactive [18F]fluoride (t1/2 = 109.7min) by a classical method with potassium carbonate and kryptofix8,9

1. Precursor Synthesis

The tosylate precursor 3 was synthesized in three steps starting from L-tryptophan 1 (Scheme 1).

Scheme 1 | Synthesis of 1-(2-tosloyloxyethyl)-tryptophan precursor (3) (precursor)

This amino acid was firstly protected with two acidic leaving groups, with a global yield of 38% for two steps. Then the intermediate 2 was alkylated with ethylenediamine diisotoluoyl, to afford the tosylate precursor 3 (yield: 49%).

2. Automated Radiosynthesis on FASTlab™

To optimize radioprotection, the whole radiosynthesis of 1-(2-[18F]fluoroethyl)-tryptophan was carried out on a GE Healthcare FASTlab™ automated system (Figure 2).

Figure 2 | GE Healthcare FASTlab™ synthesizer

The tosylate precursor 3 was labeled8,9,10 (Scheme 2) under different conditions (Table 1). The best radiolabeling yield (RCY) (57%) was obtained when the labeling was carried out in DMF, for 5 minutes at 140°C (n = 3).

Scheme 2 | Radiochemical synthesis of 1-(2-[18F]fluoroethyl)-tryptophan (18FETrp)

Table 1 | Influence of solvent and temperature on the radiochemical decay-corrected yield (RCY) of 3

Solvent | Temperature | Time | RCY
--- | --- | --- | ---
ACN | 90°C | 10 min | 28%
ACN | 120°C | 3 min | 25%
ACN | 140°C | 5 min | 57%
DMF | 90°C | 10 min | 28%
DMF | 120°C | 3 min | 25%
DMF | 140°C | 5 min | 57%

The intermediate 4 was purified on a C18 solid phase extraction cartridge (Sep-Pak®) and hydrolyzed at 90°C for 10 minutes, to give the 18FETrp 5 (Scheme 2).

Finally the labeled compound 5 was purified on semi-preparative HPLC and formulated.

Figure 3 | HPLC chromatogram of purified and formulated [18F]ETrp (in red) and cold reference [18F]ETrp (in blue)

The fully automated process takes around 40 minutes and the 18FETrp 5 was obtained, after purification on semi-preparative HPLC, with global radiochemical decay-corrected yield of 30% (n = 5). The radiochemical purity was >98%

3. Enzymatic Tests

In vitro enzymatic tests with recombinant h-IDONd) were carried out with cold reference 18FETrp, in presence of methylene blue [100µM], ascorbic acid [200mM] and sodium phosphate buffer [50mM, pH 6.5] at 37°C. Figure 4 shows the decrease of the fluorescence signal of 18FETrp, studied by HPLC, according to the time of incubation. This decrease is due to the opening of the indole ring of the substrate. For a concentration of substrates smaller than Kcat the kcat/Km – values (Table 2) were determined from curves (Figure 4). Furthermore, 18FETrp is not a substrate of recombinant h-IDONd), an enzyme expressed in liver (Table 2). Thus 18FETrp is a specific substrate of h-IDO.

Figure 4 | HPLC chromatogram superposition showing the decrease of the fluorescence signal of 18FETrp in function of incubation time

Table 2 | In vitro enzymatic tests realized with [18F]SF and some other substrates known for h-IDO and h-TDO (37°C, pH 6.5)

<table>
<thead>
<tr>
<th>Tested Substrates</th>
<th>Percentage of substrate consumed after incubation (t=1h)</th>
</tr>
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<tbody>
<tr>
<td>h-IDO</td>
<td>100%</td>
</tr>
<tr>
<td>1µM [18F]ETrp</td>
<td>90%</td>
</tr>
<tr>
<td>10µM [18F]ETrp</td>
<td>70%</td>
</tr>
<tr>
<td>100µM [18F]ETrp</td>
<td>50%</td>
</tr>
<tr>
<td>5-ido-trp</td>
<td>0%</td>
</tr>
<tr>
<td>S-HD-trp</td>
<td>0%</td>
</tr>
<tr>
<td>[18F]SF</td>
<td>0%</td>
</tr>
</tbody>
</table>

Conclusions

Herein, an automated synthesis of 1-(2-[18F]fluoroethyl)-tryptophan, with good radiochemical yields, has been developed. In vitro studies with cold reference 18FETrp show that [18F]SF is a good and specific substrate of h-IDO. Moreover, some studies with this new radiochemical compound, still under progress, could confirm that 18FETrp ([18F]SF) is a molecule of choice to bring out the presence of h-IDO, an enzyme which is located in brain and tumor cells.

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References