Development of bispecific PSMA/GRPr targeting radioligands with optimized pharmacokinetics for PET imaging of prostate cancer

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A novel class of bispecific PSMA/GRPR targeting radioligands with optimized pharmacokinetics for improved PET imaging of prostate cancer

Graphical Abstract

PSMA pharmacophore Glu-ureido-Lys

HEₙ: Pharmacokinetic spacer His (H), Glu (E), (n = 0-3)

HBED-CC chelator for $^{68}$Ga

GRPr Pharmacophore BN analogue $\text{H}_2\text{N-PEG}_2$-$[\text{D-Tyr}^6, \beta\text{-Ala}^{11}, \text{Thi}^{13}, \text{Nle}^{14}]\text{BN}(6–14)$

$^{68}$Ga-labelled compounds % ID/g 30 min p.i.

PC-3 tumors expressing GRPr

LNCap tumors expressing PSMA

1st International Electronic Conference on Medicinal Chemistry
2-27 November 2015
Abstract: A series of novel low-molecular weight bispecific radioligands were developed, which were able to target the prostate-specific membrane antigen (PSMA) and the gastrin releasing peptide receptor (GRPr), both expressed on prostate cancer cells. These bispecific radiotracers combined the peptidomimetic urea-based pseudo-irreversible inhibitor of PSMA: Glu-ureido-Lys with the bombesin (BN) analogue: H\textsubscript{2}N-PEG\textsubscript{2}-[D-Tyr\textsuperscript{6}, β-Ala\textsuperscript{11}, Thi\textsuperscript{13}, Nle\textsuperscript{14}]BN(6–14), which binds to GRPr with high affinity and specificity. The two pharmacophores were linked together through the chelating agent HBED-CC and spacers made of positively charged His (H) and negatively charged Glu (E): -(HE)\textsubscript{n}-, (n=0-3) amino acids. The positron emitter \textsuperscript{68}Ga (t\textsubscript{1/2} = 68 min, β\textsuperscript{+} 88 %, E\textsubscript{β\textsuperscript{+}} max. 1.9 MeV) was used for the radiolabelling of the bispecific radioligands and preliminary pharmacological data were collected from \textit{in vitro} assays on prostate cancer cell lines (PC-3, AR42J, LNCaP) and \textit{in vivo} experiments in normal and tumor bearing mice (biodistribution and small animal PET imaging studies). The new bispecific ligands \textit{in vitro} showed binding affinities, which essentially matched the ones of the respective monomers, while \textit{in vivo} they were able to target both PSMA (LNCaP) and GRPr (PC-3) positive tumors. In addition the charged -(HE)\textsubscript{n}-, (n=1-3), linkers improved the tracer’s pharmacokinetics by significantly reducing the normal organ uptake (i.e. kidney and spleen) and by increasing the tumor to-background ratio. In conclusion, the bispecific (PSMA and GRPr) targeting ligands, developed in this study could be considered as novel radiotracer candidates for more sensitive PET/CT-imaging of prostate cancer (PCa) in future clinical application.

Keywords: \textsuperscript{68}Ga, PET-prostate cancer diagnosis, PSMA/ GRPr bispecific radioligands, low-molecular weight heterodimer
Introduction

- **Prostate-specific membrane antigen (PSMA)**
  Membrane-bound protein overexpressed in 95-100% of human prostate cancer (PCa) cases.
  
  *Frequently PSMA (+) cases contain large areas with PSMA (-) cells!*

- **Gastrin releasing peptide receptors (GRPRs)**
  Membrane-bound protein overexpressed in 84-100% PCa cases, including small cell lung and pancreatic cancers¹,²

² Rybalov et al. *Int.J.Mol.Sc.* 2014
Aims of this study

- **Synthesis** of multimeric ligands with binding affinity for both receptors GRPr/PSMA

**HE spacers:** incorporation of PK modification spacer

- **Comparison** with monomers
- **PK Improvement** -> high tumor/normal tissue contrast ratios without losing affinity and specificity.
- **Insight** for the design of new Radioligands in the future.
- **Selection** of the optimal tracer.

**PSMA pharmacophore** Glu-ureido-Lys

$\text{HE}_n$: Pharmacokinetic spacer His (H), Glu (E), (n = 0-3)

HBED-CC chelator for $^{68}$Ga

GRPr Pharmacophore BN analogue

$\text{H}_2\text{N-PEG}_2\text{-}[\text{D-Tyr}^6, \beta\text{-Ala}^{11}, \text{Thi}^{13}, \text{Nle}^{14}]\text{BN}(6-14)$
Results and discussion

Chemical structures

Glu-ureido-Lys

Pharmacokinetic spacer His (H), Glu (E)

HBED-CC chelator

BN analogue

$H_2N$-$\text{PEG}_2$-$[\text{D-Tyr}^6, \beta\text{-Ala}^{11}, \text{Thi}^{13}, \text{Nle}^{14}]BN(6–14)$

$\text{HE}_0$

$\text{HE}_1 \ n=1$

$\text{HE}_2 \ n=2$

$\text{HE}_3 \ n=3$
Results and discussion

Table 1. High-resolution mass spectrometry data of the free ligands [M+H]⁺.

<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z calculated [M+H]⁺</th>
<th>m/z experimental [M+H]⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRPrₙᵐ</td>
<td>1800.0</td>
<td>1800.8</td>
</tr>
<tr>
<td>PSMA-11</td>
<td>947.4</td>
<td>947.4</td>
</tr>
<tr>
<td>HE₀</td>
<td>2101.3</td>
<td>2100.5</td>
</tr>
<tr>
<td>HE₁</td>
<td>2547.8</td>
<td>2547.3</td>
</tr>
<tr>
<td>HE₂</td>
<td>2814.1</td>
<td>2814.0</td>
</tr>
<tr>
<td>HE₃</td>
<td>3080.3</td>
<td>3080.3</td>
</tr>
</tbody>
</table>

68Ga-Radiolabeling
Comparative RP-HPLC analysis studies of the ligands HEₙ, n=0-3, after labelling with 68Ga (gamma-trace).
Results and discussion - *in vitro*

**Competition binding assay for GRP on PC-3 cells (10^6), AR42J (10^6) and PSMA on LNCaP cells (10^6).**

<table>
<thead>
<tr>
<th>compound</th>
<th>IC_{50} (nM) ± Std.Er</th>
<th>ANOVA vs monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRPr_m</td>
<td>3.65 ± 1.11</td>
<td>-</td>
</tr>
<tr>
<td>HE_0</td>
<td>7.72 ± 1.20</td>
<td>NS*</td>
</tr>
<tr>
<td>HE_1</td>
<td>7.28 ± 1.17</td>
<td>NS</td>
</tr>
<tr>
<td>HE_2</td>
<td>4.40 ± 1.29</td>
<td>NS</td>
</tr>
<tr>
<td>HE_3</td>
<td>7.09 ± 1.23</td>
<td>NS</td>
</tr>
<tr>
<td>AR42J</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRPr_m</td>
<td>1.29 ± 1.23</td>
<td>-</td>
</tr>
<tr>
<td>HE_0</td>
<td>3.33 ± 1.17</td>
<td>**</td>
</tr>
<tr>
<td>HE_1</td>
<td>2.58 ± 1.15</td>
<td>*</td>
</tr>
<tr>
<td>HE_2</td>
<td>5.06 ± 1.20</td>
<td>****</td>
</tr>
<tr>
<td>HE_3</td>
<td>3.68 ± 1.17</td>
<td>***</td>
</tr>
<tr>
<td>LNCaP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSMA-11</td>
<td>7.5 ± 1.29</td>
<td>-</td>
</tr>
<tr>
<td>HE_0</td>
<td>25.4 ± 1.09</td>
<td>**</td>
</tr>
<tr>
<td>HE_1</td>
<td>17.4 ± 1.07</td>
<td>*</td>
</tr>
<tr>
<td>HE_2</td>
<td>25.2 ± 1.23</td>
<td>**</td>
</tr>
<tr>
<td>HE_3</td>
<td>42.4 ± 1.09</td>
<td>****</td>
</tr>
</tbody>
</table>

\[\text{NS: not statistically significant difference. Significant differences against the monomers GRPr}_m \text{ and PSMA-11 in each assay are presented with stars (P<0.05).}\]
Results and discussion - *in vitro*

**Total cell related radioactivity** over time for $^{68}$Ga-labelled versions of monomers PSMA-11 and GRPr$_m$ and heterodimers HE$_n$, n=0-3 (30 nM) on LNCaP and PC-3 cells.

(Non-specific binding was determined by adding a blocking solution of 2-PMPA or native BN, x 1000-fold concentration as compared with the respective radioligand, 30 μM).
Results and discussion – *in vivo*

Biodistribution studies (1 h p.i.) between $^{68}$Ga-HE_0 and $^{68}$Ga-PSMA-11 and $^{68}$Ga-GRPr_m in mice.

Results are expressed as percentage of the injected dose per g (% ID/g) for each organ or tissue. Blocking experiments: co-injecting native BN (1 μL of a 100 mM solution) or 2-PMPA (15 μL of a 100 mM solution) along with the radiolabelled ligand.
Results are expressed as % ID/g (mean ± SD, n=3-4). Significant differences are presented with stars above the bars that were compared (P<0.05).
Results and discussion – *in vivo*

Biodistribution studies mice between the $^{68}$Ga-HE$_0$ and $^{68}$Ga-HE$_2$

Results are expressed as % ID/g (mean ± SD, n=3-4). Significant differences are presented with stars above the bars that were compared (P<0.05).
Results and discussion – *in vivo*

Biodistribution studies in mice between the $^{68}$Ga-HE$_0$ and $^{68}$Ga-HE$_2$

Results are expressed as % ID/g (mean ± SD, n=3-4). Significant differences are presented with stars above the bars that were compared (P<0.05).
**Results and discussion – in vivo**

Tumor uptake determined from biodistribution studies (30, 60 min p.i.) in balb/c nu/nu mice bearing: (a) LNCaP and (b) PC-3 tumors, after i.v. administration of the $^{68}$Ga-PSMA-11, $^{68}$Ga-GRPr$_m$ and heterodimers HE$_n$ (n=0-3).

Significant differences are presented with stars above the bars that were compared (P<0.05). The values are expressed as % ID/g (mean ± SD, n=3-4).
Results and discussion – *in vivo*

Whole-body μPET (axial, coronal, saggital, from top to bottom) images of male nu/nu mice bearing LNCaP tumor xenografts, for $^{68}$Ga-HE$_0$

Where: T = Tumor; K= kidneys, B = bladder as indicated with arrows.
Results and discussion – *in vivo*

Whole-body μPET (axial, coronal, sagittal, from top to bottom) images of male nu/nu mice bearing LNCaP tumor xenografts, for $^{68}$Ga-HE$_2$

Where: T = Tumor; K = kidneys, B = bladder as indicated with arrows.
Results and discussion – *in vivo*

Representative time-activity curves taken from the dynamic PET measurements (0-60 min p.i.) expressed as $\text{SUV}_{\text{mean}}$ (standardized uptake values) for $^{68}\text{Ga}$-labelled HE$_0$ (top) and HE$_2$ (bottom). The SUV time-activity curves for the organs of interest are represented with the following letters, M = muscle, T = tumor, B = bladder, K = kidneys, L = liver.
Conclusion

• A series of novel bispecific radioligands (\(^{68}\text{Ga-HE}_n\), n=0-3) were synthesized for the first time and evaluated for PSMA and GRPr targeting properties in vitro and in vivo.

• Both in vitro and in vivo studies showed that all low-molecular weight heterodimers under study (\(^{68}\text{Ga-HE}_n\), n = 0-3) could efficiently target PSMA and GRPr on LNCaP and PC-3 prostate cancer cells and tumor xenografts.

• This dual-targeting heterodimer approach can improve the sensitivity of prostate cancer detection due to the synergistic increase of binding interactions for the chosen biological targets, i.e. PSMA and GRPr.

• In addition, their biodistribution profiles were optimized by incorporation of charged linkers (\(^{68}\text{Ga-HE}_n\), n=1-3), which resulted in a significant reduction of normal organ uptake (i.e. kidneys, spleen), while tumor uptake remained at the same levels or was increased in comparison with the monomers (\(^{68}\text{Ga-PSMA-11}\), \(^{68}\text{Ga-GRPr}_m\)).

• These novel low-molecular weight heterodimers could potentially be applied in clinical practice as bispecific radiotracers for the noninvasive imaging of all stages of prostate cancer by means of PET/CT and PET/MRI.
Special thanks to:

- Martin Schäfer
- Ulrike Bauder-Wüst
- Dr. Matthias Eder
- Prof. Dr. rer. nat. Klaus Kopka