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PB183, a sigma receptor ligand, as a potential PET probe for the imaging of prostate adenocarcinoma

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Abstract—PB183, a non-selective sigma receptor ligand displaying high sigma-1 and sigma-2 receptor affinity, was evaluated in prostate tumour cell lines for its suitability as PET radiotracer. The pharmacodynamic and pharmacokinetic properties suggested **PB183** as a potential PET radiotracer to visualize prostate adenocarcinoma.

Sigma receptors, classified into sigma-1 and sigma-2 subtypes, are a distinct class of receptors.[1] Sigma-1 subtype has been cloned[2] and characterized as a mammalian homologue of the yeast sterol isomerase[3] though devoid of enzymatic activity. Sigma-2 receptor has not been cloned yet, but recently an attempt to characterize it led to hypothesize sigma-2 subtype as a class of histones receptor family.[4]

These receptors, present in many normal tissues,[5] are overexpressed in several human and rodent tumour cell lines,[6] so that they are considered potential biomarkers[7] for the evaluation of the proliferative status of tumour. Tumour grade and stage could be monitored by non invasive imaging techniques such as PET and SPECT.[8–12] In the last years, we have been developing several cyclohexylpiperazine derivatives as potent sigma-2 receptor ligands, and among them **PB28** (Fig. 1) displayed a potent agonist activity[13] and a moderate selectivity towards sigma-1 subtype (sigma-1/sigma-2 ratio = 40).[14]

Starting from these results, **PB28** was radiolabelled as [^{11}C]**PB28** by Kassiou et al.[8] and its biodistribution was evaluated in mice brain in absence and in presence of unlabelled **PB28**. [^{11}C]**PB28** showed a good uptake, but when its specific binding was determined in the presence of unlabelled **PB28** or DTG or haloperidol, high non-specific binding was found. Moreover, we studied another sigma-2 ligand, **PB167** (Fig. 1), suggesting it as a probe for PET studies in the same implanted tumour animal model.[15]

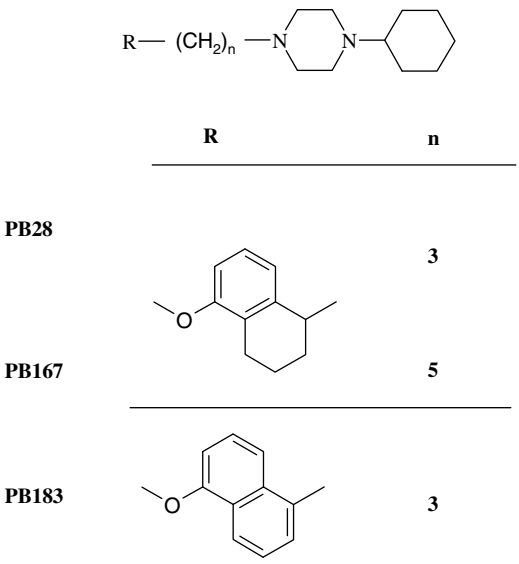

Figure 1. Cyclohexylpiperazine derivatives structures.

Although [^{11}C]**PB167** displayed a good uptake in EMT-6 implanted cells, unfortunately its uptake in CNS was remarkable. On the other hand, PB167 in CNS

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displayed high sigma-2 non-specific binding (unpublished result). The latter evidence could be due to the high lipophilicity of **PB167** (cLog$P$ = 6.24) whilst the CNS uptake could be linked to **PB167** undetected binding to P-glycoprotein (P-gp) sites. Furthermore, **PB167** uptake in CNS was an unfavourable result because **PB167** has been designed to target mainly peripheral sigma-2 receptors.

Investigating the causes for **PB28** and **PB167** failures as imaging probes, we found that these compounds inhibited P-gp, the critical efflux pump present in the Brain Blood Barrier (BBB) and for this reason they are able to cross the BBB, leading to the remarkable uptake in the CNS.

Considering such unfavourable kinetic properties of **PB28** and **PB167**, we chose **PB183** (Fig. 1), another cyclohexylpiperazine derivative **PB28**-like. **PB183** was found unable to cross BBB being a substrate of P-gp. In fact, we studied **PB183** permeability in Caco-2 cells model obtaining P_{app} (6.3×10^{-6} cm s^{-1}) which was lower than the P_{app} of verapamil, a reference P-gp modulating agent (12×10^{-6} cm s^{-1}).[16] Moreover, although compound **PB183** and verapamil displayed similar cLog$P$ (4.77 and 4.47, respectively) both were inhibited to cross the BBB by P-gp activity.[17,18]

This finding led to consider P_{app} value as a parameter for crossing the BBB, while cLog$P$ resulted as an important parameter influencing the specific binding.

The aim of this work was to find a proper molecule as PET probe for prostate cancer. Indeed it was reported that prostate cancer is the second leading cause of death in men. Several probes are under investigation for the diagnosis of this tumour by PET, but they displayed strengths and limitations.[19]

TRAnseginc Mouse Prostate (TRAMP) cell line is a well-studied murine model of prostate cancer with histopathology and disease progression that mimics the human disease. TRAMP cells exhibit similarities with human prostate cancer, including epithelial origin, progression from the PIN (prostatic intraepithelial neoplasias) stage to adenocarcinoma, and metastasis by a transgene that is hormonally regulated by androgens.[20]

Since several studies indicated sigma receptors as potential biomarkers in several solid tumours,[5–7] we initially recognized the presence of them in this cell line.

The recognition of both sigma receptors in TRAMP cells was carried out by saturation analysis[21] with radioligand (Fig. 2). We found high sigma-2 and sigma-1 receptor density (610 and 500 fmol/mg of protein). Moreover, K_d values of each radioligand (11.49 ± 4.84 nM for [^{3}H]DTG and 9.30 ± 0.25 nM for (+)-[^{3}H]pentazocine) were consistent with literature data in standard protocols.[22]

Starting from these findings, we evaluated **PB183** receptor affinity towards both sigma receptors in TRAMP

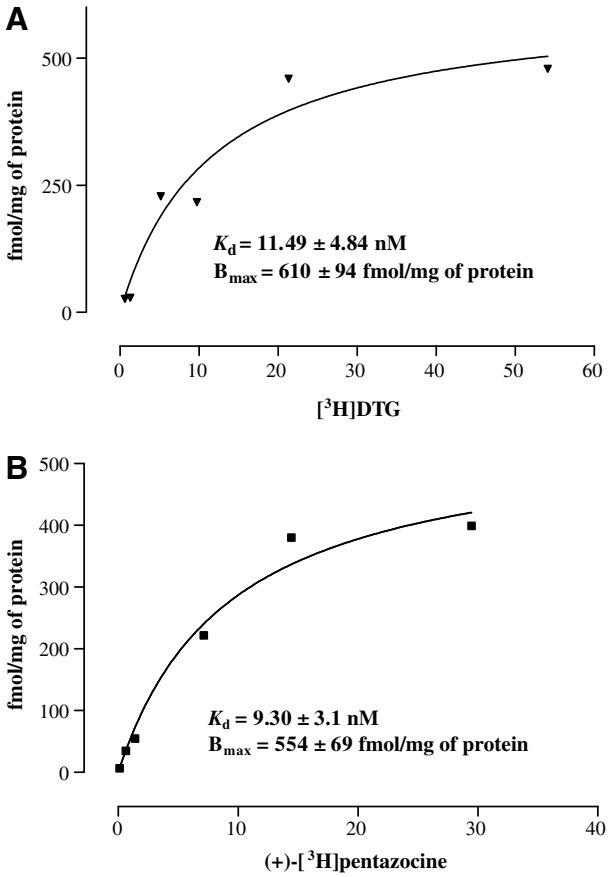

Figure 2. Saturation binding experiments with radioligands to recognize sigma-2 (A) and sigma-1 receptors (B) in TRAMP cells membranes.

cell membranes. As expected, **PB183** displayed high sigma-2 and sigma-1 receptor affinities (K_i = 0.50 ± 0.01 nM and 6.5 ± 0.5 nM, respectively).

Since sigma-2 and sigma-1 receptors agonists and antagonists, respectively, induced cytotoxic and anti-proliferative effects in several tumour cell lines,[13,23,24] we evaluated these effects in TRAMP cells. As expected, **PB183** displayed low cytotoxic (EC_{50} = 26.6 ± 0.50 μM) and anti-proliferative effects (EC_{50} = 16.9 ± 2.5 μM).[25]

On the other hand, it was very important to verify the expression level of P-gp in TRAMP cells. High levels of P-gp could inhibit **PB183** binding at the sigma receptors localized in these tumour cells. As reported in Figure 3, P-gp was moderately expressed in TRAMP cells so that the radiotracer could permeate the cell membrane to bind sigma receptors.

Since sigma receptors are potential biomarkers for monitoring the solid tumour growth, sigma ligands are suitable probes for in vivo PET technique. In the present work the presence of sigma receptors in TRAMP cells and pharmacodynamic and pharmacokinetic profiles of **PB183** were investigated. High sigma-1 and sigma-2 receptor density found in our studies elicited two important aspects: (i) these receptors are overexpressed in

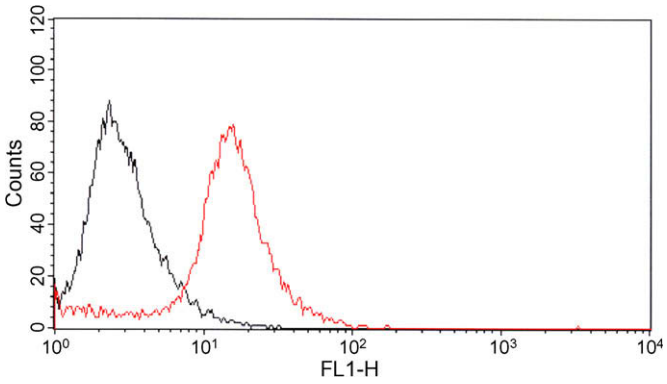

Figure 3. Analysis of P-gp expression in TRAMP cells by flow cytometry. The black histogram and the red one indicate the control and the staining with anti-P-gp antibody, respectively.

several solid tumours such as prostate carcinoma; (ii) considering that sigma-2 are ubiquitously present, their overexpression is a biological pre-requisite for PET imaging investigation. The high sigma receptors affinity of **PB183** and its favourable pharmacokinetic properties (it is transported by P-gp) suggest it as a potential PET radioligand to target peripheral solid tumours. In addition, **PB183**, bearing a methoxy group on the naphthyl moiety, an easy point to dealkylate and to obtain the corresponding [^{11}C]**PB183**, could be used as potential radiotracer in clinical PET employment.

Clinically, in PET analysis a 90-min scan was adapted for [^{11}C]probe due to the half-life of a ^{11}C-labelled tracer ($t_{1/2}$ = 20 min). The uptake of [^{11}C]probe gradually increased over 90 min or increased for 60 min and was maintained.[26,27] Conversely, a longer scan may be more preferable for PET measurement. Therefore, [^{18}F] analogues ($t_{1/2}$ = 110 min) may be more preferable for PET if they have similar pharmacokinetic properties to [^{11}C]probes.[28]

In the last years several papers reported the evaluation of [^{11}C] and [^{18}F] probes targeting sigma receptors for tumour diagnosis by PET imaging.[29–32] In some cases the failure of PET analysis is due to the pharmacodynamic and or pharmacokinetic radiotracers limitations. This paper suggests a potential strategy to limit PET analysis failures due to inadequate pharmacodynamic and pharmacokinetic properties of radiotracers. The pre-evaluation of these parameters should be a preliminary step before PET experiments.

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17. *Drug transport experiments*: Apical to basolateral permeability of drugs was measured under various conditions of incubation time (30, 60, 120 min) and drug concentrations (10–100 μM). Drugs were dissolved in Hanks' balanced

salt solution (HBSS, pH 7.4) and sterile filtered. After 21 days cell growth, the medium was removed from filter wells and from the receiver plate. The filter wells were filled with 75 μl of fresh HBSS buffer and the receiver plate with 250 μl per well of the same buffer. This procedure was repeated twice and the plates were incubated at 37 °C for 30 min. After the incubation time, the HBSS buffer was removed and drug solutions were added to the filter well (75 μl). HBSS without the drug was added to the receiver plate (250 μl). The plates were incubated at 37 °C for the desired time. After incubation time, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer and the drug concentrations were determined by HPLC analytical method.

18. *HPLC analysis*: Samples from in vitro permeation studies were analysed by using a reverse-phase HPLC equipped with a Perkin-Elmer series 200 LC pump and a Perkin-Elmer 785A UV/VIS detector. UV signals were monitored and obtained peaks integrated using a personal computer running Perkin-Elmer Turbochrom Software. The column used was a Phenomenex Prodigy ODS-3 RP-18 (250 × 4.6 mm, 5 μm particle size). The samples were eluted with ammonium formate (5 mM; pH adjusted to 5 with formic acid) and acetonitrile (95:5 v/v) at a flow rate of 1 ml/min. The wavelength for UV absorbance was set at 230 nm. The sample injection volume was 20 μl.
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21. *Saturation binding assays*: the saturation experiments were carried out as described by Vilner et al. (Ref. 6) with minor modifications. Sigma receptor membranes in TRAMP cells were radiolabelled using [^{3}H]DTG concentrations ranging from 1.0 to 150 nM. Samples containing 400 μg of membrane protein, radioligand and 1 μM (+)-pentazocine to mask sigma-1 receptors, were equilibrated in a final volume of 500 μl of 50 mM Tris–HCl, pH 8.0, for 120 min at 25 °C. The non-specific binding was determined in the presence of 10 μM DTG. Incubations were stopped by addition of 5 ml of ice-cold buffer (50 mM Tris–HCl, pH 7.4), and then the suspension was filtered through GF/C filters pre-soaked in polyethylenimine 0.5% for at least 30 min prior to use. The filters were washed twice with 5 ml of ice-cold buffer. Sigma-1 receptors were radiolabelled using (+)-[^{3}H]pentazocine concentrations ranging from 0.1 to 50 nM. Samples contained 400 μg of membrane protein and radioligand. The non-specific binding was determined in the presence of 10 μM (+)-pentazocine. Samples were incubated in a final volume of 500 μl of 50 mM Tris–HCl, pH 8.0, for 120 min at 25 °C and the following manipulations were as described above for sigma-2 receptors.
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