Improved tumour targeting of $[^{111}\text{In}/^{177}\text{Lu}]$SG5 in phosphoramidon-treated mice

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Aims and objectives

Radiolabeled gastrin and cholecystokinin (CCK) analogs have been proposed for application in the diagnostic imaging and radionuclide therapy of CCK2R-positive human tumors, like medullary thyroid cancer [1-5]. Previous studies have shown that $[^{111}\text{In-DOTA}]$MG11 ([(DOTA)DGlu$^{10}$]gastrin I(10-17)) attractive for its low renal accumulation poorly localized in CCK2R-expressing tumors due to fast in vivo degradation [6]. We have recently shown that co-injection of the neutral endopeptidase (NEP) inhibitor phosphoramidon (PA) and the radioligand markedly increased the bioavailability and tumor uptake of $[^{111}\text{In-DOTA}]$MG11 [7].

In the present study we monitor the effect of PA co-injection on the biological profile of $[^{111}\text{In}/^{177}\text{Lu}]$SG5, a [DOTA]MG11 analog whereby the oxidation-susceptible Met$^{15}$ has been replaced by Leu$^{15}$ to prevent undesired sulfoxide formation during labeling with beta emitters, such as $^{177}$Lu. We were particularly interested to evaluate the applicability of this novel approach in both tumor diagnosis and therapy.
Methods and materials

The CCK2R binding affinity of SG5 was determined by displacement studies against $^{[125]}$I-Tyr$^{12}$,Leu$^{15}$Gastrin I in A431-CCK2R(+) cell membranes at 22°C for 1 h, with Leu$^{15}$Gastrin I as reference.

Labeling with $^{111}$In or $^{177}$Lu was conducted by addition of $^{111}$InCl$_3$ or $^{177}$LuCl$_3$ in SG5 solution at pH 4.7 and heating for 40 or 20 min, respectively at 90°C. The resulting $^{[111]}$In/$^{177}$LuSG5 were obtained in >95% yield and >97% radiochemical purity, as verified by HPLC analysis.

The internalization of $^{[111]}$In/$^{177}$LuSG5 was studied by incubation at 37°C for 1 h in confluent monolayers of A431-CCK2R(+) cells; incubation in the presence of 1 µM DG2 [(N$_4$)Gly$_4$,DGlu$_5$]gastrin I(4-17) represented non-specific internalization.

To assess in vivo stability, mice were injected with 100 µL of radioligand (~1 mCi $^{111}$In or 3 mCi $^{177}$Lu, 3 nmol peptide) together with vehicle (100 µL; control) or PA (100 µL of vehicle containing 300 µg PA; PA treated) via the tail vein. Blood was collected 5 min later and analyzed by HPLC.

Cell suspensions (1-2 x 10$^7$ cells) of A431-CCK2R(+) and A431-CCK2R(-) cells were subcutaneously administered in each flank of SCID mice and 6-8 days later tumors were grown at the inoculation sites. Animals received via the tail vein a $^{[111]}$In/$^{177}$LuSG5 bolus (100 µL, up to 2 µCi $^{111}$In / up to 10 µCi $^{177}$Lu, 10 pmol total peptide in vehicle) together with vehicle (100 µL; control group) or PA (100 µL of vehicle containing 300 µg PA; PA treated group) and were sacrificed at 4 and 24 h post injection (pi). For $^{[177]}$LuSG5 two additional groups of 48 h and 72 h time intervals were also included. Animals were sacrificed and tissues of interest were excised, weighed and counted in a #-counter; values were calculated as percent injected dose per gram (%ID/g) tissue and are expressed as mean ± SD.
Results

SG5 exhibited higher binding affinity for the human CCK2R (IC$_{50}$= 0.32 nM) as compared with [Leu$^{15}$]gastrin I (IC$_{50}$= 0.86 nM) (Fig. 1).

As shown in Fig. 2, [$^{111}$In]SG5 and [$^{177}$Lu]SG5 efficiently internalized in CCK2R-expressing cells after 1 h incubation at 37$^\circ$C. In the presence of 1 µM DG2 internalization dropped below 0.4%, consistent with a CCK2R-mediated process.

PA-treatment exerted an impressive effect on in vivo stability (Fig. 3). Specifically, while 27% of [$^{111}$In]SG5 and 34% of [$^{177}$Lu]SG5 were detected intact in mouse blood at 5 min pi, these percentages rose to 85% and >90%, respectively, by PA co-injection.

The radioligand stabilization by PA translated into a significant amplification of tumor uptake in CCK2R-expressing tumors in mice. Thus, by PA co-injection tumor values increased from 3.7%ID/g to 12.8%ID/g for [$^{111}$In]SG5 (Fig. 4) and from 3.9%ID/g to 11.9%ID/g for [$^{177}$Lu]SG5 at 4 h pi while the PA effect remained evident up to 72 h pi (Fig. 5). In contrast, renal values as well as uptake in the A431-CCK2R(-) xenografts remained low and unaffected by PA.
Fig. 1: Comparison of CCK2R binding affinities of SG5 and [Leu15]gastrin I against [125I-Tyr12,Leu15]gastrin I in A431-CCK2R(+) cell membranes.

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Fig. 2: Comparative internalization as percent intracellular versus total added for [111In]SG5 (purple) and [177Lu]SG5 (red) after 1 h incubation at 37°C in A431-CCK2R(+) cells; non-specific values acquired in the presence of 1 µM DG2 are also included.

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Fig. 3: HPLC radiochromatograms of mouse blood collected 5 min after injection of [111In]SG5 (A), [111In]SG5 co-administered with PA (B), [177Lu]SG5 (C) and [177Lu]SG5 co-administered with PA (D) in mice.

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Fig. 4: Biodistribution data (%ID/g±SD) for [111In]SG5 without or with co-injection of PA 4 h and 24 h pi in SCID mice bearing CCK2R-positive xenografts (Tu+) and naïve xenografts not expressing CCK2R (Tu-); Bl= blood, Li= liver, He= heart, Ki= kidneys, St=...
stomach, In= intestines, Sp= spleen, Mu= muscle, Lu= lungs, Pa= pancreas, Fe = femur, Tu+=A431-CCK2R(+) tumor, Tu-=A431-CCK2R(-) tumor.

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Fig. 5: Tissue distribution data of [177Lu]SG5 at 4 h, 24 h, 48 h and 72 h pi with vehicle (A) or with PA co-injection (B) in SCID mice bearing CCK2R-positive xenografts (Tu+) and naïve xenografts not expressing CCK2R (Tu-); Bl= blood, Li= liver, He= heart, Ki= kidneys, St= stomach, In= intestines, Sp= spleen, Mu= muscle, Lu= lungs, Pa= pancreas, Fe = femur, Tu+=A431-CCK2R(+) tumor, Tu-=A431-CCK2R(-) tumor.

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Conclusion

Replacement of Met$^{15}$ by Leu$^{15}$ in [DOTA]MG11 in SG5 allowed for convenient labeling with $^{111}$In and $^{177}$Lu. PA treatment significantly improved the stability of circulating $[^{111}\text{In}/^{177}\text{Lu}]$SG5 in mice. Moreover, the radioligand uptake in CCK2R-expressing tumors impressively increased whereas renal values remained low. The beneficial PA-effect lasted as long as 72 h pi highlighting the potential of this new approach for tumor therapy.
References