Tumor imaging using a high relaxivity gadolinium chelate targeted to the folate receptor

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Aim : Because of its overexpression in many types of human tumors and its relative absence in most normal tissues, the folate receptor (FR) constitutes a promising target for tumor-specific contrast agents. Compounds combining folic acid to either monomeric or polymeric gadolinium chelates or to iron oxide nanoparticles have already been studied. While the proof of principle has been demonstrated, the performance of such an approach for clinical application has not been confirmed yet since its efficacy depends on the in vivo affinity of the compound for FR, its accessibility to the tumor and the sensitivity of the contrast moiety.In this study, folic acid has been coupled to a high relaxivity gadolinium chelate macromolecule in order to reach a high MR sensitivity. The in vitro affinity, the biodistribution and magnetic resonance imaging (MRI) performance were evaluated in a FR-positive tumor.

Materials and methods :

- Compounds : P866 (MW=9 kDa, r1=50 $s^{-1}.mM^{-1}$ at 20 MHz) is composed of a folic acid moiety (pharmacophor) coupled to a high relaxivity gadolinium chelate (constrastophor). The reference compound P999 is a non-targeted analog of P866 (identical contrastophor but without folic acid moiety).
- In vitro : FR-positive KB Cells (ATCC,CCL-17), were grown using folate-free RPMI medium containing 10% heat-inactivated fetal calf serum at 37°C . 10^7 KB cells were incubated for 24 hours with 5 µM of P866 in the absence or presence of folic acid 750 µM (as a competitor). After 3 washes in RPMI, cells were harvested, counted and mineralized. Gadolinium concentrations (ng Gd/10^7 cells) were measured by ICP-MS.
- Biodistribution : Nude mice (Harlan) were maintained on a folate-free diet one week before subcutaneous inoculation of 10^7 KB cells. Between day 6 and day 13 (tumor diameter : 3 to 8 mm) 15, 50 or 100 µmol./kg of P866 were injected intravenously in the presence or absence of 100 µmol./kg of folic acid (as a competitor). Four hours after injection, mice were sacrificed (3 mice/group), tumors and skeleton muscles were removed, weighed and frozen until mineralization for Gd quantification (ICP-MS). Results were expressed as tumor / muscle ratio (T/M).
- MRI : Imaging experiments were performed on a Bruker system (2.35T) using a micro-imaging gradient and a birdcage RF coil with a built-in gas mask for delivery of isoflurane/O_2 mixture. Dynamic T1w sequence (TR/TE/FA=29.3ms/3.5ms/45°, matrix=128×128×16, FOV=3×3cm^2, slice thickness: 3mm, acquisition time = 5 min/16 slices) were acquired 20 minutes before and during 4 hours after injection of 1.5, 5 or 15 µmol./kg of either P866 or P999. Signal intensities (SI) were measured on the rim, the center and the whole tumor as well as in the muscle at each time point.

Results and discussion:

- In vitro : 24 hours after incubation of P866 in FR-positive KB cells, 30.3 ± 2.3 ng Gd/10^7 cells were measured in the cell lysate corresponding to approximatively 6.10^6 molecules/cell. This accumulation is specific of the FR since it decreases to 1.2 ± 0.1 ng Gd/10^7 cells of P866 in the presence of an excess of folic acid. Furthermore, the concentration of Gd-DOTA, a non specific gadolinium chelate tested as a control, was 2.5 ± 0.4 ng Gd/10^7 cells.

- Biodistribution: A higher concentration of P866 was observed in the tumor over the muscle ratio for each of the 3 doses. In the presence of free folic acid, a marked decrease in the tumoral accumulation was observed at 15 µmol./kg and at 50 µmol./kg. For the highest dose of 100 µmol./kg, the failure of the competition with folic acid might be attributed to the saturation of the FR by the excess of free P866 (fig1).

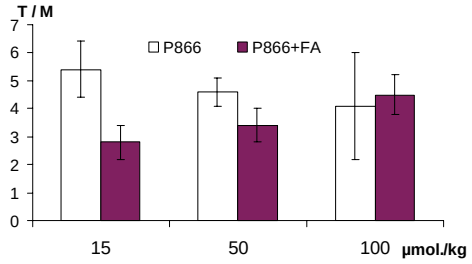
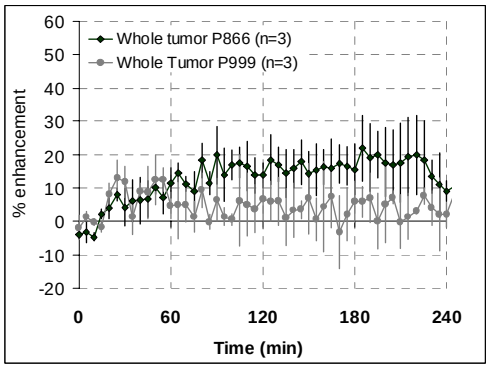
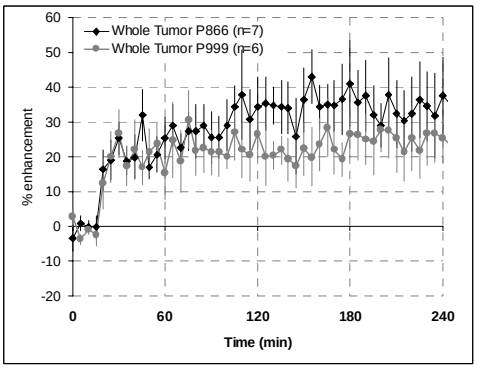


Figure 1 : (Gd) tumor over muscle ratio after injection of 15, 50 or 100 µmol./kg of P866 in the absence (left barr) or presence (right barr) of folic acid 4 hours after injection

- MRI :

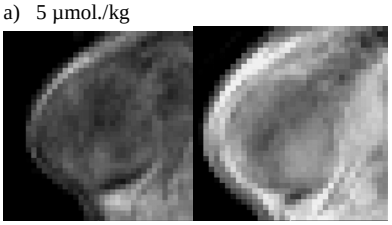


a) 5 µmol./kg



b) 15 µmol./kg

Figure 2 : a) and b) dynamic signal enhancement in the total tumor during 4 hours after injection of P866 and P999 at 5 and 15 µmol./kg, c) MRI of P866 at 15 µmol./kg 4 hours after injection



c) pre-injection 4 hours

- At 1.5 µmol/kg, the tumoral signal enhancement was too low to generate reliable quantitative SI without imaging analysis. At 5 and 15 µmol./kg, a progressive accumulation of P866 and P999 was observed in the tumor with a plateau phase starting 60 - 90 minutes post injection.

- The maximum enhancements were 20% and 40% at 5 and 15 µmol./kg in the tumor respectively. By increasing the dose, the difference between the folate compound P866 (ΔSI = 37±11%) and its analog P999 (ΔSI = 25±7%) was reduced (fig 2).

Conclusion : Specific in vitro and in vivo accumulation of the high relaxivity gadolinium chelate-coupled to the Folate moiety were demonstrated in an FR-positive tumor model. At least, a dose of 5 µmol./kg is needed to obtain sufficient signal enhancement. By increasing the dose, the biodistribution as well as the MRI experiment demonstrated that the free form of the compound hides the specific signal, probably due to a saturation of the FR.