Syntheses and preliminary application of ^{68}Ga Schiff base derivatives for *in vivo* imaging of the p-Glycoprotein status in tumours

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Objectives

Cells of the human body contain very effective transport mechanisms to transport xenobiotics actively out of the cell. Thereby the intracellular concentration of drugs is lowered and a multidrug resistant phenotype results. The most important of these ABC-transporters is p-glycoprotein (pGP), which transports neutral and cationic structures under ATP-consumption. In many tumours pGP is over expressed leading to a very low concentration of several chemotherapeutics. In order to identify multidrug resistant tumours in advance, a PET-tracer would be helpful which should fulfil two features: (1) it should enter the cell easily (by passive diffusion) and (2) the tracer should be a substrate of the pGP and this transport should be inhibitable by pGP-inhibitors such as e.g. verapamil. Using the ^{68}Ge/^{68}Ga generator, novel ^{68}Ga-based Schiff base ligands provide interesting molecules accomplishing both requirements. Furthermore such radioactive tracers are applicable for cell studies as well as µ-PET imaging.

Methods

Based on a published ligand by Sharma et al. [1] six derivatives including the reference compound were synthesized in high yields, cf. Fig. 1. Labelling of these ligands was performed by usage of the ^{68}Ge/^{68}Ga generator which provides the positron emitter Gallium-68 (T½ = 68 min) in 400 µL of an acetone/HCl mixture [2]. ^{68}Ga labelling is performed in 400 µL 0.12 M Na-HEPES buffer by adding the ^{68}Ga fraction.

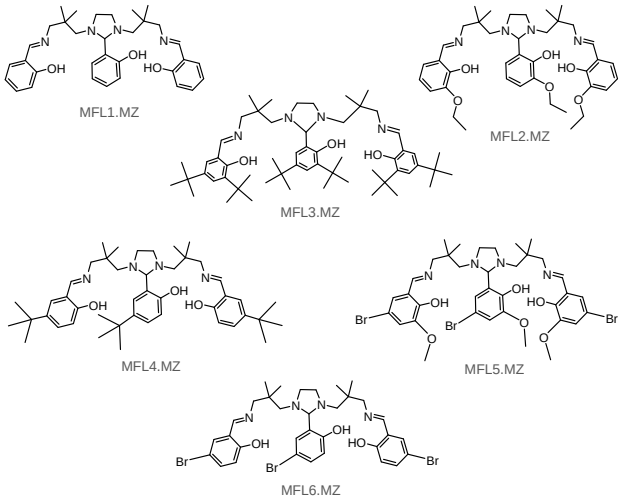

Fig. 1: ligands synthesized and investigated in this study

Through variation of reaction time, temperature and different amounts of the complex ligands, optimum reaction parameters for complex formation were tested. Analyses of radiochemical yield are carried out by TLC on silica and RP-18 phase. Cell studies on rat prostate carcinoma cells in presence or absence of verapamil where performed for all ligands to prove that the ligands are transported by pGP. The most interesting ligand MFL6.MZ was used for first in vivo and ex vivo studies concerning uptake in solid growing rat tumours and compared to the published ligand.

Results

Labelling proceeds at temperatures between 25 and 75°C within 2 to 10 min in a total volume of 800 µL. Ligands are used in nanomole amounts only and the radiochemical yields are 50 to 95%. Cell essays showed that beside one ligand, which probably was trapped in the cell membrane, all others were transported by pGP. However, the uptake into the cell by non-ionic diffusion varied broadly between the ligands. The ligand MFL6.MZ was the most promising compound. Under normal conditions, 25% of the activity was taken up into the cell. When inhibiting the pGP-mediated efflux, this amount increased to 35%. Compared to the literature ligand (4.6 % respectively 8.2 % not inhibited and inhibited, respectively) this ^{68}Ga complex was selected for in vivo studies on a µ-PET.
Imaging ^{68}Ga-MFL6.MZ revealed a 3-fold higher accumulation in tumours compared to the reference tissue (testicles), whereas the literature compound [1] is only slightly enriched in the tumour.

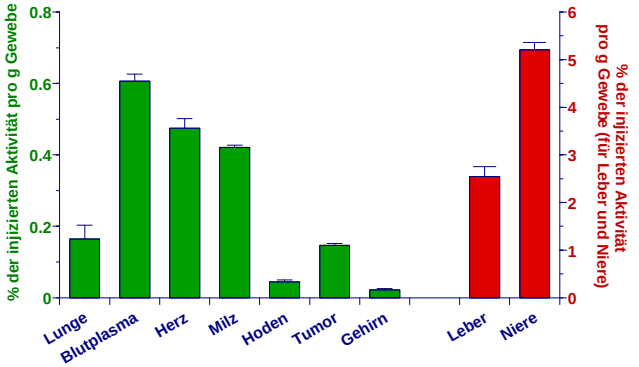

Fig. 2: biodistribution of ^{68}Ga-MFL6.MZ

Conclusions

Six Schiff base ligands were synthesized and labelled with ^{68}Ga in fast and high yielding complex formation. Tumour cell studies showed uptake in cells and transport processes of the complexes by pGP for six ligands. ^{68}Ga-MFL6.MZ was chosen for µ-PET imaging on tumour bearing rats demonstrating a high uptake in tumour compared to the literature compound. Further studies will involve blocking pGP in vivo and raising transport activity of pGP. With ^{68}Ga-MFL6.MZ it appears to be possible to identify patients with multidrug-resistant tumours pre-therapeutically in order to select adequate treatment regimes.

References

[1] Sharma et al, J Nucl Med 46: 354-364
[2] Zhernosekov et al, J Nucl Med 48: 1741-1748