Receptor Binding studies of ^{68}Ga-labelled Peptides on GIST tumour cells

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Introduction: Gastrointestinal stromal tumour (GIST) is a rare disease and frequently affects young patients, which often results in a short life expectancy of less than 3 years once diagnosed. The MITIGATE project aims to develop new approaches to diagnose and treat patients with metastatic GIST resistant to the Tyrosin Kinase Inhibitor (TK) treatment. Within this project we investigated binding of various ^{68}Ga-labelled peptides, targeting receptors reported to be overexpressed in GIST, in different cell lines.

Methods: 3 GIST cell lines were established: T1, 882 (Imatinib sensitive) and 430 (Imatinib resistant). Different DOTA-derivatized peptides were included: DOTA-NT 8-13 (targeting NTR1), DOTA-TATE (targeting SSTR2), CP04 (Targeting CCK2), VIP-DOTA (targeting VPAC1/2) and 2 DOTA-Bombesin derivatives (targeting GRP1). The peptide conjugates were radiolabelled with ^{68}Ga under standardized conditions and characterized by HPLC. ^{68}Ga-peptides were incubated with the respective tumour cell lines in 6-well plates including control by blocking with 1µM excess peptide and membrane bound activity (by Glycine buffer acid wash). The internalized activity was measured after the different incubation times. Additional controls included binding assays in receptor expressing control cells (AR42J, PC-3, HT-29)

Results: Very low or no specific binding to GIST tumour cells was found for all ^{68}Ga-DOTA labelled peptides except, for Bombesin derivatives indicating no or very low expression of respective receptors. For ^{68}Ga-labelled Bombesin a pronounced specific binding to all GIST cell lines was found, for the agonist (DOTA-AMBA) a high percentage of internalized activity, whereas for an antagonist specific binding was mainly associated with membrane bound activity (with up to >80% bound/mg protein). Binding of DOTA-Bombesin was in the following order T1<430~882.

Conclusion: Our results in the tumour cells indicate that radiolabelled Bombesin analogs may be a good candidate for targeting TK-resistant GIST for molecular imaging but also for potential radionuclide therapy approaches. The difference in binding found between TK resistant and TK sensitive tumour still cells needs to be verified. Characterisation of the tumour cells by FACS is currently ongoing and in vivo studies in GIST bearing tumour models are planned.

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