

Short Thesis for the Degree of Doctor of Philosophy (PhD)

**Synthesis, Investigation of Gallium-68 Labelled
Radiocomplexes and Their Application in Small Animal-PET
Imaging**

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Debrecen, 2017

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1 Introduction

Positron Emission Tomography (PET) is one of the most important elements of the inventory of nuclear medicine, its significance in several fields of medicine; especially in oncological diagnostics is undeniable.

Nowadays, the vast majority of PET examinations is performed with only one pharmacon, the ^{18}F -Fluoro-deoxy-glucose (^{18}F -FDG). ^{18}F -FDG enters cells with the same mechanism as glucose during its distribution in the body, then it is phosphorylated intracellularly by a hexokinase to FDG-6-phosphate. Afterwards, if the glucose-6-phosphatase enzyme concentration of the corresponding tissue is low (brain, myocardium, most malignant cells), ^{18}F -FDG is accumulated in proportionate to the carbohydrate metabolism of the tissue, it enters no further enzymatic interaction, in those tissues, where glucose-6-phosphatase enzyme content is detectable, the amount of accumulation is decreased.

Based on the mechanism of distribution and metabolism, it can be observed that ^{18}F -FDG is a metabolic tracer, which shows significant accumulation in several tissues under physiological circumstances and in several pathologic processes (tumours, inflammation), which in certain cases might lead to significant background activity and to low specificity, false positive and false negative results to the process desired to be detected.

With the occurrence of the need towards individualised medicine, the widespread of PET and the expansion of knowledge of ^{18}F -FDG-PET (and its limits), those research projects are catalysed,

which aim at the development of targeted PET-radiopharmaceuticals, which are more specific than the ^{18}F -FDG. This process is further enhanced in nuclear medicine by the need for combination of molecular diagnostics and targeted radiotherapy – theragnostic treatments reaching the same molecular target, but labelled with different isotopes.

Neuroendocrine tumours might be mentioned as an example, which are typically slow-growing tumours with low ^{18}F -FDG accumulation, however due to their somatostatin-receptor expression, their diagnostics (^{111}In -DTPA-Octreotide-SPECT, ^{68}Ga -DOTATOC/TATE-PET) and their therapy (^{90}Y -trium-DOTATOC/DOTATATE, and ^{177}Lu -tium-DOTATOC/DOTATATE/DOTANOC) are both performed with radiolabelled somatostatin-analogues, as it can be seen, with different radiometals.

Radionuclide gallium-68 – as positron-source radiometal-isotope, which is also the labelling isotope of ^{68}Ga -DOTATOC/TATE with the mentioned clinical relevance – possesses increasing interest in PET-related research. Ga-68 with its half-life of 67.7 min is excellently suitable for *in vivo* imaging of peptides, proteins with quick distribution, thus the synthesis of specific, targeted radiopharmaceuticals. Besides, for the development of appropriate ^{68}Ga -labelled radiopharmaceuticals, the synthesis and characterisation of novel ^{68}Ga -chelators and the mapping of biological vectors suitable for ^{68}Ga -PET became one of the most important tasks of the related, applied radiochemistry.

2 Aims

Ga-68 became the most important radiometal-isotope of PET imaging technique in recent decades. Its beneficial physical properties (89% β^+ ; $t_{1/2} = 67,7$ min; $E_{\text{average}}(\beta^+) = 740$ keV) and its cheap accessibility – compared to costs of cyclotron installation for the application of other, classic positron-source isotopes (^{18}F , ^{11}C) – through $^{68}\text{Ge}/^{68}\text{Ga}$ -generators makes it suitable for the introduction of novel type PET-radiopharmaceuticals synthesized at the diagnostic centre – even in a kit-based form in the future. However, careful planning, appropriately optimized synthesis and well-established preclinical testing of the ^{68}Ga -labelled radiopharmaceuticals are necessary beforehand. We intended to contribute to these processes with our research work, thus we aimed at the further examination of two important areas:

- Testing of chelator molecules suitable for ^{68}Ga -labelling, optimization of the conditions of labelling reaction for performing efficient radiosyntheses.
- Synthesis of ^{68}Ga -labelled chelator-biological vector conjugates for preclinical testing.

With these objectives in mind, experimental work was performed in the frames of three projects:

Project 1: Examination of chelators used for Ga-68 labelling with traditional, manual labelling methodology, comparative radioanalytical examination of NOTA, NOPA, NO2AP and NOPO systems.

Project 2: Besides the manual labelling methodology applied for the investigation of Ga-68 labelling, the development of alternative, microfluidic labelling methodology.

Project 3: Application of Ga-68 labelling for imaging in biological systems, for which cyclic NGR peptide as biological vector and NOTA chelator as bifunctional chelator were chosen and its suitability for PET-imaging was studied in comparison with radiopharmakon ^{68}Ga -NODAGA-[c(RGD)₂]. The expression of macromolecule Aminopeptidase N targeted by NGR peptide and the integrin targeted by RGD-peptide is known in the process of angiogenesis taking place in the environment of tumours.

3 Materials and methods

3.1 Comparative examination of ^{68}Ga -labelling properties of TACN-based chelators with differing substitution of phosphinic and carboxylic groups

Our first series of experiments were performed according to manual labelling protocols in order to reveal direct structure-labelling efficiency relationship by 1,4,7-triazacyclononane based chelators. This class of compounds were chosen as they possess more advantageous stability properties than open-chain Ga-chelators, and based on latest results from our cooperation partners the N-substitution of 3 methylene-phosphinic group to the ring enhances the affinity of the molecule towards the formation of chelate with ^{68}Ga (so called TRAP-type chelator - *Triazacyclonane Phosphinic Acid*). For further studying this effect, we started the comparative investigation of NOTA chelator known from literature with three methylene-carboxylic substituents, “mixed” chelators with differing substitution of side chains containing carboxylic and phosphinic groups (NOPA, NO2AP) and NOPO chelator possessing three phosphinic groups.

Our experiments were started with the elution of $^{68}\text{Ge}/^{68}\text{Ga}$ -generátor (iThemba) with 1M HCl-solution. The 1.25 ml fraction of the eluate with the highest activity concentration was buffered with 0.8 ml 2.7M HEPES-solution to pH 3.0. From this solution, 90 μl fractions were pipetted to the previously prepared 10 μl ligand-solutions. The reaction mixtures were kept for 5 min on 25 and 95 $^{\circ}\text{C}$, then sample was

taken and the radiochemical purity was examined by thin-layer chromatography; stationary phase: silica-impregnated paper (Varian Inc.), mobile phase 1 M NH₄OAc:MeOH/1:1. The papers were read and analysed by TLC-scanner (MiniGITA Star, Raytest).

3.2 *Microfluidic optimization of complex-formation reactions of ⁶⁸Ga-chelators*

During our further experiments, we started the development of a labelling methodology, which makes the characterisation of ⁶⁸Ga-chelators possible in a quicker and more efficient manner compared to the known manual methods. We aimed at the setting up and testing of such system suitable for microfluidic measurements, which can perform and analyse complex-formation reactions with ⁶⁸Ga in very small solution-volumes and without adding carrier; therefore the system would be able to optimise labelling conditions of ⁶⁸Ga-chelators.

The new system was compared to manual labelling of NOTA and NOPO chelators known from literature, quick multiparametric reaction-optimization became possible, which was also done with NOTA and NOPO chelators.

Microfluidic labelling was done by the co-injection of two solutions of identical volumes; from one of the lines a HEPES-buffered ⁶⁸Ge/⁶⁸Ga-generator (iThemba) eluate collected with 1M aq. HCl-solution, from the other line also HEPES-buffered chelator solution were injected. The pH of the latter solution was set based on previous titration with HCl and NaOH-solutions so that the signed pH-values of

each measurement were ensured after co-injection of identical volumes with the HEPES-buffered eluate.

While optimization experiments, 20-20 μl solutions were reacted in the PEEK-tube thermostated to 95°C and from this mixture 20 μl was injected to an online Waters HPLC system. The separation was done on an Adsorbosphere XL SCX column, mobile phase: A – water; B – 0.2 M aq. tartaric acid solution C – 5% (m/m) aq. NaCl-solution. In the case of peptides, HPLC separation was performed on a Kinetex column with water/acetonitrile gradient elution.

^{68}Ga -activity retention was studied in the PEEK-capillary as reactor of the continuous flow system with the help of “blind” solutions not containing any chelating moiety.

The potential suitability of the microfluidic system for performing complete radiochemical synthesis was tested applying a combined measurement protocol, which involved both retention and radiochemical purity examinations for the studied compounds. Syntheses were performed in 10-10 μL volumes but in this case, the reaction mixtures were not injected to the online HPLC system. By these reactions the labelled solutions were collected by dropwise fractionation from the continuous flow from the system. Radioactivity values were measured instantly with Canberra gamma spectrometer using the AUC value of the 511 keV energy peak. Afterwards, sample of the solution was injected to HPLC system for the determination of radiochemical purity.

Parameters of the syntheses (pH, ligand concentration) were chosen based on results of the multiparametric labelling optimization

experiments, and identical parameters were applied with the corresponding chelator-bearing biological vector conjugates, NODAGA-c(RGD)₂ and NOPO-RGD labelling experiments also. Reactions were performed in all cases at 95 °C, furthermore in the case of NOTA and NOPO, experiments were performed with decreasing reaction times (5; 2.5 and 1.25 min) by increasing flow rate in the system (0,066; 0,132 and 0,264 mL/min respectively)

3.3 Synthesis and application of ⁶⁸Ga-NOTA-c(NGR) for imaging Aminopeptidase N (CD13) receptors in vivo

In these series of experiments, synthesis of NGR peptide was performed as first step with Fmoc/*t*Bu strategy, after cyclization of the partially protected linear peptide. The purification of the product was done by RP-HPLC. The synthesised c[KNGRE]-NH₂ was conjugated with NOTA chelator on its Lys ε-amino group using *p*-SCN-Bn-NOTA in DMSO. The resulting NOTA conjugated NGR-analogue (NOTA-c(NGR)) was purified using semipreparative HPLC and was identified with ESI-MS.

The ⁶⁸Ga-labelling of the chelator conjugated vector was performed using 1M sodium acetate solution buffering in ⁶⁸Ge/⁶⁸Ga-generator (iThemba) eluate of 1M HCl-solution, the necessary formulation of the solution for the purpose of small animal experiments was done by adsorbing the labelled peptide on the surface of Empore® C18 SD 7 mm/3 mL extraction disc, washing the stationary phase with water, elution with EtOH, evaporation of the organic phase and solving the radiopharmakon again in isotonic salt solution.

A similar method was used for the labelling of NODAGA-[c(RGD)₂] known from literature, but with increased temperature and instead of sodium acetate labelling was done in HEPES buffered mixture.

In order to characterise the newly synthesized ⁶⁸Ga-NOTA-c(NGR), distribution of the radiolabelled compound between 1-octanol and PBS solution was measured as logP value for the determination of partition coefficient, furthermore its stability was tested in PBS solution at 95°C and in rat serum at 37°C.

Chemically induced rat mesenchymal mesoblastic nephroma (NeDe) tumor was used during our small animal experiments in a tumour models established in two different anatomical locations. In the case of subcutaneous animal model 5 x 10⁶ NeDe tumor cell in 150 µL was injected subcutaneously into the left thigh, while for the induction of the other tumour model subrenal capsule assay (SRCA) method was applied. Here, surgical operation is used for placing Gelaspon[®] disc containing tumour cells under the capsule of the left kidney. For the implantation, 5 x 10⁶ NeDe tumor cells in 20 µL saline solution is placed on a Gelaspon[®] disc. The retroperitoneum is opened by abdominal section after anesthetization of the animals with isoflurane.

12 ± 2 days after implantation of the NeDe cells the control and tumor-bearing rats are injected via the tail vein with 7.4 ± 0.2 MBq of ⁶⁸Ga-NOTA-c(NGR) or ⁶⁸Ga-NODAGA-[c(RGD)₂]. 90 min after the administration of the tracer, animals are anesthetized with isoflurane and whole body PET examinations were performed (10 min static PET imaging in all bed positions) using MiniPET-II small animal PET

scanner. CBCT was applied for the anatomical localization of the tumors.

On the following day after PET imaging, the control and tumour-bearing rats were anesthetized and injected with 7.4 ± 0.2 MBq ^{68}Ga -NOTA-c(NGR) or ^{68}Ga -NODAGA-[c(RGD)]₂-solutions via the tail vein. After 90 min incubation time, rats were euthanized with the intraperitoneal injection of 60 mg/kg pentobarbital (Nembutal) and blood samples were taken from the heart. Three tissue samples were taken from each organs (liver, spleen, kidney, intestine, heart, stomach, muscle, lung and tumour) and their activity was measured with a calibrated gamma counter.

During our *ex vivo* and *in vivo* blocking experiments the control and NeDe-tumor-bearing rats were injected with 200 μg unlabelled NOTA-c(NGR) in 100 μL saline (as a blockade, approx. 100 fold of the ^{68}Ga -labelled peptides) by intravenous injection 5 min before the administration of ^{68}Ga -NOTA-c(NGR).

APN/CD13 expression of the NeDe cell line and the tissue samples obtained during the animal experiments were assessed by Western blot analysis.

4 Results

4.1 Comparative examination of ^{68}Ga -labelling properties of TACN-based chelators with differing substitution of phosphinic and carboxylic groups

Comparative examination of a series of analogous chelators with 1,4,7-triazacyclononane ring with differing N-substitution was accomplished during this project. As a starting measurement, experiments were done at pH 3 and at 95 and 25 °C. Structural similarity was exhibited also at the labelling properties of the compounds, as expected and a curve of similar shape was presented for the examined NOTA, NOPA, NO2AP and NOPO, and a higher ligand concentration was necessary for performing quantitative labellings at room temperature than at elevated temperature. At this later series of experiments, NOPO had a better labelling efficiency compared to ligands with mixed substituents and NOTA. Interestingly, the presence of a single carboxyl-donor did not influence considerably the labelling efficiency at pH 3 when comparing with NOPO. Similarly, the monophosphinic-ligand NO2AP showed similar reactivity to NOTA at 95 °C. However at 25 °C, NO2AP had a better labelling performance than NOTA, although maximal radiochemical purity did not exceed 90% at this case, even at relatively high chelator concentrations. In summary, the highest step regarding labelling efficiency was found between chelators bearing one or two phosphinic and carboxylic groups. Comparing at 50% activity incorporation of the measurements by the

investigated temperatures, NOPA could be labelled with three times better efficiency than NO2AP, while NOPO and NOTA are separated by a factor of ten.

Almost quantitative labelling efficiency was found by all investigated compounds at reactions performed at 95 °C with ligand concentration 3 µM and at 25 °C with ligand concentration 30 µM (with 5 minute reaction time), therefore these values were chosen for further investigations, when changing pH of the reaction mixture. When performing our reactions at 95 °C, it can be observed that with the increase of phosphine-containing substituents, higher labelling yields could be reached at lower pH due to the high acidity of phosphinic acids. In accordance with previous results, NOPO could be labelled quantitatively already at pH 0.5 and even to a small extent at pH 0. On the other hand, NOTA showed better performance in the neutral and slightly acidic region. Above pH 8, none of the compounds could be labelled.

At room temperature, labelling of all chelators was restricted to a much narrower pH region. While NOPO still performed slightly better at lower pH, NOPO, NOPA and NO2AP reached their optimum between pH 3 and 4. However, ⁶⁸Ga labelling by the latter ligand again did not exceed 90%, while the first two ligands were labelled quantitatively. Above pH 4, labelling efficiency of NOPO was decreasing to a larger extent than that observed for the other chelators. By contrast, and similarly to the situation observed at 95 °C, NOTA performed better than the other ligands between pH 4 and 7, with an optimum at pH 4. Notably, some radioactivity can be clearly

incorporated by NOTA even at pH 8. Overall, radiolabelling results are in line with the previously obtained data on TRAP ligands. Due to the selectivity of phosphinate-containing TACN derivatives for gallium(III), a lower ligand excess is required for efficient radiolabelling with an increasing number of phosphinate pendant arms. A similar decrease in ^{68}Ga incorporation due to presence of the acetate pendant arms has been very recently observed for a diacetate-phosphinate TACN derivative with the P-bound $-\text{CH}_2\text{CH}(\text{PO}_3\text{H}_2)_2$ group. More phosphinate pendant arms also means a better labelling of ^{68}Ga in more acidic solutions due to the higher acidity of phosphinic acids. On the other hand, ligands with more acetate pendant arms are more suitable for ^{68}Ga labelling at $\text{pH} > 4\text{--}5$. This might be caused by competition with the hydroxide anion, as Ga-NOTA complex is more stable based on available data than Ga-NOPO complex, thus in the case of a more stable complex increase of pH has less effect on chelating properties

.4.2. Microfluidic optimization of complex-formation reactions of ^{68}Ga -chelators

Microfluidic labeling offers an alternative route for the synthesis of several well-known ^{18}F - and ^{11}C -labelled radiopharmaceuticals. Nevertheless, in the case of PET radiometals, the numbers of studies are very limited. Batch-based PDMS-chip reactor synthesis of ^{64}Cu -DOTA-c (RGDFK) was performed by Wheeler et al.

In contrast to previous studies, our current examination describes a microfluidic methodology, in which a PEEK capillary is used as a nonconventional reactor for the noncarrier added synthesis of

^{68}Ga -labelled radiopharmaceuticals. The tube is reeled up inside an air thermostat for accurate temperature control. NOTA and NOPO are well-known for their ^{68}Ga chelation properties; thus, they were chosen as model compounds for testing the operation of the new system. Moreover, new HPLC method was developed for the separation of labelled chelators and “free” ^{68}Ga . We compared the results of the manual labelling and microfluidic procedure. The methods provided slightly different results only in extremely low concentrations of NOPO. Such a deviation was considered typical for the threshold limits.

Further evaluation of the continuous flow microfluidic system was focused on the utilization of the high-throughput and minimal human interaction properties of this setup. Here, we performed sequential analysis of labelling properties of the chelators NOTA and NOPO under microfluidic conditions over a broad range of reaction parameters: 0.01–100 μM ligand concentration and 1–9 pH range. The temperature and the reaction time were maintained stably at 95 $^{\circ}\text{C}$ for 5 min.

Similarly to previous reports, NOPO with its phosphinic pendant arms exhibited an extended labelling profile in comparison with NOTA. This was observed at the lowest ligand concentration needed for achieving quantitative labelling. Additionally, radiochemical purity over 95% was obtained on a broader pH scale. NOPO preserves its affinity towards ^{68}Ga in highly acidic pH values of 1 and 2 as well.

A PEEK capillary apparatus was chosen as a non-conventional reactor, since this device has been successfully utilized as a component of multiple-use radiochemical systems due to its compatibility with

basic, acidic, and organic solvent and high temperature (up to 134 °C) conditions. Additionally, being an organic polymer, no significant reactivity was expected with the reagents used for ^{68}Ga -labelling reactions. In our system, ^{68}Ga retention was measured at carefully defined pH values, since aqueous Ga(III) can be present in many forms. In these studies, an interesting retention pattern was observed: significant ^{68}Ga retention was measured between pH values of 3–7 with the maximum at pH 3. Depending on the composition of the solution, aqueous gallium is known to form different hydroxo-complexes $[\text{Ga}(\text{OH})_n]$ in this pH region, but it is also known to be more dominant with the increase of the pH value of the solution. Other hypothesized reason for the trend towards retention may be a consequence of the buffer system. HEPES is supposed to form a weak complex with aqueous Ga(III). ^{68}Ga , as a component of an organic complex, might possess a higher affinity for adsorption than aqueous Ga(III) alone. Alteration in the charge at pH 3 can affect the existence of the proposed weak complex and, therefore, the activity retention itself. Besides – and slightly supporting our hypothesis – significant role of pH 3.0 can be observed in current labellings, as the highest labelling efficiency with the lowest necessary ligand concentration was found at pH 3.0 by both examined materials, which might originate from an easier reaction from a weaker HEPES-Ga-complex compared to different gallium-hydroxocomplexes.

Test syntheses were also performed to determine the suitability of PEEK reactor for preparative purpose also. In these experiments, activity retention was measured in the presence of different

chelators/chelator–peptide conjugates. Parameters of the system optimal for synthesis of desired conjugates were chosen with the objective of achieving required robustness since, in a continuous flow system, dilution with the carrier fluid can be expected. In these experiments, concentrations of 10 μM for NOTA and 1 μM for NOPO were selected, both at pH 3.0. These concentration values are at least one measurement step higher than the lowest ligand concentration needed for quantitative labelling. Moreover, when productivity of the system was tested at higher pH values, RCP over 95 % was still obtained. In these test runs, identical reaction parameters were also used for the analogous peptide conjugates of the chelators as well.

NOTA, NOPO, and their respective analogues conjugated with RGD peptide; NODAGA-c(RGD)₂ and NOPO-RGD were studied, and the analysis of final reaction mixtures demonstrated that only marginal or negligible retention (<5%) may be expected in the presence of the chelating moieties investigated in the present study.

4.3. Synthesis and application of ^{68}Ga -NOTA-c(NGR) for imaging Aminopeptidase N (CD13) receptors in vivo

This study aimed to investigate newly synthesized ^{68}Ga -NOTA-c(NGR) as an innovative molecular probe for the evaluation of APN expression in experimental tumors. The synthesis of the cyclic NGR peptide (c[KNGRE]-NH₂) was developed in literature with the preparation of the cyclic peptide using on resin cyclization. In these experiments, we introduced an efficient alternative route for the synthesis of c[KNGRE]-NH₂ cyclic peptide. In this case, the linear

protected peptide (Boc- Lys(ClZ)-Asn(Trt)-Gly-Arg(Pbf)-Glu(OtBu)-R) was built up on solid support by standard Fmoc/tBu strategy followed by removal of a semi-protected peptide (H-Lys(ClZ)-Asn-Gly-Arg-Glu-NH₂) from the resin using TFA cleavage mixture. The amide bond formation between the N-terminal amino group and the α -carboxyl group of glutamic acid was carried out in diluted DMF solution by the aid of coupling agents. Prior to the removal of ClZ protecting group with liquid HF, the semi-protected cyclic peptide was purified by RP-HPLC. The cyclic NGR peptide was prepared successfully by the application of this procedure and no deamidation through succinimide ring closure was observed in any stage of the synthesis. As a following step, pure c[KNGRE]-NH₂ peptide was successfully conjugated with *p*-SCN-Bn-NOTA, then the resulting NOTA-c(NGR) was easily labelled afterwards with ⁶⁸Ga with a RCP of 95% and a specific activity of 5.13–5.92 GBq/μmol.

The *in vitro* experiments demonstrated that newly synthesized ⁶⁸Ga-NOTA-c(NGR) is highly hydrophilic ($\log P = -2.77 \pm 0.12$) and was also found to be stable both in PBS at increased temperature ($T = 95\text{ }^{\circ}\text{C}$) for 1 h and in rat serum at $37\text{ }^{\circ}\text{C}$ for 2 h; these results demonstrate that ⁶⁸Ga-NOTA-c(NGR) is an appropriate candidate for further experiments.

Ex vivo and *in vivo* biodistribution studies on control animals revealed that ⁶⁸Ga-NOTA-c(NGR) was mainly excreted from the kidney, due to its hydrophilic properties that has been proved by the partition coefficient. On the other hand, the uptake of the tracer in other organs was very low, especially in the abdomen in contrast to the ⁶⁸Ga-

NODAGA-[c(RGD)]₂, where the abdominal organs (liver, spleen, intestines) showed radiotracer uptake after 90 min incubation time. This moderate accumulation of ⁶⁸Ga-NODAGA-[c(RGD)]₂ in the abdominal organs correlated well with other studies, where the biodistribution of ⁶⁸Ga-DOTA-NGR and ⁶⁸Ga-NOTA-NGR molecules were investigated. Furthermore, the low activity of other organs also allowed for high quality images with low background and high tumor-to-muscle ratios to be obtained.

In our study, we investigated the tumor specific accumulation of ⁶⁸Ga-NOTA-c(NGR) as a new molecular probe for imaging angiogenesis-marker expression, comparing with the commercially available $\alpha_v\beta_3$ integrin receptor specific ⁶⁸Ga-NODAGA-[c(RGD)]₂ on two different animal models using a small animal PET scanner. In this paper, we test different types of administrations of nephroblastoma (NeDe) tumor cells to rats to investigate the expression of APN/CD13 in primary tumors. Among the administrations, local tumor formation was induced by subcutaneous injection, and subrenal (SRCA) implantation of NeDe tumor cell line. Comparing the two different tumor models (SRCA versus subcutaneous), we found that the accumulation of both investigated radiotracers in primary tumors was higher in the SRCA model. This difference stems from the site of the tumor cell implantation. The most commonly used method for the induction of primary tumors is the subcutaneous injection of cancer cells. This kind of transplantation is ectopic and lacking orthotopic tissue microenvironments. Vascularization and tumor growing are more intensive when tumor cells are implanted at orthotopic site (e.g. renal

tumors into the kidney or subcapsular space), and the favorable microenvironment of this site may promote higher expression of angiogenic markers; furthermore subserve tumor cell proliferation and generation of metastases.

By taking the SUVmean values, the uptake of ^{68}Ga -NOTAc(NGR) of the primary tumors (subcutaneously growing NeDe tumor and tumor on the left kidney was relatively higher than that of the ^{68}Ga -NODAGA-[c(RGD)]₂ uptake, and this difference was significant ($p \leq 0.01$) in the case of SUVmax and T/M ratios at 90 min p.i.. Our results suggest that ^{68}Ga -NOTA-c(NGR) bears the potential to outperform even dimeric RGD-targeted tracers, which might originate from the different structure and stability of the cyclic NGR peptide as a homing device.

After blocking by unlabelled NOTA-c(NGR), the tumor uptake of ^{68}Ga -NOTA-c(NGR) reduced significantly ($p \leq 0.01$) in both primary tumors. Similarly to previously mentioned studies with other ^{68}Ga -labeled NGR-conjugates with human xenograft tumors, our successful blocking results suggest that ^{68}Ga -labelled NOTA-c(NGR) also specifically binds to the APN/CD13 receptors, which presence on NeDe tumors and metastases was proved by western blot experiments.

Angiogenesis is prerequisite in the multistep process of generating metastasis when cancer cells spread from primary sites to distant places. APN/CD13 may play a role in the invasion of cancer cells by enhancing their invasive capacity and metastatic behavior and by degrading the extracellular matrix to promote malignant cell invasion. Here, we used a syngenic metastasis animal model to

investigate the expression of APN/CD13 by ^{68}Ga -NOTA-c(NGR) not only in primary tumors, but also in metastatic lymph nodes. The implantation of NeDe tumor cells under the left kidney capsule caused tumor metastasis in the thoracic parathymic lymph nodes (PTNs) and in the mesenteric lymph nodes. By *ex vivo* experiments we found APN/CD13 receptor specific accumulation of ^{68}Ga -NOTA-c(NGR) in metastatic lymph nodes. These results suggest that ^{68}Ga -NOTA-c(NGR) is a promising molecular probe for the detection of metastases.

5 Summary

Radioisotope gallium-68 is a positron-source, which is available from generator, which makes quick, efficient and in the future even kit-based synthesis of PET-radiopharmaceuticals possible, as due to its metallic properties a wide scale of biological vectors can be labelled with it using a chelator moiety. Our experiments focused on the synthesis, characterization and preclinical application of ^{68}Ga -labelled complexes as they are gaining increasing relevance in nuclear medicine.

Chemical properties of the chelator moiety have a determinative role during the synthesis of ^{68}Ga -PET-radiopharmaceuticals. During one of our series of experiments we performed the comparative radioanalytical examination of TACN-based chelators. Our results reveal that at least two phosphinic substituents are essential for the excellent ^{68}Ga -complex-formation efficiency described by TRAP-type chelators, therefore one of the phosphinic group of the TRAP pattern can be changed to other donor. The presence of carboxylic group can help complex-formation at neutral and mildly acidic pHs. The income of this structure-complex-formation property relationships can be useful for planning of the chelators of the future.

For the previously described characterisation, primarily manual labellings are used in the literature, which however is time-consuming, can be hard to reproduce and can be produced with relatively high amount of substance. Contrarily, we successfully tested a microfluidic system during our research project, which is suitable for performing and

analysing ^{68}Ga -labelling reactions automatically. We were able to provide complete characterization of radiolabelling efficiency of macrocyclic chelators in an automated manner, furthermore we demonstrated that it is possible to perform ^{68}Ga -labellings in a continuous flow microfluidic system with an excellent radiochemical purity (95%<) without significant activity retention (<5%).

In our further studies, we synthesized a new, ^{68}Ga -labelled radiopharmaceutical, the ^{68}Ga -NOTA-c(NGR) with high radiochemical purity. The radioligand showed high chemical stability, and we tested it with APN/CD13+ NeDe cell line and syngeneic small animal tumour model for the detection of tumours and metastases successfully.

6 List of publications



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PhD Publikációs Lista

Candidate: Gábor Máté

Neptun ID: ORZAVL

Doctoral School: Doctoral School of Molecular Medicine

List of publications related to the dissertation

1. **Máté, G.**, Szikra, D., Simecek, J., Szilágyi, S., Trencsényi, G., Wester, H. J., Kertész, I., Galuska, L.: Multiparametric labeling optimization and synthesis of ⁶⁸Ga-labeled compounds applying a continuous-flow microfluidic methodology.
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IF: 1.942 (2015)
2. **Máté, G.**, Kertész, I., Enyedli, K. N., Mező, G., Angyal, J., Vasas, N., Kis, A., Szabó, É., Emri, M., Bíró, T., Galuska, L., Trencsényi, G.: In vivo imaging of Aminopeptidase N (CD13) receptors in experimental renal tumors using the novel radiotracer ⁶⁸Ga-NOTA-c(NGR).
Eur. J. Pharm. Sci. 69, 61-71, 2015.
DOI: <http://dx.doi.org/10.1016/j.ejps.2015.01.002>
IF: 3.773
3. **Máté, G.**, Simecek, J., Priok, M., Kertész, I., Notri, J., Wester, H. J., Galuska, L., Hermann, P.: The Influence of the Combination of Carboxylate and Phosphinate Pendant Arms in 1,4,7-Triazacyclononane-Based Chelators on Their ⁶⁸Ga Labelling Properties.
Molecules, 20 (7), 13112-13126, 2015.
DOI: <http://dx.doi.org/10.3390/molecules200713112>
IF: 2.465



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List of other publications

4. Trencsényi, G., Kertész, I., Krasznai, Z. T., **Máté, G.**, Szalóki, G., Péli-Szabó, J., Kárpáti, L., Krasznai, Z., Márián, T., Goda, K.: 2' [¹⁸F]-fluoroethylrhodamine B is a promising radiotracer to measure P-glycoprotein function.
Eur. J. Pharm. Sci. 74, 27-35, 2015.
DOI: <http://dx.doi.org/10.1016/j.ejps.2015.03.026>
IF: 3.773
5. Sipos, A., **Máté, G.**, Röth, E., Borbás, A., Batta, G., Bereczki, I., Kéki, S., Jóna, I., Ostorházi, E., Rozgonyi, F., Vanderlinden, E., Naesens, L., Herczegh, P.: Synthesis of fluorescent ristocetin aglycon derivatives with remarkable antibacterial and antiviral activities.
Eur. J. Med. Chem. 58, 361-367, 2012.
DOI: <http://dx.doi.org/10.1016/j.ejmech.2012.10.030>
IF: 3.499

Total IF of journals (all publications): 15,452

Total IF of journals (publications related to the dissertation): 8,18

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

20 September, 2016



7 List of presentations and posters

7.1 List of presentations

1. **Máté, Gábor**; Szikra, Dezső; Šimeček, Jakub; Kertész, István; Wester, Hans-Jürgen; Galuska, László: Microfluidic Labelling - an Efficient Way for the Optimization and Production of ^{68}Ga -Radiopharmaceuticals Annual Congress of the European Association of Nuclear Medicine, . Gothenburg, Sweden, 18-22 October 2014
2. Szikra, Dezső; **Máté, Gábor**; Nagy, Gábor: ^{68}Ga -microfluidics, COST meeting, Warsaw, Poland, 12 July 2014.
3. **Máté, Gábor**; Szikra, Dezső; Šimeček, Jakub; Kertész, István; Wester, Hans-Jürgen; Galuska, László: Taking Ga-68 labeling optimization to the fast line with a new microfluidic system, Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging, St. Louis, USA, 7-11 June 2014.
4. **Máté, Gábor**; Šimeček, Jakub; Notni, Johannes; Galuska, László; Wester, Hans-Jürgen; Kertész, István: Radiochemical Evaluation of Phosphinic, Carboxylic and Mixed Chelators for Gallium-68; Annual Congress of the European Association of Nuclear Medicine, Lyon, France, 19-23. October 2013. ; Abstract availability: Eur. J. Nucl. Med. 2013 40:S116-S116.

5. **Máté, Gábor**; Szikra, Dezső; Kertész, István; Galuska, László: Microfluidic Optimization of Complex-formation Reactions of ^{68}Ga -chelators; Autumn Radiochemical Days, Eger, 16-18. October 2013.
6. **Máté, Gábor**; Kertész, István; Galuska, László; Šimeček, Jakub; Notni, Johannes: Comparative study of asymmetrically substituted ^{68}Ga -chelators; XVIII. Congress of the György Hungarian Society of Nuclear Medicine, Pécs, 30 June 2013 – 02 July 2013.
7. Kertész, István; **Máté, Gábor**; Márián, Teréz; Leiter, Éva; Trencsényi, György: Labelling of siderophores with ^{68}Ga ; XVIII. Congress of the György Hungarian Society of Nuclear Medicine, Pécs, 30 June 2013 – 02 July 2013.
8. **Máté, Gábor**: Experimental Investigation of Highly-Efficient ^{68}Ga -Chelators for Radiopharmaceutical Purposes; European Medical Students' Conference, Debrecen, 19-22 October 2012.
9. **Máté, Gábor**; Kertész, István; Šimeček, Jakub; Wester, Hans-Jürgen: Investigation of Highly-Efficient ^{68}Ga -Chelators for Radiopharmaceutical Purposes; Autumn Radiochemical Days, Siófok, 8-10 October 2012.
10. Kertész, István; **Máté, Gábor**; Trencsényi, György; Márián, Teréz: Labelling of siderophores with ^{68}Ga for the Imaging of

Aspergillosis with PET technique; Autumn Radiochemical Days, Siófok, 8-10 October 2012..

11. Kertész, István; **Máté, Gábor**; Trencsényi, György; Márián, Teréz: Trial-labelling of Siderophores with Ga-68; Scientific meeting of MTA DAB Nuclear Medicine Working Committee, Debrecen, 13 September 2012.
12. Kertész, István; **Máté, Gábor**; Halmos, Gábor; Mező, Gábor: Labelling of Peptides with Positron-emitting Isotopes; Scientific Meeting of MTA Peptidchemical Committee, Balatonszemes, 30 May 2012.
13. Sipos, Attila; **Máté, Gábor**; Török, Zsolt; Róth, Erzsébet; Borbás, Anikó; Batta, Gyula; Bereczki, Ilona; Kéki, Sándor; Jóna, István; Rozgonyi, Ferenc; Ostorházi, Eszter; Evelien, Vanderlinden; Lieve, Naesens; Herczegh, Pál: Synthesis and Pharmacological Characterization of Fluorescent Teicoplanin-pseudo-aglycon and ristocetin-aglycon; MTA Carbohydrate, Nucleic Acid and Antibiotics Working Committee., Debrecen, 31 May 2012 – 01 June 2012.

7.2 *List of posters*

1. Kertész, István; **Máté, Gábor**; Enyedi, N. Katalin; Szikra, Dezső; Trencsényi, György; Márián, Teréz; Mező, Gábor: Design, synthesis and radiolabelling of NGR peptides with

- ^{68}Ga ; Annual Congress of the European Association of Nuclear Medicine, Gothenburg, Sweden, 18-22 October 2014.
2. Kertész, István; Kárpáti, Levente; **Máté, Gábor**; Péliné Szabó, Judit; Nagy, Tamás; Trencsényi, György; Márián, Teréz: Synthesis and in vitro application of 2'-[^{18}F]fluoroethylrhodamine B for detecting multidrug resistance; Annual Congress of the European Association of Nuclear Medicine, Lyon, France, 19-23 October 2013. ; Abstract availability: Eur. J. Nucl. Med. 2013 40:S283-S283.
 3. P. Szabó, Judit; Trencsényi, György; Nagy, Tamás; **Máté, Gábor**; Kertész, István; Krasznai, Zoárd T.; Márián, Teréz: The [^{18}F]fluoroethyl-rhodamine B is a PET Tracer Suitable for the Detection of Multi-drug Resistance; XVIII. Congress of the György Hungarian Society of Nuclear Medicine, Pécs, 30 June 2013 – 02 July 2013.