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Pharmacokinetic characterization of radiolabeled cyclodextrin derivatives using positron emission tomography

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
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1.Introductions and amis

Nowdays, the number of pancreatic cancer cases worldwide is highly increasing. In Hungary, it ranks fifth among cancers that cause death, and the 5-year survival rate of patients is less than 5%. In most cases, when the tumor is discovered, the disease is already in an advanced stage, and the chances of one-year survival are very low. Because the disease has no early symptoms and can quickly invade surrounding tissues and organs, it is one of the deadliest cancers. Identifying early diagnosis options is an important way to improve detection and survival rates of pancreatic cancer. Due to specific radiopharmaceuticals, PET has a high sensitivity for detecting pancreatic adenocarcinoma tumorous lesions, and early metastases. With the advancement of molecular biology and the discovery of new tumor-related targets, the development of tumor-specific radiopharmaceuticals for PET diagnostics is also progressing rapidly.

The cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway plays an important role in tumor development and formation of metastases such as pancreatic cancer, therefore they represent important diagnostic and therapeutic research targets in nuclear medicine in the field of molecular imaging. PGE2 form complexes with RAMEB (randomly-methylated-beta-cyclodextrin) with high affinity. Radioactively labeled RAMEB, can be a promising radiopharmaceutical in the diagnosis and therapy of pancreatic tumors.

Cyclodextrins are polymers composed of cyclic oligosaccharides of truncated cone shape or toroids. Cyclodextrins are widely used as pharmaceutical excipients as they have many areas of application: drug carriers, stand-alone drugs, improve the bioavailability, bioavailability, and shelf life of other preparations.It is known that cyclodextrin derivatives are effective in the therapy of several diseases. Despite of the intensive research, limited information is available on the pharmacokinetic and biodistribution of cyclodextrins. For this reason our research group started to investigate the pharmacokinetic properties of cyclodextrins using a sensitive non-invasive positron emission tomography (PET).

The aims of this studies is to produce radioactively labeled cyclodextrin derivatives - as new radiopharmaceuticals - may open a new pathway in the *in vivo* imaging and diagnosis of PGE2 positive tumors, further expanding the applications of cyclodextrins. The primary goal in this study to created radiopharmaceuticals by implementing the following projects:

The aims of the first study were to synthetize the PGE2 specific 68Ga-labeled NODAGA-randomly methylated beta-cyclodextrin (68Ga-NODAGARAMEB) and investigate its tumor-targeting properties. NODAGA-RAMEB was labeled with Gallium-68 (68Ga), and the radiochemical purity (RCP%), partition coefficient (logP values), and *in vitro-in vivo* stability of 68Ga-NODAGA-RAMEB were determined. After intravenous injection of 68Ga-NODAGA-RAMEB the accumulation in organs and

tissues was monitored *in vivo* by positron emission tomography (PET) and *ex vivo* by gamma counter in BxPC-3 and PancTu-1 tumor-bearing CB17 SCID mice.

The aim of the second project was to synthesize a PGE2-specific DOTAGA-RAMEB, which can be labeled with diagnostic and therapeutic isotopes also and binds to PGE2- positive tumors. DOTAGA-RAMEB was labeled with ^{68}Ga and $^{205/206}$Bi radionuclides and their radiochemical purity (RCP%), partition coefficient (log*P* values), and *in vitro* and *in vivo* stability were determined. For the assessment of the biological properties and the PGE2 specificity of [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi-DOTAGA-RAMEB *in vivo* PET imaging and *ex vivo* biodistribution studies were performed using healthy control and PGE2-positive BxPC-3 tumor-bearing CB17 SCID mice.

2. Materials and methods

Conjugation of the NODAGA chelator with NH2-RAMEB

The NODAGA chelator was conjugated via the amino group of NH2-RAMEB - as follows: NH2-RAMEB was dissolved in water, then the solution was cooled to 4 °C and stirred for 15 minutes. NODAGA was dissolved in DMSO and the solution was added to the cooled NH 2 -RAMEB solution with stirred. The pH of the reaction mixture was adjusted to 8.5 by adding DIPEA drop by drop. The reaction mixture was stirred at room temperature for 24 hours. The product was lyophilized, redissolved in water and purified by preparative RP-HPLC. The yield of the synthesis was 74%. Analytical RP-HPLC was used to verified the purity of NODAGA-RAMEB. The exact molecular weight of the obtained product was verified by high-resolution mass spectrometry (MS), and its structure by 1H-NMR.

Preparative and analytical RP-HPLC

The purification of RAMEB derivatives conjugated with different chelators was carried out with the help of a KNAUER RP-HPLC system, on a 150 mm x 10 mm preparative Supelco Discovery® Bio Wide Pore C18 column (particle size: 10 μm) at a flow rate of 4 ml/min using the following gradient: A eluent: 0 min 90%, 2 min 90%, 20 min 40%, 30 min 20%. The eluent system consisted of eluent A (0.1% TFA in water) and eluent B (0.1% TFA; acetonitrile/water 95:5 (v/v%)). Detection was performed with an absorbance detector at 254 nm

We also used a KNAUER RP-HPLC system was combined with radio detector and the signals were detected by both radio and absorbance detector simultaneously. Detection was performed at 254 nm on an analytical Supelco Discovery® Bio Wide Pore C18 column with a size of 250 mm x 4.6 mm and a particle size of 10 μm. The flow rate was changed to 1 ml/min, and the gradient was applied as follows: Eluent: 0 min 100%, 15 min 10%, 17 min 10%, 20 min 100%. The eluents are the same as those used for purification.

Conjugation of DOTAGA to NH2-RAMEB

The DOTAGA chelator was also conjugated through the amino group of NH2-RAMEB as follows: NH2-RAMEB was dissolved in water, then the solution was cooled to 4 °C and stirred for 15 minutes. DOTAGA was added to the cooled NH2-RAMEB solution, the pH of the reaction mixture was adjusted to 8.5 by adding DIPEA dropwise. The reaction mixture was stirred at room temperature for 24 hours. The product (DOTAGA-RAMEB) was lyophilized, then redissolved in water and purified on a preparative RP-HPLC system. The purity of the finished product was checked with an analytical RP-HPLC system, and the exact molecular weight of the obtained product A was confirmed by MS. In the last step, a 3 mM stock solution was prepared from the identified product (DOTAGA-RAMEB) with ultrapure water for radioactive labeling.

Development of suitable elution of the ^{68}Ge/^{68}Ga generator

During radiolabeling with the Ga isotope, an Eckert & Ziegler type ^{68}Ge/^{68}Ga generator was used, which was fractionally eluted with (5 ml) 0.1 M ultrapure hydrochloric acid. However, during the labeling, we started from 1 ml of generator eluate in each case. In order to obtain the most active fraction of 1-1.2 ml, fractional elution was performed for first : The generator was eluted by using a 5 ml syringe, filled 0.1 M ultrapure hydrochloric acid. 25 (200 µl) fractions were collected in 1.5 ml Eppendorf tubes. The activity of the fractions was measured with a dose calibrator. Based on our measurements, the most active part of the eluate is found in the volume between fractions 9 and 14 .

Production and purification of $^{205/206}$Bi isotopes.

The $^{205/206}$Bi isotope mixture was produced in the GE PETtrace cyclotron operating at our institute, by irradiating a lead-foil target. The lead target was placed in the solid target holder unit of the cyclotron and then irradiated with a beam current of 10 µA for 60 minutes as described by Manna et al. in 2020. After the 60-minute irradiation, we waited 24 hours decay period, then the irradiated target was dissolved with ultrapure HNO3, and then the clear solution was pipetted off the undissolved solid. The solution was then diluted to 10 mL with water and filtered through a Millipore 0.22 µm filter to completely remove undissolved solids. This $^{205/206}$Bi isotope containing solution was transferred onto a preconditioned TK 200 resin (150 mg) column. After the loading of solution, the column was washed with u.p. HNO3 to remove the remaining Pb target materials, and the $^{205/206}$Bi isotopes were eluted with u.p. HNO_3. The pure eluate which contained $^{205/206}$Bi isotopes (~30 MBq) were evaporated to dryness and were redissolved in u.p. HCl (0.1 M, 300 µL).

Gallium-68 labeling of NODAGA-RAMEB

The ^{68}Ge/^{68}Ga generator was eluted with 5 mL 0.1 M u.p. HCl. The highest activity aliquot (1 mL) was buffered with sodium acetate to ensure a pH of 4.3–4.5, followed by the addition of an aqueous solution of NODAGA-RAMEB was added. The reaction mixture was incubated at 95 °C for 10 minutes. After

10 minutes, the mixture was allowed to cool to room temperature and then applied to a Light C18 Sep-Pak column. After binding the labeled pharmacon, the column was washed with water to remove the buffer and uncomplexed free ^{68}Ga ions. The 68Ga-labeled NODAGA-RAMEB ([^{68}Ga](Ga-NODAGA-RAMEB)) product was recovered from the column with a 1:2 mixture of 96% EtOH and isotonic sodium chloride solution (0.2 mL). The product was diluted with saline solution to decrease the ethanol-content below 10% and was sterile filtered before using for animal

[^{68}Ga]Ga-DOTAGA-RAMEB radiolabeling

1 ml of ^{68}Ga eluate was buffered with sodium acetate to ensure the correct pH value. After that, the aqueous solution of DOTAGA-RAMEB was added. The reaction was incubated at 95°C for 10 minutes. The reaction mixture was then applied to an activated Light C18 Sep-Pak column. After binding, the column was washed with water. Our 68Ga labeled product ([^{68}Ga]Ga-DOTAGA-RAMEB) was also eluted with a 1:2 mixture of 96% EtOH/isotonic NaCl solution. An RP-HPLC system combined with a radio detector was used to determine the radiochemical purity. The labeled product was diluted with saline, to decrase the ethanol content below 10%, was sterile filtered before using for animals.

[$^{205/206}$Bi]Bi-DOTAGA-RAMEB radiolabeling

For the radiolabeling $^{205/206}$Bi solution was diluted to 300 μL with u.p. HCl and was added to DOTAGA-RAMEB in a reaction vial including TRIS buffer and ascorbic acid at pH 8.4. The reaction was heated to 95 ◦C and incubated for 10 min and cooled to ambient temperature for 2 min. Afterward the solution was transferred to an Oasis HLB Extraction Cartridge and was washed with 2 mL of water. The $^{205/206}$Bi labeled product [$^{205/206}$Bi]Bi- DOTAGA-RAMEB was eluted with 200 μL mixture of 96 % EtOH/ isotonic NaCl solution. In order to evaluating the radiochemical purity, the above-mentioned KNAUER RP-HPLC system was used with the Supelco Discovery® Bio Wide Pore C-18 analytical column (250 mm × 4.6 mm; particle size: 10 μm). The HPLC system was combined with radio detector and the signals were detected by both radio and absorbance detector simultaneously. Before using for animal experiments the product was sterile filtered and diluted with isotonic (0.9 %) saline solution. The product was diluted with saline solution and sterile filtered before animal experiments.

100 μL was taken from the solution containing 205/206Bi isotopes and diluted to 300 μL with ultrapure hydrochloric acid. After dilution, it was buffered with TRIS buffer to ensure the correct pH, then 20% ascorbic acid was added and DOTGA-RAMEB was added. The reaction mixture was incubated at 95 °C for 10 minutes and then allowed to cool to room temperature. After that, the solution was applied to a separation column containing an activated Oasis HLB resin. After binding of the pharmacon, the column was washed with water to remove the buffer and uncomplexed free 205/206Bi ions. The product labeled with the 205/206Bi radioisotope ([205/206Bi]Bi-DOTAGA-RAMEB) was eluted with a mixture of 96% EtOH/isotonic NaCl solution. An RP-HPLC system combined with a radio detector was used to

determine the radiochemical purity. The labeled product was diluted with saline, thereby reducing the ethanol content to less than 10%, then sterile filtered for use on animals.

LogP values: determination of octanol/PBS partition coefficient (partition coefficient) of [^{68}Ga]Ga-NODAGA-RAMEB, [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$ Bi]Bi-DOTAGA-RAMEB derivatives

The LogP value was determined for all three cyclodextrin derivatives in the same way as in the case mentioned here: the radiolabeled cyclodextrin derivative was mixed with a mixture of 1-octanol and PBS in a centrifuge tube. The mixture was mixed for 20 minutes using a Vortex, then centrifuged for 5 minutes at 20,000 rpm at 4 °C until the layers were completely separated. 3 x 100 µl samples of the separated phases were pipetted into test tubes, then their radioactivity was measured with a Perkin Elmer Packard Cobra calibrated gamma counter. The LogP value was obtained by averaging the measurement results of several parallel experiments.

Investigation of the [^{68}Ga]Ga-NODAGA-RAMEB and PGE2-conjugated resin binding reaction

H-Gly-HMPB-ChemMatrix® resin was dwelled in DCM for 10 min. Prostaglandin E2 (PGE2), PyBOP and DIPEA were added and the mixture was stirred for 2 h. After completion of the reaction, the resin was filtered by means of a glassfilter, was rinsed twice with cold DCM and evaporation until dryness yielded the conjugated resin. The overall yield of the coupling reaction was 98%. Thereafter, in parallel experiments, unmodified resin and PGE2 conjugated resin were placed into a polypropylene (PP) barrel , equipped with a PP frit.700–700 µL of PBS was introduced and after 3 min, [^{68}Ga]Ga-NODAGA-RAMEB was injected to each sample and they were incubated for 10 min at room temperature during gentle shaking. After the incubation, the resins were filtered and were rinsed with additional PBS (1 mL). The activities of the resins and the supernatants were measured.

Determination of *in vitro* metabolic stability

The *in vitro* stability of [^{68} Ga]Ga-NODAGA-RAMEB [^{68} Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$ Bi]Bi-DOTAGA-RAMEB was investigated in mouse serum in separate experiments. 10 µl of each radioisotope labeled substance was added of 0.5 ml of mouse serum, then incubated without mixing at 37 °C. Then, at 30, 60, 90 and 120 minutes, 50 µl samples were taken and ice-cold abs. EtOH added.The precipitated fraction was then separated by centrifugation for 5 minutes at 10,000 rpm at 4 °C. The supernatant was collected, diluted with water and the radiochemical purity of the radioisotope-labeled compounds was examined by analytical radio-RP-HPLC.

Determination of *in vivo* metabolic stability

The purpose of determining the *in vivo* metabolic stability is to test the stability of the radiopharmacies in the living organism. Since its blood concentration is influenced by numerous metabolic processes of

absorption, transformation and degradation of the active ingredient. Therefore, healthy SCID mice were injected with [^{68} Ga]Ga-NODAGA-RAMEB through the tail vein, and urine samples were collected 60 minutes after the injection. The resulting sample was mixed with 50 µl of ice-cold abs. mixed with ethanol and centrifuged at 10,000 rpm for 5 minutes. The supernatant was collected and analyzed by analytical radio-RP-HPLC.

Induction of tumor model

For the induction of tumor models, male CB17 SCID mice were anesthetized with a pet inhalation anesthesia machine. The skin on the left shoulder area of the experimental animals was shaved and was disinfected. Thereafter BxPC-3 or Panc-Tu1 human pancreas adenocarcinoma cells were injected subcutaneously. *Ex vivo* and *in vivo* biodistribution studies were performed 12 ± 1 days after subcutaneous injection of tumor cells at the tumor volume of 98 ± 5 mm^3.

MiniPET imaging

About 12 days after the tumor cells were implanted, the experimental animals were anesthetized with 3% isoflurane using a pet inhalation anesthesia machine, and then [^{68} Ga]Ga-NODAGA-RAMEB was injected into the lateral tail vein in the mice bearing BxPC3 and PancTu-1 tumors, as well as in the healthy control mice. . In a separate experiment, we proceeded similarly with [^{68} Ga]Ga-DOTAGA-RAMEB radioisotope-labeled compound, in addition to inhalation anesthesia, we injected the [^{68} Ga]Ga-DOTAGA-RAMEB radioisotope-labeled compound into the lateral tail vein. After injection, *in vivo* static and dynamic (0-90 min) PET imaging was performed to determine the biodistribution of the radiotracer using a small animal PET scanner under inhalation anesthesia.

In order to reveal the role of PGE2 in the process of tumor accumulation, miniPET images were taken during an *in vivo* study, in addition to the use of PGE2, namely SCID mice bearing PGE2 positive BxPC3 tumors were intravenously injected with 100 µl of [^{68} Ga]Ga-NODAGA- diluted with a saline solution containing 4% ethanol. RAMEB with a mixture of radioisotope labeled compound and 1 mg of PGE2. In order to determine the *in vivo* biodistribution of the radiotracer. as in previous studies, static and dynamic images (0-90 minutes) were taken using a PET scanner under inhalation anesthesia.

Analysis of PET data

Ellipsoid 3-dimensional volumes of interest (VOI) were manually drawn around the edge of the organ activity by visual inspection using BrainCad image analysis software. To quantify the concentration of radioactivity in the tumors, organs and tissues standardized uptake value (SUV) was determined as follows: SUV = [VOI activity (Bq/mL)]/ [injected activity (Bq)/animal weight (g)], assuming a density of 1 g/ mL. Tumor-to-muscle (T/M) ratios were assessed from the SUVmean of tumor and SUVmean of the background (muscle).

***Ex vivo* biodistribution studies**

For *ex vivo* biodistribution studies, control and tumor-bearing (BxPC- 3) SCID mice were injected intravenously with[^{68} Ga]Ga-NODAGA-RAMEB, [^{68}Ga] Ga-DOTAGA-RAMEB or [$^{205/206}$Bi]Bi-DOTAGA-RAMEB. 30, 60 and 90 min after injection of radiotracers, the animals were anesthetized with 5 % isoflurane. Three tissue samples were taken from selected organs and radioactivity and their weight were measured with a calibrated gamma counter (Perkin-Elmer Packard Cobra, Waltham, MA). Uptake of 68Ga- and 205/206Bi-labeled DOTAGA-RAMEB was expressed in the mean values of the percent injected dose per gram (% ID/g) tissue.

Immunohistochemistry

This protocol is Shortly, four μm thick sections of formaldehyde-fixed and paraffin-embedded BxPC-3 xenograft tumors were treated with rabbit anti-prostaglandin E Receptor EP2/PTGER2 monoclonal antibody at a dilution of 1:1000 after deparaffination and rehydration (pH = 6). An HRP-labeled anti-rabbit polymer antibody and the Envision DAB detection Kit were used to detect and visualize the specific antibody binding. For counterstaining hematoxylin was applied. Microscopic images were taken with a Leica DM2500 research microscope equipped with a DFC495 digital camera and LAS imaging kit.

Statistical data processing

Statistical analyses were evaluated by two-way ANOVA, Student's *t*-test (two-tailed) and Mann-Whitney *U* test and the significance level was set at $p \leq 0.05$ unless otherwise indicated. All characterization experiments were performed in triplicates at the minimum and the data are presented as mean ± SD.

3. New scientifical results

Conjugation of NODAGA to NH2-RAMEB

NODAGA-RAMEB was prepared from NH2-RAMEB by conjugation of a bifunctional chelator (p-NCS-benzyl-NODA-GA) suitable for gallium-68 labeling. The final product was obtained after HPLC purification with more than 98% purity and the structure was confirmed by 1H NMR and UHR ESI-TOF Mass Spectrometer. 1H NMR measurement was carried out in (DMSO)-d6. Chemical shifts, δ, are reported in parts per million (ppm), referenced to the residual 1H: DMSO-d6 at 2.50 ppm. The theoretical and measured mass of the synthesized products were in concordance, where NODAGA-RAMEB m/z: calculated mass:1781.7283, found mass: 1781.7289 [M]1+ (Fig. 1C

Conjugation of DOTAGA to NH2-RAMEB

The DOTAGA-RAMEB precursor was prepared by conjugation reaction of a primary amine containing RAMEB and DOTAGA bifunctional chelator. This modification allows the modified cyclodextrin derivative to be labeled with both ^{68}Ga and $^{205/206}$Bi radioisotopes. The final product was purified by HPLC and the purity was more than 98 %. The structure of DOTAGA-RAMEB was identified by high-resolution mass spectrometry (UHR ESI-TOF MS). Based on the MS measurement, the theoretical and measured mass of the synthesized products were in concordance, where the calculated mass of DOTAGA-RAMEB *m*/*z*: 1882.7721 [M]1+ and the measured mass: 1882.7764 [M]1+

Gallium-68 labeling of NODAGA-RAMEB

The bifunctional chelator-conjugated cyclodextrin (NODAGARAMEB) was radiolabeled manually with ^{68}GaCl3 using 1 M sodium acetate buffer solution to obtain 68Ga-labeled NODAGA-RAMEB. The overall reaction time was approximately 20 min. The molar activity was 15.34 ± 1.93 GBq/μmol and the radiochemical purity (RCP) of the product was found over 98.0%. The retention time of the compound was 9.13 min on the applied analytical system.

^{68} Ga and $^{205/206}$ Bi labeling of DOTAGA-RAMEB

^{68}Ga and $^{205/206}$Bi radiolabeling of the bifunctional chelator-modified cyclodextrin derivative (DOTAGA-RAMEB) was performed manually in both cases. The average reaction time of radiochemical labeling reactions was approximately 20 min. The radiochemical purity (RCP) of products was found over 98.0 % in both cases.

LogP values: determination of octanol/PBS partition coefficient (partition coefficient) of [^{68}Ga]Ga-NODAGA-RAMEB, [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$ Bi]Bi-DOTAGA-RAMEB derivatives

The value of the distribution coefficient (LogP) of [^{68}Ga]Ga-NODAGA-RAMEB was −3.63 ± 0.04, which indicates a very hydrophilic property, so, following the pharmacokinetic properties of the radiolabeled cyclodextrin molecule, it will be excreted through the kidney in the urine.

In the case when the partition coefficient value of [^{68}Ga]Ga-NODAGA-RAMEB was examined in the presence of PGE2, its hydrophilicity decreased moderately, to a value of −3.08 ± 0.03.

The value of the partition coefficient of [^{68}Ga]Ga-DOTAGA-RAMEB is -3.47 ± 0.04, which also indicates a hydrophilic property, so its elimination from the body also occurs as described above.

The octanol/PBS partition coefficient of the [$^{205/206}$Bi]Bi-DOTAGA-RAMEB derivative is -3.45 ± 0.03, so it is also very hydrophilic and is excreted in the urine through the kidneys

Investigation of the [^{68}Ga]Ga-NODAGA-RAMEB and PGE2-conjugated resin binding reaction

During the resin binding measurement the [^{68}Ga]Ga-NODAGA-RAMEB binding capacity of the resins was compared. It was found that the PGE2 conjugated resin bound 18.1 ± 1.3% more ^{68}Ga labeled RAMEB than the unmodified resin suggesting the definite formation of the inclusion aggregate.

***In vivo* stability**

In vivo stability was tested in urine collected from mice 60 min after administration of the radiotracer. Neither [^{68}Ga]Ga-NODAGA-RAMEB, [^{68}Ga]Ga-DOTAGA-RAMEB nor [$^{205/206}$Bi]Bi-DOTAGA-RAMEB showed measurable amounts of radioactive metabolite in the radio-HPLC results, which indicate excellent *in vivo* metabolic stability.

***In vivo* biodistribution studies of [^{68}Ga]Ga-NODAGA-RAMEB in healthy control mice**

For the determination of the [^{68}Ga]Ga-NODAGA-RAMEB biodistributionin healthy control CB17 SCID mice dynamic PET imaging and *ex vivo* studies were performed. Representative dynamic PET images and the mean time-activity curve (TAC) after intravenous injection of the radiolabeled probe. By the qualitative analysis of the decay-corrected PET images kidneys and bladder with urine (urinary system) were clearly visualized and very low uptake was observed in other organs and tissues. 90 min post injection radioactivity only in the kidneys and in the bladder was identifiable and no background accumulation was observed. By the quantitative analysis of the PET images, the SUVmean data and the TAC showed the rapid clearance of ^{68}Ga-NODAGA-RAMEB from the investigatedorgans. The radiotracer uptake of the investigated tissues significantly decreased after 5 min, and 90 min post injection very low ^{68}Ga-NODAGA-RAMEB accumulation was observed in the thoracic organs (SUVmean lung: 0.31 ± 0.07, SUVmean heart: 0.12 ± 0.04), in the abdominal region (SUVmean intestines: 0.13 ± 0.03, SUVmean liver: 0.16 ± 0.06, SUVmean stomach: 0.12 ± 0.04) and in the brain (SUVmean: 0.12 ± 0.04). Only the radioactivity of the urine increased significantly (SUVmean: 7.91 ± 2.92 and 55.24 ± 9.65; 5 and 90 min post injection, respectively

***In vivo* evaluation of [^{68}Ga]Ga-DOTAGA-RAMEB biodistribution in healthy control mice**

With the aim of assessing biological distribution of [^{68}Ga]Ga- DOTAGA-RAMEB dynamic PET imaging and *ex vivo* examinations were performed in healthy control CB17 SCID mice. demonstrates representative dynamic PET images and average time-activity curve (TAC) after the intravenous injection of radiolabeled probe. Although, both the kidneys and the urinary bladder filled with urine could be directly visualized by qualitatively analysing the decay-corrected PET images, in other organs and tissues faint radiopharmaceutical accumulation could be depicted. Ninety minutes postinjection measurable radioactivity could only be detected in the kidney and in the bladder, background activity was not found. After 5 min, the tracer uptake of the examined tissues notably decreased, and 90 min after the injection the thoracic organs (pulmonary SUVmean: 0.21 ± 0,05, heart SUVmean: 0.38 ± 0.08) and the abdominal regions (intestinal SUVmean:0.25 ± 0,06, liver SUVmean: 0.25 ± 0.06, stomach

SUVmean: 0.14 ± 0.03) could only be featured with very low [^{68}Ga]Ga-DOTAGA-RAMEB accumulation. However, urinary radioactivity increased significantly 5 and 90 min postinjection with SUVmean values of 3.93 ± 1.85 and 11.46 ± 2.75, respectively.

PGE2 selectivity of [^{68} Ga]Ga-NODAGA-RAMEB in BxPC3 tumor-bearing SCID mice with PGE2 administration

In order to reveal the role of PGE2 in the process of tumor accumulation, miniPET images were taken during an *in vivo* study. In addition to PGE2 administration, SCID mice bearing PGE2 positive BxPC3 tumors were injected intravenously with 7.25 ± 0.21 MBq diluted with 100 µl of 4% ethanol saline. activity [^{68} Ga]Ga-NODAGA-RAMEB with a mixture of radioisotope labeled compound and 1 mg of PGE2. Similar to previous studies, static and dynamic images (0-90 minutes) were taken using a PET scanner under inhalation anesthesia. Representative decay-corrected pet PET images are illustrated in Fig. By qualitative analysis of the PET images, we found that subcutaneously growing BxPC3 tumors were clearly identifiable 30 minutes after injection, however, high background radioactivity was detected. Furthermore, as the incubation time increased, the background activity decreased from 30 to 90 min and the tumor became more prominent. This observation was confirmed by quantitative SUV data analysis (Figure 4B), where we found that T/M SUV mean data increased from 10 min (T/M SUV mean: 7.80 ± 1.64) to 90 min (T/M SUV mean: 18.57 ± 2.64) after injection. The SUV values of BxPC3 tumors decreased very slowly 10 min after injection. (SUV mean: 0.45 ± 0.06). Despite the relatively low radiotracer uptake of BxPC3 tumors (SUV mean: 0.14 ± 0.04) at 90 minutes, the result of the high tumor-to-muscle ratio (T/M) provided excellent image contrast. Different pharmacokinetic properties were observed when dynamic PET imaging was performed after co-injection of [68 Ga]Ga-NODAGA-RAMEB and PGE2 in BxPC3 tumor-bearing mice. In the presence of PGE2, the BxPC3 tumor cannot be identified in the first 5-10 minutes after injection. Thereafter, radiotracer accumulation increases in BxPC3 tumor with significantly high SUV values (SUV mean: 0.95 ± 0.20) at 30 min and 90 min post-injection (SUV mean: 1.12 ± 0.21). These mean SUV values are about 8-9 times higher (significance: $p \leq 0.01$) than the [68 Ga]Ga-NODAGA-RAMEB accumulation with simultaneous injection of PGE2. Furthermore, [^{68} Ga]Ga-NODAGA-RAMEB showed significant accumulation in background tissues (muscle, chest and abdominal organs) (Fig.…C). This is indicated by a significantly ($p \leq 0.01$) approximately 10 times lower tumor-to-muscle ratio (T/M SUV average: 1.36 ± 0.15) at 30 minutes and (T/M SUV average: 1.56 ± 0.23) at 90 minutes than that observed in co-injected PGE2 molecule.

PGE2 selectivity of [^{68} Ga]Ga-NODAGA-RAMEB in PancTu-1 tumor-bearing SCID mice

The PGE2 selectivity of [^{68} Ga]Ga-NODAGA-RAMEB was attested by using the low prostaglandin E2 receptor expressed PancTu-1 tumors. Representative decay-corrected PET images were made, 80-90 min post injection. By the qualitative analysis of the miniPET images it was found, that the BxPC3 tumors were clearly visualized however, the PancTu-1 tumor - with lower prostaglandin E2 receptor

(EP2) expression - did not differ sharply from the background tissues. 80–90 min after the i.v. injection of 68Ga-NODAGA-RAMEB the SUVmean, SUVmax, T/M SUVmean and T/M SUVmax values of BxPC3 tumors were 0.15 ± 0.04, 0.25 ± 0.03, 18.85 ± 2.64 and 18.32 ± 3.21, respectively By the assessment of the PET images at 80–90 min, we found significantly ($p \leq 0.01$) lower accumulation in the PancTu-1 tumors after using [^{68} Ga]Ga-NODAGA-RAMEB. The SUVmean (0.04 ± 0.01), SUVmax (0.08 ± 0.02), T/M SUVmean (1.33 ± 0.19) and T/M SUVmax (1.66 ± 0.22) values of PancTu-1 tumors were approximately 3–14- fold lower, than that of BxPC3 tumors, confirming the high PGE2 selectivity of the ^{68}Ga-labeled probe

***In vivo* assessment of [^{68}Ga]Ga-DOTAGA-RAMEB biodistribution in BxPC-3 tumor-bearing mice** During the next part of our study the tumor targeting potential of [^{68}Ga]Ga-DOTAGA-RAMEB was defined using *in vivo* PET imaging approximately 12 days after the subcutaneous injection of PGE2 positive BxPC-3 cancer cells. By the qualitative image analysis we found that the BxPC-3 tumors were clearly identified in the PET images using the [^{68}Ga]Ga-DOTAGA-RAMEB radiopharmaceutical (Fig. 4A). After the quantitative analysis of the decay-corrected PET images we found that from the 3rd minute (SUVmean: 0.36 ± 0.04) post injection SUVmean values of BxPC-3 tumors were depicted to show a continuous decrease until the 50th minute (SUVmean: 0.20 ± 0.04), then an equilibrium state has been reached (as shown in Fig. 4B). Based on these data we draw the conclusion that tumor-to-background (muscle) ratio, which is of remarkable importance regarding the evaluation of PET images was the highest 90 min after the injection of [^{68}Ga]Ga-DOTAGA-RAMEB, when T/M ratio was detected to be 2.5 ± 0.2

***Ex vivo* biodistribution studies of[^{68} Ga]Ga-NODAGA-RAMEB in tumor-bearing SCID mice**

For the assessment of PGE2 selectivity of [^{68} Ga]Ga-NODAGA-RAMEB *ex vivo* biodistribution studies were performed 30, 60 and 90 min post injection using BxPC3 tumor-bearing mice. demonstrates, that –except for the urinary system – the accumulation of [^{68} Ga]Ga-NODAGA-RAMEB in PGE2 positive BxPC3 tumors and the tumor-to-muscle ratios (T/M) were significantly ($p \leq 0.01$) higher at each investigated time point, than that of other organs and tissues, confirming the strong PGE2 binding property of the radiolabeled cyclodextrin probe. It was also found that the [^{68} Ga]Ga-NODAGA-RAMEB accumulation significantly ($p \leq 0.01$) reduced from 30 min to 90 min post injection in most of the investigated thoracic and abdominal organs and BxPC3 tumors Similarly to the *in vivo* PET data, when BxPC3 and PancTu-1 tumors were compared, significantly ($p \leq 0.01$) lower [^{68} Ga]Ga-NODAGA-RAMEB accumulation was observed in the PancTu-1 tumors by *ex vivo* measurements90 min post injection (Table 2). The %ID/g data revealed that approximately 5-fold lower radiotracer uptake was found in PancTu-1 tumors, than that of the accumulation of 68Ga-NODAGARAMEB in BxPC3 tumors 90 min post injection. In addition, after the co-injection of 68Ga-NODAGA-RAMEB and PGE2, approximately 20- fold higher tumor uptake (%ID/g) and 6-fold lower T/M ratio were found in BxPC3

tumors at 90 min post injection, than it was observed in the absence of the co-injected PGE2, and this differences were significant ($p \leq 0.01$).

Ex vivo biodistribution analysis of [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi-DOTAGA-RAMEB in BxPC-3 tumor-bearing mice

PGE2 positive tumor-bearing BxPC-3 mice were dissected 30, 60 and 90 min after the injection of the radiopharmaceuticals and the radioactivity of the organs and the tissues were determined by gamma-counter. Statistically no significant difference was found between the %ID/g values of the examined organs and tissues in case of either radiotracers 30 and 60 min post injection, in the mentioned table. 90 min after the injection of [^{68}Ga]Ga- DOTAGA-RAMEB low concentration levels were detected in the examined organs and tissues. In contrast, [$^{205/206}$Bi]Bi-DOTAGA-RAMEB showed high uptake in the spleen, colon, stomach and in adipose tissue at the same investigation time point (Fig. 5). By analyzing the accumulation of [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206Bi}$]Bi-DOTAGA-RAMEB in the prostaglandin E2 positive BxPC-3 tumors, it was found that there was no significant ($P \leq 0.05$) difference between the two radiopharmaceuticals at any investigated time points.

Immunhistochemistry

After the *ex vivo* tests, the presence of the prostaglandin E receptor (EP2) could be confirmed by immunohistochemical tests in the subcutaneously growing BxPC3 and PancTu-1 tumors. The 7B. shows strong EP2 receptor positivity and intense cytoplasmic/membrane expression in the xenograft pancreatic carcinoma cells. In contrast, we could observe a lower signal intensity in PancTu-1 tumors. These results correlated well with the results of *ex vivo* and *in vivo* studies, further confirming the strong binding affinity of [^{68}Ga]Ga-NODAGA-RAMEB to the PGE2 molecule. In separate experiments, we examined the presence of the PGE2 receptor in subcutaneously growing BxPC3. Strong receptor positivity characterized the tumors both in the cytoplasm and in the cell membrane. These results confirm the high binding affinity of the PGE2 receptor in the case of [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi-DOTAGA-RAMEB in accordance with the *ex vivo* and *in vivo* studies.

4. Discussion

The first project in this study, NH2-RAMEB was modified with NODAGA chelating agent and was labeled with Gallium-68 radioisotope to produce a promising radiotracer[^{68}Ga]Ga-NODAGA-RAMEB for *in vivo* PET imaging of PGE2 positive tumors. *in vivo* PET imaging of PGE2 positive tumors.

For the synthesis of [^{68}Ga]Ga-NODAGA-RAMEB molecule, NODAGA chelator was selected, as it is able to form a stable thioureido linkage during the coupling reaction. In this study NODAGA was conjugated to the primary amine of the RAMEB using conventional robust and reproducible coupling protocol The radiolabeling procedure was performed similarly to our previous study, and this well-

established protocol resulted in a composite material with high radiochemical purity (above 98.0%) and the molar activity of 15.34 ± 1.93 GBq/ μmol. Similar results were found when [^{68}Ga] Ga-NODAGA-HPβCD was synthesized and characterized by our research group. The radiochemical purity of the ^{68}Ga-NODAGA-HPβCD was also higher than 98%, and the specific activity was approximately 17 GBq/μmol, confirming that our method was optimized for the radiolabeling of cyclodextrin derivatives with [^{68}Ga]Ga-NODAGA (Hajdu et al., 2019). The applicability of the radiotracer was evaluated by *in vitro* and *in vivo* stability studies. The *in vitro* metabolic stability was monitored after 30, 60, 90 and 120 min incubation time in order to match the timeframe with the residence of the tracer in the body. The stability test showed that there was no 68Ga loss during the 120 min evaluation period in mouse serum. These results demonstrated that the [^{68}Ga]Ga-NODAGA-RAMEB tracer is stable in the presence of serum, which play an important role to consider for intravenous administration. The *in vivo* metabolic stability was determined at 60 min post injection after intravenous administration. The *in vivo* stability results correlated well with the *in vitro* results, as no radio-metabolite was found in the urine. These observations indicate that the newly prepared radiotracer remained stable in the body during the circulation and excretion, and this has been abundantly sufficient for further *in vivo* experiments. By the determination of partition coefficient, we found that the logP value of ^{68}Ga-labeled NODAGA-RAMEB was −3.63 ± 0.04, suggesting that the tracer is highly hydrophilic. The dynamic *in vivo* PET imaging and *ex vivo* biodistribution studies revealed that the [^{68}Ga]Ga-NODAGA-RAMEB was mainly excreted by the kidney due to its hydrophilic properties that has been proved by the partition coefficient, in alignment with our other radiolabeled cyclodextrin ([^{68}Ga]Ga-NODAGAHPβCD), which was also highly hydrophilic (logP:-3.07 ± 0.11) (Hajdu et al., 2019). The accumulation of the tracer in other organs of SCID mice was negligible, and the timeactivity curves demonstrated that a fast elimination occured from the body. These results are consistent with those of other researchers, where rapid elimination was also observed through the

By comparing the ex vivo (ID%/g) and *in vivo* (SUVs) biodistribution data of the two 68Ga-labeled cyclodextrin probes, relatively lower [^{68}Ga]Ga-NODAGA-RAMEB accumulation was observed in the investigated abdominal and thoracic organs and tissues, than that of the [^{68}Ga]Ga-NODAGA-HPβCD (Hajdu et al., 2019).However, the uptake ratio of organs comparing to each other is approximately the same. Furthermore, in case of the [^{68}Ga]Ga-NODAGA-RAMEB relatively high lung accumulation was also observed at each investigated time point by *ex vivo* measurements. The possible reason for this has been already described (Hajdu et al., 2019) for [^{68}Ga]Ga-NODAGA-HPβCD, and based on this the highly hydrophilic RAMEB may also accumulate in the water compartments of the lung, and it takes time to return into the circulatory system in mice. However, when biodistribution studies were performed in the presence of PGE2 [^{68}Ga]Ga-NODAGA-RAMEB was co-injected intravenously with 1 mg PGE2), we found that the radiotracer was not excreted rapidly from the body, abundance of the radioactivity remained in the body after 90 min post injection. One explanation for this observation can

be that the [^{68}Ga]Ga-NODAGA- RAMEB-PGE2 complex is a moderately enlarged molecule, less hydrophilic (logP: approx. −3) and these properties prevent the rapid renal elimination and increases the circulation time in blood vessels. Another reason can be the effect of the interaction of PGE2 with the blood plasma proteins (Raz, 1972), which also can increase the circulation time.

We wanted to assess if this complexation takes place also *in vivo* and if this complexation has an influence on the accumulation of the radiolabeled RAMEB in the cancerous tissues, which showed elevated density of PGE receptors. For this reason, we have compared the radiotracer-binging ability of a solid phase resin and a resin conjugated with PGE2. Our measured results support the published hypothesis the PGE2 conjugated resin has bound significantly higher activity than the unmodified resin.This result may prove that PGE2 plays a significant role in the relationship between [^{68}Ga]Ga-NODAGA-RAMEB and tumor.

In this present study we have focused on *in vivo* PET imaging to attest the PGE2 selectivity of the [^{68}Ga]Ga-labeled NODAGA-RAMEB molecule. For tumor induction in CB17 SCID mice BxPC3 and PancTu-1 cancer cell lines were used. It was earlier reported, that PGE2 production is elevated in BxPC3 human pancreatic adenocarcinoma cell line. Furthermore, it was verified, that the activation of EP1 and EP2 receptors requires significantly higher levels of PGE2. Other research group also found strong secretion of PGE2 and high expression of EP2 receptor in BxPC3 cell line, when different human pancreatic cancer cells were characterized by *in vitro* assays (Takahashi et al., 2015). The PGE2 production of PancTu-1 cell line was also investigated. In 2015, Gonnermann's research group verified by flow cytometry and Western Blot analysis, that the COX-2 expression and PGE2 production is very low in PancTu-1 cell line, when different pancreatic ductal adenocarcinoma cells were investigated (Gonnermann et al., 2015). In this study we also found remarkable presence of EP2 receptors in BxPC3 tumors, and lower receptor expression in PancTu-1 tumors, which was verified by immunohistochemistry, confirming that subcutaneously growing BxPC3 tumors retained this property *in vivo*. These differences between the BxPC3 and PancTu-1 tumors was clearly visualized by *in vivo* PET imaging (Fig. 5) and *ex vivo* gamma counter measurements after intravenous injection of ^{68}Galabeled NODAGA-RAMEB. Since our preliminary experiments with BxPC3 tumors showed that the highest tumor-background ratio (T/M) was observed 80–90 min after the injection of [^{68}Ga]Ga-NODAGA-RAMEB, PancTu-1 tumors and the effect of the co-injected PGE2 were investigated during this time period in comparative studies. We found, that the accumulation of 68Ga-NODAGA-RAMEB was significantly ($p \leq 0.01$) higher in BxPC3 tumors than in the PancTu-1 tumors characterized by lower EP2 receptor expression and PGE2 production. By analyzing the dynamic PET images, we found, that the highest tumor-background ratio (T/M) was obtained at 80–90 min post injection, and this high T/M value greatly influenced the contrast and evaluation of the PET images, and the identification of the tumors. The T/M SUV values of PancTu-1 tumors were approximately 10-fold lower, than those of BxPC3 tumors, confirming the high PGE2 selectivity of the ^{68}Ga-labeled cyclodextrin. In our other

experiments, after the co-injection of [^{68}Ga]Ga-NODAGARAMEB and PGE2 different pharmacokinetic properties were observed. We found that the SUVmean values of BxPC3 tumors were significantly ($p \leq 0.01$) higher (approx. 8–9-fold), than that of the [^{68}Ga]Ga-NODAGA-RAMEB accumulation without the co-injected PGE2. In addition, significant radiotracer accumulation was observed in the background tissues. From these data we concluded, that [^{68}Ga]Ga-NODAGA-RAMEB-PGE2 complex prevents a rapid renal clearance and increases the circulation time of the radiolabeled RAMEB-PGE2 in blood vessels, resulting a slower, but higher accumulation of [^{68}Ga]Ga-NODAGARAMEB in BxPC3 tumors.

Overall, our results suggest that there is a strong connection between the PGE2-EP2 and the [^{68}Ga Ga-NODAGA-RAMEB, but the exact targeting mechanism is still not clear. Further investigations are needed to clarify if the radiotracer binds first to PGE2 and then together to the EP2 receptor or [^{68}Ga Ga-NODAGA-RAMEB targets the already EP2 receptor-bound PGE2, or it is also possible that the two processes take place in simultaneously, however, their dynamics are not fully determined. Furthermore, these processes can be greatly influenced by the microenvironment of the tumor, its properties (e.g.: prostaglandinproduction, hypoxia, etc.), and EP2 receptor density. Overall, with the methods used in our study, the following results confirmed the close connection between the PGE2 molecule and [^{68}Ga]Ga-NODAGA-RAMEB:

In our second project we aimed to synthesize a RAMEB cyclodextrin derivative that binds with high affinity to PGE2-positive tumors and can be radiolabeled with both diagnostic and therapeutic isotopes. For this reason, the DOTAGA chelator was chosen for the synthesis of ^{68}Ga and $^{205/206}$Bi-labeled radiopharmaceuticals. DOTA and their derivatives represent a significant group of complexing agents in biomedical applications due to their excellent complexing properties against many metal ions and the high kinetic stability of their metal complexes In this study DOTAGA bifunctional chelator was conjugated via the amino group of NH2-RAMEB.DOTAGA contains four free acidic groups and an activated group, which was able to react specifically with an amino group For these types of bifunctional chelators that contain a pentafluorophenyl ester or succinimidyl ester group, no protection/deprotection reactions and time-consuming chromatographic work are required. The in this way newly synthesized DOTAGA-RAMEB is suitable for radiolabeling with both ^{68}Ga and $^{205/206}$Bi isotopes ablished protocols, similar to our previous work, resulting in products with high radiochemical purity (above 98.0 %). In case of ^{68}Ga labeling generator was eluted with ultra pure HCl (c = 0.1 M, pH = 1). The strongly acidic milieu (pH < 2) is inadequate for radiolabeling, therefore the eluates were buffered using 1 M sodium acetate to provide a pH of 4.3–4.5. In all preparations, the ^{68}Ga-eluate was buffered and was added directly to the DOTAGA-RAMEB which resulted an efficient robust radiolabeling reaction. In case of $^{205/206}$Bi labeling the radiolabeling reaction must be done at a pH of 8.2–8.5. This pH values reducing precipitation of bismuth hydroxide and results an effective labeling reaction. In this case the $^{205/206}$Bi-solution was added directly to a pre-buffered DOTAGA-RAMEB precursor. The applicability of our

radiolabeled products was then evaluated by *in vitro* and *in vivo* stability tests. The *in vitro* metabolic stability was monitored after 30, 60 and 90 min incubation time, based on our previous studies, because this interval corresponds to the time residence of the tracer in the body. The stability test showed there were no ^{68}Ga and $^{205/206}$Bi isotopes leakage or tracer fragmentation during the 90 min investigation time period. Results demonstrated that the ^{68}Ga and $^{205/206}$Bi labeled derivatives were stable in the presence of serum, similarly to our previous compounds, which is important for intravenous administration. *In vivo* metabolic stability was evaluated 60 min after intravenous administration of radiotracers. As no radioactive metabolite was found in the urine, it was determined that the *in vivo* stability results are consistent with the *in vitro* data. These experimental results demonstrated that the newly prepared [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi-DOTAGA-RAMEB are stable during the circulation and excretion, which is essential for further *in vivo* experiments. During the determination of partition coefficient, it was found that the log*P* value of ^{68}Ga-labeled derivative was 3.47 ± 0.04, and the $^{205/206}$Bi-labeled derivative was 3.45 ± 0.03 suggesting that these tracers are highly hydrophilic. In the first of the preclinical studies, biodistribution of [^{68}Ga]Ga- DOTAGA-RAMEB was determined in healthy control CB17 SCID mice applying *in vivo* PET imaging. Urinary excretion of [^{68}Ga]Ga-DOTAGA-RAMEB could be visualized on the decay-corrected PET images. Average log*P* values of 3.5 indicating highly hydrophilic property could underpin this observation. This result is consistent with the biodistribution data of [^{68}Ga]Ga-NODAGA-RAMEB previously examined by our research group, where renal excretion was revealed as well according to the log*P* values. As for other organs and tissues, low tracer accumulation was depicted by *in vivo* PET imaging both in case of the previously examined [^{68}Ga]Ga-NODAGA-RAMEB and [^{68}Ga]Ga-DOTAGA-RAMEB produced by the exchange of the chelator. Time-activity curves demonstrate rapid tracer clearance. Our elimination results are in line with the outcomes of experiments with other types of cyclodextrins (HPβCD) done by different laboratories, which reported renal excretion during similar examinations on humans and rodents performed with unlabeled and radiolabeled HPβCD. During the second phase of the preclinical research, tumor-targeting capability of [^{68}Ga]Ga-DOTAGA-RAMEB was assessed in PGE2-positive tumor-bearing mice by *in vivo* PET imaging. Already confirmed by other research teams, PGE2 overexpression and EP2 receptor upregulation characterized those BxPC-3 cancerous cells that were used in our experiments We also verified the presence of PGE2 receptors of subcutaneously growing BxPC-3 tumors applying immunohistochemical methods. Due to the strong receptorial overexpression tumors could be accurately localized by *in vivo* PET imaging after the intravenous injection of [^{68}Ga]Ga-DOTAGA-RAMEB Highest T/M ratio (2.5 ± 0.2) could be experienced 90 min post injection, which is of considerable significance regarding the evaluation of PET images. Comparing this with our previous examinations using BxPC-3 tumors, the highest tumor-to-background ratio was also observed at 80–90 min post injection. Summarizing the results of *in vivo* imaging, we concluded that the exchange of the chelator NODAGA for DOTAGA did not influence either the biological behavior of ^{68}Ga-labeled RAMEB or its PGE2-positive tumor-targeting ability Since due to its physical characteristic features

$^{205/206}$Bi could not be detected by PET scanner, the comparative analysis of the accumulation of [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi-DOTAGA-RAMEB was performed using a gamma counter. Based on the %ID/g results of the *ex vivo* experiments, no statistically significant difference was detected between the %ID/g values of the examined organs and tissues 30 and 60 min after the injection in case of either radiotracers. As for some organs, the uptake of the two radiopharmaceuticals differed at 90th minutes post injection. The [^{68}Ga]Ga-DOTAGA-RAMEB accumulation was low in the given organs at this time of measurement. These data are in accordance with the results of *in vivo* PET studies and those of previous research with [^{68}Ga]Ga-NODAGA-RAMEB and [^{68}Ga]Ga-NODAGA-HPβCD. Conversely, intensive accumulation of [$^{205/206}$Bi]Bi-DOTAGA-RAMEB was noticed in the spleen, colon, and adipose tissue, that could not be explained by the 3.45 log*P* value of the molecule. Although, physicochemical features of $^{205/206}$Bi may underpin our result, large scale future studies are required for the correct interpretation of this finding. Considering the tumor-targeting capability of the tracers, we revealed that both [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi]Bi- DOTAGA-RAMEB were taken up by PGE2-positive BxPC-3 neoplasms and no remarkable difference was experienced between the %ID/g values of the two radiopharmaceuticals at either time of the analysis ($p \leq 0.05$).

Both newly synthesized [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$Bi] Bi-DOTAGA-RAMEB showed specific binding in PGE2-positive BxPC-3 tumors. Due to its high selectivity and rapid elimination, [^{68}Ga]Ga-DOTAGA-RAMEB seems to be a promising radiotracer in the PET diagnostics of PGE2-positive tumors. Furthermore, [$^{205/206}$Bi]Bi- DOTAGA-RAMEB, as a model compound of [^{213}Bi]Bi-DOTAGA-RAMEB, may promotes the biological studies and radiochemical developments in the field of radionuclide therapy of PGE2-positive malignancies.

5. Summary

The application of cyclodextrin derivates in the medical and pharmaceutical industres is very diverse. CD-s have excellent drug carrier properties It is known that cyclodextrin derivatives are effective in the therapy of several diseases. During my doctoral studies, we aimed to synthetize radiolabeled-randomly methylated beta-cyclodextrin,and investigate its tumor-targeting properties. These derivatives can potentially serve as new radiopharmaceuticals in pancreatic tumor diagnostics and therapy.

In our first project, we produced a radiolabeled cyclodextrin derivative ([^{68} Ga]Ga-NODAGA-RAMEB), which proved to be suitable for the detection of BxPC3 pancreatic adenocarcinoma tumors. The NODAGA- RAMEB was successfully labeled with the ^{68} Ga isotope,and then subjected to analytical tests. We obtained a robust, reproducible radiopharmaceutical with high radiochemical purity, which was extensively tested in preclinical *in vitro*, *in vivo* and *ex vivo* studies. Based on these tests, we found that the metabolic stability of [^{68} Ga]Ga-NODAGA-RAMEB is excellent, it can accumulate specifically in the tumor, it is eliminated from the body through the urinary system, and it does not accumulate in other organs.

The aim of our second study, we replaced the diagnostic isotope with a therapeutic isotope. We produced a cyclodextrin derivative that may can suitable for BxPC3 pancreatic adenocarcinoma tumor therapy. DOTAGA-RAMEB was labeled with ^{68}Ga and $^{205/206}$Bi radionuclides. We produced two reproducible, robust radiopharmaceuticals with high radiochemical purity: [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$ Bi]Bi-DOTAGA-RAMEB. Both radiopharmaceuticals were subjected to extensive biological tests, during which we found that they accumulate specifically in pancreatic tumor cells. The [^{68}Ga]Ga-DOTAGA-RAMEB and [$^{205/206}$ Bi]Bi-DOTAGA-RAMEB eliminated renally, and do not show specific accumulation in other organs.

Based on our results, radiolabeled cyclodextrins further expanding the applications of cyclodextrins in pharmaceutical and medical industry.

6. Publications related to the dissertation tot he candidate

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Subject: PhD Publication List

Candidate: Katalin Csige
Doctoral School: Doctoral School of Pharmacy

List of publications related to the dissertation

1. **Csige, K.**, Péli-Szabó, J., Kálmán-Szabó, I., Dénes, N., Szikra, D. P., Képes, Z., Opposits, G., Méhes, G., Kertész, I., Fenyvesi, F., Trencsényi, G., Hajdu, I.: In vivo investigation of Gallium-68 and Bismuth-205/206 labeled beta cyclodextrin for targeted alpha therapy of prostaglandin E2 receptor-expressing tumors in mice.
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DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár

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