P001 FOLATE RECEPTOR TARGETING WITH DENDRIMER 99MTc-PAMAM-FOLIC ACID CONJUGATE

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Objectives: Poly(amidoamine)(PAMAM) dendrimer was first to be synthesized between 1979 and 1985.It is possible to precisely control their molecular properties by choosing different building/branching units and surface functional groups. The large numbers of surface functional groups on dendrimer's outer shell can be modified or conjugated with a variety of interesting guest molecules. Over the last several years, increasing interest has been attracted to the application of dendrimer as targeting carriers in cancer therapy and image. The system of dendrimer-folic acid conjugate labeled by fluorescein has been investigated widely for targeted drug Over the last several years, increasing interest has been attracted to the application of dendrimers as targeting carriers in cancer therapy and MR imaging. Recent studies have demonstrated that the conjugation of special targeting moieties with dendrimers can lead to preferential distribution of the cargo in the targeted tumor cells delivery.

Methods: dendrimer PAMAM G5 reacted with folic acid, the DTPA was conjugated in the second step. ^{99m}Tc labeled the conjugate by coordinated with DTPA. The in vitro/in vivo stability was test according to reported method. Micro-SPECT image study was performed in another laboratory in our institute.

Results: At 6h post administration, much accumulation was observed in the tumor cells via SPECT image and biodistribution study. Relatively good tumor selectivity was shown by the dendrimer folic acid conjugate.

Conclusions: We synthesized ^{99m}Tc labeled dendrimer-folic acid with excellent in vitro/in vivo stability, its biodistribution and micro-SPECT image was study in KB tumor bearing nude mice.

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P002 IN VIVO ANTIBODY UPTAKE AND PHYSIOLOGICAL MEASUREMENTS IN MOUSE LUNG AND MUSCLE: A COMPARTMENTAL PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING APPROACH

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Objectives: Pharmacokinetic properties of therapeutic antibodies in plasma and tissues are key determinants of both pharmacodynamic response and level of toxicity. The ability to quantitatively assess drug concentrations in plasma, interstitial, and cellular compartments can enhance the understanding of many aspects of drug delivery. Measurable tissue physiological parameters such as fractional interstitial and blood volumes can be used in a physiologically-based pharmacokinetic model to allow parameter estimation and to describe antibody uptake more thoroughly than traditional tissue distribution studies alone.

Methods: Fractional vascular and interstitial spaces of normal mouse tissues, including muscle and lung, were measured using in vivo ^{99m}Tc red blood cell labeling and ^{111}In-DTPA infusion, respectively. A separate group of mice was administered unlabeled trastuzumab or a control IgG at three dose levels (0.1, 1, or 10 mg/kg) spiked with ^{125}I-labeled antibody that was labeled indirectly via succinimidyl [^{125}I]-iodobenzoate. For both studies, plasma and tissue samples were collected by retroorbital bleed and terminal organ harvest, respectively, at designated time points and counted for radioactivity in the appropriate gamma energy window. A compartmental model presented in Cancer Research 52 377-384 (1992) was adapted to describe the muscle and lung uptake of antibody in mice. Two model parameters, the blood-to-tissue transport (k) and the interstitial fluid flow (L), were estimated from plasma and tissue interstitial concentrations at selected time points. A third parameter, the product of two constants characterizing in vivo antigen binding properties (K_aB_o), was assumed zero due to a lack of antigen expression in either tissue.

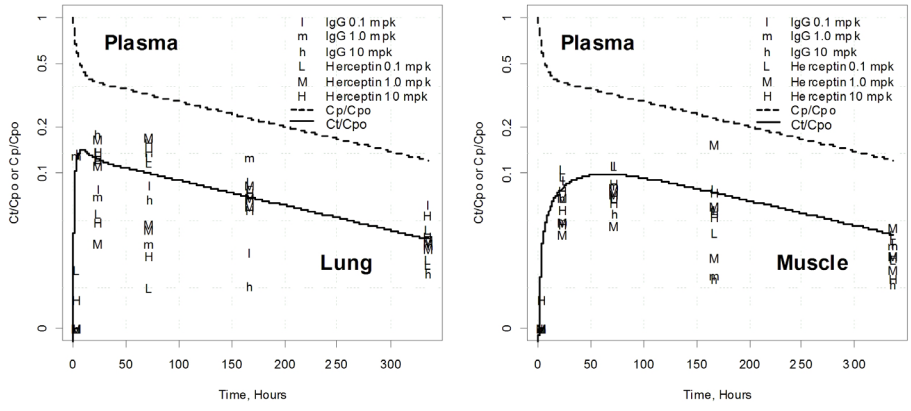
Results: A much higher vascular volume was found for lung relative to muscle (181 ± 44 versus 12 ± 6 μL/g). The difference in interstitial volume for lung and muscle was not significant (203 ± 72 versus 134 ± 50 μL/g). This data can also be expressed in percentages:

Percent Tissue Composition by Volume (*assuming 1 g tissue = 1 mL*)

	muscle	lung
% Vascular	1.23	18.1
% Interstitial	13.4	20.3
% Intracellular	85.3	61.6

Greater antibody accumulation was observed for lung compared to muscle at early time points, and both k and L were estimated to be higher for lung than muscle by an order of magnitude; higher standard errors in the lung data likely reflect greater experimental variability due to blood content upon tissue harvest. No dose dependence in uptake or significant differences between trastuzumab or IgG uptake in lung or muscle were observed, consistent with low HER2 expression in both organs.

Tissue Interstitial (C_t) and Plasma Concentration (C_p) / Initial Plasma Concentration (C_{po}) vs Time
dose levels (H/high, M/medium, and L/low) expressed in mg/kg (mpk)



Conclusions: The combination of a compartmental model of antibody uptake coupled with measurement of physiological parameters using selected radioprobes offered a powerful platform for studying tissue disposition and PK of an antibody therapeutic. This strategy may also be useful in studying the mechanism of actions in tumor tissues and possible toxicities related to uptake of antibody-drug conjugates in normal organs.

P003 IN VIVO STABILITY OF RADIOLABELED BOMBESIN ANALOGS IN MICE

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Objectives: Selections of peptides for peptide receptor targeting, either for scintigraphy or therapy are currently based, among others, on their good bombesin receptor-targeting characteristics and in vitro stability. Accordingly, we selected 4 bombesin analogs from literature and included a DOTA-conjugated non-stabilized bombesin as control (DOTA-Pro-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-Ala Val-Gly-His-Leu-Met-NH_2, BP2346). In vitro serum stability data (t½) for the stabilized analogs are reported in the range of 3 hours to days. Our aim was to investigate and compare this with the t½ in vivo, therefore, we labeled the DOTA- and DTPA-bombesin analogs with ^{111}In, and Demobesin-1 with ^{99m}Tc, and determined t½ in vivo in a comparative study in control mice. In addition, tissue distribution was performed to correlate bombesin recepptor-mediated uptake versus serum stability.

Methods: BP2346 and BP2653 (DTPA-aCMpip-Tha-Gln-Trp-Ala-Val-βAla-His-Tha-Nle-NH_2 are from BioSynthema, St Louis, MO, USA. DOTA-CH_2CO-Gly-(4-aminobenzyl)-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (Amba) and DOTA-PESIN (DOTA-PEG4-BN(7–14)) are from Prof H.R. Mäcke, University Hospital Basel, Switzerland). Demobesin-1 (N4-Phe-Gln-Trp-Ala-Val-Gly-His-NHEt) is from Dr. T. Maina, Demokritos, Athens, Greece. Mice were injected with 30 MBq (1 nmol peptide) of ^{111}In-labeled analogs or 60 MBq (1nmol) ^{99m}Tc Demobesin-1. Blood samples were collected in test tubes at 5 or 15 min post injection, n=2 per timepoint. Samples were mixed with ethanol to a final ethanol concentration of ≤ 50 % (v/v), vortexed and centrifuged (9500g). Supernatant was filtered (0.22µm Millex-GV) and recentrifuged prior to HPLC (Waters Breeze), based on a 1525 binary HPLC pump connected to a Unispec MCA γ-detector (Canberra, Zellik, Belgium). Stationary phase: Symmetry C_{18} column (5µm, 4.6 mm×250 mm, Waters). Biodistribution after the adminstration of 10 pmol radiolabeled analogs was performed at 1 h pi.

Results: Extraction of activity from serum was ≥ 90%.In contrast to reported long in vitro t½ values, all ^{111}In-labeled DOTA- and DTPA-conjugated bombesin analogs had t½ in vivo in the range of 2-3 min, with no mutual significant differences, and not significantly different from native bombesin. In vivo t½ of ^{99m}Tc-Demobesin was 11 min. Although, the here determined in vivo t½ values are relatively short, high and specific bombesin receptor-mediated uptakes for these analogs are reported, and also confirmed in this study. The relevance of increased in vivo stability of bombesin analogs was not confirmed in vivo. Since the receptor-mediated uptakes are high, the interactions between the receptor and bombesin must be rapid. Further experiments on the hypothetical need of increased in vivo t½ stability to increase specific receptor-mediated uptake need to be performed. However, with increased in vivo t½ stability prolonged pharmacological side effects must be anticipated.

Conclusions: In vitro serum t½ of bombesin analogs have no predictive value for the t½ in vivo in mice.

P004 A POTENTIAL METHOD TO MONITOR ENZYME REPLACEMENT THERAPY FOR TREATMENT OF GAUCHER DISEASE USING PET

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Objectives: Gaucher disease is the most common lysosomal storage disorder and results from a lack in the catalytic activity of Glucocerebrosidase. The most successful treatment of this disease is enzyme replacement therapy where a recombinant, modified form of the enzyme is intravenously injected into Gaucher patients. Lowering the costs and improving the efficacy of enzyme replacement therapy are of great interest to the medical and scientific community. Such efforts would greatly benefit by the development of a method to noninvasively and directly monitor the biodistribution of the infused enzyme. We have sought to develop a mechanism-based approach to radioactively label Glucocerebrosidase in the active site of the enzyme. Positron emission tomography will be used to monitor the biodistribution of the injected enzyme, thus molecular imaging at the protein level.

Methods: We radiochemically synthesized various ^{18}F-fluorosugar glycosides that act as mechanism-based inactivators of β-glucosidases. One of these labeling agents was used to radioactively tag glucocerebrosidase in vitro and a method to isolate radiochemically pure enzyme was developed. Preliminary biodistribution data in mice was obtained using microPET and postmortem tissue activity counts.

Results: We developed the radiochemical synthesis of several mechanism-based ^{18}F-fluorosugars, one of which was successfully used to radioactively label glucocerebrosidase within reasonable reaction times. Subsequent control experiments demonstrated that the incorporation of the ^{18}F isotope was in the active site of the enzyme via a covalent ^{18}F-fluoro-glycosyl-enzyme intermediate. Preliminary biodistribution data in mice was determined with microPET and correlated well with previously known distribution data based on crude tissue assays. In addition, it was demonstrated that tracer uptake in the liver was a mannose receptor-dependent process, providing support that tracer uptake is labeled enzyme and not some other ^{18}F labeled species.

Conclusions: We have developed a method to radioactively tag Glucocerebrosidase through the use of mechanism-based inactivators and used the labeled enzyme and PET for biodistribution studies in mice. Preliminary results support the proposal that PET can be a useful method for monitoring enzyme replacement therapy in treatment of lysosomal storage diseases.

P005 TIME–DEPENDENT PREFERENTIAL IN VIVO D2 OCCUPANCY BY AMISULPRIDE IN THE MEDIAL STRIATUM – CONTINUOUS MEASUREMENT USING A BETA MICROPROBE SYSTEM

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Objectives: Recently, our group 1 and others 2 have described the radiosyntheses of 18F- based bifunctional molecules that include 2-substituted pyridines, activated towards efficient nucleophilic heteroaromatic [18F]fluorination, and robust terminal alkyne groups. In our case, [18F]FPy5yne (1) was used to 18F label an azide- modified peptide by way of a CuI- mediated Huisgen [3+2] cycloaddition reaction. In the hopes of expanding this technique to include nucleic acid- based molecular probes, we now report the radiobioconjugation of 1 to a model DNA antisense sequence (18F-ODN), which is antisense to mdr1 mRNA.

Methods: All studies were carried out with the approval of the local ethical committee. 0.5mm diameter beta microprobes were implanted in the medial striatum of one hemisphere and in the lateral striatum of the opposite hemisphere, with a further probe implanted in the cerebellum as a reference region. D2/D3 receptor binding was measured using the high-affinity D2/D3 radioligand ^{18}F-fallypride. ^{18}F-fallypride binding was measured for 60 minutes before the injection of amisulpride.

Results: Non-decay-corrected, collected yield from start of bioconjugation reaction was 43.2% (57.8% decay- corrected) after HPLC purification. 18F-ODN (4.03 mCi) was formulated in 1 mL nanopure water. Starting from 92.6 mCi at end-of-bombardment, 4.4% non- decay corrected yield (24.9% decay- corrected) of 18F-labelled antisense oligonucleotide was obtained. Total unoptimized synthesis time was 276 minutes from end-of-bombardment. [IMG]

Conclusions: We hypothesize that this preferential action on a subset of striatal dopaminergic neurons may play a role in the reduced side-effect profile of amisulpride compared to typical antipsychotics. This research was supported by FRS-FNRS grant no. 3.4593.09 and by Johnson & Johnson Pharmaceutical Research & Development.

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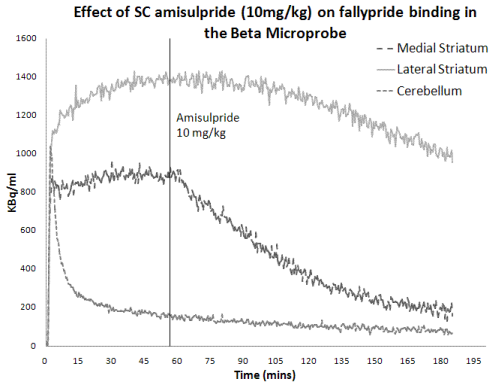

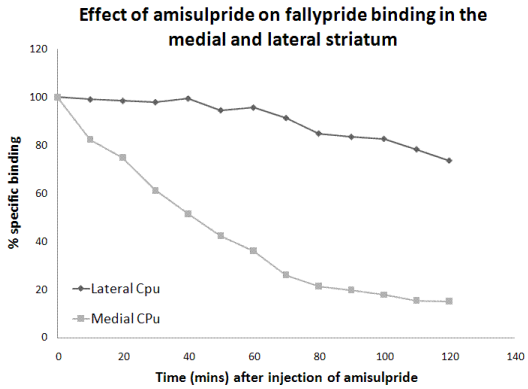

P006 THE USEFULNESS OF AN ARTERIOVENOUS SHUNT COMBINED WITH A BETA MICROPROBE FOR THE MEASUREMENT OF INPUT FUNCTION IN RATS

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Objectives: An accurate arterial input function is essential for many kinetic models with PET and beta microprobe data. This includes functional data such as glucose metabolism, measured using FDG, and receptor binding using radioligands. The usual method for determining input function is manual blood sampling at specific time points followed by counting of the radioactivity in a given volume. However, the measurement of input function via arterial blood sampling generally has poor time resolution and can lead to significant blood loss in small rodents. Beta microprobe systems have recently become available as a tool for measuring the concentration of PET ligands in specific brain regions. These systems utilise implantable probes consisting of a small (0.25-1mm diameter, 1mm length) scintillation crystal from which impulses are carried by fibre optic to a photomultiplier tube, allowing the generation of a time-activity curve with a very high (1 second) temporal resolution. Pain et al (2004) used two beta microprobes to measure input function with one probe directly in the femoral artery. However, in this method the activity in surrounding tissues influenced the accuracy of the input function. Weber et al (2002; 2003) used an arteriovenous shunt and coincidence counter to measure input function while using beta microprobes in the brain.

Methods: We have combined these two techniques to accurately measure input function with high temporal resolution (1 second) and no loss of blood. All studies were carried out with the approval of the local ethical committee. Under isoflurane anaesthesia, catheters were implanted in the femoral artery and vein for connection of an arteriovenous shunt. Using our apparatus a beta microprobe can be reproducibly positioned directly in the flow of blood. To reduce cardiovascular load on the animal the shunt is driven by a peristaltic pump. Via the shunt it is also possible to infuse tracer or treatment compounds, and to continuously measure blood pressure. Blood samples can easily be taken in a controlled manner where necessary for the determination of metabolites. This is demonstrated for the case of ^{18}F-fallypride.

Results: We have used our apparatus to measure input function in beta microprobe studies of the brain, and furthermore have combined the measurement of input function in this way with PET scanning in rats.

Conclusions: Thus, kinetic modelling can be carried out without the need for a reference region or less accurate estimation of input function. This research was supported by FRS-FNRS grant no. 3.4593.09 and by Johnson & Johnson Pharmaceutical Research & Development.

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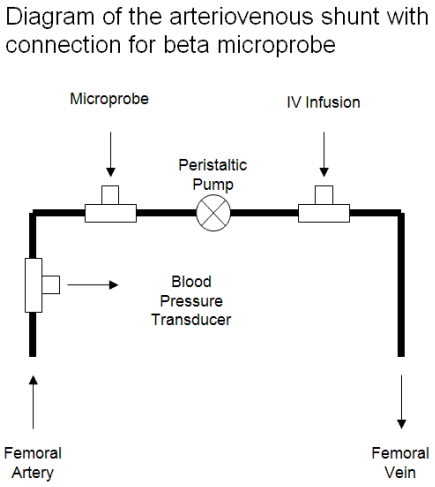

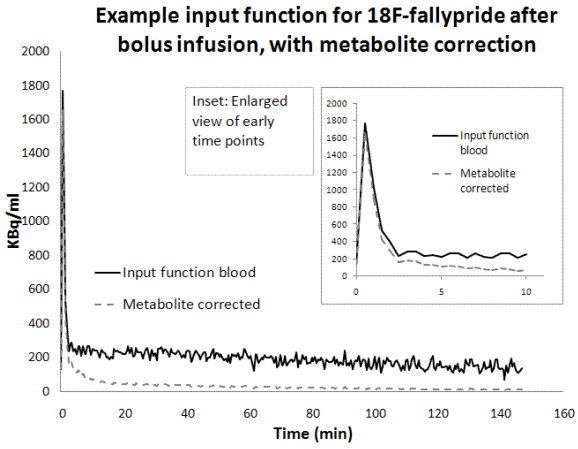

P007 EXPERIMENTAL ANIMAL STUDIES IN DEVELOPMENT AND EVALUATION OF NEW RADIOLABELLED MOLECULES

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Objectives: Current regulations impose severe constrains on the use of experimental radiopharmaceuticals in human subjects. However, the possibility to investigate the in vivo biological behavior of novel radiocompounds is mandatory to clearly establish which type of diagnostic information the new tracer is able to deliver and, therefore, to assess its true diagnostic benefit. A common solution to this problem involves the use of animal models that may replace and efficiently mimic the human situation. Obviously, a critical step in this approach is to identify the most appropriate animal model. The current molecular imaging paradigm requires the design and development of a new generation of radiolabelled molecules that can facilitate in vivo mapping of the biochemistry inner molecular biology of cell function. Interestingly, this type of highly selective recognition of disease-target sites at the molecular level may receive a higher benefit from animal studies than it was with previous generations of imaging agents because of the continuous improvement in our ability to provide disease-specific animal models through the manipulation of genetic information.

Methods: In our laboratory, we developed a number of rat and mouse models specifically devoted to evaluate the diagnostic accuracy of new SPECT radiopharmaceuticals in some pathological processes: a) receptor – mediated deep vein thrombosis (DVT), b) induced amyloidosis, c) sterile and bacterial inflammation, d) apoptosis in organ transplantation, e) induced IgA secretion at the genito-urinary mucosa, f) athymic nude mice tumor bearning to demonstrate specifity of pretargeting technique.

Results: These models have been progressively implemented to allow capturing of the basic molecular mechanisms underlying the interaction of the tracer with its biological target. The results for the quality of all designed animal studies were obtained after administration of specific radiolabelled molecules, visualization of the specific pathological regions of interest and biodistribution studies compared with in vitro binding and histopathological interpretation. All used radiolabelled preparations had high radiochemical purity and the specificity of experimental induced diseases performed as ratio pathological v.s. normal tissue was between 1.23 +/- 0.23 and 5.06 +/-0,86 respectively.

Conclusions: In this work, beside practical considerations including definition of the control groups, random assignment of animals to control/treatment groups, determination of the number of animals needed for each group and identification of the most appropriate statistical analysis, the effectiveness of animal models in providing biological information at the molecular level that can be actually translated to the human subject and the intrinsic limitations of this approach will be discussed by using different examples of experimental tracers.

P008 COMPARISON OF REFERENCE TISSUE MODEL VERSUS TWO COMPARTMENT MODEL WITH INPUT FUNCTION FOR [18F]FALLYPRIDE MODELLING. A COMBINED MICROPET - BETA MICROPROBE STUDY IN RATS

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Objectives: The quantification of in vivo receptor kinetics with PET tracer experiments is an intricate and challenging problem. This process not only requires measurement of the uptake and washout of the radioligand in tissue as a function of time (time-activity curve or tissue response function), but also of its delivery to the tissue (arterial input function). Today, in order to avoid arterial input function measurements, most of the receptors studies conducted with PET rely on the "Reference Tissue Model" (RTM, Lammertsma 1996a) or on its simplified version (SRTM, Lammertsma 1996b). Our laboratory has developed an accurate input function measurement technique for rats that can be used during microPET studies. The system consists of an arteriovenous shunt combined with a beta microprobe (Warnock 2009). This setup was used to compare the RTM and SRTM versus the two compartment model with input function for the D2/D3 ligand [18F]fallypride modelling.

Methods: Four rats were scanned for 120 min (list mode) with a Focus 120 (Siemens) microPET machine after [18F] fallypride injection (117-130 MBq iv, specific activity 22.9-35.9 GBq/μmol time of injection). Input functions were obtained (temporal resolution of 1 sec) with a beta microprobe (Swisstrace) placed directly in the blood flow of an arteriovenous shunt (Warnock 2009). Six blood samples were taken during the time course of the study for metabolite measures (HPLC). Data were reconstructed using FBP with all corrections. A rat MRI template (Schweinhardt 2003) was used for coregistration. Image processing and kinetic analysis were performed using PMOD (3.0 for kinetics and 2.7 for image processing) software. Time activity curves were extracted for striatum (L & R) and for cerebellum. Binding potentials were calculated with RTM and SRTM using cerebellum as reference tissue. For the two compartment model, the ratio k3/k4 was taken as the binding potential (BP).

Results: The binding potential values obtained for each study are summarized in the table presented below. RTM and SRTM gave exactly the same results with BP values around 2. The two tissue compartment model with the metabolite corrected input curve led to much higher BP of about 10.

Binding potential values.

	RTM (4-parameter)	SRTM (3-parameter)	2-Tissue compartment model with metabolite corrected input (k3/k4)
#1401	2.07	2.01	10.30
#2701	2.00	1.96	11.00
#0602	2.51	2.46	9.94
#2202	2.57	2.54	10.93

The Figure presents: (A) microPET-MRI template corregistrated; (B) microPET image of [18F]fallypride (summed image 0 - 120 min); (C) microPET image and ROIs used for quantification.

Conclusions: These results highlight the fact that quantification of in vivo receptor kinetics with [18F]fallypride calls for careful evaluation and serious validation before any use. The much higher BP results obtained with the metabolite corrected input curve raise the question of the actual usefulness of cerebellum as reference tissue for [18F]fallypride modelling. This research was supported by FRS-FNRS grant n°3.4593.09 - 3.4551.08 and by Johnson & Johnson Pharmaceutical Research & Development.

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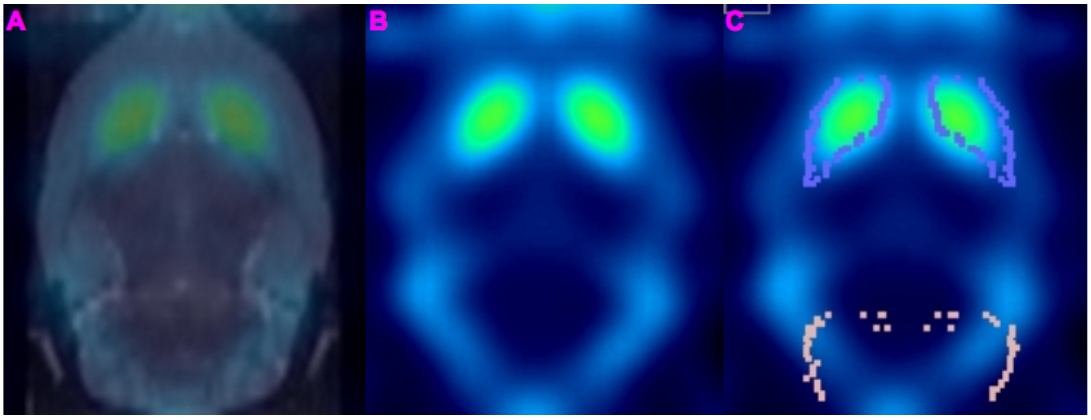

P009 RADIOCHEMICAL LIMITATIONS ENCOUNTERED DURING PREPARATIONS OF RADIOPEPTIDES FOR PEPTIDE RECEPTOR-MEDIATED PROCESSES IN ANIMAL SPECT

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Objectives: Radiolabeled peptides such as analogs of octreotide, bombesin, substance P and CCK are investigated in nuclear medicine. Targeting of these peptides are via high affinity, and frequently low capacity receptors. The receptor-mediated uptake are optimal (in % of the dose) in the low nanomolar range, since, among others, the amounts of receptor are limited. Thus only picomole of peptide can be administered. Currently, radiopeptides are investigated by animal SPECT, and images at high resolution can be achieved. For this application, however, high activities are required, while only small amounts of peptide (picomoles) can be administered. As a consequence, high specific activities of these radiopeptides are needed. Therefore, the radiochemical limitations encountered during preparations for animal SPECT were investigated.

Methods: DOTA-peptides were labeled with $^{111}InCl_3$, a typical example: 50 MBq of a ^{111}In-labeled DOTA-bombesin analog is injected in a 25 gram mice and at an optimized amount of peptide of 0.01 nmol thus a specific activity of 5 GBq ^{111}In per nmol DOTA-bombesin is required. However, this is 2.5-times above theoretical maximal specific activity of ^{111}In, and 6-times above highest achieved and reported specific activity for ^{111}In-DOTA-peptides, conflict 1. Additions like buffer and quenchers need to be done to adjust the pH post radiolabeling and to protect the radiopeptide versus radiolysis (due to high amounts of activity in a small volume), conflict 2. These additions increases osmolarity rapidly above physiological osmolarity ($\approx$ 300 mosmol) and not conform required isotonic physiological conditions, conflict 3. Biodistributions by dissection are preferably performed at optimized amounts of peptide, e.g. 0.01 nmol DOTA-bombesin, and thus not concordant with conditions applied during SPECT studies, conflict 4. Purifications and concentrations, also by RP C_{18} HPLC were investigated in order to find solutions for these conflicts.

Results: Radiometal-labeled DOTA-peptides could be separated from non-labeled DOTA-peptides, e.g. by RP C_{18} HPLC, and confirmed by MS. In the here discussed picomole range the total amount of peptide could not be quantified accurately. HPLC purification of ^{111}In-labeled DOTA-peptides from $^{111/112}$Cd-labeled was not successful. Metal ions of $^{111/112}$Cd and ^{111}In incorporated in the DOTA of the peptide, but could not be separated from each other, as confirmed by MS. ^{111}Cd and ^{112}Cd are present in $^{111}InCl_3$, ^{112}Cd as the target material for the production of ^{111}In and ^{111}Cd is formed by decay of ^{111}In. Moreover, during the process of purification and concentrating, increased radiolysis and variation in recoveries were encountered. As a consequence, at this level of radioactivity, in small volumes and in this range of mass the total amount of intact peptide can be estimated, but only with large uncertainty. The specific activity could only be determined, accordingly.

Conclusions: Radiochemical limitations hamper visualization at high resolution of peptide receptor-mediated processes under optimal physiological conditions by animal SPECT.

P010 SERUM STABILITY STUDIES OF F-18-ETHYL-RHODAMINE B

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Objectives: Rhodamine dyes are lipophilic cations that share several biological properties with ^{99m}Tc-MIBI including accumulating in the myocardium and being substrates for Pgp, the protein implicated in MDR1 multidrug resistance. The synthesis of ^{18}F-ethyl-rhodamine B was previously published by our group[1]. Initial microPET studies of the biodistribution of this compound in mice showed that it was rapidly excreted through the gall bladder with minimal accumulation in the heart, which led us to suspect that there might be in vivo hydrolysis of the ester bond of the ^{18}F-ethyl group resulting in loss of the ^{18}F label. The objective of this project was, therefore, to investigate the stability of ^{18}F-ethyl-rhodamine B in the serum of several species to measure possible in vivo enzymatic hydrolysis of ^{18}F-ethyl-rhodamine B.

Methods: The radiosynthesis of ^{18}F-ethyl-rhodamine B was accomplished using the same procedure as previously described[1]. Briefly, rhodamine B lactone was treated with 2-^{18}F-ethyltosylate in acetonitrile to produce crude 2'-^{18}F-ethylrhodamine B, which was purified by semi-preparative RP-HPLC, dried and dissolved in 10% ethanol in PBS. For each stability experiment, 40-100 μCi (in 20 μL) of ^{18}F-ethyl-rhodamine B was added to plasma (0.20 mL) in a borosilicate culture tube equilibrated to 37°C in a water bath. The contents were shaken and incubation was continued for 15, 30, 60, and 120 minutes. Enzymatic hydrolysis was stopped by the addition of cold (4°C) absolute ethanol (1 mL), and the samples were cooled in an ice bath to precipitate the serum proteins. The samples were then centrifuged (15 mm, 2500 x g, 4°C) and the supernatant was analyzed by RP-HPLC.

Results: The results (Figure 1) clearly show that ^{18}F-ethyl-rhodamine B is hydrolyzed rapidly in mouse serum, decreasing from 87% intact ^{18}F-ethyl-rhodamine B after 15 min. to only 29% after 2 h. In contrast, the ^{18}F-ethyl-rhodamine B was 95% intact in human serum and 86% intact in rat serum at the end of the 2-h incubation period. The control sample, ^{18}F-ethyl-rhodamine B in PBS, showed no hydrolysis of the ester linkage after 2 h.

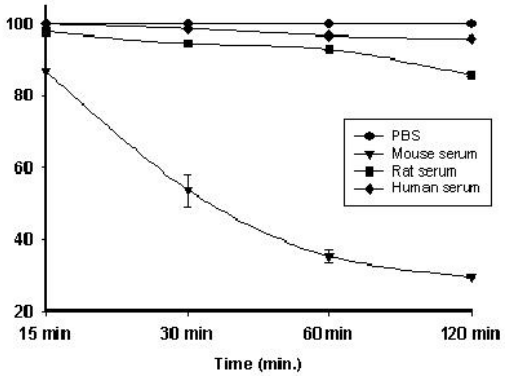

Figure 1: Stability of ^{18}F-Ethyl-Rhodamine B in Different Sera

Conclusions: In summary, the rapid hydrolysis of ^{18}F-ethyl-rhodamine B was probably caused by esterases in mouse serum that are present in much lower quantities rat and human serum: the ^{18}F-ethyl-rhodamine B is quite stable both in rat and human serum. Additional microPET imaging and biodistribution studies are planned to determine these differences in the in vitro stability of the compound result in improved in vivo stability and thus improved myocardial uptake. Efforts are also underway to characterize the product(s) of the hydrolysis reaction.

Research Support: This research was partially supported by grant number R01 CA94338 (NCI)

References: 1. Heinrich TK, Treves ST, Packard AB, Development of fluorine-18-labeled rhodamine B derivatives for myocardial perfusion imaging with positron emission tomography.Presented at the SMI/AMI Joint Molecular Imaging Conference, Providence, RI, September 2007.

P011 RADIO-ACTIVATION OF TiO2 NANOPARTICLES FOR USE IN NANOTOXICOLOGY STUDIES, AND AS A MODEL SUBSTANCE FOR ACTIVATION OF PHARMACEUTICALLY RELEVANT NANOPARTICLES

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Objectives: Radioactive nanoparticles are of great interest for the development of innovative radio-therapeutic applications and for toxicological studies. Activation procedures of such particles necessitate a careful examination of possible structural changes that might alter their biological effect. The purpose of this study was to determine if manufactured TiO_2 nanoparticles can be activated by high energy proton irradiation without causing significant matrix structural damage, and whether the activated nanoparticles behave in a biologically similar fashion to the non-activated material.

Methods: Specially designed capsules were filled with TiO_2 nanoparticle powders, and inserted into a target holder. Proton irradiations were performed with a Cyclotron in order to produce ^{48}V as a radiotracer ($T_{1/2}$=15,97d). Possible structural damage to the nanoparticles was investigated by X-ray diffraction. The activated TiO_2 powder was recovered in a colloidal solution of 12mg of TiO_2 powder and 2ml water. The solution was then subjected to ultracentrifugation at 41000rpm at a temperature of 4°C for 1h in order to determine the stability of the ^{48}V within the nanoparticles. Calu-3 cells, a human bronchial cell line, were seeded at the apical side of the transwell at a density of 105cells/cm^2. Seven days post-seeding the air-interfaced culture was established by aspirating the apical volume and after 14 days in culture, Calu-3 cells were exposed for 72h to cold TiO_2 or [^{48}V]-TiO_2 nano-sized particles in dispersion at concentrations of 50, 200, 500μM. After exposure the cells were gently detached from the transwell membrane, harvested and washed twice with PBS solution and one time with Percoll gradient and the cell number was determined. the uptake of cold nanoparticles was determined by ICPMS while that of the radioactive nanoparticles was determined by gamma-ray spectrometry.

Results: An activity of 850kBq of ^{48}V was obtained at the end of the cyclotron bombardement. A slight change in the X-ray diffraction pattern after irradiation indicates minor changes to the crystal structure in terms of a slightly reduced anatase to rutile ratio. The exposure of the Calu-3 cell cultures to 200 and 500mM solutions yielded an uptake of 1.15pg/cell and 2.25pg/cell respectively for cold TiO_2 particles, and of 0.96pg/cell and 1.98pg/cell respectively for [^{48}V]-TiO_2 activated nanoparticles. This difference is not significant for the present experimental procedure.

Conclusions: Manufactured TiO_2 nanoparticles can be successfully radiolabelled by proton irradiation. All the produced radioactivity stays within the nanoparticles and the activation process does not significantly damage the nanoparticle structure. In the present experiments the uptake in Calu-3 cell cultures did not differ between activated and cold nanoparticles. From this we conclude that their size distribution and their physical and chemical surface properties are not modified by the proton activation to an extent that significantly affects their biological uptake.

P012 FEASIBILITY OF USING C-14 LABELLED MP4A FOR THE ASSESSMENT OF ACETYLCHOLINESTERASE INHIBITOR IN TISSUE SAMPLES WITH MINIMAL DILUTION

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Objectives: Acetylcholinesterase (AChE) inhibitors have been used as medicals, pesticides and chemical warfare agents. Exposure assessments of such chemicals have been performed by measurement of AChE activity in a tissue by traditional method using acetylthiocholine (ATCh) and [^{3}H/^{14}C]acetylcholine ([^{3}H/^{14}C]ACh). However, the substrates used in these methods are not specific for AChE, and these methods with the photometry detection require at least dozen times of tissue dilution. The dilution could affect the equilibrium between protein-bound and unbound inhibitor, and the change of behavior of inhibitor by dilution could cause misestimating of the actual inhibition in tissues. N-[^{11}C]Methylpiperidin-4-yl acetate ([^{11}C]MP4A), a lipophilic acetylcholine analog, is used for in vivo measurement of cerebral AChE activity with PET. [^{14}C]MP4A would be expected as an agent for AChE specific measurement in tissue samples with minimal dilution in vitro. We now evaluate the feasibility of using [^{14}C]MP4A for the measurement of acetylcholinesterase activity in minimally diluted tissue sample with a simple radio-TLC analysis.

Methods: Initially, we validated the method for measuring the AChE activity with [^{14}C]MP4A comparing the traditional methods in a pure enzyme, and thereafter the effect of tissue dilution on the IC_{50} value of donepezil (AChE specific inhibitor) in monkey brain was examined with [^{14}C]MP4A. Furthermore, the feasibility of simultaneous measurement of AChE and butyrylcholinesterase (BChE) activity in a human blood sample were examined with a mixture of [^{14}C]MP4A and (R)-N-[^{14}C]methylpiperidin-3-yl butyrate ([^{14}C]MP3B_R), a BChE specific substrate.

Results: The hydrolysis rate of ATCh, [^{14}C]ACh and [^{14}C]MP4A were proportional to the AChE concentration (r^2>0.99). The ratio of hydrolysis rate of ATCh, ACh and [^{14}C]MP4A in the solution of AChE to that of BChE were 1.5, 5.3 and 40, respectively, indicating a markedly high AChE selectivity of [^{14}C]MP4A among the substrates. The estimated IC_{50} values of donepezil at 0.20, 0.084 and 0.00065 g/mL of the brain tissue concentration were 390±31, 95±7.3, 13±0.60 nM (estimate±SE), respectively, revealing that the tissue dilution strongly influences the inhibitory effect of donepezil. In simultaneous measurement of AChE and BChE activity in a human blood sample, [^{14}C]MP4A, [^{14}C]MP3B_R and their alcoholic metabolites were well separated by TLC and individually measurable. Thus, the measured hydrolysis rate with the mixture of both substrates ([^{14}C]MP4A, 1.2±0.012 $min^{-1}mL^{-1}$; [^{14}C]MP3B_R, 2.5±0.059 $min^{-1}mL^{-1}$; mean±SE) in a human blood was comparable to that with each [^{14}C]MP4A (1.1±0.011 $min^{-1}mL^{-1}$) and [^{14}C]MP3B_R (2.9±0.057 $min^{-1}mL^{-1}$) alone.

Conclusions: By use of the radio-labelled specific substrates, [^{14}C]MP4A and [^{14}C]MP3B_R, AChE and BChE activity were measured specifically and easily with lowly diluted multi-samples. Thus, this method is feasible for the quantitative exposure assessment of medicals, pesticides and chemical warfare agents against AChE and BChE.

P013 TOXICITY DETERMINATION OF COPPER LABELING THIOSEMICARBAZONES USING BRINE SHRIMP TEST

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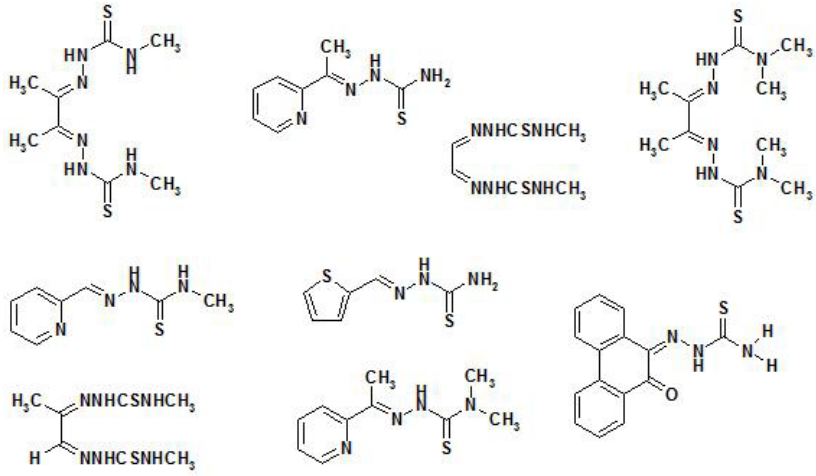
Objectives: The production of new copper radiopharmaceuticals has been initiated in last 2 decades and many radiolabeled thioseicarbazone copper complexes have shown to exhibit interesting imaging properties via various mechanisms such as perfusion, hypoxia and DNA proliferation etc.. In this study the toxicity of various well-known and new thiosemicarbazones (TSCs) used in the preparation Cu-radiopharmaceuticals has been tested using brine shrimp toxicity tests.

Methods: The dilutions (1ppm, 10ppm and 100 ppm) of TSC ligands were prepared in brine of (using 1% dimethylsulfoxide as the co-solvent). The Artemian Californica cysts were hatched after incubation at 25C for 24h at the well ventilated brine medium. The hatched shrimps were gathered using sun light and micropipets and the equal group numbers were pooled in different glass tubes containing test compounds in 1,10 and 100 ppm final dilutions in brine. Control samples were also checked for 1% DMSO in brine (negative control) and 1 ppm of Taxol (as positive) controls. The number of the dead animals in each tube for each compound dilution (n=3) were reported as LD, when controlled by positive and negative controls.

Results: Even at 1 ppm all the thiosemicarbazones showed lethality of more than 90%, showing the high toxicity of this starting materials. The tests were repeated several times and the results were reproducible.

Conclusions: The use of radiolabeled copper complexes is limited possibly due to the toxicity of starting un-labeled ligand s and the toxicity of these compounds must be well considered when administered to animals and human. Brine shrimp test is a valuable probe for the safety determination of these compounds. new techniques must be emplyed to decrease the amount of un-reacted starting materials



P014 THE IMAGING PROBE DEVELOPMENT CENTER: FACILITATING INTERDISCIPLINARY IMAGING RESEARCH THROUGH THE PRODUCTION AND SUPPLY OF MOLECULAR PROBES

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Objectives: The Imaging Probe Development Center (IPDC) was established as part of the NIH Roadmap for Medical Research Initiative with the realization that molecular imaging will be a key technology for advancing biomedical research in the 21st century. The center serves as a synthetic chemistry core facility dedicated to the production of known and novel molecular imaging probes. The IPDC's resources are currently being used by the NIH intramural community with plans to expand to the broader scientific community now being discussed.

Methods: IPDC scientists use their diverse backgrounds in organic, inorganic, radiochemical and conjugation chemistries to produce requested agents for all imaging modalities including the major ones of MRI, optical and PET/SPECT. IPDC produces radiolabeled agents and their custom precursors and improvements in methods and yields are continuously being sought. Our approaches include use of new automated synthesis systems and microwave-based chemistry.

Results: The variety of the radiolabeled agents produced at the time of writing is exemplified by the following list. They include I-124- and I-125-radiolabeled T3 and T3 analogs prepared for thyroid hormone action studies in CNS, I-125-labeled and tritiated vasopressin peptide analogs for receptor binding studies, I-125-labeled secreted frizzle-related protein also for receptor binding studies, [125I]MRS1898, a high-affinity, selective radioligand for studies on the rat adenosine A3AR receptor, F-18-radiolabeled Aposense, an apoptosis marker, and Lapatinib, a HER2 tyrosine kinase inhibitor. The latter two are under preparation for total synthesis of precursors and subsequent radiolabeling. A titanium dioxide-based Ge-68/Ga-68 generator system is also on site, coupled to an automated synthesis module and is being studied for radiolabeling of Ga-68-labeled Affibody molecules initially for pre-clinical small animal imaging and ultimately intended for clinical imaging of HER2 receptors. It will also serve as a resource for other projects involving Ga-68 radiolabeling.

Conclusions: IPDC is producing multiple diverse probes for use by molecular imaging scientists at NIH. For more information, or to make enquiries about assistance with probe availability please visit our website at: http://www.ipdc.nih.gov

Research Support: IPDC is supported by the NIH Roadmap for Medical Research Initiative

P015 PREPARATION OF 99mTc-ISOSULFAN BLUE FOR USE IN AXILLARY LYMPH NODE LOCALIZATION IN RATS

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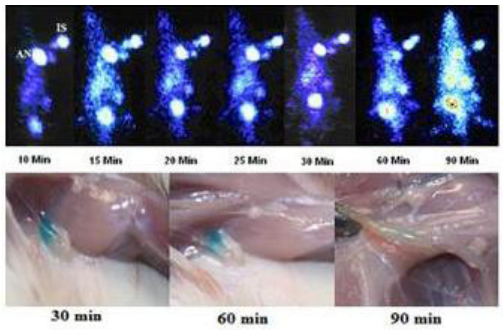
Objectives: So far, for SLN detection three strategies have been proposed; noncarcinogenic inert blue dye injection, radiocolloidal labeling (^{99m}Tc-nanocolloids) and combination of both (1). The purpose of this study is to investigate ^{99m}Tc-Isosulfan Blue as a new single agent that combines both detection methods. It travels quickly through lymphatic channels and is retained in the sentinel lymph node for a significant period of time.

Methods: Preparation of ^{99m}Tc- Isosulfan blue was carried out by reduction of ^{99m}Tc-pertechnetate in the presence of stannous chloride. Ascorbic acid was used as antioxidant. The final pH of solution was obtained as 6.8. The radiochemical purity and stability of the product was analyzed by Thin Layer Chromatography by using silica gel coated plastics sheets which developed in acetone and saline systems. Experiments performed with the rats were according to a protocol approved by Ethical Committee for Animal Research, University of Ege, and Faculty of Pharmacy. 3 male rats were used in study. Each one was anesthetized with a cocktail 0.65 ml of ketamine and 0.35 ml of xylazine. ^{99m}Tc-Isosulfan Blue (0.1 mCi, 0.1 ml) was injected subcutaneously in to the inside of the center of the paw. The animals were placed in supine position and static images were acquired in a 256 × 256 matrix for 300 sec each, at 10, 15, 20, 25, 30, 90 min after administration of ^{99m}Tc-Isosulfan Blue. After the imaging study, abdominal cavity and extremity was opened, axillary lymph nodes were scanned for the radioactivity by using a gamma probe. The percentage of ^{99m}Tc- Isosulfan blue that passed through the lymph node was calculated.

Results: Radiolabeling efficiency of ^{99m}Tc-Isosulfan Blue was found to be greater than 95% and stable for up to 4 h. Scintigraphic images clearly demonstrated uptake and deposition of ^{99m}Tc-Isosulfan Blue dye in axillary node.



Conclusions: ^{99m}Tc-Isosulfan Blue has several potential advantages over the current sentinel node protocols for surgeons; the requirement of only one procedure instead of two separate procedures, the potential for decreasing patient time in the operating room. These characteristics of the ^{99m}Tc-Isosulfan Blue makes it suitable for sentinel node detection.

References: 1. Albertini, J., Cruse, C., Rapaport, D., et al. (1996). Intraoperative radiolymphoscintigraph improves sentinel node identification for patients with melanoma, Ann Surg Oncol, 223:217-24

P016 ESTIMATES FOR THE BIODISTRIBUTION AND DOSIMETRY OF 68GE IN 68GA PET IMAGING

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Objectives: Recent years have shown a strong increase within Europe in the use of ^{68}Ge / ^{68}Ga generators for in vivo PET imaging. Generally ^{68}Ge will be present in a small activity admixture in the ^{68}Ga eluate by some breakthrough of the generator. Dosimetric consequences of small activities of ^{68}Ge in patients have not been well determined and form therefore a subject for speculative discussions. Evaluation of the existing biodistribution models for ionic ^{68}Ge with the ^{68}Ga decay product released during its uptake included. Estimates for the effective dose from ^{68}Ge and its daughter ^{68}Ga are calculated using the latest dosimetry models.

Methods: The biodistribution of ionic ^{68}Ge is described in ICRP30 (1982), but does not include the distribution of ^{68}Ga that is formed during its uptake in the body. For ionic ^{68}Ga there is a good description of the biodistribution as gallium-citrate in ICRP53 (1987). Both radionuclide biodistributions have been combined by using a pharmacokinetic compartment model. The radioactivity uptake kinetics in each organ was simulated and residence times were calculated by integration. The dosimetry code Olinda was used to calculate the organ dosimetry and the effective dose.

Results: Germanium leaves the blood circulation with a half-life of 6h. The urinary to fecal clearance pathway ratio is 1:1. The tissue clearance half life for both germanium and gallium is 34 h. The effective dose per injected activity is 0.034 mSv/MBq (0.016 mSv/MBq by ^{68}Ge and 0.018 mSv/MBq by ^{68}Ga). The highest dose is in the colon with 0.15mGy/MBq. Based on data of the currently available generators, breakthrough is at a level of $\leq$ 0.01 %, expressed as % of Bq ^{68}Ge per Bq ^{68}Ga. Suppose an administration of 100 MBq of a ^{68}Ga-labeled compound, and 0.01 % breakthrough this will imply 10 kBq ^{68}Ge. This breakthrough will cause an additional effective dose of 0.34 mSv, which is negligible in comparison to the effective dose from ^{68}Ga: 32 mSv (when injected as gallium-citrate).

Conclusions: Small amounts of ^{68}Ge activity present in ^{68}Ga elutions by breakthrough of the generator do not form a problem for the patient's dosimetry. The effective dose from ^{68}Ge including the ^{68}Ga formed during its body uptake is 0.034 mSv/MBq.