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Original Article

Organ biodistribution of Germanium-68 in rat in the presence and absence of [^{68}Ga]Ga-DOTA-TOC for the extrapolation to the human organ and whole-body radiation dosimetry

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Abstract: Positron Emission Tomography (PET) and in particular gallium-68 (^{68}Ga) applications are growing exponentially worldwide contributing to the expansion of nuclear medicine and personalized management of patients. The significance of ^{68}Ga utility is reflected in the implementation of European Pharmacopoeia monographs. However, there is one crucial point in the monographs that might limit the use of the generators and consequently expansion of ^{68}Ga applications and that is the limit of 0.001% of Germanium-68 (^{68}Ge(IV)) radioactivity content in a radiopharmaceutical. We have investigated the organ distribution of ^{68}Ge(IV) in rat and estimated human dosimetry parameters in order to provide experimental evidence for the determination and justification of the ^{68}Ge(IV) limit. Male and female rats were injected in the tail vein with formulated [^{68}Ge]$GeCl_4$ in the absence or presence of [^{68}Ga]Ga-DOTA-TOC. The tissue radioactivity distribution data was extrapolated for the estimation of human organ equivalent doses and total effective dose using Organ Level Internal Dose Assessment Code software (OLINDA/EXM). ^{68}Ge(IV) was evenly distributed among the rat organs and fast renal excretion prevailed. Human organ equivalent dose and total effective dose estimates indicated that the kidneys were the dose-limiting organs (185±54 μSv/MBq for female and 171±38 μSv/MBq for male) and the total effective dose was 15.5±0.1 and 10.7±1.2 μSv/MBq, respectively for female and male. The results of this dosimetry study conclude that the ^{68}Ge(IV) limit currently recommended by monographs could be increased considerably (>100 times) without exposing the patient to harm given the small absorbed doses to normal organs and fast excretion.

Keywords: Positron emission tomography, ^{68}Ga, ^{68}Ge, dosimetry, ^{68}Ge/^{68}Ga generator

Introduction

The Positron Emission Tomography (PET) field and, in particular, the utilization of ^{68}Ga radiometal is exponentially growing. The development and application of ^{68}Ga-based imaging agents for targeted, pre-targeted, and non-targeted imaging is expanding with acceleration [1], covering a broad range of tracers from small molecules to macromolecules and further to particles. The development of macrocyclic chelators, availability of ^{68}Ge/^{68}Ga generators, development of methods for ^{68}Ga pre-concentration and purification, and broad clinical experience with somatostatin analogues have considerably contributed to the growth of ^{68}Ga applications. ^{68}Ga has demonstrated the potential of becoming a PET analogue of generator produced gamma emitting ^{99m}Tc with added value of higher sensitivity and resolution, and importantly allowing in vivo quantification of tissue radioactivity and dynamic scanning. ^{68}Ga-labeling chemistry is amenable to kit type as well as automated radiopharmaceutical production, and is becoming a cost-effective complement to cyclotron-based tracers.

^{68}Ga has the potential to facilitate development of routine clinical PET imaging and promote the personalised medicine concept with PET for earlier and better diagnosis. The worldwide

appreciation of ^{68}Ga in clinical applications is reflected in the increasing variety of commercially available generators and publication of two European Pharmacopeia monographs, "Gallium (^{68}Ga) edotreotide injection" [2] and "Gallium (^{68}Ga) chloride solution for radiolabeling". One of the major concerns is the breakthrough from the ^{68}Ge/^{68}Ga generator of long-lived parent radionuclide, ^{68}Ge(IV), with a half-life of 270.8 d. The radionuclidic purity specified in the monographs as not more than 0.001% ^{68}Ge(IV) restricts the use of the generators and might limit clinical applications of ^{68}Ga. However, until now the dosimetry of ^{68}Ge(IV) has not been studied and the limit defined in the European Pharmacopeia monographs is based on a hypothetical assumption of total accumulation of ^{68}Ge(IV) radioactivity in the bone marrow with an infinite retention [3]. Experimental dosimetry data is needed urgently in order to set scientifically justified requirement for radionuclidic purity with regard to ^{68}Ge(IV) and avoid unnecessary hindrance of the worldwide use of ^{68}Ga-based radiopharmaceuticals.

While the dosimetry investigation of ^{68}Ge has not been performed, the metabolism, toxicity, carcinogenicity, mutagenicity, teratogenicity as well as myopathy and nephropathy of germanium in its anionic, cationic, organic and particulate chemical forms such as germanic acid ($Ge(OH)_4$), germanium dioxide (GeO_2), sodium metagermanate (Na_2GeO_3), spirogermanium, carboxyethylgermanium sesquioxide (Ge-132), germanium sulfide, colloids have been studied previously [4-9]. Oral, intramuscular, subcutaneous, intraperitoneal as well as intracardiac puncture administration routes were used.

Germanium dioxide is the most carefully investigated chemical form, especially for inhalation and ingestion because of the health issues of workers in electronic industry of semiconductors. The kinetics of the distribution of GeO_2 was studied in mice [10, 11]. The concentration of the compound in blood and tissues peaked within one hour after administration. Germanium could hardly be detected in any tissue 24 h after administration. The biological half-life in blood was 1.2 h. The kinetics of orally administered germanium dioxide distribution in rat revealed the half-life of absorption to be 0.7 h and that of elimination to be 2.3 h [12]. The half-life of the elimination in humans has been reported as 1.5 days [13]. In general, the inhaled and ingested inorganic germanium compounds are readily absorbed and excreted via kidneys with physiological half-life of 1-4 days [11, 14, 15]. It should be mentioned that the inorganic compounds of germanium were administered in high doses such as tens to hundreds of mg per kilogram animal weight. Only high doses of germanium dioxide orally administered during 8 months to rats produced myopathy [8].

Moreover, anticarcinogenic effect of germanium in the form of sodium metagermanate fed to rodents for 15-24 months as well as anticancer properties of organic germanium compound such as spirogermanium and carboxyethylgermanium sesquioxide in animals and man has been reported [4-6]. The blood clearance of spirogermanium was fast without accumulation in tissues. Low toxicity of orally administered inorganic germanium compounds with the highest concentration in the kidney was found also in humans [16]. Again only extremely high doses of germanium dioxide, sodium germanate or Ge-132 (100-2000 times exceeding average estimated dietary intake) ingested during prolonged period of time as elixirs in Japan resulted in renal failure and even death [9, 17]. The above mentioned administration routes (oral, intramuscular, subcutaneous, intraperitoneal, intracardiac puncture) result in rapid elimination from the blood circulation with predominant renal excretion and no deposition in tissue after 24 h [4, 17].

The studies with intravenous administration are fewer. Germanic acid administered to rats intravenously and intraperitoneally demonstrated delayed elimination in the latter case most probably reflecting the time required for the penetration into the blood stream as well as longer deposition in the spleen and kidney [18, 19]. The highest uptake amongst the organs was detected in kidney but the elimination was fast within a few hours. Fast distribution and elimination of amorphous germanium dioxide, germanium sulfide, colloidal and elemental germanium administered parentally and intravenously were observed in dogs and rabbits [15]. No significant tissue localization could be detected after 72 h. Even particulate germanium was readily dissolved in vivo and excreted.

In summary, germanium as a chemical is an element of low risk to man. Germanium is not

pharmacologically active and in vivo it acts as a non-toxic foreign material which is readily eliminated. The toxicity, carcinogenicity, mutagenicity of germanium and its compound are low or absent. Thus with regard to radioactive ^{68}Ge(IV) where the amounts of the element are negligible, the safety issue is reduced to ionizing radiation and, in particular the buildup of ^{68}Ga(III) at the sites of deposition of the ^{68}Ge(IV). It should also be taken into consideration that most of the radiation dose with positron-emitting radionuclides is due to the positrons rather than to annihilation photons.

The limit defined in the monograph for ^{68}Ge(IV), not more than 0.001% of the total radioactivity in a ^{68}Ga preparation, was calculated assuming high and infinite accumulation of the radionuclide in sensitive organs such as bone marrow [3]. Unfortunately, this model is not based on experimental data and does not reflect the actual biodistribution pattern. Our study was initiated with the aim to provide experimental data for the determination of the ^{68}Ge(IV) dosimetry and respective acceptable limit of ^{68}Ge(IV) content in the ^{68}Ge/^{68}Ga generator eluate and ^{68}Ga-based radiopharmaceuticals. The results of this experimental work are of strong interest to the clinical community using ^{68}Ga/PET, and the work has been conducted for the benefit of patients.

Materials and methods

Chemicals

The purchased chemicals were used without further purification: acetate buffer (Sigma-Aldrich), 30% HCl (Merck). DOTA-TOC (Anaspec, USA) was dissolved in deionised water to give 1 mM stock solution. [^{68}Ge]$GeCl_4$ (58 MBq) was obtained from IDB Holland BV in 0.1M HCl solution of 50μL. The radionuclide solution was diluted to 1 ml with 0.1M HCl for the further use.

Preparation of ^{68}Ge in the presence and absence of [^{68}Ga]Ga-DOTA-TOC

^{68}Ga was eluted with 0.6 M HCl from a 50 mCi ^{68}Ge/^{68}Ga generator (1850 MBq, IDB Holland BV, ^{68}Ge breakthrough: 0.0002%-0.5% within 1 year). The first fraction of 1 mL was discarded and the second fraction of 1 mL was collected and buffered with acetate buffer (200 μL) containing 60 μL of 10 M NaOH to yield pH of 4.6-5.0. Then ^{68}Ge(IV) hydrochloric solution (375 μL) and 15nmol of DOTA-TOC were added and the reaction mixture was incubated at 90-95 °C for 5-10 min. In the absence of [^{68}Ga]Ga-DOTA-TOC the eluent was used instead of the eluate and the rest of the procedure was kept the same. In all cases, after the completion of the reaction the solution was diluted with phosphate buffered saline (PBS) up to total volume of 9 mL in order to provide pH of 7.4 and the concentration of ^{68}Ge radioactivity of 1 MBq per injection. The final formulation was sterile filtered.

Analytical methods

High-performance liquid chromatography (HPLC) (Beckman, USA) with UV and radioactivity detectors coupled in series was used with C-5 reversed phase column (Discovery BIO Wide Pore, Supelco, USA). The conditions were as followed: A=10 mM TFA; B=80% acetonitrile, 20% H_2O, 10mM TFA; gradient elution: 0–2 min at 80% A, 2-13min at 80–2% A, 13-15min at 10-80% A; flow rate: 1.0 mL/min; sample solution with EDTA. Retention time (R_t) for the UV- and radio-HPLC signals of ^{Nat}Ga-DOTA-TOC and [^{68}Ga]Ga-DOTA-TOC was respectively 4.87±0.02 and 4.90±0.02 min. Retention time for ionic ^{68}Ge(IV) was 1.0±0.02 min. The recovery of the radioactivity from the column was determined performing analysis with and without column and collecting the fractions for the subsequent measurement of the radioactivity in a well-type NaI(Tl) scintillation counter.

Instant thin layer chromatography (ITLC-SG, Pall Life Sciences) with sodium citrate as mobile phase was used for the detection of ionic ^{68}Ge(IV) that moved with R_f of 1. The ITLC strips were exposed to a phosphor imager plate that was then scanned with PhosphorImager SI unit (Molecular Dynamics, UK) and analyzed using ImageQuant 5.1 software.

Radionuclide identity determination

The HPLC fractions corresponding to the ionic ^{68}Ge and [^{68}Ga]Ga-DOTA-TOC were collected and the radioactivity decay was monitored, respectively during 273 days for ^{68}Ge and 6 hours for ^{68}Ga for the accurate determination of the half-lives of the radionuclides. The ^{68}Ga emitted radioactivity was measured in an ion-

Table 1. Animal weights and injected doses

Tracer	N	Animal weight, [g]	Injected dose, [MBq]
[d][68Ga]$GaCl_3$	3	327.5±3.54	7.85±0.26
[d][68Ga]Ga-DOTA-TOC	4*	322.4±48.3	9.10±2.69
[c][68Ga]Ga-DOTA-TOC	1*	320	5.09
[c][68Ga]Ga-DOTA-TOC	1**	220	6.06
[a][68Ge]$GeCl_4$	5*	278.6±11.0	1.29±0.12
[a][68Ge]$GeCl_4$	5**	204.0±10.2	1.31±0.17
[b][68Ge]$GeCl_4$	5*	280.0±25.5	0.57±0.06
[b][68Ge]$GeCl_4$	5**	206.4±11.4	0.73±0.06

*Male; **Female; [a]In the absence of [68Ga]Ga-DOTA-TOC; [b]In the presence of [68Ga]Ga-DOTA-TOC; [c]In the presence of [68Ge]$GeCl_4$; [d]Ref. [27].

ization chamber (VDC-405, Netherlands) every 15 min. The ^{68}Ge decay was monitored via ^{68}Ga in the transient equilibrium with the former measured in the well counter. The radioactivity readings were converted to their logarithms and the values were plotted against the corresponding time: $ln(A_i) = ln(A_0)\lambda t$ for the subsequent determination of the half-life ($T_{1/2} = \frac{ln(2)}{slope}$).

Organ distribution

Healthy female and male Sprague-Dawley rats were kept at a constant temperature (25 °C) and humidity (50 %) in a 12 h light-dark cycle, and given free access to food and water. The animal permission was granted by the local Research Animal Ethics Committee C 38/9.

Ten female and ten male rats were divided into two groups of ten rats each. One group (five male and five female) was given [68Ge]$GeCl_4$ and the other group received [68Ge]$GeCl_4$ together with [68Ga]Ga-DOTA-TOC. The radiopharmaceuticals were injected into the tail vein of unsedated animals as a bolus in 0.5-0.6 ml of phosphate buffered saline (pH 7.4) as the vehicle. For each group one rat of each gender was sacrificed after 1, 6, 24, 48, and 168 h, respectively. Organs were immediately extracted, weighted and their radioactivity measured in a well-type NaI(Tl) scintillation counter, applying correction for dead-time and for decay. To discriminate between contribution from the radioactivity of ^{68}Ga and ^{68}Ge, at the 1 and 6 h time points, in the group co-injected with [68Ge]$GeCl_4$ and [68Ga]Ga-DOTA-TOC, measurements were done twice; immediately after dissection of organs and >48 h post injection. The samples were stored refrigerated. At all time points the remaining corpus was also measured to allow monitoring of radioactivity elimination and allowing estimation of recovery. The organ radioactivity readings were decay-corrected to the time of injection, and results were expressed as standardized uptake values (SUV).

Dosimetry calculations

Human absorbed dose estimates were calculated with the OLINDA/EXM package (Version 1.1, Vanderbilt University, USA, 2007) [20], using the measured residence times and the dose rate S-values. The SUV values in rat were first multiplied with the appropriate decay factor dependent on the time point of the data post-injection, then multiplied by standard organ masses and divided by the standard total-body weight for the standard adult (female) man phantom obtained from the OLINDA/EXM software. The values obtained correspond to the fraction of injected radioactivity (%IA) per organ in man as a function of time. The residence time, which is the cumulative radioactivity for the organs, was determined by integrating the area under the time-radioactivity curves for each organ using the trapetzoid method where a simple exponential decay was assumed to occur from the last data point to eternity (as if there was no further biological decay, but only physical decay). The residence time for the remaining carcass was calculated in the same way as for the other organs and this value was used as input in the OLINDA/EXM software as “Remainer”. The biokinetic model of OLINDA/EXM dosimetry software takes into account the contribution of the daughter ^{68}Ga.

Statistics

Average values (mean) and their corresponding standard deviation (SD) were calculated with Excel (Microsoft) or GraphPad Prism version 5.0 (GraphPad Software, San Diego, California, USA). Average of several measurements were presented as mean±SD, and the number of measurements was given as (N=number of measurements). Non-linear regression analyses were made with GraphPad Prism and the resulting fitted variables were reported as the

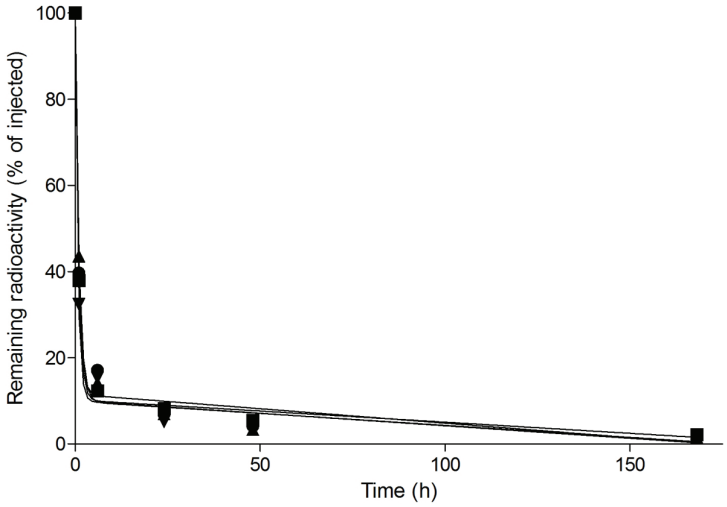

Figure 1. Graph (R^2=0.994±0.003; N=4), showing the elimination kinetics of [^{68}Ge]$GeCl_4$, administered intravenously, alone or in combination with [^{68}Ga]Ga-DOTA-TOC. A total of 20 animals were used – 5 of each gender receiving one of the two combinations of radionuclides. ● female Ge; ■ male Ge; ▲ female Ga/Ge; ▼ male Ga/Ge. As shown in the graph, the elimination kinetics was practically identical in the four different groups.

mean±SD. Goodness of fit was presented as the R^2 value.

Results

Organ distribution

A total of 20 rats (10 female and 10 male) were injected with [^{68}Ge]$GeCl_4$ alone (N=10) or in combination with [^{68}Ga]Ga-DOTA-TOC (N=10). Detailed descriptions of animal weights and amounts of radioactivity which were administrated are found in **Table 1**. In order to investigate if the presence of a radiopharmaceutical might influence the distribution and organ residence time of ^{68}Ge(IV) in rat, two sets of experiments were performed. In one [^{68}Ge]$GeCl_4$ was co-injected with [^{68}Ga]Ga-DOTA-TOC. The radioactivity content of ^{68}Ge in relation to [^{68}Ga]Ga-DOTA-TOC was 15.10±0.25%. The specific radioactivity of [^{68}Ga]Ga-DOTA-TOC at the end of the synthesis was 27±2 MBq/nmol. The injected peptide amount was 1±0.1 nmol. In the other set of experiments, solely [^{68}Ge]$GeCl_4$ of similar amount was administered.

The distribution of radioactivity for a number of organs was determined together with the remaining radioactivity (not excreted) for each animal and time point and it was found that radioactivity rapidly was eliminated/excreted in both genders and for both combinations of radionuclides (**Figure 1**). The elimination kinetics was best described by a single exponential phase, accounting for 89.5±0.8% of the signal, followed by a steady-state phase. In average, only 1.8±0.3% of the germanium remained in the animals 168 h after administration and the half-life for the elimination was 36±5 min.

The distribution of radioactivity was determined for 17 organs and is presented as decay-corrected SUV values in **Figure 2**. ^{68}Ge(IV) showed a rapid clearance from blood to tissues (SUV in blood was ~0.5 already 1 h post injection) and displayed a pronounced washout pattern from all organs. Essentially, the only organ displaying SUVs above 1 was kidney indicating renal excretion both in male and female rats.

Dosimetry

The rat SUV data was extrapolated to the human species for the dosimetry calculations assuming similarity of biodistribution pattern in human and rat. The radiation dose to the organs was calculated assuming homogeneous distribution of radioactivity throughout the organ. The theoretical residence time for ^{68}Ge(IV), assuming retention in the organism for its whole physical life-span, is very long (~9377 h), while we found from the recovery measurements that due to elimination this decreased to 193±12 h, i.e. only ~2% of the theoretical value. The longest residence times were observed for the remaining body, followed by kidneys, liver and bone and values were similar for male and female (**Figure 3**).

The determined residence times were entered into the OLINDA/EXM program to calculate organ doses as well as the total effective dose, the latter using the recommended tissue weighting factors from International Commission on Radiological Protection (ICRP, 2007). The whole body (effective) and organ equivalent doses (μSv/MBq) obtained from

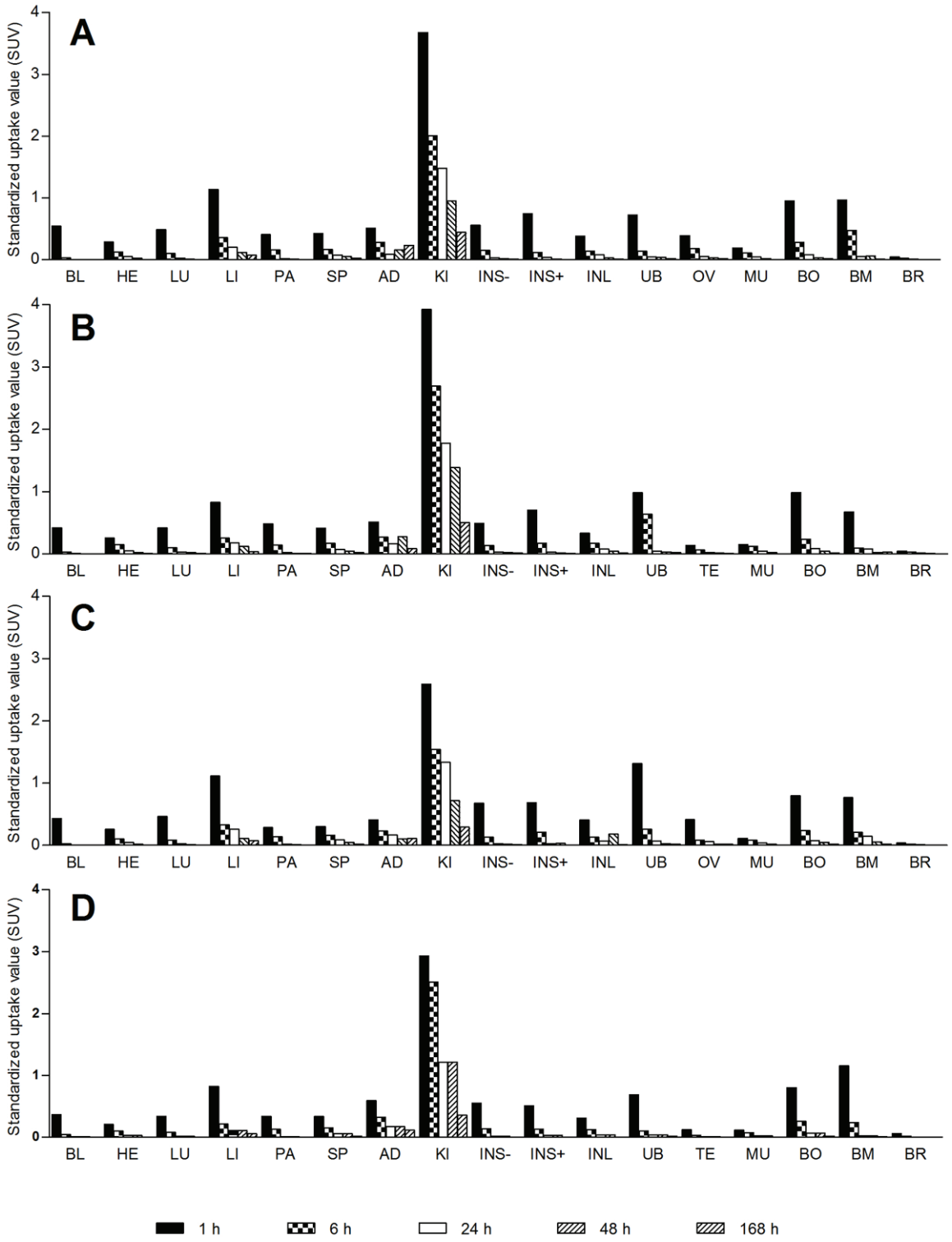

Figure 2. Graph, summarizing the distribution in 17 rat organs of [^{68}Ge]$GeCl_4$, administered intravenously, alone or in combination with [^{68}Ga]Ga-DOTA-TOC. A total of 20 animals were used – 5 of each gender receiving one of the two combinations of radionuclides. BL – blood; HE – heart; LU – lungs; LI – liver; PA – pancreas; SP – spleen; AD – adrenals; KI – kidneys; INS- – small intestine without its content; INS+ – small intestine with its content; INL – large intestine; UB – bladder; OV – ovaries; TE – testes; MU – muscle; BO – bone; BM – red bone marrow; BR – brain. A: distribution of [^{68}Ge]$GeCl_4$ in female rats; B: distribution of [^{68}Ge]$GeCl_4$ in male rats; C: distribution in female rats of [^{68}Ge]$GeCl_4$ co-injected with [^{68}Ga]Ga-DOTA-TOC, and D: distribution in male rats of [^{68}Ge]$GeCl_4$ co-injected with [^{68}Ga]Ga-DOTA-TOC.

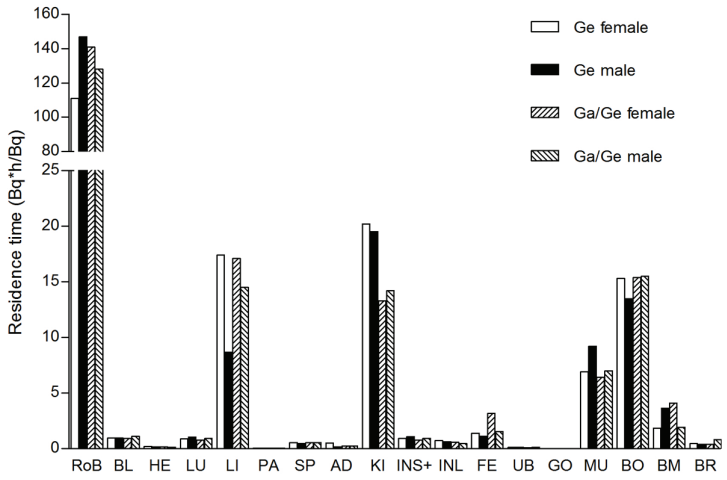

Figure 3. Graph showing the residence times in different organs for [^{68}Ge]$GeCl_4$ administered alone or in combination with [^{68}Ga]Ga-DOTA-TOC. The values were extrapolated to human female and male from rat *ex vivo* determinations. These values were used for dosimetry calculations in order to obtain organ and total doses using software OLINDA/EXM. It should be noted that the values for "Rest of body" were determined for all animal-associated radioactivity that was not in the excised and measured organs. RoB – rest of body; BL – blood; HE – heart; LU – lungs; LI – liver; PA – pancreas; SP – spleen; AD – adrenals; KI – kidneys; INS+ –small intestine with its content; INL – large intestine; UB – bladder; GO – gonades; MU – muscle; BO – bone; BM – red bone marrow; BR – brain.

OLINDA/EXM calculations are presented in **Table 2**. Noticeable was that for practically all organs, calculated doses were slightly higher for females than for males and, consequently, the effective doses were also higher for females (15.5 μSv/MBq) as compared to males (10.7 μSv/MBq).

Thus, four animals were sacrificed at each time point. The data on the elimination kinetics of the four groups of animals (5 individuals in each group) was fitted to a single exponential function, accounting for ~90% of the radioactivity, followed by a linear elimination phase (R^2 = 0.994±0.003; N=4). The resulting values of elimination half-life were consistent for all groups ($T_{½}$=36±5 min, N=4). These consistent results allowed us to limit the number of animals considering ethical aspects.

Discussion

Preparation and quality assessment of the radioactive tracers

Metalloid germanium belongs to the group IVa demonstrating chemical properties similar to carbon and silicon. It exhibits oxidation state of +2 and +4 with the latter dominating under normal environmental conditions [21]. It should be mentioned that the hydrochloric acid solution of ^{68}Ge(IV) produced for the utilization in ^{68}Ge/^{68}Ga generators (iThemba/IDB) was used in this study in combination with the same type of the generator. This assured the identical source and treatment of ^{68}Ge(IV) production.

Ge(IV) has chemistry different from Ga(III) that not only allows the separation on the generator chromatographic column but also excludes the complexation of Ge(IV) with DOTA based bifunctional chelator which is frequently used in peptide based bioconjugates, e.g. DOTA-TOC. Our experiments clearly demonstrated the absence of the complex formation. The product of DOTA-TOC labeling with ^{68}Ga(III) in the presence of ^{68}Ge(IV) was analyzed by Radio-HPLC and monitored for 24 hours (**Figure 4B-G**). The retention time for the ^{68}Ge(IV) and [^{68}Ga]Ga-DOTA-TOC was respectively 1.0±0.02 and 4.90±0.02 min. The Radio-HPLC signal associated with [^{68}Ga]Ga-DOTA-TOC decreased with the time and fully disappeared after 24 hours while the signal related to ^{68}Ge(IV) stayed unchanged within this time frame. Moreover the corresponding fractions of the HPLC analysis were collected and the fade of radioactivity was monitored and recorded for the determination of the half-lives. The fraction corresponding to ^{68}Ge(IV) was monitored for 273 days and resulted in half-life of 271.8±1.1 d thus confirming the origin of the signal from ^{68}Ge. The radioactivity of the fraction related to [^{68}Ga]Ga-DOTA-TOC was monitored for 260 min and resulted in half-life of 68.8±0.13 min characteristic to ^{68}Ga. The formation of radioactive colloidal particles was not observed. Firstly, the recovery from the HPLC column was over 98% and secondly no radioactive signal was detected at the origin of the strip in the ITLC analysis of the product indicating the presence of radioactivity only in the ionic form of ^{68}Ge(IV) moving with the front (**Figure 4H**).

Table 2. Organ- and effective doses ([^{68}Ge]$GeCl_4$; [μSv/MBq]) for human females and males*

	FEMALE**	MALE***
Kidneys	185±54	171±38
Adrenals	83±41	40±10
Liver	38±0.4	19±7
LLI wall	23±7	15±0.2
ULI wall	17±5	12±0.1
Red marrow	13±4	12±4
Spleen	11±0.1	8.5±0.8
Osteogenic cells	11±3	6.9±1.6
Small intestine	10±0.8	9.7±1
Ovaries/Testes	9.2±1	1.8±0.5
Urinary Bladder Wall	7.7±1	7.0±0.5
Breasts	7.4±1	NA
Uterus	7.4±1	NA
Stomach wall	7.4±1	6.4±0.6
Thymus	7.4±1	6.4±0.6
Thyroid	7.4±1	6.4±0.6
Gall bladder wall	7.1±1	6.3±0.6
Skin	7.1±1	6.0±0.3
Lungs	3.2±0.2	3.2±0.3
Heart wall	2.6±0.9	2.1±0.2
Muscle	2.0±0.1	1.5±0.2
Pancreas	1.9±0.1	1.9±0.1
Brain	1.2±0.1	1.4±0.7
Total effective dose	15.5±0.1	±1.2

*Values are given as mean±standard deviation. **Average of OLINDA calculations for [^{68}Ge]$GeCl_4$ administrated alone and in combination with [^{68}Ga]Ga-DOTA-TOC. ***Average of OLINDA calculations for [^{68}Ge]$GeCl_4$ administrated alone and in combination with [^{68}Ga]Ga-DOTA-TOC.

Human absorbed dose estimates based on rat biodistribution

[^{68}Ge]$GeCl_4$ administrated alone or together with [^{68}Ga]Ga-DOTA-TOC was rapidly eliminated presumably through renal excretion and none of the measured organs accumulated ^{68}Ge(IV) during the span of the experiment of 7 days. These results are in agreement with literature reports on germanium biodistribution and converge into the same conclusions: irrespectively of administration route and chemical form of germanium, fast blood clearance, renal excretion, and no deposition in tissue after 24 h occur. Thus the biodistribution of ^{68}Ge(IV) in its various chemical forms taken in by inhalation and ingestion by workers was considered in ICRP Publications 30 and 68 [22-24]. It was assumed that germanium was rapidly distributed to the kidneys and excreted in the urine with half-life of 0.02 days. This estimated half-life is practically identical with what we found in the present study, namely 0.025 days, despite the different routes of administration and chemical forms of Ge(IV). Furthermore, the remaining rather small fraction of germanium was considered to be distributed throughout all organs and retained with a half-life of 1 day. Based on this data it was concluded that the bone dosimetry was not required [23]. In another study [25], intravenously injected [^{68}Ge]$GeCl_4$ in rats also demonstrated fast renal excretion, low blood and liver uptake with retention in kidneys up to 48 h. All available data indicates that the biological half-life of germanium is extremely short compared to its physical life span and that it was sufficient to follow its kinetics in rat for one week only, for the purpose of assessing ^{68}Ge(IV) dosimetry, despite of the nuclide's much longer half-life of 270.8 days. The results obtained in the present study also corroborated this assumption. The publications from ICRP [22-24] and studies on rat biodistribution [11], where the route of administration was other than intravenous and the chemical form was germanium dioxide and sodium germinate, served as a basis for the calculations of effective dose of ^{68}Ge(IV) and ^{68}Ga(III) [26]. The higher kidney uptake in the early phase was assumed. It was concluded that permitting 0.01% of ^{68}Ge(IV) radioactivity content in ^{68}Ga(III) preparation would result in an additional radiation dose of less than 1 μSv. The effective dose was estimated to 0.034 mSv/MBq. However, it should be stressed that the chemical form of the germanium was different and specifically not [^{68}Ge]$GeCl_4$ which is used in the current methodologies for the preparation of ^{68}Ga-based radiopharmaceuticals, and the administration route was not intravenous, but either inhalation or ingestion. Thus these calculations required relevant experimental data for clear evidence.

The toxicity and carcinogenic effect of intraperitoneally injected $GeCl_4$ was studied in rats and demonstrated lack of carcinogenic potential, very low degree of toxicity of cationic tetravalent germanium, renal excretion, and fast blood clearance [7]. Only 1.08% of the injected radioactivity per gram was found in the

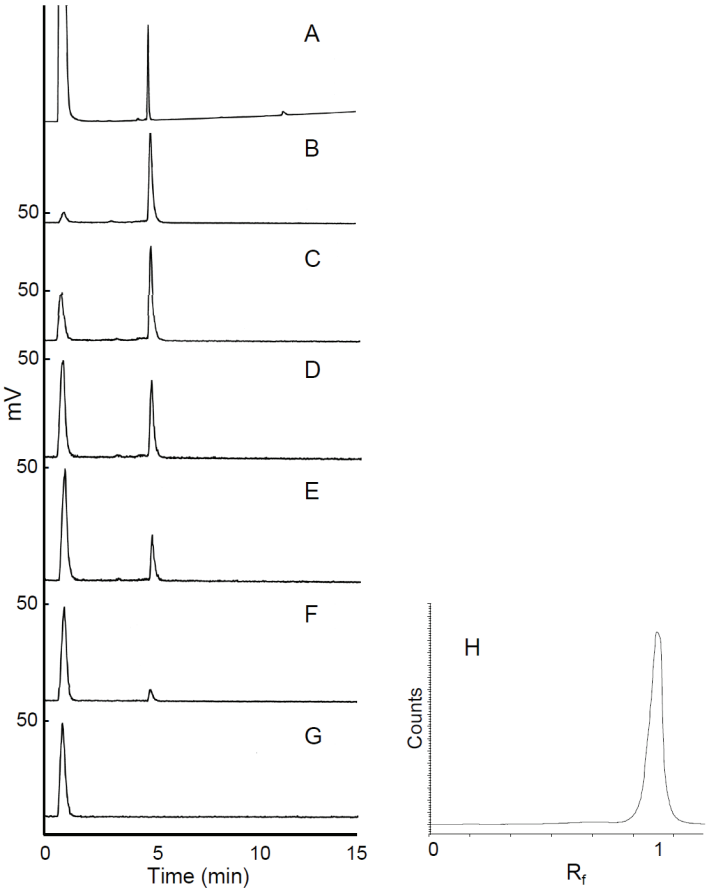

Figure 4. A: UV-HPLC chromatogram of the authentic reference, [^{Nat}Ga]-DOTA-TOC (the void signal corresponds to the buffer); B-F: Radio-HPLC chromatograms of the [^{68}Ga]Ga-DOTA-TOC labeled in the presence of [^{68}Ge]$GeCl_4$. The analysis was conducted respectively at 25, 89, 147, 215, and 344 min time points. The signals with R_t of 1.0±0.02 min and 4.90±0.02 min correspond respectively to the ionic ^{68}Ge(IV) and [^{68}Ga]Ga-DOTA-TOC; G: Radio-HPLC chromatogram taken 24 h after the production of [^{68}Ga]Ga-DOTA-TOC in the presence of [^{68}Ge]$GeCl_4$. The signal with R_t of 1.0±0.02 min corresponds to the ionic ^{68}Ge(IV) and the signal at 4.90±0.02 min corresponding to [^{68}Ga]Ga-DOTA-TOC was not detected; H) Radiochromatogram of ITLC analysis conducted 24 h after the production and demonstrating a single signal corresponding to the ionic ^{68}Ge(IV) moving with the front of the running buffer.

kidney 24 h post administration, which is in good agreement with our findings, where kidney uptake after 24 h was in the order of 0.6 ± 0.1%. The highest uptake by kidney was followed by liver, large intestine, femur, spleen and heart. The blood uptake was very low (0.003%). The tissue retention was low with fast renal excretion so that after seven days post injection the radioactivity in the most of the tested tissues was at the detection limit. Moreover, it is worth mentioning that we showed that no accumulation in red bone marrow occurred and retention was low (0.008 ± 0.004% ID/g, 7 days after injection).

This dosimetry investigation concludes that the kidney is the dose-limiting organ in both male and female and indicates that the maximum total amount of radioactivity of ^{68}Ge(IV) that can be given to a subject, if it was solely restricted by this organ dose, is as high as ~880 MBq for male and ~810 MBq for female. This amount of injected activity would result in a total effective dose of ~9.4 mSv (male) and ~12.5 mSv (female), i.e. close to or slightly higher than the maximum allowed dose of radioactivity to healthy volunteers, which is the general limit most authorities adhere to. Hence, the effective dose becomes the limiting parameter, and the corresponding maximal amounts of radioactivity are 645 and 935 MBq, respectively, for females and males. To put this in perspective, a fresh ^{68}Ge/^{68}Ga generator with loaded radioactivity of 1850 MBq would allow for a breakthrough of ^{68}Ge(IV) of 35 and 50% respectively for female and male before reaching the limit doses. This amount exceeds the limit recommended by European Pharmacopoeia monographs (0.001%) by 35000-50000 times. However, the limit for ^{68}Ge(IV) should be set in the context of the total dose from ^{68}Ga-labeled tracer and ^{68}Ge(IV). Assuming 1 GBq of ^{68}Ga(III) used for the preparation of a radiopharmaceutical and allowing for a breakthrough of ^{68}Ge(IV) of 0.1%, the contribution to the effective dose would be as low as 16 and 11 μSv for female and male, respectively. In this putative scenario, the additional effective dose associated with ^{68}Ge(IV) would be 625-910 (depending on the gender) times lower than the general rule of a dose to healthy volunteers of not more than 10 mSv, which compared with the radiation dose from the PET tracer itself must be regarded as a negligible contribution. Moreover, this assumption of

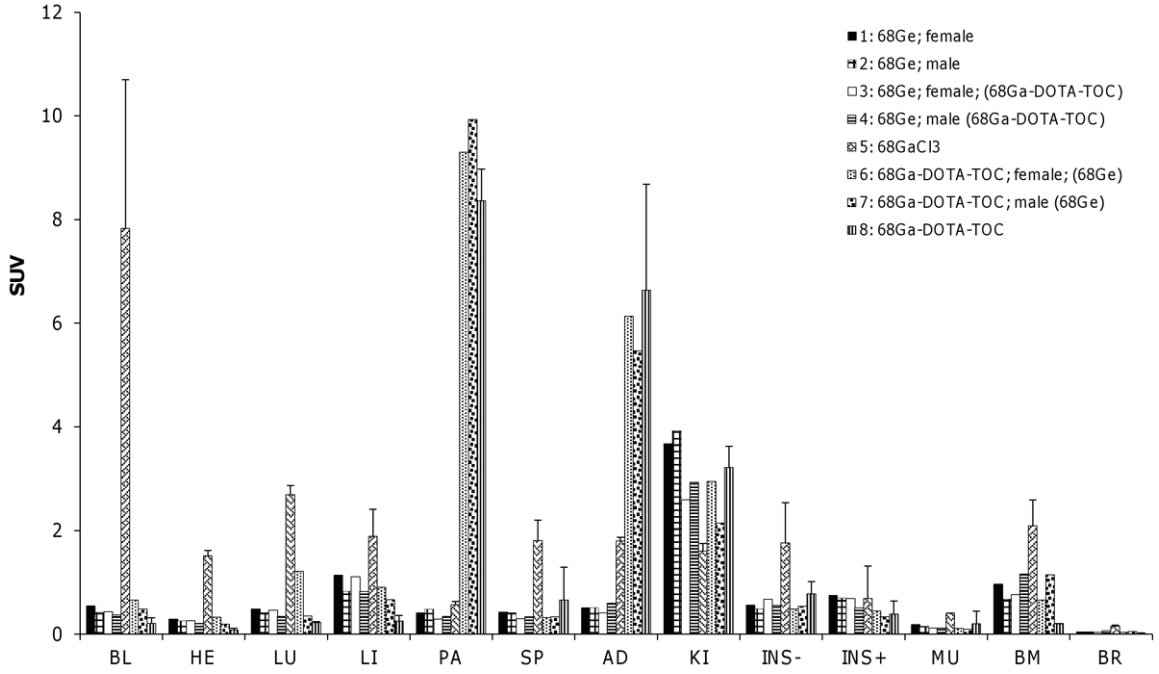

Figure 5. Organ distribution of [^{68}Ga]$GaCl_3$ [27], [^{68}Ge]$GeCl_4$, [^{68}Ga]Ga-DOTA-TOC in healthy male and female Sprague-Dawley rats. 1: [^{68}Ge]$GeCl_4$ (female); 2: [^{68}Ge]$GeCl_4$ (male); 3: [^{68}Ge]$GeCl_4$ (female) injected in combination with [^{68}Ga]Ga-DOTA-TOC; 4: [^{68}Ge]$GeCl_4$ (male) injected in combination with [^{68}Ga]Ga-DOTA-TOC; 5: [^{68}Ga]$GaCl_3$ (male, N=4) [*27*]); 6: [^{68}Ga]Ga-DOTA-TOC (female) injected in combination with [^{68}Ge]$GeCl_4$; 7: [^{68}Ga]Ga-DOTA-TOC (male) injected in combination with [^{68}Ge]$GeCl_4$; 8: [^{68}Ga]Ga-DOTA-TOC (male, N=3) [27]). BL – blood; HE – heart; LU – lungs; LI – liver; PA – pancreas; SP– spleen; AD – adrenals; KI – kidneys; INS- – small intestine without its content; INS+ – small intestine with its content; MU – muscle; BM – red bone marrow; BR – brain.

0.1% ^{68}Ge(IV) breakthrough is 100 times higher than the 0.001% limit of the content of ^{68}Ge(IV) in a ^{68}Ga-labeled tracer preparation, which is currently recommended by European Pharmacopoeia monograph.

Comparative biodistribution of radioactive components at one hour time point

In addition, the biodistribution of [^{68}Ga]$GaCl_3$ [27], [^{68}Ge]$GeCl_4$, [^{68}Ga]Ga-DOTA-TOC as well as combination of [^{68}Ge]$GeCl_4$ and [^{68}Ga]Ga-DOTA-TOC at 1 h time point was compared (**Figure 5**). In contrast to ^{68}Ge(IV), ^{68}Ga(III) after intravenous injection demonstrates highest uptake in the blood with rather uniform distribution amongst the organs reflecting the binding of Ga(III) to the serum proteins such as transferrin, ferritin, lactoferrin, and prolonged circulation in blood. It is also reflected in the biodistibution data in brain SUV values. The latter makes 4-5% of the blood uptake. The eventual build up in such organs as liver, kidney, spleen, and skeleton requires 24 h [22]. This time frame corresponds to over 21 half-lives of ^{68}Ga and consequently absence of radiation danger. Ge(IV) does not bind irreversibly to plasma proteins or other macromolecules and the distribution to the tissues is attributed to the passive diffusion through capillary walls [11]. The assumption of the retention of Ge(IV) is also based on the demonstrated rapid elimination. Even frequent and prolonged administration does not result in tissue deposition. [^{68}Ge]$GeCl_4$ was evenly distributed amongst all organs. It should be stressed that free ^{68}Ge(IV) is excreted much faster as compared to ^{68}Ga(III) and without deposition in any organ. The distribution of [^{68}Ga]Ga-DOTA-TOC was not influenced by the presence of [^{68}Ge]$GeCl_4$ with highest accumulation of the tracer in the pancreas and adrenals that physiologically express somatostatin receptors (**Figure 5**). This implies that the higher limit for the ^{68}Ge(IV) content would present no concern not only from radiation point of view but also biodistribution of the imaging agents.

Conclusions

The biodistribution of ^{68}Ge(IV) in rat was studied as a function of time in the absence and

presence of [^{68}Ga]Ga-DOTA-TOC. The data was used for the calculation of radiation dosimetry in humans. The elimination was fast with a half-life of ~0.6 h and no accumulation was observed in any organ including bone marrow. The dose-limiting organ was kidney, and maximum amount of ^{68}Ge(IV) of 645 MBq and 935 MBq, respectively for female and male may be administered before reaching an effective dose of 10 mSv. These results imply that the ^{68}Ge(IV) limit currently recommended by European Pharmacopoeia monograph could be increased at least 100 times without exposing the patient to harm. The findings may also open possibilities for the kit type preparation of ^{68}Ga-based imaging agents.

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