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IN VIVO INVESTIGATION OF TUMOR HYPOXIA USING PET RADIOTRACERS

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Background: Hypoxia is a common characteristic of all solid tumors. The oxygen deficiency in tumor cells induces the expression of proteins that promote the formation of metastases, angiogenesis, apoptotic resistance, and metabolic adaptation. Hypoxic tumor cells have an increased glycolytic activity. Glucose transporters and glycolytic enzymes (hexokinase) have frequently been found to be overexpressed in hypoxic tumor cells. These observations suggest that 18FDG can be a suitable tracer for the detection of hypoxic regions, but in this respect, however, the literature is contradictory. 18FAZA ([18F]-fluoro-azomycinarabino-furanoside) is a widely used PET hypoxia-specific tracer. In our work we have investigated the application of 18FAZA and 18FDG for the detection of hypoxic areas of heterotopic xenograft tumors in SCID mice.

Material and methods: Ten female CB17 SCID mice were used in these experiments. A2780 ovarian carcinoma (3 × 106), and KB-3-1 cervix karcinóma (3 × 106) cells were injected subcutaneously into the dorsal region of the thigh. The development of tumors and the locations of hypoxic areas were examined by MiniPET-II scanner. Radiopharmaceuticals (4 ± 0.3 MBq 18FDG, or 7 ± 1 MBq 18FAZA) were injected via the lateral tail vein. 40 min after 18FDG injection animal were anaesthetized by 3% isoflurane with a dedicated small animal anaesthesia device. 20-min static single-frame PET scans were acquired using a small animal PET scanner (MiniPET-II, Department of Nuclear Medicine, Debrecen) to visualize the tumors. In case of 18FAZA we used static (120 min after tracer injection) and dynamic scans. The PET data was evaluated with BrainCAD software. The 18FDG and 11C-methionine uptake were expressed in terms of standardised uptake values (SUVs) and tumor to muscle (T/M) ratios.

Results: 18FDG-MiniPET images showed heterogeneous radiopharmaceutical accumulation in the investigated tumor xenografts. These data indicated intensive glucose metabolism within the tumors. By taking the SUV values we found differences between the highly positive (SUVmean: 2.72 ± 0.23) and negative (SUVmean: 1.2 ± 0.36) tumor areas. Significant differences were found in the case of 18FAZA. The SUVmean values of the 18FAZA positive areas were 6–7 fold higher than that of the negative areas. In the examined xenograft tumors there were certain areas which were highly positive with both tracers (18FDG and 18FAZA).

Conclusion: Investigation of tumor hypoxia plays an important role in the preclinical and clinical studies. Our in vivo MiniPET studies demonstrated the specificity of 18FAZA in xenograft tumor models that we used. Within the tumors the heterogenity of 18FDG accumulation were similar to that observed with the use of 18FAZA, but the 6–7 fold higher difference in the accumulation of 18FAZA between the hypoxic and non-hypoxic areas demonstrated that for the detection of hypoxia 18FAZA is a more appropriate and specific radiotracer.

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SIMULTANEOUS DETERMINATION OF RADIOCHEMICAL AND CHEMICAL PURITY OF [18F] FDG BY NEW NORMAL PHASE HPLC METHOD

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Background: [18F]FDG produced by nucleophilic substitution method followed by an acidic hydrolysis may contain two radiochemical byproducts beside the active ingredient, namely free [18F]-ions and partially acetylated [18F]FDG as well as inactive impurities FDG and ClDG. To avoid the use of separate chromatographic methods for the determination of radiochemical and chemical purity of the product as recommended by Ph.Eur.6.2, in this work we report a new single normal phase HPLC method.

Material and methods: The HPLC system used for method development consisted of an HPLC pump (Jasco), a syringe-loading sample injector valve (Rheodyne), a radiometric detector (atomki, Hungary) and a refractive index detector (Knauer). Standards of FDG and ClDG were purchased from ABX GmbH.

Results: Using Inertsil NH2 column (4.6 mm × 250 mm; GL science, Japan) at 1 ml/min flow rate with mobile phase acetonitrile/water 85/15 V/V% we achieved the separation of FDG and ClDG with resolution of 1.74 ± 0.07, and the retention was 1.09 ± 0.04. The concentration of FDG and ClDG standards was 100 μg/mL. The injected volume was 20 μL. The retention times of FDG and ClDG were 7.2 min and 8.2 min, respectively. Under the same chromatographic conditions partially acetylated [18F]FDG and [18F]FDG were eluted with retention times of 2.0 min and 7.0 min, respectively. In order to make free [18F]-ions eluted we used gradient elution, with a flow rate 1 ml/min, and the eluent composition was 85/15 V/V% acetonitrile/water for 8 minutes. After that the flow rate was 2 ml/min and the eluent composition was 5/95 V/V% acetonitrile/0.1 mM sodium acetate solution (pH6) for 7 minutes. Due to gradient elution [18F] was eluted with a retention time of 12.4 min. The resolution between partially acetylated [18F]FDG/[18F]FDG and [18F]/[18F]FDG were 2.2 and 4.8, respectively. To validate the analytical method, we determined the linearity, precision and limit of quantification. According to our results the RSD% of FDG peak height was 1.9% (n = 3) and the [18F]FDG peak area was 0.07% (n = 8). The detector response was linear for [18F]FDG in the range of 2-840 MBq/ml with a coefficient of determination (r2) of 0.998. The LOQ for [18F] and partially acetylated [18F]FDG are 2.0 MBq/ml and 2.7 MBq/ml, respectively. LOQ for FDG and ClDG is 100 μg/mL.

Conclusion: Our HPLC method gives an opportunity to decrease the workload during the routine quality control of [18F]FDG at 5 ml of maximum recommended dose of injection.

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18F-FLUOROETHYLRHODAMINEB A PET TRACER FOR DETECTING MULTIDRUG RESISTANCE

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Background: Chemotherapy failure due to multidrug resistance (MDR) is a common problem in cancer treatment, because of the overexpression of the drug efflux pump P-glycoprotein (Pgp). Detection of the Pgp pump functions is an essential aspect in the treatment of cancer patients. One of the promising methods in diagnostic imaging for in vivo

detection of Pgp is the usage of radiolabeled substrates of Pgp. The rhodamine family is a well-known substrate of Pgp that is widely used in in vitro measurements. 18F-labeled rhodaminB (18FRB) gives a possibility for the in vivo experiment of the multidrug resistance of tumors. In our study the application of the18F-labeled rhodaminB was tested on 3 different type multidrug resistance and sensitive cancer cell line pairs.
Material and methods: For in vitro study human cervix carcinoma KB-3.1 Pgp negative and KB-V-1 Pgp positive, human ovarian carcinoma A2780 Pgp negative and A2780AD Pgp positive and a mouse fibroblast 3T3 and 3T3MDR1 Pgp positive cell lines were used. The accumulation of the 18FRB was measured by a calibrated gamma-counter. The Pgp functions were tested with rhodamine 123 by flow-cytometry.
Results: All of the three Pgp positive cell lines were demonstrated that the 18FRB is a potent Pgp substrate. The accumulation rates in the Pgp negative cell lines were 52 (cervix carcinoma) 85 (ovarian carcinoma) and 20 (fibroblast) fold higher in contrast to the Pgp positive cell lines. The treatment with cyclosporin A (CSA) — which is a Pgp inhibitor — increased the 18FRB uptake of Pgp positive cells close to the level of their negative pairs. The study of the accumulation kinetics showed that the 18FRB uptake reached a steady state level after a few minutes (~10 minutes). In the presence of verapamil the 18FRB uptake was also higher in Pgp positive cells in contrast to the Pgp negative cells where a slightly lower uptake was observed. (not Pgp effect) The Pgp functions were confirmed with a validated fluorescent dye (rhodamine 123) by flow-cytometry. The experiments in cell suspension were completed with measurement of adherent cells in petri-dish by MiniPET-II scanner. The results showed that 18FRB is a suitable tracer for the demonstration of Pgp pump functions in both of suspension and monolayer samples. Our results suggested that the 18FRB is proper for detection of the changes of the mithocondrial membrane potential.
Conclusion: The 18F-labeled rodaminB sensitively indicates the function of the Pgp pump in the multidrug resistant cancer cells. Further biological studies are needed to demonstrate the applicability of the 18FRB in in vivo by PET imaging.
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APPLICATION OF MEDI-MEDIA-FILL KIT FOR VERIFICATION AND VALIDATION OF ASEPTIC COMPOUNDING PROCEDURE IN CLINICAL PREPARATION SITES

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Our goal was to develop and provide a reagent and device kit which makes possible to verify and validate directly the process on the spot of the production of parenterally administered therapeutic and diagnostic radiopharmaceuticals in nuclear medicine.
The principles of aseptic pharmaceutical production and sterile product compounding are discussed in the USP Chapter <797> in details. The simulation test described in the kit is based on it. The aseptic compounding process, which involves injecting, mixing, dispensing, of sterile solutions, was simulated with sterile TSB microbiological medium and sterile sealed vials in various volume sizes. The aseptic process simulation test was combined with microbiological hygienic monitoring of the environment and personnels using contact agar plates. The result of the simulation test was visually assessed in microbiological tests after incubation of test vials and plates for two weeks. The test vials containing sterile TSB solution prepared in the simulation test were examined for sterility. The hygienic conditions were evaluated by examining the changes on contact agar plates used for sampling. The results of the tests are supplemented with the quantitative analysis of bacterial endotoxin, the most aggressive and common pyrogenic agent. The execution of the aseptic simulation test was considered to be conform to the requirements if each sample containing TSB medium, which is a clear, transparent, light amber solution in sterile conditions, retained its original properties, colony formation on agar plates was not seen, the quantity of bacterial endotoxin in TBS solution did not exceed the specified limit value. To document the whole process in an easily archivable form a data log sheet is attached to the kit where the data and results can be recorded and summarized.
The Medi-Media-Fill kit is suitable and applicable for all sterile medicinal products produced in situ, in pharmacy laboratories including the simulation of sterile aseptic compounding and dispending procedure of SPECT and PET radiopharmaceuticals intended for diagnostic use. The aseptic conditions of radiopharmaceutical production can be checked, the operational steps can be validated, the person's experience involving in the compounding procedure can be demonstrated. The simulation tests carried out by Medi-Media-Fill kit can also demonstrate and confirm the good operation of the laboratory that in aseptic pharmaceutical production meets the requirements of the authorities and inspectors, and thus, it can ensure the safe diagnostical examinations.
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FIRST CLINICAL EXPERIENCES IN SPECT/CT IMAGING OF FOLATE RECEPTOR OVEREXPRESSING TUMORS IN SPONTANEOUSLY DISEASED ANIMALS, USING TC-99M LABELED TARGETED NANOPARTICLES

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Background: In our previous examinations, a new biocompatible, biodegradable self-assembled nanoparticulate product was successfully synthesized and radiolabelled with 99mTc, and studied as a potential new SPECT or SPECT/CT imaging agent for diagnosis of folate receptor overexpressing tumors. Besides this new polyelectrolyte was published as a novel nanoscale drug carrier system and then presented as a new folate receptor targeting MRI contrast agent. In present study we examined possibility and conditions of the clinical veterinarian application of these 99mTc-labeled nanoparticles in early diagnosis of spontaneously diseased animals using a human SPECT/CT.
Material and methods: In course of labeling methods of folate targeted nanoparticles, radiochemical purity was examined by thin layer chromatography, particle sizes were checked by dynamic light scattering. Intracellular localization and toxicity of the nanoparticles have been reported in our previous papers.
In vivo experiments were performed on spontaneous diseased dog and feline patients. Biodistribution values were determined by SPECT/CT imaging method (Mediso Medical Imaging Systems), organ uptakes were estimated by quantitative ROI analysis.
Results: The in vivo biodistribution examinations of nanoparticles had close correlation to earlier described products which have similar particle size distributions. Images and calculated injected dose percentage values validated that in vivo radiolabeling efficiency and particle diameters were relative stable and constant after IV application.
The results confirmed that the nanoparticles accumulated in tumour cells overexpressing folate receptors, contrast agent revealed higher