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EVALUATION OF COLLOID RADIOPHARMACEUTICALS USED FOR SENTINEL NODE EVALUATION

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In our experimental work five different radiopharmaceutical kit (A,B,C,D,E) were evaluated by dynamic light scattering method (DLS). Besides labelling efficiency, median particle size, particle size range and potential chemical, physico-chemical interaction were determined Dynamic Light Scattering properties on Zeta Sizer Nano Zs (Malvern, USA) type instrument. The labelling efficiency and radiochemical stability measured by ITLC method.

The migration properties and sentinel node uptake after s.c. injection measured by animal experiment separately in rats and Beagle dogs.

During the comparative evaluation of the five compounds we experiment that the median particle size (MPS) and the particle size range were different. In case of compounds "A" and "B" MPS were around 10 nm "C" and "D" showed MPS around 30 nm and "E" sample product to be slightly above 100 nm. Radiochemical purity in all case complied with specification. The time, labelling volume and stability during preparation showed good correlation to each other.

The DLS method was very sensitive to detect particles, particles-interaction migrated. During our chemical experiment we experienced that, if aluminium (Al3+) content of generator eluate exceeds 10 μg/ml content the radioactive particles showed significant association. The resulted size increase well observable on the data sheet of DLS results.

We can conclude from our results that clinically used colloid radiopharmaceuticals showed different median particle size, which one support different pharmacokinetic properties observed during clinical trial.

The established new quality control method is useful for measure possible colloid particle interaction as well may occur after labelling.

In summary we can concluded that the DLS method used determination of precise median particle size of the various radiocolloid provide useful information for the fast way to find sentinel nodes during minutes in surgery rooms.

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COMPARATIVE STUDY OF ASYMMETRICALLY SUBSTITUTED 68GA-CHELATORS

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Background: Radionuclide 68Ga and its related chemistry have been in the focus of several nuclear medical research projects in the past years. Although 11C- and 18F-labelled radiopharmaceuticals are used most widely in almost any indications (including tumour diagnosis, visualisation of inflammatory processes, etc.), which require Positron Emission Tomography as a diagnostic tool, they lack a very important advantage the 68Ga possesses: 68Ga can be obtained as a product of a 68Ge/68Ga-generator. Since the synthesis of 68Ga-labelled molecules does not presume nearby cyclotron installed close to the PET-camera, it can facilitate the development of kit-based PET radiopharmaceutical production, and thus it might reduce the cost of these examinations resulting in a better accessibility for PET diagnosis of patients. Furthermore, 68Ga with its half-life of 67.7 min and negligible accompanying gamma radiation is very close to an "ideal" radionuclide for these types of medical use.

The labelling reactions involving 68Ga are achieved via complex formation between the metal ion and a chelator. Ideally, these reactions can be performed quickly and easily without the need for further purification, although they make it essential that a highly efficient chelator-moiety is conjugated to the target molecule. At present, the clinically available precursors (like 68Ga-DOTA-Toc) contain the not too specific, heterocyclic DOTA-chelator for this purpose. As a new generation of heterocyclic chelators containing phosphinic group (TRAP-chelators) have been developed for 68Ga recently, the aim of our research project is the investigation of the effect of phosphinic groups on the gallium-binding properties of heterocyclic chelators.

Material and methods: Chelators involved in our experiments were synthesized and purified by our co-operators at TUM, Munich, Germany (except the commercially available NOTA chelator). 68Ga activity was eluted from a 68Ge/68Ga-generator by ultra pure (UP) HCl solution. All reactions were performed in ultra pure solvents using standard circumstances (room temperature or 95°C, 100 μl volumes, buffered with concentrated HEPES solution), for pH-dependency examinations UP-NaOH was added to the reactions. Efficiency of 68Ga-labelling was analysed by paper chromatography and a TLC-scanner.

Results: For our investigations, NOTA and NOPO chelators were used as a standard, as their binding abilities have been published in the literature. By examining structurally similar, alternative chelators, a strong correlation can be identified between the number of phosphinic groups and the binding efficiency of these molecules. Increasing number of phosphinic groups also changes the pH optimum for labelling of these chelating units to more acidic conditions, which makes labelling reactions from generator eluates significantly more effective. Additionally, an almost 100% radiochemical purity can be achieved even from the concentration of 0.3 μM at the very short reaction time of 5 min.

Conclusions: Our examinations support the assumption that the new generation of TRAP-chelators — containing the maximum of three phosphinic groups — might be the best known tools for the synthesis of innovative 68Ga-labelled radiopharmaceuticals.

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LABELLING OF SIDEROPHORES WITH 68GA

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Background: Positron Emission Tomography (PET) is a modern, powerful imaging technique that enables accurate, non-invasive investigation of physiologic changes inside the body. During PET examination a chemical compound labelled with a short-lived positron-emitting radionuclide is injected into the body. The radiopharmaceutical should be selected so that the substance participates in the physiological process to be monitored. Therefore the future of this technique is highly dependent on the number of the new, innovative radiopharmaceuticals. A possible, new diagnostic application of PET can be the visualization of invasive pulmonary aspergillosis. Aspergillus fumigatus is a typical ubiquitous saprophytic mold. However, it causes life-threatening invasive disease especially in consequence of the expanding number of immunosuppressed patients, and thus it has become the most common airborne fungal pathogen of humans. An accurate diagnosis of invasive

pulmonary aspergillosis (IPA) is difcult due to the lack of a specic and sensitive method.

Iron is an essential nutrient for virtually every organism. Also, iron plays an important role in the pathophysiology of A. fumigatus, and siderophore production is crucial for the virulence of A. fumigatus, which excretes the siderophores fusarinine C (FsC) and triacetylfusarinine C (TAFC) to mobilize extracellular iron. Another, structurally similar siderophore, Ferrioxamine (FOXE) was also investigated in the literature.

Material and methods: Desferri-siderophores (TAFC and FOXE) were obtained from Genaxxon Bioscience. They were dissolved in ultra-pure water, and the stock solutions were stored in a fridge. Dilution series were applied to investigate the concentration dependence of the labelling process. 68Ga was gained from a 68Ge/68Ga generator (iThemba) with 1M HCl solution. For adjusting the necessary pH of the chelation, the reaction mixture was mixed with a concentrated HEPES solution. The reaction mixtures were incubated, and the yields were monitored by means of paper chromatography and HPLC. After the termination of the reaction, the radiolabelled siderophores were bound to a reverse phase disks. They were rinsed with water, and the radioactivities were recovered with ethanol. The organic phases were diluted with isotonic NaCl solution until the alcoholic content was lower than 10%. The stabilities of the labelled siderophores were investigated in mouse serum, and the octanol-water partition coefficients were also determined. For the uptake assays, 68Ga-labelled siderophores were incubated with iron-decient A. fumigatus at room temperature. A biodistribution study was also performed with non-infected animals where different organs and tissues of the mouse were removed, and the amount of radioactivity was determined with a gamma-counter. Results were expressed as percentage of injected dose per gramm of tissue.

Results and discussion: We have successfully synthetized 68Ga-TAFC and 68Ga-FOXE, resulting in high radiochemical purity — better than 95% — and very high specific activity. These compounds display high stability in biologic systems. Based on the recent literature these compounds might be useful tools for the diagnosis of the infection of A. fumigatus, and monitoring of the efficacy of the treatment with PET. The gallium-binding properties of the siderophores are comparable with the commercially available chelators, so using them as a lead-structure can also be promising.

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SAFETY ASPECTS OF WHOLE BODY HYBRID IMAGING AND THERAPEUTICAL RADIOPHARMACEUTICAL APPLICATIONS IN DOGS

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Background: The clinical and investigational use of whole body hybrid imaging (SPECT/CT, PET/CT) and therapeutical radiopharmaceutical applications is increasing worldwide. Meanwhile there is still no perfect consensus in low-dose effects and concerns are still present in human and veterinary nuclear medicine and radiological clinics.

Material and methods: We evaluated the radiotoxicological data of Beagles and spontaneously occuring tumor bearing dogs before and after whole body PET/CT (18FDG and 68Ga-ligands), SPECT/CT (99mTc-ligands) and radiopharmaceutical treatments (131I, 90Y, 177Lu-ligands). Beyond basic hematological and biochemical parameters micronucleus frequency, chromosome aberrations, sperm morphology and motility tests and proliferation assay, DNA repair in isolated lymphocytes.

Results: Decreased bone marrow (PLT, WBC) and renal function (CARB, CREA, PHOSP) only was realized after extreme-high therapeutical radiopharmaceutical application (170Tm-EDTMP and 90Y-DOTATOC) in dogs. The more sensitive assays however sometimes showed a slight, transitory radiotoxicological effect even after diagnostical whole body hybring imaging.

Conclusion: Conventional hematological and biochemical panel is generally not available to detect radiotoxicological effects after applying normal clinical-dose of therapeutical radiopharmaceuticals. Controversaly, micronucleus frequency, chromosome aberrations in cultured lymphocytes, sperm morphology and motility tests, the proliferation assay and DNA repair test is sensitive enough to measure the radiotoxicological effects after whole body hybrid imaging (SPECT/CT, PET/CT) and therapeutical radiopharmaceutical applications.

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USING MULTIPLE XENOGRAFTS TO MULTIPLY THE DATA GETTING FROM A SINGLE NUDE MOUSE

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Background: There is an increasing demand from different occasions to decrease the animal use in biomedical research and paralelly to obtain as many as possible valuable data from animal tests. These considerations raise the possibility to insert two or more (4, 6 ...) different xenografts into the same host animal and to use these multiple xenograft modells for radiopharmaceutical investigations (SPECT, PET and hybrid images and biodistributions).

Material and methods: Five-six weeks old (18–22 gram weight) Nude mice (BALB-C nu/nu) altogether over 100 were used for the xenograft inoculations. Xenografts were inoculated either as simple cell-line suspensions (107 cells/0.1 ml) or as pre-cultured them into an in-laboratory developed biodegradable matrix scaffold. Different cancer cell lines (MKN-45, A431, C6) and tumorigen non-cancer cell lines (mesenchymal originated stem cells, fibrocytes) were used to develope the novel models. Non-specific (18FDG, 99mTc MIBI) and specific (68Ga, 177Lu, 99mTc-labelled receptor-affin analogues) tumor targeting agents were injected intravenously into mice and standard biodistribution studies SPECT/CT and PET/CT image were done at different time postapplications.

Results: There were slight but not significant differencies between 18FDG and 99mTcMIBI uptakes (SUV) by non-malignant laesions and the malignant xenografts. However we were able to detect significant