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During the last two years the division of Radiochemistry and Radiopharmacology covered very different disciplines:

1. Production of short-lived radiopharmaceuticals for the application in positron emission tomography (PET) E0301.
2. Production of radiopharmaceuticals for the application at conventional γ-cameras or single photon emission computer tomography (SPECT) systems E0301.
3. Quality control and quality guarantee of the produced radiopharmaceuticals as well as sample securing and documentation E0301.
4. On going development of established preparation procedures and transfer of experiences on new systems. Determination of biokinetic data of new compounds for the deposit at the competent authority, to get the approval from the local ethic commision for clinical applications E0301/E0302.

Parallel to these activities extensive experiences from biokinetic investigations broaden the diagnostic and therapeutic spectrum of macromolecular compounds which show high tumor uptake rates. This property was used with the consent of the competent ethic commissions for different experimental and clinical applications:

- Detection of solid tumors and metastases E0302/E0303.
- Intraoperative fluorescence diagnosis of tumors - boundaries, -residues and sentinel lymph nodes E0303.
- Chemotherapy in a clinical Phase-I/II study E0303.
- Fluorescence diagnosis of rheumatoid arthritis E0303.
- Chemotherapy of rheumatoid arthritis E0303.

Synthesis of Short Lived Radiopharmaceuticals for The Positron Emission Tomography (PET) (E0301)

M. Eisenhut

Cooperations: Internal: Prof. Dr. U. Haberkorn (E0600); Prof. Dr. L. Strauss (E0105); Priv.DoZ. Dr. J. Debus (E0500); Dr. William E. Hull (R0400); Prof. Dr. W.D. Lehmann (R0400).

External: Prof. Dr. F. Rösch (Inst. für Nuklearchemie, Universität Mainz).

Among the imaging procedures used in diagnostic nuclear medicine the positron emission tomography (PET) holds a privileged position, which is to due to the high spatial image resolution as compared to conventional scintigrams and single photon emission tomography (SPECT). In this context the research group develops radiolabeled new compounds which are investigated as potential radiopharmaceuticals for PET. In addition new compounds useful for single-photon-emissions tomography (SPECT) are on the research program, since time scale problems inherently associated with the „traditional“ positron emitters \([{}^11\text{C}]\) and \([{}^18\text{F}]\) exhibit unavoidable experimental limitations.

Radiopharmaceuticals are useful for the detection of tumors and their metastases. In addition, radiopharmaceuticals are developed for the follow up of cancer treatments like radiation therapy and chemotherapy. Among the above mentioned objectives PET measurements of organ function are also part of the scientific program. Finally, routine productions of radiopharmaceuticals like 2’-\([{}^18\text{F}]\)-fluoro-2’-deoxy-D-glucose (\([{}^18\text{F}]\)-FDG), L-6-\([{}^18\text{F}]\)fluoro-3,4-dihydroxy-phenylalanin (\([{}^18\text{F}]\)-Dopa), \([{}^15\text{O}]\)-water, \([{}^11\text{C}]\)-ethanol, \([{}^11\text{C}]\)-AIB and \([{}^11\text{C}]\)-acetate are provided for the Clinical Cooperation Unit - Nuclear Medicine and the Medical PET Group - Biological Imaging.

One of the currently performed research activities focusses on the automated syntheses of 3’-deoxy-3’-\([{}^18\text{F}]\)fluorothymidine, \([{}^18\text{F}]\)FLT. The clinical background for these efforts is dependent on the opportunity to image cell proliferation with PET. In addition to \([{}^18\text{F}]\)FDG PET which is commonly used for the imaging of cellular glucose demand an other important parameter would be available for the characterization of tumor growth. \([{}^11\text{C}]\)-labeled thymidine has been applied earlier for the measurement of cell proliferation with PET. Although this radiopharmaceutical was useful for that purpose the complicated radiosynthesis, the short physical half life and the metabolic instability prevented a more general application. \([{}^18\text{F}]\)FLT is more resistant than \([{}^11\text{C}]\)thymidine against in vivo catabolism and its radioisotope has a longer physical half-life (\(t_{1/2} = 110\) min). The cellular uptake and initial metabolism of FLT is comparable with \([{}^11\text{C}]\)thymidine. Phosphorylation at the 5’-position mediated by the thymidine kinase-1 causes intracellular trapping of the tracer. Due to these promising characteristics experiments are performed which should improve the radiosynthesis of \([{}^18\text{F}]\)FLT. A series of new precursor
molecules are, therefore, developed to improve the automa-
tion of the synthesis.

In addition O-[18F]fluorethly-L-tyrosine, [18F]FET, is cur-
rently introduced for routine PET application of brain tu-
mors. On the basis of a collaboration project the synthesis
is adapted from procedures established at the Institut für
Kernchemie in Mainz (Prof. F. Rösch). As indicated above
the setup of an automated synthesis module will also be
part of our service efforts. For preclinical investigations we
are currently investigating α-[2-{18F}fluorethly]tyrosin
([18F]FAET). This new radiofluorinated amino acid will be
compared as an alternative with [18F]FET. Therefore, cell
and animal experiments are used to trace differences in
the metabolic fate of the two compounds. It is expected,
that substitution at the α-position will lead to an improved
tumor uptake mediated by metabolic trapping via protein
incorporation. Finally the development of substrates for the
HSV-thymidinkinase (HSV-tk) which can be used as re-
porter molecules in gene therapy are part of the program.
To begin with porter molecules in gene therapy are part of the program.

HSV-thymidinkinase (HSV-tk) which can be used as re-
tumor uptake mediated by metabolic trapping via protein
with [18F]F2 leading to
deoxyuridine will be synthesized and subsequently labeled
that substitution at the
compared as an alternative with [18F]FET. Therefore, cell
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Pre-targeted Immunoscintigraphy and
Peptide / Receptor Imaging for Tumor
Localization with Positron Emission
Tomography (E0302)
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In cooperation with: S. Kaul, G. Bastert, Department of Gyneco-
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A pivotal point of nuclear medicine is the use of specific ra-
diotracers showing a high accumulation at the target site
and a fast clearance from nontarget sites. Monoclonal anti-
odies (MAbs) as well as small regulatory peptides can
specifically target malignant cells expressing the corre-
sponding tumor associated antigens and receptors and
thus are potential carriers for diagnostic and therapeutic
radionuclides. Positron emission tomography (PET) is the
most efficient imaging method in nuclear medicine be-
cause of its higher detection efficiency and its better con-
trast resolution compared with conventional gamma cam-
eras. Consequently, MAbs and regulatory peptides labeled
with a positron emitter should enable a specific and sensi-
tive scintigraphic tumor localization with PET.

Our approaches for PET imaging of tumors are based on
Gallium metal chelates of high in vivo stability. These che-
lates can either be used as a hapten for targeting of tu-
mors in pretargeted immunoscintigraphy or covalently
bound to oligopeptides for receptor imaging and can be la-
beled with the short-lived positron emitter Gallium-68 (t1/2
68 min, β+ 88%) which is obtained from a Germanium-68 /
Gallium-68 radionuclide generator.

Pretargeted immunoscintigraphy
Immunoscintigraphic tumor localization with labeled MAbs
often resulted in false negative findings due to the low ra-
dioactivity contrasts between the tumor and the surround-
normal tissues. As a promising technique to improve
sensitivity of immunoscintigraphic tumor localization multi-
step targeting methods have been emerged using antitu-
mor MAbs additionally conjugated with a high-affinity noncovalent binding site for a small, diffusible, radiolabeled hapten, which is given after the localization of the MAbs on the tumor. These methods circumvent the limitations of high bloodpool and liver background activity as well as of macromolecule targeting of solid tumors related to the administration of MAbs labeled prior to injection and allow optimization of tumor-to-normal tissue contrasts by reducing background activity.

To combine the specificity of antitumor MAbs with the improved tumor contrast of pretargeting and the sensitivity of PET we developed the reagents and a treatment schedule for a threestep targeting method. The first step is the administration of a bispecific monoclonal antibody (BS-MAb) prepared from the F(ab’)_2 fragments of an antitumor MAb and the F(ab’)_2 fragments of an anti-Ga chelate MAb via a mixed functional chemical linker.

After a localization period of 18 - 24 h a blocker was given which consists of the nonradioactive Ga chelate covalently coupled to human apotransferrin serving as a high molecular weight carrier with slow extravasation. This blocker saturates the anti-Ga chelate binding sites of the BS-MAb still present in the circulation. Shortly after the blocker the free ⁶⁸Ga labeled Ga chelate is injected which rapidly penetrates into the tumor tissue and binds to the prelocalized BS-MAb. Excess ⁶⁸Ga chelate is rapidly cleared from the circulation via the kidneys. Activity distribution was examined 1 h postinjection of the Ga chelate. Using three different antitumor MAbs and the same anti-Ga chelate MAb for BS-MAb preparation multistep targeting resulted in tumor-to-blood ratios of 3.6, 4.7 and 2.6 in rat pancreas carcinoma, human colon carcinoma and human mammary carcinoma bearing nude mice respectively. This compares to ratios of 0.6 - 0.8 24 h postinjection obtained with the corresponding native MAbs labeled with Iodine-131 prior to injection.

In a preliminary clinical trial we attempted to assess the efficacy of pretargeting and PET for breast cancer localization in patients. For that reason the antitumor MAB 12 H 12 was included as an antitumor part in BS-MAb preparation. MAB 12 H 12 is a mouse IgG1, which recognizes the carbohydrate side chains of the tumor associated glycoprotein TAG 12, which differs in glycosylation from the MUC1 mucin on normal epithelial cells. TAG 12 is overexpressed by the vast majority of epithelial cell adenocarcinomas and is shed into the circulation due to proteolytic cleavage from the cell membrane.

Ten patients with biopsy proven primary breast carcinoma were infused with 10 mg of the BS-MAb. Eighteen hours later 10.7 mg of the blocker were injected intravenously, followed 15 min later by 9.6 µg of the Ga chelate with ~ 260 MBq of ⁶⁸Ga. PET imaging was started 60 - 90 min after Ga chelate injection. Fourteen of 17 known lesions, averaging 25 ± 16 mm in size were clearly visualized as foci of increased activity with PET. Uptake of the Ga chelate by intraductal tumor sites was much higher than by infiltrating tumor sites suggesting a potential of the antimucin BS-MAb for detecting breast carcinoma in situ. Nevertheless, PET was able to detect infiltrating tumor sites 10 mm in size and contrasting only by a factor of two from surrounding normal breast tissue (Fig. 1). This demonstrates the better sensitivity of PET for detection of breast cancer at low tumor contrasts compared to conventional immunoscintigraphy. Since the low affinities of antimucin MAbs and antigen shedding proved to be not optimal for increasing tumor-to-tissue ratios, pretargeting with anti-CEA and anti-EpCAM BS-MAbs which are available with much higher affinities and the absence of antigen shedding might further increase the sensitivity for breast cancer detection.

Receptor imaging of neuroendocrine tumors with PET

Most tumors of endocrine origin like gastric carcinoids, pancreatic tumors, meningiomas and small cell lung cancers show a highly increased expression of somatostatin receptors. Somatostatin is a small, cyclic peptide hormone consisting of 14 aminoacids which is synthesized and released from endocrine cells. It exerts a wide variety of antiserotonin actions on the central nervous system, the hypothalamus and the gastrointestinal tract. Especially, the inhibitory action of somatostatin on the release of growth hormone (Somotropin) from receptor positive tumor cells, resulting in an antiproliferative effect, suggested its potential as a therapeutic agent. Due to the rapid proteolytic degradation of somatostatin in blood synthetic analogs were developed. The most efficient analogons, an octapeptide designated Octreotide, is highly resistant to degradation and retained an affinity of > 10^10 M^-1 to type II and V receptors similar to that of the native somatostatin. Conjugated with the chelator DTPA and labeled with Indium-111 Octreotide proved to be of high specificity for the localization of neuroendocrine tumors.

Sensitivity of the ¹¹¹In Octreotide is restricted, however, to a tumor size of ≥ 25 mm, due to the biokinetics of the compound and due to single photon detection with conven-
tional gamma cameras. Derivatization with the macrocyclic ligand DOTA enables peptid labeling with metallic radionuclides other than \(^{111}\text{In}\). We have developed a fast and efficient technique for labeling of DOTA-Octreotide with the short-lived positron emitter \(^{68}\text{Ga}\) resulting in a \(> 85\%\) labeling yield and in a recovery of \(~ 50\%\) of the initial radioactivity. \(^{68}\text{Ga}\)-DOTA-Octreotide proved to be of high in vivo stability and was cleared from the circulation more rapidly because of its increased hydrophilicity compared to \(^{111}\text{In}\)-DTPA-Octreotide. In a first preliminary clinical trial with menigioma and gastric cancer patients PET imaging with \(^{68}\text{Ga}\)-DOTA-Octreotide resulted in high contrast images of lesions 7-8 mm in size 1 - 2 h postinjection (Fig. 2).

Publications (* = external co-author)


Synthesis of Macromolecular Compounds for the Diagnosis and Therapy of Solid Tumors and Inflammatory Processes (E0303)

H. Sinn

Cooperations

DKFZ: Dr. E. Frei, C 0300; Dr. T. Haase, E 0400; Dr. H. Wesch, E 0100; Dr. U. Zilfmann, Zentrales Tierlabor.


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Since we had discovered that solid tumors show a high protein (predominantly serum albumin) uptake and consumption, we tried to use this fact to channel active agents into proliferating tumor tissues. From the radioactive experiments we had learned that during the labeling procedure the native character of the protein must be maintained, otherwise the circulating time of the loaded protein will be considerably reduced and instead of a tumor uptake a rapid accumulation in the liver can be registered. The first trial to transfer our experiences on other compounds was focused on Doxorubicine and Methotrexate (MTX), well known drugs in oncology. The result of extensive experiments with tumor bearing rats was an improvement in efficacy, accompanied with a considerable reduction of side effects. These positive findings initiated us to start, together with the oncologic division of the Klinikum Mannheim, a Phase-I study with MTX-albumin, approved by the local ethic commission and under the control of the Arbeitsgemeinschaft internistischer Onkologen (AIO). The required amounts of MTX-albumin was produced under GMP conditions by Impfstoffwerk Dessau. The patent was licenced out in 1998 to Klingen Pharma, München, and the clinical studies continued, now under the leadership of this company.

Apart these activities we always tried to broaden the spectrum of available compounds. Very soon it became evident that the coupling procedure of MTX was applicable only to a few cytostatic drugs, so that the greater part of our work was directed towards finding mild coupling reaction conditions to conserve the native character of albumin also under these new conditions. Today we dispose on a variety of biologically active compounds e.g. Aminopterin, Aureomycine, Doxorubicin, Ellipticine, Mitoxantrone, Quercetine, a new cis-Platinum complex and Tetracyclines covalently linked to albumin following different coupling reactions.
Parallel to the coupling of chemotherapeutic agents a series of fluorescent dyes were also linked to albumin. The application of these conjugates takes place from very different reasons e.g. for demonstration that albumin is taken up in solid tumors and inflammatory processes (fig. 1, 2, 3), for intraoperative laser induced fluorescence diagnosis (LIFD) to show tumor boundaries or lymphatic nodes to the surgeon and last but not least in an increasing number of cases of photodynamic therapy (PDT) of solid tumors. The range of fluorescence wave lengths reaches from 526 nm of fluorescein to about 660 nm of a tetracarboxyphenyl-chlorine. Whereas the fluorescein-albumin has a bright fluorescence without any photoactivity and is preferred therefore by neurosurgeons, in PDT the chlorine conjugate is preferred, due to its high photoactivity and the comparably high penetration depth of the red light. The successful coupling of different porphyrins to albumin initiated us to try it with Fe³⁺ containing porphyrins e.g. hemin with the ulterior motive to create a non toxic, tumor seeking magnetic resonance contrast medium (MRK). Animal experiments confirmed this speculation (fig. 4) and showed simultaneously that albumin can be used as a very potent carrier for a variety of compounds for diagnostic or therapeutic purposes.

Fig.1: Two neighboured sections of a rat brain with an implanted C6-Gliom. The animal was treated 24 h before brain resection with 2 mg AFlc-HSA/kg body weight. Left side: the HE colored section (white light). Right side: untreated section, only exposed to laser light at 488 nm.

Fig.2: A rat brain with an implanted C6-Gliom. The animal was treated 24 h before brain resection with 2 mg Chlorine-HSA/kg body weight. Left side: white light photo. Right side: photo during an exposure to laser light at 654 nm.

Fig.3: Distribution of AFlc-HSA in a paw of a mouse with collagen induced (CIA) as determined by laseroptical imaging. White light (left side) and fluorescence image (right side) of a paw with three arthritic toes (arrows) 3 h after an intravenous injection of AFlc-HSA. Only inflamed toes show strong fluorescence when exposed to laser light at 488 nm.

Fig.4: Magnet Resonance (MR) images of a rat brain with an implanted C6-Gliom. The animal was treated 24 h before brain resection with 2 mg Hemine-HSA/kg body weight.
New projects:
- Visualization and therapy of neoangiogenesis with makromolecular compounds
- Visualization and therapy of glomerulonephritis with makromolecular compounds

Publications (* = external co-author)

Patents