

Szent István University
Postgraduate School of Veterinary Science

**Biological evaluation of new beta-emitting
radiopharmaceuticals for therapy of malignant and
chronic diseases**

PhD Theses

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az Országos „Frédéric Joliot-Curie” Sugárbiológiai és Sugáregészségügyi

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nyerte el végleges formáját.

Dr. Máthé Domokos

Introduction

The scope of the PhD work is the presentation of the concept of **dual, molecular imaging and therapy** in animal models using radioisotopes during the development of novel dual-use (that is, human and veterinary medical applications) radiopharmaceuticals and **matching pairs** of therapeutic and diagnostic pharmaceuticals. This scope extends to development of radiopharmaceuticals labelled with ^{177}Lu and ^{188}Re isotopes – both emitting gamma rays for imaging and beta-particles for localized bone and joint therapy.

^{177}Lu is a widely used and promising radioisotope with short range beta emission and gamma radiation suitable to imaging. Its radiation properties are well suited to palliative therapy of metastases located in the bone compartment as the absorption of beta particles is confined to the immediate cells surrounding the uptake site of the radioisotope. Bone remodeling is characterised by free calcium-hydroxy-apatite surfaces. Phosphonates, such as EDTMP are readily able to distribute from blood to bone compartment and adsorb

onto available calcium hydroxyapatite on remodeling bone. The remodeling pace of osteoblastic metastases exceeds normal bone remodeling thus the agent is preferentially localized in this type of cancer metastasis to bone. A new pharmaceutical formulation of the organic phosphonate molecule ethylene-diamine tetra-methyl phosphonate has been labelled with ^{177}Lu . As interspecies differences are highlighting the mechanisms behind uptake and distribution into bone, we estimated pharmacokinetic parameters of the compound in mice and rabbits. For biodistribution imaging, rats, rabbits and dogs equally have been injected with non-therapeutic doses of activity. Scintigraphic and tomographic imaging was performed (together with CT in the rodent species) to study biodistribution over time. For any radiation dosimetric analysis it is important to establish data on the kinetics of the agent. To this end we have chosen the noncompartmental model and analysed interspecies differences in pharmacokinetics. Finally in dogs we have conducted a study to achieve data for the definition of Maximal Tolerated Dose (MTD) and to examine toxic side effects of different, gradually increasing doses of intravenously injected activity. These studies have provided the direct link to design human

clinical trials. Radionuclides of a given decay chain produced by fixing their mother elements on a chromatography column in a shielded container (a „generator”) and collected by elution of the here described „generator” are offering an attractive logistics advantage of on-the spot and on-demand availability. One of the actually proposed beta-emitting radionuclides for internal radioisotope therapy, ^{188}Re is available from a generator. Besides its beta emission, just like ^{177}Lu , ^{188}Re also emits gamma rays that allow imaging. We designed a series of experiments to produce a local therapeutic agent, ^{188}Re -tin colloidal particles for intra-articular use. We have chosen the animal model of rabbit antigen-induced arthritis for the proof-of-concept testing of the effects of the radiopharmaceutical, and we characterized the labelling reaction, the size distribution and the consequent radiation dosimetry of the particles while imaging its intra-articular application. We also examined the effects of irradiation delivered by the particles onto the synovial surface using histological specimens of the animal model. The results obtained in our study provided a basis of safe application of ^{188}Re -tin colloid in human knee joint in a clinical trial setting that

has since been made reality by prof. Jae Min Jeong and his co-workers in the Republic of Korea.

In the course of development of molecular imaging and molecular therapy using radioisotopes targeted on tumor cells peptides and especially somatostatin receptors have a prominent role. Many tumor cell types express somatostatin receptors on their surface and in a lot of cases an over-expression has been found. We have been developing the use of spontaneous animal tumors as models to study molecular imaging and therapy based on peptide receptors (dubbed peptide receptor scintigraphy and peptide receptor radionuclide therapy). In this field exploiting expression and over-expression of somatostatin 2A receptors on tumor cells has the most important role as a well circumscribed subset of neuroendocrine tumors over-expresses this surface receptor. To our knowledge the overexpression of somatostatin 2A receptors is also a phenomenon in naturally occurring dog tumors. As in humans, in dogs also tumor cells mainly of neuroendocrine origin express the receptor subtype. Thus the search of such tumors is reduced to a relatively smaller subgroup of dog patients with mostly very infrequent occurrence. We have selected insulinoma of dogs to model human disease

and to be the subject of our studies. This decision was taken with regard to dog insulinoma's relatively less infrequent incidence and former data on proven receptor expression in dogs with this type of tumor. We examined the possibilities of imaging somatostatin 2A receptors with novel targeting peptides previously not utilized in the dog. The peptides are derivatives of somatostatin-analogue octreotide and contain unnatural amino acids to prevent fast enzymatic cleavage and excessively short blood half-life. As the peptides are agonists of the somatostatin receptor, they will be internalized after having bound the cell surface protein. Their „load“ coupled to the peptide with a partly covalently attached chelator system will thus remain in the cell.

These peptides are capable of being labelled with different isotopes, using the same peptide vector but a different chelator system for ^{99m}Tc and for trivalent metal ions like ^{90}Y , ^{111}In and ^{177}Lu .

The author's studies have been performed in two main organ systems, bone and joint and somatostatin receptor system, the dissertation as well is divided into two parts, bone and joint therapeutic radiopharmaceuticals and somatostatin 2a receptor ligand radiopharmaceuticals. The publications appeared in these field by the author

represented the base for human clinical trials in two novel radiopharmaceuticals.

Aims

a) Defining the place and showing the consequences of the application of different animal models (healthy, induced disease and spontaneous disease) in the pipeline of radiopharmaceutical development.

b) Describing effects of beta irradiation to distinct, localized parts or organ systems of the body with different energies and thus different tissue penetration for the use of human and veterinary clinical therapy within the appropriate animal species exploiting the full potential of isotopic imaging with tomographic imaging techniques such as SPECT.

c) Presenting the applicability of radionuclide therapy in veterinary medicine and observing its effects.

d) Presenting the concept of isotope therapy from „molecules to models in animals” with the use of

immunohistochemistry as the reference basis of molecular imaging.

Studies on ^{177}Lu -EDTMP for bone therapy

Experimental design

In the course of an Indo-Hungarian joint research programme we have prepared ^{177}Lu by thermal neutron irradiation of natural ^{176}Lu , and thereafter we synthesized EDTMP molecule. We have formulated the lyophilized kit necessary for labelling based on the ingredients of an actually used clinical radiopharmaceutical. The biodistribution of the ^{177}Lu -EDTMP molecule thus prepared was examined with several methods in many species. In the mouse we performed a traditional bio-assay with five animals per time point. In addition we performed a multiplexed multipinhole SPECT/CT in vivo imaging study and we examined the details of bone biodistribution using digital micro-autoradiography. In the rat we also made biodistribution and imaging studies with both gamma camera and SPECT/CT. After rabbit gamma camera imaging and biodistribution (n=3 per time point) we have performed imaging in dogs, too. In the dog, we analysed blood cell counts and biochemistry in a dose escalation study of groups of 3 dogs with 9.5,

19, 28.5, 37.5 MBq/bwkg dose). Besides this we modelled pharmacokinetics by the biodistribution data in rabbits and mice with the noncompartmental model.

Results and discussion

In all the species used we could present that the radiopharmaceutical has a high affinity for bone, and there is no detectable radioactivity in other organs 24 hours post injection than the skeleton. Maximal skeletal binding was 40.62% in mouse and 36.52% in rabbit. All other imaging studies showed that the critical organ besides the bone marrow is the kidney as excretion of the radiopharmaceutical is by the urinary tract. The other finding in imaging studies is that EDTMP binds preferably to remodeling bone surfaces that are present in a healthy animal mostly at joints. Using mouse digital microautoradiography we could observe radiopharmaceutical binding to cortical bone without the presence of it in the dose-risky area of bone marrow. According to pharmacokinetic analysis, mouse species binds the radiopharmaceutical more effectively, while there is less radiopharmaceutical bound to rabbit bone surfaces due to probably different calcium homeostasis of the rabbit. As mostly mouse data are used to

extrapolate into adults and rabbit data rather for pediatric dosimetry calculations (rabbit epiphysis nutrition is similar to humans). This could later lead to an extended use of the radiopharmaceutical. With dog dose escalation we could not reach MTD and only in the highest dose group could we observe a transient platelet number decrease, while reactivity of the marrow remained. These healthy animal studies were a necessary requisite of further clinical trials.

Application of ^{188}Re -tin colloid in a rabbit model of rheumatoid arthritis

Experimental design

We have prepared ^{188}Re -tin colloid with two processes, at room temperature and at 100 °C. We have examined the diametrical spectrum of the particles using dynamic light scattering and the radiochemical purity and labelling yield using thin layer chromatography.

Biodistribution of the radiopharmaceutical thus prepared was imaged with a gamma camera by comparing the biodistribution of soluble perrhenate with the radiocolloid in the rabbit. Effects of the developed new radiopharmaceutical were studied in an antigene-

induced arthritis rabbit model after three weeks of induction by injecting 50 MBq of radiocolloid into the joint space. Four weeks post injection histopathology was performed in the knee joints.

Results and discussion

A higher labelling yield of the room temperature produce (66.7%) and appropriate size (99% > 3 μ m, mean 4.535 μ m) According to imaging 72 hours post injection there was no radioactivity in the joint of the injected rabbits with perrhenate while the colloid injection did not leak out from the joint in the same time. In the body of rabbits receiving perrhenate eluate predilection sites of stomach and thyroid were present whereas only the knee was visible in colloid injected animals not even the popliteal nor the inguinal lymph node. Histopathology revealed a thick pannus-like inflammatory tissue in the knee joint of saline-treated animals while radiocolloid treatment had an effect of thinning of synovial membrane to a thin fibrous membrane. We concluded that the tin radiocolloid developed by us is applicable after room temperature production and filtration in the knee to treat rheumatoid arthritis and its effect is the modification of the pannus to fibrous tissue in synovium. Further

decisions on development were centrally based on this artificially induced animal model results.

Somatostatin receptor radionuclide therapy in naturally occurring dog insulinoma

Experimental designs

We have applied the dual strategy of diagnosing the receptor expression using radiolabelled peptides with a suitable isotope for diagnostics, and thus exploring the possibilities of subsequent radiopeptide therapy. We also applied a radiopeptide first labelled with a diagnostic isotope (^{111}In) in the subsequent therapeutical setting where the same peptide was labelled with a therapeutic beta-emitting isotope (^{90}Y).

We signal referred animals from the Dept. Internal Medicine Faculty of Veterinary Sciences by Animal 1, 2 and 3. In all animals diagnostic suspicion of insulinoma was based on physical signs (hindlimb weakness, seizures, unconsciousness), low glucose levels and abdominal ultrasound. In all animals imaging was done using radiopeptide SPECT and we performed an in-

house developed immunohistochemistry for processing of postmortem samples. In the case of Animal 2 with therapy we measured plasma insulin levels to monitor therapy, too.

This diagnosis was based on ^{111}In -DOTATOC SPECT that later was ensured by immunohistochemistry. In Animal 2, we performed therapy in two, one month-distanced cycles with 185 and 370 MBq of ^{90}Y -DOTATOC activity. The effects of therapy were monitored by ultrasound and plasma insulin measurements, later by $^{99\text{m}}\text{Tc}$ -HYNIC-TOC SPECT, too. This latter method was also used in Animal 3 as well to diagnose tumor.

Besides this we examined the biodistribution of each radiopeptide in 1-1 healthy Beagle dog.

Results and discussion

In all animals we could present the tumors in more foci.

The day after therapy a moribund state was suspended in Animal 2 with a spectacular amelioration of the symptoms, Glucose levels rose from 1.4 mM to 2.4 mM.

We have followed the status and side effect profile of the dog treated during the course of 19 months and found no abnormalities and signs of side effects. The effects of therapy included total symptom-free period for 17 months, decreases in plasma insulin levels below the normal limit immediately after therapy and stable decrease of insulin level to half of the originally present high level.

In all animals we could prove the expression of somatostatin 2a receptors on the tumor cells in the foci identified by SPECT/scintigraphy. Healthy dog studies have presented some interesting differences with humans in organ uptake pattern (heart and stomach avidly taking up peptides whereas spleen not visible).

NEW SCIENTIFIC RESULTS

1. We have synthesized ^{177}Lu -EDTMP using carrier-free ^{177}Lu and formulated it pharmaceutically according to a previously not applied formulation with ^{177}Lu labels that is easy to tolerate *in vivo*, too.
2. We have shown the bone-seeking behaviour of ^{177}Lu -EDTMP in the mouse, rat, rabbit and dog species using nuclear imaging equipment. The application of a novel, world-unique SPECT system using multiplexed multipinhole collimation has played a significant role in the determination and identification of ^{177}Lu -EDTMP depositing in remodeling rodent bone areas and not in „inactive” bone.
3. We were the first to provide direct imaging proof that ^{177}Lu -EDTMP is preferentially binding to the cortical areas and not the spongiosa of the remodelling bones. Thus, the radiation dose of the isotope is confined to these areas rather than into bone marrow.
4. We have determined the differences between mouse and dog imaging of ^{177}Lu -EDTMP in the same time points post injection where dog uptake of radioactivity is present in a later maximum than in the mouse.

5. We have explored and defined the pharmacokinetics of ^{177}Lu -EDTMP in mouse and rabbit and we have determined that an important difference exists between mouse and rabbit bone Area Under the Data values representing differences in calcium bone turnover. Rabbit ^{177}Lu -EDTMP bone fixation has been proven to be less active upon comparison of Area Under the Curve values than turnover in the mouse. This has very important implications for human therapeutic dose planning.

6. We have presented that in dogs, ^{177}Lu -EDTMP is not exerting toxic effects to bone marrow after application in a therapeutic dose and we set the minimal values of injected activity in this species.

7. We have developed a new method of preparation of a radiosynoviorthesis agent, ^{188}Re -tin particles at room temperature reaction.

8. We have shown that the agent ^{188}Re -tin particle is not leaking out of rabbit arthritic knee joint at any time after application thus it is suitable for human intra-articular therapy as well.

9. We have determined that ^{188}Re -tin particle radiation dose is confined to the articular cartilage in human knee joint in extrapolative calculations of dosimetry.

10. We have shown using histology that beta particles arising from ^{188}Re are anti-inflammatory and have a beneficial effect against synovial thickening in the antigen induced arthritic rabbit knee model.

11. We have prepared the radiopharmaceutical $^{99\text{m}}\text{Tc}$ -HYNIC-TOC that shows binding to somatostatin 2A receptors in the dog, too.

12. We have shown that in the dog species the expression pattern of somatostatin receptors in the organs is different from that of man. We have shown that of ^{111}In and $^{99\text{m}}\text{Tc}$ -labelled somatostatin analogue peptides are differently binding to heart, stomach and spleen in the dog and in man. Dogs present receptors in the stomach and heart and we could not detect those receptors in spleen whereas in humans the situation is vica versa.

13. We have proven that dog spontaneous insulinomas express somatostatin 2A receptors with a direct immunohistochemistry method previously not presented in the literature.

14. We have also proven that dog somatostatin receptor peptide radionuclide SPECT is feasible with both ^{111}In and $^{99\text{m}}\text{Tc}$ using DOTA-TOC as targeting peptide.

15. We have shown that dog insulinoma can be used as a spontaneous model of PRRT and have achieved an outstanding case survival result with repeated ⁹⁰Y-PRRT. This survival is the first proof of targeted peptide radiation therapy in the dog.

LIST OF PUBLICATIONS OF THE AUTHOR NOT RELATED TO THE THESES

Mathe D., Balogh L., Polyak A., Kiraly R., Marian T., Pawlak D., Zaknun J.J., Pillai M.R.A., Janoki Gy. A. (2009): Multi-species animal investigation on biodistribution, pharmacokinetics and toxicity of ¹⁷⁷Lu-EDTMP, a potential bone pain palliation agent. Nucl Med Biol, in press **IF:2.419**

Chakraborty S., Das T., Banerjee S., Balogh L., Chaudhari PR., Sharma V., Polyak A., Mathe D., Meera V., Janoki G.A., Pillai M.R.A. (2008): ¹⁷⁷Lu-EDTMP: a viable bone pain palliative in skeletal metastasis. Cancer Biother Radiopharm. 23, 202-213 **IF:1.318**

Hauser P., Hanzély Z., **Máthé D.**, Szabó E., Barna G., Sebestyén A., Jeney A., Schuler D., Fekete G., Garami M. (2009): Effect of somatostatin analogue octreotide in medulloblastoma in xenograft and cell culture study. Pediatr Hematol Oncol. 26, 363-374 **IF:0.897**

Máthé D., Chaudhari P.R., Balogh L., Polyák A., Király R., Andócs G., Perge E., Glávits R., Jánoki Gy. A. (2002): Preliminary studies with ¹⁸⁸Rhenium-tin colloid for radiation synovectomy: preparation, size determination, in-vivo distribution, effects and dosimetry studies. Cent East Eur Rev Nucl Med. 5, 99-104.

Máthé D., Polyák A., Király R., Perge E., Mészáros Á., Haller K., Jakab Cs., Jánoki Gy. A. (2009): Receptor izotóp-diagnosztikai és -terápiás vizsgálatok szomatostatin analóg peptidekkel kutyákban. Magy Allatorvosok, *közlés alatt* **IF:0.104**

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