USE OF RADIOACTIVE ION BEAMS FOR BIOMEDICAL RESEARCH

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SUMMARY

A short status report and review is given on the experimental work carried out during 1993 in collaboration between CERN-ISOLDE and the University of Geneva.

i. It has been shown that CERN-ISOLDE can produce radionuclides suitable for PET studies (PET = Positron Emission Tomography)

ii. The ISOLDE preparation can be radiochemically transformed into suitable radiopharmaceuticals, which meet the requirements for nuclear medical application.

iii. 86-Y EDTMP and 142-Sm EDTMP are a suitable radiopharmaceuticals for quantitative bone imaging using PET technique. This is of practical clinical importance for improving the radionuclide therapy of bone metastases.

iv. Low molecular weight chelating ligands give a wide scope for influencing the biodistribution of carrier free radiolanthanides. Already tumor-to-liver ratios of about 10 have been obtained for tumor-bearing mice.

v. Aminobenzyl-DTPA anti-CEA monoclonal antibodies have been successfully labeled with radiolanthanides. They show high in vivo stability and high tumor uptake.

The results obtained during 1993 justify our moving to the first clinical human studies, and to continue the animal experiments with different labeled bioconjugates. We request additional 8 shifts of beam time, distributed over 3 different targets for 1994.
1. Review on radionuclide production at CERN-ISOLDE within the medical experimental program

Three targets were used at the ISOLDE facility for our experiments in 1993 to produce several radionuclides for our experiments:

- Nb-foil target with surface ionization ion source May 1993 24 h beam time
- U-carbide target with surface ionization ion source August 1993 30 h beam time
- Ta-foil target with surface ionization ion source Oct. 1993 40 h beam time

The following mass-separated radionuclides were collected:

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>half life</th>
<th>activity</th>
<th>intensity</th>
<th>remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>86-Y</td>
<td>14.7 h</td>
<td>2 * 100 uCi</td>
<td>1.0 exp+7</td>
<td>Nb-target, PET study with RPT</td>
</tr>
<tr>
<td>87-Y</td>
<td>80.3 h</td>
<td>88 uCi</td>
<td>6.2 exp+7</td>
<td>Nb-target, yield determination</td>
</tr>
<tr>
<td>141-Ce</td>
<td>32.5 d</td>
<td>200 uCi</td>
<td>6.2 exp+9</td>
<td>UC-target, mice studies</td>
</tr>
<tr>
<td>147-Nd</td>
<td>10.98 d</td>
<td>100 uCi</td>
<td>5.5 exp+8</td>
<td>&quot;</td>
</tr>
<tr>
<td>147-Eu</td>
<td>24.6 d</td>
<td>12 uCi</td>
<td>1.16 exp+8</td>
<td>&quot;</td>
</tr>
<tr>
<td>153-Sm</td>
<td>46.75 h</td>
<td>22.7 uCi/10 min</td>
<td>2.35 exp+8</td>
<td>test, yield determination</td>
</tr>
<tr>
<td>225-Ac</td>
<td>10.0 d</td>
<td>120 uCi</td>
<td>UC-target, mice studies</td>
<td></td>
</tr>
<tr>
<td>169-Yb</td>
<td>32.0 d</td>
<td>25 uCi</td>
<td>Ta-target, mice studies</td>
<td></td>
</tr>
<tr>
<td>167-Tm</td>
<td>9.25 d</td>
<td>100 uC</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>153-Gd</td>
<td>242 d</td>
<td>10 uCi</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>149-Gd</td>
<td>9.5 d</td>
<td>20 uCi</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>147-Eu</td>
<td>24.6 d</td>
<td>8 uCi</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>145-Sm</td>
<td>340 d</td>
<td>4 uCi</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>143-Pm</td>
<td>265 d</td>
<td>6 uCi</td>
<td>Ta-target, on stock</td>
<td></td>
</tr>
<tr>
<td>142-Sm</td>
<td>72.4 min</td>
<td>2*20 mCi</td>
<td>Ta-target, PET with RPT</td>
<td></td>
</tr>
</tbody>
</table>

The radionuclides were radiochemically processed and delivered for the experiments in carrier-free form, in small volumes (each about 10 μl) of weak (0.05 M) HCl solution.

2. Experimental program in 1993

2.1. PET-studies (IS 331)

Two isotopes (86-Y and 142-Sm) were used in PET studies with rabbits in the rotating PET scanner in the Department of Radiology, Nuclear Medicine of the University of Geneva.

<table>
<thead>
<tr>
<th>Date</th>
<th>Radiopharmaceutical</th>
<th>PET-Study performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 May</td>
<td>50 uCi 86-Y-EDTMP (2 ml 10mM)</td>
<td>static PET scan with rabbit</td>
</tr>
<tr>
<td>10 May</td>
<td>82 uCi 86-Y-EDTMP (2 ml 0.5mM)</td>
<td>sequence with attenuation correction</td>
</tr>
<tr>
<td>12 Oct</td>
<td>1 mCi 142-Sm-EDTMP (2 ml 1mM)</td>
<td>sequence with attenuation correction</td>
</tr>
<tr>
<td>13 Oct</td>
<td>1 mCi 142-Sm-EDTMP (1 ml 1mM)</td>
<td>sequence with attenuation correction</td>
</tr>
<tr>
<td>13 Oct</td>
<td>2 mCi 142-Sm-EDTMP (2 ml 1mM)</td>
<td>static, no attenuation correction</td>
</tr>
</tbody>
</table>

(EDTMP = Ethylenediaminetetramethylene phosphonic acid)

2.1.1. PET studies with 86-Y

The study performed with 86-Y early in May were the first PET studies ever performed in Geneva using radionuclide produced by CERN-facilities!
Although the available activity was low, good bone images were obtained. The expected behavior of the 86-Y-EDTMP was obtained: blood clearance is fast and no uptake in the liver could be detected. The overall 86-Y activity obtained at ISOLDE was too small to perform human studies, but the same target provides the production of large quantities of the positron emitter 83-Sr, which is very suitable to be used in human PET studies.

### 2.1.1. Studies with 142-Sm

The PET-scans carried out by this collaboration were the first ever using this positron emitting isotope. In the rabbits used in our studies the blood clearance was very fast. The kidneys are clearly imaged in the first series (10 min p.i.) but already 30 min p.i. clear bone images can be obtained. The excreted activity is accumulated in the bladder. The liver cannot be seen in the images a most important and promising result. EDTMP was used as chelating ligand in this study as well.

The positron abundance in 142-Sm is 145 % (50% for 142-Sm and an additional 95% for the short lived daughter 142-Pm).

### 2.2. Biomedical animal studies using tumor-bearing mice

#### 2.2.1. Overview

In all, 9 series of animal studies were performed so far using tumor bearing mice.

<table>
<thead>
<tr>
<th>Serie</th>
<th>Date</th>
<th>Number of mice</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.08.-03.09.93</td>
<td>6 mice</td>
<td>225-Ac, 141-Ce, 147-Nd and 147-Eu, i.p.-injection comparison CIT, NTA and EDTMP concentration: 10, 2.5 and 2.5 mM gamma ray spectroscopy (75 spectra)</td>
</tr>
<tr>
<td>2</td>
<td>13.09.-17.09.93</td>
<td>4 mice</td>
<td>225-Ac / 145-Sm, 1 mM EDTMP two window measurement and gamma spectroscopy</td>
</tr>
<tr>
<td>3</td>
<td>28.09.-30.09.93</td>
<td>3 mice</td>
<td>225-Ac / 145-Sm, 0.5 mM EDTMP, i.v.injection two window measurement and gamma spectroscopy</td>
</tr>
<tr>
<td>4</td>
<td>03.10.-05.10.93</td>
<td>7 mice</td>
<td>225-Ac, 141-Ce, 145-Sm [EDTMP] = 0.1 - 10 mM gamma ray spectroscopy (50 spectra)</td>
</tr>
<tr>
<td>5</td>
<td>27.10.-29.10.93</td>
<td>3 mice</td>
<td>225-Ac / 145-Sm [EDTMP] = 0.1, 1 and 2 mM other batch 2 window measurement and gamma spectroscopy</td>
</tr>
<tr>
<td>6</td>
<td>30.10.-02.11.93</td>
<td>12 mice</td>
<td>225-Ac, 141-Ce, 149-Gd, 167-Tm [EDTMP] = 0 - 10 mM gamma spectroscopy, 130 spectra</td>
</tr>
<tr>
<td>7</td>
<td>06.12.-09.12.93</td>
<td>7 mice</td>
<td>same isotopes as in serie 7, [EDTMP] = 0.001 - 30 mM gamma spectroscopy, 50 spectra</td>
</tr>
<tr>
<td>8</td>
<td>17.01.-25.01.94</td>
<td>10 mice</td>
<td>comparison of 153-Gd and 111-In in bioconjugates (aminobenzyl-DTPA anti-CEA monoclonal antibodies)</td>
</tr>
<tr>
<td>9</td>
<td>21.02.-11.03.94</td>
<td>8 mice</td>
<td>comparison of 169-Yb and 141-Ce in bioconjugates (aminobenzyl-DTPA anti-CEA monoclonal antibodies)</td>
</tr>
</tbody>
</table>

CIT = citrate
NTA = nitrilotriacetic acid
DTPA = diethylenetriaminepentaacetic acid
EDTMP = ethylenediaminetetramethylene phosphonic acid
2.2.2. Results

1. Experiment:
   EDTMP reduces liver uptake by a factor of 10 in comparison to CIT. Tumor-uptake similar. Highest tumor uptake for Ac, an important result, NTA showed no significant differences from CIT.

2. Experiment:
   full standard organ distribution measured for Sm and Ac simultaneously with 0.5 mM EDTMP, under this conditions liver uptake for Sm very low (<2 %/g), for Ac still high (30 %/g), femur identical (30 %/g). tumor accumulates factor 2 (at least) higher for Ac compared to Sm urine for Sm 50 %, Ac 4 %.

3. Experiment:
   same program as 2 but i.v. injection (i.v. = intra venous)
   approximately the same results

4. Experiment:
   variation of EDTMP concentration, biodistribution of main compartments: liver, femur, urine and tumor very clear dependency on the EDTMP-concentration

5. Experiment:
   a second EDTMP batch was tested, previous results have been confirmed, both EDTMP preparations show identical results.

6. Experiment: (Fig. 1 - 3)
   main run. 12 animals, 6 of them having a tumor implanted.
   full dependency on EDTMP-concentration (0 - to 10 mM) measured.
   The change of the biodistribution is very impressive: clear decreasing of liver uptake with growing EDTMP concentration, in contrary the urine excretion grows simultaneously with the decreasing liver uptake, tumor uptake is less influenced by the EDTMP-concentration, Ac shows highest tumor uptake. Tumor-to-liver ratios show a maximum at around 5 mM for Tm, in the case of Ac the same ratio is about 1, the tumor-to-blood ratios are up to 100.

7. Experiment:
   More data accumulated higher EDTMP concentrations have been used (up to 30 mM). Results from experiment Nr.6 were confirmed.

8. Experiment: (Fig. 4)
   First experiment with the bioconjugates. The aminobenzyl-DTPA was bound to the bifunctional ligand on the carbon backbone so to retain the maximum number of metal-ion binding ligands. The Gd was incorporated into the protein with high yield. The biodistribution of 111-In and 153-Gd labeled antibodies was practically identical. High tumor uptake was obtained (up to 30 %/g), the tumor to liver ratio was found to be up to 3.

9. Experiment:
   This is an preliminary experiment with the aim to design a study, where monoclonal antibodies will be simultaneously injected, labeled with about 5-6 different radionuclides of the full rare earth region as well as 225-Ac and 111-In. Study Nr. 9 already demonstrated that we have to expect a dependency of the in vivo stability of injected labeled bioconjugates on the complex stability of the chelating group used in the bioconjugate and the corresponding metal ion.

3. Conclusion

* Rare earth isotopes bound in low molecular weight chelate complexes are bone and tumor-affinity agents.
* The heavy lanthanides including Y bound with CIT show a fast blood clearance, an acceptably low liver uptake, and a tumor - liver ratio of about 1.
* The light lanthanides (Gd - Eu - Sm - Nd - Ce) and Ac show under the same conditions a fast blood clearance as well but an unacceptable high liver uptake and nearly no renal excretion. Consequently the tumor - liver ratios are very small.
However, when injected in solutions with chelating ligands which form more stronger complexes (NTA, EDTMP) then the biodistribution is changed dramatically: instead of high liver accumulation one can obtain reproducibly high renal excretion in combination with the biodistribution usually found for heavy lanthanides injected in CIT. However, there is a strong concentration effect: for Sm, EDTMP-concentrations above 1 mM are required to reproduce a biodistribution suitable for diagnosis and (maybe) for therapy.

The 142-Sm injected as EDTMP complex is very suitable to measure individually the uptake of Sm-radionuclides in bone metastases quantitatively. The half-life of 73 min is sufficient to perform human studies. The production yield at ISOLDE is sufficient to study several patients during the production day.

It has been shown that radiopharmaceuticals based on radionuclides produced at the facility at CERN are suitable to be used in human PET studies. In case of 142-Sm Geneva is so far the only place in the world, where such studies can be performed.

Radionuclides of rare earth elements are useful isotopes for labeling of monoclonal antibodies. The bioconjugates used in our studies seem to show high enough in-vivo stability to make them suitable for practical use in immunoscintigraphy.

3. Outlook and program for 1994

The results obtained during 1993 and early 1994 are promising. During 1994 we will be able to perform the first clinical patient studies using 83-Sr and 142-Sm (in vivo dosimetry, bone metastases). For these investigations we need to have, four times access to the beam when the foil targets of Nb and Ta are in operation (two times each 1/2 - 1 shift).

The biochemical experiments with animals will be continued in two main directions:

- Animal studies using low molecular chelating ligands
  - variation of species (rats, rabbits)
  - variation of ligands (NTA and others)

- experimental work with bioconjugates in mice.
  - relationship between complex stability and in vivo stability of labeled bioconjugates,
  - variation of chelating group in the bioconjugates.

In order to make this kind of study very efficient, a certain number of long-lived radionuclides must be collected at ISOLDE (see Chapter 1). As it has been shown during 1993 several µCi could be sufficient to perform such studies, if careful planned. The collaboration between the Department of Medical Biochemistry of the University of Geneva and ISOLDE is now el established. Technicians and students have been successfully involved in the experiments.

In total we request 8 shifts of beam time in addition to the remaining 8 shifts distributed to three different target units: the foil targets of Ta and Nb and to U-carbide with W-surface ionization ion source.
higher uptake compared to Gd or Tm. Sm shows smaller liver uptake compared to the lighter lanthanides (Ce as representative) and EDTMP—concentrations (above 1 μMol).

The radiolanthanides with the smallest ionic radius showed the smallest liver uptake, radionuclides used in the experiment.

A strong dependency of the uptake on the EDTMP concentration is observed for all radionuclides simultaneously.

100 μl of a solution, containing several of the carrier-free radionuclides 167-Tm, 149-Gd, 145-Sm, 141-Ce and 225-Ac were injected into the tail vein of the mice. The organ distribution was measured 20 hours after injection. Gamma-spectroscopy was applied to measure the concentration of the different radionuclides simultaneously.

* A strong dependency of the uptake on the EDTMP concentration is observed for all radionuclides used in the experiment.
* The radiolanthanides with the smallest ionic radius showed the smallest liver uptake, lanthanides with larger ionic radius and Ac show acceptable small liver uptake only at high EDTMP-concentrations (above 1 μMol).
* Sm shows smaller liver uptake compared to the lighter lanthanides (Ce as representative) and higher uptake compared to Gd or Tm.

**Fig.1.** Liver uptake of radiolanthanides and actinium in tumor-bearing mice as function of the EDTMP concentration (EDTMP = ethylenediaminetetramethylene phosphonic acid).
The behavior of Gd and Ce is in-between according to the ionic radius. At 0.1 mMol EDTMP concentration the urine excretion reaches the same level which was measured for Tm without EDTMP. Ac is practically not excreted via the kidney as long as the EDTMP concentration is below 0.1 mMol. At 0.5 to 1 mMol EDTMP concentration the urine excretion reaches the same level which was measured for Tm without EDTMP.

In case of Tm the urinary excretion grows from about 25% for a solution, were no EDTMP was added, up to nearly 70% for 10 mMol EDTMP concentration. A strong EDTMP concentration effect is observed.

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* The behavior of Gd and Ce is in-between according to the ionic radius.

**Fig. 2.** Urine excretion of radiolanthanides and actinium in tumor-bearing mice as function of the EDTMP concentration (compare with Fig. 1.)

100 µl of a solution, containing the mixture of the four carrier-free radionuclides 167-Tm, 149-Gd, 141-Ce and 225-Ac chelated with EDTMP in different concentrations were injected into the tail vein of tumor-bearing mice. The urine was carefully collected individually for each animal. Gamma-spectroscopy was applied to measure the concentration of the different radionuclides simultaneously.
In Fig. 1, it is seen that the liver uptake decreases significantly with increasing EDTMP concentration. In the same way the tumor accumulation is influenced significantly only at high EDTMP concentrations (above 5 mil). For 167-Tm the tumor-to-liver ratio increases from about 0.3 (without EDTMP) to 5 - 10. The corresponding ratios for the other radioelements are smaller according to their ionic radius. However, in case of 225-Ac the ratio is still growing at EDTMP concentration of 20 - 30 mMol reaching approximately the same value measured for Tm without EDTMP.
100 μl of a solution, containing the mixture of modified monoclonal antibodies labeled with 153-Gd and 111-In were injected into the tail vein of tumor bearing mice. After corresponding time intervals the animals were sacrificed, the organs taken and the excretions individually carefully collected. The In and Gd content of the samples were measured using gamma spectroscopy technique. In this experiment the chelating group DTPA (diethylenetriaminepentaacetic acid) was bound on the carbon backbone to retain maximum of complex stability for binding metal ions. The behavior of 111-In labeled antibodies is well known, thus the 111-In acts as an inner standard in this experiment. 153-Gd as an representative of the rare earth elements showed nearly the same biokinetic behavior. The conclusion is: radiolanthanides are promising candidates in the whole field of immunoscintigraphy and immunotherapy, an very important applied research field in nuclear medicine today and in future. A very high tumor uptake was observed.

Fig. 4. Biokinetic of monoclonal antibodies, labeled with 153-Gd and 111-In