Evaluation of carrier added and no carrier added 
$^{90}\text{Y}$-EDTMP as bone seeking therapeutic radiopharmaceutical

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Abstract: The optimum conditions to label ethylenediaminetetramethylene phosphonate (EDTMP) compound with $^{90}\text{Y}$ as a potential candidate for bone metastases therapy were investigated. Yttrium-90 is a pure $\beta$-emitter and can be obtained by $^{90}\text{Y} (n,\gamma)^{90}\text{Y}$ nuclear reaction in a reactor or from an in-house generator system ($^{90}\text{Sr} \rightarrow^{90}\text{Y}$). The preparation of $^{90}$Y-EDTMP is described using $^{90}\text{Y}$, which was obtained from neutron irradiation of $\text{Y}_2\text{O}_3$ as well as from a laboratory scale organic resin-based $^{90}\text{Sr} \rightarrow^{90}\text{Y}$ generator. Because of the radiolabeling yield of $^{90}$Y-EDTMP on ligand/metal molar ratio, incubation time and $\text{pH}$ was evaluated. Under optimum parameters, the radiolabeling yields of $^{90}$Y-EDTMP were $<95\%$ for no-carrier-added as well as carrier-added $^{90}\text{Y}$. The biodistribution of no-carrier-added and carrier-added $^{90}$Y-EDTMP complexes in rats was identical. The results indicate that $^{90}$Y (carrier-added)-edtmp is also an effective bone pain palliation agent because of its rapid blood clearance, greater uptake in bones and little absorption in soft tissues.

Keywords: Carrier-added Yttrium-90; No-carrier-added Yttrium-90; Strontium-90/Yttrium-90 generator; EDTMP; Biodistribution in rats.

INTRODUCTION

Nuclear medicine is a medical modality that uses radiopharmaceuticals to diagnose and treat diseases. Fruitful radiotherapy depends upon matching the radionuclide with chemistry and biological kinetics of carrier molecule. Radionuclides which decay with the emission of $\beta$-particles are rapidly becoming of great interest for cancer therapy. The debilitating and intractable pair that may accompany cancer is often interest for cancer therapy. The debilitating and intractable pair that may accompany cancer is often produced by bone metastases, while radiotherapy is the first choice for palliative treatment for patients with limited number of lesions. Systemic radionuclide therapy with radiopharmaceuticals is preferable for extensive bone metastases with multifocal sites of pain and remains a most widely used and effective modality (Serafini, 2001). Strategies for the management of bone metastases include opiate analgesia, steroids, bisphosphonates, cytotoxic chemotherapy, external beam radiotherapy and unsealed source radiotherapy. Unsealed source radiotherapy for skeletal metastases was one of the first applications of radioisotopes in medicine and dates from more than five decades ago, when phosphorus-32 was first used. Strontium-89 was introduced in 1942 as a targeted radiotherapeutic agent for bone metastases (Pecher, 1942).

Ethylenediaminetetramethylene phosphonate is a tetraphosphonate ligand and show great affinity to skeleton and osteoblastic bone metastases and various EDTMP chelates possess a significantly high stability (Goeckeler et al., 1987). Subsequently a variety of radioactively labeled EDTMP compounds have been developed and used clinically for diagnosis and treatment of osteoblastic lesions. The synthesis of EDTMP chelates with $\beta$-emitters reported in literature includes yttrium-90 [Keeling et al., 1989], rhodium-105 (Ando et al., 2000), samarium-153 (Goeckeler et al., 1987; Mushtaq et al., 1997), erbium-165 (Hassfjell et al., 1998),holmium-166 (Louw et al., 1996), lutetium-177 (Ando et al., 1998; Chakraborty et al., 2002), rhenium-188 (Oh et al., 2002; Pervez et al., 2003; Mushtaq et al., 2007). Diagnostic radionuclides, yttrium-86 (Roesch et al., 1996), technetium-99m (Garnuszek et al., 2003; Bokhari et al., 2012; Faheem et al., 2013) and indium-111 have been reported as well (Lazniecek et al., 1994). Yttrium-90 is believed to be the most useful among the radionuclides that have been employed for radiotherapeutic purposes. Yttrium-90 with half-life of 64.1 h has no accompanying gamma-ray radiation in its decay, high-energy $\beta$ rays of ($E_{\text{max}}=2.3$ MeV), and a stable Zirconium-90. $^{90}\text{Y}$ is mostly obtained from $^{90}\text{Sr}/^{90}\text{Y}$ the chromatographic generator of system. Different procedures for clean separation of $^{90}$Y from high yielded fission product $^{90}$Sr have been reported, the solvent extraction and ion exchange was mostly employed for its separation (Chinol et al., 1987). The $^{90}$Y, high specific activity is achieved by the methods for radioimmunotherapy (Hnatowich et al., 1985). However users must note that parent strontium-90 ($T_{1/2}=28$ y) gives bone marrow depression and its

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permissible dosage is only 74 kBq (Cember, 1983). Therefore, developing a strontium-90 free method for getting $^{90}\text{Y}$ is mandatory. $^{90}\text{Y}$ may be produced by the bombardment of neutrons on yttrium metal or its oxide in the nuclear reactor. As a result the specific activity is low, but free of strontium-90 and carrier added $^{90}\text{Y}$ can be employed for therapeutic purposes.

In this study, we standardized the optimal reaction conditions for labeling of $(n,\gamma)\ Y^{90}$ with EDTMP kit and in vitro stability of $^{90}\text{Y}$-EDTMPcomplexes. In addition, biodistribution studies of this radio pharmaceutical with carrier and no-carrier-added yttrium in rats were performed. The behavior of carrier-added and no-carrier-added $^{90}\text{Y}$-EDTMPcomplexes was compared in vitro and in vivo conditions.

**MATERIALS AND METHODS**

**Material**

All the chemicals used in this study were of analytical reagent grade. Pyridine, ethanol, sodium hydroxide, sodium hydrogen phosphate, ethylenediaminetetraacetic acid (EDTA) and hydrochloric acid (37%) were obtained from E. Merck (Germany). $\text{Y}_2\text{O}_3$ powder was taken from International Hospital Supply Corp (New York). The cation exchange resin AG 50 WX 8 having 100-200 mesh size was product of Bio-Rad. Commercially available EDTMP injection was purchased by Dojin Laboratories (Japan). Sprague-Dawley rats were purchased from National Institute of Health, Islamabad.

**Production of $^{90}\text{Y}$ by $(n,\gamma)$ Reaction**

Known quantities of $\text{Y}_2\text{O}_3$ targets were encapsulated in quartz ampoules. These ampoules were sealed into aluminum cans by cold welding technology. These yttrium oxide targets were irradiated in the core of Pakistan research reactor-I for time period up to 120 hours at a neutron flux of $\sim 1.5 \times 10^{14}$ cm$^{-2}$ s$^{-1}$. After dissolving the irradiated $\text{Y}_2\text{O}_3$ material in concentrated HCl, it was evaporated and then taken in distilled H$_2$O.

**Production of no-carrier-added $^{90}\text{Y}$**

No-carrier-added $^{90}\text{Y}$ was attained from $^{90}\text{Sr} \rightarrow ^{90}\text{Y}$ generator system described by Chinol and Hnatowich (Chinol and Hnatowich, 1987). The generator contains a glass column with a 1 cm diameter and 15 cm long with a frit at the bottom. The column is mounted vertically and is surrounded with 6 cm lead. The cation exchange resin AG 50 WX 8 having 100-200 mesh size was conditioned with 1/M NaOH to convert the resin to the sodium form and was then washed free of excess base with triple distilled water. The solution of $^{90}\text{Sr}$ (10 mCi) in 6/mL of 1 M HNO$_3$, with 0.72/mL of 0.03 M EDTA was adjusted to pH 4.6 by adding concentrated NaOH. Five grams of pretreated resin was then added and suspension stirred slowly overnight. The glass column was then loaded, first with 1 g of the resin without radioactivity followed by the 5/g of resin containing the $^{90}\text{Sr}$. The washing was given to generator column by 50 mL of 0.003 M EDTA, pH 4.6, to remove the traces of unbound $^{90}\text{Sr}$. During each elution, 5 mL of 0.003 M EDTA, pH 4.6 was forced through the generator at a flow rate of 0.5/mL per minute. Solution was evaporated, then 8/mL of 1:1 conc. H$_2$SO$_4$: HNO$_3$ was added and the contents evaporated to dryness to destroy the EDTA. Finally the $^{90}\text{Y}$ activity is dissolved in dilute HCl. A rapid estimate of $^{90}\text{Sr}$ breakthrough was determined by the paper chromatographic technique 28. The $\beta$-rays were monitored with an alpha/beta counting devices and $\gamma$-ray spectra were analyzed with HpGe detector. When the activities of Yttrium-90 (no-carrier-added or with carrier) handled were more than few MBq, measurements were made in calibrated ionization chamber (Capintec).

**Preparation of $^{90}\text{Y}$-EDTMP radiopharmaceutical**

Ethylenediaminetetramethylene phosphonate was dissolved in distilled H$_2$O. $^{90}\text{Y}$Cl$_3$ solution was added to the EDTMP injection. Various parameters such as ligand and metal molar ratio, pH and incubation time were standardized to get maximum labelling yield. All experiments were carried out at room temperature (22± 2°C) while volume of reaction mixture was fixed 2/mL. For comparison purpose carrier added and no-carrier added $^{90}\text{Y}$-EDTMPcomplexes were prepared.

**Quality Control Procedures**

The radiolabeling yield of $^{90}\text{Y}$-EDTMPcomplexes was determined by ascending chromatographic technique. 5µL of the test solution was spotted at 2 cm from one end (bottom end) of Whatman 3 MM paper strips (14x2 cm). The strips were developed in pyridine/ethanol/water (1:2:4), dried, cut into 1 cm pieces and the strips were scanned by 2π Scanner (Germany).

**Paper electrophoresis**

Electrophoresis of $^{90}\text{Y}$-EDTMP was studied by using Deluxe electrophoresis chamber (Gelman USA) system. 5µL test solution was spotted on Whatman No.1 paper of 30cm was used marked with L at the left side of the strip and R at the right side of the strip. The strip was placed in the electrophoresis chamber containing buffer in such a way that the left side dipped at anode and the right side at cathode. Electrophoresis was carried out for 1/h under a voltage gradient of 10 Vcm$^{-1}$. The phosphate buffer (0.025 M) of pH 7.5 was used in this experiment. The strips were dried, cut into 1 cm pieces and the strips were scanned by using 2π scanner to know the charge on $^{90}\text{Y}$-EDTMP. Locally made apparatus for electrophoresis was used during the experiments.

**Biodistribution studies**

Biodistribution of $^{90}\text{Y}$-EDTMPcomplexes (carrier added and no-carrier added) were achieved in 200-225/g Male Sprague-Dawley rats. 200µL of $^{90}\text{Y}$-EDTMP were injected into the tail veins of the anesthetized rats. After a definite...
time, the rats were sacrificed after ether anesthesia. They were killed by cervical dislocation at 0.5h, 1h and 4h post injection. A 1/mL blood sample was taken from the heart and weighed instantly after killing. The rats were then weighed and dissected with care being taken for isolating the urine and blood on the kill papers, the tissue washing and the urine collected from the cages. Four rats were used for each time point. Counting was performed using a well type gamma counter/ionization chamber. Rats were anaesthetized by use of ether, when required.

RESULTS

Production of 90Y

The natural yttrium comprises of 100% 89Y and nuclear reaction cross-section for 89Y (n,γ) 90Y is 1.28±0.02 barn (Erdtman, 1976). The typical yields for different time durations of irradiation in PARR-I (~1.5×10^14 n cm^-2 s^-1) were in excess of theoretically calculated values. This could perhaps be recognized to the contribution from epithermal neutrons (resonance integral for 90mY=880 could perhaps be recognized to the contribution from epithermal neutrons (resonance integral for 90mY=880 ± 80 mb and for 90Y=1.0±0.2 b). The 120 hours irradiation of Y_2O_3 (15 mg) resulted in the formation of ~ 12 GBq (324 mCi) of 90Y activity at the end of irradiation and the corresponding specific activity was ~10^17 GBq/g of Yttrium. Although high purity target material Y_2O_3 were used for neutron irradiation. The peaks corresponding to 175Yb (T 1/2=4.185 d) and 177Lu (T 1/2=6.734 d) were used for neutron irradiation. The peaks corresponding to 175Yb (T 1/2=4.185 d) and 177Lu (T 1/2=6.734 d) were measured by gamma spectrometry. The estimated activities of these produced radionuclides were 1 kBq. Beta emitting strontium-89 (T 1/2=50.5 d) may be present as an impurity in 90Y (estimated value =2.22 kBq or 0.06 µCi/mg), since it is produced via 89Y (n, p) nuclear reaction with fast neutrons.

Quality Control and Labeling Yield

Ascending paper chromatography on Whatman 3 MM assessed labeling efficiency and radiochemical purity. Using pyridine/ethanol/water (1:2:4) as the solvent, 90Y-EDTMPmoved towards the solvent front, while free 90Y retained at place of spotting (fig. 1).

Electrophoresis pattern in phosphate buffer presented the movement of 90Y-EDTMPcomplex in direction of anode representing that the complex is negatively charged, similar to other rare earth-EDTMP complexes. On the other hand, 90YCl_3 did not show any movement from the point of application under similar parameters. Fig. 2 represents electrophoresis analyses of 90Y-EDTMPAs well as 90YCl_3. The results of paper chromatography and electrophoresis were employed to determine both the radiochemical purity and labeling yield of 90Y-EDTMP. It was observed that pH was an important factor for complexation of (n,γ) 90Y with EDTMP. In acidic media (2-6 pH) the EDTMP solution turned turbid upon the addition of 90Y (with carrier). High yield of complexation was possible at pH 7-9. Hence pH ~7.5 was selected for further studies.

Table 1: Biodistribution of carrier-added (CA) and no carrier added (NCA) 90Y-EDTMP in Sprague-Dawley rats expressed as the percentage of the injected dose per organ.

<table>
<thead>
<tr>
<th>Organ</th>
<th>0.5h</th>
<th>1h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
<td>NCA</td>
<td>CA</td>
</tr>
<tr>
<td>Blood</td>
<td>0.48±0.12</td>
<td>0.42±0.10</td>
<td>0.52±0.12</td>
</tr>
<tr>
<td>Heart</td>
<td>0.03±0.15</td>
<td>0.05±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.22±0.12</td>
<td>0.98±0.03</td>
<td>0.27±0.11</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.55±0.15</td>
<td>0.65±0.11</td>
<td>0.63±0.16</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.11±0.23</td>
<td>0.99±0.05</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>0.10±0.03</td>
<td>0.07±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.02±0.02</td>
<td>0.02±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.06±0.02</td>
<td>0.08±0.03</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.21±1.52</td>
<td>2.15±1.75</td>
<td>1.10±0.27</td>
</tr>
<tr>
<td>Bone</td>
<td>50.94±4.25</td>
<td>48.82±3.82</td>
<td>51.11±3.80</td>
</tr>
<tr>
<td>Urine</td>
<td>36.85±4.25</td>
<td>40.62±4.33</td>
<td>40.55±4.56</td>
</tr>
</tbody>
</table>

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Ligand molar ratio was varied in range of 1 to 12. With
the increase of molar ratio of ligand labeling yield was
increased. These results indicate that $^{90}$Y-EDTMP
can be formed with radiochemical purity over 95% using
an EDTMP/Yttrium molar ratio from 5 to 12 at pH 7.5.
The molar ratio effect is shown in fig. 3. The rate of
complexation of $^{90}$Y with EDTMP is quite rapid at room
temperature, within few minutes maximum labeling
efficiency is achieved. The complex of $^{90}$Y-EDTMPs is
also quite stable and up to 7 d ~95% labeling efficiency is
retained (fig. 4). The complexation of no-carrier-added
$^{90}$Y was easily achieved with 5mg/ml EDTMP at pH ~7.5.
Within few minutes labeling efficiency was <96% at room
temperature. The paper chromatographic/electrophoresis
behavior of carrier-added or no-carrier-added $^{90}$Y-
EDTMP and $^{90}$YCl$_3$ was same. In this research work we
validated that $^{90}$Y-EDTMP stable complex could be
prepared at a ligand-to-metal ratio of 5:1. Approximately
1GBq/mg specific activity of the $^{90}$Y was used in this
work. Mixing 3 mg of yttrium ($^{90}$Y) solution with 75 mg
EDTMP solution, a therapeutic dose of $^{90}$Y-
EDTMP (~3GBq) can be prepared, which is quite
sufficient for the treatment of a 70 kg patient. However, it
is possible to get an image of Bremsstrahlung of $^{90}$Y
to validate that the palliative radiotherapy is reaching the
target actually. Like $^{32}$P and $^{89}$Sr, $^{90}$Y is a pure beta-
emitter, which is beneficial for bone cancer therapy. Due
to the lack of $\gamma$- radiations there is possibility to get a
qualitative dosimetric measurement by the combination of
quantitative $^{99m}$Tc-MDP bone scintigraphy with $^{90}$Y
Bremsstrahlung images.

**Biodistribution**

The biodistribution of $^{90}$Y-EDTMP with and without
carrier is compared in table 1. The biological distribution
behavior of radioactivity in selected organs of rats after
$^{90}$Y-EDTMP administrations at different time intervals (0.5
h, 1 h and 4 h) is also presented. Biodistribution results
showed that $^{90}$Y-EDTMP stable complex could be
prepared at a ligand-to-metal ratio of 5:1. Approximately
1GBq/mg specific activity of the $^{90}$Y was used in this
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Bremsstrahlung images.
lack of γ- radiations there is possibility to get a qualitative dosimetric measurement by the combination of quantitative 99mTc-MDP bone scintigraphy with 90Y Bremsstrahlung images. Skeletal uptake of 90Y-EDTMP in rats is comparable to 153Sm-EDTMP (Goeckeler et al., 1985). The radiochemical purity of no-carrier-added 90Y-EDTMP remained unchanged up to 4 d. The results showed that 90Y-EDTMP, carrier added as well as no-carrier added showed long-term retention in the skeleton, high uptake in bone, and had efficient and rapid clearance from the blood. No specific accumulation in non-osseous tissues and organs was observed.

**CONCLUSION**

Under the suitable conditions, the radiolabeling yields of carrier-free and carrier-added 90Y-EDTMP were >96% and >95 % respectively. It was found that the yttrium carrier did not alter the skeletal uptake in vivo as compared to no-carrier-added 90Y-EDTMP. 90Y-EDTMP (carrier-added) is a potential radiopharmaceutical for bone palliation because of relatively low soft-tissue absorption, greater skeletal uptake and rapid blood clearance. No significant change in vivo/vitro behavior of both 90Y-EDTMP complexes (carrier-added or no-carrier added) could be observed in present study. Mixing 3 mg of yttrium (90Y) solution with 75 mg EDTMP solution, a therapeutic dose of 90Y-EDTMP (~3GBq) can be prepared, which is quite sufficient for the treatment of a 70 kg patient.

**REFERENCES**


