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

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Abstract: The optimum conditions to label ethylenediaminetetramethylene phosphonate (EDTMP) compound with ^{90}y as a potential candidate for bone metastases therapy were investigated. Yttrium-90 is a pure β-emitter and can be obtained by ^{89}y (n,γ) ^{90}y nuclear reaction in a reactor or from an in-house generator system (^{90}sr→^{90}y). The preparation of ^{90}Y-EDTMPis described using ^{90}y, which was obtained from neutron irradiation of y_2o_3 as well as from a laboratory scale organic resin-based ^{90}sr→^{90}y generator. Because of the radiolabeling yield of ^{90}Y-EDTMP on ligand/metal molar ratio, incubation time and ph was evaluated. Under optimum parameters, the radiolabeling yields of ^{90}Y-EDTMPwere <95% for no-carrier-added as well as carrier-added ^{90}y. The biodistribution of no-carrier-added and carrier-added ^{90}Y-EDTMPcomplexes 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 β-particles are rapidly becoming of great 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 β-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 (Laznicek *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 β rays of ($E_{\beta max}$=2.3 MeV), and a stable Zirconium-90. ^{90}Y is mostly obtained from ^{90}Sr/^{90}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}Y is mandatory. ^{90}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}Y can be employed for therapeutic purposes.

In this study, we standardized the optimal reaction conditions for labeling of (n,γ) ^{90}Y with EDTMP kit and in vitro stability of ^{90}Y-EDTMPcomplexes. In addition, biodistribution studies of this radiopharmaceutical with carrier and no-carrier-added yttrium in rats were performed. The behavior of carrier-added and no-carrier-added ^{90}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). Y_2O_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}Y by (n,γ) Reaction

Known quantities of Y_2O_3 targets were encapsulated in quartz ampoules. These ampoules were sealed into aluminum cans by cold welding technology. These yettrium oxide targets were irradiated in the core of Pakistan research reactor-I for time period up to 120 hours at a neutron flux of ~$1.5x10^{14}$ cm^{-2} s^{-1}. After dissolving the irradiated Y_2O_3 material in concentrated HCl, it was evaporated and then taken in distilled H_2O.

Production of no-carrier-added ^{90}Y

No-carrier-added ^{90}Y was attained from $^{90}Sr \rightarrow {}^{90}Y$ 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 W X 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}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}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}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_2SO_4: HNO_3 was added and the contents evaporated to dryness to destroy the EDTA. Finally the ^{90}Y activity is dissolved in dilute HCl. A rapid estimate of ^{90}Sr breakthrough was determined by the paper chromatographic technique 28.The β-rays were monitored with an alpha/beta counting devices and γ-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}Y-EDTMPradiopharmaceutical

Ethylenediaminetetramethylene phosphonate was dissolved in distilled H_2O. $^{90}YCl_3$ 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}Y-EDTMPcomplexes were prepared.

Quality Control Procedures

The radiolabeling yield of ^{90}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}Y-EDTMPwas studied by using Deluxe electrophoresis chamber (Gelman USA) system. 5µL of 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}Y-EDTMP. Locally made apparatus for electrophoresis was used during the experiments.

Biodistribution studies

Biodistribtion of ^{90}Y-EDTMPcomplexes (carrier added and no-carrier added) were achieved in 200-225/g Male Sprague-Dawley rats. 200µL of ^{90}Y-EDTMPwere 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 was 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 ^{90}Y

The natural yttrium comprises of 100% ^{89}Y and nuclear reaction cross-section for ^{89}Y (n,γ) ^{90}Y is 1.28±0.02 barn (Erdtman, 1976). The typical yields for different time durations of irradiation in PARR-I (~$1.5x10^{14}$ 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 ^{90m}Y=880±80 mb and for ^{90}Y=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 ^{90}Y activity at the end of irradiation and the corresponding specific activity was ~1017 GBq/g of Yttrium. Although high purity target material Y_2O_3 were used for neutron irradiation. The peaks corresponding to ^{175}Yb ($T_{1/2}$=4.185 d) and ^{177}Lu ($T_{1/2}$=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 ^{90}Y (estimated value =2.22 kBq or 0.06 μCi/mg), since it is produced via ^{89}Y (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, ^{90}Y-EDTMPmoved towards the solvent front, while free ^{90}Y retained at place of spotting (fig. 1).

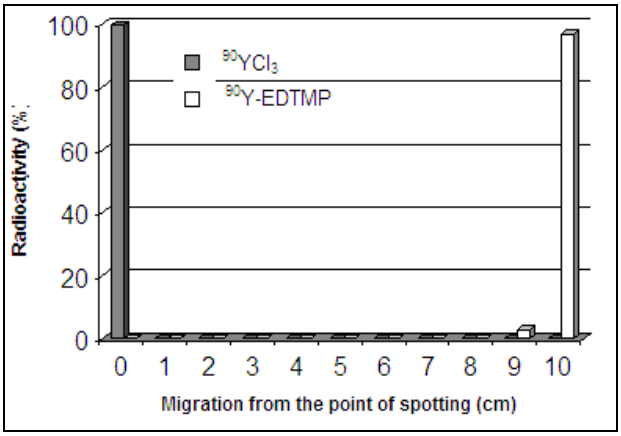

Fig. 1: Paper chromatography pattern of the ^{90}Y-EDTMPcomplex and $^{90}YCI_3$ in pyridine: ethanol: water (1:2:4).

Electrophoresis pattern in phosphate buffer presented the movement of ^{90}Y-EDTMPcomplex in direction of anode representing that the complex is negatively charged, similar to other rare earth-EDTMP complexes. On the other hand, $^{90}YCl_3$ did not show any movement from the point of application under similar parameters. Fig. 2 represents electrophoresis analyses of ^{90}Y-EDTMPas well as $^{90}YCl_3$. The results of paper chromatography and electrophoresis were employed to determine both the radiochemical purity and labeling yield of ^{90}Y-EDTMP. It was observed that pH was an important factor for complexation of (n,γ) ^{90}Y with EDTMP. In acidic media (2-6 pH) the EDTMP solution turned turbid upon the addition of ^{90}Y (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) ^{90}Y-EDTMP in Sprague-Dawley rats expressed as the percentage of the injected dose per organ.

Organ	Time after injection (mean ± SD, n=4)					
	0.5h		1h		4h	
	CA	NCA	CA	NCA	CA	NCA
Blood	0.48±0.12	0.42±0.10	0.52±0.12	0.33±0.09	0.05±0.02	0.04±0.02
Heart	0.03±0.15	0.05±0.01	0.02±0.01	0.02±0.01	0.01±0.00	0.01±0.00
Stomach	1.22±0.12	0.98±0.03	0.27±0.11	0.17±0.06	0.06±0.03	0.04±0.02
Intestine	0.55±0.15	0.65±0.11	0.63±0.16	0.40±0.12	0.42±0.16	0.39±0.10
Kidneys	1.11±0.23	0.99±0.05	0.23±0.02	0.20±0.03	0.12±0.06	0.14±0.06
Liver	0.10±0.03	0.07±0.01	0.03±0.01	0.02±0.01	0.05±0.02	0.04±0.02
Spleen	0.02±0.02	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.00	0.00±0.00
Lung	0.06±0.02	0.08±0.03	0.02±0.01	0.01±0.01	0.00±0.00	0.00±0.00
Muscle	3.21±1.52	2.15±1.75	1.10± 0.27	1.05±0.62	0.28±0.20	0.32±0.18
Bone	50.94±4.25	48.82±3.82	51.11±3.80	48.81±3.51	50.25±4.45	46.80±4.18
Urine	36.85±4.25	40.62±4.33	40.55±4.56	45.55±3.99	44.45±3.37	48.25±4.44

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-EDTMPcomplex 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-EDTMPis 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-EDTMPand $^{90}YCl_3$ was same. In this research work we validated that ^{90}Y-EDTMPa 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 γ- radiations there is possibility to get a qualitative dosimetric measurement by the combination of quantitative ^{99m}Tc-MDP bone scintigraphy with ^{90}Y Bremsstralung images.

Biodistribution

The biodistribution of ^{90}Y-EDTMPwith and without carrier is compared in table 1. The biological distribution behavior of radioactivity in selected organs of rats after ^{90}Y-EDTMPadministrations at different time intervals (0.5 h, 1 h and 4 h) is also presented. Biodistribution results showed that ^{90}Y-EDTMPusing carrier-added or no-carrier-added ^{90}Y skeleton uptake was significant. At the initial time intervals the radioactivity of the complex was highest in the kidneys. This is because of partial elimination of the complex in the urine shortly after induction. In other organs the time decrease of radioactivity is due to very rapid decrease of radioactivity in blood. The ^{90}Y-EDTMPuptake in heart, liver, lungs is not significant (<0.2%). As compare to other organs, the maximum radioactivity was found in bone after 30 min and the radioactivity in bone remained almost unchanged at longer time intervals (~50% of injected dose).The most of administered radioactivity was rapidly removed in urine while a very small portion in faeces. It may be due to retention of significant activity in metastatic lesion site as compared to normal cells due to the hypoxic nature of the cells.

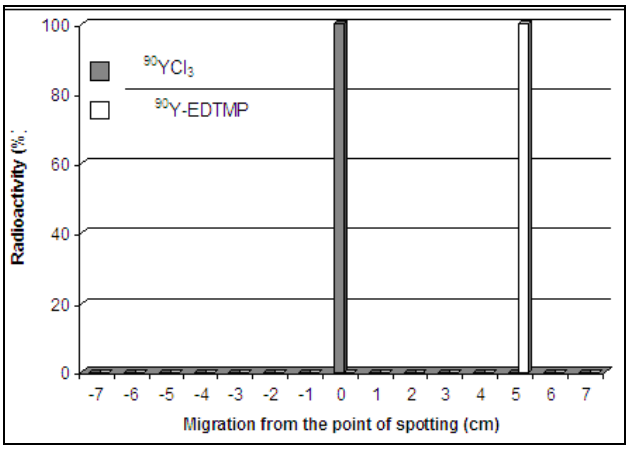

Fig. 2: Paper electrophoresis pattern of the ^{90}Y-EDTMPcomplex and $^{90}YCI_3$ in 0.025 M phosphate buffer (PH 7.5) at a potential gradient of 10 V cm^{-1} for 1 h.

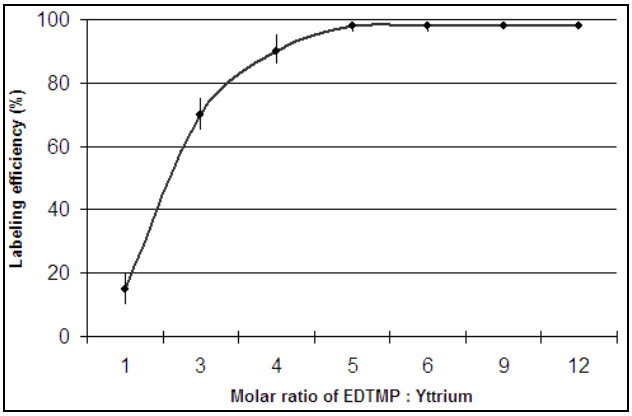

Fig. 3: Effect of increasing molar ratio of EDTMP on complexation yield of ^{90}Y-EDTMP(Y molarity =1)

DISCUSSION

It is quite significant that ^{90}Y may be available as a radionuclide generator product by decay of its 28 y parent strontium-90. Various ^{90}Y generator systems of different types have been reported; most of the clinical studies of ^{90}Y-labeled compounds have been performed with activity obtained not from in-house ^{90}Sr→^{90}Y generators, but from commercial sources. Before its use in clinical practices, the ^{90}Y must be free of ^{90}Sr which cause bone marrow suppression and trace elements causing hindrance with the radiolabeling procedures by competing with ^{90}Y (carrier-free) for binding sites must be removed. Using 0.003 M EDTA as eluant for separation of ^{90}Y from in-house made ^{90}Sr→^{90}Y generator, elution efficiency averages 95% and ^{90}Sr breakthrough averages 0.002%. Up to 3 months no evidence of radiolytic degradation was noted to AG 50 W X 8 having 100-200 mesh cation exchange resin, which serves as the solid support for the generator. 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 γ- radiations there is possibility to get a qualitative dosimetric measurement by the combination of quantitative ^{99m}Tc-MDP bone scintigraphy with ^{90}Y Bremsstralung images. Skeletal uptake of ^{90}Y-EDTMPin rats is comparable to ^{153}Sm-EDTMP (Goeckeler *et al*., 1985). The radiochemical purity of no-carrier-added ^{90}Y-EDTMP remained unchanged up to 4 d. The results showed that ^{90}Y-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 ^{90}Y-EDTMPwere >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 ^{90}Y-EDTMP. ^{90}Y-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 ^{90}Y-EDTMPcomplexes (carrier-added or no-carrier added) could be observed in present study. 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.

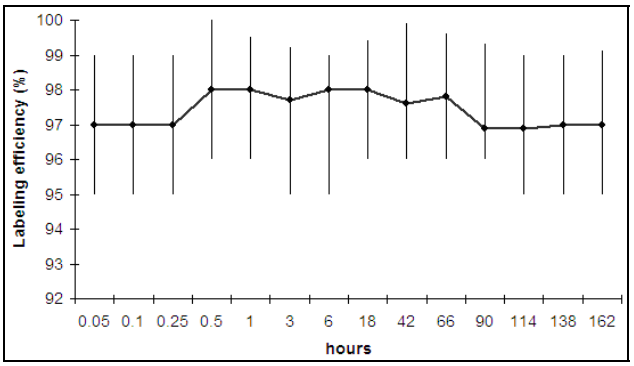

Fig. 4: Rate of complexation of ^{90}Y with EDTMP and stability of ^{90}Y-EDTMPat room temperature.

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