Titanium-45 as a Candidate for PET Imaging: Production, Processing & Applications

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Introduction

The 80kD glycoprotein transferrin (TF) and its related receptor (TFR1) play a major role in the recruitment by cancer cells of factors for their multiplication, adhesion, invasion and metastatic potential. Though primarily designed to bind iron and then be internalised into cells with its receptor, TF can also bind most transition metals such as Co, Cr, Mn, Zr, Ni, Cu, V, In & Ga. Under certain conditions TF binds Ti (IV) even more tightly than it does Fe and that this occurs at the N-lobe (as distinct from C) of apoTF. Further, under physiological conditions the species Fe(C)Ti(N)-TF may provide the route for Ti entry into cells via TFR1 (1). Thus, the radiometal PET reporter isotope ^{45}Ti with an 'intermediate' (~hrs) half-life suited to tracking cell-focused biological mechanisms is an attractive option for elucidating cellular mechanisms involving TF binding and internalisation, at least in (preclinical) animal models.

^{45}Ti ($T_{½}$ = 3.08 hr; β^+ branching ratio = 85 %; mean β^+ energy = 439keV, no significant dose-conferring non-511keV γ-emissions) was produced using the reaction ^{45}Sc(p,n)^{45}Ti by irradiating (monoisotopic) scandium discs with an energy-degraded proton beam produced by an 18MeV isochronous medical cyclotron. Separation and purification was achieved with an hydroxylamine hydrochloride functionalised resin. Comparative microPET imaging was performed in an immunodeficient mouse model, measuring biodistributions of the radiolabels ^{45}Ti-oxalate and ^{45}Ti-human-TF (^{45}Ti-h-TF), out to 6hr post-injection.

Materials and Methods

High purity 15mm diameter scandium disc foils (99.5%, Goodfellow, UK) each thickness 0.100 ± 0.005 mm (55 mg) were loaded into an in-house constructed solid-targetry system mounted on a 300mm external beam line utilising helium-gas and chilled water to cool the target body (2). The proton beam was degraded to 11.7 MeV using a graphite disc integrated into the graphite collimator. This energy abolishes the competing 'contaminant' reactions ^{45}Sc(p,n+p)^{44}Sc and ^{45}Sc(p,2n)^{44}Ti. Beam current was measured using the well documented ^{65}Cu(p,n)^{65}Zn reaction. Calculations showed that the chosen energy is close to the optimal primary energy (~12 MeV) for maximising the (thin-target) yield from a 0.100 mm thick target.

For separation of Ti from the Sc target two methods were examined; (i) ion exchange column separation using 2000 mg AG 50W-X8 resin conditioned with 10mL 9M HCl. Disc is dissolved in 1 mL of 9M HCl, which at completion of reaction is pipetted into column. Successive 1 mL volumes of 9M HCl are added, and subsequent elutions collected. (ii) Following Gagnon et al., (3) a method employing hydroxylamine hydrochloride functionalised resin ('hydroxamate method') was applied, similar to its use in our hands for purification and separation of ^{89}Zr (2) following its original description for ^{89}Zr by Holland et al., (4). Disc dissolved in 2mL 6M HCl, then diluted to 2M. Elute through column to waste fraction 1 (w1 – see FIG. 1). Then elute 6 mL of 2M HCl through column to w2, followed by 6 mL of traceSELECT H_2O to w3. Finally, elute Ti into successive 1 mL product fractions (p1, 2 etc.) using 5 mL of 1M oxalic acid. This procedure takes approximate 1 hr. ^{45}Ti in elution vials was measured using γ-spectroscopy. Sc in the same vials was determined later using ICP-MS.

Results

A typical production run using a beam current of 40 μA for 60min on a 0.100mm-thick disc produced an activity of 1.83 GBq. Radionuclidic analysis of an irradiated disc using calibrated cryo-HPGe γ-spectroscopy revealed $T_{½}$ = 2.97–3.19 hr (95% CI) for ^{45}Ti, and with contaminant ^{44}Sc < 0.19 %, with no other isotopes detected.

Despite systematic adjustments to column conditions satisfactory chemical separation was not achieved using the ion exchange column method (i), despite previous reports of its success (5). Typical results of separation using the successful hydroxamate method (ii) are shown on the FIGURE 1.

It is seen that significant portion of ^{45}Ti is lost in the initial washing steps leading to waste collection. N = 4 replicate experiments showed a variation (SD) of 10 % of the mean in each elution fraction. Subsequent ICP-MS of the same elutions for (cold) Sc showed approximately

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80 % by mass appeared in w1 and 20 % in w2, with negligible total mass (total fraction ~1/6000) of Sc in product (p1–4) vials. However, the FIG. 1 shows that a total of only 30% of the original activity of ^{45}Ti (corrected to EOB) is available in the product vials, with the vial of highest specific activity (p1) containing 14 %. However, using a stack of 2×0.100mm thick Sc discs as a target yields isotope of adequate specific activity without need for concentration, for subsequent labelling and small-animal imaging purposes.

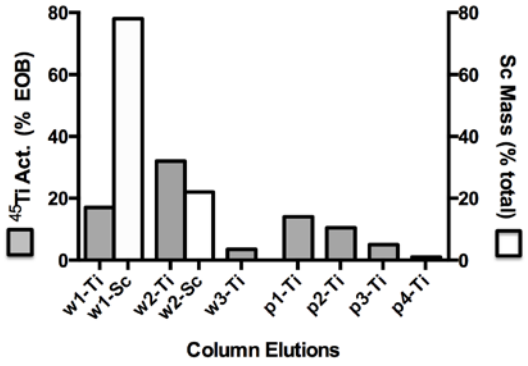

FIGURE 1. Profile of ^{45}Ti activity, from γ-spectroscopy, expressed as a percentage of total ^{45}Ti EOB activity. Elution fractions w1-Ti to w3-Ti were collections from the 3 waste stages. Elution fractions p1-Ti to p4-Ti were the collected product, each of 1 mL. Elution profile for (cold) Sc target mass (ICP-MS) is also shown, with very low detection in p-fractions.

In a 'proof-of-principle' experiment, two groups of healthy Balb/c-nu/nu female adult mice were administered with ^{45}Ti radiotracers. The first group (N = 3) received approximately 20 MBq IP of ^{45}Ti-oxalate buffered to pH = 7.0, and underwent microPET/CT imaging (Super Argus PET, Sedecal, Spain) out to 6hr post-injection, plus biodistribution analysis of radioactivity by dissection at sacrifice (6hr). The second group (N = 3) received approximately 20 MBq IP of ^{45}Ti-h-TF and were also studied to 6hr post-injection, followed by radioactive analysis after dissection at sacrifice. Organ and tissue biodistributions of the two groups at 6hr were similar but with ^{45}Ti-oxalate showing slightly greater affinity for bone. Biodistribution by dissection results broadly confirmed the findings from PET images. However, TLC results suggested that similarity of radiolabel biodistributions of the two groups may be due to contamination of the TF radiolabel with non-conjugated Ti at time of injection. An alternative explanation is dechelation *in vivo* of ^{45}Ti from ^{45}Ti-h-TF.

Conclusion

Despite significant loss of ^{45}Ti to the waste fractions of the separation process (total 53 %, corrected to EOB), ^{45}Ti of acceptable specific activity and high radionuclidic purity has been produced from the reaction ^{45}Sc(p,n)^{45}Ti, with separation and purification of the product by hydroxamate column chemistry, confirming an earlier report. Though microPET in vivo imaging using ^{45}Ti-based radiolabels was shown to be feasible, the similarity in the results for the label ^{45}Ti-h-TF compared with 'raw' ^{45}Ti-oxalate suggests further investigations. These may include a direct comparison of in vivo ^{45}Ti-h-TF small-animal imaging plus post-dissection biodistribution with the same procedures using ^{89}Zr labelled h-apotransferrin (6).

References

1. Y. Nuevo-Ordoñez et al.: *Metallomics* **3,** pp. 1297–1303, 2011.
2. R.K. Scharli, S. Chan, R.I. Price et al.: *AIP Conf Proc* **1509**, pp. 101–107; 2012.
3. K. Gagnon, G.W. Severin, T.E. Barnhart et al.: *AIP Conf Proc* **1509**, pp. 211–215, 2012.
4. J. Holland et al.: *Nucl. Med. Biol.* **36,** pp. 729–739, 2009.
5. A.L. Vavere et al.: *Nucl. Med. Biol.* **32**, pp. 117–122, 2005.
6. J.P. Holland et al.: *Nat. Med.* **18**, pp. 1586–1591, 2012.

Acknowledgements

RAPID Lab team members are gratefully acknowledged for technical & scientific support.

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