Applied Radiation and Isotopes 70 (2012) 63–68

Contents lists available at SciVerse ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Liquid scintillation based quantitative measurement of dual radioisotopes (^{3}H and ^{45}Ca) in biological samples for bone remodeling studies

Susanta K. Hui [a,*], M. Sharma [a], M.H. Bhattacharyya [b]

[a] Department of Therapeutic Radiology, University of Minnesota, 420 Delaware Street SE, Mayo Mail Code 494, Minneapolis, MN 55455, USA
[b] Department of Medicine and Orthopaedic Surgery, Medical College of Georgia, Augusta, GA 30912, USA

ARTICLE INFO

Article history:
Received 23 November 2010
Received in revised form
1 August 2011
Accepted 5 August 2011
Available online 25 August 2011

Keywords:
^{3}H
^{45}Ca
Liquid scintillator
Scintillation cocktail
Dual-label
Bone remodeling

ABSTRACT

Acute and prolonged bone complications associated with radiation and chemotherapy in cancer survivors underscore the importance of establishing a laboratory-based complementary dual-isotope tool to evaluate short- as well as long-term bone remodeling in an *in vivo* model. To address this need, a liquid scintillation dual-label method was investigated using different scintillation cocktails for quantitative measurement of ^{3}H-tetracycline (^{3}H-TC) and ^{45}Ca as markers of bone turnover in mice. Individual samples were prepared over a wide range of known ^{45}Ca/^{3}H activity ratios. Results showed that ^{45}Ca/^{3}H activity ratios determined experimentally by the dual-label method were comparable to the known activity ratios (percentage difference ~2%), but large variations were found in samples with ^{45}Ca/^{3}H activity ratios in range of 2–10 (percentage difference ~20–30%). Urine and fecal samples from mice administered with both ^{3}H-TC and ^{45}Ca were analyzed with the dual-label method. Positive correlations between ^{3}H and ^{45}Ca in urine (R=0.93) and feces (R=0.83) indicate that ^{3}H-TC and ^{45}Ca can be interchangeably used to monitor longitudinal *in vivo* skeletal remodeling.

1. Introduction

1.1. Radioisotopes for bone remodeling studies

Liquid scintillation has been used for the non-invasive study of bone remodeling with multiple radioisotopes (^{3}H, ^{47}Ca, ^{45}Ca) (Bates et al., 1996; Fricke, 1975; Hanes et al., 1999; Mahin and Lofberg, 1966; Shahnazari et al., 2010; Zhao et al., 2010). The method of dual radioisotope labeling in animals is widely used to verify (a) the same physiological process with different methods, or (b) two different physiological processes with respective radioisotopes in a single experiment. Literature is available for determination of dual radioisotopes viz. ^{32}P and ^{45}Ca (Bem and Reimschüssel, 1979), ^{3}H and ^{14}C (Los Arcos and Barquero, 1996; Reddy et al., 2009), ^{3}H and ^{125}I (Thibodeau et al., 1981) and ^{90}Sr and ^{90}Y (Lee et al., 2002). ^{3}H-Tetracycline (^{3}H-TC) (DeMoss and Wright, 1997) is individually used to quantify the resorptive phase of bone calcium metabolism while ^{45}Ca is a bone seeking tracer generally used to investigate endocrine metabolism (Shahnazari et al., 2010).

With a single intravenous injection, ^{3}H-TC can best be used for short-term (days) kinetic studies due to its short biological half-life (~2.8 h) (DeMoss and Wright, 1997), whereas ^{45}Ca can be used for relatively longer-term (several months) kinetic studies due to its longer biological half-life (~187 days) (ICRP (1980)). Classically, ^{3}H-TC is used to monitor bone resorption, because it is not readily incorporated into newly formed bone (Muhlbauer and Fleisch, 1990), whereas urinary excretion of calcium tracers is thought to reflect net bone turnover, because calcium tracer is reabsorbed through the kidney. Dual isotope studies have been conducted to compare calcium, the endogenous constituent of bone, with respect to ^{3}H-TC, a known marker for bone resorption. For example, in animal models using ^{3}H-TC and ^{45}Ca (Zhao et al., 2010) and ^{3}H-TC and ^{41}Ca (Cheong et al., 2011), the authors show that both isotopes measured in urine can be used interchangeably to screen dietary and other interventions for beneficial effects on bone (Zhao et al., 2010). Due to the low kidney re-absorption upon turnover in animals, ^{3}H-TC may also be a useful complementary assay technique to ^{45}Ca for studying cancer treatment modalities (example, radiation) that may have a role in kidney damage. Finally, due to the much larger endogenous excretion of ^{45}Ca in feces compared to urine in both mice and rats (Wang and Bhattacharyya, 1993; Zhao et al., 2010), feces can be used for a much longer period than urine to track bone turnover via ^{45}Ca excreta measurements. To date, the kinetics of these two bone markers (^{3}H-TC and ^{45}Ca or ^{41}Ca) have not been correlated in feces and urine.

1.2. Dual-isotope approach using liquid scintillation spectrometry

Liquid scintillation is a simple and expedited technique used for measuring radioisotopes. The important benefits are ease of sample preparation and high counting efficiency with low-levels of nuclide. The dual-isotope liquid scintillation technique can be highly

* Corresponding author. Tel.: +612 626 4484; fax: +612 626 7060.
E-mail address: huixx019@umn.edu (S.K. Hui).

0969-8043/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2011.08.007

beneficial, but the measurement is critically dependent on an accurate assessment of the actual radioactivity of both radioisotopes (Dodson, 2002). Because the β-particle energy is a continuum extending from zero to some maximum energy, the energy spectrum of a strong β emitter may overlap the spectrum of a weak β emitter. Depending on the activity of both the radioisotopes and the beta energy difference, this overlap may lead to an error in the measurements. Additional difficulties include variable efficiency of one radioisotope compared to the other radioisotope at a particular energy, which may occur due to quench. Quench reduces the number of photons observed for a given amount of energy. With an increase in quench, the spectra generally shift towards the lower energy. Proper quench determination is crucial for accurate measurement of dual-labeled samples, as it helps in automatic detection of the shift.

Equally important is the use of an appropriate scintillation cocktail. The scintillation cocktails can be categorized by their phasing properties and ability to dissolve aqueous and non-aqueous samples. In the present study, we compared two categories of scintillation cocktails: cocktails that develop a gel phase with the sample (Universol™, Insta-Gel Plus and HI-Fluor) and cocktails that do not develop a gel phase with the sample (Ecolite™, Ecolume™).

Radioisotopes commonly used in biomedical research have low energy and a short range of air or fluid penetration. The liquid scintillation detection efficiency of radioisotopes in biological samples depends on direct contact with the scintillation cocktail and the sample (Medeiros et al., 2003). The liquid scintillation efficiency is in turn highly constrained by interferences such as inhomogeneity, chemiluminescence, phosphorescence, micro precipitation, adsorption and chemical (impurities) or color quench, all of which are in a way related to the scintillation cocktail composition (Medeiros et al., 2003) and the sample preparation technique (L'Annunziata et al., 2003).

The three main objectives of the present liquid-scintillation-based study for dual radioisotope estimation in different biological samples were to investigate the (a) impact of different scintillation cocktails on dual-label radioisotope counting in different biological samples, (b) accuracy of the dual-label method in determining the actual amounts of both radioisotopes at known concentrations and (c) association of dual radioisotope excretion in urine and feces after *in vivo* administration of both bone markers to mice. Our aim in optimizing conditions to simultaneously quantitate both isotopes is to apply the dual-isotope approach to evaluate bone remodeling changes that occur in cancer patients after radiation and chemotherapy.

2. Materials and methods

2.1. Reagents and scintillation cocktails

The dual-label samples were prepared from ^{3}H-tetracycline [7-^{3}H(N)] (American Radiolabeled Chemicals Inc., St. Louis, MO) and $^{45}CaCl_2$ (Perkin Elmer, Waltham, Massachusetts) solutions. These are beta emitting nuclides with average and maximum energy being, respectively, 5.7 keV and 18.6 keV for ^{3}H and 77 keV and 257 keV for ^{45}Ca. Five different types of scintillation cocktails were used, namely Ecolite™, Ecolume™ and Univer-Sol™ (MP Biomedicals, Irvine, CA), and Insta-Gel Plus and HI-Fluor (Perkin Elmer, Boston, MA).

2.2. Apparatus

An alpha/beta liquid scintillation detector (Beckmann Colter LS 6500) with an energy range of 0–2000 keV was used in the present study. It had a logarithmic amplification, a 32,768 channel multi-channel analyzer (MCA) having an effective resolution of 0.0625 keV per channel (2000 keV/32,728 channels) and an automated background subtraction option. Different micro-pipettes (Eppendorf, CA) were used for precise measurement of ^{3}H and ^{45}Ca radioisotope solutions. Dilutions were conducted with deionized water (Fischer Scientific, NJ). All the liquid scintillation measurements were carried out in 20 ml polyethylene scintillation vials (Perkin Elmer, MA).

2.3. Quench calibration for ^{3}H and ^{45}Ca

The counting efficiency of ^{3}H and ^{45}Ca was determined by measuring a series of quenched standards for both the radioisotopes having a fixed dpm value. Quenched standards are generally prepared by adding variable amounts of a quenching agent, like nitromethane, to a fixed radioisotope of fixed activity. In the present study, an active stock solution of ^{45}Ca containing 55,000 dpm/0.5 ml was prepared from the standard $^{45}CaCl_2$ of 62.7×10^6 dpm/μl and deionized water. Then, 0.5 ml of this active stock solution was added to 15 ml of the Ecolite scintillation cocktail. After an initial precision measurement on 30 samples of ^{45}Ca, a set of 10 samples were selected with less than $\pm 1\%$ deviation from the prepared dpm value of 55,000. A set of samples with a wide quenching range was then obtained by adding increasing volumes of nitromethane (0, 15, 30, 45, 60, 75, 90, 105, 120 and 135 μl) in those ten samples. For ^{3}H radioisotope, the standard kit (Lot #HGG0608, Perkin Elmer) containing 10 samples of $\sim$50,000 dpm values and varying levels of quench was used.

The quench-indicating parameter used by LSC 6500 is called the Horrocks number or the H#. This parameter is determined by calculation of the difference in channels between inflection points of the Compton edge of a quenched sample vs. an unquenched sample. The quench curve, or the plot of efficiency of the radioisotope as a function Horrocks number, was used for efficiency determination of both the radioisotopes. In dual-label mode, the quench curves were generated for each individual radioisotope in wide mode and then in two window settings viz. window1 and window2. Window1 mainly ranged from 0–15 keV and consisted of most of the beta spectra of ^{3}H with a small tailing contribution of the ^{45}Ca beta spectra. Window2 mainly ranged from 15–270 keV and consisted of mainly ^{45}Ca beta spectra. LSC 6500 uses H# and a 32,768 channel multi-channel analyzer to provide an automated window adjustment as a function of quench using automatic quench compensation (AQC). This approach reduces the error from spill of the high energy isotope into the low energy isotope. An advantage of the use of H# is that any sample can have only one H#, which reflects the efficiency of counting the two radioisotopes in that sample. Fig. 1 shows the spectral distribution of a typical ^{3}H and ^{45}Ca radioisotope in single- and dual-label mode. The efficiency of ^{3}H and ^{45}Ca in both the single- and dual-label windows vs. H# is shown in Fig. 2.

2.4. Biological sample preparation

A total of 18 skeletally mature (15 weeks old) BALB/c mice were injected intravenously with 15 μCi of ^{3}H-tetracycline and 15 μCi of $^{45}CaCl_2$. This study was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC). Following the tail vein IV injection, metabolic cages were used to collect 24 h excreta (feces and urine) from each mouse individually at days 3, 6, 9, 13 and 16. Fecal samples for each mouse on a given collection day were transferred to pyrex beakers. The urine samples were retrieved by rinsing the cage with 15 ml of deionized water. Feces digestion was a three day process. The oven-dried (80 °C) feces samples were weighed and powdered, and 0.2 g of that sample was solubilized in 2 ml of conc. HNO_3 on the first day, followed by second day addition of 1 ml of hydrogen