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Report

Long-Term Stability Studies Using Radiopharmaceuticals: Consequence of Using Multiply Tritiated Compounds, with an Application to Fluocinolone Acetonide

Michael F. Powell,[1,2] Adina Magill,[1] and Allyn R. Becker[1]

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The use of multiply tritiated radiopharmaceutical compounds for long-term stability studies should be avoided because different radiolabeled products are produced by radioactive decay than by chemical degradation. This is demonstrated by showing that doubly tritiated fluocinolone acetonide and ^{14}C-labeled fluocinolone acetonide have different shelf lives and that the tritiated compound affords additional degradation products not seen in reaction of the ^{14}C-labeled drug after 26 months.

KEY WORDS: tritium decay; radiochemical degradation; radiolabeled degradation products.

INTRODUCTION

Drug stability in homogeneous solutions at room temperature (RT) can be predicted accurately by carrying out kinetic studies at elevated temperatures and then extrapolating the rate constants to room temperature by the Arrhenius equation. Unfortunately, this predictive method cannot be used routinely for determining drug stability in most heterogeneous drug formulations because of physical changes that occur at higher temperatures. For example, at elevated temperatures (>RT), some drugs are not necessarily distributed in the same phase as they are at room temperature, and therefore, predictions based on extrapolations from data at higher temperatures may not prove fruitful. Such conditions force one to monitor drug stability for an extended period of time to determine the room-temperature shelf life (or expiration dating period).

One of the more elegant ways of determining long-term drug stability is to employ radiolabeled drug and follow the slow change in labeled drug concentration with time. The tracers of choice are tritium (^3H) and carbon-14 (^{14}C), each with its own relative advantages and disadvantages, and these are discussed elsewhere (1). The radioassay method has a singular advantage over cold-assay techniques in that exact product yields and mass balance can be readily determined, since the response factors for product detection are known to be unity. Radiolabeled drug of a high specific activity is sometimes used for such studies, where the highly labeled drug may be multiply labeled, i.e., have two or more radioactive atoms per drug molecule (1). It is usually assumed that these multiply labeled compounds behave similarly as singly labeled species in that the drug degradation rate and decomposition products should be independent of the specific activity used. It is also generally thought that radioimpurity formation by primary radioactive decay of one of the radionuclei (e.g., tritium) of a multiply labeled compound is insignificant, as evidenced in a recent review: "In multiply-labeled molecules containing radionuclides of long half-life, such as carbon-14 or tritium, the contribution of radioactive impurities on storage from primary (internal) decay is also very small" (2). This statement is generally true for facile reactions, or when carbon-14 is employed as the radiotracer, but not when highly tritiated compounds are used for long-term stability studies. In this case radiolabeled degradation products, formed by radiodecay of one of the tritium atoms on a multiply labeled compound, are produced at a comparable rate as the radioactive products produced by the chemical degradation reaction.

Although concurrent radiodecay and chemical degradation are not unexpected in long-term studies, they are rarely accounted for in stability studies carried out with multiply tritiated drug. Based on the fact that the tritium radiodecay process ($t_{1/2}$ ~12.3 years) and chemical degradation both yield radiolabeled degradation products, we make the recommendation herein that long-term stability studies not be carried out using multiply tritiated compounds. We have determined the chemical degradation of fluocinolone acetonide (1) in a cream formulation to give the major degradation products 2–5. By monitoring the drug concentration using both doubly tritiated and ^{14}C-labeled drug, we have unequivocally established that the use of doubly tritiated species can complicate long-term stability studies.

EXPERIMENTAL

Materials. Fluocinolone acetonide (1) and the ketoaldehyde (2), keto acid (3), etianic acid (4), and tetrol (5) derivatives of 1 were synthesized by the Institute of Organic Chemistry (Syntex Research) (3). Tritiated 1 was prepared with a specific activity of 30.7 mCi/mmol and was labeled at

[1] Institute of Pharmaceutical Sciences, Syntex Research, Palo Alto, California 94303.

[2] To whom correspondence should be addressed.

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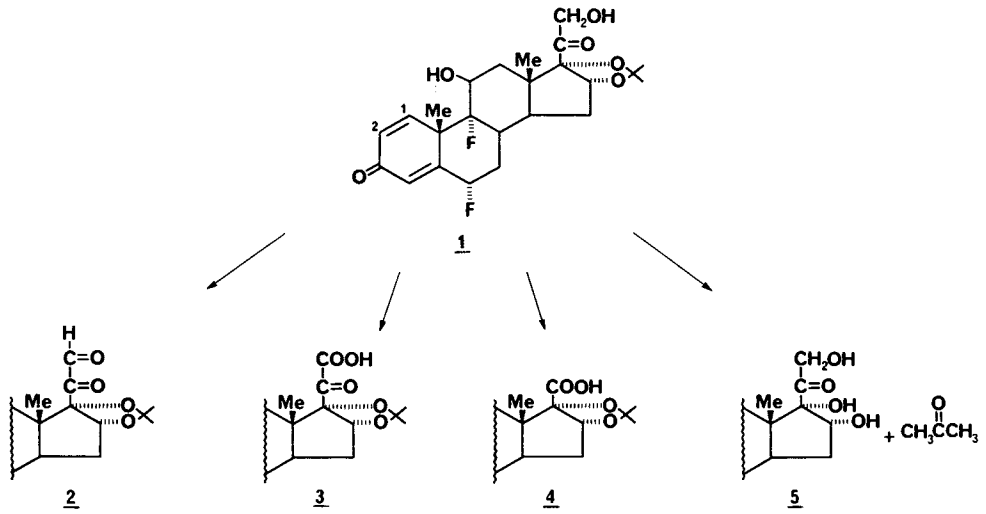

Scheme I

the steroid 1 and 2 positions (4). Approximately 20% of 1 was doubly labeled, as shown by the change in compound activity upon oxidation of the 1,2-tritio saturated precursor (45.8 Ci/mmol) to give 1,2-tritio-1 and by the yield of radiodecay products after 26 months of reaction time (vide infra). The ^{14}C-labeled 1 contained carbon-14 in the acetonide moiety and had a specific activity of 49.7 mCi/mmol (4).

The cream formulation consisted largely of propylene glycol, stearyl alcohol, water, and combined radiolabeled and unlabeled 1 at 0.01% concentration. A synopsis of the properties of a similar cream has been published (5). The mobile phase was prepared from high-performance liquid chromatography (HPLC)-grade acetonitrile (Burdick and Jackson) and doubly distilled water. HPLC-grade tetrahydrofuran (Burdick and Jackson) was used throughout. Oxifluor scintillation cocktail (New England Nuclear) was used for liquid scintillation counting.

Apparatus. The separation and product analysis of tritiated water (TOH), acetone, 1, 2, 3, 4, and 5 were carried out using an HPLC system consisting of a Rheodyne Model 725 injector, a Spectra Physics Model SP 8700 pump system, a Schoffel Model 770 spectrophotometric detector, and an SP 4000 computing integrator. The following reverse-phase (RP) HPLC conditions provided separation of 1 from its degradation products: column—Altex Ultrasphere ODS (25 × 0.46-cm ID); and mobile phase—95% water/5% acetonitrile for 5 min, a linear gradient to 70% water/30% acetonitrile for 10 min, and then a final linear gradient to 50% water/50% acetonitrile for another 25 min. The other HPLC parameters were as follows: flow rate—1 ml/min; detection—238 nm at 0.04 aufs; injection loop size—100 μl; and typical retention times—tritiated water (3 min), acetone (6 min), 3 (18 min), 4 (21 min), 5 (25 min), 2 (32 min), and fluocinolone acetonide (1) (36 min) (Fig. 1). Sample fractions were collected in 1.5-ml portions directly into 20-ml scintillation vials, and the ^{14}C and ^{3}H activities were determined using a Beckman Model LS 860 scintillation counter in the ^{3}H–^{14}C dual-channel mode. Samples were counted for a sufficient time to reduce the standard counting error to approximately 1% or less.

Product Studies. Aliquots of radiolabeled cream containing 0.05 mCi ^{14}C-labeled 1 and 0.05 mCi ^{3}H-labeled 1 were placed in 10-ml amber vials, flame-sealed, and stored at room temperature for 26 months. At this time, 0.50 mg of degraded cream was dissolved in 2.0 ml of tetrahydrofuran. A small portion of the clear tetrahydrofuran solution was added to excess water and, after the mixture was suspended using a vortex mixer for ~30 sec, 100 μl was removed for radioassay. The remaining suspension was centrifuged for 20 min at 2000 rpm, yielding an opaque aqueous layer and a heavier residue. The aqueous portion was analyzed for drug and degradation products directly by RP HPLC. Control experiments demonstrated that, after washing with water, the residue fraction was not radioactive, and so its composition was not investigated further. The HPLC effluent of the aqueous fraction was monitored at 238 nm and also by continuous collection of sample fractions and subsequent sample radioassay by liquid scintillation counting. Mass balance was established by determining the radioactivity of a portion of the degraded radiolabeled cream corresponding to the amount of material injected onto the HPLC column and comparing the counts per minute of this sample with the total counts obtained from the sum of the HPLC eluents of the individual peak fractions. Identification of the radioactive HPLC peaks was made by comparison of the chromatographic retention times with known compounds (Fig. 1) and, in the case of 3 and 5, also by extraction properties from acidic and basic solutions. Product identity was also verified by comparing the chromatographic retention times by an independent reverse-phase method, the details of which are discussed in another paper (6).

RESULTS AND DISCUSSION

Reaction Products of Radiolabeled Fluocinolone Acetonide. In this study, two different types of radiolabeled drug were employed: approximately one-half of the radioactivity was due to molecules containing ^{3}H (largely ditritiated at the 1,2-positions); the remaining radioactivity was due to molecules labeled with ^{14}C at the tertiary carbon of the acetonide group, as shown in Scheme I. After reaction at room temperature for 26 months, the cream formulation con-

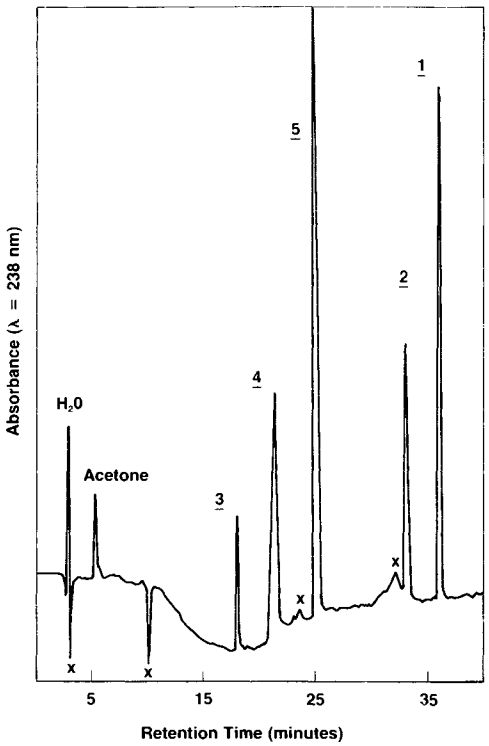

Fig. 1. HPLC gradient chromatogram of a standard solution of 1–5. The distorted baseline is due to the HPLC gradient method, and the peaks marked "x" are due to impurities or changes in mobile-phase composition.

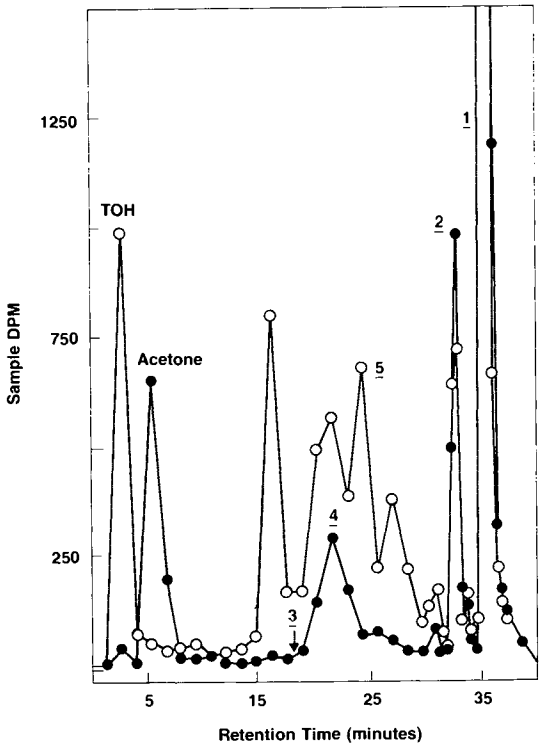

Fig. 2. HPLC radiochromatograms for ^{3}H-labeled (○) and ^{14}C-labeled (●) **1** in a cream-based formulation stored at room temperature for 26 months. The disintegrations per minute (dpm) have been corrected for ^{3}H and ^{14}C channel overlap and background counts. The peak widths were determined primarily by sample collection time, and not by resolution associated with HPLC separation of **1** from the products. Sample fractions between 28 and 32 min were collected for 30 sec per fraction to permit chromatographic resolution of **1** and **2**.

taining ^{3}H- and ^{14}C-labeled **1** showed only 3–4% drug degradation, as assayed by reverse-phase HPLC and liquid scintillation counting. The ^{3}H and ^{14}C radiochromatograms for **1** and the degradation products are shown in Fig. 2. Generally, the degradation of ^{14}C-labeled **1** agreed within experimental error with the amount of drug loss observed for nonradiolabeled **1**, as assayed by HPLC. Product yields, with the exception of **5**, were calculated from the ^{14}C data. Since **5** is missing the acetonide group which contains the ^{14}C isotope, the concentration of **5** had to be determined by tritium assay. Comparison of the peak retention times for the standards in Fig. 1 with the radiochromatograms in Fig. 2 shows that the major products for degradation of ^{14}C-labeled drug are acetone, **2**, and **4**, whereas for ^{3}H-labeled drug, tritiated water, **2**, **4**, and **5** are easily identified. Excellent mass balance was obtained, in that the sample radioactivities of the ^{3}H and ^{14}C radiochromatograms were nearly equal to the ^{3}H and ^{14}C activities injected onto the HPLC column. For example, the yields of **2**, **3**, **4**, and **5** in Fig. 2 are 1.6, 0.2, 0.6, and 0.8%, respectively, and the sum of these is in good agreement with the observed extent of reaction (≥96% drug remaining). An appropriate amount of ^{14}C-labeled acetone (~0.7%) was detected in the ^{14}C radiochromatogram which was essentially equivalent to the amount of ^{3}H-labeled **5** formed (~0.8%). The keto-acid derivative **3** was also detected in small amounts in some of the radioassays and therefore, is only a minor degradation product of **1**. Additionally, tritiated water was detected in the degraded cream formulation and is due to tritium isotope exchange under the reaction conditions used. The small amount of tritiated water found after 26 months demonstrates that tritium isotope exchange is slow, yet is significant when compared to the rate of degradation of **1**, and so must be included in the ^{3}H mass balance calculation. Such tritium isotope exchange is relatively common when tritiated steroids are stored in neutral hydroxylic solvents (2,7).

Consequence of Using Multiply Tritiated Drugs for Long-Term Stability Studies. The HPLC radiochromatograms in Fig. 2 show that **1**, **2**, and **4** are present in both the ^{3}H and the ^{14}C traces. Degradation product ^{3}H-labeled **5** is also readily identified in the cream formulation. However, when comparing the ^{3}H and the ^{14}C radiochromatograms, at least two major peaks are found in the ^{3}H radiochromatogram in Fig. 2 that do not correspond to any of the peaks in the ^{14}C radiochromatogram. One might argue that these "extra" peaks could be due to further degradation of **5** since the derived compounds would not contain ^{14}C but would still contain ^{3}H. This is not the case, however, because only a small amount of **5** is formed and because there are similar yields of acetone and ^{3}H-labeled **5** formed in the hydrolysis of **1**.

The extra ^{3}H-labeled degradation products observed in this 26-month study are due to radiodecay products formed from doubly labeled **1**. Approximately 20% of the ^{3}H-labeled **1** in the formulation contains two tritium atoms per molecule, so that when one of these tritium atoms decays to give ^{3}He and a β particle, a reactive intermediate with a single

tritium atom per molecule remains. This ^{3}H-labeled intermediate probably reacts with molecular oxygen or the solvent (such as water or propylene glycol) to form radiodecay products, perhaps the 1- or 2-hydroxylated derivatives of **1**. To date, the extra ^{3}H peaks in Fig. 2 have not been identified. The yield of ^{3}H-labeled radiodecay products can be estimated from the initial activity, the amount of doubly labeled **1** in the formulation, and the reaction time. For example, a drug containing two tritium atoms per molecule would show 100 exp[2(−0.0046m^{-1}) (26m)] or 78.36% remaining of doubly labeled drug after 26 months, whereas the total remaining radioactivity in the sample would be 100 exp[(−0.0046m^{-1}) (26m)] or 88.52%. This implies that 12.96% [(88.52 − 78.36)/78.36] of the remaining sample radioactivity would be due to monotritiated radiodecay products. Since tritiated **1** was only ~20% doubly labeled in this investigation, approximately 2.6% of the ^{3}H radioactivity after 26 months of reaction is due to radiodecay products. Thus, for an ~10^5-dpm sample injected onto the HPLC (as was done for the radiochromatograms in Fig. 2), approximately 2600 dpm should be due to radiolabeled decay products of doubly tritiated molecules. This value is close to the ^{3}H radioactivity (~2500 dpm) found in the extra radiochromatogram peaks, i.e., those peaks that do not correspond in retention time to ^{14}C-labeled products or to ^{3}H-labeled **5**.

Is Tritium Still Useful as a Tracer for Long-Term Drug Stability Studies? Even for drugs whose half-life due to chemical degradation is similar to the half-life of tritium, singly tritiated drugs can be used without complication for long-term stability studies because the total sample radioactivity can always be determined at the same time as the HPLC drug assay. This comparative method of mass balance determination works well because, even though the total sample radioactivity decreases over the years, the remaining radioactivity can be assayed quite accurately. Additionally, interference by the formation of radiodecay products does not occur when using singly tritiated molecules since the radiodecay product does not contain tritium and is undetectable in the radioassay. Similarly, ^{14}C-labeled drugs usually do not show ^{14}C-labeled radiodecay products [except for multiply labeled macromolecules (1,2)] because of the long half-life of carbon-14 (t_{50} ~5000 years). Thus, long-term stability studies using multiply tritiated drug should be avoided, especially if the rate of chemical degradation is comparable to the rate of tritium decay. Otherwise singly tritiated compounds having nonexchangeable tritium are equally satisfactory.

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