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Research Article

Radiosynthesis of [^{11}C]paclitaxel

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Summary

[^{11}C]paclitaxel, a potential solid tumor imaging agent, was synthesized by reacting [α-^{11}C]benzoyl chloride with the primary amine precursor of paclitaxel. The time for synthesis, purification, and formulation was 38 min from end of bombardment with an average specific radioactivity of 49.9 GBq/μmol (1349 mCi/μmol) at end of synthesis. The average decay corrected radiochemical yield was 7% with greater than 99% radiochemical purity.

Key Words: paclitaxel; anticancer; microtubule; carbon-11; positron emission tomography

1. Introduction

Paclitaxel (Figure 1) is an effective anti-cancer drug against solid tumors. Paclitaxel binds to tubulin creating stable nonfunctioning microtubules thereby interfering with mitosis and inducing apoptosis.[1] The increased medical use of paclitaxel has resulted in a number of biological questions including: determination of adequate dosing

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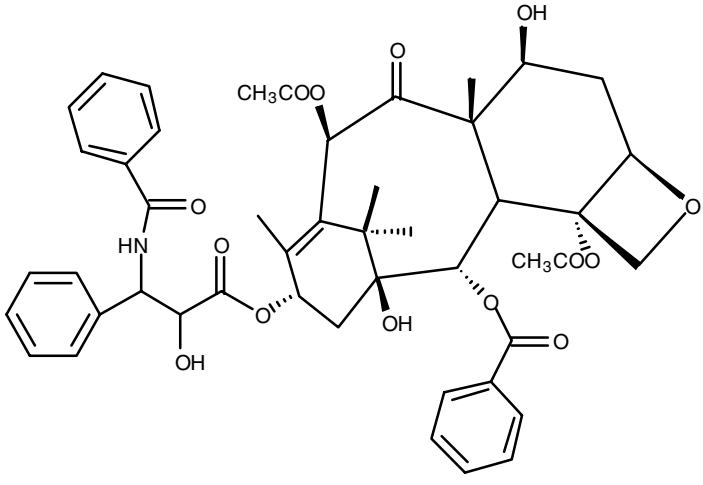

Figure 1. Structure of paclitaxel

regime, therapeutic tissue concentration, cellular/intracellular accumulation, and resistance to treatment. The availability of a suitably radiolabeled ligand for these sites may help begin answering some of these biological questions.

Imaging agents, including ^{99m}Tc-Sestamibi,[2,3] ^{201}Tl,[2] and ^{11}C-colchicine[4] are currently being utilized to evaluate the efficacy of paclitaxel. Potential imaging analogs of paclitaxel including ^{111}In-,[5] ^{123}I-[6] and ^{18}F-[7] labeled compounds may also prove useful in addressing the aforementioned biological questions.

This paper describes the synthesis of [^{11}C]paclitaxel by reacting [^{11}C]benzoyl chloride with the primary amine precursor of paclitaxel. The synthetic procedure, purification, formulation, and characterization are described.

Results and discussion

[^{11}C]Paclitaxel was synthesized in a multi-step procedure (Figure 2). First [α-^{11}C]benzoyl chloride was synthesized as previously described.[8,9] Briefly, [^{11}C]carbon dioxide was bubbled slowly into a solution of phenylmagnesium chloride in tetrahydrofuran (THF). To the resulting [^{11}C]benzoate solution was added phthaloyl dichloride. The [^{11}C]benzoyl chloride produced was injected onto a normal phase semi-preparative high pressure liquid chromatography (HPLC) column and

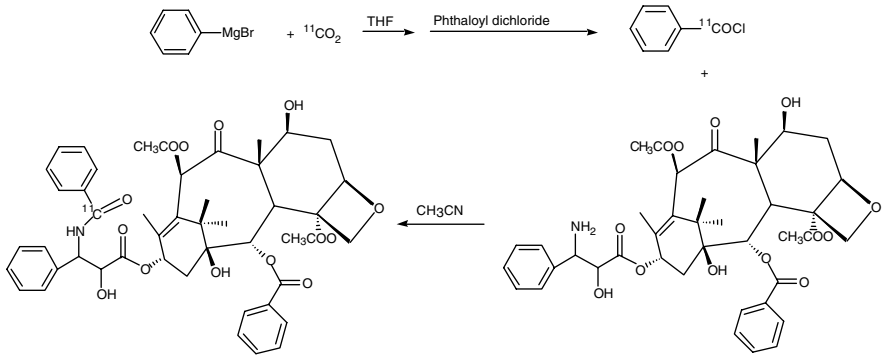

Figure 2. Radiosynthesis of [^{11}C]paclitaxel

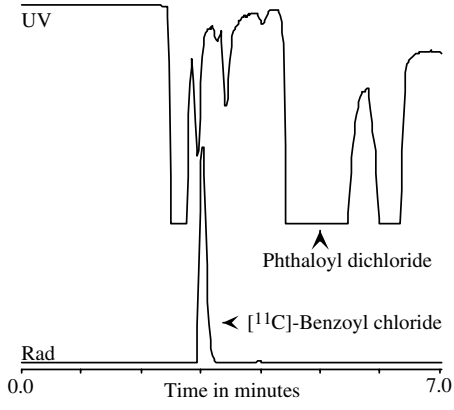

Figure 3. Normal phase semi-preparative chromatogram of [^{11}C]benzoyl chloride reaction mixture

eluted with *n*-hexane (Figure 3). The normal phase procedure removes the excess phthaloyl dichloride and other contaminants that interfere in the benzoylation of the primary amine precursor of paclitaxel. These interfering impurities are not easily removed by other methods such as distillation or solid-phase extraction. The radiosynthesis and purification of the [^{11}C]benzoyl chloride took an average of 17 min from EOB to complete with an average radiochemical yield of 41% (determined from [^{11}C]carbon dioxide collected in the Grignard vial).

To the collected [^{11}C]benzoyl chloride in *n*-hexane was added the primary amine of paclitaxel in acetonitrile (CH_3CN). The *n*-hexane was evaporated by gentle flow of argon at room temperature with a minor loss of [^{11}C]benzoyl chloride. After the volume was reduced, the

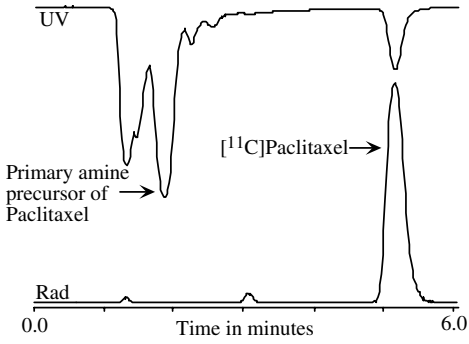

Figure 4. Reverse phase semi-preparative chromatogram of [^{11}C]paclitaxel reaction mixture

solution was allowed to sit for 2 min at room temperature. The solution was then diluted with reverse phase HPLC solvent and injected onto the reverse phase semi-preparative column.

A typical semi-preparative chromatogram (Figure 4) indicates only a minor radiolabeled side product and unreacted [^{11}C]benzoyl chloride ($R_T = 1.3$ min) are present with good separation of [^{11}C]paclitaxel from the precursor. The [^{11}C]paclitaxel product was collected, evaporated to dryness and formulated. The determination of purity was performed as previously described[8] and the product co-elutes with a genuine sample of paclitaxel.

The syntheses, double semi-preparative HPLC, and formulation were completed in an average time of 38 minutes ($n = 3$) from (EOB) with an average decay corrected radiochemical yield of 7% based on [^{11}C]carbon dioxide collected in the Grignard vial. An average of 19 mCi of final product had an average specific radioactivity of 49.9 GBq/μmol (1349 mCi/μmol) at end of synthesis (EOS). The final formulated solution was radiochemically pure (> 99%) as determined by analytical HPLC and did not contain the amine precursor.

Experimental

Paclitaxel, [2aR-[2aα,4β,4aβ,6β,9α(αR*,βS*),11α,12α,12aα,12bα]]-β-(benzoylamino)-β-hydroxybenzenepropanoic acid 6,12b-*bis*(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz-[1,2-b]oxet-9-yl ester, and Cremophor EL were obtained from Sigma.

The primary amine precursor of paclitaxel, [2aR-[2aα,4β,4aβ,6β,9α(αR*,βS*),11α,12α,-12aα,12bα]]-β-(amino)-β-hydroxybenzenepropanoic acid 6,12b-*bis*(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester, was purchased from Hauser Laboratories (Boulder, CO). [^{11}C]Carbon dioxide was produced by 18 MeV proton bombardment of a nitrogen gas target using a GE PETtrace biomedical cyclotron. Phenylmagnesium chloride (2.0 M in THF) was acquired from Aldrich Chemical Co. All other reagents were ACS or HPLC purity. Normal phase semi-preparative HPLC was performed using a Beckman 110B pump, a Bioscan Flow Count PIN diode radioactive detector, and a Waters 441 fixed wavelength (254 nm) UV detector. The normal phase HPLC purification was performed on a Waters 10 μm μPorasil column (7.8 × 300 mm) using a mobile phase of *n*-hexane at a flow rate of 5 ml/min. Reverse phase HPLC analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single 2 in NaI crystal radioactive detector. All HPLC chromatograms were recorded with a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax, version 1.4). Reverse phase HPLC semi-preparative purification were performed on an Waters Novapak 6 μm C-18 column (7.8 × 300 mm) using a mobile phase of 50% acetonitrile /50% water (0.1 M ammonium formate) at a flow rate of 7 ml/min. Chemical and radiochemical purity were determined using a Waters Novapak 4 μm C-18 HPLC column (3.9 × 150 mm) with a mobile phase of 50% acetonitrile /50% water (0.1 M ammonium formate) at a flow rate of 3 ml/min. A dose calibrator (Capintec 12R) was used for all radioactivity measurements.

Radiosynthesis and purification of [^{11}C]paclitaxel

[^{11}C]carbon dioxide was released with heating (350°C) from molecular sieves (Alltech, 4 Å, size 80/100) contained in a GE PETtrace ^{11}C radiochemistry module using a nitrogen gas flow rate of 15–30 ml/min into 300 μl phenylmagnesium chloride solution (1.0 M, diluted with THF under argon) in a sealed v-vial. When the radioactivity in the vial reached a plateau, 50 μl of phthaloyl dichloride was added. The solution was heated for 1 min at 80°C, then placed at room temperature for 4 min. The solution was evaporated (argon flow) to approximately 100 μL and injected onto the normal phase column. The [^{11}C]benzoyl

chloride peak ($R_T = 3.1$ min.) was collected in a 10 ml v-vial. The primary amine precursor (1 mg) dissolved in 200 µl CH_3CN was added to the [^{11}C]benzoyl chloride solution. After reducing the volume to about 200 µL using argon flow, the solution was left at room temperature for 2 min. Reverse phase HPLC solvent (200 µl) was added prior to applying the solution to the reverse phase semipreparative HPLC column. The capacity factor, k', for [^{11}C]paclitaxel was 3.3 and for the primary amine of paclitaxel was 0.7. After collection and evaporation to dryness, the product was redissolved in a mixture of 0.5 ml of Cremophor EL and 0.75 ml absolute ethanol. The solution was subsequently diluted with 8.75 ml of normal saline. The chemical and radiochemical purity of the final solution were determined by analytical HPLC[8].

Conclusion

[^{11}C]paclitaxel was synthesized from the primary amine of precursor of paclitaxel and [^{11}C]benzoyl chloride. The yield and specific radioactivity are sufficient for *in vivo* animal biodistribution studies, with the ultimate goal of imaging humans by positron emission tomography.

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References

1. Blagosklonny M, Fojo T. *Int J Cancer* 1999; **83**: 151–156.
2. Oriuchi N, Jibu T, Milas L, Choe JG, Kuang LR, Kim EE, Hunter NR, Wallace S, Podoloff DA. *Nucl Med Biol* 2000; **27**: 127–133.
3. Luker GD, Fracasso PM, Dobkin J, Piwnica-Worms D. *J Nucl Med* 1997; **38**: 369–372.
4. Levchenko A, Mehta BM, Lee JB, Humm JL, Augensen F, Squire O, Kothari PJ, Finn RD, Leonard EF, Larson SM. *J Nucl Med* 2000; **41**: 493–501.
5. Li C, Yu DF, Yang DJ, Tansey W, Liu CW, Milas L, Hunter NR, Kim EE, Wallace S. *J Nucl Med* 1997; **38**: 1042–1047.

6. Roh EJ, Park YH, Song CE, Oh SJ, Choe YS, Kim BT, Chi DY, Kim D. *Bioorg Med Chem* 2000; **8**: 65–68.
7. Keisewetter DO, Eckleman WC. *J Label Compd Radiopharm* 2001; **44** (Suppl. 1): S903–S905.
8. Mathews WB, Burns HD, Dannals RF, Ravert HT, Naylor EM. *J Label Compd Radiopharm* 1995; **36**: 729–737.
9. Luthra SK, Pike VW, Brady F. *Int J Radiat Appl Instrum, Part A* 1990; **41**: 471–476.

