(N-[^{11}C]-Methyl)Doxepin: Synthesis of a Radiotracer for Studying the Histamine H-1 Receptor

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Summary

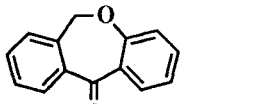
Doxepin (3-dibenz [b,e] oxepin - 11(6H) - ylidene - N,N - dimethyl - 1-propanamine), a potent H_1 histamine antagonist, was labeled with ^{11}C-iodomethane by N-alkylation of normethyldoxepin. The synthesis, purification, and formulation were performed in approximately 20 minutes with an average specific activity of 1630 mCi/μmol (EOS).

Key Words: Doxepin, carbon-11, histamine, positron emission tomography

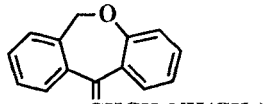
Introduction

The neurotransmitter histamine is known to participate in the control of a number of biological responses (1). In the central nervous system, histamine modulates through the H_1, H_2 and H_3 histamine receptor subtypes. These receptors have a wide distribution throughout the brain (2). Pyrilamine, a potent antagonist to the histamine H_1 receptor, has recently been labeled with carbon-11 and studied *in vivo* in animals and humans using positron emission tomography (PET) (3,4).

Doxepin (Figure 1), a tricyclic antidepressant, is also one of the most potent histamine H_1 antagonists (5). The equilibrium dissociation constant (K_D) for doxepin in the autopsied human frontal cortex is 0.24 nM compared to a K_D for pyrilamine of 5.0 nM (6). In therapeutic doses, doxepin is also known to inhibit the binding of tritiated pyrilamine *in vivo* in mice brain (7).



Doxepin



Nordoxepin

Figure 1: Structures of doxepin and nordoxepin.

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In this study, doxepin was labeled with carbon-11 by alkylation of normethyldoxepin (Figure 1) with ^{11}C-iodomethane. The synthetic procedure, purification, formulation and characterization are described.

Results and Discussion

(N-[^{11}C]-Methyl)doxepin was synthesized by N-methylation of the free base nordoxepin with ^{11}C-iodomethane. In our hands, a variety of reversed phase chromatographic separation techniques would not separate (N-[^{11}C]-methyl)doxepin from nordoxepin effectively. To facilitate the chromatographic separation of nordoxepin and (N-[^{11}C]-methyl)doxepin, decanoyl chloride was added to the reaction mixture (with excess triethylamine) to derivatize the secondary amine precursor, nordoxepin. The semipreparative uv chromatogram (Figure 2) displays the almost complete removal of the nordoxepin (R_t = 5.6 min, k' = 4.6) from the chromatogram providing pure (N-[^{11}C]-methyl)doxepin (R_t = 6.2 min, k' = 5.2). The effective specific activity improved from 1274 mCi/μmol (n = 4) to 2002 mCi/μmol (n = 4) with the removal of the nordoxepin. Also it is important to note that the peak for (N-[^{11}C]-methyl)doxepin in the radioactivity chromatogram shows saturation of the detection system due to paralyzing of the photomultiplier tube with a large quantity radioactivity flowing past the detector.

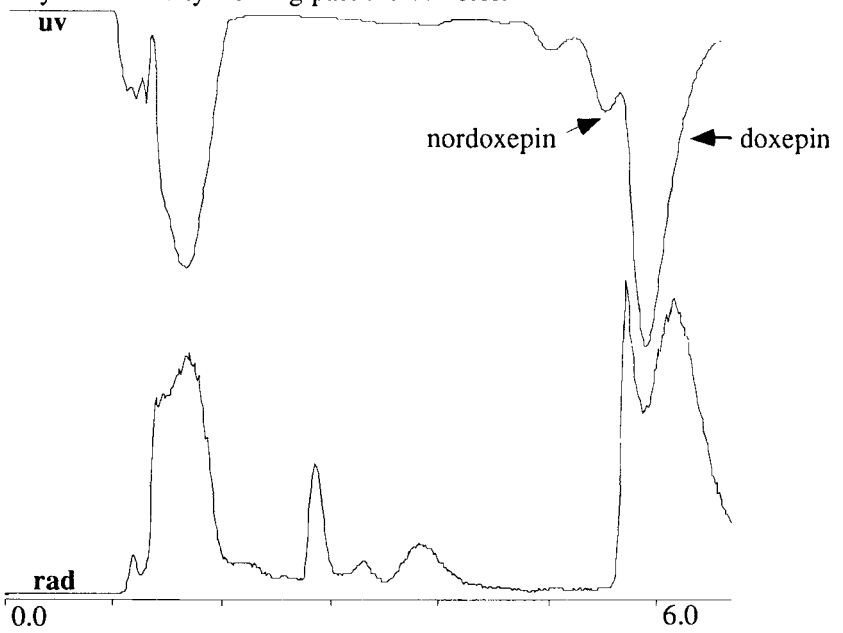

Figure 2: Semipreparative ultraviolet and radioactive chromatograms of the (N-[^{11}C]-methyl)doxepin reaction mixture.

The synthesis, semipreparative HPLC, and formulation was completed in an average run time of 20.3 minutes (n = 8) with an average radiochemical yield of 15 % based on $^{11}CH_3I$. The average specific activity was 1638 ± 708 mCi/μmole at end of synthesis. The final formulated solution was chemically and radiochemically pure as determined by analytical HPLC (Figure 3).

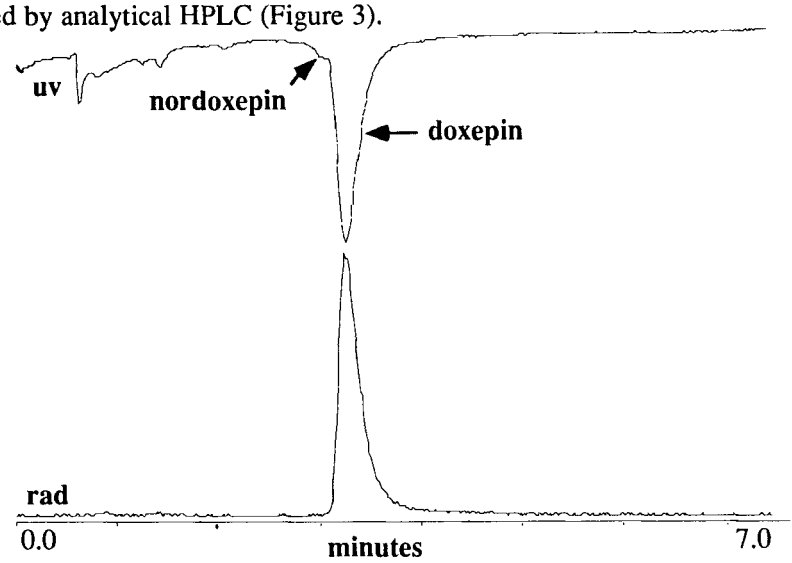

Figure 3: Analytical HPLC of (N-[^{11}C]-methyl)doxepin

Experimental

Normethyldoxepin (nordoxepin) hydrochloride and doxepin hydrochloride were obtained from Sigma Chemical Company. Dimethylformamide (DMF) was stirred over BaO overnight and vacuum distilled prior to use. All other reagents were A.C.S. or HPLC purity. High performance liquid chromatographic analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single two inch NaI crystal radioactive detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax - version 1.2). HPLC semipreparative purifications were completed on an Alltech 10 μ C-18 Econosil column (10 x 250 mm) using a mobile phase of 50% acetonitrile / 50% water (0.1 M ammonium formate) at a flow rate of 11 mL/min. Chemical and radiochemical purity were determined using an Alltech 10 μ C-18 HPLC column (0.46 x 250 mm) with the same mobile phase as in the semipreparative HPLC at a flow rate of 4 mL/min. A dose calibrator (Capintec 12R) was used for all radioactivity measurements.

Radiosynthesis and purification of (N-methyl-[^{11}C])-doxepin.

Nordoxepin hydrochloride (1 mg, 3.31 μmoles) was dissolved in 500 μL of water. Following the addition of 50 μL of 1 N NaOH, the solution was extracted with diethyl ether. The ether layer was passed through a small column of K_2CO_3 and evaporated to dryness under a gentle stream of argon gas. $^{11}CH_3I$ was synthesized from cyclotron produced $^{11}CO_2$ as previously described (8). The $^{11}CH_3I$ was bubbled into a sealed vial containing the extracted nordoxepin dissolved in 200 μL of DMF cooled to -78 °C. After the ^{11}C radioactivity reached a plateau, the vial was heated to 80° C for 3 minutes. Triethylamine (10 μL, 7.26 mg, 72 μm) and decanoyl chloride (5 μL, 4.60 mg, 24 μm) were added. The vial was heated at 80° C for 1 minute and 200 μL of HPLC solvent was added prior to applying the solution to the semipreparative HPLC column. After collection and vacuum evaporation to dryness, the product was redissolved in 7 mL of sterile normal saline. Following sterile filtration into a sterile evacuated vial, 3 mL of sterile sodium bicarbonate was added.

The chemical and radiochemical purity of the final solution was determined as described previously (9).

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