Preparation of [^{125}I]-3H2-Iopiperidol-A by Exchange Radioiodination

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SUMMARY

Preparation of radiolabeled [^{125}I]-N,N'-bis(2,3-dihydroxypropyl)-5-(3-hydroxy-2-oxo-1-piperidinyl)-2,4,6-triiodo-1,3-benzenedicarboxamide, ([^{125}I]-3H2-iopiperidol-A), a nonionic radiographic contrast agent, was achieved by $HAuCl_4$ mediated exchange radioiodination. [^{125}I]-Iopiperidol-A was isolated by reversed-phase HPLC in 83% radiochemical yield. The radiochemical purity of isolated [^{125}I]-3H2-iopiperidol-A was 98.7% and the specific activity was 6.6 mCi/μmol. The role of $HAuCl_4$ in facilitating exchange radioiodination of 3H2-iopiperidol-A, however, appears to be a complex process not limited to the production of electrophilic iodine ion.

Keywords: 3H2-Iopiperidol-A, Iopamidol, Radiographic Contrast Agent, Radiolabel

INTRODUCTION

A research program to identify a second generation nonionic radiographic contrast agent to supplement the nonionic agent Iopamidol (1) has resulted in several potential lead candidates (1). One of these candidates, N,N'-bis(2,3-dihydroxypropyl)-5-(3-hydroxy-2-oxo-1-piperidinyl)-2,4,6-triiodo-1,3-benzenedicarboxamide, (3H2-iopiperidol-A) (2), has been advanced toward preclinical development. As part of this preclinical development, the need was identified for radioisotopically labeled 3H2-Iopiperidol-A for use in biodistribution, metabolism and pharmacokinetic studies.

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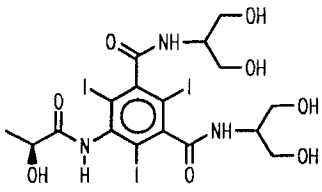

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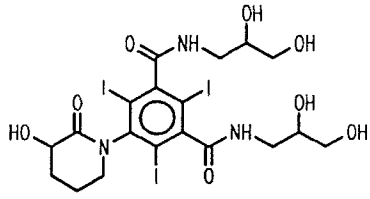

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Whereas ^{3}H or ^{14}C-labeled drug entities are typically employed in metabolism and pharmacokinetic protocols, the aromatic triiodoisophthalate core offered the potential to use [^{125}I]-labeled 3H2-iopiperidol-A for these studies. The fully substituted aromatic ring is believed to impart considerable steric and electronic stability toward loss of iodine from these radioopaque agents (2). Nonionic radiographic contrast media based on this core have been shown to be stable toward *in vitro* and *in vivo* deiodination (3,4). In the absence of *in vivo* deiodination, the envisioned [^{125}I]-radiolabel should provide an accurate assessment of the *in vivo* biodistribution and pharmacokinetic properties of 3H2-iopiperidol-A. This report describes the preparation [^{125}I]-3H2-iopiperidol-A suitable for use in biodisposition, metabolic and pharmacokinetic studies.

DISCUSSION

The strategy for preparation of radiolabeled 3H2-iopiperidol-A envisioned introduction of [^{125}I]-iodide into the aromatic core by a direct radioiodine exchange reaction with the drug candidate. There are several reported examples of radioiodine exchange reactions on ionic radiographic contrast agents (5,6). These exchange reactions can be achieved by nucleophilic aromatic substitution of [^{125}I]-iodide into the ionic isophthalic acid core. Unlike ionic radiographic agents, radioexchange reactions on nonionic radiographic contrast agents have not been widely reported. One approach of exchange radioiodination on the nonionic agent iopamidol was described by Sinn et al. (7). In this example, the radioiodination reaction employed $HAuCl_4$ to facilitate the exchange reaction.

The reported radioiodination procedure for the preparation of [^{125}I]-iopamidol used specially designed glassware and involved removal of H_2O from the reaction by inclusion of a desiccant within a separate chamber of the glassware (7). We modified the conditions for preparation of [^{125}I]-3H2-iopiperidol-A to accommodate the reaction in a 1 mL conical vial, reduced the mass from 10 mg to 50 ug to facilitate

HPLC purification, and made no effort to remove H_2O during the reaction. The initial solvent composition of 0.35 M HCl, 60 mM NaCl generated by the addition of $HAuCl_4$ in 1 M HCl to Na[^{125}I] in 0.1 M NaOH was maintained. This modified exchange radioiodination procedure was also used to prepare [^{125}I]-iopamidol.

Under these modified conditions high incorporation of radioiodine into 3H2-iopiperidol-A was observed upon heating at 110 °C. The integrated radiochemical yield of [^{125}I]-3H2-iopiperidol-A, determined by HPLC was 25% at 30 min, 65% at 1 h and 82% at 2 h (Figure 1). Only marginal improvement in integrated radiochemical yield was observed by continued heating at 110 °C up to 5 hours. After 5 hours at 110 °C, there were several minor radioimpurities (15%) but essentially no free iodide in the 3H2-iopiperidol-A exchange reaction. Similarly, the integrated radiochemical yield of [^{125}I]-iopamidol was 45% at 30 min and 64% at 1 h. No improvement in integrated radiochemical yield was observed by continued heating to 5 hours. After 5 hours at 110 °C, the iopamidol exchange reaction showed integrated yields of 65% for [^{125}I]-iopamidol, 23% for radioiodide and 11% for a minor radioimpurity. The UV chromatograms of 3H2-iopiperidol-A and iopamidol showed negligible decomposition.

Following exchange radioiodination by this procedure, [^{125}I]-3H2-Iopiperidol-A was isolated in 83% radiochemical yield by reversed phase HPLC. Recovery of radioactivity was quantitative under the HPLC conditions used for the analysis and isolation of [^{125}I]-3H2-iopiperidol-A. The radiochemical purity of

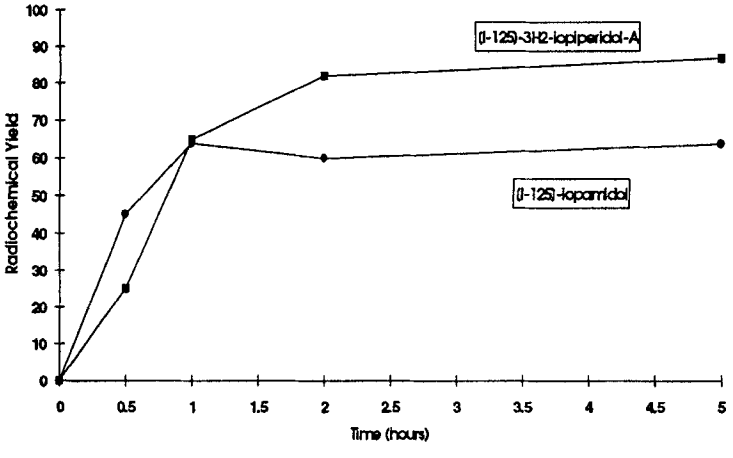

Figure 1. Integrated radiochemical yield of [^{125}I]-3H2-iopiperidol-A and [^{125}I]-iopamidol for $HAuCl_4$ mediated exchange radioiodination.

isolated [^{125}I]-3H2-iopiperidol-A was 98.7% as determined by analytic HPLC. The specific activity of [^{125}I]-3H2-iopiperidol-A was 6.6 mCi/μmole. This specific activity was suitable for use in preclinical studies, as the radiotracer was to be further diluted with carrier 3H2-iopiperidol-A to achieve pharmaceutical dosage typical of radiographic contrast agents. Stability studies on [^{125}I]-3H2-iopiperidol-A have not been done, however, stability studies on related nonionic [^{125}I]-triiodoisophthalate (8) analogues prepared by $HAuCl_4$ mediated radioiodine exchange have been completed. These stability studies showed a decrease of only 7% radiochemical purity upon storage in phosphate buffer pH 7 at -20 ^{o}C for 63 days. This result would indicate that radioiodine has been incorporated into the aromatic core, as desired, and is not present as the N-iodo or similar by-product. The exchange radioiodination is not likely to be regiospecific, thus the [^{125}I]-3H2-iopiperidol-A radiolabel would be expected to consist of a mixture of 2,4- and 6-substituted isotopic regioisomers.

These results are consistent with results observed by Sinn et al. who reported the isolation of [^{125}I]-iopamidol in 80% radiochemical yield with a specific activity up to 0.8 mCi/umol (7). This isolated radiochemical yield is similar to the 83% isolated radiochemical yield observed for the preparation of [^{125}I]-3H2-iopiperidol-A, and it is slightly improved relative to the integrated radiochemical yield of 65% that we report for [^{125}I]-iopamidol. The low specific activity of 0.8 mCi/umol reported by Sinn et al. relative to the specific activity of 6.6 mCi/umol determined for isolated [^{125}I]-3H2-iopiperidol-A was a direct result of the amount of substrate (10 mg iopamidol relative to 50 ug of 3H2-iopiperidol-A) subjected to exchange radioiodination.

In a control experiment no radiochemical incorporation into 3H2-iopiperidol-A was observed in the absence of $HAuCl_4$ oxidant. The $HAuCl_4$ mediated exchange radioiodination reaction proceeds through the putative intermediacy of electrophilic iodide (7). Elding and Olsson (9) have shown, under conditions nearly identical to those employed in the exchange radioiodination reaction, that $HAuCl_4$ oxidizes NaI to afford ICl_2^-. The oxidation of Na[^{125}I] would be complete within seconds and the resultant [^{125}I]Cl would be stable under the reaction conditions of 0.35 M HCl, 60 mM NaCl. The hypothesis that [^{125}I]Cl was the reactive species mediating exchange radioiodination was tested by use of [^{125}I]ICl_2^- prepared by exchange of Na[^{125}I] into ICl (10,11). At the optimal [^{125}I]/ICl ratio of 0.75 (11), heating at 110 ^{o}C for 3 hours afforded 3% integrated radiochemical yield of [^{125}I]-3H2-iopiperidol-A. Similar exchange reactions with [$^{125}I^-$]/ICl ratios of 0.01 and 3.7 failed to incorporate radioiodine into 3H2-iopiperidol-A. The failure of the exchange radioiodination by reaction of 3H2-iopiperidol-A with directly prepared [^{125}I]ICl_2^- suggests that the involvement of $HAuCl_4$ may be more complex than simple oxidative formation of ICl_2^-.

EXPERIMENTAL

Reagents

N,N'-Bis(2,3-dihydroxypropyl)-5-(3-hydroxy-2-oxo-1-piperidinyl)-2,4,6-triiodo-1,3-benzenedicarboxamide (3H2-iopiperidol-A) (batch 003/005) was obtained from Diagnostics Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute. N,N'-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-1,3-benzenedicarboxamide (iopamidol) was obtained from Squibb Diagnostics Manufacturing. $HAuCl_4$ and ICl were obtained from Aldrich Chemical Company, Milwaukee, WI. Na[^{125}I] in 1.0 mM NaOH (2200 mCi/umol, 948 mCi/mL, pH 10) was obtained from Nordion International Inc., Kanata, Ontario, Canada. Trifluoroacetic acid (TFA) was obtained from Pierce, Rockford, IL. All other chemicals were ACS reagent grade. HPLC grade MeOH was obtained from Baxter Healthcare Corp., McGaw Park, IL. HPLC grade H_2O was obtained by deionization and reverse osmosis (Milli-Q, Millipore Corp). Aqueous HPLC solvents were filtered through a 0.45 μm nylon-66 filter prior to use.

General Procedures

Radioactivity of exchange reactions and HPLC fractions were routinely quantified with use of an Atomlab 100 dose calibrator (Atomic Products Corp.). An attenuation factor of 1.7-2.0 was noted for dose calibrator determinations made in Reacti-vials relative to glass test tubes used for collecting HPLC fractions. A LKB Wallace gamma counter was also used to quantify and corroborate the radioactivity of isolated [^{125}I]-3H2-iopiperidol-A. Counting efficiency for the gamma counter was determined to be 65% by standard methods. Isolated radiochemical yields were calculated from measurements obtained in glass test tubes with the dose calibrator.

The HPLC apparatus used for preparative isolation and analysis of [^{125}I]-3H2-iopiperidol-A consisted of a Rainin liquid chromatograph with two model HPXL pumps, a Rheodyne model 7125 injector, a Dynamax two channel data acquisition and analysis module, and a Gilson model FC-80K fraction collector. The HPLC system used for radiotracer isolation had an in-line Beckman model 170 radiometric detector. The HPLC system used for analytic work had a Knauer model 87 variable wavelength UV detector operating at 254 nm and a Beckman model 170 radiometric detector connected in series.

Isolation of [^{125}I]-3H2-iopiperidol-A was achieved with a Nucleosil 5 C18 (4.0 x 250 mm) column operating at a flow rate of 1.0 mL/min with an isocratic mobile phase composed of 0.1% aqueous TFA/MeOH (94:6). The retention time for 3H2-iopiperidol under the conditions used in the preparative isolation was 47.5 min. Analytic HPLC was performed with a Zorbax ODS (4.6 x 150 mm) column operating either with a flow rate of 1.5 mL/min and mobile phase composition of 0.1% aqueous TFA/MeOH (92:8) or with a flow rate of 1.0 mL/min and a mobile phase composition of 0.1% aqueous TFA/MeOH (94:6). The retention times of 3H2-iopiperidol-A under these analytic conditions were 13.1 min (8% MeOH, 1.5 mL/min) and 32.4 min (6% MeOH, 1.0 mL/min), respectively. The retention time for iopamidol under the latter analytic HPLC conditions (6% MeOH, 1.0 mL/min) was 5.5 min. Specific activity of [^{125}I]-3H2-iopiperidol-A was determined by HPLC by comparison of UV peak areas to a response curve from 3H2-iopiperidol-A standards of known concentration. HPLC recovery for [^{125}I]-3H2-iopiperidol-A was determined by comparison of the sum of radioactivity contained within all of the isolated fractions of HPLC eluent to the total radioactivity for that identical injection volume contained in the same type of test tube. HPLC recovery of [^{125}I]-3H2-iopiperidol-A was quantitative.

[^{125}I]-3H2-Iopiperidol-A (2)

HAuCl$_4$ Procedure: To a 1 mL Reacti-Vial containing 40 μL of 0.35 M HCl, 60 mM NaCl was added 3H2-iopiperidol-A (50 μg, 6.2x10^{-2} μmol) dissolved in 5 μL of H_2O. To this solution was added 2 μL (0.5 mCi, 2.3x10^{-4} μmol) of Na[^{125}I] followed by 5 μL (5 μg, 1.47x10^{-2} μmol) of a 2.9 mM solution of HAuCl$_4$ dissolved in 1 M HCl. The reaction was placed in a heating block maintained at 110 ^{o}C. The radiochemical yield of [^{125}I]-3H2-iopiperidol-A was determined by HPLC analysis at 30 min, 1 h, 2 h and 5 h in duplicate radioexchange reactions. In a separate exchange reaction prepared as described, 320 uCi of the 500 uCi reaction solution was directly subjected to reversed-phase HPLC purification. Based on HPLC integrations from this preparative chromatogram, the integrated radiochemical yield was 87.6%. From this HPLC separation, 263 uCi (47 uCi/mL) of [^{125}I]-3H2-iopiperidol-A was isolated. The isolated radiochemical yield of [^{125}I]-3H2-iopiperidol-A based on the activity (dose calibrator) of the injected sample was 83%. The radiochemical purity of this isolated [^{125}I]-3H2-iopiperidol-A was 98.7% while the specific activity was 6.6 mCi/μmole.

Control Procedure: To a 1 mL Reacti-Vial containing 40 μL of 0.35 M HCl, 60 mM NaCl was added 3H2-iopiperidol-A (50 μg, 6.2x10^{-2} μmol) dissolved in 5 μL of H_2O. To this solution was added 5 μL of 1 M

HCl (no catalyst) followed by 2 μL (0.5 mCi, 2.3x10^{-4} μmol) of Na[^{125}I]. The reaction was placed in a heating block maintained at 110 °C. The radiochemical yield of [^{125}I]-3H2-iopiperidol-A was determined by HPLC analysis at 1 h and 3 h in duplicate radioexchange reactions. The integrated radiochemical yield of [^{125}I]-3H2-iopiperidol-A was 0% at both time points in the duplicate samples.

ICl Procedure: To a 1 mL Reacti-Vial containing 40 μL of 0.35 M HCl, 60 mM NaCl was added 3H2-iopiperidol-A (50 μg, 6.2x10^{-2} μmol) dissolved in 5 μL of H_2O. To this solution was then added ICl (4x10^{-2} μg, 2.4x10^{-4} μmol) dissolved in 5 μL of 1 M HCl, 0.5 M NaCl. This was followed by addition of 2 μL (0.4 mCi, 1.8x10^{-4} μmol) of Na[^{125}I]. The reaction was placed in a heating block maintained at 110 °C. The integrated radiochemical yield of [^{125}I]-3H2-iopiperidol-A, determined by HPLC analysis at 1 h and 3 h, was 0% and 3%, respectively. Similar experiments with [^{125}I]/ICl ratios of 0.01 and 3.7 failed to incorporate radioiodine into 3H2-iopiperidol-A.

[^{125}I]-Iopamidol (1)

To a 1 mL Reacti-Vial containing 30 μL of 0.35 M HCl, 60 mM NaCl was added iopamidol (50 μg, 6.4x10^{-2} μmol) dissolved in 5 μL of H_2O. To this solution was added 2 μL (1.0 mCi, 4.5x10^{-4} μmol) of Na[^{125}I] followed by 5 μL (5 μg, 1.47x10^{-2} μmol) of a 2.9 mM solution of $HAuCl_4$ dissolved in 1 M HCl. The reaction was placed in a heating block maintained at 110 °C. The radiochemical yield of [^{125}I]-iopamidol was determined by HPLC analysis at 30 min, 1 h, 2 h and 5 h in duplicate radioexchange reactions.

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