SYNTHESIS OF [^{195m}Pt]-TETRAPLATIN

Steven D. Wyrick and Stephen G. Chaney*
Radiosynthesis Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy and Department of Biochemistry and Biophysics*, School of Medicine, University of North Carolina, Chapel Hill, N.C. 27599

SUMMARY

(Trans d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV) (tetraplatin) is a second generation platinum antitumor agent which exhibits less toxicity than cisplatin and is effective in cell lines with acquired resistance to cisplatin. We previously reported the synthesis of tritium labelled tetraplatin which was utilized in both tissue culture and *in vivo* studies. Loss of the labelled diaminocyclohexane carrier moiety during the *in vivo* studies necessitated the synthesis of [^{195m}Pt]-tetraplatin from [^{195m}Pt]-potassium hexachloroplatinate as described herein.

Key words: tetraplatin, trans-(d,l)-1,2-diaminocyclohexanetetrachloroplatinum-(IV), platinum-195m

INTRODUCTION

Cis-diamminedichloroplatinum(II) (cisplatin) is a very widely used anticancer drug.[1,2] However, both toxicity and acquired resistance to cisplatin are significant clinical problems. Thus, there has been considerable effort directed toward development of safer second generation drugs. One of these drugs, (trans-d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV) (tetraplatin), is about to enter phase I/II clinical trials. Tetraplatin has similar effectiveness[3-5] and reduced nephrotoxicity[6,7] compared to cisplatin in a variety of tumor screens. It is also effective against a variety of murine[3,4] and human[8-11] cell lines with acquired resistance to cisplatin.

We have previously reported the synthesis of [4,5-$^{3}H_2$(n)](trans-d,l)-1,2-diaminocyclohexanedichloroplatinum(II) and [4,5-$^{3}H_2$(n)](trans d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV)[12] and have used these compounds for biotransformation studies in tissue culture.[13-15] While lability of the diaminocyclohexane carrier ligand was not a problem in those studies, loss of the carrier ligand has proven to be a significant problem for *in vivo* studies

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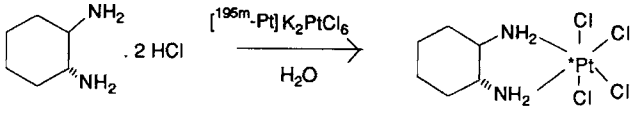
(manuscript in preparation). Thus, in order to carry out accurate pharmacokinetic and biotransformation studies *in vivo* it has proven necessary to synthesize tetraplatin with a ^{195m}Pt label. In addition, we have recently discovered that 1,2-bis-azidocarbonylcyclohex-4-ene, an intermediate in the synthesis of trans-1,2-diaminocyclohex-4-ene which is used in our tritiation procedure,[12] explodes violently in the presence of peroxide containing ether. We strongly recommend that handling of this intermediate should be avoided if possible and the ^{195m}Pt labelled derivative used as an alternative.

DISCUSSION

We previously reported the synthesis of a number of tritium labelled platinum antitumor agents, including tetraplatin, which contain the trans-(d,l)-1,2-diaminocyclohexane carrier ligand.[12] In order to label tetraplatin at a site other than the carrier moiety, ^{195m}Pt was used. As shown below, trans-(d,l)-1,2-diaminocyclohexane dihydrochloride (1) was obtained by resolution of a commercial mixture of the cis and trans isomers as the nickel complexes by the procedure of Saito[16] and was reacted with [^{195m}Pt]-potassium hexachloroplatinate (obtained from Oak Ridge National Laboratory) in water at reflux for 10 h to afford the labelled product (2) in 54% chemical yield. The previous larger



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scale preparation of the tritium labelled product involved a two step synthesis in which labelled trans-(d,l)-1,2-diaminocyclohexanedichloro-platinum(II) was first prepared and then further oxidized with chlorine gas in 0.5 N HCl to to afford tetraplatin in 61% overall yield. It was decided to employ the one step procedure of Kepler[17] to prepare the ^{195m}Pt labelled product because it affords better yields with small amounts of starting material (2.4 mg of the diaminocyclohexane·2HCl and 6.2 mg of [Pt^{195m}]-potassium hexachloroplatinate). Results of elemental analysis[18] for the unlabelled tetraplatin prepared by this procedure are as follows: Calc. C=15.66, H=3.29, Cl=30.82, N=6.09, Pt=42.04;

Found C-15.86, H-3.24, Cl-30.79, N-6.19, Pt-42.27, K<0.05.

EXPERIMENTAL PROCEDURES

All chemicals were used as received from the manufacturers. Radioactivity was quantitated using a Nuclear Chicago Gamma Counter model 1185.

[^{195m}Pt]-Trans-(d,l)-1,2-diaminocyclohexanetetrachloroplatinum(IV) (Tetraplatin) (2). A solution of [^{195m}Pt]-potassium hexachloroplatinate (6.2 mg, 0.0128 mmol, 0.2 mCi/mg) in 2.9 mL of 1.0 N HCl was evaporated nearly to dryness *in vacuo* to afford a yellow-orange solid and a solution of 2.4 mg (0.013 mmol) of trans-(d,l)-1,2-diaminocyclohexane dihydrochloride in 2.0 mL of water was added. This solution was protected from light and stirred at reflux for 10 h. The water was then evaporated *in vacuo* and the solid product was separated from starting material by dissolving in 2.0 mL of acetone. The acetone solution was filtered through a cotton plug, concentrated to approximately 0.2 mL and diluted with 2.0 mL of diethyl ether. The resulting suspension was centrifuged and the supernatant pipeted off. Addition of ether, centrifugation and removal of the supernatant was repeated twice and the yellow, solid residue was vacuum dried to afford 3.1 mg (54%) of product. Unlabelled tetraplatin prepared by this method was assessed for purity by two different HPLC systems. Tetraplatin was resolved from its corresponding platinum(II) analog, $PtCl_2$(dach), as described by Anderson et al.[19] A Zorbax 7 ODS column (Phenomenex) was used with isocratic water elution at 0.5 ml/min (retention time=24-25 min). Resolution of tetraplatin from more polar contaminants was obtained with a Partisil 5 ODS-3 column (Whatman) using isocratic elution with 80% 5 mM heptanesulfonate, pH 3.4/ 20% methanol at 1 ml/min (retention time=8-9min). Purity was greater than 95% by both methods. Purity of the ^{195m}Pt labelled product was assessed by HPLC with a Partasil 5 ODS-3 column using the gradient elution method previously described.[20] Buffer A was 5.0 mM heptanesulfonate, pH 3.4 and Buffer B was 90% methanol. The elution profile was 10 min of Buffer A, a 30 min linear gradient from A to B, and 10 min of Buffer B at a flow rate of 1 ml/min. The retention time was 8 minutes which corresponds to that for the ^{3}H-tetraplatin. Purity was greater than 95% by this method.

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