^{11}C-LABELED KETANSERIN: A SELECTIVE SEROTONIN S_2 ANTAGONIST

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SUMMARY

A selective serotonin S_2 receptor antagonist, ketanserin, was prepared labeled with carbon-11 by a rapid synthesis which uses no-carrier-added phosgene as the labeled precursor. The ring-closure reaction in toluene of phosgene with the substituted 2-aminobenzamide precursor gives a nearly quantitative yield of ketanserin. The 30 min procedure yields 150 mCi of HPLC purified ketanserin at a specific activity of 250 mCi/μmol. The product's tissue distribution in mice shows a brain uptake and cerebrum to cerebellum ratio that encourages further *in vivo* receptor binding studies.

Key Words- ^{11}C-Phosgene, Ketanserin, Serotonin, Receptors

INTRODUCTION

Ketanserin 2 is a relatively new compound that was reported to bind selectively to serotonin S_2 receptor sites *in vitro*.[1] When labeled with tritium it was shown to effectively label the S_2 receptors both *in vitro* and *in vivo*,[2,3] and to be the most specific molecule available for labeling the S_2 receptor site. The study of these receptors in human brain using positron tomography is of interest due to their possible role[4,5,6] in certain brain disorders, and ketanserin is at present the molecule of choice for this study.

This paper describes a rapid, no carrier added synthesis of carbon-11 labeled ketanserin which uses labeled phosgene and produces a sterile, apyrogenic product. The specific activity obtained is sufficient to allow injection of a lower dose than was successfully used in studies of the tritium-labeled material.[3] The tissue distribution of the labeled ketanserin in mice is also presented as an indication of radiation dose distribution and the suitability of labeled ketanserin for brain receptor studies.

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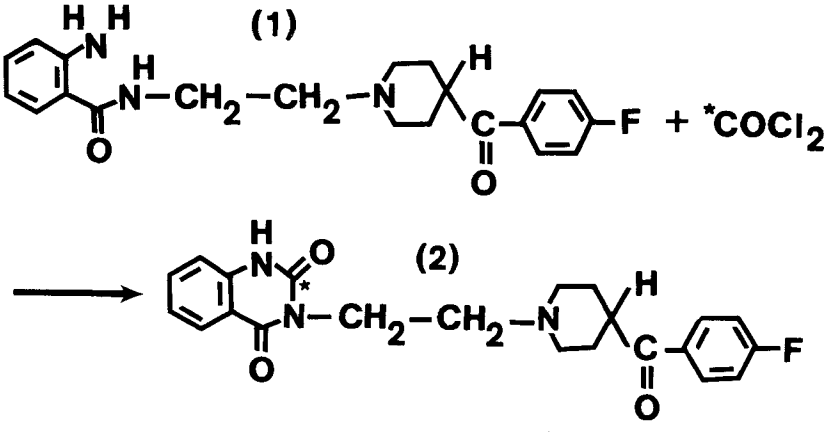
EXPERIMENTAL

The labeled ketanserin (3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl] ethyl]-2,4-(1H,3H)-quinazolinedione-[2-^{11}C], 2) is produced by the ring closure reaction of ^{11}C-phosgene with the substituted o-aminobenzamide precursor, N-(2-amino-benzoyl)-2-(4-(4-fluorobenzoyl)-1-piperidinyl) ethylamine, 1.



Scheme 1- Preparation of ^{11}C-Ketanserin, 2

The labeled phosgene was first produced as has been described previously.[7,8] Briefly, $^{11}CO_2$ was reduced over hot zinc to ^{11}CO and then chlorinated with heated platinum tetrachloride to give phosgene. Liberated water and chlorine were trapped in the flow system by diphosphorous pentoxide and antimony.

About 1 mg (3 μmol) of the amine 1* was dissolved in 0.3 ml toluene or chloroform, and placed in the 3 ml reaction vessel at room temperature. The labeled phosgene was delivered in a helium gas stream (10 ml/min) after its preparation and was allowed to bubble through the solution. After the phosgene activity was collected the toluene was evaporated to dryness using a heating bath at 130°C. The vessel was then cooled to room temperature using a water bath and 1 ml chloroform was added to dissolve the residue. Although the yields in both solvents were similar, toluene was preferred for mechanical reasons since heating of the chloroform solution was necessary to complete the reaction.

The chloroform solution was then injected on the chromatography system described below and the radioactive fraction was collected. The pear-shaped flask was immersed in a water bath at 80°C and the chromatography solvent was evaporated to dryness with the aid of a moderate nitrogen flow directed to the

bottom of the flask. When the flask was visibly dry, 5 ml of sterile physiological saline solution, buffered to pH 3 with 10^{-3}M phosphate and pre-warmed to 55°C, was added to dissolve the purified product. To this point in the synthesis all manipulations were performed remotely in a shielded hood.

The saline solution containing the labeled ketanserin was transferred to a sterile evacuated dosage vial and the radioactivity was counted. The required amount was then drawn up through a sterile 0.22 μm (Millipore) filter into a sterile syringe for injection.

Chromatography Systems

Purification of labeled ketanserin was accomplished using a high performance liquid chromatography (HPLC) system with ultraviolet absorbtion and radioactivity detectors. A Waters μ-porasil analytical column was eluted at 6 ml/min with a mixture (v/v) of 98% methylene chloride and 2% of a solution of water (2%) and ethylamine (2%) in ethanol. On this system toluene eluted at 0.75 min, the amine 1 at 1.6 min, and ketanserin at 3.6 min with excellent resolution. Monitoring of the absorbance at 254 nm with a Waters flow monitor allowed estimation of the specific activity of the product.

Thin layer chromatography (TLC) was done on 20 cm Merck 0.25 mm silica gel 60 F_{254} plates and the product detected under 254 nm light. Radioactivity was detected using a Numelec Corp. Chromolec 101 multi-channel thin-layer plate reader. Solvents used were (v/v) benzene/ethanol/NH_4OH 90/10/1 (BEA), and ethyl acetate/hexane/ethyl amine 90/10/1 (EHE). R_f values for ketanserin were: BEA- 0.17, EHE- 0.43; and for the amine were: BEA- 0.25, EHE- 0.56.

In Vivo Uptake Experiments

The tissue distribution of labeled ketanserin was determined at four time intervals using male albino mice. Weighed portions of the sterilized injection solution were given by tail vein injection (about 0.2 ml of 1 mCi/ml dilution) without anesthesia. After the desired time interval the mice were sacrificed by cervical dislocation and organ samples were placed in pre-weighed vials for counting and weighing. Similarly treated portions of the injection solution were

diluted to 50 ml and weighed samples were counted as standards to determine the radioactivity contained per gram of injection solution. All samples were decay-corrected and the activity expressed as percentage of the injected dose found per gram of tissue.

RESULTS AND DISCUSSION

The radiochemical yields of labeled ketanserin ranged from 25-50% based on labeled CO_2, which is within the same range as the variable yield of phosgene itself when produced by this method.[7,8,9] This implies an excellent yield for the reaction of phosgene with 1 to produce ketanserin. The reaction is therefore sufficiently rapid so that there is no need to allow time specifically for reaction. The remaining 50-75% of the carbon-11 which is not converted to phosgene does not dissolve in the reaction solvent and is therefore not trapped. The single radioactive product was identified as ketanserin by its retention on the HPLC purification system and by its behavior on the TLC systems described previously. In all cases the radioactivity behaved identically to an authentic sample of ketanserin.*

The final product, taken up in sterile saline solution and filtered, was ready for injection 30 min after the end of bombardment. The solution was tested and found to be sterile and apyrogenic. The actual yields of labeled ketanserin ready for injection were 130-170 mCi with a specific activity of 250 mCi/ mol using a bombardment on the nitrogen target of 30 A of 20 MeV protons for 30 min. This represents an excessive amount of product, however the ultimate goal of receptor binding studies demands the highest possible specific activity. Since the major source of stable carbon contamination in our preparation is the platinum tetrachloride,[10] it may be possible to increase the specific activity of the product by using a photochemical chlorination[11] and high purity chlorine gas. Our surplus of labeled product is not a problem with sufficient radiation protection and remote handling. The successful use of lower specific activity material for study of serotonin S_2 receptor binding in rat brain[3] indicates that this material should be sufficient for human use.

In Vivo Distribution

The tissue distribution of ^{11}C-ketanserin is given in table 1. Each entry gives the average for five mice.

A rapid clearance from the blood is observed, with the highest uptake in the lungs, liver, and kidney. After the initial uptake, a rapid decline is observed in all organs. The cerebellum was removed and counted separately, and shows consistently lower values than the cerebrum. Also, the initial decline in cerebellum activity is more rapid than in the cerebrum.

Table 1: Tissue Distribution of ^{11}C-Ketanserin in Mice (% dose/gram)

	5 min	15 min	30 min	45 min
Blood	1.33 ± 0.14	1.00 ± 0.11	0.91 ± 0.06	0.75 ± 0.26
Heart	3.93 ± 0.23	2.67 ± 0.36	2.05 ± 0.33	1.69 ± 0.28
Lung	15.30 ± 2.0	11.9 ± 1.9	6.66 ± 0.87	7.64 ± 1.2
Liver	10.82 ± 0.84	9.10 ± 0.64	8.71 ± 0.69	8.14 ± 1.5
Kidney	11.55 ± 0.91	10.97 ± 0.25	8.02 ± 0.60	8.90 ± 2.0
Spleen	4.31 ± 0.27	5.02 ± 0.65	3.00 ± 0.38	2.80 ± 0.40
Cerebrum	1.68 ± 0.12	1.47 ± 0.18	1.13 ± 0.09	0.95 ± 0.07
Cerebellum	1.47 ± 0.11	0.96 ± 0.06	0.72 ± 0.08	0.60 ± 0.14

Comparison of these results with those reported for in vivo receptor studies using tritium labeled ketanserin[3] are encouraging for further work with ^{11}C-ketanserin. A similar brain uptake and cerebellum/cerebrum ratio were observed. The brain uptake, while low, is expected to be sufficient for positron tomography studies in view of the previous use in this laboratory of another molecule for receptor studies which had similar uptake characteristics in mice.[10]

CONCLUSIONS

Carbon-11 labeled phosgene can be used to label ketanserin in high yield following a rapid procedure. The pure labeled radiopharmaceutical is quickly obtainable in a form suitable for injection in sufficient amount and with a suitable specific activity to allow positron tomographic study of serotonin S_2 receptors in vivo in human brain.

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