HIGH YIELD PREPARATION OF PURE 2-RADIOIODO-KETANSERIN OF HIGH SPECIFIC ACTIVITY, A SEROTONIN S2 RECEPTOR TRACER FOR SPECT.

J. Mertens, M. Gysemans, C. Bossuyt-Piron, M. Thomas.

VUB Cyclotron, Vrije Universiteit Brussel.

Laarbeeklaan 103, 1090 Brussels, Belgium.

SUMMARY

The synthesis of pure and high specific activity 2-radioiodo-Ketanserin is described. The Cu(I) assisted nucleophilic non-isotopic exchange applied on 0.5 mg 2-Br-Ketanserin allows to obtain a labelling yield ≥ 90%. After appropriate semi-preparative HPLC separation the tracer is further purified by means of a mini RP column technique, thus avoiding the inconveniences of evaporation of eluent. An overall radiochemical yield exceeding 75% of 2-^{123}I-Ketanserin with a purity ≥ 99% ready for i.v. injection is obtained.

Keywords: 2-^{123}I-Ketanserin, Cu(I), Non-isotopic exchange, Preconcentration.

INTRODUCTION

3(2-(4-(2-^{123}I-4-fluorobenzoyl)piperidinyl)ethyl-2,4,(1H,3H) quinazolinedione, 2-^{123}I-Ketanserin, shows high affinity and selectivity for serotonin S2 receptors in the living brain and seems to be a suitable tracer for mapping S2 receptors with SPECT (1). The 2-, i.e. ortho-benzoyl, position was premised to be the most favorable for introduction of the iodine atom as due to clustered lipophilic compensation the lipophilicity and binding characteristics of the native compound are maintained for the larger part (2).

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As the 2-position in the (4-fluorobenzoyl)piperidinyl group is deactivated for direct electrophilic substitution, 2-radioiodo-Ketanserin of high specific activity (n.c.a.) can only be obtained by nucleophilic radioiodide for bromo exchange on 2-bromo-Ketanserin followed by both appropriate HPLC and purification. Before intravenous administration the radiopharmaceutical has to be made up with isotonic saline and sterilized through a 0.22 μ filter.

This paper describes the application of our Cu(I) assisted nucleophilic exchange method (3) and an original purification step after adequate separation as well as the final make-up and quality control of the radiopharmaceutical.

MATERIALS AND METHODS

HPLC set-up :

Semi-preparative : the system consists of a Rheodyne injector (2 ml loop), a Waters M 6000A pump provided of a semi-prep pumphead, a Waters Lambdamax Model 480 UV detector with variable wavelength, a γ-scintillation detector (a coiled PTFE capillary - contained in a 13 mm tick lead capsule for high radioactivity) mounted above the surface of a NaI(Tl) crystal detector (Horshow) connected to Ortec electronics (high voltage supply, single channel analyzer, amplifier, ratemeter), a HP 3580 and a Intersmat ICR-1B integrator respectively connected to the radioactivity and UV detection system output.

Analytical : the set-up consists of a Rheodyne injector (50 μl loop), a Hitachi 655A pump equipped with a L-6000 II controller, a 655A variable wavelength UV monitor, a NaI(Tl) detector (Horshow) and appropriate electronics (Canberra), a D2000 Chromato-integrator Hitachi and a Ankerschmidt R40 1-channel recorder.

UV spectrometry: UV-spectra are recorded by means of a Perkin-Elmer Lambda 5 UV/VIS spectrometer using a 1 cm path cell.

TLC : is performed on Merck Kieselgel 60 F_{254} 5x10 cm plates using $CHCl_3$/MeOH - 9/1 (v/v) as mobile fase. The spots are recovered, extracted after measurement of activity and HPLC analysed.

Reagents : the reagents used were HPLC grade (Lichrosolv quality Merck) or p.a. grade (Merck).

3(2-(4-(2-bromo-4-fluorobenzoyl)piperidyl)ethyl-2,4,(1H,3H) quinazolinedione, 2-bromo-Ketanserin :

3-(2-Chloroethyl)-2,4(1H,3H)-quinazolindione 1 and (2-bromo-4-fluorophenyl)(4-

piperidinyl) methanone 2 were initially obtained from Janssen Research Foundation and later custom synthetised as described by C. G. M. Janssen et al. (4) and W. Wouters et al.(5). Coupling of 1 and 2 to obtain 2-bromo-Ketanserin is achieved as described in (4). The crude product was purified using a Silica 60 column and eluted with $CHCl_3$/MeOH - 95/5 (v/v) resulting in a 99.5% HPLC and TLC pure product.

^{1}H NMR (270 MHz,$CDCl_3$) : δ 1.75 -1.85 (m, 4, piper-N[$CCH_2]_2$); 2.17 (m, 2, $NCCH_2N$); 2.71 (m, 2, piper-N[$CH_2C]_2$; ax), 3.00 - 3.14(m, 3, piper-N[$CH_2C]_2$; eq and CHCO); 4.23 (t, 2, NCH_2CN); 7.02 - 8.13 (7, ArH); 9.25 (s, 1, NH).

MS (FAB) (m/e, %RA) : 476 (M+2, 20.4); 474 (M, 20.8); 396(13.9); 189(57.2); 146(37.4); 57(87.1)

LABELLING REACTION

^{123}I is applied for in vivo baboon and human SPECT studies, ^{131}I and ^{125}I are used for rodent and in vitro studies. To obtain low specific activity appropriate amounts of I^- are added to the reaction mixture.

A reaction mixture of 0.5 mg 2-bromo-Ketanserin, 5 mg gentisic acid, 7 mg citric acid, 0.2 mg $SnSO_4$, 30 µl of a 1.3 10^{-2} M $CuSO_4$ solution and 500 µl of a 10% v/v glacial acetic acid contained in a septum closed vial (Chrompack 12 mm Septum Blue) is respectively ultrasonicated (Bromosonic 12) and N_2 flushed during 5 minutes. After addition of 1 - 100 µl of radioiodide solution (3.7 - 740 MBq in 10^{-2} M NaOH) the vial is heated in boiling water during 40 minutes.

An appropriate aliquot (depending on the radioactivity present in the vial) is taken, allowing the determination of the labelling yield by means of HPLC and TLC.

PURIFICATION AND RECOVERY

The content of the reaction vial followed by 1.2 ml of rinsing solution (i.e. 0.5 ml MeOH and 0.7 ml HPLC eluent) are sucked through a 0.45 µ filter into a syringe. The content of that syringe is injected for HPLC separation using a Lichrochart 250x10 mm 10 µ RP select B Merck column and a MeOH/ACN/ H_2O//TMA/HOAC - 15/17/68//1.5/2.0 mixture of pH = 4.9 as eluent at a flow rate of 6 ml/min. Once the recorderpen reaches the baseline after the 2-bromo-Ketanserin peak, the UV detector is set at the highest sensitivity (0.002 AUFS) to check residual bromo compound and to estimate the specific activity of

the 2-radioiodo-Ketanserin. The radioactive peak is collected once the radioactivity signal reaches a count rate exceeding 10 times the baseline.

A typical HPLC run, i.e., UV and radioactive trace, is represented in Fig. 1.

The eluent fraction containing the radioactive peak, collected in a separatory funnel, is brought to 1.5 times its volume with H_2O and the pH is adjusted to 9.5 with 1N NaOH . Mixing is obtained by N_2 bubbling. The resulting solution is passed through a Baker Bond octadecyl 100 mg mini column - mounted on a 3-way minivalve on the funnel outlet - at a flow rate of 4 ml/min. by applying N_2 pressure . The funnel is rinsed with 20 ml of H_2O. After switching the 3-way valve the column is rinsed with another 10 ml H_2O and 300 µl 10^{-1} N HCl. A small N_2 flush is applied to obtain apparent dryness. The septum closed mini column is disconnected, wiped with 70% isopropanol and transferred to the laminar flow. The radiopharmaceutical is slowly eluted with 200 µl of ethanol and recovered in a vial containing 20 µl of 10^{-2} N HCl. After gently swirling during 1 min. 300 µl of 9 $^{0}/_{00}$ NaCl is added, the content mixed and sterilized by means of a 0.22 µ Millipore filter. Another 1.6 ml of isotonic saline - in order to rinse the vial, syringe and filter - is passed through the filter for final make-up of the radiopharmaceutical preparation (pH ~ 4). The described procedure can be automatically performed.

A fast quality control by means of analytical HPLC, using a 250x4 mm 7 µ Lichrochart Merck column and a MeOH/ACN/ H_2O//TMA/HOAC - 18/22/60//0.8/1.2 mixture as eluent at a flow rate of 1 ml/min., is carried out on the residual solution in the sterile vial after filling the syringe for injection. A typical quality control HPLC run is shown in Fig. 2.

RESULTS AND DISCUSSION

As shown in Fig. 3 applying the Cu(I) assisted nucleophilic exchange in acidic medium (pH of the reaction mixture 2.0) at 100 °C a steady state labelling yield of >90% is reached within 30 minutes. If a considerable amount of radioiodate is present in the radioiodide solution, after consumption of a large part of the radioiodide, the labelling reaction is no longer ruled by nucleophilic exchange but by the reduction of $^{*}IO_3^- \rightarrow {}^{*}I^-$ [1]. This mostly happens when using ^{123}I and ^{131}I with a specific activity approaching the theoretical value. As the labelling occurs in acidic reducing conditions, at lower specific activity - i.e., non radioactive I^- present - the following reaction occurs:

$$^{*}IO_3^- + I^- \rightarrow {}^{*}I\text{-}I_2 \qquad [1]$$

$$^{*}I\text{-}I_2 + Sn^{2+} \rightarrow {}^{*}I^- + I^- \qquad [2]$$

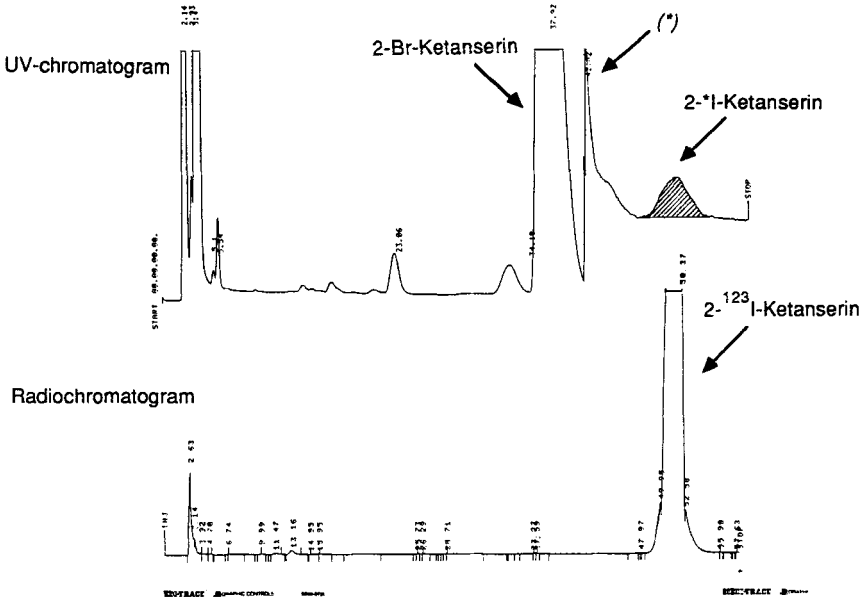

Figure1 : *Semi-preparative HPLC separation of a reaction mixture containing 2-^{123}I-Ketanserin (Low Specific Activity) and 0.5 mg 2-Br-Ketanserin . (*) : the UV-sensitivity is changed from 0.05 AUFS to 0.002AUFS*

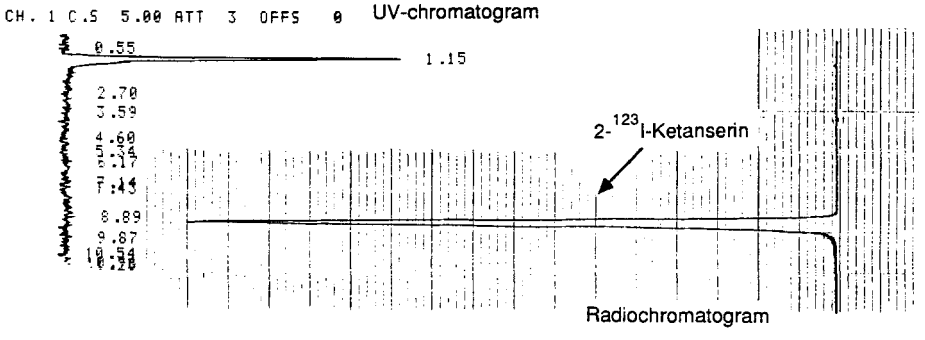

Figure 2 : *Quality control of the injection ready radiopharmaceutical*

In n.c.a. conditions $*IO_3^-$ can only be reduced by Sn^{2+}. To render the labelling yield almost independent of the radiochemical purity of the $^{123}I^-$ solution purchased, a reaction time of 40 minutes is preferred.

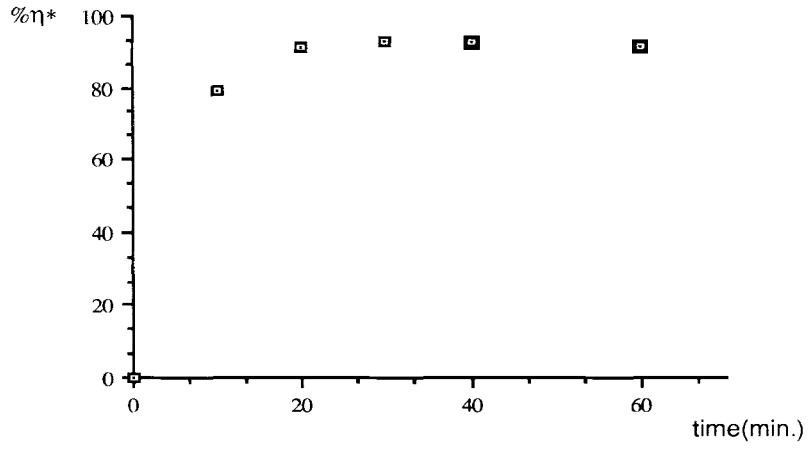

Figure 3 : η : *labelling yield (activity 2-*I-ketanserin/initial activity) as a function of time.*

At low specific activity (~10^5 MBq/ mol) the labelling yield is decreased to 85% which agrees with theory, as the back reaction Br^- + I-Ketanserin $\rightarrow$ Br-Ketanserin is not negligible anymore as it is the case at high specific activity.

As a labelling yield of > 90% is obtained already and for reason of HPLC separation efficiency, 0.5 mg of 2-Br-Ketanserine is proposed.

As shown in Fig.1, 2-radio-Ketanserin is apparently totally separated of 2-bromo-Ketanserin since the baseline is reached before the recovery of the radioactive peak starts. Whereas 2-Br-Ketanserin also shows high affinity for S2 receptors, the radiopharmaceutical preparation must be totally free of that substrate. This parameter was checked by submitting the collected eluent of at least five typical runs to the described preconcentration procedure and injecting the ethanolic concentrate in the analytical HPLC system operated at high UV sensitivity(λ = 229 nm i.e. λ_{max} of Br-ketanserin in eluent). No peaks corresponding to 2-bromo-Ketanserin or any other impurity have been observed, which means that the radiopharmaceutical is of high purity (detectionlimit of 2-bromo-Ketanserin is 5.10^{-12} mol). When applying an evaporation technique, impurities are concentrated. Therefore we prefer preconcentration on a mini RP column, allowing to remove the water soluble alkaline and acid impurities.

For reason of lipophilicity, 2-I-Ketanserin is preferentially sterilized and injected as its salt. Elution of the radiopharmaceutical from the mini column with acidified ethanol should allow

to obtain the salt in situ, but the aggressive acidified ethanol interacts with the alkane silanol group of the particles causing degradation of the column and turbidity of the saline solution after sterilization. For this reason the strong acid is added after recovery of the 2-radioiodo-Ketanserin from the column. The use of a commercial mini RP column has the advantage that the packing and back-pressure are about constant which make them more suitable for incorporation into an automated system than a custom made column.

With a little routine pure, 2-radioiodo-Ketanserin can be obtained with an overall radiochemical yield of at least 75% (not corrected for decay) within 2 hours, with a radiochemical purity ≥ 99%. The injection ready preparation was found to be stable - i.e. free $^{123}I^-$ ≤ 1 % - for at least 20 hours at ambient temperature.

CONCLUSION

2-radioiodo-Ketanserin of high specific activity can be obtained with a high labelling yield (≥ 90 %) by means of the Cu(I) assisted nucleophilic exchange in acidic medium.

A radiopharmaceutical of high purity is obtained applying efficient HPLC separation coupled to a purification on a mini RP column rather than evaporation. By adding a strong acid to the ethanol in which the radiopharmaceutical is recovered, the Ketanserin analogue is obtained as its salt, which makes it more suitable for sterilization and intravenous injection. The stability of the tracer in the final formulation makes it suitable for ex muros distribution.

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