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A convenient method for the preparation of radioiodinated meta-iodobenzylguanidine at a no-carrier-added level

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Radioiodinated meta-iodobenzylguanidine (MIBG) in high effective specific activity was prepared using 3-tributylstannylbenzylguanidine as the precursor. The labeling was carried out in aqueous solution with the insoluble and lyophilized precursor suspended in the solvent. Simply by filtration, the starting material and by-products were readily separated from the labeled solution. Less than 1.15 ppb tin has remained in the filtrate as determined by the atom fluorescence spectrometry. By this approach, high specific activity (3.4 GBq/μmol) [^{131}I]MIBG was obtained in 72.3 ± 3% (n = 3) radiochemical yield and 97.3 ± 2% (n = 3) radiochemical purity. The whole preparation could be finished in less than 10 min. According to this method, a kit for the preparation of ^{123}I-MIBG and ^{131}I-MIBG is currently being developed.

Keywords: meta-iodobenzylguanidine; destannylation; no-carrier-added; lyophilization

Introduction

Meta-iodobenzylguanidine (MIBG) is an analogue of the adrenergic neurotransmitter norepinephrine, which shows high uptake not only in normal sympathetically innervated tissues, such as the heart and salivary glands, but also in neural crest tumors, such as neuroblastoma.[1,2] Nowadays, radioiodinated MIBG has been used extensively in the clinic either as an imaging agent for diagnosis or a therapeutic agent for tumors, especially ^{123}I-MIBG and ^{131}I-MIBG.[2–4]

At present, the radioiodinated MIBG used in the clinic is prepared on the basis of the isotopic exchange reaction between unlabeled MIBG and radioactive iodine.[1,2] However, the products synthesized this way contains large amount of unlabeled MIBG molecules, or 'cold carriers'. These unlabeled molecules would competitively inhibit the uptake of radiolabeled MIBG, greatly lower the drug's effectiveness, and might cause a side effect.

Up until now, several methods to synthesize MIBG at a no-carrier-added level have been developed with reported specific activities ranging from 9.2 to 59 GBq/μmol.[1] The most preferred precursor of no-carrier-added radioiodinated MIBG so far is 3-tributylstannylbenzylguanidine, or its derivatives for the tributylstannyl group could be easily replaced by iodine.[2] Most often, preparative HPLC would be employed during purification after labeling, which is time consuming and not readily available at most hospital radiopharmacy departments.

Many efforts have been made to avoid the use of preparative HPLC. Among these, the Hunter group has developed a method on the basis of electrophilic radioiodination reaction and solid-phase technology by using dibutylstannyl benzylguanidine precursor linked to polymers.[5] It is simple and convenient, as purification is just involving filtration. However, the synthesis of the polymer precursor is not that simple for most laboratories, and it is still not commercially available. The Donovan group has reported a highly fluorinated (fluorous) tin precursor of MIBG, and purification was easy by chemoselective filtration using a fluorous solid-phase extraction cartridge.[6] But the elution used to elute the radiolabeled MIBG from the cartridge including 80% methanol and further purification and re-formulation is required before a suitable preparation ready for injection is obtained.

We herein present a new method for the no-carrier-added MIBG synthesis using 3-tributylstannylbenzylguanidine as the precursor. The labeling reaction was carried out in aqueous solution with the lyophilized and insoluble precursor suspended in the solvent. The unreacted precursor and by-products were readily separated by filtration through a 0.22-μm membrane syringe filter. The filtrate contained only water solvent and thus, after dilution with an isotonic physiological buffer or saline, had the potential be administered intravenously into patients. The tin concentration in the filtration was less than 1.15 ppb as determined by atom fluorescence spectrometry (AFS). The whole preparation could be finished in less than 10 min with 72.3 ± 3% (n = 3) radiochemical yield and 97.3 ± 2% (n = 3) radiochemical purity.

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Materials and methods

Reagents

All chemicals were purchased from Sigma-Aldrich (St louis, Missouri, USA) unless otherwise noted. The sodium [^{131}I]iodide solution with specific activities of approximate 3.7 GBq/μmol was obtained from Chengdu Gaotong Isotope Co., Ltd (Chengdu, Chongqing province, China). Non-radioactive MIBG was synthesized according to literature procedure.[7] The 717 anion exchange resin (exchange capacity = 1.2 meq/mL) was obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Prior to use, the resin was washed three times with 20 mL of sterile water for injection per milliliter resin.

Instrumentation

The preparative HPLC was performed on a Waters (Milford, Massachusetts, USA) 2545 Binary Gradient Module equipped with a Waters 2998 Photodiode Array Detector, a Waters Xbridge prep OBD column (C18, 5 μm, 19 * 150 mm). The elution condition was as follows: solvent A = H_2O, solvent B = CH_3OH; 0–10 min 90% B; 10–15 min 90% B to 100% B; 15–30 min 100% B. The flow rate was 10 mL/min. The detection wavelength was 230 nm.

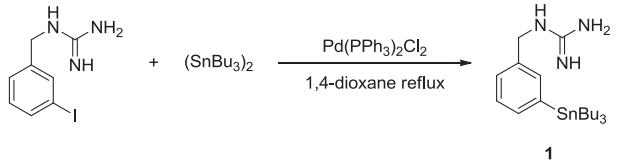
The analytical HPLC of the precursor was performed on a Waters 1525 Binary HPLC Pump equipped with a Waters 2487 Dual λ Absorbance Detector and a Waters SunFire column (C18, 5 μm, 4.6 * 150 mm). The elution condition was as follows: solvent A = H_2O (0.1%CF_3COOH), solvent B = CH_3OH; 0–10 min 90% B; 10–15 min 90% B to 100% B; 15–30 min 100% B. The flow rate was 1 mL/min. The detection wavelength was 230 nm.

The analytical HPLC of final [^{131}I]MIBG product was performed on a Waters 1525 Binary HPLC Pump equipped with a Waters 2487 Dual λ Absorbance Detector, a Perkin Elmer γ-counter, a Waters SunFire column (C18, 5 μm, 4.6 * 250 mm). The elution condition was as follows: solvent A = H_2O (0.1% H_3PO_4), solvent B = CH_3CN (0.1% H_3PO_4); 0–20 min 23% B. The flow rate was 1 mL/min. The detection wavelength was 230 nm.

NMR spectra were recorded on an Avance III 400 MHz Digital NMR spectrometer (Billerica, Massachusetts, USA). ESI mass spectra were acquired on a Waters Acquity instrument fitted with a Waters SQ Detector 2. The lyophilization was performed on a Labconco Tray Dryer (version 10417A) (Kansas, Missouri, USA). The tin concentration of the purified product was measured on an AFS-9700 atomic fluorescence photometer (Made by Haiguang Instrument corporation, Beijing, China). The 0.22-μm membrane syringe filter (13 mm Nylon 100/pk) was purchased from Dikma Technologies Inc (Beijing, China).

3-Tributylstannylbenzylguanidine (1)

A mixture of MIBG (500 mg, 1.82 mmol), bis(tributyltin) (1.27 g, 2.18 mmol), $Pd(PPh_3)_2Cl_2$ (127.6 mg, 0.18 mmol) in 30 mL 1,4-dioxane, and 10 mL dimethylformamide was reflux at 100 °C until the solution turned black. After cooling down to room temperature, the solution was filtrated and evaporated. The residues were then resolved in 50 mL methanol and washed with 20 mL hexane three times. Finally, the preparative HPLC was applied to yield light yellow liquid 576.7 mg (72.4%). ^{1}H NMR (400 M, CD_3OD) δ 0.88–0.91(m, 9H), 1.08–1.11(m, 6H), 1.32–1.37 (m, 6H), 1.55–1.58 (m, 6H), 4.39 (s, 2H), 7.24–7.26 (m, 1H), 7.33–7.37 (m, 1H), 7.41 (m, 2H); mass spectrometry (MS) (+): $[M+H]^+$ 439.4 (Scheme 1).



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Scheme 1. Synthesis of (**1**) [3-tributylstannylbenzylguanidine].

Non-radioactive (cold) iodination

Into the lyophilized mixture of 3-tributylstannylbenzylguanidine (0.05 mg, 0.114 μmol) and KH_2PO_4 (1.36 mg, 10 μmol) was added 100 μL of 0.1 mg/mL KI solution, 50 μL of a solution 0.5 mol/L in acetic acid, and 0.3 mol/L in H_2O_2, and 1 mL sterile water for injection. The mixture was reacted for 5 min at room temperature and then ended by adding 1 mL of 10 mg/mL sodium metabisulphite solution. After that, the 717 anion exchange resin (0.75 mg, 1 mL) was added into the solution for 2 min. Finally, the solution was separated from the resin and precipitations through a 0.22-μm membrane syringe filter. HPLC: retention time (RT) = 7.0 min; MS (+): $[M+H]^+$ 276.0, $[M\text{-}C_2N_3H_4]^+$ 205.0, peak at 439.4 $[\mathbf{1}+H]^+$ absent.

Preparation of [^{131}I]MIBG

Into the lyophilized mixture of 3-tributylstannylbenzylguanidine (0.05 mg, 0.114 μmol) and KH_2PO_4 (1.36 mg, 10 μmol) was added 1 mL (~0.185 GBq) of $Na^{131}I$ solution and 50 μL of a solution 0.5 mol/L in acetic acid and 0.3 mol/L in H_2O_2. The mixture was reacted for 5 min at room temperature and then ended by adding 1 mL of 10 mg/mL sodium metabisulphite solution. After that, the 717 anion exchange resin (0.75 mg, 1 mL) was added into the solution for 2 min. Finally, the solution was separated from the resin and precipitations through a 0.22-μm membrane syringe filter. The filtrate was analyzed by analytical HPLC equipped with both ultraviolet (UV) detector and γ-ray counter. The UV detector showed a small yet clearly observed peak at the RT of MIBG (RT = 7.0 min) and two larger peaks near the solvent front (RT < 3.5 min). The corresponding radioactivity trace showed one main peak at the RT of MIBG (RT = 7.0 min), which was coordinated with the UV signal and another small peak at 3.1 min, which was confirmed by coinjection to be iodine. The radiochemical purity calculated from the γ-ray signal spectrum was 97.3 ± 2% (n = 3).

Results and discussion

The precursor 3-tributylstannylbenzylguanidine could be synthesized by reaction of MIBG and bis(tributyltin) catalyzed by bis (triphenylphosphine)palladium(II) dichloride in one step.[8,9] Owing to the strong polarity of the guanidino group, the product could not be separated by normal-phase chromatography.[10,11] As a result, the preparative HPLC was employed. Reversed-phase preparative HPLC was found to give 3-butylstannylbenzylguanidine in nearly 100% percent chemical purity as determined by analytical HPLC. The identity of the product was confirmed by ^{1}H NMR and comparison with literature spectrum. The peaks at δ = 0.88–1.58, 4.39, and 7.24–7.41 coordinated with the hydrogen atoms linked in the tributyl, benzyl, phenyl groups, respectively. The corresponding integrated area ratio of the peaks was 27:2:4, which was coordinated with the numbers of hydrogen atoms linked.

The 3-tributylstannylbenzylguanidine was lyophilized into white solid powder together with KH_2PO_4. When the powder

was resolved into water during the labeling, the insoluble 3-tributylstannylbenzylguanidine was suspended in the solvent. The unreacted starting material and by-product could be easily separated by filtration through a 0.22-μm membrane. Furthermore, the lyophilized white solid powder had a looser structure, which could increase the interacting surface between 3-tributylbenzylguanidine and iodine, and further improve the chemical yield.

The H_2O_2/HOAc was chosen to be oxidant because there was no need for further purification after labeling. As compared, the chloramine-T could reach the similar radiochemical yield, but its by-product could not be easily removed. Another potential oxidant was iodogen, as it could be coated on the vial bottom as solid phase and removed easily. Furthermore, it could be added into the mixture of 3-tributylstannylbenzylguanidine and KH_2PO_4 previously and lyophilized into white solid powder altogether as a kit, and then only the sodium [^{131}I]iodine solution should be added during radiolabeling. This method would increase the labeling efficiency and feasibility in the clinic and was under evaluation.

The unreacted tin precursor and its by-products that remained in the purified product are toxic, and the tin concentration must be determined. The previous document[6] has reported a similar [*I]MIBG injection using 3-tris[2-perfluorohexylethyl]stannylbenzylguanidine as the precursor in which the tin concentration was determined to be less than 1 ppm by inductively coupled plasma–MS. In our case, the tin concentration in the purified product was determined by AFS to be under the detection limit (1.15 ppb), which is far more less than 1 ppm.

As verified, the purified product was free of the tin precursor and its by-products. The oxidant H_2O_2/HOAc was reduced to water, which needed no further purification. The purified product already had KH_2PO_4 buffer in it, and the PH was measured to be around 5. The metabisulphite was indexed in the Chinese Pharmacopeia to be used as pharmaceutical excipient. Thus, the purified product, after dilution with an isotonic physiological buffer or saline, had the potential to be administered intravenously into patients and was fit for the clinic to prepare temporarily. The toxicity, sterility, and endotoxicity of the purified product were under evaluation.

The high specific activity of no-carrier-added (nca) [*I]MIBG can reduce the total MIBG molecular amount injected, which would improve the drug's effectiveness and lower the possible side effect. The specific activity of ca [*I]MIBG and nca [*I]MIBG reported before ranged from 0.037 to 0.185 and 9.25 to 74 GBq/μmol, respectively. The specific activity of our purified product was measured by HPLC equipped with both UV detector and γ-ray counter to be 3.4 GBq/μmol. It was much higher than the previously reported ca [*I]MIBG but lower than the nca [*I]MIBG. With regard to the fact that the specific activity of the sodium [^{131}I]iodide solution we used as the starting material was merely 3.7 GBq/μmol, the relatively low specific activity of our purified product was acceptable. Once the specific activity of the sodium [^{131}I]iodide solution was improved, the specific activity of our purified product would increase accordingly.

Conclusion

A convenient method of nca [*I]MIBG synthesis in high specific activity using 3-tributylstannylbenzylguanidine as the precursor has been developed. The whole synthesis process can be finished in less than 10 min with good radiochemical yield and high radiochemical purity. Purification only includes filtration. MS and AFS data indicate that the precursor and its by-products can be readily separated from the desired product.

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Supporting information

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