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^{125}I-Labelled 2-Iodoestrone-3-sulfate: synthesis, characterization and OATP mediated transport studies in hormone dependent and independent breast cancer cells

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ABSTRACT

Introduction: Organic Anion Transporting Polypeptides (OATP) are a family of membrane associated transporters that facilitate estrone-3-sulphate (E3S) uptake by hormone dependent, post-menopausal breast cancers. We have established E3S as a potential ligand for targeting hormone dependent breast cancer cells, and in this study sought to prepare and investigate radioiodinated E3S as a tool to study the OATP system.
Methods: 2- and 4-Iodoestrone-3-sulfates were prepared from estrone *via* aromatic iodination followed by a rapid and high yielding sulfation procedure. The resulting isomers were separated by preparative HPLC and verified by ^{1}H NMR and analytical HPLC. Transport studies of 2- and 4-[^{125}I]-E3S were conducted in hormone dependent (i.e. MCF-7) and hormone independent (i.e. MDA-MB-231) breast cancer cells in the presence or absence of the specific transport inhibitor, bromosulfophthalein (BSP). Cellular localization of OATP1A2, OATP2B1, OATP3A1 and OATP4A1 were determined by immunofluorescence analysis using anti-Na^+/K^+ ATPase-α (1:100 dilution) and DAPI as plasma membrane and nuclear markers, respectively.
Results: Significantly ($p < 0.01$) higher total accumulation of 2-[^{125}I]-E3S was observed in hormone dependent MCF-7 as compared to hormone independent MDA-MB-231 breast cancer cells. In contrast 4-[^{125}I]-E3S did not show cellular accumulation in either case. The efficiency of 2-[^{125}I]-E3S transport (expressed as a ratio of V_{max}/K_m) was 2.4 times greater in the MCF-7 as compared to the MDA-MB-231 breast cancer cells. OATP1A2, OATP3A1 and OATP4A1 expression was localized in plasma membranes of MCF-7 and MDA-MB-231 cells confirming the functional role of these transporters in radioiodinated E3S cellular uptake.
Conclusion: An efficient method for the preparation of 2- and 4-[^{125}I]-E3S was developed and where the former demonstrated potential as an in vitro probe for the OATP system. The new E3S probe can be used to study the OATP system and as a platform to create radiopharmaceuticals for imaging breast cancer.

1. Introduction

Estrone-3-sulphate (E3S), a circulating inactive plasma estrogen, has been reported to serve as the predominant source for tumour estradiol in patients with post-menopausal, hormone dependent, breast cancer [1–3]. It has previously been demonstrated that the Organic Anion Transporting Polypeptides (OATPs), a family of membrane associated uptake transporters belonging to the solute carrier (SLC) superfamily, substantially contribute to specific carrier mediated E3S cellular uptake in breast cancer cells, with up to 10 times higher transport efficiency in hormone dependent (MCF-7) breast cancer cells [4]. Furthermore, the potential of E3S to serve as a novel ligand for targeting hormone dependent breast cancers was established based on tumour uptake, tumour-to-muscle and tumour-to-blood ratios of E3S, as observed in our previous pharmacokinetic and bio-distribution study conducted in mice bearing hormone dependent (MCF-7) and independent (MDA-MB-231) human breast tumour xenografts [5].

Amongst the 11 human OATP isoforms identified [6], seven (OATP1A2,1B1,1B3,1C1,2B1,3A1 and 4A1) have been reported to recognize E3S as a substrate [7,8]. OATPs transport a wide range of structurally independent, amphipathic compounds including bile salts, hormones and their conjugates, toxins and various xenobiotics [9–12]. Based upon a comparison of known substrates, Hagenbuch et al. proposed that OATP substrates are higher molecular weight (>450) compounds and

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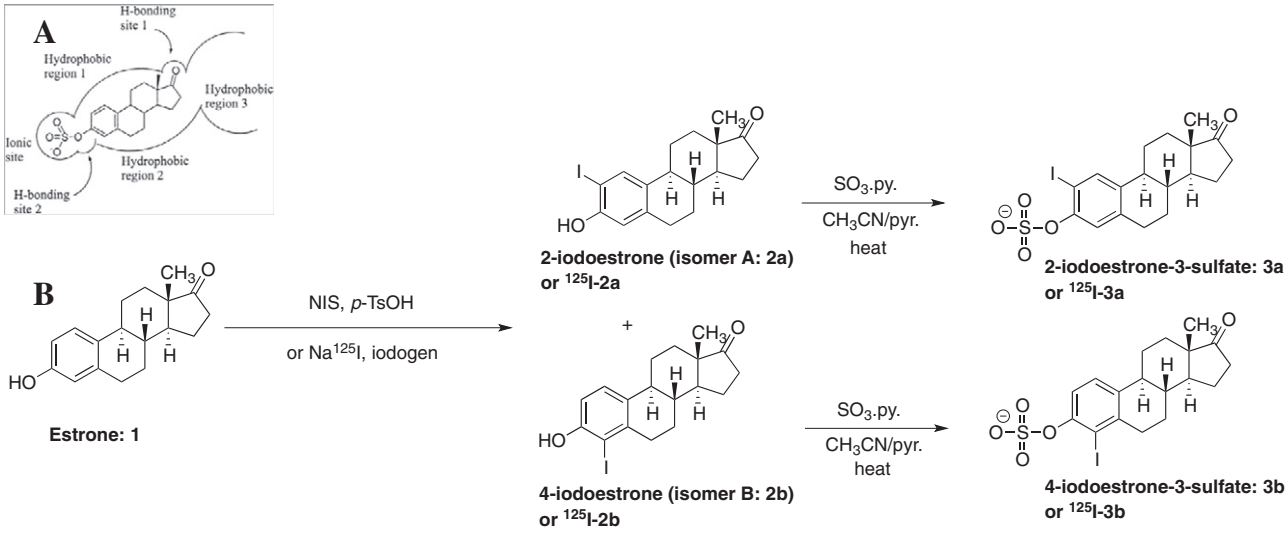

Fig. 1. Chemical structure and schematic OATP binding of E3S (A), and the synthesis scheme for radioiodinated E3S and 2- and 4-[^{125}I]-E3S (B).

emphasized that OATPs preferentially transport compounds that have a steroid nucleus (e.g. E3S) or are small linear/ cyclic peptides [10]. It was also reported that OATP substrates have three conserved hydrophobic regions, two hydrogen bonding sites and one ionic site [10,13] (Fig. 1A). Based on the nature of the known binding ligands, it was hypothesized that E3S labeled with a radionuclide at the 2 or 4-positions of the steroid could serve as an OATP substrate. Potent sterioid sulfatase inhibitors based on comparable A ring modification have been developed [14–19] including 2/4 derivatives of oestrone-3-O-sulphamate [20] (Journal of Steroid Biochemistry and Molecular Biology (1998), 64(5–6), 269–275). This suggested that it should be possible to prepare potent iodinated substrates as radiolabeled compounds to support the investigation of the OATP system.

In this study, 2-[^{125}I]-iodo-estrone-3-sulphate (2-[^{125}I]-E3S) and 4-[^{125}I]-iodo-estrone-3-sulphate (4-[^{125}I]-E3S) were synthesized, along with the corresponding non-radioactive analogues, and OATP mediated transport characterized in hormone dependent (MCF-7) and hormone independent (MDA-MB-231) human breast cancer cells. The cellular uptake and affinity kinetics (K_m and V_{max}) of the iodinated E3S compounds were compared between hormone dependent and independent breast cancer cells to determine if greater transport efficiency (V_{max}/K_m) is observed in hormone dependent breast cancers cells, as was previously observed for E3S. In order to further confirm the role of OATPs in the transport of iodinated-E3S, localization of OATP1A2, OATP2B1, OATP3A1 and OATP4A1, four predominant OATP isoforms reported to facilitate E3S cellular uptake in breast cancers [15,21–23], was assessed in both MCF-7 and MDA-MB-231 cells. The results provide key insight into the feasibility of using radioiodinated E3S as a probe for the OATP and to assess the potential use of analogous compounds labelled with iodine-123/131 or iodine-124 as radiopharmaceuticals for SPECT or PET imaging [24–26].

2. Materials and methods

2.1. Chemicals, general procedures and equipment

Chemicals were purchased from Sigma-Aldrich Canada unless otherwise stated. All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise specified. [^{125}I]NaI was obtained from McMaster Nuclear Reactor as a 1.48 GBq/mL solution in aqueous NaOH (pH 8 ~ 11). Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F-254) using UV light and an ethanolic solution of phosphomolybdic acid and cerium sulfate followed by heating as visualizing strategies. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVIII 700 MHz NMR spectrometer. Chemical shifts were calibrated using residual non-deuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, br = broad.

Analytical HPLC-MS of non-radioactive materials was performed using a Waters Acquity HPLC-MS system including a Binary Solvent Manager, Sample Manager (samples cooled to 10°C), Column Manager (column temperature 30°C), Photodiode Array Detector (monitoring at 254 nm and 214 nm), TQD with electrospray ionization and a Waters Acquity BEH C18, 2.1 × 100 mm or 2.1 × 50 (1.7 μm) column. Preparative HPLC of non-radioactive and radioactive materials and analytical HPLC of radioactive materials were performed using a Waters 1525 Binary HPLC pump, a Waters 2489 UV/Visible Detector (monitoring at 254 nm and 214 nm), Bioscan Flow Count radiodetector (FC-3300) and a Waters XBridge Prep C18 19 × 100 mm (5 μm) column (non-radioactive) or a Waters XBridge Prep C18 10 × 100 mm (5 μm) column (radioactive-preparative) or a Waters XBridge C18 4.6 × 100 mm (5 μm) column (radioactive- analytical). HPLC elution method 1: Waters XBridge Prep C18 19 × 100 mm (5 μm) column; mobile phase A: H_2O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; 0 → 10 minutes, 50% → 0%A. HPLC elution method 2: Waters XBridge Prep C18 19 × 100 mm (5 μm) column; mobile phase A: H_2O (0.4% w/v ammonium formate); mobile phase B: methanol; flow rate: 10 mL/min; 0 → 10 minutes, 80% → 30%A. HPLC elution method 3: Waters XBridge Prep C18 10 × 100 mm (5 μm) column; mobile phase A: H_2O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 5 mL/min; 50%A isocratic. HPLC elution method 4: Waters XBridge Prep C18 10 × 100 mm (5 μm) column; mobile phase A: H_2O (0.4% w/v ammonium formate); mobile phase B: methanol; flow rate: 5 mL/min; 45%A isocratic. HPLC elution method 5: Waters Acquity BEH C18 2.1 × 50 (1.7 μm) column; mobile phase A: H_2O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate = 0.3 mL/min; 0 → 8 min, 90 → 0% A; HPLC elution method 6: Waters XBridge C18 4.6 × 100 mm (5 μm) column; mobile phase A: H_2O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 1 mL/min; 0 → 10 minutes, 90% → 0%A.

*2.2. Synthesis of 2- and 4- iodoestrones (**2a** and **2b**)*

To a solution of estrone (135 mg, 0.5 mmol) in acetonitrile (10 mL) were added *N*-iodosuccinimide (115 mg, 0.5 mmol) and *p*-TsOH