J. Steroid Biochem. Molec. Biol. Vol. 39, No. 5A, pp. 729–734, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

RADIOTOXICITY OF 17α-[^{125}I]IODOVINYL-11β-METHOXYESTRADIOL IN MCF-7 HUMAN BREAST CANCER CELLS

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(Received 4 March 1991)

Summary—Therapeutic strategies for human breast cancer using ^{125}I-labeled steroid hormones are clinically attractive in light of the estrogen dependence of many human breast cancers and the favorable microdosimetry resulting from ^{125}I decay. We determined the uptake, specific estrogen receptor binding and radiotoxicity of 17α-[^{125}I]iodovinyl-11β-methoxyestradiol (^{125}IVME2) *in vitro* using cultured MCF-7 human breast carcinoma cells. ^{125}IVME2 rapidly enters MCF-7 cells and reaches a plateau in the presence of competing 10^{-7} M 17β-estradiol. In the absence of competitor, uptake is substantially greater before reaching a plateau. Efflux of ^{125}IVME2 from cells incubated in the absence of estradiol decreases to levels corresponding to specific binding. Under equilibrium conditions and in the absence of competitor, ^{125}IVME2 binds to both specific and nonspecific sites but, in the presence of excess 17β-estradiol, the observed binding is nonspecific. ^{125}IVME2 is cytotoxic to exponentially growing MCF-7 cells and produces a survival curve typical of those observed for [^{125}I]iododeoxyuridine and 16α-[^{125}I]iodoestradiol.

INTRODUCTION

Strategies using ^{125}I-labeled steroid hormones and their analogues as tumor seeking carriers for systemic radiotherapy are particularly attractive in light of the estrogen dependence of many human breast cancers [1]. The estrogen receptor (ER) is an ideal mechanism for selectively concentrating ^{125}I-labeled hormones within nuclei of target cells [2–4]. ^{125}I is one of the most promising internally emitting radionuclides because of its high yield of low energy Auger electrons and its virtual lack of associated high-energy β- and γ-radiations [3]. The exquisite radiotoxicity of ^{125}I in association with DNA is well-known [5]. Other investigators have proposed bromine-80*m*, another Auger-electron emitting radionuclide with a 4.4 h half-life, and astatine-211, an α-emitter, as potential radionuclides for systemic radiotherapy with steroid hormone carriers [6, 7].

We previously reported an unequivocal relationship between ER binding of 16α-[^{125}I]iodoestradiol (^{125}IE2) and the clonogenic survival of cultured MCF-7 human breast cancer cells [8]. At ER saturation, the dose of ^{125}IE2 necessary to reduce survival 37% was 80 disintegrations/cell and nonspecific cytotoxicity was minimal. Unfortunately, radioiodinated 17β-estradiol is a poor diagnostic imaging agent because it is cleared rapidly by the liver into the enterohepatic circulation where it is metabolized [9, 10]. The addition of an 11β-methoxy group to estradiol and related estrogens increases target selectivity of the derivatives relative to the parent compounds by reducing plasma binding and metabolism [11–17]. Estrogen derivatives with alkyl derivatives at the 17α-position have increased target organ activity and retention because steric hindrance decreases catabolism [14]. Furthermore, the 17α-iodovinyl derivatives of estradiol appear to be more stable than the corresponding acetylenic derivatives [18, 19].

We have prepared no-carrier-added 17α - [^{125}I]iodovinyl - 11β - methoxyestradiol (^{125}IVME2) and investigated its uptake, binding and radiotoxicity in cultured MCF-7 human breast cancer cells. Other investigators have shown that ^{125}IVME2 binds specifically and with

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high-affinity cytosolic ER in MCF-7 cells [20]. Although investigations *in vivo* have demonstrated high uterus/blood and uterus/muscle ratios, little is known about its *in vitro* and *in vivo* radiotoxicity [20–23].

EXPERIMENTAL

Chemicals

The synthesis of ^{125}IVME2 from moxestrol was performed similarly to the procedure described by Hochberg *et al.* [24] for the synthesis of [^{125}I]iodovinyl-19-nortestosterone. A mixture of 50 mg moxestrol and 50 mg tri-*n*-butyltin hydride in dry tetrahydrofuran (THF) was heated at reflux in an atmosphere of argon for 24 h. The mixture was cooled to room temperature, treated with ice-cold water and the organic material isolated with ethyl acetate. Purification of the product by preparative layer chromatography on silica gel using a hexane–ethyl acetate mixture (4:1) as the developing solvent afforded 7 mg pure crystalline E-17α-(2-tri-*n*-butylstannylvinyl)-11β-methoxyestradiol. This was radiolabeled by iododestannylation [25] by mixing 100 μg of the tributylstannyl derivative in 100 μl THF, 100 μl 5% sodium acetate in glacial acetic acid and 6 mCi Na^{125}I (Amersham). This solution was treated with 100 μl 30% hydrogen peroxide–glacial acetic acid mixture (2:1) (prepared in the cold 24 h before use) in a reactivial containing a magnetic stir-bar. The vial was stoppered and the mixture stirred at room temperature for 30 min. Water (2 ml) was added to the reaction mixture and the organic product isolated with ethyl acetate. The organic layer was washed with a solution of 5% sodium metabisulfite in water followed by evaporation of the solvent. The product was purified using a reverse phase 7.8 mm × 30 cm C-18*u*-NOVA-PAK HPLC column (Waters Chromatography) and methanol–water solvent (80:20, v/v) at a flow rate of 1.0 ml/min. The major radioactive peak (^{125}IVME2), eluted at 18–20 min, was evaporated and dissolved in 1 ml of reagent alcohol. The theoretical specific activity of ^{125}IVME2 is 2200 Ci/mmol.

Cell cultures

Stock monolayer cultures of MCF-7 cells (Michigan Cancer Foundation) were maintained by weekly passage in RPMI-1640 medium (Gibco Laboratories) supplemented with 2 ml glutamine, 10 μg of penicillin/ml, 10 μg of streptomycin/ml, 1% nonessential amino acids, 10 mM HEPES buffer, 1 mM sodium pyruvate and 5% Calf Supreme (Gibco Laboratories). Cell cultures were harvested using Dispase (Boehringer Mannheim), collected in 50 ml centrifuge tubes, and washed twice in media by centrifugation at 450 *g* for 10 min. Cell aggregates were disrupted by passing the suspension through a 10 ml syringe with a 21 G needle 5 times. Single-cell suspensions prepared in this manner were used for uptake and binding studies and assessment of radiolabeled hormone cytotoxicity. The cells were allowed to incubate for 3 h at 37°C to allow recovery from the harvesting procedure.

Specific binding

Cellular uptake and specific binding of ^{125}IVME2 in the presence or absence of excess nonradioactive 17β-estradiol (Steraloids) were determined with the oil microcentrifuge assay [26]. Uptake was determined by combining a 5 ml suspension of 5×10^5 cells with 5 ml culture medium containing 2 μCi/ml of ^{125}IVME2 (that concentration of ^{125}IVME2 which saturates ER sites) in the presence or absence of 1×10^{-7} M 17β-estradiol. Triplicate 100 μl aliquots were sampled over 1 h and assayed by the oil-microcentrifuge method. For efflux measurements, tubes were incubated with 2 μCi/ml ^{125}IVME2 in the presence or absence of 1×10^{-7} M 17β-estradiol for 2 h at 37°C. Triplicate 100 μl aliquots were assayed to determine saturation binding levels. Tubes were then centrifuged for 10 min at 900 *g*, and the supernatant was replaced with an equal volume of medium (1:1000 dilution). The suspension was agitated gently in a vortex mixer and passed through a 5 ml pipette 5 times to disrupt cell aggregates. Three replicate 100 μl samples were assayed at varying times over the subsequent 2 h.

We determined the equilibrium binding of ^{125}IVME2 by placing 2×10^5 cells in 0.5 ml culture medium containing 0.05–2.0 μCi/ml ^{125}IVME2 with or without 1×10^{-7} M nonradioactive 17β-estradiol. After a 2 h incubation at 37°C, suspensions were chilled in crushed ice and assayed by the oil-microcentrifuge method [26]. Subtraction of nonspecific binding from total binding yields the specific binding of ^{125}IVME2. Using the back extrapolate of the plateau to the ordinate and the known specific activity of ^{125}IVME2, we calculated the number of ER/cell.

Radiotoxicity

The radiotoxicity of ^{125}IVME2 was determined in the presence or absence of nonradioactive 17β-estradiol with a standard colony-forming assay [27]. MCF-7 cells were plated in four-well Limbro culture dishes (Flow Laboratories) at 1500 cells/well until they were adherent (18 h); they were then exposed to graded doses of ^{125}IVME2 in the presence or absence of 1×10^{-7} M 17β-estradiol under equilibrium conditions for 24 h at 37°C. The culture medium was then aspirated, the plates were washed, and fresh medium was added. Culture medium was replaced twice a week until colonies formed. Colonies were fixed and stained; only those with >50 cells were scored.

RESULTS

Uptake of ^{125}IVME2 by MCF-7 cells, which is very rapid in the presence of excess nonradioactive 17β-estradiol, reaches a plateau by 4 min and represents nonspecific binding of ^{125}IVME2 (Fig. 1). In the absence of competing 17β-estradiol, uptake is initially rapid and then dampened for 30 min at which time binding levels off. The difference between the two curves represents specific ER binding. Efflux of ^{125}IVME2 in the presence of competing ligand is rapid with background levels reached in 10 min (Fig. 2). In the absence of competitor, efflux of ^{125}IVME2 levels off after 40 min. This plateau represents residual specific ER binding.

Equilibrium binding was determined as a function of medium concentration. Cell binding

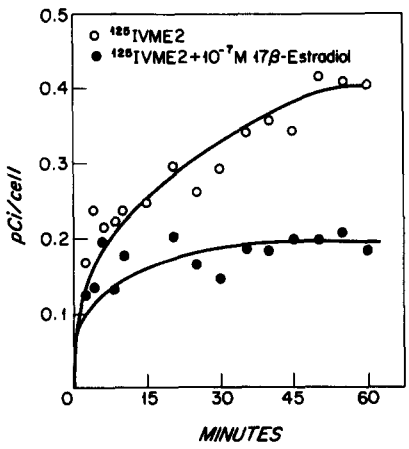

Fig. 1. ^{125}IVME2 uptake by 5×10^5 MCF-7 cells at 37°C in the presence or absence of 1×10^{-7} M 17β-estradiol.

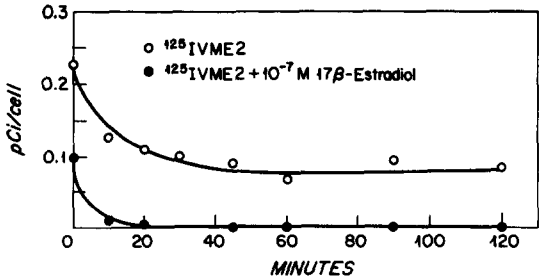

Fig. 2. Efflux of ^{125}IVME2 in the presence or absence of 1×10^{-7} M 17β-estradiol from 5×10^5 MCF-7 cells exposed for 2 h at 37°C.

in the absence of nonradioactive 17β-estradiol represents total cellular binding to ER (specific as well as nonspecific sites). In the presence of competing ligands, any observed binding is nonspecific. Specific binding is the difference between total cellular binding and nonspecific binding. Specific binding was saturated at 0.18 pCi/cell (Fig. 3). The back extrapolate of this plateau to the ordinate, when converted for the specific activity of ^{125}IVME2, corresponds to 4.9×10^4 ER/cell.

A colony-forming assay was used to determine the clonogenic survival of MCF-7 cells exposed to graded doses of ^{125}IVME2 in the presence or absence of nonradioactive 17β-estradiol (Fig. 4). The surviving fraction is expressed as a function of cellular radioactivity which was calculated using the data from Fig. 2 and the specific activity of ^{125}IVME2 (2200 Ci/mmol). ^{125}IVME2 by itself is very toxic at low doses where the ER is not saturated. At higher doses where the ER is saturated, radiotoxicity is nonspecific and much less pronounced. The radiotoxicity observed at low

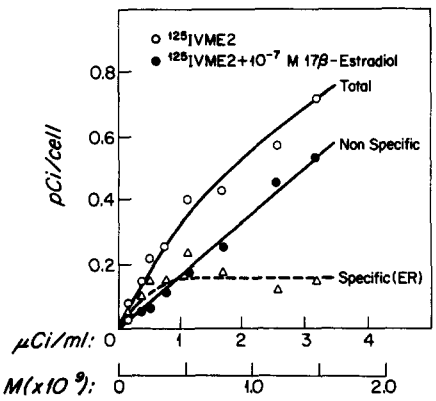

Fig. 3. Equilibrium binding of ^{125}IVME2 in the presence or absence of 1×10^{-7} M 17β-estradiol. MCF-7 cells (2×10^5) were exposed to varying doses of ^{125}IVME2 for 2 h at 37°C. Total binding is measured in the presence of carrier-free ^{125}IVME2. Nonspecific binding is measured in the presence of ^{125}IVME2 plus 1×10^7 M 17β-estradiol.

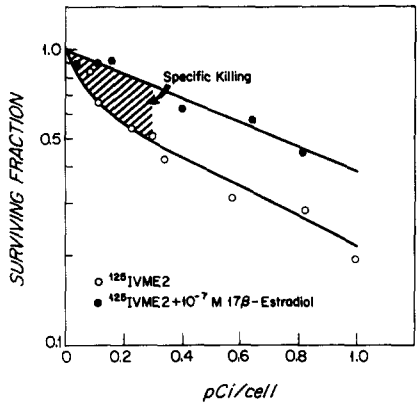

Fig. 4. *In vitro* cytotoxicity of ^{125}IVME2 on MCF-7 cells. MCF-7 cells were exposed to varying doses of ^{125}IVME2 for 24 h at 37°C in the presence or absence of 1×10^{7} M 17β-estradiol. Survival was determined by colony formation and was independent of cell plating density.

doses is similar to that of nuclear-bound 5-[^{125}I]iododeoxyuridine (^{125}IUdR) [27], while the high dose radiotoxicity bears a resemblance to survival curves for membrane-bound ^{125}I [28]. When cells were exposed to ^{125}IVME2 in the presence of 1×10^{-7} M nonradioactive 17β-estradiol, the survival curve was linear with the slope paralleling that of cells exposed to high doses of ^{125}IVME2. The separation between these two curves represents specific ER-mediated cytotoxicity. Cytotoxicity beyond the dose when there is no further increase in ER specific cell killing (0.3 μCi/ml) is nonspecific. No cytotoxicity was observed when cells are exposed to 10^{-7} M 17β-estradiol alone. Heterogeneity of ER expression by MCF-7 cells is the most probable reason for the incomplete sensitivity of MCF-7 cells to ^{125}IVME2. We have shown previously that 1×10^{-7} M 17β-estradiol and the nonradioactive counterparts of carrier-free ^{125}I-labeled ER ligands are nontoxic under these conditions [8].

DISCUSSION

By virtue of their interactions with specific nuclear receptors, steroid hormones are ideally suited to be selective tumor carriers for systemic radiotherapy using ^{125}I. The numerous Auger electrons associated with ^{125}I decay are extremely cytotoxic, but only when released in close proximity to the genome [28]. Unfortunately, the design of ^{125}I radiopharmaceuticals which localize specifically within the cell nucleus affords limited options.

Many of the physical and biological requirements for treating cancer with internally emitting radionuclides are met by ^{125}IUdR, a thymidine analogue readily incorporated into DNA. No-carrier-added ^{125}IUdR can be administered by i.p. injection to mice bearing ascites tumor cells in effective therapeutic doses without producing overt signs of normal tissue toxicity [29, 30]. Unfortunately, effective systemic administration is hampered by rapid metabolic degradation and dose-limiting systemic toxicity to the bone marrow and gastrointestinal tract [31, 32].

We previously reported the radiotoxicity of ^{125}IE2 and [^{125}I]tamoxifen, an antiestrogen, in cultured MCF-7 cells. Radiotoxicity is related to ER content and physiology and, in the case of ^{125}I tamoxifen, is a complex function of ER and antiestrogen binding sites and nonspecific binding [8, 33–35]. These studies demonstrate that uptake, efflux, and saturation binding are similar for both ^{125}IVME2 and ^{125}IE2.

Clinical results in patients injected with ^{125}IE2 have yielded positive tumor scans [36]. Unfortunately, high levels of hepatic activity may preclude effective therapy if the radiolabeled estrogen must be i.v. injected. On the other hand, many ovarian cancers are ER-positive and the disease has a high proclivity to disseminate within the abdominal cavity. In such cases, i.p. injections could be used, thereby preventing the first pass uptake by the liver which occurs after i.v. injections. Optimization of dose fractionation schedules to overcome cell cycle changes in ER expression and tumor heterogeneity and the use of more metabolically stable estrogens may allow successful treatment of i.p. ER-positive ovarian tumors with radiolabeled estrogens.

Acknowledgements—We thank Anna Miller for technical assistance. This work was supported by USPHS Grant CA37553 from the National Cancer Institute, DHHS, and by the Claude Worthington Benedum Foundation.

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