The Role of 17β-Hydroxysteroid Dehydrogenases in Modulating the Activity of 2-Methoxyestradiol in Breast Cancer Cells

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Abstract

The bis-sulfamoylated derivative of 2-methoxyestradiol (2-MeOE2), 2-methoxyestradiol-3,17-O,O-bis-sulfamate (2-MeOEtbisMATE), has shown potent antiproliferative and antiangiogenic activity in vitro and inhibits tumor growth in vivo. 2-MeOEtbisMATE is bioavailable, in contrast to 2-MeOE2 that has poor bioavailability. In this study, we have examined the role of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 2 in the metabolism of 2-MeOE2. In MDA-MB-231 cells, which express high levels of 17β-HSD type 2, and in MCF-7 cells transfected with 17β-HSD type 2, high-performance liquid chromatography analysis showed that a significant proportion of 2-MeOE2 was metabolized to inactive 2-methoxyestrone. Furthermore, MCF-7 cells transfected with 17β-HSD type 2 were protected from the cytotoxic effects of 2-MeOE2. In contrast, no significant metabolism of 2-MeOE2bisMATE was detected in transfected cells and 17β-HSD type 2 transfection did not offer protection against 2-MeOEtbisMATE cytotoxicity. This study may go some way to explaining the poor bioavailability of 2-MeOE2, as the gastrointestinal mucosa expresses high levels of 17β-HSD type 2. In addition, this study shows the value of synthesizing sulfamoylated derivatives of 2-MeOE2 with C17-position modifications as these compounds have improved bioavailability and potency both in vitro and in vivo. (Cancer Res 2006; 66(1): 324-30)

Introduction

Estrogens are thought to have a role in the promotion and progression of hormone-dependent tumors. Levels of estrogens within these tumors are higher than in the normal breast tissue and the peripheral circulation (1, 2), indicating that these hormones may be synthesized in situ. These metabolic pathways are catalyzed by several enzymes, among which steroid sulfatase, which catalyzes the biotransformation of estrone sulfate to estrone (E1), and the 17β-hydroxysteroid dehydrogenases (17β-HSD), which catalyze the interconversion of the weak estrogen E1 and the biologically active estrogen estradiol (E2), are important. 17β-HSD type 1, using the reduced form of the cofactor NADPH, catalyzes the reduction of E1 to E2. Conversely, 17β-HSD type 2 requires the cofactor NAD and catalyzes the oxidation of E2 to E1 (3). Studies using primary cultures of cells derived from breast tumors have shown an increased level of expression of 17β-HSD type 1 compared with that in cells derived from normal tissue (4). Furthermore, in a case-control study of patients diagnosed with breast cancer, increased expression of 17β-HSD type 1 correlated with an increased risk of relapse (5).

2-Methoxyestradiol (2-MeOE2; Fig. 1, 2) is generated in vivo by hydroxylation and subsequent methylation of E2. 2-MeOE2 has been shown to be an antiangiogenic and antiproliferative agent in vitro (6, 7). 2-MeOE2 also inhibits the in vivo growth of xenografts derived from human breast MDA-MB-435 carcinoma cells, Meth A sarcomas, B16 melanomas, and the multiple myeloma cell line KAS-6/1 in immunodeficient mice. However, comparatively high p.o. (75 mg/kg/d) or i.p. (150 mg/kg/d) doses of 2-MeOE2 are necessary to reduce the growth of breast or myeloma tumors, respectively (8, 9). In a recent study, we administered 2-MeOE2 (10 mg/kg) either p.o. or i.v. to female rats to investigate the metabolism of the agent in vivo (10). 2-MeOE2 was below the limit of detection in plasma following p.o. dosing and was rapidly removed from the plasma following administration of a single i.v. dose, which suggested that the agent was undergoing rapid biotransformation in vivo. In contrast, the antiangiogenic/antitumor agent 2-methoxyestradiol-3,17-O,O-bis-sulfamate (2-MeOE2bisMATE; Fig. 1, 3; refs. 11–17) could still be detected in plasma 24 hours after a single p.o. dose (10).

The difference between the plasma levels of 2-MeOE2 and 2-MeOE2bisMATE in both human and rodent may be a result of the differing metabolic fate of these compounds. In the present study, we test the hypothesis that 2-MeOE2 is metabolized and inactivated by 17β-HSD enzymes in estrogen receptor (ER)–positive (MCF-7) and ER-negative (MDA-MB-231) breast cancer cells. Furthermore, using both radiometric enzyme assays and analysis of the metabolism of unlabeled substrates by high-performance liquid chromatography (HPLC), we measured the interconversion of E1 and E2 in wild-type and transfected MCF-7 and MDA-MB-231 cells. Finally, we examined the metabolism of 2-MeOE2bisMATE, a compound expected to be resistant to 17β-HSD metabolism due to a sulfamate moiety at the C17 position. These studies will provide further information on the metabolic fate of 2-MeOE2 and identify new antitumor compounds, such as 2-MeOE2bisMATE, which are more potent in vitro and in vivo than 2-MeOE2.

Materials and Methods

Drug synthesis. The syntheses of 2-methoxyestrone (2-MeOE1; Fig. 1, 1) and 2-MeOE2 (Fig. 1, 2) have been reported (18). 2-MeOE2bisMATE (Fig. 1, 3) was synthesized by reaction of 2-MeOE2 with excess sulfamoyl chloride in dimethyl acetamide. 2-Methoxyestradiol-17-O-sulfamate (2-MeOE2-17MATE; Fig. 1, 4) was synthesized from 2-MeOE2 in three steps by first benzylating the 3-hydroxyl group, then sulfamoylating the 17-hydroxyl...
unlabeled product (50 µg) for visualization, and the diethyl ether phase was evaporated to dryness. The extracted steroids were resuspended in diethyl ether and spotted onto TLC plates/silica gel 60 F254 (Merck, West Drayton, United Kingdom). The TLC plates were developed using dichloromethane/ethyl acetate (4:1 v/v) as the solvent system. UV light (254 nm) was used to visualize the positions of the E1 and E2; the areas corresponding to the product were cut out, placed in scintillation vials, and the product was eluted in methanol (0.5 mL) before the addition of scintillation fluid (10 mL). Product and recovery radioactivities were counted using a liquid scintillation counter.

In vitro metabolism of unlabeled steroids. Twenty-four hours after transfection, cells were incubated with either vehicle (THF) or 10 µmol/L of E2, E1, 2-MeOE2, 2-MeOE1, or 2-MeOE2bisMATE in medium for 48 hours. The compounds were also incubated with medium alone. After incubation, cells were removed from the flask by addition of trypsin. 17β-Epistosterone was used as an internal standard. Medium (3 mL) and cells were combined and extracted twice with diethyl ether (4 mL) and the lower aqueous layer frozen in a methanol/solid carbon dioxide mixture. The upper organic phase from each extraction was decanted, combined, and evaporated to dryness under a stream of air at room temperature. The residues were stored at −20°C until they were analyzed by HPLC.

To confirm that 2-MeOE1 had been generated from 2-MeOE2, the residue was reconstituted in 58% methanol and 0.02 mol/L ammonium sulfate (500 µL) and incubated with an excess of sodium borohydride for 1 hour at room temperature. 2-MeOE2 and 2-MeOE1 were extracted from the reaction mixture with diethyl ether (4 mL) and the organic phase was evaporated to dryness. HPLC analysis was done as described by Ireson et al. (10). Briefly, 2-MeOE2bisMATE was separated from 2-MeOE2, 2-MeOE1, and 2-methoxyestradiol-17-β-sulfamate (2-MeOE2bisMATE; Fig. 1, f) using a reversed-phase HPLC method. Extracted samples were reconstituted in mobile phase and injected on to a C3-phenyl column (250×5 mm, 5 µmol/L) purchased from Phenomenex (Cheshire, United Kingdom). The agents were separated from other medium components using an isotropic mobile phase consisting of 58% methanol in 0.02 mol/L ammonium sulfate containing 1 mol/L sodium azide. Analysis was done with a photodiode array detector set at 285 nm.

Statistics. All experiments were done in triplicate and data presented are representative of one of three such experiments, all errors shown are the mean ± SD. Student’s t test was used to assess the significance of the differences in cell proliferation.

Results

17β-HSD activity in MCF-7 and MDA-MB-231 cells. MCF-7 (ER positive) breast cancer cells have relatively low endogenous 17β-HSD reductive (type 1) and oxidative (type 2) activities (20, 21), and are, therefore, a good model into which to transf ect and study 17β-HSD types 1 and 2 enzyme activities. The 17β-HSD type 1 activity was 12 fmol E2/2/h/106 cells and the corresponding 17β-HSD type 2 activity was 10 fmol E1/1/h/106 cells following mock transfection. Seventy-two hours after transf ecting MCF-7 cells with the 17β-HSD type 1 cDNA, the type 1 activity was 2,817 fmol E2/h/106 cells, a 235-fold increase relative to mock-transfected cells. After transf ection with the cDNA for 17β-HSD type 2, the type 2 activity increased 51-fold relative to mock-transfected cells to 512 fmol E1/h/106 cells.

MDA-MB-231 breast cancer cells have a greater 17β-HSD oxidative activity than 17β-HSD reductive activity (20, 21), and are, thus, a good model to study metabolism by endogenous 17β-HSD type 2. Following mock transf ection, MDA-MB-231 cells 17β-HSD enzyme activities were 11 and 54 fmol product/h/106 cells for types 1 and 2, respectively. The type 1 activity increased over 11-fold to 128 fmol E2/h/106 cells 72 hours after transf ection with the cDNA for 17β-HSD type 1. MDA-MB-231 cells were not transfected with cDNA for 17β-HSD type 2 as they already express high levels of this enzyme.
E1 and E2 metabolism. In addition to measuring enzyme activities with labeled substrates, the metabolism of E1 and E2 by MCF-7 cells was also examined using unlabeled substrates and subsequent HPLC analysis in wild-type and transfected cells. No discernable peaks were detected (Fig. 2A) in mock-transfected MCF-7 cells to which vehicle (THF) was added. In mock-transfected cells incubated with E2 (10 μmol/L), a small peak was detected that eluted with the same Rt as the E1 standard (Fig. 2B, peak 2). In contrast, as shown in Fig. 2C, metabolism in cells transfected with the cDNA for 17β-HSD type 2 resulted in the oxidation of E2 to E1. Similar experiments with the cDNA for 17β-HSD type 1 revealed that E1 was almost completely reduced to E2. The results from this series of experiments are quantified in Table 1 and clearly show that whereas wild-type MCF-7 cells have low intrinsic reductive and oxidative activity, transfection with the cDNAs for 17β-HSD type 1 or 2 greatly enhances their ability to activate E1 to E2 or inactivate E2 by conversion to E1.

2-MeOE1 and 2-MeOE2 metabolism. As 17β-HSD types 1 and 2 interconvert E1 and E2, the ability of these enzymes to reduce and oxidize 2-MeOE1 and 2-MeOE2, respectively, was examined using unlabeled substrates and subsequent HPLC analysis. In mock-transfected MCF-7 cells incubated with 2-MeOE2 (10 μmol/L), a small peak was detected (Fig. 3A, peak 2), which eluted with the same Rt as the 2-MeOE1 standard. Following transfection with the cDNA for 17β-HSD type 2, 2-MeOE2 was below the limit of detection and oxidized to 2-MeOE1 (Fig. 3B, peak 2). Similar experiments with the cDNA for 17β-HSD type 1 revealed that 2-MeOE1 was reduced to 2-MeOE2. The results from these series of experiments are quantified in Table 2.

We have shown that the breast cancer cell line MDA-MB-231 has endogenously high levels of 17β-HSD type 2 enzyme activity relative to 17β-HSD type 1 enzyme activity and is, therefore, a good model to look at the oxidation and inactivation of 2-MeOE2. No discernable peaks were detected in wild-type mock-transfected MDA-MB-231 cells to which vehicle (THF) was added (Fig. 3C). A significant peak was detected (Fig. 3D, peak 2) in wild-type cells incubated with 2-MeOE2 (10 μmol/L), which eluted with the same Rt as the 2-MeOE1 standard. Table 2 shows that 72% of the 2-MeOE2 was oxidized to the biologically inactive 2-MeOE1. When these cells were transfected with the 17β-HSD type 1 cDNA, 2-MeOE2 was protected from oxidation and only 44% was oxidized to the inactive 2-MeOE1 (Table 2).

2-MeOE2bisMATE metabolism. We have previously shown that bis-sulfamoylation of 2-MeOE2 to generate 2-MeOE2bisMATE offers considerable advantages by increasing both the potency and bioavailability of this class of compound. Due to the presence of the sulfamate group at the C17 position, it was postulated that this compound would not be a substrate for either 17β-HSD type 1 or 2. To test this hypothesis, MCF-7 cells were transfected with either 17β-HSD type 1 or 2 and incubated with 2-MeOE2bisMATE (10 μmol/L) for 48 hours. Figure 4 shows that the metabolism of 2-MeOE2bisMATE is identical in mock-, 17β-HSD type 1–, and 17β-HSD type 2–transfected cells, with a significant peak eluting at the same Rt as 2-MeOE2bisMATE, indicating that this compound is resistant to metabolism in MCF-7 cells. A small peak was also detected in extracts from all three transfections, which elutes at the same Rt as 2-MeOE2-17MATE (Fig. 1, 4), a breakdown product of 2-MeOE2bisMATE. In the absence of cells, in medium-only control experiments the proportion of the breakdown product 2-MeOE2-17MATE doubles relative to the parent compound 2-MeOE2bisMATE (data not shown).

Cell proliferation. Observation of mock-transfected MCF-7 cells incubated with 2-MeOE2 (10 μmol/L) for 48 hours indicated that cell proliferation was inhibited. A large number of apoptotic-like cells were also observed relative to vehicle-treated cells (Fig. 5A and B). However, MCF-7 cells transfected with 17β-HSD type 2; 24 hours before incubation with 2-MeOE2 (10 μmol/L), seemed to be resistant to the inhibition of cell proliferation and induction of cell death by 2-MeOE2 (Fig. 5C). In contrast, transfection with 17β-HSD type 2 offered no apparent protection from the inhibition of cell proliferation and induction of cell death by 2-MeOE2bisMATE (Fig. 5D and E). Cell proliferation was quantified to confirm these observations. The addition of 2-MeOE2 (10 μmol/L) to MCF-7 cells resulted in a 60% reduction in cell proliferation in both mock-transfected and 17β-HSD type 1–transfected MCF-7 cells (Fig. 6). However, in MCF-7 cells transfected with 17β-HSD type 2, there was only a 16% inhibition of proliferation relative to mock-transfected cells. Treatment of mock-transfected and 17β-HSD type 2–transfected MCF-7 cells with 2-MeOE1 (10 μmol/L) had no significant effect on proliferation with respect to untreated cells. However, in 17β-HSD type 1–transfected cells, a 57% reduction in cell proliferation was measured after incubation with 2-MeOE1 (10 μmol/L; Fig. 6).

Table 1. Metabolism of E2 and E1 in MCF-7 cells transfected with 17β-HSD type 1 or 2

<table>
<thead>
<tr>
<th>Transfection</th>
<th>E2 to E1</th>
<th>E1 to E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>10 ± 2</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>17β-HSD type 1</td>
<td>8 ± 3</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>17β-HSD type 2</td>
<td>98 ± 3</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

NOTE: Values are expressed as a percentage of total steroid ± SD (n = 3).

Figure 2. High-performance chromatograms of extracted medium and cells showing the metabolism of E2 in MCF-7 cells mock-transfected or transfected with 17β-HSD type 2. A, mock-transfected MCF-7 cells were incubated with vehicle (THF) for 48 hours. B, mock-transfected MCF-7 cells were incubated with E2 (10 μmol/L) for 48 hours. C, MCF-7 cells transfected with 17β-HSD type 2 were incubated with E2 (10 μmol/L) for 48 hours. Peaks 1 and 2 were identified as E2 and E1, respectively, by cochromatography with authentic standards (I.S., internal standard, 17β-epitestosterone). AU, arbitrary unit.
Treatment of MCF-7 cells with 2-MeOE2bisMATE (10 μmol/L) resulted in an 85% inhibition of cell proliferation. Transfection with either 17β-HSD type 1 or 2 had no significant effect on the inhibition of proliferation by 2-MeOE2bisMATE (10 μmol/L; Fig. 7).

**Discussion**

In this study, we used two breast cancer cell lines to explore the roles of the reductive and oxidative 17β-HSD enzymes in the interconversion of E1 and E2, and in the metabolic fate of the anticancer agent 2-MeOE2. The ER-positive MCF-7 cells have both low 17β-HSD types 1 and 2 activities and as such provide a suitable model into which to transfect either 17α-HSD type 1 or 2. To look at endogenous 17β-HSD type 2 activity, we used the ER-negative MDA-MB-231 cells, which have higher oxidative than reductive 17β-HSD activity (20, 21). In this study, we showed that MCF-7 cells only converted 8% of the added E1 to E2. This is not surprising, as when assayed both the 17β-HSD types 1 and 2 enzyme activities were low and approximately equal. This result suggests that in vivo ER-positive tumors, which lack the pathways to synthesize E2 themselves, may be reliant on the peripheral tissue to generate the E2 required for their growth.

In addition to their role in E2 metabolism in tumors, the 17β-HSD enzymes have an important role to play in the metabolism of other steroids, and in addition may affect the bioavailability of steroid-based drugs. The natural metabolite of E2, 2-MeOE2, is generated in vivo from 2-hydroxyestradiol by catechol-O-methyl transferase, an enzyme that is expressed in a wide range of mammalian tissues (23). 2-MeOE2 has been shown to be an antiproliferative and antiangiogenic agent in vitro and to inhibit tumor growth in vivo and is currently in phase I/II trials for breast cancer (24, 25). In this study, we showed that 2-MeOE2 is oxidized to the inactive metabolite 2-MeOE1 (26) by MCF-7 cells transfected with the 17β-HSD type 2 enzyme. After just 48 hours, 95% of the added 2-MeOE2 was metabolized to 2-MeOE1. Wild-type MDA-MB-231 cells have a 5-fold greater oxidative 17β-HSD activity than reductive activity and were able to inactivate 72% of the 2-MeOE2 after 48 hours. This result suggested that in hormone-independent tumors, in which 17β-HSD type 2 is the prominent activity (27), the anticancer agent 2-MeOE2 would be rapidly inactivated. This hypothesis is supported by

![Figure 3](image1.png)

**Figure 3.** High-performance chromatograms of extracted medium and cells showing the metabolism of 2-MeOE2 in MCF-7 and MDA-MB-231 cells. A, mock-transfected MCF-7 cells were incubated with 2-MeOE2 (10 μmol/L) for 48 hours. B, MCF-7 cells transfected with 17β-HSD type 2, 24 hours before incubation with 2-MeOE2 (10 μmol/L) for 48 hours. C, wild-type MDA-MB-231 cells were incubated with vehicle (THF) for 48 hours. D, wild-type MDA-MB-231 cells were incubated with 10 μmol/L 2-MeOE2 for 48 hours. Peaks 1 and 2 were identified as 2-MeOE2 and 2-MeOE1, respectively, by cochromatography with authentic standards (I.S, internal standard, 7β-epitestosterone).

![Figure 4](image2.png)

**Figure 4.** High-performance chromatograms of extracted medium and cells showing the metabolism of 2-MeOE2bisMATE by 17β-HSD types 1 and 2. A, mock-transfected cells were incubated with 2-MeOE2bisMATE (10 μmol/L) for 48 hours. B, MCF-7 cells were transfected with 17β-HSD type 1, 24 hours before incubation with 2-MeOE2bisMATE (10 μmol/L) for 48 hours. C, MCF-7 cells were transfected with 17β-HSD type 2, 24 hours before incubation with 2-MeOE2bisMATE (10 μmol/L) for 48 hours. Peaks 3 and 4 were identified as 2-MeOE2bisMATE and 2-MeOE2-17MATE, respectively, by cochromatography with authentic standards (I.S, internal standard, 7β-epitestosterone).

### Table 2. Metabolism of 2-MeOE2 and 2-MeOE1 in MCF-7 and MDA-MB-231 cells transfected with 17β-HSD type 1 or 2

<table>
<thead>
<tr>
<th>Transfection</th>
<th>2-MeOE2 to 2-MeOE1</th>
<th>2-MeOE1 to 2-MeOE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 wild type</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>MCF-7 17β-HSD type 1</td>
<td>ND</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>MCF-7 17β-HSD type 2</td>
<td>95 ± 2</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>MDA-MB-231 wild type</td>
<td>72 ± 4</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>MDA-MB-231 17β-HSD type 1</td>
<td>44 ± 2</td>
<td>56 ± 0</td>
</tr>
</tbody>
</table>

**Note:** Values are expressed as a percentage of total drug ± SD (n = 3). Abbreviation: ND, not detectable.
research from our group (12), which showed that although C17-
position modified 2-MeOE2 derivatives were potent inhibitors of
MDA-MB-231 proliferation (IC_{50} < 0.40 \mu mol/L), 2-MeOE2 was a
relatively weak inhibitor with an IC_{50} in excess of 4.0 \mu mol/L.

The inactivation of 2-MeOE2 in MDA-MB-231 cells was partially
reversed by overexpressing the 17β-HSD type 1 enzyme. This
increased the reductive metabolism within the cell. MCF-7 cells
transfected with 17β-HSD type 1 were able to convert 95% of added
2-MeOE1 to the active 2-MeOE2. These two results indicated that in
tumors with a greater reductive 17β-HSD activity, 2-MeOE2 may
not be metabolized to 2-MeOE1 and any circulating unconjugated
2-MeOE1 could be reduced to the active 2-MeOE2. Further evidence
showing the vital balance between oxidative and reductive
17β-HSD activities, in relation with the efficacy of 2-MeOE2, was
the observation that MCF-7 cells overexpressing 17β-HSD type 2
were significantly protected from the cytotoxic effects of 2-MeOE2.
Conversely, proliferation of MCF-7 cells overexpressing type 1 was
inhibited by treatment with the inactive metabolite 2-MeOE1 (26).

In both breast and myeloma xenograft models in vivo, 2-MeOE2
has successfully inhibited tumor growth, although in both cases
relatively high doses of 2-MeOE2 were required, 75 and 150 mg/kg/d,
respectively (8, 9). In phase I trials, a clinical benefit was shown in
only two patients who were receiving 1,600 to 3,200 mg/d of
2-MeOE2; further dose escalations of up to 6,000 mg/d are planned
(28). The ability of some tumor cell lines to inactivate 2-MeOE2
does not fully explain the high doses of 2-MeOE2 required to show
efficacy in these trials. Previously, our group (10) showed that when
a single p.o. dose of 2-MeOE2 (10 mg/kg) was administered to
rats, 2-MeOE2 could not be detected in the plasma 1 hour after ad
ministration. After i.v. administration, however, 2-MeOE2 could be
detected in the plasma, although its half-life was only 14 minutes.
In a recent phase I trial, a daily p.o. dose of 1,000 mg 2-MeOE2 was
given to 24 patients with advanced metastatic breast cancer (24,
25). Metabolism studies showed that 80% to 95% of the 2-MeOE2
was oxidized to 2-MeOE1 and furthermore 80% to 90% of both 2-
MeOE2 and 2-MeOE1 were conjugated to glucuronides/sulfates.

17β-HSD type 2 activity but not that of type 1, has been detected
in the human gastrointestinal tract. Immunohistochemistry
has localized this expression to the absorptive epithelium of the
stomach, duodenum, ileum, colon, and rectum (29). It is now
proposed that 17β-HSD type 2 expression in the gastrointestinal
tract has both an important role in regulating exposure to p.o.
administered sex steroids and protecting the human body against
exposure to environmental and/or bacterially synthesized sex
steroids (29–31). Evidence for this role is provided by the obser-
vation that p.o. administered E2 and testosterone are inactivated
rapidly and do not enter the circulation in significant amounts
(32, 33). Furthermore, studies have shown that 17β-HSD type 2 can
metabolize several p.o. administered steroidal compounds, includ-
ing those used as oral contraceptives and hormone replacement
therapy (34). The metabolism of 2-MeOE2 to 2-MeOE1 by 17β-HSD
type 2, in conjunction with high E1 sulfotransferase immunoreac-
tivity that has been detected in epithelial cells of the gastrointes-
tinal tract (35), may explain both the high levels of 2-MeOE1 and
sulfate conjugates detected in the recent phase I study (25).

The metabolic inactivation of 2-MeOE2 has led to the search for
2-MeOE2 derivatives, which are not so rapidly inactivated. One of
these derivatives, 2-MeOE2bisMATE, has shown promise as an
antitumor, antiangiogenic agent and has good bioavailability
(10–17). In this current study, no metabolism of 2-MeOE2
bisMATE was detected when cells overexpressing either 17β-HSD
type 1 or 2 were treated with this compound. In all experiments where
2-MeOE2bisMATE was incubated with cells, ~20% of the

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**Figure 5.** Representative micrographs showing mock-transfected MCF-7 cells (A) incubated with vehicle alone (THF); mock-transfected MCF-7 cells (B) incubated with 2-MeOE2 (10 \mu mol/L); MCF-7 cells transfected with 17β-HSD type 2 (C) incubated with 2-MeOE2 (10 \mu mol/L); mock-transfected MCF-7 cells (D) incubated with 2-MeOE2bisMATE (10 \mu mol/L); and MCF-7 cells transfected with 17β-HSD type 2 (E) incubated with 2-MeOE2bisMATE (10 \mu mol/L). MCF-7 cells were seeded in T-25 flasks and transfected 24 hours later with the relevant cDNA. Following a further 24 hours, the medium was replaced with fresh growth medium containing the compounds and the cells were incubated for another 48 hours.

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**Figure 6.** Effects of 2-MeOE2 and 2-MeOE1 on cell proliferation in transiently transfected MCF-7 cells. MCF-7 cells were seeded in T-25 flasks and transfected 24 hours later with the relevant cDNA. Following a further 24 hours, the medium was replaced with fresh growth medium containing the compounds and the cells were incubated for a further 72 hours. Cell numbers were counted every 24 hours. Results are expressed as number of cells per milliliter. Columns, mean; bars, SD. a, P < 0.001 versus mock-transfected untreated cells.
Representative of one of three such experiments (n with 17α-ethyl, Coulter Counter. wt, mock-transfected MCF-7; 2, MCF-7 cells transfected with 17α-HSD type 2; 1, MCF-7 cells transfected with 17β-HSD type 1. Representative of one of three such experiments (n = 3). Columns, mean; bars, SD. a, P < 0.001 versus mock-transfected untreated cells.

compound was detected as the inactive breakdown product 2-MeOE2-17MATE. In contrast, when incubated with medium alone, this percentage increased significantly (P < 0.001) to 33% of the compound detected. This observation implies that 2-MeOE2-bisMATE may be stabilized in cells, possibly by binding to tubulin, as this class of compound is known to disrupt tubulin dynamics (36). Alternatively, the acidification of the medium by the cells may make the loss of the 3-sulfamate moiety less probable. Further evidence for the stability of 2-MeOE2bisMATE was that overexpression of type 2 offered no protection from the cytotoxicity of 2-MeOE2bisMATE in cell proliferation experiments in contrast to 2-MeOE2.

Modification of the C17 position not only imparts improved resistance to metabolism but can also increase the potency of 2-MeOE2-like compounds (37). Furthermore, Utsumi et al. (13) showed that 2-MeOE2bisMATE and another C17 position-modified compound, 2-methoxyestradiol-17β-cyanomethyl-3-O-sulfamate, inhibited both in vitro cell proliferation (MCF-7 and MDA-MB-231 cells) and caused significant tumor regression (20 mg/kg p.o.) in a MCF-7 xenograft model.

In conclusion, we have shown that the anticancer agent 2-MeOE2 can be oxidized by the 17β-HSD type 2 enzyme to an inactive metabolite, 2-MeOE1, and this may go some way to explaining the poor bioavailability of this compound observed in clinical trials. In contrast, 2-MeOE2bisMATE, the promising antitumor/antiangiogenic agent, is resistant to metabolism by both the 17β-HSD type 1 and type 2 enzymes.

Acknowledgments

Received 7/8/2005; revised 9/14/2005; accepted 10/18/2005.

Grant support: Sterix Ltd., a member of the Ipsen group.

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