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Therapeutic Promises of 2-Methoxyestradiol and Its Drug Disposition Challenges

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Abstract

2-Methoxyestradiol (2MeO-E2) is an endogenous metabolite of estrogen which was initially considered to be inactive. During the last few decades it has been shown that 2MeO-E2 is a promising anticancer drug. *In vitro* experiments have demonstrated that it has several anticancer activities, and potential to alleviate hypertension, glomerulosclerosis, hypercholesterolemia, and other disorders. However, due to its low solubility and extensive glucuronidation, to achieve effective concentrations large doses of 2MeO-E2 would be required. Clinical studies reflected very high inter- and intrapatient variability and oral bioavailability of 1 to 2%. Thus, this review paper highlights the origin of this compound, its therapeutic promises, and possible mechanisms of action. It also discusses the pharmacokinetic properties of 2MeO-E2 as well as current developments to overcome low drug solubility and its extensive first pass metabolism.

Keywords

2-methoxyestradiol; anticancer activity; glucuronidation; metabolism; pharmacokinetics; dosage form; structural modifications; sulfamates

Introduction

2-Methoxyestradiol (2MeO-E2) is endogenously formed from estradiol, and was previously thought to be inactive. However, 2MeO-E2 may have potential as an anti-cancer drug, thus interest in this molecule has been renewed. As a result, herein we review the endogenous formation and metabolism of 2MeO-E2, its pharmacology and toxicity, its pharmacokinetics in rodents and humans, as well as the associated biopharmaceutical challenges. Furthermore, we discuss attempts to overcome these challenges, including new formulations and synthesis of analogs or prodrugs of 2MeO-E2.

Endogenous Origin of 2MeO-E2

2-Methoxyestradiol (2MeO-E2) is an endogenous compound and a nonpolar metabolite of estradiol (E2). Generally, formation and metabolism of estrogens are rather complex; thus, Scheme I illustrates only 2MeO-E2 formation and its possible pathways of elimination.[1–4] Estrone (E1) and E2 are substrates for the phase I enzymes, CYP1A1 and 3A4. Oxidative metabolism by these enzymes leads mainly to the formation 2-hydroxyestrogens with 4-hydroxyestrogens being minor products of CYP-mediated metabolism.[5] Catechol-O-methyltransferase or (COMT) subsequently O-methylates the 2-hydroxyl group to produce

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2MeO-E2 or 2-methoxyestrone (2MeO-E1). In the presence of CYP enzymes[6] methoxyestrogens can be transformed back to the catechol. It is believed that glucuronidation is a major pathway of 2MeO-E2 elimination. The presence of hydroxylated and dimethylated metabolites of 2MeO-E2 was also detected *in vitro*, but as a minor fraction.[7]

Many tissues can actively produce 2MeO-E2, since COMT is a ubiquitous enzyme found in different organs such as liver, kidney, intestine, stomach, spleen, brain, pancreas, lung, etc.[8] The exact endogenous 2MeO-E2 concentrations in tissues are not known and even the serum concentrations of 2MeO-E2 were frequently reported in combination with 2-methoxyestrone.[9] Recently, Xu et al. (2007) have developed an analytical method to detect different methoxyestrogens and reported total concentration of 2MeO-E2 in the blood serum to be about 9.4 ± 1.34, 10.6 ± 7.91, and 2.5 ± 0.57 pg/ml in luteal, follicular and postmenopausal women, respectively.[10] The concentrations of unconjugated form of 2MeO-E2 were about half of the above values. 2MeO-E2 concentrations however, might increase by 1000-fold during last months of pregnancy.[9]

Historically 2MeO-E2 was considered an inert metabolite and the action of COMT was believed to be a secondary pathway of E2 inactivation. However, the activity of COMT was found to be greater in tumor tissues.[6] Thus, the amount of 2MeO-E2 formed in benign breast tumor was up to 2.9 times higher than in normal tissues and up to 5.4 times higher in malignant tumor tissues. Furthermore, lower levels of 2MeO-E2 as the consequence of low activity of COMT were found in pregnant women with preeclampsia in comparison with normotensive controls.[11,12] This suggests about an important role of COMT and 2MeO-E2 in preventing preeclampsia.

Pharmacologic Activities of 2MeO-E2

During the last decade 2MeO-E2 has received attention due to its anticancer activities. Phase I and II clinical trials revealed that orally administered 2MeO-E2 is well tolerated by patients with only grade 2 and 3 toxicities observed.[13–16] No maximum tolerated dose however, was determined. It was also observed that during the treatment 2MeO-E2 concentrations in plasma remained in the nanogram per milliliter range, partially due to its extensive presystemic metabolism. As for *in vitro* antiproliferative properties, the majority of 60 cell lines from NCI are found to be sensitive to 2MeO-E2 with inhibitory concentrations between 0.08 to 5.0 μM.[17,18] The list of the tumor cells that respond to treatment with 2MeO-E2 continues to increase. For instance, recent studies on esophageal (WHCO3), nasopharyngeal (CNE2) carcinoma cells, and tumor-derived uterine leiomyoma cell lines (ELT3 and huLM) revealed to be an effective antiproliferative agent with inhibitory concentrations between 1 to 20 μM.[19–21]

It is believed that 2MeO-E2 acts on tumor growth directly by reducing cell proliferation or by inducing apoptosis, and also by inhibiting angiogenesis.[17] Apoptosis induced by 2MeO-E2 occurs by upregulating death receptor 5 (DR 5), increasing expression of STAT1 protein, and activating p53, caspases 9, 8, and 3.[22–24,17] The increase in STAT1 expression and activation of caspase are required as apoptosis is often blocked by caspases inhibitor Z-VAD-FMK. The interference in tumor microtubule formation takes place via inhibiting the tubulin polymerization rate by competitively inhibiting colchicine-binding sites. In comparison with other competitive inhibitors of colchicine-binding sites, 2MeO-E2 is atypical since its IC_{50} was found to be one or two magnitudes lower than other inhibitors.[17] The K_i value 2MeO-E2 inhibition of tubulin polymerization is 22 μM.[18] Thus, by binding to the colchicine-binding site, 2MeO-E2 inhibits the tubule formation or alters its stability.

Generally, apoptosis requires much lower 2MeO-E2 concentrations than those to attain interference in tubulin polymerization.[22]

The antiproliferative activity of 2MeO-E2 is apparently time- and concentration-dependent, whereas it is estrogen receptor (ER) independent. The affinity to ER α and β was established to be 2MeO-E2 about 0.5% and 0.008% of estradiol, respectively.[17,18, 25] However, Lui and Zhu (2004) observed that 2MeO-E2 affinity to both ER might be much higher than what was estimated earlier, about 1 to 2% of estradiol.[26] Moreover, they detected both mitogenic and antiproliferative properties of 2MeO-E2. In agreement with previous findings antiproliferative properties were ER independent, whereas mitogenic ones were ER dependent. The latter were attributed to the residual 2MeO-E2 estrogenic activity which was observed only at very low 2MeO-E2 concentrations, up to 750 nM, and when 2MeO-E2 antiproliferative activity was not yet observed.

Besides antitumor properties, 2MeO-E2 possesses other biological activities. As such, it was reported that 2MeO-E2 induces the expression of endothelial nitric oxide synthase and production of nitric oxide.[27,28] Besides being a vasodilator, nitric oxide is also known to have antithrombogenic properties, thereby preventing platelet aggregation and adhesion, and it also enhances endothelial barrier properties via decreasing infiltration of monocytes and macrophages. Therefore, 2MeO-E2 may alleviate the symptoms of atherosclerosis via decreasing plaque formation.[29] In addition to barrier-improving properties, 2MeO-E2's ability to decrease proliferating cells and inhibit collagen synthesis may attenuate hypertension, pulmonary hypertension, glomerulosclerosis, brain injury, and possibly other conditions.[3,30]

Toxicology of 2MeO-E2

Several studies with oral administration of 2MeO-E2 to humans have been reported, and the typical toxicities associated with anti-cancer drugs such as myelosuppression appear to be absent. Dahut et al reported that upon oral dosing of 3g twice daily, no dose-limiting toxicitiese were observed, and a maximum tolerated dose was not reached.[13] They reported some evidence of estrogenic effects including hot flashes, thrombosis, and fluid retention. More recently, Tevaarwerk et al reported that the maximum tolerated dose of a nanocrystalline dispersion of 2MeO-E2 with a higher bioavailability was 1g orally every 6 hours.[48] Dose limiting toxicities in this study were fatigue, muscle weakness, hypophosphatemia, hyponatremia, increased alanine aminotransferase and γ-glutamyltransferase, and anorexia. Overall, 2MeO-E2 appears to have minimal toxicities even upon administration of high doses, especially in the context of cancer treatment.

2MeO-E2 Pharmacokinetics

2MeO-E2 PK in Rodents

Tests in mice revealed that a large portion of orally administered 2MeO-E2 (100 mg/kg) was absorbed by both male (85%) and female (76%) mice. Low plasma concentrations of 2MeO-E2 and, therefore low bioavailability (1.5%), were observed and attributed to extensive metabolic transformations rather than its poor absorption.[31] From tissue analysis 60 minutes after intravenous (IV) bolus injection of 11C-2MeO-E2, this compound was highly accumulated in the liver, followed by lung and kidneys of the mice, consistent with hepatic uptake and elimination..[32] However, no determinations of 2MeO-E2 distribution in mice intestine were performed, although this organ could be vital in 2MeO-E2 metabolism. PK analysis has shown that 2MeO-E2 elimination followed a biexponential pattern with a terminal half-life ($t_{½}$) of 19 minutes, which was in agreement with the findings of other investigators, $t_{1/2}$ of 14 and 20.2 minutes.[33,34] 2MeO-E2 clearance (CL) and volume

distribution (V_d) were estimated to be 0.36 or 14.3 ml/min and volume of distribution of 52.9 or 427.5 ml for mice and rats, respectively. Lee et al. (2007) also have indicated that AUC and 2MeO-E2 plasma concentration were dose proportional between intravenous doses of 0.18 to 8.5 μg, corresponding to 0.025–0.3mg/kg body weight.[32]

2MeO-E2 PK in Humans

To achieve limited positive response on tumor treatment with 2MeO-E2, large orally administered doses were required, up to 3000 mg bid.[13–15] Even at these doses, the plasma concentration of 2MeO-E2 remained in the lower nanogram per milliliter range, unphysiologically high apparent CL and V_d were estimated with median $t_{½}$ of about 1 to 2 days. Such a long half-life in comparison to $t_{½}$ obtained in rodents may be attributed to a much higher extent of binding of 2MeO-E2 to plasma proteins. However, the plasma protein binding in rats has not been reported in the literature. Additionally, it has been suggested that the long half-life may be due to extensive enterohepatic recycling in humans,[35] but this remains to be established. Consequently, the drug binding to plasma proteins in humans was 97.4±0.22%, which was also concentration independent at 2MeO-E2 concentrations of 3.3 to 100 nM.[35] The binding association constant for 2MeO-E2 was highest for albumin (4.29 (±0.420) × 10^4/mol/l), then α_1-acid glycoprotein (4.41 (±0.139) × 10^4/mol/l) and sex-hormone-binding globulin (1.5 (±0.26) × 10^6/mol/l). Taking their reciprocals provides dissociation constants of approximately 23.3μM, 22.7μM, and 0.67μM for albumin, α1-acid glycoprotein, and sex-hormone binding globulin, respectively. Furthermore, at clinically relevant protein concentrations, the fraction unbound for albumin (4g/dl), α1-acid glycoprotein (0.25g/dl), and sex-hormone binding globulin (20μg/ml) were 3.75%, 27.6%, and 73.3%, respectively, indicating that despite its low apparent affinity albumin is the greatest contributor to plasma protein binding of 2MeO-E2.

In addition, clinical trials revealed that a linear relationship was not found between C_{max} or AUC and the 2MeO-E2 dose in doses ranging from 400mg to 3000mg administered twice daily, or 400mg to 1200mg administered once daily.[13,14] The doses administered in these studies (approximately 6–40mg/kg) were much larger and given by a different route; thus they cannot be readily compared to the results of Lee et al[32] in which linear kinetics were reported in mice upon a much smaller intravenous dose.

Lakhani et al. (2003) found less than 0.01% of the unchanged 2MeO-E2 in urine and about 1% as glucuronides after oral administration of 800 or 2200 mg bid.[2] The latter indicates that glucuronidation might be a major pathway of 2MeO-E2 metabolism. The estimated hepatic clearance of 2MeO-E2 could be as high as 712 ml/min, about half of hepatic blood flow.[7] This value includes known Phase I and II metabolism of 2MeO-E2 but not biliary excretion. Hence, 2MeO-E2 clearance could be as high as hepatic blood flow which makes 2MeO-E2 a high extraction ratio drug. This leads to the conclusion that first pass metabolism is also major player in 2MeO-E2 disposition.[1,7] Lakhani (2005) also reported that 2MeO-E2 solubility in phosphate buffer (pH 6–7) is only about 2.3 to 3.3 μg/ml, whereas its high apparent permeability (P_{app}) of 28.8 ± 4.9·10^{-6} cm/s allows the drug to easily penetrate gastrointestinal wall.[36] This makes 2MeO-E2 to fall into Class 2 of Biopharmaceutical Classification System (BCS) - a drug with high permeability but poor GI solubility.

2MeO-E2 Metabolism

2MeO-E2 contains two hydroxyl groups at the positions C-3 and C-17 and thus, it is a potential substrate for UDP-glucuronosyltransferases (UGTs). Using transfected cell lines, Basu et al. (2004) and Lépine et al. (2004) have reported that several UGT 1A and some 2B isoforms extensively transformed 2MeO-E2 to the glucuronidated forms, as summarized in

Table 1.[37,38] The relative contribution to the metabolism of 2MeO-E2 by some UGTs is somewhat controversial. The exact reason is unclear; however, the assay methods they used differed in terms of the expression system, buffer and cofactor concentrations, detergents, as well as substrate concentrations and incubation times. For example, Basu et al expressed the enzymes in COS-1 cells, using 200μM substrate concentrations and a four hour incubation, whereas Lepine et al used HEK293 cells, 25μM substrate, and 16 hour incubation. These different assay methods may have led to differing results. Basu et al. (2004) implied that UGT1A10 is the main isozyme contributing to 2MeO-E2 glucuronidation. However, Lépine et al. (2004) reported catalytic efficiencies for 2MeO-E2 3-glucuronidation for UGT1A8, 1A1 and 1A3 of 15.9, 4.5, and 3.1 μl/min.mg protein, respectively. Although K_m (12 μM) for UGT1A10 implies that this isozyme has a high affinity to 2MeO-E2, its intrinsic clearance was estimated to be quite low.[38] Glucuronidation of 2MeO-E2 took place at higher rates than methoxylated or hydroxylated estrones.[37,38] Both groups agreed that UGT2B7 was significantly not involved in the formation of 2MeO-E2 glucuronides, and there was no detectable glucuronidation occurring at the C-17 position. Even for primary estrogens like E2, the involvement of UGT2B7 or other isozymes in the formation of estradiol-17β-glucuronide is not quite clear.[38–40] On the other hand, incubation with pooled human liver microsomal proteins resulted in formation of two types of glucuronides, presumably 2MeO-E2-17- and -3-glucuronides.[7] Therefore, the mechanism of formation, conditions, and type of the enzymes participating in the formation of 17-glucuronide still need to be clarified.

In vitro experiments have shown that glucuronidation of 2MeO-E2 might not be limited to the liver. Hence, UGT1A1 mRNA is expressed (mRNA) in duodenum : ileum : stomach at relative levels of approximately 10:4:1.[37] UGT1A7 is also present in nearly every tissue with high levels in esophagus, thyroid and adrenal glands, with highest being in esophagus.[41] UGT1A8 was found at moderately to high expression levels in esophagus, duodenum ileum, colon, and stomach. UGT1A9 appeared to be highly expressed in distal parts of the GI tract such as jejunum, ileum, colon and rectum. The highest amounts however, were found in kidney and liver.[41] UGT1A10 appeared to be present in duodenum : ileum : colon : stomach : esophagus at relative levels of 9:8:3:2:1.[37] Therefore, orally administered 2MeO-E2 can theoretically undergo extensive metabolism starting in esophagus by UGT1A7, followed by UGT1A8 in stomach (empty), then in duodenum by UGT1A1, 1A7, 1A9, and 1A10, in ileum by UGT1A1, 1A9 and 1A10, colon by UGT1A7, 1A9, and 1A10, and of course, in liver by UGT 1A1, 1A3, 1A9. Of course, the actual contribution of these enzymes to 2MeO-E2 metabolism would be strongly influenced by tissue surface area, residence time of the drug in the organ, enzyme expression level, and drug concentration, all of which determine the exposure of the drug to the enzyme(s).Lakhani et al. (2007) have also emphasized that Phase I metabolism can play an important role in metabolism of 2MeO-E2, but not as extensively as glucuronidation.[7] Although no oxidative metabolites were found in urine samples of patients taking 2MeO-E2, *in vitro* experiments showed the presence of five Phase I metabolites such as 2MeO-E1, 2-hydroxyestradiol, 2-hydroxyestrone, 2,3-dimethoxyestrone, and 2,3-dimethoxyestradiol. The incubation of 2MeO-E2 with different recombinant CYP450 enzymes demonstrated that the following isoforms are responsible for hepatic oxidation of the drug: CYP 1A1, 1A2, 3A4, 3A5 and 2E1.[36] Neither the urine samples nor *in vitro* studies showed the presence of sulfated metabolites of 2MeO-E2 indicating that 2MeO-E2 metabolism by sulfotransferase is limited or does not take place. However, it was reported that 2MeO-E2 can undergo sulfonation catalyzed by SULT1A1.[42] Being a low-capacity and high-affinity enzyme, SULT1A1 may be effective in 2MeO-E2 biotransformation in nanomolar range; at very high concentrations UGTs become the major route of 2MeO-E2 biotransformation. The additional bulk of the C-2 methoxy group may also inhibit SULT activity.[7]

2MeO-E2 Transport

To our knowledge, no studies have been published so far to reveal the role of transporters, especially ATP-binding cassette (ABC) transporters, on the disposition of 2MeO-E2. Some research however, was done to identify an ABC transporter that might be responsible for the movement of E2 though the lipid bilayer. Considering the fact that E2 and 2MeO-E2 are structurally similar, one might also apply the results obtained with E2 to 2MeO-E2. Because E2 is a very hydrophobic compound it would be reasonable to assume that E2 is a substrate for Pgp (ABCB1), known to transport variety of hydrophobic compounds.[43] However, Fujise et al. (2002) have shown via *in vitro* experiments that estradiol is not a P-glycoprotein substrate, whereas uptake experiments with inside-out membrane vesicles and BCRP-transduced cells showed that E2 is a competing substrate for breast cancer resistance protein (BCRP), known also as ABCG2.[44–46] In any case, diffusional permeabilities for these compounds would likely overwhelm any contribution of ABC efflux transporters to overall transport of E2 or 2MeO-E2. However, they may result in inhibition of certain transporters if sufficiently high local concentrations of the parent compounds or their metabolites are achieved.

Pharmacokinetic-Pharmacodynamic Relationships

The pharmacokinetic-pharmacodynamic relationships for 2MeO-E2 have not been clearly established in humans, since phase three clinical trials have not yet been published. However, as clinical trials proceed, one may expect more data to be published to elucidate this issue. In phase 1 clinical trials of 2MeO-E2, one ovarian cancer patient showed a partial response despite low circulating 2MeO-E2 concentrations, leading Lakhani et al to suspect an active metabolite of 2MeO-E2.[36] This possibility remains to be investigated. However, an alternative explanation could be reversible metabolism. In such a case, 2MeO-E2-3-glucuronide could enter a tissue and be cleaved by cellular glucuronidases, releasing “free” 2MeO-E2. Glucuronidase activity in breast cancer tissue was demonstrated long ago by Fishman et al.[47] (JBC 1947). More recently the presence of such glucuronidases in lung cancer tissue inspired the design of a glucuronidase-activated prodrug of doxorubicin which would attempt to selectively target cancer tissues in which this enzyme was highly expressed (Murdter Cancer Res 1997). Thus the release of 2MeO-E2 from its glucuronide within the tumor, if it occurs, could explain the observation by Lakhani et al. Further research is needed to fully relate the pharmacokinetics of 2MeO-E2 (and its metabolites) to their pharmacodynamics in humans.

Attempts to Conquer Challenges

New Formulations

There are several approaches to solve the problem of low solubility of 2MeO-E2, including improved formulations. For instance, encapsulation of 2MeO-E2 within biodegradable and biocompatible layers of polyelectrolyte led to an increase in the drug solubility and stability for up to 9 months in aqueous solution.[49] This multilayered matrix easily released the active ingredient and showed to produce slightly better or similar effect as free 2MeO-E2 in *in vitro* experiments. These microcrystals however need to be further tested *in vivo* to determine their efficacy.

Generally, particles of smaller diameter allow a drug to dissolve at higher rates. Thus, Tevaarwerk et al. (2009) have conducted Phase I clinical trials for the purpose of evaluating the efficacy of nanocrystal dispersion of 2MeO-E2.[50] It was observed that there was a dose dependent increase in the systemic exposure within the dose range of 250 – 1000 mg, and minimal increase at higher doses. The maximally tolerated dose was found to be 1000 mg every 6 or 8 hrs. Based on the published data, this nanocrystal drug formulation led to an

increase in 2MeO-E2 bioavailability to approximately 3 – 4%, which is three to four-fold higher than those observed with 'typical' formulations, but the bioavailability remains very low.

Coadministration with inhibitors

It may be possible to formulate 2MeO-E2 with other agents in an attempt to decrease its extensive presystemic elimination, thus enabling more of the drug to reach systemic circulation. Such inhibitors would need to be effective at inhibiting the major enzymes metabolizing 2MeO-E2 including several UGT isoforms, CYP1A1, and SULT1A1 as discussed above, but without significant toxicities of their own. Such an approach, if effective, may increase the toxicity of 2MeO-E2 and may have problems with drug-drug interactions. We are currently investigating a novel, proprietary combination approach.

Alternative routes of administration

In addition to oral formulations, parenteral formulations may be explored. These non-oral routes of administration would diminish problems with the extensive presystemic metabolism of 2MeO-E2, although solubility remains problematic. For example, intravenous dosing would almost certainly require a non-aqueous solvent such as propylene glycol or polyethylene glycol, or pharmaceutical surfactants such as Cremophor EL. Also, long-term intravenous dosing would be difficult to maintain. Non-aqueous depot injection of 2MeO-E2 directly into the area of the tumor is a possibility. For transdermal administration, which is commonly used for many contraceptives containing estrogens and progestins, it may be difficult to achieve desired plasma concentrations of 2MeO-E2.

Synthesis of Active Analogs or Prodrugs

Syntheses of 2MeO-E2 analogs with similar or better anticancer activities and lower rates of metabolism or prodrugs with improved solubility are another way to resolve low bioavailability of 2MeO-E2. An introduction of various functional groups at different locations on the structure of 2MeO-E2 structure yielded a variety of compounds.[51,52] However, it is of interest to synthesize a compound with other than hydroxyl and amine (NH_2) groups at C-3 and C-17 which would still possess similar or improved antitumor properties.

The anticancer activity of 2MeO-E2 is primarily associated with the methoxy moiety at C-2. Therefore, any changes at this location resulted in the reduction in efficacy to inhibition of tubulin polymerization.[53] The compounds with different functional groups at C-2 are listed in Table 2, compounds 2 through 10. These drugs were tested in several cancer cell lines, but for the sake of comparison between analogs only a few of cell lines were selected and the results are listed in Table 2. Antiproliferative properties (IC_{50}) or growth inhibition (GI_{50}) were reported as IC_{50} (or GI_{50}) ratio of 2MeO-E2 to 2MeO-E2 analogs. Values larger than 1 are indicative of higher potency of 2MeO-E2 analog than 2MeO-E2. As can be seen, the analogs with bulky side groups or electronegative atoms at C-2 lost their anticancer activities; therefore only drugs with small and nonpolar moieties such as propynyl (compound 9) and 3-hydroxy-1-propenyl (compound 2) possessed comparable properties to 2MeO-E2. Hence, modifications at C-3 or C-17 would be more desirable.

Table 2 also contains a list of compounds and their antiproliferative properties where a new group was introduced to C-17, C-3 or at both locations simultaneously. Only 3 out of 25 compounds (compounds 14, 15 and 16) with substituted group at C-3 were added to the table because they seemed to be more potent than 2MeO-E2 itself.[54] All 3 compounds are H-donors, not bulky and contained π electrons as a part of nitrile or carbonyl. When the hydroxyl group at C-17 was substituted by other moieties such as methylene and ethylene

(compound 24 and 28) the potency of the drug increased in comparison with 2MeO-E2. However, as it was noticed earlier, the substitution with groups of longer chains caused antiproliferative properties to decline (e.g. compounds 33 vs. 36 – 38). An introduction of amine (40), carboxamide (44), and hydroxyl amide (42) decreased the potency of the 2MeO-E2 analogs.[55]

Double modification at C-3 and C-17 tended to decrease the potency not only in comparison to 2MeO-E2[56] but, in some cases, also in comparison to the compounds with only one modified group at C-17 (Table 2), despite the fact that each group substituted at a single location showed improvement in antiproliferative properties (e.g. compounds 16, 24, and 27). The only exception is compound 46, the potency of which is actually increased fivefold in comparison to 2MeO-E2. The drugs with improved therapeutic properties should also have better pharmacokinetic properties to be more advantageous than 2MeO-E2. Some PK parameters of the selected 2MeO-E2 analogs measured via cassette dosing in rats are listed in Table 3.[55,56] Bioavailabilities of all compounds were estimated to be between 14 to 84%. C_{max} and AUC values were possible to estimate unlike for 2MeO-E2. Compound 27 had the highest C_{max}. However, this drug and also 30, and 35 have formamides at C-3 which might have aniline-like toxicity if it is cleaved[56] and thus might be not suitable as an anticancer drug. The addition of ethylene or methylene groups to C-17 affected the compound solubility and glucuronidation rate by reducing them both. Instead of a single first order reaction, *in vitro* glucuronidation of 2MeO-E2 analogs occurred via a slow initial phase and a faster second phase..[55] Perhaps due to the slow initial metabolic phase the AUC for compounds 24 and 28 was larger than 2MeO-E2; however their metabolic rate during the second phase was much faster than for 2MeO-E2. Thus, compounds 46 and 25 that could be potential anticancer drugs. Compound 46 is currently in Phase I clinical trials.[56]

The introduction of other groups to C-17 or C-3 had variable success. For instance, the addition of sulfamate to C-17 (compound 47) or to both C-17 and C-3 (compound 48) resulted in drastic improvement of the antiproliferative properties of the compound and it was attributed to the compound itself rather than its actions as a prodrug.[57,58] On the other hand, the addition of phosphates to the 2MeO-E2 structure (compounds 11–13, Table 2) diminished the therapeutic properties in most of the tested cancer cell lines and it was concluded to be inefficient as prodrugs.[59] The most effective phosphate prodrug however, was determined to be 3-phosphate-2-MeO-E2 but it possessed high toxicity in comparison to 2MeO-E2 itself. Sulfamoylation at either C-3 alone or C-3 and C-17 (compounds 47 and 48, respectively) substantially improved the antiproliferation potency.[57,58] Finally, although replacing the 2-methoxy group with an ethyl group (compound 49) had decreased potency as discussed earlier, sulfamoylation (compound 50) had drastically improved potency.[57,58]

Conclusions

2MeO-E2 is an active metabolite of estrogen which is found to possess anticancer activities on several cancer cell lines. *In vivo* studies have revealed that 2MeO-E2 is well tolerated but due to its extensive first pass metabolism and low solubility, subtherapeutic plasma concentrations of 2MeO-E2 were observed despite large orally administered doses. Pharmacokinetic analysis revealed nonlinear dose proportionality in addition to the low oral bioavailability of 2MeO-E2. 2MeO-E2 is extensively metabolized not only in the liver but also in the GI tract. Although glucuronidation is considered to be a major route of 2MeO-E2 inactivation, 2MeO-E2 is also largely converted into 2MeO-E1 and is also metabolized by several CYPs. The attempts to tackle the problems related to 2MeO-E2 low solubility or/and extensive metabolism not always resulted in desired improvement. Thus, new formulations only modestly improved the oral bioavailability. Several different 2MeO-E2 analogs have been synthesized that would be more metabolically stable than the parent drug, but only a

few of them have shown some promise. Although 2MeO-E2 is a promising anticancer drug, the resolution of its current problems associated with solubility and metabolism are far from being completed for a successful launch of 2MeO-E2 into the market. Further studies will be required to identify the receptors targeted by 2MeO-E2 and mediating its therapeutic effects. The additional compounds can be designed to bind these receptors.

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Abbreviations

2MeO-E1	2-Methoxyestrone
2MeO-E2	2-Methoxyestradiol
ABCB1	ATP-binding cassette transporter isoform B1
ABCG2	ATP-binding cassette transporter isoform G2
AUC	Area under the plasma drug concentration-time curve
C_{max}	Maximum drug concentration
BCRP	Breast cancer resistance protein
CL	Clearance
COMT	Catechol-O-methyltransferase
CYP or CYP450	Cytochrome P450
E1	Estrone
E2	Estradiol
ER	Estrogen receptor
Pgp	P-glycoprotein
SULT	Sulfotransferase
$t_{½}$	Half-time
V_d	Volume of distribution
UGT	UDP-glucuronosyltransferase

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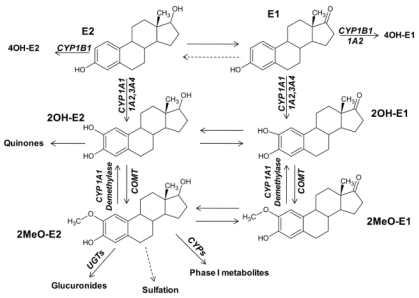

Scheme 1.

Pathways of 2MeO-E2 formation and elimination. Compounds and enzymes are previously defined. Estrogen metabolism is notoriously complex; readers should note that other phase 1 metabolites, glucuronides, and sulfates can be formed from any of these. However, the emphasis of this figure is to highlight the endogenous formation and metabolism of 2MeO-E2.

Table 1

Rates of 2-methoxyestradiol (2MeO-E2) glucuronidation by human UGT isoforms.

	pmol/h/mg protein	
UGTs	**[37] Basu et al., 2004**	**[38] Lépine et al., 2004**
1A1	160 ± 30	1306 – 2612
1A3	621 ± 21	261 – 1305
1A7	2504 ± 95	-
1A8	2045 ± 77	1306 – 2612
1A9	~52.5	261 – 1305
1A10	7933 ± 267	261 – 1305
2B4	-	131 – 260
2B7	-	-
2B17	-	26 – 130

Table 2

2MeO-E2 analogs and their relative anticancer activities

Compound	Position			Effect/Cell Line	Relative Potency	Reference
	C-2	C-3	C-17			
2MeO-E2	CH_3O	OH	OH	MDA-MB-435 Cytotoxicity	1	[53] Cushman et al., 2002
2	C_3H_4OH	OH	OH		1.53	
3	EtO-OH	OH	OH		0.01	
4	$CHNC_2H_4N(Me)_2$	OH	OH		<0.01	
5	$CHNC_2H_4OH$	OH	OH		<0.01	
6	$(MeO)_3Ph\text{-}C_2H_2$	OH	OH		0.02	
7	$Ph\text{-}C_2$	OH	OH		0.01	
8	C_2H	OH	OH		0.04	
9	CH_3C_2	OH	OH		0.62	
10	CN	OH	OH		≪0.01	
11	CH_3O	PO_3H_2	OH		0.73	[59] Edsall et al., 2007
12	CH_3O	OH	PO_3H_2		<0.01	
13	CH_3O	PO_3H_2	PO_3H_2		≪0.01	
14	CH_3O	NHCN	OH		1.44	[54] Suwandi et al., 2009
15	CH_3O	$NHCONH_2$	OH		1.15	
16	CH_3O	NHCOH	OH		9.7	
17	CH_3O	OH	H		0.64	[55] Shah et al., 2009
18	CH_3O	C_2H_3	H	HUVEC Antiproliferation	1.17	[56] Agoston et al., 2009
19	CH_3O	CONHEt	H		0.02	
20	CH_3O	$CONH_2$	H		0.57	
21	CH_3O	NH_2	H		0.14	
22	CH_3O	NHCOH	H		0.12	
23	CH_3O	NHCOMe	H		0.4	

Compound	Position			Effect/Cell Line	Relative Potency	Reference
	C-2	C-3	C-17			
24	CH_3O	OH	CH_2		4.42	[55] Shah et al., 2009
25	CH_3O	$CONH_2$	CH_2		2.72	[56] Agoston et al., 2009
26	CH_3O	NH_2	CH_2		0.68	
27	CH_3O	NHCOH	CH_2		3.09	
28	CH_3O	OH	$CHCH_3$		3.23	[55] Shah et al., 2009
29	CH_3O	NH_2	$CHCH_3$		0.94	[56] Agoston et al., 2009
30	CH_3O	NHCOH	$CHCH_3$		1.17	
31	CH_3O	OH	$CHCH_2CH_3$		0.26	[55] Shah et al., 2009
32	CH_3O	OH	$CHCH_2CH_2CH_3$		0.24	
33	CH_3O	OH	CH_3		0.35	
34	CH_3O	NH_2	CH_3		0.26	[56] Agoston et al., 2009
35	CH_3O	NHCOH	CH_3		1.17	
36	CH_3O	OH	CH_2CH_3	HUVEC Antiproliferation	0.23	[55] Shah et al., 2009
37	CH_3O	OH	$CH_2CH_2CH_3$		0.02	
38	CH_3O	OH	$CH_2CH_2CH_2CH_3$		0.07	
39	CH_3O	OH	$N_2HSO_2Ph\text{-}CH_3$		0.011	[55] Shah, et al., 2009
40	CH_3O	OH	NH_2		0.06	
41	CH_3O	OH	NH-Pr		0.04	
42	CH_3O	OH	NOH		0.2	
43	CH_3O	OH	COOH		0.02	
44	CH_3O	OH	$CONH_2$		0.18	
45	CH_3O	OH	16,17-Olefin		1.18	
46	CH_3O	$CONH_2$	16,17-Olefin		5.23	[56] Agoston et al., 2009
47	CH_3O	NH_2SO_3	OH		10	[58] Newman et al., 2004

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Compound	Position			Effect/Cell Line	Relative Potency	Reference
	C-2	C-3	C-17			
48	CH_3O	NH_2SO_3	NH_2SO_3		60	
49	CH_3CH_2	OH	OH		<0.3	[58] Newman et al., 2004
50	CH_3CH_2	NH_2SO_3	OH		>300	
47	CH_3O	NH_2SO_3	OH		6.3	
48	CH_3O	NH_2SO_3	NH_2SO_3	MCF7 Antiproliferation	6.3	[58] Newman et al., 2004
48	CH_3O	NH_2SO_3	NH_2SO_3		6.25	[57] Raobaikady et al., 2003

Table 3

Pharmacokinetic summary of selected 2MeO-E2 analogs

Compound	C max nM	AUC nM h	BA %	Reference
1	BLOQ[1]	BLOQ	-	[55] Shah et al., 2009
24	40	572	32.8	
25	62	467	22.8	[56] Agoston et al., 2009
27	127	1703	39.5	
28	80	1163	84.2	[55] Shah et al., 2009
30	83	659	24.7	[56] Agoston et al., 2009
35	73	673.2	20.6	
46	42	320	14.3	

[1] BLOQ – below the limit of quantification