Role of 2-methoxyestradiol, an Endogenous Estrogen Metabolite, in Health and Disease

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Abstract: Estradiol (E2) is a steroid hormone whose physiological actions are mainly mediated by its interaction with intracellular estrogen receptors (ER) leading to modification on the mRNA and protein synthesis in its target cells. However, estrogens can also activate several intracellular signal transduction cascades by non-genomic mechanisms. Estrogens must be inactivated and removed from blood through its conversion to soluble compounds with an apparent low estrogenic activity and decreased affinity for ER. In this context, 2-methoxyestradiol (2ME2) is generated by a sequential hydroxylation of E2 via the enzyme cytochrome P450 isofrom 1A1 to produce 2-hydroxyestradiol (2OHE2) followed by a conjugation reaction catalyzed by the enzyme Catechol-O-Methyltransferase generating 2ME2 from 2OHE2. Recent evidence indicates that physiological concentration of 2ME2 may regulate several biological processes while high concentrations of this metabolite may induce pathophysiological alterations in several tissues. In the last years, 2ME2 has also been described as a promising anticancer drug although its cellular and molecular mechanisms are still being disclosed. Herein, we will review the available literature concerning the role of 2ME2 in health and disease. We will focus on to describing the intracellular mechanisms by which 2ME2 exerts its effects on reproductive and non-reproductive tissues. The promising anticancer effects of 2ME2 and its synthetic derivatives will also be discussed. Finally, a group of 2ME2-target genes that could be used as biomarkers of 2ME2 under physiological or pathophysiological conditions will be reviewed.

Keywords: Biomarkers, estradiol, estrogen receptor, gene expression, 2-methoxyestradiol.

1. INTRODUCTION

Estradiol (E2) is a pleiotropic hormone that regulates a wide variety of physiological functions in the reproductive tract, mammary gland, bones, brain, blood vessels and heart [1-6]. The canonical mechanism by which E2 regulates its target cells, involves binding to estrogen receptor α (ESR1) and/or β (ESR2) and modification of gene expression [7]. However, it has also been reported that E2 may modulate the physiology of its target cells activating several intracellular signal transduction cascades by non-genomic mechanisms [8, 9].

Estradiol is removed from the blood by a metabolic inactivation that requires biotransformation to less estrogenic compounds. Although conversion of E2 to inactive metabolites occurs mainly in the liver, some peripheral organs including breast, uterus, placenta, oviduct and brain express the enzymes required to inactive estradiol [10, 11]. The subsequent conversion of E2 to hydroxyestradiols and then to methoxyestradiols is one of the most recognized enzymatic pathways that inactive E2 [12]. When methoxyestradiols were characterized they were catalogued as inactive molecules without a biological role. However, it has also been demonstrated that 2-methoxyestradiol (2ME2) may exert physiological actions in different organs and tissues and that an unbalanced E2 metabolization to 2-hydroxyestradiol (2OHE2) and 2ME2 could be the responsible factor for several diseases including infertility, cancer or preeclampsia [13]. In this context, pharmacological doses of 2ME2 have been tested as a promissory anticancer therapeutic strategy based on its effects on the angiogenesis and cellular proliferation of tumour cells [14]. The present review will describe the available literature concerning the role of 2ME2 in health and disease. The intracellular mechanisms by which 2ME2 exerts its effects on reproductive and non-reproductive tissues and the promising anticancer effects of 2ME2 and its synthetic derivatives will be discussed. Finally, a group of 2ME2-target genes that could be used as biomarkers of 2ME2 under physiological or pathophysiological conditions will be reviewed.
2. GENERATION OF 2-METHOXYESTRADIOL

Estradiol is mostly inactivated to 17β-estrone through an oxidative reaction catalyzed by the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) [15]. Furthermore, other enzymatic modifications to inactive E2 have been described in the last years [12]. These reactions include sulfonation catalyzed by several estrogen sulfotransferases, O-methylations catalyzed by the enzyme Catechol-O-Methyltransferase (COMT), hydroxylations catalyzed by several cytochrome P450s isoforms enzymes and glucuronidations catalyzed by UDP-glucuronosyltransferases. Although, these reactions were firstly characterized in the liver, some enzymes that mediate these effects have been found in several peripheral tissues such as breast, uterus, placenta and brain [10] suggesting that these organs have also the ability to inactive E2 by these pathways.

Estradiol is mainly inactivated through its conversion to 2OHE2 and subsequent generation of 2ME2 in extra hepatic tissues (Fig. 1). These reactions involve a first oxidation in the carbon 2 inside the aromatic A-ring of E2, catalyzed by the enzyme Cytochrome P450 isoform 1A1 (CYP1A1), generating a molecule of 2OHE2. Then, the hydroxyl group previously added is replaced by a methyl group through a conjugation reaction catalyzed by the enzyme COMT to generate a molecule of 2ME2 [12]. As CYP1A1 and COMT are expressed in the oviduct [11]), ovary [16], endometrium [17], placenta [18, 19], prostate [20] and testis [21] it is feasible to postulate that 2ME2 could also be locally generated from E2 in these organs.

CYP1A1 has the ability to metabolize several exogenous and endogenous compounds as E2 and xenobiotics via an NADPH-dependent oxidative metabolism reaction [22]. CYP1A1 is normally expressed at low levels in different tissues and its expression is regulated by different biological compounds such as 6-Formylindolo(3,2-b)carbazole (a tryptophan-derived), bilirubin or eicosanoids [23]. However, an increased expression of this enzyme has been reported in cancer cells [24, 25] suggesting a role of CYP1A1 in this pathology. It has been documented that several toxic compounds associated with cancer etiology are able to increase CYP1A1 expression as for instance cigarette smoke condensate [26, 27] and dioxin [28, 29].

On the other hand, COMT enzyme has the ability to add a methyl group (provided from S-adenosyl-l-methionine) to several molecules such as hydroxysteradiols and catecholamines [30] by an enzymatic reaction that requires Mg2+. This enzyme has two active isoforms encoded by the same gene and they are known as the cytoplasmic soluble form (S-COMT) and a membrane-bound form (MB-COMT). Both isoforms are identical except by an N-terminal extension of 50 amino acids in the amino-terminal in M-COMT, which is responsible for its anchorage to the endoplasmic reticulum membranes [30].

3. MECHANISMS OF ACTION OF 2-METHOXYESTRADIOL

A variety of biological effects of 2ME2 reported in the literature are ER independent. However, in some few cases, these effects require a functional ER [11, 31, 32]. Here, the most well known cellular and molecular mechanisms exerted by 2ME2 on its target cells will be briefly reviewed.

3.1. Depolarization of Microtubules

One of the first characterized effects of 2ME2 was its ability to inhibit tubulin polymerization [33-35]. The molecular mechanism consists in a direct interaction between 2ME2 and tubulin protein to inhibit the phases of nucleation and propagation during tubulin assembly [33]. Thus, 2ME2 induces mitotic perturbations and death in cells with a high proliferative rate [36]. It has been proposed that this effect is the most important mechanism exerted by 2ME2 to induce cell death in cancer cells [37].

3.2. Reactive Oxygen Species (ROS)

The ability of 2ME2 to induce ROS or block its production seems to depend on the cell types. Thus, 2ME2 induced ROS production to decrease cell viability in the
MCF7 [38], nasopharyngeal carcinoma [39], ovarian cancer [40] or human neuroblastoma cells [41, 42]. On the other hand, 2ME2 blocked ROS production in non-cancer cells as the normal mouse spleen or swine granulosa cells [43, 44].

3.3. HIF-1α Downregulation

One of the most characterized physiological effects of 2ME2 is its ability to down-regulate the expression of the hypoxia-inducible factor 1 alpha (HIF-1α) protein. This is a transcription factor induced under hypoxic conditions that regulates the expression of several molecules related to cell proliferation, angiogenesis and metabolism such as the vascular endothelial growth factor (VEGF) [45, 46]. Despite HIF-1α expression is generally associated with cell survival, high levels of this transcription factor have been related with several pathological conditions such as allergic rhinitis [47, 48], traumatic brain injury [49, 50] or preeclampsia [51, 52]. Protein stability and nuclear location of HIF-1α are decreased by 2ME2 [50] leading to changes both in the microtubules polymerization and in the intracellular free radical levels [53-55]. This 2ME2 effect is related with a successful placentation process described later in this review.

3.4. Modulation of Protein Phosphorylation and Kinases Activity

It has been reported that 2ME2 modulates phosphorylation status of different proteins to exert its biological or pharmacological actions. Thus, 2ME2 induces ERK 1/2 phosphorylation to down-regulate synthesis of Angiotensin type 1 receptor and endothelin 1 in liver epithelial cells [56] and coronary artery endothelial cells [57], respectively. Furthermore, 2ME2 decreases ERK1/2 phosphorylation to inhibit cell growth in human aortic smooth muscle cells [58].

On the other hand, 2ME2 activates different protein kinases to induce cell death in cancer cells. For example, 2ME2 activates JNK kinases in prostate cancer cells [59], Ewing sarcoma cells [60, 61] and nasopharyngeal carcinoma cells [62] to induce cell death by mitochondrial-dependent apoptotic pathways and/or autophagy. Furthermore, 2ME2 activates a RNA-dependent protein kinase (PKR) [63] to induce autophagy in osteosarcoma cells and induces apoptosis in esophageal carcinoma cells by increasing cdc2 kinase activity [64].

3.5. Smooth Muscle Contraction

A poorly explored effect of 2ME2 is its ability to modulate contraction of vascular and non-vascular smooth muscle cells. As 2ME2 inhibits phenylephrine-induced tension in aorta [65] and KCl-induced contraction in coronary artery [66] it is probable that 2ME2 has a relaxing effect on the vascular smooth muscle cells. Interestingly, 2ME2 exerts these effects by ER independent mechanisms although it requires an intact endothelium. On the other hand, 2ME2 attenuates KCl-induced uterine contraction by a non-genomic mechanism in non-vascular smooth muscle cells [67].

4. REPRODUCTIVE EFFECTS OF 2-METHOXY-ESTRADIOL

The biological role of estrogens in the male and female reproductive systems involves regulation of the gametogenesis, transport of gametes and embryos, blastocyst implantation, pregnancy, lactation and sexual development [68]. In the testis, expression and enzymatic activity of the proteins responsible to produce 2ME2 has been reported in several mammalian species. CYP1B1 expression was reported in human [69-71] and rat [72, 73] while CYP1A1 has been detected in human [71], mouse [74], and rat [75]. Furthermore, expression and/or activity of COMT have been reported in mouse [76] and human testes [77]. The biological and/or pathophysiological effects of 2ME2 in the mammalian testis are unknown although it has recently been reported that high concentration of 2ME2 decreases viability of Sertoli [78] and Leydig cells [79]. This suggests that an increased intratesticular concentration of 2ME2 could have implications for male infertility. Interestingly, 2ME2 exerts these deleterious effects inducing DNA fragmentation by caspase-independent mechanisms [78, 79].

In contrast to the testicular cells, there are more abundant data on the physiological role of 2ME2 in the mammalian ovary. According with the literature, CYP1A1 and COMT are present in the human ovary [12] and it has been reported that during the menstrual cycle, plasma levels of 2ME2 are higher in the luteal than in the follicular phase [80]. Furthermore, levels of 2ME2 in the follicular fluid are directly correlated with the follicle size in the pig [81]. On the other hand, an excessive production of 2ME2 could be involved in the follicular arrest found in women with polycystic ovarian syndrome since high levels of 2ME2 inhibited proliferation of the granulose cells [82].

In the rat oviduct, the effect of 2ME2 on the oviductal egg transport is dependent of the occurrence of mating. Thus, it has been reported that 2ME2 shortens the time of permanence of the eggs from 72 h to less of 24 h in the oviduct of unmated although it had no effect in mated rats [11]. Moreover, the effect of 2ME2 on the oviductal egg transport is through a non-genomic mechanism involving participation of the ER and the signaling cascades of cAMP-PKA in the oviductal cells [11, 83, 84]. It has recently been reported that the cytokine TNF-α is able to shutdown this 2ME2 non-genomic signaling in the rat oviduct [84] indicating an interaction between cytokines and estrogen metabolites in the female reproductive tract. The physiological relevance of this phenomenon is associated with the prevention of the deleterious effects of 2ME2 on the first stages of the embryo development [85]. In this context, we have recently found that a pharmacological dose of 2ME2 administered to pregnant mice is able to inhibit embryo implantation (unpublished observations). It is postulated that this effect of 2ME2 is mediated by an increase in the uterine secretion of the extracellular matrix protein F-spondin blocking the interaction between endometrial integrin αβ3 and its ligand localized in the blastocyst [86].

In ovariectomized rats, 2ME2 delivered at a 1 µg/h rate by a subcutaneously implanted osmotic pump did not exert uterotrophic effects although it regulated bone metabolism.
proposed that a decreased 2ME2 production during mild invasion and incomplete spiral artery remodelling during antiangiogenic factors, which generates a poor trophoblastic restriction [93]. It is postulated that preeclampsia is produced it is related with maternal and perinatal morbidity and affects approximately 5% of all pregnancies worldwide and placental hypoxia during pregnancy [93]. This condition dysfunction associated with hypertension, proteinuria and is implicated in the occurrence and it is necessary hormone replacement therapy [105]. The beneficial effects of E2 on the vasculature are associated with modifications of lipoproteins, inhibition of atherosclerosis and regulation of the vascular tone [106]. A role for estrogen metabolites on the effect of E2 in the vascular system was proposed because E2 prevents atherosclerosis by inhibiting the neointima formation in double knockout ESR1 and ESR2 mice [107]. Furthermore, 2ME2 is associated with inhibition of vaso-occlusive disorders because it inhibited synthesis of endothelin-1 in porcine coronary artery endothelial cells [57]. Moreover, 2ME2 down-regulated the expression of Angiotensin type I receptor that is involved in blood vessels inflammation [108]. This effect is mediated by previous binding of 2ME2 with a newly discovered membrane estrogen receptor GPR30 and not requires activation of the classical ER [108]. On the other hand, it has also been reported that CYP1A1, CYP1B1 and COMT were expressed in vascular tissues of several mammalian species [57].

The role of 2ME2 on the brain physiology is mainly associated with its inhibitory effect on the HIF-1α expression. Increased expression of HIF-1α is implicated in many cerebrovascular disorders that may be induced by an acute hypoxic environment in the neuronal cells [109]. In this context, inhibition of the HIF-1α activity or expression imparts neuroprotection to adult rodents followina cerebral ischemia [110, 111]. Thus, it is now suggested that pharmacological targeting of HIF-1α activity may be a promising therapeutic strategy to attenuate the secondary
brain damage following a cerebral stroke or traumatic brain injury. Considering that 2ME₂ is able to inhibit HIF-1α expression in cancer cell lines and in HUVEC cells [53, 112] several laboratories have conducted investigations to determine the beneficial effects of 2ME₂ following brain injury. Intraperitoneal administration of 2ME₂ (10-20 mg/kg body weight) 30 min after brain trauma reduced the progression of secondary brain damage in adult mice [50]. Using a rat pup hypoxic-ischemic model, Chen et al. [111] showed that 2ME₂ administered 5 min after a hypoxic stimulus reduced brain damage as shown by increased preservation of the blood-brain barrier, attenuation of brain edema and inhibition of neural apoptosis. The molecular basis by which 2ME₂ influences the nervous system could be related with changes on COMT activity since genetic and/or epigenetic alterations in the COMT expression are associated with mental disorders [113, 114].

The effects of 2ME₂ on renal tissues are associated with an attenuation of glomerulocoe sclerosis since 2ME₂ stimulates synthesis of nitric oxide (NO) in glomerular endothelial cells and inhibits abnormal growth of glomerular mesangial cells via regulation of cAMP production [115, 116].

Altogether, these data reinforce the concept that the protective effects of E₂ on the female health may be in part mediated by a previous conversion of E₂ to 2ME₂ in its target organs.

6. EFFECTS OF 2-METHOXYESTRADIOL ON TUMOR CELLS

It is now well recognized that 2ME₂ can inhibit cell growth and induce cell death in a variety of malignant cell lines including lung and colon carcinoma, melanoma, and cancers from the reproductive system [12, 80, 117-120]. The antitumoral effects of 2ME₂ are normally independent of ER activation and involve suppression of tumor cells growth by different molecular mechanisms. These include inhibition of angiogenesis [34, 35, 120], induction of apoptosis through caspases-dependent [121] or caspases-independent mechanisms [122], and inhibition of β-tubulin polymerization [33-35]. The ability of 2ME₂ to inhibit β-tubulin polymerization is correlated with its inhibitory effect on HIF-1α activity in tumor cells [112]. On the other hand, various reports suggest a correlation between COMT polymorphisms and cancer risks that could be associated with patient ethnicity. Thus, a specific val158 met COMT polymorphism has been associated with a decreased risk of uterine leiomyoma while a COMT Val/Val genotype is correlated with an increased endometrial cancer risk in some Asian populations [123, 124].

7. CLINICAL USE OF 2-METHOXYESTRADIOL

Clinical use of 2ME₂ is mainly limited by its poor water solubility and low bioavailability [125, 126]. Indeed, a clinical trial of 2ME₂ was cancelled because its plasma concentrations after oral administration were lower than the effective dose [127]. Experiments in rats demonstrated that 2ME₂ could be detected in the plasma until 120 minutes following intravenous administration while 2ME₂ was undetectable when it was given by an oral route. This suggests that 2ME₂ may be rapidly metabolized in the gastrointestinal tract [128]. According to this assumption, Guo et al. [129] performed an in situ rat intestinal recirculation perfusion technique and demonstrated that the absorption rate constant of 2ME₂ was independent of the different 2ME₂ concentrations used in this experiment. This corroborates that the low bioavailability of 2ME₂ when it is administered orally is related to its metabolic transformation rather than its poor absorption rate in the intestinal epithelium. In this context, 2ME₂ levels found in the urine of cancer patients treated with an oral dose of 2ME₂ were close to 0.002% from the total dose administered [130]. On the other hand, plasma levels of 2-methoxyestrone (2ME₁) were 10-20 fold higher than 2ME₂ after a continuous oral administration of 2ME₂ in patients with different types of cancer [131-135]. This indicates that 2ME₂ is mainly metabolized and inactivated through its conversion to 2ME₁ probably by the enzyme 17β-HSD [131-135]. This hypothesis is supported by the fact that two breast cancer cell lines insensitive to the anti-proliferative and cytotoxic effects of 2ME₂ (ZR75 and MDA-MB-231 cells) have a higher 17β-HSD activity than other breast cancer cell lines sensitive to 2ME₂ (MCF-7, T-47D or MDA-MB-435 cells) [136, 137]. Furthermore, MCF-7 cells transfected with the enzyme 17β-HSD were insensitive to 2ME₂ [136]. Interestingly, the enzyme 17β-HSD is also highly expressed in the human gastrointestinal tract [138].

It has been shown that only 1% from 2ME₂ given orally to cancer patients was found as glucuronides in the urine whereas oxidative or sulfated metabolites of 2ME₂ were very low or undetectable [130]. This could indicate that glucuronidations might be the major pathway by which 2ME₂ is inactivated before to be excreted.

Attempts to overcome the limitation of the 2ME₂ low bioavailability have involved different strategies to develop new drug formulations. The formulation of 2ME₂ as a nano-suspension in conjunction with poly-(organophosphazenes) has been recently designed [139]. The hydrogel containing a relatively low concentration of 2ME₂ demonstrated improved antitumor and antiangiogenic activity in a mouse breast tumor model compared with the original delivery method [125]. A further approach to overcome the problem of low plasma availability could be to develop engineer modifications into the structure of 2ME₂ that increase its half-life and decrease the time-course of excretion. In this context, preparation of 2ME₂ encapsulated or adsorbed in magnetic nanoparticles (i.e. aluminoisilicate zeolites) may be a good experimental strategy to deliver this drug specifically into the tumor cells [140].

On the other hand, Stubelius et al. [31] have found that 2ME₂ increased the Natural Killer (NK) and T cells population from the bone marrow, spleen and liver in ovariectomized mice indicating a probable pharmacological role of 2ME₂ on the immune system. Furthermore, it has been reported that mice lacking COMT have an altered immune phenotype as shown by an increase in the number of T and B-lymphocytes and a high frequency of neutrophils. Furthermore, NK cell population shifted toward less mature...
Therefore, some cautions must be taken to administer pharmacological doses of 2ME₂ since unwanted effects on the system immune could occur in some patients.

8. SPECIAL FORMULATIONS OF 2-METHOXYESTRADIOL OR ITS DERIVATIVES AS PROMISSORY DRUGS IN CANCER TREATMENT

2ME₂ has already been tested as a pharmacological treatment in patients with hormone-refractory prostate cancer [131] and with different solid tumors [127]. In both cases, 2ME₂ administered as oral capsules was well tolerated by patients although it showed modest anticancer activity probably by its rapid metabolic degradation to inactive compounds (especially to 2ME₁), which significantly decreased its bioavailability. For this, several attempts have been performed in order to produce formulations of 2ME₂ or its analogs that conserve its pharmacological properties, but with a longer bioavailability than natural 2ME₂. Here, one formulation and two 2ME₂ derivatives (Fig. 2) that have been extensively studied are described.

-Panzer™: The first commercially available preparation of 2ME₂ was Panzer™ (Entremed Inc, now called CASI Pharmaceuticals Inc. Rockville, MD, USA). In this formulation, 2ME₂ is reduced to nanometer-sized particles (nanocrystal colloidal dispersion) to delay its degradation. This compound is administered orally and the maximum tolerated dose has been estimated in 1000 mg administered four times daily [132]. Panzer™ has been assayed in several phase I and II clinical trials in patients with breast cancer [133], different refractory solid tumors [134], ovarian cancer, primary peritoneal carcinomatosis [132], metastatic prostate cancer [135], metastatic kidney cancer [142] or advanced carcinoid tumors [143]. In these studies, Panzer™ was generally well tolerated although fatigue was the main side effect (55%). According with the results obtained in this study, ENMD-1198 may be a promissory 2ME₂ derivative drug in cancer treatment since it prolonged disease stabilization in some patients [147].

8.1. ENMD-1198 ([3-carboxyamide-2-methoxyestra-1,3,5(10)16-tetraene])

This 2ME₂ derivative also known as C24-883 or ENMD-0998 was developed by CASI Pharmaceuticals Inc. (Rockville, MD, USA) as an oral formulation. This compound has the ability to prevent microtubule polymerization, decrease HIF-1α expression and induce apoptosis in cancer cells. Furthermore, it shows a significant increase in its bioavailability compared with 2ME₂ [144]. ENMD-1198 also inhibits growth and vascularization of human hepatocellular carcinoma cells *in vitro* and *in vivo* [145] and reduces breast tumor burden *in vivo* [146]. This compound was tested in a clinical trial in patients with different advanced cancers [147]. The results obtained showed that ENMD-1198 was generally well tolerated although fatigue was the main side effect (55%). According with the results obtained in this study, ENMD-1198 may be a promissory 2ME₂ derivative drug in cancer treatment since it prolonged disease stabilization in some patients [147].

8.2. STX140 (2-Methoxyestradiol-3,17-O,O-bis-sulfamate)

This 2ME₂ derivative consists of a 2ME₂ molecule with a sulfamate group added in carbons 3 and 17. Firstly tested in 2003, this compound inhibited proliferation of breast cancer cell lines that were resistant and non-resistant to doxorubicin and mitoxantrone [148]. Moreover, the required dose of

Fig. (2). Molecular structures of 2ME₂ and its derivatives STX140 and ENMD1198.
STX140 was significantly lower than the 2ME2 dose to inhibit breast cancer cells growth in vitro [149] and in vivo [150, 151]. The mechanisms by which STX140 induces apoptosis and/or autophagia in cancer cells are similar to 2ME2 and involve an increase in the intracellular reactive oxygen species [152] and cell cycle blockade at G2M stage [153]. Interestingly, STX140 did not affect cell viability in a non-tumorigenic human breast cell line [154]. This compound also inhibited initiation and progression of mammary tumors in adult mice [155] suggesting that STX140 is a promissory 2ME2 analog that can be potentially used in human breast cancer.

Independent of these promissory compounds, watersoluble 2ME2 prodrugs that can be endogenously metabolized to 2ME2 has recently been synthesized. Edsall et al. [156] developed and tested the 2ME2 prodrugs 3-phosphate (2ME2-3P), 17-phosphate (2ME2-17P) and 3,17-diphosphate (2ME2-3,17DP). They demonstrated that 2ME2-3P and 2ME2-17P were metabolized to 2ME2. Furthermore, 2ME2-3P also had cytotoxic effects like 2ME2 in different cancer cell lines and in an in vivo lung carcinoma metastasis model [156]. Moreover, Kambhampati et al. [128] recently synthesized and studied the biological effects of 2-methoxy-3-yloxymethyl phosphate-17-acetoxyestradiol (2-ME2-PD1), a second generation of 2ME2 prodrugs. They found that 2-ME2-PD1 was transformed to 2ME2 and is more effective than 2ME2 to induce antiproliferative and anticancer effects in esophageal cancer as assessed in a xenograft mouse model [128].

9. POTENTIAL BIOMARKERS OF 2-METHOXY-ESTRADIOL IN HEALTH AND DISEASE

Micro array analysis is a useful tool to establish gene expression profiling in several tissues and organs. This experimental approach has been used to explore possible molecular markers involved in the antiangiogenic and proapoptotic effects of 2ME2. The apoptotic effect of 2ME2 on multiple myeloma cells is associated with the expression of genes related to the unfold protein response in the endoplasmic reticulum, heat shock proteins and the ubiquitin-proteasome pathway, and structural/cytoskeleton genes [157]. Utilizing 2ME2-treated female human aortic smooth muscle cells, Barchiesi et al. [158] reported changes in the gene expression including inhibition of molecules relevant to the cell cycle, tubulin polymerization, cholesterol and steroid synthesis, and induction of metalloproteases and cиклооксигеназа-2. Rincon-Rodriguez et al. [86] have recently described for the first time a group of genes induced by 2ME2 in the prepuberal mouse uterus. As this group of genes only correspond to the 0.23% from the total of analysed genes it is probable that the effects of 2ME2 involve a fine-tuning on the uterine tissues. Interestingly, a group of genes (0.04%) was regulated by 2ME2 but not by E2 suggesting that 2ME2 uses intracellular signalling pathways independently of E2. On the other hand, with the purpose to find new molecular markers that could be useful as a prognostic of the anticancer effects of 2ME2 we compared the effect of an antitumoral or non-antitumoral concentration of 2ME2 on the mRNA level of stearoyl-coenzyme A desaturase 2 (scd2) [159], sorting nexin 6 (snx6) [160] and F-spondin (spon1) [161] in the cancer cell line Ishikawa. Interestingly, spon1 and snx6 were increased following treatment with a anti-tumoral concentration of 2ME2, indicating that spon1 and scd2 could participate in the signaling pathway by which 2ME2 exerts apoptotic activity in tumoral cell lines [14]. Both transcripts could also be used as biomarkers in the diagnostic of cancer patients with potential probabilities for treatment with 2ME2.

10. CONCLUDING REMARKS

Given the crucial role of E2 in the human physiology, a new research avenue has now been open related with the role of estrogen metabolites as responsible for the E2 physiological and pathophysiological actions. Recent evidence indicates that physiological and pathophysiological levels of 2ME2 may regulate various biological processes on reproductive tissues, where it changes profile gene expression in the uterus, alters egg transport in the oviduct and affects male infertility. Furthermore, imbalance in the production of 2ME2 from catechol estrogens could cause several diseases as endometriosis, preeclampsia or cardiovascular disorders. Altogether, it highlights a new concept in the endocrine physiology postulating that the protective effects of E2 could be in part mediated by a previous conversion of E2 to 2ME2 in its target organs. This also demonstrates that 2ME2 is an important component in the intracellular E2 signaling pathway. On the other hand, 2ME2 has been described as a promising anticancer drug although the molecular mechanisms by which this E2 metabolite induces apoptotic and antiangiogenic activity in tumor cells are recently being disclosed. The potential therapeutic applications of 2ME2 in the anticancer strategy have been limited by its poor water solubility and low bioavailability preventing to reach the adequate plasma concentrations relative to its effective dose. For this, several attempts have been performed to produce preparations of 2ME2 or its analogs, which conserve its pharmacological properties although with a longer bioavailability than natural 2ME2. Panzem™, ENMD-1198 and STX140 are new formulations that are actually being developed in clinical trails although with modest results. A further approach to overcome 2ME2 low plasma bioavailability could be to develop engineer modifications into the structure of 2ME2, which permits its incorporation in polymeric or magnetic nanoparticles delivering this drug specifically into the tumor cells. In addition, the search for new biomarkers associated with the physiological and pathophysiological effects of 2ME2 could provide a new impulse in our understanding of the molecular basis of E2 actions on its target organs. Interestingly, transcripts that respond to 2ME2 in primary cell cultures from cancer patients could also be used as therapeutic biomarkers to enhance the probabilities for 2ME2 treatment in these patients.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.
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REFERENCES


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