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

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Abstract: Estradiol (E_2) 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 ($2ME_2$) is generated by a sequential hydroxylation of E_2 via the enzyme cytochrome P450 isoform 1A1 to produce 2-hydroxyestradiol ($2OHE_2$) followed by a conjugation reaction catalyzed by the enzyme Catechol-O-Methyltransferase generating $2ME_2$ from $2OHE_2$. Recent evidence indicates that physiological concentration of $2ME_2$ may regulate several biological processes while high concentrations of this metabolite may induce pathophysiological alterations in several tissues. In the last years, $2ME_2$ 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 $2ME_2$ in health and disease. We will focus on to describing the intracellular mechanisms by which $2ME_2$ exerts its effects on reproductive and non-reproductive tissues. The promising anticancer effects of $2ME_2$ and its synthetic derivatives will also be discussed. Finally, a group of $2ME_2$-target genes that could be used as biomarkers of $2ME_2$ under physiological or pathophysiological conditions will be reviewed.

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

1. INTRODUCTION

Estradiol (E_2) 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 E_2 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 E_2 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 E_2 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 E_2 to hydroxyestradiols and then to methoxyestradiols is one of the most recognized enzymatic pathways that inactive E_2 [12]. When methoxyestradiols were characterized they were catalogued as inactive molecules without a biological role. However, it has also been demonstrated that 2-methoxyestradiol ($2ME_2$) may exert physiological actions in different organs and tissues and that an unbalanced E_2 metabolization to 2-hydroxyestradiol ($2OHE_2$) and $2ME_2$ could be the responsible factor for several diseases including infertility, cancer or preeclampsia [13]. In this context, pharmacological doses of $2ME_2$ 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 $2ME_2$ in health and disease. The intracellular mechanisms by which $2ME_2$ exerts its effects on reproductive and non-reproductive tissues and the promising anticancer effects of $2ME_2$ and its synthetic derivatives will be discussed. Finally, a group of $2ME_2$-target genes that could be used as biomarkers of $2ME_2$ under physiological or pathophysiological conditions will be reviewed.

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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 E_2 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 E_2 by these pathways.

Estradiol is mainly inactivated through its conversion to $2OHE_2$ and subsequent generation of $2ME_2$ in extra hepatic tissues (Fig. **1**). These reactions involve a first oxidation in the carbon 2 inside the aromatic A-ring of E_2, catalyzed by the enzyme Cytochrome P450 isoform 1A1 (CYP1A1), generating a molecule of $2OHE_2$. 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 $2ME_2$ [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 $2ME_2$ could also be locally generated from E_2 in these organs.

CYP1A1 has the ability to metabolize several exogenous and endogenous compounds as E_2 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 hydroxyestradiols and catecholamines [30] by an enzymatic reaction that requires Mg^{2+}. 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-METHOXYE-STRADIOL

A variety of biological effects of $2ME_2$ 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 $2ME_2$ on its target cells will be briefly reviewed.

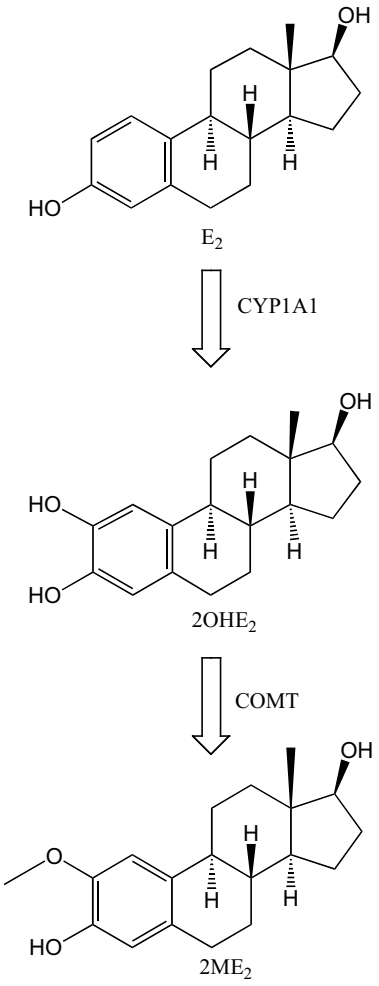

Fig. (1). Generation of 2-methoxyestradiol ($2ME_2$) from estradiol (E_2). In hepatic and extrahepatic tissues, E_2 is metabolized to 2-hydroxyestradiol ($2OHE_2$) by CYP1A1 and then $2OHE_2$ is metabolized to $2ME_2$ by COMT.

3.1. Depolarization of Microtubules

One of the first characterized effects of $2ME_2$ was its ability to inhibit tubulin polymerization [33-35]. The molecular mechanism consists in a direct interaction between $2ME_2$ and tubulin protein to inhibit the phases of nucleation and propagation during tubulin assembly [33]. Thus, $2ME_2$ 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 $2ME_2$ to induce cell death in cancer cells [37].

3.2. Reactive Oxygen Species (ROS)

The ability of $2ME_2$ to induce ROS or block its production seems to depend on the cell types. Thus, $2ME_2$ 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, $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ [50] leading to changes both in the microtubules polymerization and in the intracellular free radical levels [53-55]. This $2ME_2$ 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 $2ME_2$ modulates phosphorylation status of different proteins to exert its biological or pharmacological actions. Thus, $2ME_2$ 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, $2ME_2$ decreases ERK1/2 phosphorylation to inhibit cell growth in human aortic smooth muscle cells [58].

On the other hand, $2ME_2$ activates different protein kinases to induce cell death in cancer cells. For example, $2ME_2$ 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, $2ME_2$ 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 $2ME_2$ is its ability to modulate contraction of vascular and non-vascular smooth muscle cells. As $2ME_2$ inhibits phenylephrine-induced tension in aorta [65] and KCl-induced contraction in coronary artery [66] it is probable that $2ME_2$ has a relaxing effect on the vascular smooth muscle cells. Interestingly, $2ME_2$ exerts these effects by ER independent mechanisms although it requires an intact endothelium. On the other hand, $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ in the mammalian testis are unknown although it has recently been reported that high concentration of $2ME_2$ decreases viability of Sertoli [78] and Leydig cells [79]. This suggests that an increased intratesticular concentration of $2ME_2$ could have implications for male infertility. Interestingly, $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ are higher in the luteal than in the follicular phase [80]. Furthermore, levels of $2ME_2$ in the follicular fluid are directly correlated with the follicle size in the pig [81]. On the other hand, an excessive production of $2ME_2$ could be involved in the follicular arrest found in women with polycystic ovarian syndrome since high levels of $2ME_2$ inhibited proliferation of the granulose cells [82].

In the rat oviduct, the effect of $2ME_2$ on the oviductal egg transport is dependent of the occurrence of mating. Thus, it has been reported that $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ on the first stages of the embryo development [85]. In this context, we have recently found that a pharmacological dose of $2ME_2$ administered to pregnant mice is able to inhibit embryo implantation (unpublished observations). It is postulated that this effect of $2ME_2$ is mediated by an increase in the uterine secretion of the extracellular matrix protein F-spondin blocking the interaction between endometrial integrin $\alpha_v\beta_3$ and its ligand localized in the blastocyst [86].

In ovariectomized rats, $2ME_2$ delivered at a 1 μg/h rate by a subcutaneously implanted osmotic pump did not exert uterotrophic effects although it regulated bone metabolism

[87, 88]. Rincon-Rodriguez *et al*. [86] showed that $2ME_2$ (0.1-10 µg/mice) injected intraperitoneally during 7 days to prepuberal mouse did not change the uterine wet weight and height of epithelial cells in endometrium. In contrast, Sibonga *et al.* [88] observed that $2ME_2$ (20 - 40 mg/kg/day) administered subcutaneously to ovariectomized rats produced uterine hypertrophy and increased uterine epithelial thickness. These divergent results could be explained by a lower binding affinity of $2ME_2$ for ER as compared with E_2. It also indicates that murine endometrium is responsive to $2ME_2$.

In human endometrial tissue, S-COMT and M-COMT are expressed during the menstrual cycle. In this context, high immunoreactivity of S-COMT is observed in the proliferative phase while low expression of S-COMT occurs in the secretory phase suggesting up-regulation of COMT by estrogen and down-regulation by progesterone. This could be important to maintain the homeostasis of this tissue [82]. Endometriosis affects 10% of premenopausal women [89, 90] although the pathogenesis of this condition is not clearly understood. Singh *et al.* [91] reported the expression of CYP1A1 and CYP1B1 in eutopic and ectopic endometria from women with endometriosis suggesting participation of the estrogen metabolic pathway in this pathology. In a female mice endometriosis model, $2ME_2$ induced suppression of endometriotic lesions [92] suggesting that decreased local $2ME_2$ production in the ectopic endometrium may be one of the factors that promotes the survival of endometriotic tissue [92].

On the other hand, preeclampsia is a maternal endothelial dysfunction associated with hypertension, proteinuria and placental hypoxia during pregnancy [93]. This condition affects approximately 5% of all pregnancies worldwide and it is related with maternal and perinatal morbidity and mortality [94, 95], preterm birth or intrauterine growth foetal restriction [93]. It is postulated that preeclampsia is produced by an imbalance between expression and/or action of pro and antiangiogenic factors, which generates a poor trophoblastic invasion and incomplete spiral artery remodelling during early pregnancy [96, 97]. In the last years, it has also been proposed that a decreased $2ME_2$ production during mild pregnancy is one of the first factors associated with preeclampsia [19, 35, 98]. The mechanisms by which $2ME_2$ levels are decreased in the preeclampsia may involve reduced expression and/or activity of M-COMT and S-COMT in the placenta [19, 52]. The human COMT gene is located on chromosome 22 where it encodes for S-COMT and M-COMT [30]. COMT gene has several polymorphic sites that are associated with a high or low expression and/or enzymatic activity of COMT. For instance, COMT gene contains several single nucleotide polymorphisms as the SNPs rs4680, which determines a G/A transition (codon 158 in M-COMT and codon 108 in S-COMT gene sequence) leading to a substitution of valine by methionine. It has been determined that this methionine substitution generates a COMT enzyme with a 3-fold reduction in its protein activity compared with native COMT [30]. Some studies have found that women with preeclampsia have a high prevalence of Val158 substitution compared to normotensive pregnant women [99, 100]. This could indicate that a decreased production of $2ME_2$ observed in preeclamptic women is due to the presence of this polymorphism. On the other hand, some preeclamptic women also have decreased COMT expression in their placental tissue although the mechanisms involved in this phenomenon are not well elucidated [19]. The role of $2ME_2$ on the human placentation may be associated with the regulation of the expression and/or activity of HIF-1α in the placental tissue as shown in the human cytotrophoblast cell line HTR-8 [51]. Expression of HIF-1α is highly increased by the hypoxic environment present during the early placentation [51, 101]. However, HIF-1α levels decrease during mild placentation stage so that increased levels of this transcription factor can prevent a normal placentation. This is supported by the fact that HIF-1α overexpression in the uterus of pregnant mice is associated with fetal intrauterine growth restriction, decreased placental weight and placental abnormalities as observed in preeclamptic women [102]. Moreover, HIF-1α is highly increased in the placenta of preeclamptic women compared with normotensive pregnant women [103]. The mechanism by which high HIF-1α expression is associated with preeclampsia may involve inhibition of the VEGF signalling pathway and impaired placental perfusion [104].

5. NON-REPRODUCTIVE EFFECTS OF 2-METHOXYESTRADIOL

Estradiol may induce beneficial effects on the female physiology. Compared with age-matched men, the rate of progression of cardiovascular, renal, bone or neural diseases in premenopausal women is low. However, the progression of these pathologies is accelerated with the menopause occurrence and it is necessary hormone replacement therapy [105]. The beneficial effects of E_2 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 E_2 in the vascular system was proposed because E_2 prevents atherosclerosis by inhibiting the neointima formation in double knockout ESR1 and ESR2 mice [107]. Furthermore, $2ME_2$ is associated with inhibition of vaso-oclusive disorders because it inhibited synthesis of endothelin-1 in porcine coronary artery endothelial cells [57]. Moreover, $2ME_2$ 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 $2ME_2$ 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 CYPA1A1, CYP1B1 and COMT were expressed in vascular tissues of several mammalian species [57].

The role of $2ME_2$ 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 followinga 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 $2ME_2$ 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 $2ME_2$ following brain injury. Intraperitoneal administration of $2ME_2$(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 $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ on renal tissues are associated with a attenuation of glomeruloe sclerosis since $2ME_2$ 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 E_2 on the female health may be in part mediated by a previous conversion of E_2 to $2ME_2$ in its target organs.

6. EFFECTS OF 2-METHOXYESTRADIOL ON TUMOR CELLS

It is now well recognized that $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ 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 $2ME_2$ is mainly limited by its poor water solubility and low bioavailability [125, 126]. Indeed, a clinical trial of $2ME_2$ was cancelled because its plasma concentrations after oral administration were lower than the effective dose [127]. Experiments in rats demonstrated that $2ME_2$ could be detected in the plasma until 120 minutes following intravenous administration while $2ME_2$ was undetectable when it was given by an oral route. This suggests that $2ME_2$ 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 $2ME_2$ was independent of the different $2ME_2$ concentrations used in this experiment. This corroborates that the low bioavailability of $2ME_2$ when it is administered orally is related to its metabolic transformation rather than its poor absorption rate in the intestinal epithelium. In this context, $2ME_2$ levels found in the urine of cancer patients treated with an oral dose of $2ME_2$ 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 $2ME_2$ after a continuous oral administration of $2ME_2$ in patients with different types of cancer [131-135]. This indicates that $2ME_2$ 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 $2ME_2$ (ZR75 and MDA-MB-231 cells) have a higher 17β-HSD activity than other breast cancer cell lines sensitive to $2ME_2$ (MCF-7, T-47D or MDA-MB-435 cells) [136, 137]. Furthermore, MCF-7 cells transfected with the enzyme17β-HSD were insensitive to $2ME_2$ [136]. Interestingly, the enzyme 17β-HSD is also highly expressed in the human gastrointestinal tract [138].

It has been shown that only 1% from $2ME_2$ given orally to cancer patients was found as glucuronides in the urine whereas oxidative or sulfated metabolites of $2ME_2$ were very low or undetectable [130]. This could indicate that glucuronidations might be the major pathway by which $2ME_2$ is inactivated before to be excreted.

Attempts to overcome the limitation of the $2ME_2$ low bioavailability have involved different strategies to develop new drug formulations. The formulation of $2ME_2$ as a nano-suspension in conjunction with poly-(organophosphazenes) has been recently designed [139]. The hydrogel containing a relatively low concentration of $2ME_2$ 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 $2ME_2$ that increase its half-life and decrease the time-course of excretion. In this context, preparation of $2ME_2$ encapsulated or adsorbed in magnetic nanoparticles (i.e. alluminosilicate 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 $2ME_2$ 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 $2ME_2$ 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

cells [141]. Therefore, some cautions must be taken to administer pharmacological doses of $2ME_2$ since unwanted effects on the system immune could occur in some patients.

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

$2ME_2$ 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, $2ME_2$ 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 $2ME_2$ or its analogs that conserve its pharmacological properties, but with a longer bioavailability than natural $2ME_2$. Here, one formulation and two $2ME_2$ derivatives (Fig. **2**) that have been extensively studied are described.

-Panzem™: The first commercially available preparation of $2ME_2$ was Panzem™ (Entremed Inc, now called CASI Pharmaceuticals Inc. Rockville, MD, USA). In this formulation, $2ME_2$ 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]. Panzem™ has been assayed in several phase I and II clinical trialsin 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, Panzem™ was generally well tolerated although fatigue was the most common adverse effect in almost all patients. This compound provided better bioavailability than $2ME_2$ and also had some antitumor actions. However, its effect at clinical level was rather modest probably because it was tested in very aggressive metastatic cancer or by its even low bioavailability. With the purpose to enhance its clinical effects, Panzem™ has been administered concomitantly with a multitargeted receptor tyrosine kinase inhibitor (Sunitinib) in patients with metastatic renal cell carcinoma [142] or with an angiogenesis inhibitor (Bevacizumab) in patients with advanced carcinoid tumors [143]. However, the results obtained at clinical level were not very conclusive to demonstrate efficacy in these patients.

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

This $2ME_2$ 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 $2ME_2$ [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 $2ME_2$ derivative drug in cancer treatment since it prolonged disease stabilization in some patients [147].

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

This $2ME_2$ derivative consists of a $2ME_2$ 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

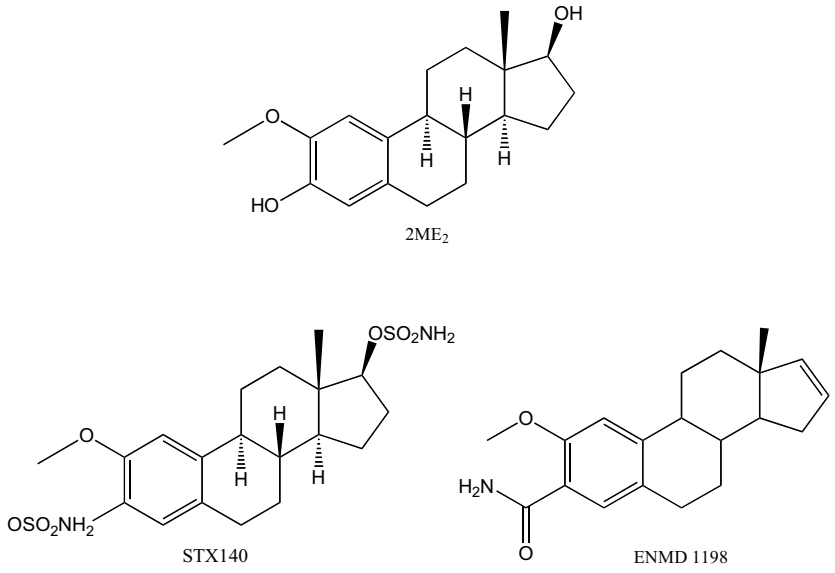

Fig. (2). Molecular structures of $2ME_2$ and its derivatives STX140 and ENMD1198.

STX140 was significantly lower than the $2ME_2$ 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 $2ME_2$, 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 $2ME_2$ analog that can be potentially used in human breast cancer.

Independent of these promissory compounds, water-soluble $2ME_2$ prodrugs that can be endogenously metabolized to $2ME_2$ has recently been synthesized. Edsall *et al.* [156] developed and tested the $2ME_2$ prodrugs 3-phosphate ($2ME_2$-3P),17-phosphate ($2ME_2$-17P) and 3,17-diphosphate ($2ME_2$-3,17DP). They demonstrated that $2ME_2$-3P and $2ME_2$-17P were metabolized to $2ME_2$. Furthermore, $2ME_2$-3P also had cytotoxic effects like $2ME_2$ 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 $2ME_2$ prodrugs. They found that 2-ME2-PD1 was transformed to $2ME_2$ and is more effective than $2ME_2$ 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 proapototic effects of $2ME_2$. The apoptotic effect of $2ME_2$ on multiple myeloma cells are 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 $2ME_2$-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 ciclooxygensae-2. Rincon-Rodriguez *et al.* [86] have recently described for the first time a group of genes induced by $2ME_2$ 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 $2ME_2$ involve a fine-tuning on the uterine tissues. Interestingly, a group of genes (0.04%) was regulated by $2ME_2$, but not by E_2 suggesting that $2ME_2$ uses intracellular signalling pathways independently of E_2. On the other hand, with the purpose to find new molecular markers that could be useful as a prognostic of the anticancer effects of $2ME_2$ we compared the effect of an antitumoral or non-antitumoral concentration of $2ME_2$ 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 $2ME_2$, indicating that *spon1* and *scd2* could participate in the signaling pathway by which $2ME_2$ 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 $2ME_2$.

10. CONCLUDING REMARKS

Given the crucial role of E_2 in the human physiology, a new research avenue has now been open related with the role of estrogen metabolites as responsible for the E_2 physiological and pathophysiological actions. Recent evidence indicates that physiological and pathophysiological levels of $2ME_2$ 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 $2ME_2$ 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 E_2 could be in part mediated by a previous conversion of E_2 to $2ME_2$ in its target organs. This also demonstrates that 2ME is an important component in the intracellular E_2 signaling pathway. On the other hand, $2ME_2$ has been described as a promising anticancer drug although the molecular mechanisms by which this E_2 metabolite induces apoptotic and antiangiogenic activity in tumor cells are recently being disclosed. The potential therapeutic applications of 2ME 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 $2ME_2$ or its analogs, which conserve its pharmacological properties although with a longer bioavailability than natural $2ME_2$. 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 $2ME_2$ low plasma bioavailability could be to develop engineer modifications into the structure of $2ME_2$, 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 2ME could provide a new impulse in our understanding of the molecular basis of E_2 actions on its target organs. Interestingly, transcripts that respond to 2ME in primary cell cultures from cancer patients could also be used as therapeutic biomarkers to enhance the probabilities for $2ME_2$ 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

[1] Carreau, S.; Bouraima-Lelong, H.; Delalande, C. Estrogens: new players in spermatogenesis. *Reprod Biol.*, **2011**, *11*(3), 174-193.

[2] Drummond, A.E.; Fuller, P.J. Ovarian actions of estrogen receptor-beta: an update. *Semin Reprod Med.*, **2012**, *30*(1), 32-38.

[3] Groothuis, P.G.; Dassen, H.H.; Romano, A.; Punyadeera, C. Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. *Hum. Reprod. Update*, **2007**, *13*(4), 405-417.

[4] Khosla, S.; Oursler, M.J.; Monroe, D.G. Estrogen and the skeleton. *Trends Endocrinol Metab.*, **2012**, *23*(11), 576-581.

[5] Brown, C.M.; Suzuki, S.; Jelks, K.A.; Wise, P.M. Estradiol is a potent protective, restorative, and trophic factor after brain injury. *Semin Reprod Med.*, **2009**, *27*(3), 240-249.

[6] Leung, S.W.; Teoh, H.; Keung, W.; Man, R.Y. Non-genomic vascular actions of female sex hormones: physiological implications and signalling pathways. *Clin. Exp. Pharmacol. Physiol.*, **2007**, *34*(8), 822-826.

[7] Cheskis, B.J.; Greger, J.G.; Nagpal, S.; Freedman, L.P. Signaling by estrogens. *J. Cell Physiol.*, **2007**, *213*(3), 610-617.

[8] Coleman, K.M.; Smith, C.L. Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. *Front Biosci.*, **2001**, *1*(6), D1379-1391.

[9] Orihuela, P.A.; Parada-Bustamante, A.; Zuniga, L.M.; Croxatto, H.B. Inositol triphosphate participates in an oestradiol nongenomic signalling pathway involved in accelerated oviductal transport in cycling rats. *J. Endocrinol.*, **2006**, *188*(3), 579-588.

[10] Tsuchiya, Y.; Nakajima, M.; Yokoi, T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett*, **2005**, *227*(2), 115-124.

[11] Parada-Bustamante, A.; Orihuela, P.A.; Rios, M.; Navarrete-Gomez, P.A.; Cuevas, C.A.; Velasquez, L.A.; Villalon, M.J.; Croxatto, H.B. Catechol-o-methyltransferase and methoxyestradiols participate in the intraoviductal nongenomic pathway through which estradiol accelerates egg transport in cycling rats. *Biol. Reprod.*, **2007**, *77*(6), 934-941.

[12] Zhu, B.T.; Conney, A.H. Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis*, **1998**, *19*(1), 1-27.

[13] Pinto, M.P.; Medina, R.A.; Owen, G.I. 2-methoxyestradiol and disorders of female reproductive tissues. *Horm Cancer*, **2014**, *5*(5), 274-283.

[14] Parada-Bustamante, A.; Orihuela, P.A.; Molina, C.; Cardenas, H.; Reuquen, P.; Valencia, C.; Rincon, R. *In: Estradiol: synthesis, health effects and drug interactions.* R. Palmieri; S. Grimaudo, Ed. Nova Science Publishers: New York, **2013**; Vol 1, pp. 121-142.

[15] Vihko, P.; Isomaa, V.; Ghosh, D. Structure and function of 17beta-hydroxysteroid dehydrogenase type 1 and type 2. *Mol. Cell Endocrinol.*, **2001**, *171*(1-2), 71-76.

[16] Kohen, P.; Henriquez, S.; Rojas, C.; Gerk, P.M.; Palomino, W.A.; Strauss, J.F., 3rd; Devoto, L. 2-Methoxyestradiol in the human corpus luteum throughout the luteal phase and its influence on lutein cell steroidogenesis and angiogenic activity. *Fertil Steril.*, **2013**, *100*(5), 1397-1404.

[17] Hevir, N.; Ribic-Pucelj, M.; Lanisnik Rizner, T. Disturbed balance between phase I and II metabolizing enzymes in ovarian endometriosis: a source of excessive hydroxy-estrogens and ROS? *Mol. Cell Endocrinol.*, **2013**, *367*(1-2), 74-84.

[18] DuBois, B.N.; O'Tierney-Ginn, P.; Pearson, J.; Friedman, J.E.; Thornburg, K.; Cherala, G. Maternal obesity alters feto-placental cytochrome P4501A1 activity. *Placenta*, **2012**, *33*(12), 1045-1051.

[19] Kanasaki, K.; Palmsten, K.; Sugimoto, H.; Ahmad, S.; Hamano, Y.; Xie, L.; Parry, S.; Augustin, H.G.; Gattone, V.H.; Folkman, J.; Strauss, J.F.; Kalluri, R. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. *Nature*, **2008**, *453*(7198), 1117-1121.

[20] John, K.; Ragavan, N.; Pratt, M.M.; Singh, P.B.; Al-Buheissi, S.; Matanhelia, S.S.; Phillips, D.H.; Poirier, M.C.; Martin, F.L. Quantification of phase I/II metabolizing enzyme gene expression and polycyclic aromatic hydrocarbon-DNA adduct levels in human prostate. *Prostate*, **2009**, *69*(5), 505-519.

[21] Vargas, C. *Expression of the enzymes CYP1A1, CYP1B1 and COMT in human testicular tissue.* Bs Thesis, Metropolitan Technological University of Chile (UTEM) and University of Chile, June **2013**.

[22] Lee, A.J.; Cai, M.X.; Thomas, P.E.; Conney, A.H.; Zhu, B.T. Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology*, **2003**, *144*(8), 3382-3398.

[23] Bock, K.W. Homeostatic control of xeno- and endobiotics in the drug-metabolizing enzyme system. *Biochem. Pharmacol.*, **2014**, *90*(1), 1-6.

[24] Badal, S.; Delgoda, R. Role of the modulation of CYP1A1 expression and activity in chemoprevention. *J. Appl. Toxicol.*, **2014**, *34*(7), 743-753.

[25] Androutsopoulos, V.P.; Tsatsakis, A.M.; Spandidos, D.A. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. *BMC Cancer*, **2009**, 9, 187.

[26] Esakky, P.; Hansen, D.A.; Drury, A.M.; Moley, K.H. Cigarette smoke condensate induces aryl hydrocarbon receptor-dependent changes in gene expression in spermatocytes. *Reprod. Toxicol.*, **2012**, *34*(4), 665-676.

[27] Raza, H.; John, A.; Nemmar, A. Short-term effects of nose-only cigarette smoke exposure on glutathione redox homeostasis, cytochrome P450 1A1/2 and respiratory enzyme activities in mice tissues. *Cell Physiol. Biochem.*, **2013**, *31*(4-5), 683-692.

[28] Mandal, P.K. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *J. Comp. Physiol. B.*, **2005**, *175*(4), 221-230.

[29] Korashy, H.M.; Abuohashish, H.M.; Maayah, Z.H. The role of aryl hydrocarbon receptor-regulated cytochrome P450 enzymes in glioma. *Curr. Pharm. Des.*, **2013**, *19*(40), 7155-7166.

[30] Mannisto, P.T.; Kaakkola, S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol. Rev.*, **1999**, *51*(4), 593-628.

[31] Stubelius, A.; Erlandsson, M.C.; Islander, U.; Carlsten, H. Immunomodulation by the estrogen metabolite 2-methoxyestradiol. *Clin. Immunol.*, **2014**, *153*(1), 40-48.

[32] Sutherland, T.E.; Schuliga, M.; Harris, T.; Eckhardt, B.L.; Anderson, R.L.; Quan, L.; Stewart, A.G. 2-methoxyestradiol is an estrogen receptor agonist that supports tumor growth in murine xenograft models of breast cancer. *Clin. Cancer Res.*, **2005**, *11*(5), 1722-1732.

[33] D'Amato, R.J.; Lin, C.M.; Flynn, E.; Folkman, J.; Hamel, E. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*(9), 3964-3968.

[34] Klauber, N.; Parangi, S.; Flynn, E.; Hamel, E.; D'Amato, R.J. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Res.*, **1997**, *57*(1), 81-86.

[35] Pribluda, V.S.; Gubish, E.R., Jr.; Lavallee, T.M.; Treston, A.; Swartz, G.M.; Green, S.J. 2-Methoxyestradiol: an endogenous antiangiogenic and antiproliferative drug candidate. *Cancer Metastasis Rev.*, **2000**, *19*(1-2), 173-179.

[36] Thaver, V.; Lottering, M.L.; van Papendorp, D.; Joubert, A. *In vitro* effects of 2-methoxyestradiol on cell numbers, morphology, cell cycle progression, and apoptosis induction in oesophageal carcinoma cells. *Cell Biochem. Funct.*, **2009**, *27*(4), 205-210.

[37] Chua, Y.S.; Chua, Y.L.; Hagen, T. Structure activity analysis of 2-methoxyestradiol analogues reveals targeting of microtubules as the major mechanism of antiproliferative and proapoptotic activity. *Mol. Cancer Ther.*, **2010**, *9*(1), 224-235.

[38] Stander, B.A.; Marais, S.; Vorster, C.J.; Joubert, A.M. *In vitro* effects of 2-methoxyestradiol on morphology, cell cycle progression, cell death and gene expression changes in the tumorigenic MCF-7 breast epithelial cell line. *J. Steroid Biochem. Mol. Biol.*, **2010**, *119*(3-5), 149-160.

[39] Ting, C.M.; Lee, Y.M.; Wong, C.K.; Wong, A.S.; Lung, H.L.; Lung, M.L.; Lo, K.W.; Wong, R.N.; Mak, N.K. 2-Methoxyestradiol

induces endoreduplication through the induction of mitochondrial oxidative stress and the activation of MAPK signaling pathways. *Biochem. Pharmacol.*, **2010**, *79*(6), 825-841.

[40] Kato, S.; Sadarangani, A.; Lange, S.; Delpiano, A.M.; Vargas, M.; Branes, J.; Carvajal, J.; Lipkowitz, S.; Owen, G.I.; Cuello, M.A. 2-methoxyestradiol mediates apoptosis through caspase-dependent and independent mechanisms in ovarian cancer cells but not in normal counterparts. *Reprod Sci.*, **2008**, *15*(9), 878-894.

[41] Zhang, Q.; Ma, Y.; Cheng, Y.F.; Li, W.J.; Zhang, Z.; Chen, S.Y. Involvement of reactive oxygen species in 2-methoxyestradiol-induced apoptosis in human neuroblastoma cells. *Cancer Lett.*, **2011**, *313*(2), 201-210.

[42] Fussell, K.C.; Udasin, R.G.; Smith, P.J.; Gallo, M.A.; Laskin, J.D. Catechol metabolites of endogenous estrogens induce redox cycling and generate reactive oxygen species in breast epithelial cells. *Carcinogenesis*, **2011**, *32*(8), 1285-1293.

[43] Stubelius, A.; Andreasson, E.; Karlsson, A.; Ohlsson, C.; Tivesten, A.; Islander, U.; Carlsten, H. Role of 2-methoxyestradiol as inhibitor of arthritis and osteoporosis in a model of postmenopausal rheumatoid arthritis. *Clin Immunol.*, **2011**, *140*(1), 37-46.

[44] Basini, G.; Santini, S.E.; Grasselli, F. 2-Methoxyestradiol inhibits superoxide anion generation while it enhances superoxide dismutase activity in swine granulosa cells. *Ann. N.Y. Acad. Sci.*, **2006**, 34-40.

[45] Liu, W.; Shen, S.M.; Zhao, X.Y.; Chen, G.Q. Targeted genes and interacting proteins of hypoxia inducible factor-1. *Int. J. Biochem. Mol. Biol.*, **2012**, *3*(2), 165-178.

[46] Brocato, J.; Chervona, Y.; Costa, M. Molecular responses to hypoxia-inducible factor 1alpha and beyond. *Mol. Pharmacol.*, **2014**, *85*(5), 651-657.

[47] Zhou, H.; Chen, X.; Zhang, W.M.; Zhu, L.P.; Cheng, L. HIF-1 alpha inhibition reduces nasal inflammation in a murine allergic rhinitis model. *PLoS One*, **2012**, *7*(11), 1.

[48] Mo, J.H.; Kim, J.H.; Lim, D.J.; Kim, E.H. The role of hypoxia-inducible factor 1alpha in allergic rhinitis. *Am. J. Rhinol. Allergy*, **2014**, *28*(2), e100-106.

[49] Shenaq, M.; Kassem, H.; Peng, C.; Schafer, S.; Ding, J.Y.; Fredrickson, V.; Guthikonda, M.; Kreipke, C.W.; Rafols, J.A.; Ding, Y. Neuronal damage and functional deficits are ameliorated by inhibition of aquaporin and HIF1alpha after traumatic brain injury (TBI). *J. Neurol. Sci.*, **2012**, *323*(1-2), 134-140.

[50] Schaible, E.V.; Windschugl, J.; Bobkiewicz, W.; Kaburov, Y.; Dangel, L.; Kramer, T.; Huang, C.; Sebastiani, A.; Luh, C.; Werner, C.; Engelhard, K.; Thal, S.C.; Schafer, M.K. 2-Methoxyestradiol confers neuroprotection and inhibits a maladaptive HIF-1alpha response after traumatic brain injury in mice. *J. Neurochem.*, **2014**, *129*(6), 940-954.

[51] Lee, S.B.; Wong, A.P.; Kanasaki, K.; Xu, Y.; Shenoy, V.K.; McElrath, T.F.; Whitesides, G.M.; Kalluri, R. Preeclampsia: 2-methoxyestradiol induces cytotrophoblast invasion and vascular development specifically under hypoxic conditions. *Am. J. Pathol.*, **2010**, *176*(2), 710-720.

[52] Perez-Sepulveda, A.; Espana-Perrot, P.P.; Norwitz, E.R.; Illanes, S.E. Metabolic pathways involved in 2-methoxyestradiol synthesis and their role in preeclampsia. *Reprod Sci.*, **2013**, *20*(9), 1020-1029.

[53] Mabjeesh, N.J.; Escuin, D.; LaVallee, T.M.; Pribluda, V.S.; Swartz, G.M.; Johnson, M.S.; Willard, M.T.; Zhong, H.; Simons, J.W.; Giannakakou, P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell.*, **2003**, *3*(4), 363-375.

[54] Carbonaro, M.; O'Brate, A.; Giannakakou, P. Microtubule disruption targets HIF-1alpha mRNA to cytoplasmic P-bodies for translational repression. *J. Cell Biol.*, **2011**, *192*(1), 83-99.

[55] Banerjee, S.; Randeva, H.; Chambers, A.E. Mouse models for preeclampsia: disruption of redox-regulated signaling. *Reprod Biol Endocrinol*, **2009**, 7, 4.

[56] Koganti, S.; Snyder, R.; Gumaste, U.; Karamyan, V.T.; Thekkumkara, T. 2-methoxyestradiol binding of GPR30 down-regulates angiotensin AT(1) receptor. *Eur. J. Pharmacol.*, **2014**, 723, 131-140.

[57] Dubey, R.K.; Jackson, E.K.; Keller, P.J.; Imthurn, B.; Rosselli, M. Estradiol metabolites inhibit endothelin synthesis by an estrogen receptor-independent mechanism. *Hypertension*, **2001**, *37*(2 Pt 2), 640-644.

[58] Barchiesi, F.; Jackson, E.K.; Fingerle, J.; Gillespie, D.G.; Odermatt, B.; Dubey, R.K. 2-Methoxyestradiol, an estradiol metabolite, inhibits neointima formation and smooth muscle cell growth via double blockade of the cell cycle. *Circ. Res.*, **2006**, *99*(3), 266-274.

[59] Chang, I.; Majid, S.; Saini, S.; Zaman, M.S.; Yamamura, S.; Chiyomaru, T.; Shahryari, V.; Fukuhara, S.; Deng, G.; Dahiya, R.; Tanaka, Y. Hrk mediates 2-methoxyestradiol-induced mitochondrial apoptotic signaling in prostate cancer cells. *Mol. Cancer Ther.*, **2013**, *12*(6), 1049-1059.

[60] Lorin, S.; Borges, A.; Ribeiro Dos Santos, L.; Souquere, S.; Pierron, G.; Ryan, K.M.; Codogno, P.; Djavaheri-Mergny, M. c-Jun NH2-terminal kinase activation is essential for DRAM-dependent induction of autophagy and apoptosis in 2-methoxyestradiol-treated Ewing sarcoma cells. *Cancer Res.*, **2009**, *69*(17), 6924-6931.

[61] Lorin, S.; Pierron, G.; Ryan, K.M.; Codogno, P.; Djavaheri-Mergny, M. Evidence for the interplay between JNK and p53-DRAM signalling pathways in the regulation of autophagy. *Autophagy*, **2010**, *6*(1), 153-154.

[62] Lee, Y.M.; Ting, C.M.; Cheng, Y.K.; Fan, T.P.; Wong, R.N.; Lung, M.L.; Mak, N.K. Mechanisms of 2-methoxyestradiol-induced apoptosis and G2/M cell-cycle arrest of nasopharyngeal carcinoma cells. *Cancer Lett.*, **2008**, *268*(2), 295-307.

[63] Yang, C.; Shogren, K.L.; Goyal, R.; Bravo, D.; Yaszemski, M.J.; Maran, A. RNA-dependent protein kinase is essential for 2-methoxyestradiol-induced autophagy in osteosarcoma cells. *PLoS One*, **2013**, *8*(3), 19.

[64] Joubert, A.; Marais, S. *In vitro* effects of 2-methoxyestradiol on cell morphology and Cdc2 kinase activity in SNO oesophageal carcinoma cells. *Cell Biochem. Funct.*, **2007**, *25*(3), 357-362.

[65] Gui, Y.; Zheng, X.L.; Zheng, J.; Walsh, M.P. Inhibition of rat aortic smooth muscle contraction by 2-methoxyestradiol. *Am. J. Physiol. Heart Circ. Physiol.*, **2008**, *295*(5), H1935-1942.

[66] Hill, B.J.; Gebre, S.; Schlicker, B.; Jordan, R.; Necessary, S. Nongenomic inhibition of coronary constriction by 17ss-estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol. *Can. J. Physiol. Pharmacol.*, **2010**, *88*(2), 147-152.

[67] Goyache, F.M.; Gutierrez, M.; Hidalgo, A.; Cantabrana, B. Non-genomic effects of catecholestrogens in the *in vitro* rat uterine contraction. *Gen. Pharmacol.*, **1995**, *26*(1), 219-223.

[68] Croxatto, H. *Gamete transport* J.R. EY Adashi, and Z Rosenwaks Z. *,* Lippincott-Raven Publishers: Philadelphia, **1996**; 1, pp. 385-402.

[69] Sutter, T.R.; Tang, Y.M.; Hayes, C.L.; Wo, Y.Y.; Jabs, E.W.; Li, X.; Yin, H.; Cody, C.W.; Greenlee, W.F. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. Biol. Chem.*, **1994**, *269*(18), 13092-13099.

[70] Shimada, T.; Hayes, C.L.; Yamazaki, H.; Amin, S.; Hecht, S.S.; Guengerich, F.P.; Sutter, T.R. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.*, **1996**, *56*(13), 2979-2984.

[71] Nishimura, M.; Yaguti, H.; Yoshitsugu, H.; Naito, S.; Satoh, T. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi*, **2003**, *123*(5), 369-375.

[72] Leung, G.S.; Kawai, M.; Tai, J.K.; Chen, J.; Bandiera, S.M.; Chang, T.K. Developmental expression and endocrine regulation of CYP1B1 in rat testis. *Drug Metab. Dispos.*, **2009**, *37*(3), 523-528.

[73] Deb, S.; Kawai, M.; Chang, T.K.; Bandiera, S.M. CYP1B1 expression in rat testis and Leydig cells is not inducible by aryl hydrocarbon receptor agonists. *Xenobiotica*, **2010**, *40*(7), 447-457.

[74] Choudhary, D.; Jansson, I.; Schenkman, J.B.; Sarfarazi, M.; Stoilov, I. Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. *Arch. Biochem. Biophys.*, **2003**, *414*(1), 91-100.

[75] Revel, A.; Raanani, H.; Younglai, E.; Xu, J.; Han, R.; Savouret, J.F.; Casper, R.F. Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. *Reprod. Toxicol.*, **2001**, *15*(5), 479-486.

[76] Overbye, A.; Seglen, P.O. Phosphorylated and non-phosphorylated forms of catechol O-methyltransferase in rat liver, brain and other tissues. *Biochem. J.*, **2009**, *417*(2), 535-545.

[77] Axelrod, L.R.; Goldzieher, J.W. Formation of 2-methoxyestradiol-17beta in human steroid-producing tissues by a transmethylation reaction. *Endocrinology*, **1962**, 70, 943-945.

[78] Molina, C. *Effects of Hydroxyestradiols and Methoxyestradiols on viability and transcriptional response to testosterone in isolated Sertoli cells.* Bs Thesis, Metropolitan Technological University of Chile (UTEM) and University of Chile, March **2013**

[79] Valencia, C. *Effects of Hydroxyestradiols and Methoxyestradiols on viability of isolated Leydig cells.* Bs Thesis, University of Frontier of Chile (UFRO) and University of Chile, January **2014**

[80] Mueck, A.O.; Seeger, H. 2-Methoxyestradiol--biology and mechanism of action. *Steroids*, **2010**, *75*(10), 625-631.

[81] Bianchi, F.; Careri, M.; Mangia, A.; Musci, M.; Santini, S.E.; Basini, G. Porcine follicular fluids: comparison of solid-phase extraction and matrix solid-phase dispersion for the GC-MS determination of hormones during follicular growth. *J. Pharm. Biomed. Anal.*, **2007**, *44*(3), 711-717.

[82] Salih, S.M.; Salama, S.A.; Fadl, A.A.; Nagamani, M.; Al-Hendy, A. Expression and cyclic variations of catechol-O-methyl transferase in human endometrial stroma. *Fertil Steril.*, **2008**, *90*(3), 789-797.

[83] Parada-Bustamante, A.; Orihuela, P.A.; Rios, M.; Cuevas, C.A.; Orostica, M.L.; Velasquez, L.A.; Villalon, M.J.; Croxatto, H.B. A non-genomic signaling pathway shut down by mating changes the estradiol-induced gene expression profile in the rat oviduct. *Reproduction*, **2010**, *139*(3), 631-644.

[84] Orostica, M.L.; Lopez, J.; Rojas, I.; Rocco, J.; Diaz, P.; Reuquen, P.; Cardenas, H.; Parada-Bustamante, A.; Orihuela, P.A. Estradiol increases cAMP in the oviductal secretory cells through a nongenomic mechanism. *Reproduction*, **2014**, *148*(3), 285-294.

[85] Lattanzi, M.L.; Santos, C.B.; Mudry, M.D.; Baranao, J.L. Exposure of bovine oocytes to the endogenous metabolite 2-methoxyestradiol during *in vitro* maturation inhibits early embryonic development. *Biol. Reprod.*, **2003**, *69*(6), 1793-1800.

[86] Rincon-Rodriguez, R.J.; Orostica, M.L.; Diaz, P.; Reuquen, P.; Cardenas, H.; Orihuela, P.A. Changes in the gene expression pattern induced by 2-methoxyestradiol in the mouse uterus. *Endocrine*, **2013**, *44*(3), 773-783.

[87] Martucci, C.P.; Fishman, J. Impact of continuously administered catechol estrogens on uterine growth and luteinizing hormone secretion. *Endocrinology*, **1979**, *105*(6), 1288-1292.

[88] Sibonga, J.D.; Lotinun, S.; Evans, G.L.; Pribluda, V.S.; Green, S.J.; Turner, R.T. Dose-response effects of 2-methoxyestradiol on estrogen target tissues in the ovariectomized rat. *Endocrinology*, **2003**, *144*(3), 785-792.

[89] Davis, C.J.; McMillan, L. Pain in endometriosis: effectiveness of medical and surgical management. *Curr. Opin. Obstet. Gynecol.*, **2003**, *15*(6), 507-512.

[90] Bulun, S.E. Endometriosis. *N Engl J Med*, **2009**, *360*(3), 268-279.

[91] Singh, M.N.; Stringfellow, H.F.; Taylor, S.E.; Ashton, K.M.; Ahmad, M.; Abdo, K.R.; El-Agnaf, O.M.; Martin-Hirsch, P.L.; Martin, F.L. Elevated expression of CYP1A1 and gamma-SYNUCLEIN in human ectopic (ovarian) endometriosis compared with eutopic endometrium. *Mol. Hum. Reprod.*, **2008**, *14*(11), 655-663.

[92] Becker, C.M.; Rohwer, N.; Funakoshi, T.; Cramer, T.; Bernhardt, W.; Birsner, A.; Folkman, J.; D'Amato, R.J. 2-methoxyestradiol inhibits hypoxia-inducible factor-1{alpha} and suppresses growth of lesions in a mouse model of endometriosis. *Am. J. Pathol.*, **2008**, *172*(2), 534-544.

[93] Sibai, B.; Dekker, G.; Kupferminc, M. Pre-eclampsia. *Lancet*, **2005**, *365*(9461), 785-799.

[94] Chesley, L.C. Hypertensive disorders in pregnancy. *J. Nurse Midwifery*, **1985**, *30*(2), 99-104.

[95] Roberts, J.M.; Taylor, R.N.; Musci, T.J.; Rodgers, G.M.; Hubel, C.A.; McLaughlin, M.K. Preeclampsia: an endothelial cell disorder. *Am. J. Obstet. Gynecol.*, **1989**, *161*(5), 1200-1204.

[96] Dragun, D.; Haase-Fielitz, A. Low catechol-O-methyltransferase and 2-methoxyestradiol in preeclampsia: more than a unifying hypothesis. *Nephrol. Dial. Transplant*, **2009**, *24*(1), 31-33.

[97] Shenoy, V.; Kanasaki, K.; Kalluri, R. Pre-eclampsia: connecting angiogenic and metabolic pathways. *Trends Endocrinol Metab.*, **2010**, *21*(9), 529-536.

[98] Jobe, S.O.; Ramadoss, J.; Wargin, A.J.; Magness, R.R. Estradiol-17beta and its cytochrome P450- and catechol-O-methyltransferase-derived metabolites selectively stimulate production of prostacyclin in uterine artery endothelial cells: role of estrogen receptor-alpha versus estrogen receptor-beta. *Hypertension*, **2013**, *61*(2), 509-518.

[99] Lim, J.H.; Kim, S.Y.; Kim do, J.; Park, S.Y.; Han, H.W.; Han, J.Y.; Lee, S.W.; Yang, J.H.; Ryu, H.M. Genetic polymorphism of catechol-O-methyltransferase and cytochrome P450c17alpha in preeclampsia. *Pharmacogenet Genomics*, **2010**, *20*(10), 605-610.

[100] Liang, S.; Liu, X.; Fan, P.; Liu, R.; Zhang, J.; He, G.; Liu, Y.; Bai, H. Association between Val158Met functional polymorphism in the COMT gene and risk of preeclampsia in a Chinese population. *Arch. Med. Res.*, **2012**, *43*(2), 154-158.

[101] Caniggia, I.; Mostachfi, H.; Winter, J.; Gassmann, M.; Lye, S.J.; Kuliszewski, M.; Post, M. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J. Clin. Invest.*, **2000**, *105*(5), 577-587.

[102] Tal, R.; Shaish, A.; Barshack, I.; Polak-Charcon, S.; Afek, A.; Volkov, A.; Feldman, B.; Avivi, C.; Harats, D. Effects of hypoxia-inducible factor-1alpha overexpression in pregnant mice: possible implications for preeclampsia and intrauterine growth restriction. *Am. J. Pathol.*, **2010**, *177*(6), 2950-2962.

[103] Nishi, H.; Nakada, T.; Kyo, S.; Inoue, M.; Shay, J.W.; Isaka, K. Hypoxia-inducible factor 1 mediates upregulation of telomerase (hTERT). *Mol. Cell Biol.*, **2004**, *24*(13), 6076-6083.

[104] Vuorela, P.; Helske, S.; Hornig, C.; Alitalo, K.; Weich, H.; Halmesmaki, E. Amniotic fluid--soluble vascular endothelial growth factor receptor-1 in preeclampsia. *Obstet. Gynecol.*, **2000**, *95*(3), 353-357.

[105] Sood, R.; Faubion, S.S.; Kuhle, C.L.; Thielen, J.M.; Shuster, L.T. Prescribing menopausal hormone therapy: an evidence-based approach. *Int. J. Womens Health*, **2014**, 6, 47-57.

[106] Resanovic, I.; Rizzo, M.; Zafirovic, S.; Bjelogrlic, P.; Perovic, M.; Savic, K.; Patti, A.M.; Isenovic, R.E. Anti-atherogenic effects of 17beta-estradiol. *Horm Metab. Res.*, **2013**, *45*(10), 701-708.

[107] Karas, R.H.; Hodgin, J.B.; Kwoun, M.; Krege, J.H.; Aronovitz, M.; Mackey, W.; Gustafsson, J.A.; Korach, K.S.; Smithies, O.; Mendelsohn, M.E. Estrogen inhibits the vascular injury response in estrogen receptor beta-deficient female mice. *Proc. Natl. Acad Sci. U.S.A.*, **1999**, *96*(26), 15133-15136.

[108] Koganti, S.; Snyder, R.; Thekkumkara, T. Pharmacologic effects of 2-methoxyestradiol on angiotensin type 1 receptor down-regulation in rat liver epithelial and aortic smooth muscle cells. *Gend. Med.*, **2012**, *9*(2), 76-93.

[109] Semenza, G.L. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell*, **2001**, *107*(1), 1-3.

[110] Chang, Y.; Hsiao, G.; Chen, S.H.; Chen, Y.C.; Lin, J.H.; Lin, K.H.; Chou, D.S.; Sheu, J.R. Tetramethylpyrazine suppresses HIF-1alpha, TNF-alpha, and activated caspase-3 expression in middle cerebral artery occlusion-induced brain ischemia in rats. *Acta Pharmacol. Sin.*, **2007**, *28*(3), 327-333.

[111] Chen, W.; Jadhav, V.; Tang, J.; Zhang, J.H. HIF-1alpha inhibition ameliorates neonatal brain injury in a rat pup hypoxic-ischemic model. *Neurobiol. Dis.*, **2008**, *31*(3), 433-441.

[112] Mooberry, S.L. Mechanism of action of 2-methoxyestradiol: new developments. *Drug Resist. Updat*, **2003**, *6*(6), 355-361.

[113] Matsumoto, C.; Shinkai, T.; Hori, H.; Ohmori, O.; Nakamura, J. Polymorphisms of dopamine degradation enzyme (COMT and MAO) genes and tardive dyskinesia in patients with schizophrenia. *Psychiatry Res.*, **2004**, *127*(1-2), 1-7.

[114] Abdolmaleky, H.M.; Cheng, K.H.; Faraone, S.V.; Wilcox, M.; Glatt, S.J.; Gao, F.; Smith, C.L.; Shafa, R.; Aeali, B.; Carnevale, J.; Pan, H.; Papageorgis, P.; Ponte, J.F.; Sivaraman, V.; Tsuang, M.T.; Thiagalingam, S. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Hum. Mol. Genet*, **2006**, *15*(21), 3132-3145.

[115] Xiao, S.; Gillespie, D.G.; Baylis, C.; Jackson, E.K.; Dubey, R.K. Effects of estradiol and its metabolites on glomerular endothelial nitric oxide synthesis and mesangial cell growth. *Hypertension*, **2001**, *37*(2 Pt 2), 645-650.

[116] Tofovic, P.S.; Rosado, B.M.; Dubey, K.R.; Mi, Z.; Jackson, E.K. Effects of estradiol metabolites on cAMP production and degradation. *Prilozi*, **2009**, *30*(1), 5-24.

[117] Schumacher, G.; Kataoka, M.; Roth, J.A.; Mukhopadhyay, T. Potent antitumor activity of 2-methoxyestradiol in human pancreatic cancer cell lines. *Clin. Cancer Res.*, **1999**, *5*(3), 493-499.

[118] Arbiser, J.L.; Panigrathy, D.; Klauber, N.; Rupnick, M.; Flynn, E.; Udagawa, T.; D'Amato, R.J. The antiangiogenic agents TNP-470 and 2-methoxyestradiol inhibit the growth of angiosarcoma in mice. *J. Am. Acad. Dermatol.*, **1999**, *40*(6 Pt 1), 925-929.

[119] Dubey, R.K.; Gillespie, D.G.; Keller, P.J.; Imthurn, B.; Zacharia, L.C.; Jackson, E.K. Role of methoxyestradiols in the growth inhibitory effects of estradiol on human glomerular mesangial cells. *Hypertension*, **2002**, *39*(2 Pt 2), 418-424.

[120] Fotsis, T.; Zhang, Y.; Pepper, M.S.; Adlercreutz, H.; Montesano, R.; Nawroth, P.P.; Schweigerer, L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature*, **1994**, *368*(6468), 237-239.

[121] LaVallee, T.M.; Zhan, X.H.; Johnson, M.S.; Herbstritt, C.J.; Swartz, G.; Williams, M.S.; Hembrough, W.A.; Green, S.J.; Pribluda, V.S. 2-methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway. *Cancer Res.*, **2003**, *63*(2), 468-475.

[122] Lambert, C.; Apel, K.; Biesalski, H.K.; Frank, J. 2-methoxyestradiol induces caspase-independent, mitochondria-centered apoptosis in DS-sarcoma cells. *Int. J. Cancer*, **2004**, *108*(4), 493-501.

[123] Feng, Y.; Zhao, X.; Zhou, C.; Yang, L.; Liu, Y.; Bian, C.; Gou, J.; Lin, X.; Wang, Z. The associations between the Val158Met in the catechol-O-methyltransferase (COMT) gene and the risk of uterine leiomyoma (ULM). *Gene*, **2013**, *529*(2), 296-299.

[124] Lin, G.; Zhao, J.; Wu, J.; Andreevich, O.R.; Zhang, W.H.; Zhang, Y.; Yu, L. Contribution of catechol-O-methyltransferase Val158Met polymorphism to endometrial cancer risk in postmenopausal women: a meta-analysis. *Genet Mol. Res.*, **2013**, *12*(4), 6442-6453.

[125] Cho, J.K.; Hong, K.Y.; Park, J.W.; Yang, H.K.; Song, S.C. Injectable delivery system of 2-methoxyestradiol for breast cancer therapy using biodegradable thermosensitive poly(organophosphazene) hydrogel. *J. Drug Target*, **2011**, *19*(4), 270-280.

[126] Verenich, S.; Gerk, P.M. Therapeutic promises of 2-methoxyestradiol and its drug disposition challenges. *Mol. Pharm.*, **2010**, *7*(6), 2030-2039.

[127] Dahut, W.L.; Lakhani, N.J.; Gulley, J.L.; Arlen, P.M.; Kohn, E.C.; Kotz, H.; McNally, D.; Parr, A.; Nguyen, D.; Yang, S.X.; Steinberg, S.M.; Venitz, J.; Sparreboom, A.; Figg, W.D. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. *Cancer Biol. Ther.*, **2006**, *5*(1), 22-27.

[128] Kambhampati, S.; Rajewski, R.A.; Tanol, M.; Haque, I.; Das, A.; Banerjee, S.; Jha, S.; Burns, D.; Borrego-Diaz, E.; Van Veldhuizen, P.J.; Banerjee, S.K. A second-generation 2-Methoxyestradiol prodrug is effective against Barrett's adenocarcinoma in a mouse xenograft model. *Mol. Cancer Ther.*, **2013**, *12*(3), 255-263.

[129] Guo, X.H.; Zhang, N.; Cui, F.D.; Du, B.; Zhang, Z.Z. An investigation on intestinal absorption of a new anticancer drug, 2-methoxyestradiol. *Pharmazie*, **2009**, *64*(11), 748-751.

[130] Lakhani, N.J.; Sparreboom, A.; Xu, X.; Veenstra, T.D.; Venitz, J.; Dahut, W.L.; Figg, W.D. Characterization of *in vitro* and *in vivo* metabolic pathways of the investigational anticancer agent, 2-methoxyestradiol. *J. Pharm. Sci.*, **2007**, *96*(7), 1821-1831.

[131] Sweeney, C.; Liu, G.; Yiannoutsos, C.; Kolesar, J.; Horvath, D.; Staab, M.J.; Fife, K.; Armstrong, V.; Treston, A.; Sidor, C.; Wilding, G. A phase II multicenter, randomized, double-blind, safety trial assessing the pharmacokinetics, pharmacodynamics, and efficacy of oral 2-methoxyestradiol capsules in hormone-refractory prostate cancer. *Clin. Cancer Res.*, **2005**, *11*(18), 6625-6633.

[132] Matei, D.; Schilder, J.; Sutton, G.; Perkins, S.; Breen, T.; Quon, C.; Sidor, C. Activity of 2 methoxyestradiol (Panzem NCD) in advanced, platinum-resistant ovarian cancer and primary peritoneal carcinomatosis: a Hoosier Oncology Group trial. *Gynecol. Oncol.*, **2009**, *115*(1), 90-96.

[133] James, J.; Murry, D.J.; Treston, A.M.; Storniolo, A.M.; Sledge, G.W.; Sidor, C.; Miller, K.D. Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer. *Invest. New Drugs*, **2007**, *25*(1), 41-48.

[134] Tevaarwerk, A.J.; Holen, K.D.; Alberti, D.B.; Sidor, C.; Arnott, J.; Quon, C.; Wilding, G.; Liu, G. Phase I trial of 2-methoxyestradiol NanoCrystal dispersion in advanced solid malignancies. *Clin Cancer Res.*, **2009**, *15*(4), 1460-1465.

[135] Harrison, M.R.; Hahn, N.M.; Pili, R.; Oh, W.K.; Hammers, H.; Sweeney, C.; Kim, K.; Perlman, S.; Arnott, J.; Sidor, C.; Wilding, G.; Liu, G. A phase II study of 2-methoxyestradiol (2ME2) NanoCrystal(R) dispersion (NCD) in patients with taxane-refractory, metastatic castrate-resistant prostate cancer (CRPC). *Invest. New Drugs*, **2011**, *29*(6), 1465-1474.

[136] Newman, S.P.; Ireson, C.R.; Tutill, H.J.; Day, J.M.; Parsons, M.F.; Leese, M.P.; Potter, B.V.; Reed, M.J.; Purohit, A. The role of 17beta-hydroxysteroid dehydrogenases in modulating the activity of 2-methoxyestradiol in breast cancer cells. *Cancer Res.*, **2006**, *66*(1), 324-330.

[137] Liu, Z.J.; Lee, W.J.; Zhu, B.T. Selective insensitivity of ZR-75-1 human breast cancer cells to 2-methoxyestradiol: evidence for type II 17beta-hydroxysteroid dehydrogenase as the underlying cause. *Cancer Res.*, **2005**, *65*(13), 5802-5811.

[138] Sano, T.; Hirasawa, G.; Takeyama, J.; Darnel, A.D.; Suzuki, T.; Moriya, T.; Kato, K.; Sekine, H.; Ohara, S.; Shimosegawa, T.; Nakamura, J.; Yoshihama, M.; Harada, N.; Sasano, H. 17 beta-Hydroxysteroid dehydrogenase type 2 expression and enzyme activity in the human gastrointestinal tract. *Clin. Sci.* (*Lond)*, **2001**, *101*(5), 485-491.

[139] Du, B.; Li, X.T.; Zhao, Y.; A, Y.M.; Zhang, Z.Z. Preparation and characterization of freeze-dried 2-methoxyestradiol nanoparticle powders. *Pharmazie*, **2010**, *65*(7), 471-476.

[140] Gutierrez, M.; Escudey, M.; Escrig, J.; Denardin, J.C.; Altbir, D.; Fabris, J.D.; Cavalcante, L.C.D.; Garcia-Gonzalez, M.T. Preparation and Characterization of Magnetic Composites Based on a Natural Zeolite. *Clays and Clay Minerals*, **2010**, *58*(5), 589-595.

[141] Stubelius, A.; Wilhelmson, A.S.; Gogos, J.A.; Tivesten, A.; Islander, U.; Carlsten, H. Sexual dimorphisms in the immune system of catechol-O-methyltransferase knockout mice. *Immunobiology*, **2012**, *217*(8), 751-760.

[142] Bruce, J.Y.; Eickhoff, J.; Pili, R.; Logan, T.; Carducci, M.; Arnott, J.; Treston, A.; Wilding, G.; Liu, G. A phase II study of 2-methoxyestradiol nanocrystal colloidal dispersion alone and in combination with sunitinib malate in patients with metastatic renal cell carcinoma progressing on sunitinib malate. *Invest. New Drugs*, **2012**, *30*(2), 794-802.

[143] Kulke, M.H.; Chan, J.A.; Meyerhardt, J.A.; Zhu, A.X.; Abrams, T.A.; Blaszkowsky, L.S.; Regan, E.; Sidor, C.; Fuchs, C.S. A prospective phase II study of 2-methoxyestradiol administered in combination with bevacizumab in patients with metastatic carcinoid tumors. *Cancer Chemother Pharmacol.*, **2011**, *68*(2), 293-300.

[144] LaVallee, T.M.; Burke, P.A.; Swartz, G.M.; Hamel, E.; Agoston, G.E.; Shah, J.; Suwandi, L.; Hanson, A.D.; Fogler, W.E.; Sidor, C.F.; Treston, A.M. Significant antitumor activity *in vivo* following treatment with the microtubule agent ENMD-1198. *Mol. Cancer Ther.*, **2008**, *7*(6), 1472-1482.

[145] Moser, C.; Lang, S.A.; Mori, A.; Hellerbrand, C.; Schlitt, H.J.; Geissler, E.K.; Fogler, W.E.; Stoeltzing, O. ENMD-1198, a novel tubulin-binding agent reduces HIF-1alpha and STAT3 activity in human hepatocellular carcinoma(HCC) cells, and inhibits growth and vascularization *in vivo*. *BMC Cancer*, **2008**, 8, 206.

[146] Snoeks, T.J.; Mol, I.M.; Que, I.; Kaijzel, E.L.; Lowik, C.W. 2-methoxyestradiol analogue ENMD-1198 reduces breast cancer-induced osteolysis and tumor burden both *in vitro* and *in vivo*. *Mol. Cancer Ther.*, **2011**, *10*(5), 874-882.

[147] Zhou, Q.; Gustafson, D.; Nallapareddy, S.; Diab, S.; Leong, S.; Lewis, K.; Gore, L.; Messersmith, W.A.; Treston, A.M.; Eckhardt, S.G.; Sidor, C.; Camidge, D.R. A phase I dose-escalation, safety and pharmacokinetic study of the 2-methoxyestradiol analog ENMD-1198 administered orally to patients with advanced cancer. *Invest New Drugs*, **2011**, *29*(2), 340-346.

[148] Suzuki, R.N.; Newman, S.P.; Purohit, A.; Leese, M.P.; Potter, B.V.; Reed, M.J. Growth inhibition of multi-drug-resistant breast cancer cells by 2-methoxyoestradiol-bis-sulphamate and 2-ethyloestradiol-bis-sulphamate. *J. Steroid Biochem. Mol. Biol.*, **2003**, *84*(2-3), 269-278.

[149] Raobaikady, B.; Purohit, A.; Chander, S.K.; Woo, L.W.; Leese, M.P.; Potter, B.V.; Reed, M.J. Inhibition of MCF-7 breast cancer cell proliferation and *in vivo* steroid sulphatase activity by 2-methoxyoestradiol-bis-sulphamate. *J. Steroid Biochem. Mol. Biol.*, **2003**, *84*(2-3), 351-358.

[150] Ireson, C.R.; Chander, S.K.; Purohit, A.; Perera, S.; Newman, S.P.; Parish, D.; Leese, M.P.; Smith, A.C.; Potter, B.V.; Reed, M.J. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxyoestradiol-bis-sulphamate *in vivo* in rodents. *Br. J. Cancer*, **2004**, *90*(4), 932-937.

[151] Utsumi, T.; Leese, M.P.; Chander, S.K.; Gaukroger, K.; Purohit, A.; Newman, S.P.; Potter, B.V.; Reed, M.J. The effects of 2-methoxyoestrogen sulphamates on the *in vitro* and *in vivo* proliferation of breast cancer cells. *J. Steroid Biochem. Mol. Biol.*, **2005**, *94*(1-3), 219-227.

[152] Visagie, M.H.; Joubert, A.M. *In vitro* effects of 2-methoxyestradiol-bis-sulphamate on reactive oxygen species and possible apoptosis induction in a breast adenocarcinoma cell line. *Cancer Cell Int.*, **2011**, *11*(1), 43.

[153] Vorster, C.; Joubert, A. *In vitro* effects of 2-methoxyestradiol-bis-sulphamate on cell growth, morphology and cell cycle dynamics in the MCF-7 breast adenocarcinoma cell line. *Biocell*, **2010**, *34*(2), 71-79.

[154] Visagie, M.H.; Joubert, A.M. 2-Methoxyestradiol-bis-sulphamate refrains from inducing apoptosis and autophagy in a non-tumorigenic breast cell line. *Cancer Cell Int.*, **2012**, *12*(1), 37.

[155] Meyer-Losic, F.; Newman, S.P.; Day, J.M.; Reed, M.J.; Kasprzyk, P.G.; Purohit, A.; Foster, P.A. STX140, but not paclitaxel, inhibits mammary tumour initiation and progression in C3(1)/SV40 T/t-antigen transgenic mice. *PLoS One*, **2013**, *8*(12), e80305.

[156] Edsall, A.B.; Agoston, G.E.; Treston, A.M.; Plum, S.M.; McClanahan, R.H.; Lu, T.S.; Song, W.; Cushman, M. Synthesis and *in vivo* antitumor evaluation of 2-methoxyestradiol 3-phosphate, 17-phosphate, and 3,17-diphosphate. *J. Med. Chem.*, **2007**, *50*(26), 6700-6705.

[157] Chauhan, D.; Li, G.; Auclair, D.; Hideshima, T.; Richardson, P.; Podar, K.; Mitsiades, N.; Mitsiades, C.; Li, C.; Kim, R.S.; Munshi, N.; Chen, L.B.; Wong, W.; Anderson, K.C. Identification of genes regulated by 2-methoxyestradiol (2ME2) in multiple myeloma cells using oligonucleotide arrays. *Blood*, **2003**, *101*(9), 3606-3614.

[158] Barchiesi, F.; Lucchinetti, E.; Zaugg, M.; Ogunshola, O.O.; Wright, M.; Meyer, M.; Rosselli, M.; Schaufelberger, S.; Gillespie, D.G.; Jackson, E.K.; Dubey, R.K. Candidate genes and mechanisms for 2-methoxyestradiol-mediated vasoprotection. *Hypertension*, **2010**, *56*(5), 964-972.

[159] Miyazaki, M.; Gomez, F.E.; Ntambi, J.M. Lack of stearoyl-CoA desaturase-1 function induces a palmitoyl-CoA Delta6 desaturase and represses the stearoyl-CoA desaturase-3 gene in the preputial glands of the mouse. *J. Lipid Res.*, **2002**, *43*(12), 2146-2154.

[160] Parks, W.T.; Frank, D.B.; Huff, C.; Renfrew Haft, C.; Martin, J.; Meng, X.; de Caestecker, M.P.; McNally, J.G.; Reddi, A.; Taylor, S.I.; Roberts, A.B.; Wang, T.; Lechleider, R.J. Sorting nexin 6, a novel SNX, interacts with the transforming growth factor-beta family of receptor serine-threonine kinases. *J. Biol. Chem.*, **2001**, *276*(22), 19332-19339.

[161] Hoe, H.S.; Wessner, D.; Beffert, U.; Becker, A.G.; Matsuoka, Y.; Rebeck, G.W. F-spondin interaction with the apolipoprotein E receptor ApoEr2 affects processing of amyloid precursor protein. *Mol. Cell Biol.*, **2005**, *25*(21), 9259-9268.

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