Cell PRESS

Potential vascular actions of 2-methoxyestradiol

Raghvendra K. Dubey[1,2,3] and Edwin K. Jackson[3]

[1] Clinic for Reproductive Endocrinology, Department of Obstetrics and Gynecology
[2] Zurich Center for Integrative Human Physiology, University Hospital Zurich, Frauenklinikstrasse, Zurich, Switzerland
[3] Center for Clinical Pharmacology, Departments of Pharmacology and Chemical Biology and of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

2-Methoxyestradiol (2-ME) is a biologically active metabolite of 17β-estradiol that appears to inhibit key processes associated with cell replication *in vitro*. The molecule has been suggested to have potent growth-inhibitory effects on proliferating cells, including smooth muscle cells and endothelial cells, and may be antiangiogenic. Because of these potential roles for 2-ME, its lack of cytotoxicity and low estrogenic activity, we hypothesize that 2-ME could be a valuable therapeutic molecule for prevention and treatment of cardiovascular diseases. Whether 2-ME is as effective *in vivo* as it is *in vitro* at modulating vascular processes remains controversial. Here we discuss recent developments regarding mechanisms by which 2-ME might regulate vascular activity and angiogenesis and speculate on the therapeutic implications of these developments.

Pharmacokinetics of 2-methoxyestradiol

2-Methoxyestradiol (2-ME) is a major endogenous metabolite of estradiol formed via the sequential conversion of estradiol to 2-hydroxyestradiol (2-HE) and 2-ME by cytochrome P450s (CYP450s) and catechol-O-methyltransferase (COMT), respectively (Figure 1) [1]. Both CYP450s and COMT are ubiquitous enzymes responsible for the oxidative metabolism and catechol methylation, respectively, of both endogenous and exogenous molecules. Hence many tissues that produce estradiol, or are exposed to estradiol, may generate 2-ME, although organs synthesizing estradiol (e.g. ovary) would be expected to be the most active in this regard. While tissue levels of 2-ME are unknown, reported plasma concentrations in men, non-pregnant women and pregnant women are 10 to 35 pg/ml, 18 to 138 pg/ml and 216 to 10,690 pg/ml, respectively [2,3]; however, one should view these values as tentative until confirmed by state-of-the-art mass spectrometry. Although rapidly cleared via hydroxylation, demethylation can reconvert 2-ME to 2-HE [4] (Figure 1b).

2-Methoxyestradiol receptors

The receptors that mediate the biological effects of 2-ME remain ill-defined. 2-ME has little or no affinity for classical estrogen receptors (ERs) but does bind to tubulin (IC_{50} of 2 μM) [2]. Moreover, 2-ME binds to an uncharacterized 92 kDa protein [5]. Clearly, the identification of 2-ME receptors is highly pertinent to the development of 2-ME analogues.

Potential effects of 2-methoxyestradiol

Cardiovascular and renal protection

In vitro (rat and human cells) and *in vivo* studies (rat models) suggest that 2-ME induces cardiovascular and renal protective action (Figure 2) and inhibits abnormal cellular growth in vascular smooth muscle cells, cardiac fibroblasts and glomerular mesangial cells that contribute to vaso-occlusive disorders, cardiac hypertrophy and glomerulosclerosis, respectively [2,4,6,7,8].

Plasma cholesterol

2-ME may influence plasma lipid levels in a beneficial manner [2,4,8]. Both 2-ME and 2-HE significantly reduce cholesterol levels in rats, including genetically-obese ZSF1 rats [2,4] (Figure 2). Interestingly, a recent study found that 2-ME reduces the formation of atherosclerotic lesions in female apolipoprotein-E deficient mice [8].

Inflammation

Invasion of tissues by monocytes/macrophages also contributes to vascular disease. *In vitro* experiments show that 2-ME inhibits the motility, migration and adhesion of circulating breakpoint cluster region-Abelson (BCR-ABL)-transformed cells to fibronectin [9], suggesting that 2-ME inhibits the ability of circulating inflammatory cells to adhere to and infiltrate vascular lesions (Figure 2). Indeed, evidence shows that 2-ME inhibits adhesion of monocytes to aortic endothelial cells, a prerequisite for atherosclerosis [10]. 2-ME also inhibits hypoxia inducible factor-1α (HIF-1α), a transcription factor that mediates inflammation [11]. Hence, 2-ME may protect against atherosclerosis by inhibiting key inflammatory processes in the vascular wall.

Endothelial function

2-HE improves endothelium-dependent relaxation in obese ZSF1 rats [2,4]. In vascular endothelial cells, both 2-HE and 2-ME induce COX-2 expression leading to production of prostacyclin, a vasoprotective molecule [12]. Moreover, in coronary artery endothelial cells, 2-ME and 2-HE inhibit the synthesis of endothelin-1, a vasoconstrictor associated with vaso-occlusive disorders [7]. In rat aortic segments, 2-ME counteracts phenylephrine-induced

Corresponding author: Dubey, R.K. (Raghvendra.Dubey@usz.ch).

1043-2760/$ – see front matter doi:10.1016/j.tem.2009.04.007 Available online 4 September 2009

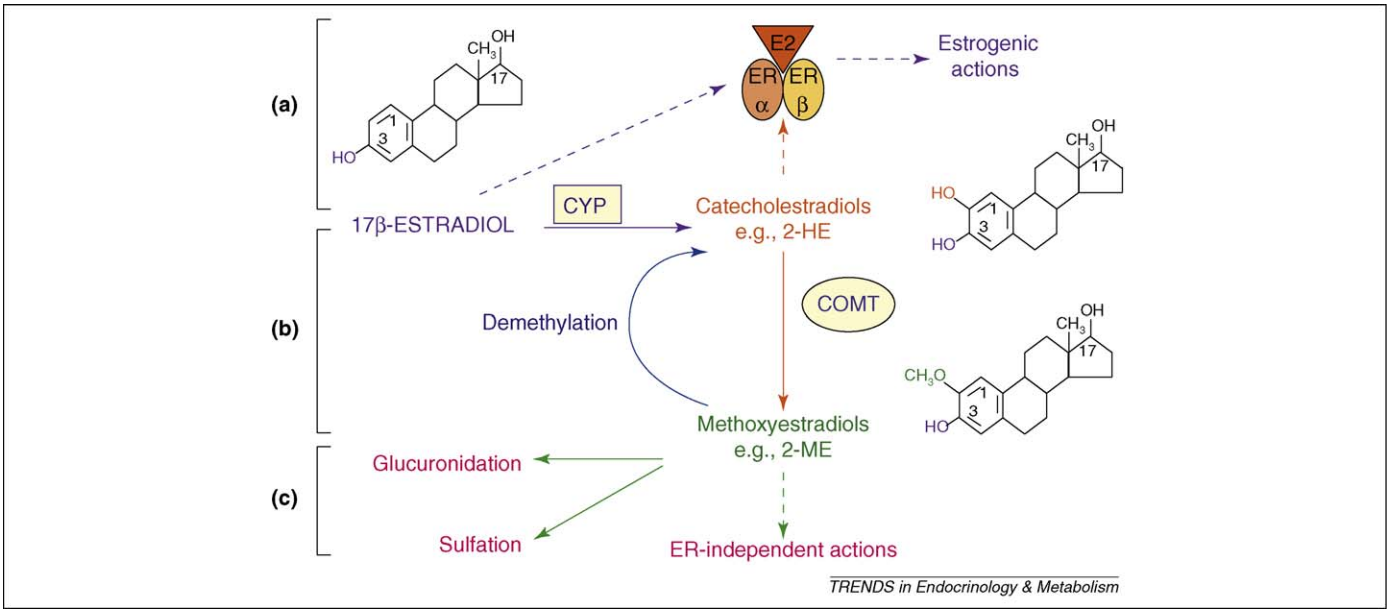

Figure 1. Endogenous formation and metabolism of 17β-estradiol (E2). **(a)** E2 interacts with ERα and ERβ to mediate its estrogenic actions in multiple tissues. **(b)** Endogenous E2 is metabolized to 2-hydroxyestradiol (2-HE) and 2-methoxyestradiol (2-ME) via the sequential actions of cytochrome P450 (CYP) and catechol-O-methyltransferase (COMT). CYP exists in multiple isoforms that are capable of metabolizing estradiol to catecholestradiols (e.g. CYP1A1, CYP1A2, CYP1B1 and CYP3A4). 2-HE has mild estrogenic actions and is rapidly metabolized to 2-ME. 2-ME has little or no affinity for ERs and mediates its biologic actions via ER-independent mechanisms. **(c)** 2-ME is rapidly cleared from the body via phase II conjugation enzymes involving glucuronidation and sulfation (hepatic clearance: 712 ml/min in humans). Moreover, demethylation can reconvert 2-ME to 2-HE. ER, estrogen receptor.

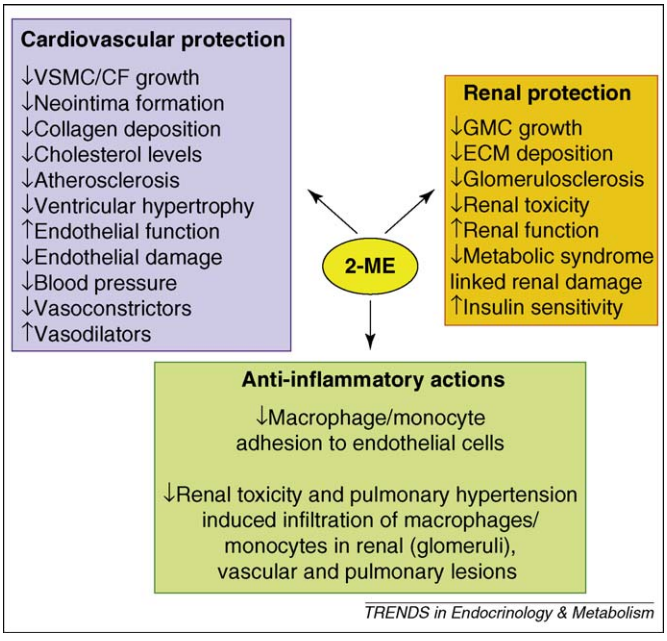

Figure 2. Potential beneficial effects of 2-methoxyestradiol (2-ME). 2-ME induces cardiovascular protection and attenuates pulmonary hypertension by improving endothelial function, by inhibiting abnormal growth of vascular smooth muscle cells (VSMCs), by inhibiting the synthesis of extracellular matrix (ECM) proteins including collagen, by inhibiting the infiltration of inflammatory cells (monocytes/ macrophages) in vascular lesions, and by lowering cholesterol levels. As in VSMCs, 2-ME inhibits left ventricular hypertrophy by inhibiting abnormal growth and ECM synthesis/deposition by cardiac fibroblasts (CF). 2-ME reduces cardiovascular and renal damage associated with metabolic syndrome and obesity. 2-ME may also protect against renal disease/toxicity by attenuating glomerulosclerosis/glomerular remodeling by inhibiting the abnormal growth of glomerular mesangial cells (GMC), the deposition of EMC proteins including collagen by GMCs, and the infiltration of inflammatory cells (monocytes/ macrophages). ↑ induction; ↓ inhibition.

contraction in the presence, but not absence, of endothelium [13]; nitric oxide synthase (NOS) inhibitors block this effect, suggesting that 2-ME abrogates vascular contraction via endothelium-dependent NO production [13]. Additionally, 2-ME increases the redistribution [14] and expression [15] of endothelial NOS (eNOS), resulting in localized NO production within the plasma membrane, and potentially contributing to endothelium-dependent relaxation [16]. 2-HE and 2-ME are also potent anti-oxidants (more potent than vitamin E and estradiol) [2,12]; therefore they may likewise potentiate the vasodilatory activity of NO by preventing its oxidation. Detailed electron microscopic studies provide evidence that 2-ME prevents structural damage of vascular endothelial cells during pre-eclampsia [15]. Additionally, both 2-HE and 2-ME prevent low density lipoprotein (LDL) oxidation [2,7] and may protect endothelial cells against free radicals and oxidized LDL-induced injury. *In vitro*, at concentrations >100 nM, 2-ME is antiangiogenic [17] and induces apoptosis in proliferating endothelial cells; whether this occurs *in vivo* remains unknown. Additional studies using COMT knockout mice and/or pharmacological inhibitors of 2-ME are required to address this issue.

The putative ability of 2-ME to enhance endothelial vasodilation may contribute to several beneficial actions of 2-ME. 2-ME has been suggested to protect against systemic hypertension [18], pulmonary hypertension [19,20], pre-eclampsia [15], renal disease [7,21], and ischemia-induced brain injury [22]. Because abnormal endothelial barrier function is a feature of these diseases, 2-ME could help preserve endothelial barrier function. For example, superfluous production of soluble Fms-like tyrosine kinase-1 (sFLT-1) disrupts the endothelial barrier in capillaries [23], and 2-ME reduces circulating sFLT-1

levels in mice with pre-eclampsia [15]. Furthermore, hypoxic conditions are associated with endothelial barrier disruption, and 2-ME inhibits hypoxia-induced sFLT-1 production and HIF-1α expression in cultured cells [15]. Further studies are warranted to investigate the effects of 2-ME on endothelial barrier function.

Vascular smooth muscle cell (VSMC) proliferation

Migration, proliferation, and extracellular matrix production by VSMCs contributes to the pathophysiology of vascular diseases including atherosclerosis, restenosis and neointimal hyperplasia [4]. In cultured human and rat aortic VSMCs, estradiol metabolites differentially inhibit migration, proliferation and collagen synthesis in the following order of potency: 2-ME > 2-HE > 4-ME ≥ estradiol [24,25]. Subjecting cells to the dual ERα and ERβ antagonist ICI 182,780, or to ER antisense constructs, does not block the growth-inhibitory effects of catecholestradiols and/or methoxyestradiols on VSMCs [7,25], suggesting ERαβ-independent effects.

How does 2-ME inhibit VSMC growth? As mentioned, both 2-HE and 2-ME are potent antioxidants [4,7,12]; they also inhibit free radical (peroxyl-radical)-induced proliferation and migration of VSMCs [26]. Flow cytometry indicates that 2-ME inhibits VSMC proliferation at both the G_0/G_1 and G_2/M phases of the cell cycle [6] both *in vitro* (VSMC cultures) and *in vivo* (balloon injury-induced neointima formation in rats). For example, 2-ME downregulates hyperphosphorylated retinoblastoma protein (pRb), cyclin D_1, cyclin B_1, phosphorylated-ERK1/2 (MAPK) and phosphorylated-Akt (all positive regulators of VSMC growth) [6] (Figure 3). In addition, 2-ME induces p27 expression (a negative regulator of VSMC growth), downregulates vascular expression of proliferating cell nuclear antigen (PCNA) and c-myc, and upregulates vascular COX-2 expression [6]. It remains unclear whether the modulatory effects of 2-ME on the cell cycle and signal transduction pathways in VSMCs contribute to 2-MEs inhibitory actions, or whether these effects are simply 'bystander' manifestations (i.e. are simply a consequence rather than a cause of cell cycle arrest). Both 2-HE and 2-ME may also influence VSMCs indirectly by increasing the levels of endogenous compounds that inhibit growth (e.g. NO, cAMP, and prostacyclin) or by decreasing the levels of endogenous compounds that stimulate proliferation (e.g. endothelin-1 and catecholamines) [2].

2-Methoxyestradiol potentially mediates the antiproliferative effects of estradiol and catecholestradiols

As mentioned earlier, 2-ME is generated by the sequential metabolism of estradiol to 2-HE and 2-ME by CYP450s and COMT, respectively (Figure 1). VSMCs contain aromatase activity [27] and are capable of synthesizing estradiol locally [27]. Moreover, VSMCs are enzymatically equipped with CYP450 and COMT enzymes, and incubation of VSMCs with estradiol or 2-HE results in 2-ME formation [28,29]. In as much as endogenous estradiol and 2-HE are metabolized to 2-ME (Figure 1), it is plausible that the antiproliferative effects of estradiol and 2-HE on VSMCs are also mediated in part by 2-ME. Furthermore, endogenous factors competing for the same pathway may compromise the beneficial effects of estradiol and 2-HE. For example, catecholamines and medroxyprogesterone (a synthetic progestin used for combined hormone therapy with estradiol) block the conversion of estradiol and 2-HE to 2-ME, and interfere with their antimitogenic effects [30,31].

ER-independent mechanisms probably contribute to the antiproliferative effects of estradiol, catecholestradiols and methoxyestradiols

Ample evidence suggests that the antiproliferative actions of 2-ME and 2-HE are in part ER-independent. For example, even though 2-HE and 2-ME have little or no binding affinity for ERs, they inhibit growth of cardiovascular cells. Moreover, these antimitogenic effects are not blocked by ICI 182,780 and are present in cells cultured from ERαβ double-knockout mice [2,7,29,32]. However, whether the antiproliferative effects of estradiol are mediated via classical ERs or via 2-HE/2-ME-linked ER-independent mechanisms remains unclear and is discussed below.

Estradiol inhibits VSMC proliferation in injury-induced vascular lesions in mice with reduced activity of ERα [33], ERβ [34] and both ERα and ERβ [35], suggesting that the inhibitory effects of estradiol itself are ER-independent or involve an unidentified ER. In contrast, the inhibitory effects of estradiol on injury-induced lesion formation and VSMC proliferation are abrogated in complete ERα knockout mice, suggesting that ERα mediates the protective effects of estradiol against lesion formation and VSMC proliferation [36]. Interestingly, compared to wild-type mice, injury-induced lesion formation and VSMC proliferation are also dramatically reduced in complete ERα knockout mice. Whether lack of robust VSMC growth in complete ERα knockout mice contributes to the lack of protective effects of estradiol remains unclear. Also, in studies with mice lacking both ApoE and ERα, both ERα-dependent and ERα-independent mechanisms appear to account for the plaque-reducing and atheroprotective effects of estradiol [37]. Importantly, the atheroprotective effects of estradiol are not lost in mice lacking ApoE and ERβ [38]. Together these findings suggest that ERα-independent effects are not ERβ-mediated and may involve an ER or an ER-independent mechanism. Because 2-ME inhibits both injury-induced neointima formation and atherosclerosis [6,8], it is possible that endogenous conversion of estradiol to 2-ME contributes to the anti-vaso-occlusive effects of estradiol via an ER-independent mechanism. *In vitro* evidence for this possibility exists: antimitogenic effects of estradiol and 2-HE are blocked in VSMCs from COMT knockout mice [39] that express both ERα and ERβ and by pharmacological inhibitors of estradiol metabolism [29]. Future studies in COMT knockout mice may help dissect the exact role of ERα and estradiol-derived 2-ME in inhibiting lesion-induced neointima formation and VSMC proliferation.

Pharmacological evidence for a role of ERs in mediating the inhibitory effects of estradiol in VSMCs is inconclusive. Some investigators [40], but not others [41], report that ICI 182,780 attenuates estradiol's ability to reduce injury-induced neointima formation. ICI 182,780 not only binds to ERs but also blocks the metabolism of estradiol to hydroxyestradiols [25,29]. In VSMCs, ICI 182,780 blocks

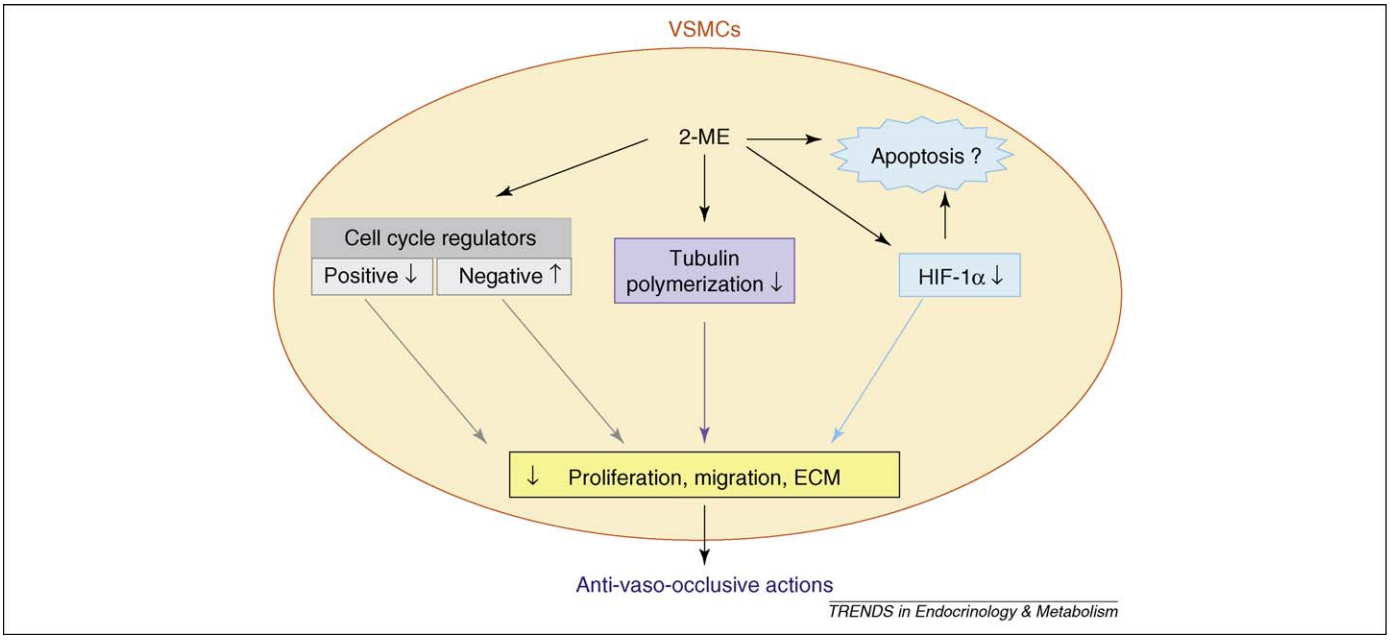

Figure 3. Schematic representation of the cellular mechanisms in vascular smooth muscle cells (VSMCs) via which 2-methoxyestradiol (2-ME) potentially inhibits proliferation, migration and extracellular matrix (ECM) synthesis, and mediates anti-vaso-occlusive actions. 2-methoxyestradiol, 2-ME; positive cell cycle regulators include phosphorylated mitogen activated kinase, hyperphosphorylated retinoblastoma protein, phosphorylated-Akt, cyclin D_1, cyclin B_1; negative regulators of cell cycle include p27; HIF-1α, hypoxia-induced factor-1α; ↓, inhibition; ↑, activation;?, unknown.

the antimitogenic effects of estradiol only at concentrations that block the metabolism of estradiol to hydroxyestradiols [25,29]. The abrogatory effects of ICI 182,780 in one study therefore may be due to the high concentrations used; indeed, estradiol levels are increased by more than 2-fold in rats given ICI 182,780, perhaps due to inhibition of estradiol metabolism. Because ICI 182,780 is both a dual antagonist of ERα and ERβ and an inhibitor of estradiol metabolism, it is difficult to determine whether it blocks the antimitogenic effects of estradiol by inhibiting ERs or by attenuating estradiol metabolism. However, results from both molecular and pharmacological studies suggest that antiproliferative effects of estradiol are mediated via both ERα-dependent and ER-independent mechanisms.

2-Methoxyestradiol and angiogenesis

Studies by Fotsis *et al.* [17] demonstrate that oral administration of 2-ME inhibits angiogenesis *in vivo* and suppresses tumor growth by limiting blood supply. Antiangiogenic effects of 2-ME at high concentrations may explain its beneficial effects in pulmonary hypertension [19,20], rheumatoid arthritis [42], neoplasia [43], eye diseases associated with neovascularization [44], and endometriosis [45].

The proposed mechanisms by which high concentrations of 2-ME inhibit angiogenesis are depicted in Figure 4. As in VSMCs (Figure 3), 2-ME interacts with endothelial tubulin dynamics and HIF-1α to affect target proteins (e.g. VEGF) and genes involved in endothelial proliferation, apoptosis, survival and invasion, processes that are central to the regulation of angiogenesis [6,43,45,46].

2-Methoxyestradiol and apoptosis

Caspase-3 cleavage assay, FACS analysis of 2-ME-treated VSMCs, and terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) staining of carotid segments from rats treated with 2-ME do not support the hypothesis that 2-ME induces apoptosis in VSMCs [6]. However, some investigators report that 2-ME can induce apoptosis in VSMCs [47]. Although the reasons for these disparate findings remain unclear, it is possible that differences in methodologies used and experimental conditions (different treatment times and serum concentrations) are contributory factors, and this potential role of 2-ME needs to be further investigated.

In endothelial cells it appears that 2-ME induces apoptosis, as reflected by increases in expression of death receptor 5 (DR5; a member of tumor necrosis factor (TNF) death receptor family) and Fas (another member of the death receptor family) [48] (Figure 4). Specifically, DR5 activation by 2-ME renders endothelial cells more sensitive to the cytotoxic activities of the DR5 ligand, TNF-related apoptosis-inducing ligand (TRAIL) [48]. 2-ME-induced apoptosis requires the sequential activation of caspase-8, caspase-9, and caspase-3 [48]. Death receptors signal apoptosis by recruiting Fas-associated death domain (FADD) to the oligomerized DR complex, where it facilitates the binding and activation of procaspase-8 [49]. In endothelial cells, expression of dominant-negative FADD inhibits apoptosis induced by 2-ME by approximately 75% [48], suggesting that in endothelial cells 2-ME induces apoptosis via the extrinsic pathway.

The intrinsic or mitochondrial apoptotic pathway may also play a role in mediating the apoptotic effects of 2-ME in endothelial cells. Treatment of endothelial cells with low concentrations of 2-ME rapidly activates c-Jun NH_2-terminal kinase (JNK)/stress-activated protein kinase (SAPK) [50] and upregulates Fas, triggering programmed cell death (Figure 4). Although the apoptotic effects of 2-ME are evident, whether these effects are specifically induced in pathologically-proliferating cells or in normal cells remains unknown. Finally, whether conversion of estradiol

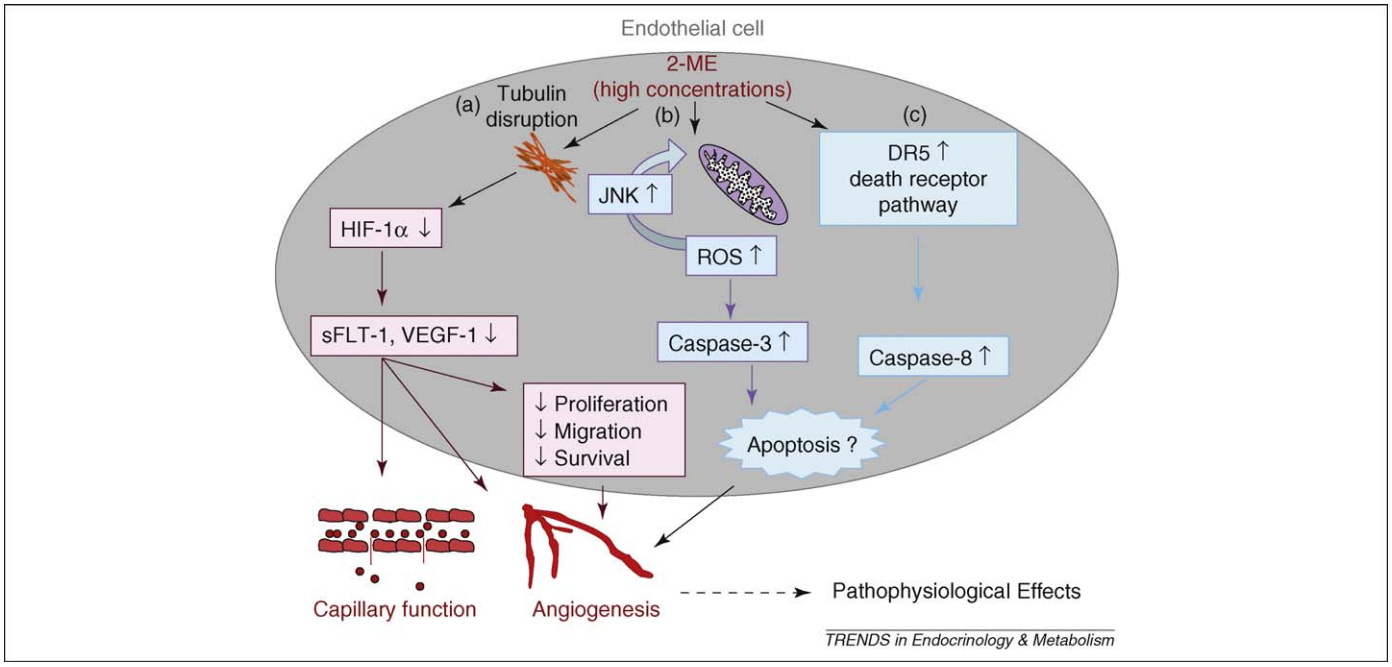

Figure 4. Schematic representation of the cellular mechanisms in endothelial cells mediating the antiangiogenic and capillary barrier actions of 2-ME. **(a)** Disruption of tubulin dynamics by 2-ME inhibits cellular accumulation of hypoxia-induced factor-1α (HIF-1α), thereby inhibiting its translocation to the nucleus and resulting in the inhibition of expression and secretion of angiogenic factors including vascular endothelial growth factor-1 (VEGF-1). Decreased generation of VEGF-1 and Fms-like tyrosine kinase-1 (sFLT-1) protects against endothelial cell proliferation, migration and survival as well as against endothelial barrier dysfunction. 2-ME can also induce antiangiogenic effects by causing endothelial cell apoptosis. **(b)** 2-ME can generate reactive oxygen species (ROS) by activating c-Jun N-terminal kinase (JNK) or by interacting directly with mitochondria to activate caspase-3 and apoptosis. **(c)** 2-ME can also trigger apoptosis by upregulating the extrinsic pathway by increasing the expression of death receptor (DR5) and activating caspase-8. The collective effects of these mechanisms confer protection against endothelial barrier disruption and capillary leakage, inhibit angiogenic activity and neovascularisation, and potentially may protect against tumor progression, plaque development, pulmonary hypertension, obesity, pre-eclampsia, renal disease, eye disease, endometriosis and rheumatoid arthritis. ↓, inhibition; ↑, increase; ———— hypothesized effects.

to 2-ME also induces apoptotic effects remains unknown and needs to be investigated.

Clincial implications and future directions

That 2-ME potentially inhibits cell growth via common signaling pathways suggests that it may be a useful therapeutic agent for various proliferative diseases. 2-ME is also presumed to lower cholesterol [2,8], inhibit inflammatory processes associated with vascular and renal diseases, and attenuate atherosclerosis [9,10,19,20,21], angiogensis/neovascularization and capillary formation [17,43,44] (Figure 2). Because 2-ME inhibits key mechanisms associated with capillary leakage and angiogenesis, it may be of therapeutic use in diseases such as pulmonary hypertension, endometriosis, pre-eclampsia, renal diseases, solid tumors and angiogenic eye disorders.

A drawback of current estrogen therapies is the increased risk of developing breast and endometrial cancers. Because 2-ME appears relatively non-toxic and potentially has both anti-vaso-occlusive and anti-carcinogenic actions, it could be used clinically to prevent cardiovascular disease in women without increasing risk of cancer. Being non-feminizing, 2-ME could also be used to treat cardiovascular disease in men. However, several issues need to be investigated. Although 2-ME may protect against disease-associated angiogenesis, it may also interfere with biological processes such as follicular development and intestinal epithelial cell growth that are regulated by physiological angiogenesis and cell proliferation. It is also important to determine whether 2-ME mimics estrogenic effects in hot flushes and osteoporosis, and whether 2-ME is devoid of pro-thrombotic effects. Further, 2-ME increases thymus weight and uterine growth in mice [8], although the pathological consequences of these effects are unclear.

Another major challenge facing the development of 2-ME as a drug is to overcome its undesirable pharmacokinetic properties: poor oral bioavailability and short half-life [3]. Future studies therefore should also focus on developing 2-ME analogs or 2-ME delivery systems that increase its bioavailability and extend its half-life. To develop more potent 2-ME analogs, the receptor via which 2-ME induces its biological effects needs to be identified. 2-ME may be less potent *in vivo* compared with its actions *in vitro*, and nanotechnology and drug modeling will be crucial in resolving the pharmacokinetic/pharmacodynamic issues that reduce the therapeutic potential of 2-ME. In this regard, modification of the molecule to target specific tissues will also be helpful to enhance its therapeutic potential and reduce adverse effects.

In conclusion, research thus far suggests that 2-ME blocks mechanisms that regulate cell proliferation and classifies this estrogen derivative as a potential therapeutic tool in proliferative diseases. Nonetheless, additional studies are warranted to address more carefully its safety and efficacy.

Acknowledgments

The authors are supported by grants from Swiss National Science Foundation (3200B0-106098/1 and 320000-117998/1), Oncosuisse (OCS-01551-08-2004), EMDO Stiftung, and the NIH (HL69846 and DK68575).

References

1 Zhu, B.T. and Conney, A.H. (1998) Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res.* 58, 2269–2277
2 Dubey, R.K. *et al.* (2004) Cardiovascular pharmacology of estradiol metabolites. *J. Pharmacol. Exp. Therapeutics* 308, 403–409
3 Lakhani, N.J. *et al.* (2007) Pharmacokinetics, pharmacodynamics and drug metabolism – Characterization of in vitro and in vivo metabolic pathways of the investigational anticancer agent, 2-methoxyestradiol. *J. Pharm. Sci.* 96, 1821–1831
4 Dubey, R.K. *et al.* (2007) 2-Methoxyestradiol: A potential treatment for multiple proliferative disorders. *Endocrinology* 148, 4125–4127
5 Ho, A. *et al.* (2006) SAR studies of 2-methoxyestradiol and development of its analogs as probes of anti-tumor mechanisms. *Bioorg. Med. Chem. Lett.* 16, 3383–3387
6 Barchiesi, F. *et al.* (2006) 2-methoxyestradiol, an estradiol metabolite, inhibits neointima formation and smooth muscle cell growth via double blockade of the cell cycle. *Circ. Res.* 99, 266–274
7 Dubey, R.K. and Jackson, E.K. (2001) Cardiovascular protective effects of 17beta-estradiol metabolites. *J. Appl. Physiol.* 91, 1868–1883
8 Bourghardt, J. *et al.* (2007) The endogenous estradiol metabolite 2-methoxyestradiol reduces atherosclerotic lesion formation in female apolipoprotein E-deficient mice. *Endocrinology* 148, 4128–4132
9 Sattler, M. *et al.* (2003) 2-methoxyestradiol alters cell motility, migration, and adhesion. *Blood* 102, 289–296
10 Kurokawa, A. *et al.* (2007) 2-methoxyestradiol reduces monocyte adhesion to aortic endothelial cells in ovariectomized rats. *Endocr. J.* 54, 1027–1031
11 Cramer, T. *et al.* (2003) HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* 112, 645–657
12 Seeger, H. *et al.* (1999) Effect of estradiol metabolites on prostacyclin synthesis in human endothelial cell cultures. *Life Sci.* 65, PL167–PL170
13 Gui, X-L. *et al.* (2008) Inhibition of rat aortic smooth muscle contraction by 2-methoxyestradiol. *Am. J. Physiol. Heart Circ. Physiol.* 295, H1935–H1942
14 Tsukamoto, A. *et al.* (1998) 2-methoxyestradiol, an endogenous metabolite of estrogen, enhances apoptosis and beta-galactosidase expression in vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 248, 9–12
15 Kanasaki, K. *et al.* (2008) Deficiency in catechol-O-methyltransferase and 2-methoxyestradiol is associated with pre-eclampsia. *Nature* 453, 1117–1121
16 Tofovic, S.P. *et al.* (2001) 2-hydroxyestradiol attenuates the development of obesity, the metabolic syndrome, and vascular and renal dysfunction in obese ZSF1 rats. *J. Pharmacol. Exp. Ther.* 299, 973–977
17 Fotsis, T. *et al.* (1994) The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumor-growth. *Nature* 368, 237–239
18 Masi, C.M. *et al.* (2006) Estrogen metabolites and systolic blood pressure in a population-based sample of postmenopausal women. *J. Clin. Endocrinol. Metab.* 91, 1015–1020
19 Tofovic, S.P. *et al.* (2006) 2-Methoxyestradiol mediates the protective effects of estradiol in monocrotaline-induced pulmonary hypertension. *Vasc. Pharmacol.* 45, 358–367
20 Tofovic, S.P. *et al.* (2005) Estradiol metabolites attenuate monocrotaline-induced pulmonary hypertension in rats. *J. Cardiovasc. Pharmacol.* 46, 430–437
21 Tofovic, S.P. *et al.* (2005) Estradiol metabolites attenuate renal and cardiovascular injury induced by chronic nitric oxide synthase inhibition. *J. Cardiovasc. Pharmacol.* 46, 25–35
22 Chen, C.H. *et al.* (2007) Multiple effects of 2ME2 and D609 on the cortical expression of HIF-1 alpha and apoptotic genes in a middle cerebral artery occlusion-induced focal ischemia rat model. *J. Neurochem.* 102, 1831–1841
23 Luttun, A. and Carmeliet, P. (2003) Soluble VEGF receptor Flt1: the elusive preeclampsia factor discovered. *J. Clin. Invest.* 111, 600–602
24 Barchiesi, F. *et al.* (2002) Methoxyestradiols mediate estradiol-induced antimitogenesis in human aortic SMCs. *Hypertension* 39, 874–972
25 Dubey, R.K. *et al.* (2000) Methoxyestradiols mediate the antimitogenic effects of estradiol on vascular smooth muscle cells via estrogen receptor-independent mechanisms. *Biochem. Biophys. Res. Commun.* 278, 27–33
26 Dubey, R.K. *et al.* (1999) Estrogen and tamoxifen metabolites protect smooth muscle cell membrane phospholipids against peroxidation and inhibit cell growth. *Circ. Res.* 84, 229–239
27 Harada, N. *et al.* (1999) Localized expression of aromatase in human vascular tissues. *Circ. Res.* 84, 1285–1291
28 Zhao, W. *et al.* (1998) Constitutive and inducible expression of cytochrome P4501A1 and P4501B1 in human vascular endothelial and smooth muscle cells. *In Vitro Cell Dev. Biol. Anim.* 34, 671–673
29 Dubey, R.K. *et al.* (2003) CYP450- and COMT-derived estradiol metabolites inhibit activity of human coronary artery SMCs. *Hypertension* 41, 807–813
30 Zacharia, L.C. *et al.* (2001) Catecholamines abrogate antimitogenic effects of 2-hydroxyestradiol on human aortic vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 21, 1745–1750
31 Dubey, R.K. *et al.* (2008) Medroxyprogesterone abrogates the inhibitory effects of estradiol on vascular smooth muscle cells by preventing estradiol metabolism. *Hypertension* 51, 1197–1202
32 Dubey, R.K. *et al.* (2005) Cytochromes 1A1/1B1-and catechol-O-methyltransferase-derived metabolites mediate estradiol-induced antimitogenesis in human cardiac fibroblast. *J. Clin. Endocrinol. Metab.* 90, 247–255
33 Iafrati, M.D. *et al.* (1997) Estrogen inhibits the vascular injury response in estrogen receptor alpha-deficient mice. *Nature Med.* 3, 545–548
34 Karas, R.H. *et al.* (1999) Estrogen inhibits the vascular injury response in estrogen receptor beta-deficient female mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15133–15136
35 Karas, R.H. *et al.* (2001) Effects of estrogen on the vascular injury response in estrogen receptor alpha,beta (double) knockout mice. *Circ. Res.* 89, 534–539
36 Pare, G. *et al.* (2002) Estrogen receptor-α mediates the protective effects of estrogen against vascular injury. *Circ. Res.* 90, 1087–1092
37 Hodgin, J.B. *et al.* (2001) Estrogen receptor α is a major mediator of 17β-estradiol's atheroprotective effects on lesion size in Apoe-/- mice. *J. Clin. Invest.* 107, 333–340
38 Hodgin, J.B. and Maeda, N. (2002) Estrogen and mouse models of atherosclerosis. *Endocrinology* 143, 4495–4501
39 Zacharia, L.C. *et al.* (2003) Methoxyestradiols mediate the antimitogenic effects of 17 beta-estradiol – Direct evidence from catechol-O-methyltransferase-knockout mice. *Circulation* 108, 2974–2978
40 Bakir, S. *et al.* (2000) Estrogen-induced vasoprotection is estrogen receptor dependent – Evidence from the balloon-injured rat carotid artery model. *Circulation* 101, 2342–2344
41 Finking, G. *et al.* (2002) Reduction of post injury neointima formation due to 17beta-estradiol and phytoestrogen treatment is not influenced by the pure synthetic estrogen receptor antagonist ICI 182, 780 in vitro. *BMC Cardiovasc. Disord.* 2, 13
42 Josefsson, E. and Tarkowski, A. (1997) Suppression of type II collagen-induced arthritis by the endogenous estrogen metabolite 2-methoxyestradiol. *Arthritis Rheum.* 40, 154–163
43 Mabjeesh, N.J. *et al.* (2003) 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* 3, 363–375
44 Robinson, M.R. *et al.* (2002) Safety and pharmacokinetics of intravitreal 2-methoxyestradiol implants in normal rabbit and pharmacodynamics in a rat model of choroidal neovascularization. *Exp. Eye Res.* 74, 309–317
45 Becker, C.M. *et al.* (2008) 2-methoxyestradiol inhibits hypoxia-inducible factor-1 alpha and suppresses growth of lesions in a mouse model of endometriosis. *American J. Pathol.* 172, 534–544
46 Semenza, G.L. (2003) Targeting HIF-1 for cancer therapy. *Nat. Rev. Cancer* 3, 721–732
47 Gui, Y. and Zheng, X.L. (2006) 2-methoxyestradiol induces cell cycle arrest and mitotic cell apoptosis in human vascular smooth muscle cells. *Hypertension* 47, 271–280
48 LaVallee, T.M. *et al.* (2003) 2-methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway. *Cancer Res.* 63, 468–475
49 Kim, P.K.M. *et al.* (2001) The role of caspase-8 in resistance to cancer chemotherapy. *Drug Resist. Updates* 4, 293–296
50 Chauhan, D. *et al.* (2003) JNK-dependent release of mitochondrial protein, Smac, during apoptosis in multiple myeloma (MM) cells. *J. Biol. Chem.* 278, 17593–17596