Potential Role of Estrogen Metabolite 2-Methoxyestradiol in Health and Disease

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ABSTRACT

2-Methoxyestradiol (2-ME2) is an endogenous metabolite of 17β-estradiol (E2) that was originally thought to be an inert end product of estrogen metabolism. However, studies conducted over the past two decades have shown 2-ME2 to be a promising anticancer agent. Reports suggest that 2-ME2 directly influences tumor growth through mechanisms which reduce cell proliferation or induce apoptosis as well as through the inhibition of angiogenesis. Incidentally, 2-ME2 as an anticancer agent has poor bioavailability, and this has led to the development of several analogs and derivatives, which currently have had limited success. Thus, it is imperative that we re-evaluate our understanding of 2-ME2-mediated effects in order to innovatively derive, or generate, more efficacious cancer treatment options. In this review, the roles of 2-ME2 in cancer as well as the highly variable mechanisms of action reported for this metabolite are discussed.

Keywords: 2-Methoxyestradiol, Cancer, Mechanism of action, Microtubule disruption, Angiogenesis, Tumor suppressor protein, Estrogen receptor.

INTRODUCTION

17β-estradiol (E2) is a mitogenic molecule that enhances proliferation in target cells. While several endogenous E2 metabolites have been shown to be more potent estrogenic compounds than their precursor, others, such as 2-methoxyestradiol (2-ME2) (Fig. 1) are nonestrogenic, yet still retain biological activity.1,2 The formation of 2-ME2 occurs from the catechol O-methyltransferase (COMT)-mediated O-methylation of the catechol estrogen 2-hydroxyestradiol (2-OHE2), a major metabolite formed in humans by the hydroxylation of E2.3 2-Methoxyestradiol has been shown to have promising therapeutic potential as it displays anti-proliferative, antiangiogenic and proapoptotic properties in various cancers while having limited effects on normal cells and tissues.

2-ME2 and Cancer

Unlike the growth enhancing estrogen metabolites, 2-ME2 has been shown to have potent anti-angiogenic, anti-proliferative and pro-apoptotic properties in vitro as well as in vivo.4 In contrast to its effects on malignant and transformed cells, 2-ME2 has minimal to no significant effects on the growth of normal cells including lymphocytes.5-9 There are also indications that 2-ME2 affects actively proliferating cells (with no effect on quiescent cells) possibly due to disruption of cellular events associated with proliferation.10 For example, Van Zijl et al reported that 2-ME2 disrupted mitotic spindle formation and enhanced Cdc2 kinase activity leading to persistence of the spindle checkpoint. Thus, prolonged metaphase arrest may have resulted in the induction of apoptosis in MCF-7 cells, but not in normal MCF-12A cells.11

The most commonly reported effects of 2-ME2 are microtubule disruption, cell cycle arrest, inhibition of angiogenesis, and induction of apoptosis. Several mechanisms have been proposed for 2-ME2 action, but there is a lack of evidence for a common pathway for all of the cells sensitive to this metabolite. The variation in the anti-tumor effects of 2-ME2 reported in the literature is extensive and appears to heavily rely on factors, such as cell type, 2-ME2 concentration, culture conditions, genotype and gene expression profiles. Numerous studies have been conducted in an effort to better understand the biochemical, cellular and molecular mechanisms for the actions of 2-ME2. A portion of these reported effects and mechanisms of action are summarized in the sections below.

MECHANISM OF 2-ME2 ACTION

Estrogen Receptor

Despite being a natural metabolite of E2, the antiproliferative and cytotoxic effects induced by 2-ME2 are independent of estrogen receptor (ER) status and are not considered to be ER-mediated.12,13 The relative binding affinity of 2-ME2 for ERα and β varies depending on assay conditions.
Receptor binding assays are a common investigative tool to ascertain the binding specificity and high affinity that would be expected of a hormone receptor. However, factors, such as time, temperature, salt and pH can induce variation and have a detrimental impact on receptor assays in vitro.\textsuperscript{14} The reported binding affinity of 2-ME2 (relative to estradiol binding) ranges from 0.3 to 2\% for ER\(	extalpha\) and 0.008 to 1\% for ER\(\beta\).\textsuperscript{12,13,15} It is important to note that the biological activity of a steroid hormone may not be accurately determined by receptor binding alone.\textsuperscript{14} Liu and Zhu observed both mitogenic and anti-proliferative properties of 2-ME2 in breast cancer cells.\textsuperscript{15} The former was reported by the authors to be ER-dependent and occurred at low, nanomolar concentrations (10-750 nM) in the absence of E2 and other growth factors. The observed anti-proliferative effects of 2-ME2 in this study agree with previous findings by other investigators and are reported to be ER-independent. In contrast to the observed results by Liu and Zhu, a previous report from our laboratory has shown that micromolar concentrations of 2-ME2 (1-10 \mu M) reduces cell number with no observable effects occurring with 1-100 nM 2-ME2 in the ER+ T47D breast cancer cell line.\textsuperscript{16}

**Microtubule Disruption**

Unlike its growth enhancing precursor, 2-ME2 has been reported to interact with microtubules (MTs) to induce mitotic arrest, inhibit cell proliferation and induce apoptosis in tumor cells in vitro and in vivo.\textsuperscript{4,17,18} 2-ME2 binds, in a competitive manner (\(K_i = 22 \mu M\)), at or near the colchicine-binding site of B-tubulin. At high 2-ME2 concentrations, this results in inhibition of tubulin polymerization and MT assembly and subsequent cell cycle arrest at the G2-M transition.\textsuperscript{19} In contrast, at low concentrations, 2-ME2-induced mitotic block involves kinetic stabilization of MT dynamics rather than alteration of MT polymerization.\textsuperscript{20,21} In fact, it has been shown that low concentrations of 2-ME2 could induce mitotic cell arrest via suppression of MT dynamics and not the depolymerization of MTs.\textsuperscript{22}

**Inhibition of Angiogenesis**

Studies have shown that 2-ME2 is a potent inhibitor of proliferation of transformed and endothelial cells, as well as angiogenesis in vivo.\textsuperscript{21,24} 2-ME2 is an inhibitor of endothelial cell migration in vitro and inhibits the neovascularization of solid tumors, suppressing their growth in mice.\textsuperscript{23} The anti-angiogenic effect of 2-ME2 is mediated primarily through inhibition of protein expression, nuclear accumulation and transcriptional activity of hypoxia-inducible factor-1\alpha (HIF-1\alpha). HIF-1\alpha is a transcription factor that stimulates hypoxia-induced secretion of vascular endothelial growth factor (VEGF).\textsuperscript{25-27} It was recently reported that 2-ME2 inhibited HIF-1\alpha protein translation by inducing argonate 2 (Ago2)-mediated association between HIF-1\alpha mRNA and HIF-targeting miRNAs in the cytoplasm. This effect was reported to occur after microtubule disruption and led to the targeted translocation of these complexes to cytoplasmic P-bodies in a microtubule dynamicity-dependent and reversible manner.\textsuperscript{28} However, in another report, 2-ME2 was shown to inhibit complex I of the mitochondrial electron transport chain leading to the generation of reactive oxygen species (ROS), which inhibited HIF-1\alpha protein stabilization and mitochondrial respiration in both intact cells and sub-mitochondrial particles.\textsuperscript{29}

**Cell Cycle Distribution Alterations and Arrest**

2-ME2 has been observed to arrest the growth of many human cancer cell lines representing several cell types including Jurkat cells, multiple myeloma, epithelial, melanoma, medulloblastoma cancer cells and transformed fibroblasts at the G2-M transition.\textsuperscript{5,9,12,20,30,31} At the biochemical and molecular levels, 2-ME2-induced G2-M cell cycle arrest has been characterized by the induction of cyclin B and Cdc2 kinase activity.\textsuperscript{5,20,32} 2-ME2 has also been reported to induce G2-M arrest in breast cancer cell lines regardless of hormone receptor status.\textsuperscript{6,32} In other reports, however, the anti-tumor effects of 2-ME2 were not associated with G2-M cell cycle arrest. For example, reports have indicated that 2-ME2 inhibited the growth of pancreatic cancer cells by prolonging S-phase or by inducing both G1-S and G2-M arrest in human osteosarcoma cells and pancreatic cell lines.\textsuperscript{33-35} In the ER-positive MCF-7 breast cancer cell line, 2-ME2 (10 nM) induced an increase in cAMP concentration in early S-phase that decreased during mitosis, and phosphorylation of S-phase proteins was enhanced in 2-ME2-exposed cells with no effect on protein synthesis during G2-M transition.\textsuperscript{24}

**Induction of Apoptotic Cell Death**

The induction of apoptosis by 2-ME2 in tumor cells is reported to involve different molecular mechanisms. LaVallee et al have shown that 2-ME2 may utilize the extrinsic pathway for induction of apoptosis.\textsuperscript{36} In these studies, the authors reported that 2-ME2 treatment of breast, cervical and prostate carcinoma cells as well as glioma cells and HUVECs...
resulted in up-regulation of death receptor 5 (DR5) protein expressions in vitro and in vivo. This rendered the cells more sensitive to the cytotoxic activities of the DR5 ligand, the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). In this study, it was found that 2-ME2-induced apoptosis required sequential activation of caspase-8, caspase-9, and caspase-3. The phosphatidylinositol-3-kinase (PI3K) pathways have also been implicated in 2-ME2-induced activation of the extrinsic apoptotic pathway in prostate cancer cells, and the role of the Akt pathway in the response to 2-ME2 was also explored in human leukemia cells as well.

Several studies also suggest that 2-ME2 can induce apoptosis both by tumor suppressor protein p53-dependent and p53-independent mechanisms in various tumor cell types. We have previously shown that in T47D breast cancer cells containing mutant p53, 2-ME2 (1-10 μM) significantly decreases breast cancer cell number and this effect may not be mediated by signaling pathways that are directly influencing, or dependent upon the levels of p53. Interestingly however, concentrations of 2-ME2 had no significant effect on T47D cell number (1-100 nM) induced p53 protein accumulation which is believed to be due to the possible sequestration of p53 within nucleolar compartments within the cell. In a report by Mukhopadhyah et al, treatment with 5 μM 2-ME2 caused significant growth inhibition of human lung cancer cell lines containing wild-type (wt) p53 (H460 and A549), while having little to no effect on the p53 negative H358 and p53 mutated H322 cell lines. In these studies, the authors found that 2-ME2 treatment up-regulated the endogenous wt p53 protein and subsequently, the cells bypassed the G1-S checkpoint and underwent apoptosis (no change in mutant p53 levels was observed). In four pancreatic cancer cell lines harboring mutant p53, 2 μM 2-ME2 induced S phase arrest and apoptosis that was suggested to occur through a p53-independent mechanism. In another report, 2-ME2 induced G2-M arrest, up-regulated p53 protein levels, and induced micronuclei formation and apoptosis in SV40 T antigen transformed HSF43 lymphoblast cells (line E8T4). In these studies, apoptosis and G2-M block were also observed in two lymphoblast cell lines expressing either low levels of wt p53, or high levels of temperaturesensitive mutant p53, but this was to a much lesser extent than in E8T4 cells and without observed alteration in p53 protein levels. However, when the authors cultured the cells at the permissive temperature, an increase in apoptosis and a prominent G2-M-phase block were present in the mutant p53 cells, suggesting that the high levels of mutant p53 became functional, enhancing the apoptotic effects initiated by 2-ME2.

Further studies have implicated c-jun NH2-terminal kinase (JNK) signaling cascades, including phosphorylation of the anti-apoptotic Bcl-2 family members in 2-ME2-induced apoptosis. In the ER-negative MDA-MB-435s human breast cancer cell line, it was reported that 2-ME2 induced the activation of JNK which was associated with the induction of apoptosis through the mitochondrial pathways as a result of increased phosphorylation (inactivation) of the anti-apoptotic Bcl-2 and Bcl-xL proteins. In comparison, this same study also reported 2-ME2-induced activation of ERK and p38 in these cells, which was found to have a protective effect against 2-ME2-induced apoptosis. In several cell lines derived from prostate, breast, liver and colorectal carcinomas, 2-ME2 treatment led to an activation of JNK and phosphorylation of Bcl-2, which preceded the induction of apoptosis. Thus, it appears that 2-ME2 induces apoptosis in epithelial carcinomas by causing phosphorylation of JNK, which appeared to be correlated with phosphorylation of Bcl-2. However, the stimulus type, regulatory pathways involved and the degree and duration of phosphorylation at specific Bcl-2 residues produce different outcomes. For example, 2-ME2 inhibited the proliferation of Jurkat leukemia cells by up-regulating p16(INK4A) and markedly suppressing the levels of cyclins D3 and E, p21(Cip1/Waf1) and E2F1. Further, 2-ME2-induced apoptosis of Jurkat cells was associated with both expression down-regulation as well as JNK-mediated inactivation of Bcl-2, up-regulation of Bak protein levels, activation of caspases-9 and -3 and also PARP-1 cleavage. However, the overexpression of Bcl-2 prevented the 2-ME2-induced apoptotic response by orchestrating a p27(Kip1)-dependent G1-S phase arrest which was associated with NF-κB activation. p38/JNK-dependent activation of NF-κB has also been reported to be required for 2-ME2-induced apoptosis in prostate cancer cells, however, a reduction in NF-κB transcriptional and DNA binding activity was observed in 2-ME2-induced apoptosis of medulloblastoma cells.

CONCLUSION

The reports summarized within this review clearly demonstrate not only the cell-specific nature of 2-ME2 action, but also genotypic influence on the cellular response to 2-ME2. While this metabolite shows great potential for therapeutic intervention, it is limited by drug disposition challenges. 2-ME2 has poor bioavailability as the rate of oxidation of 2-ME2 is higher than its absorption, limiting the action of the metabolite in vivo. Several analogs and derivatives have recently been developed, and while some show promise, they currently lack the ability to induce effects without unwanted cytotoxicity. The specificity for actively
proliferating cancer cells with minimal to no toxicity is an intrinsic property of 2-ME2 that increased its appeal as a treatment modality in the first place. Due to this, it has become imperative that we re-evaluate our understanding of the molecular mechanisms governing the cellular responses to 2-ME2. The need is paramount to more accurately define the necessary factors that are required to be targeted by a drug candidate, in order to successfully achieve desired effects that are similar to those observed with this metabolite. Thus, continued elucidation of the mechanisms of 2-ME2 action is warranted.

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