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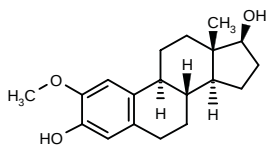
2-Methoxyestradiol

Anticancer Agent
Angiogenesis Inhibitor

2-ME
2ME2
NSC-659853
Panzem®

2-Methoxyestra-1,3,5(10)-triene-3,17β-diol



$C_{19}H_{26}O_3$
Mol wt: 302.4114
CAS: 000362-07-2
EN: 279172

Abstract

2-Methoxyestradiol is a potent anticancer agent, currently undergoing phase I and II studies for the treatment of several different cancers. Chemically, 2-methoxyestradiol is a naturally occurring metabolite of estradiol, but it does not act as an agonist at the estrogen receptor binding sites. 2-Methoxyestradiol acts to inhibit both tumor growth and angiogenesis. 2-Methoxyestradiol therefore has actions both within the tumor cell compartment and within the tumor vasculature. In this way, 2-methoxyestradiol targets the tumor itself, as well as its blood supply, thereby combining the effects of chemotherapy and antiangiogenesis. It also plays a role in the inhibition of metastatic spread, widening even further its implications for the treatment of cancer. Based on the significant anticancer efficacy and favorable toxicity profile of the drug in preclinical studies, it was advanced into the clinic as a novel hemotherapeutic/antiangiogenic agent. Results from early phase I and II clinical trials have shown that 2-methoxyestradiol has a good toxicity profile in cancer patients. Synergistic antitumor activity has been reported with other available chemotherapeutic agents. Problems arising from the reduced bioavailability of the currently available formulation are expected to be rectified soon, as this promising anticancer agent continues through clinical development.

Introduction

Cancer arises from cells with a genetic dysfunction that results in excess cell growth and a subsequent decline in death of the cells. These features allow cancer cells to rapidly grow and divide – eventually taking the place of normal parenchyma. These characteristics of uncontrolled growth are properties of all neoplasms, and cancer is therefore more common in tissues with a high cell turnover. Currently used drugs for the treatment of cancer focus principally on the correction of this uncontrolled growth.

The rationale of chemotherapy is to target and destroy some aspect of the tumor cell that can be differentiated from normal cells. Tumor cells are distinct from normal cells in that they divide more rapidly and are associated with inadequate cell death. Therefore, treatments can either target the cell division process to limit proliferation of cells, or can work through the early induction of cell death. Anticancer drugs aimed at these differences are termed antiproliferative or proapoptotic, respectively.

Many anticancer drugs target cell division, as it is well known that malignant cells divide much more rapidly than cells from normal tissue. The mechanism of cell division is invariable in all dividing cells. Rapidly dividing cells are more likely to be influenced by antiproliferative agents, as there is a greater chance that these cells will be dividing when the drug treatment is given.

Normal cells undergo programmed cell death (or apoptosis) after a specified number of divisions. Apoptosis is essential for normal development, as it is a process that removes older cells threatening the integrity of the organism. In this way, apoptosis helps to maintain the function of normal cells, as older ones are programmed to die off before they are likely to succumb to

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DNA disruption or damage. Apoptosis is mediated by a family of proteins known as caspases. Tumor cells are characterized by a dysregulation of programmed cell death and can therefore continue to grow and divide at the expense of other tissue, apparently by interfering with the activity of the caspase protein.

Tumor cells also need blood and nutrients to grow, just like cells from any other tissue. Tumor growth is therefore dependent on blood supply to that region, which is largely determined by the growth of new blood vessels (or angiogenesis). Drugs that inhibit angiogenesis therefore limit the rate of growth of the tumor, as the tumor can potentially outgrow its blood supply. It is generally held that tumors cannot grow beyond 1 mm in diameter without requiring some level of new vessel formation. Angiogenesis is also an essential requirement for the development of metastases.

2-Methoxyestradiol (2ME2, Panzem®) is a naturally occurring estrogen metabolite. It is formed through the hydroxylation and subsequent methylation of estradiol. Though originally considered to be an inactive end product of estradiol metabolism, recently reported studies of 2-methoxyestradiol have presented this molecule as a potential treatment in the field of oncology, as it has been shown to selectively destroy cancer cells in both animals and humans.

2-Methoxyestradiol exerts its anticancer effects through three main actions: firstly, by inhibiting cellular proliferation; secondly, by inducing programmed cell death in tumor cells; and finally, by decreasing angiogenesis (blood supply to the tumor).

2-Methoxyestradiol is therefore described as having a dual mode of action characterized by antitumor (antiproliferative and proapoptotic) and antiangiogenic actions. It thus exerts effects both within the tumor cell compartment and within the tumor vasculature (1).

Pharmacological Actions

The mechanism by which 2-methoxyestradiol produces its antitumor and antiangiogenic effects has not been clearly defined. It would seem logical to presume that the compound might have some action at estrogen receptor sites, being that it is an endogenous metabolite of estradiol. A recent study by LaVallee *et al.*, however, showed that the actions of 2-methoxyestradiol are in fact independent of estrogen receptor activation. 2-Methoxyestradiol was shown to have low binding affinity for both estrogen receptor ERα and ERβ sites, with K_i values of nM respectively, being reported, as compared with K_i values for 21 nM and 417 estradiol of 0.042 nM and 0.132 nM, respectively. Furthermore, the estrogen antagonist ICI-182780 had no effect on the action of 2-methoxyestradiol. It can therefore be inferred that 2-methoxyestradiol does not act as an agonist at ER sites (2). The effects of 2-methoxyestradiol were also shown in this study to be related to the action of cytochrome P-450, the enzyme that catalyzes the oxidation of estradiol. Furthermore, 2-methoxyestradiol exerted feedback inhibition of cytochrome P-450 in a recently reported study by Dawling et al. It is therefore likely that the effects of 2-methoxyestradiol are mediated via P-450 mechanisms (2, 3).

A plethora of molecular targets have been proposed for the activities of 2-methoxyestradiol, but an unequivocal molecular target capable of accounting for both the antitumor and antiangiogenic effects of 2-methoxyestradiol has not yet been elucidated. It is conceivable that the compound has more than one molecular target, and that its mechanism of action depends on the cellular environment. It is this versatility of action that allows for its use in a wide range of potential therapeutic indications.

2-Methoxyestradiol upregulates the death receptor 5 (DR5) in both tumor and endothelial cell lines. The DR5 receptor is intricately involved in apoptosis mediated via the extrinsic pathway. Results from *in vitro* and *in vivo* studies showed that 2-methoxyestradiol produced an increase in the expression of DR5 protein, and a subsequent increase in caspase 8, 9 and 3 levels. These results strongly suggest that the proapoptotic effects of 2-methoxyestradiol are mediated via the extrinsic pathway, through the activation of DR5 and caspase systems (4, 5).

There is some evidence that 2-methoxyestradiol interacts with microtubules and superoxide production (6).

Drugs that inhibit cell proliferation at mitosis by an action on mitotic spindle microtubules have become increasingly valuable in the treatment of various forms of cancer. Microtubules are cylindrrical structures that make up part of the cellular skeleton. Found in the cytoplasmic ground substance of nearly all cells, they are intricately involved in cell division. Microtubule-targeting agents are a common class of anticancer agents, as the stabilization of these structures has an antiproliferative effect – thereby decreasing the rate of growth of tumors. 2-Methoxyestradiol provokes inhibition of microtubule dynamics, leading to mitotic arrest, which subsequently leads to apoptosis. It is thought that this cytotoxic activity is mediated through the ability of 2-methoxyestradiol to bind to tubulin (via the colchicine binding site), thereby altering the stability of the microtubule (7-9).

Hypoxia-inducible factor-1α (HIF-1α) activity is also inhibited following 2-methoxyestradiol administration. This protein is overexpressed in over 70% of human cancers, and is therefore a recognized target for rational drug design in oncology. HIF-1α activity is increased when tumors outgrow their blood supply. Increased activity of HIF results in the increased expression of vascular endothelial growth factor (VEGF), which ultimately leads to the promotion of angiogenesis. Mabjeesh *et al.* Reported that 2-methoxyestradiol produced a decrease in HIF-1α activity following microtubule disruption. 2-Methoxyestradiol is therefore purported to exert its antiangiogenic effects through inhibition of these pathways. Results from this study also provided a mechanistic link between the microtubule and antiangiogenic effects of 2-methoxyestradiol (8, 10, 11).

2-Methoxyestradiol is said to be a potent inhibitor of superoxide dismutase. These claims are controversial, however, with other researchers publishing results purporting that 2-methoxyestradiol does not act through this pathway. Superoxide dismutase normally acts to prevent apoptosis by protecting cells from damage due to free radicals. Superoxide dismutases are enzymes that actively eliminate reactive oxygen species – the potentially dangerous byproducts of cellular metabolism. Tumors characteristically have low levels of superoxide disumutase, and many tumors are therefore dependent on superoxide dismutase for survival. Some studies have shown 2-methoxyestradiol to induce apoptosis in cancer cells by blocking the action of the protective superoxide disumutase, thereby increasing the level of superoxide anions in the cellular environment (12-15).

The anticancer efficacy of 2-methoxyestradiol has been demonstrated in many different tumor and nontumor models. *In vitro* studies have shown 2-methoxyestradiol to inhibit the growth of tumor and nontumor cell lines. It has antiproliferative, antiangiogenic and proapoptotic effects in both human and animals models of malignancy. *In vivo* studies have further shown 2-methoxyestradiol to inhibit tumor growth and angiogenesis in xenograft and metastatic disease models, as well as metastatic spread. Perhaps most importantly, 2-methoxyestradiol appears to produce its anticancer effects in the context of limited toxicity (2, 16).

Using multiple myeloma cell lines from both animals and humans, 2-methoxyestradiol was shown to inhibit growth and induce apoptosis. 2-Methoxyestradiol was also shown to have anticancer efficacy in cells that were resistant to other chemotherapeutic agents. Apoptosis was related to the activation of caspase proteins. The percentage of cells undergoing apoptosis in multiple myeloma cell lines was, however, relatively small (< 25%). On the other hand, 2-methoxyestradiol had no effect on the survival of peripheral blood lymphocytes (17, 18).

2-Methoxyestradiol dose-dependently inhibited tumor growth by 50-90% in a murine model of pancreatic cancer (19).

2-Methoxyestradiol has also been shown to be a potent inhibitor of human osteosarcoma cell growth. Investigators showed that this effect was due to cell cycle arrest at two separate points. Its effects on both cell cycle and osteosarcoma cell growth were concentration-dependent, whereas it did not affect the proliferation of normal osteoblasts (9, 20, 21).

Human breast and prostate cancer cell lines underwent apoptosis in the presence of 2-methoxyestradiol. The authors commented that these effects appeared to be mediated by an activation of c-Jun *N*-terminal kinase (JNK), which then led to apoptosis. 2-Methoxyetsradiol was also effective in inhibiting breast cancer cell growth in combination with tamoxifen (22, 23).

Some investigators have suggested that the mechanism for the antiproliferative efficacy of 2-methoxyestradiol is cell line-dependent. For example, while apoptosis appears to be due to mitochondrial damage in sarcoma cells, programmed cell death is more often related to the action of reactive oxygen species in leukemia cells (5, 24).

Xenograft growth was inhibited by i.p. injection of 2-methoxyestradiol in a murine model of multiple myeloma. No tumor regrowth was seen, indicating that the effects of 2-methoxyestradiol are maintained following discontinuation of treatment (18).

Results from a recent preclinical study showed that 6-week administration of 2-methoxyestradiol 150 mg/kg/day inhibited tumor growth by 60% in an animal model that resembles the development of breast cancer in humans. Investigators reported that the antiproliferative action of 2-methoxyestradiol appeared to be mediated through upregulation of DR5 and activation of caspase 3, resulting in the induction of apoptosis in this context. No toxicity was observed in this model (25).

Oral administration of 2-methoxyestradiol (100 mg/kg) decreased tumor size in mice inoculated with human multiple myeloma cells. Angiogenesis was decreased and survival increased following drug administration (17).

2-Methoxyestradiol has also been proposed as a potential treatment for noncancer conditions. The drug was recently shown to have some effect on cancellous bone mass in ovariectomized rats. Administration of 2-methoxyestradiol led to maintenance of baseline cancellous bone, preventing the skeletal changes normally observed in this animal model. Investigators suggested that the compound may therefore represent a potential alternative therapy in the treatment of osteoporosis. Further studies are needed to verify these claims (26).

Investigators have also considered that antiangiogenic medications may have potential benefits in the treatment of blindness. Results with ocular implants have demonstrated that 2-methoxyestradiol inhibits the neovascularization associated with age-related macular degeneration.

Clinical Studies

2-Methoxyestradiol was advanced to phase I clinical trials following overwhelming preclinical evidence of its antitumor and antiangiogenic effects, in the absence of major toxicity. Phase I studies in patients with breast cancer and phase II studies in patients with prostate cancer and multiple myeloma have been carried out to date (26).

The first phase I efficacy study of 2-methoxyestradiol was performed in patients with advanced refractory breast cancer. The drug was administered at doses of 200-1000 mg/day once or twice daily to 31 patients with treatment-resistant breast cancer for 28 days. All patients had received prior treatment for their disease, with a median of 3 treatments being reported in this cohort. After a 14-day observation period, responding or stable patients continued oral 2-methoxyestradiol administration until a 50% reduction in tumor size was observed. The objectives of this study were to define maximum tolerated

dose (MTD), along with other tolerability data. Toxicity data were taken from the first 28 days of dosing only. Pharmacokinetic and efficacy data were measured as secondary outcome variables. 2-Methoxyestradiol was well tolerated and appeared to be effective in the treatment of patients with refractory breast cancer. Seventeen patients experienced stable disease after the 28-day treatment period and continued with 2-methoxyestradiol therapy for variable amounts of time. The MTD was not identified by the end of the 28-day treatment period. The most commonly reported adverse events were fatigue, myalgia and nausea. No grade 4 toxicities were reported. 2-Methoxyestradiol did not have any effect on hormone status in these patients. Pharmacokinetic analysis showed a $t_{1/2}$ of 10 h, with peak serum levels being reached after 2-4 h. 2-Methoxyestradiol showed significant interpatient variability and accumulation was seen in patients after twice- but not once-daily dosing (1, 27).

2-Methoxyestradiol has been shown to have synergistic activity together with the anticancer agent docetaxel (Taxotere®) in *in vivo* and *in vitro* analyses. The safety and tolerability of the combination were therefore assessed in a phase I study in patients with metastatic breast cancer. Fifteen patients with advanced breast cancer were administered once-daily oral 2-methoxyestradiol 200-1000 mg/day in combination with i.v. docetaxel 35 mg/m^2 once a week for 4 of 6 weeks. 2-Methoxyestradiol was administered for 28-days, followed by a 13-day observation period. Investigators measured efficacy, tolerability and pharmacokinetic data following this combination regimen. The MTD was based on the toxicity data from the first 42 days of 2-methoxyestradiol + docetaxel administration. Changes in hormone levels (*i.e.*, estrogen, testosterone, progesterone, luteinizing hormone and follicle-stimulating hormone) were measured as secondary variables in these patients. The combination resulted in complete and partial tumor responses in 1 and 2 patients, respectively. Overall response rate was 20%, with 47% of patients maintaining stable disease over the course of the study. Median time to treatment failure was 203 days in this setting. The MTD was not reached in this dose-finding study. The most commonly reported side effects included fatigue, diarrhea, alopecia, myalgia and dyspnea. No grade 4 toxicities were reported. Moreover, side effects were typically associated with weekly dosing of docetaxel. Hormone status showed no significant alterations throughout the study. 2-Methoxyestradiol therefore shows promise as an anticancer agent both alone and in combination with other anticancer agents. Results from the aforementioned phase I trials failed to identify the MTD. The current 2-methoxyestradiol formulation limits exposure to levels below the target range specified by preclinical trials. A new formulation with increased bioavailability and efficacy is currently undergoing preclinical trials, and is expected to be the subject of future clinical analysis (28).

The anticancer efficacy of 2-methoxyestradiol is currently being assessed in a dose-escalation phase I study in patients with solid tumors. Oral doses of 800-6000 mg/day are administered to patients twice daily. The aim of this study will be to determine the tolerability profile, as well as the MTD of 2-methoxyestradiol in this setting. The MTD is defined as the dose preceding that which causes dose-limiting toxicity in 2 of 6 patients. From this finding, a recommendation for an optimal biological dose in patients with solid tumors will be made. Pharmacokinetic data will also be collected. An interim analysis from the first 14 enrolled patients was recently reported at the Annual Conference of the American Society of Clinical Oncology. Dahut et al. reported that 2-methoxyestradiol is well tolerated in patients with solid tumors. Mild nausea, vomiting, fatigue and hypoalbuminemia were among the most commonly reported adverse events in these patients. One patient experienced grade 4 angioedema 38 days into the study. The MTD had not yet been reached at the time of presentation. Preliminary efficacy data were also very promising, with 1 patient experiencing a 67% and 78% reduction in lymph node metastases and CA125 concentration, respectively. Complete tolerability, efficacy and pharmacokinetic data are expected soon (29).

Results from a phase II study of 2-methoxyestradiol in men with prostate cancer were recently reported at the American Society of Clinical Oncology Annual Meeting. Thirty-three patients with refractory prostate cancer were administered a single oral dose of 2-methoxyestradiol of 400 or 1200 mg/day in this randomized, double-blind trial. Refractory prostate cancer was defined as prostate cancer unresponsive to luteinizing hormone-releasing hormone (LHRH) agonist therapy, estrogen therapy or orchiectomy for the purposes of this study. Doses used in this trial were based on recommendations made by investigators from the previously reported trial in patients with refractory breast cancer. 2-Methoxyestradiol was administered in 28-day cycles until treatment failure or unacceptable toxicity was reported. Anticancer efficacy was measured by tumor response (via radiological examination, clinical examination and patient interview) and prostate-specific antigen (PSA) levels in this study. Antiangiogenic efficacy was measured by levels of vascular endothelial growth factor (VEGF). Pharmacokinetic and pharmacodynamic data were also assessed. Finally, treatment-emergent adverse events were recorded throughout the study. Patients received 2-methoxyestradiol for a median of 16 weeks. During this time, no tumor response or serological response was observed. Prostate-specific antigen was stabilized or decreased in some patients, however, and VEGF levels were also decreased in this study. Mean C_{max} was 5.5 and 9.6 ng/ml on day 28 following doses of 400 and 1200 mg/day, respectively. Mean tmax was 1.2 and 6.7 h, respectively, and gastrointestinal dissolution was identified as the rate-determining step following oral administration of this preparation. Bioavailabilities were not significantly different between groups, indicating that absorption was limited with this formulation. Steady-state concentrations were reached on day 8 of the 28-day cycle in this study. Pharmacodynamic data revealed that oxidation at

position 17 of 2-methoxyestradiol results in an 80-95% conversion to the metabolite, which is 10 times less biologically active when compared with 2-methoxyestradiol. Liver function abnormalities were detected in 3 of 22 evaluable patients; AST and ALT levels were elevated after 3-5 weeks of 2-methoxyestradiol administration. These changes rapidly returned to normal following discontinuation of the study drug, however. There was no correlation between plasma levels of 2-methoxyestradiol and liver function abnormalities. No grade 4 toxicities were reported. 2-Methoxyestradiol was therefore well tolerated in this patient population. Evidence of anticancer efficacy was observed, despite the poor bioavailability of the preparation used. The authors noted that the plasma levels of 2-methoxyestradiol were much lower than expected, suggesting that efficacy and tolerability profiles will need to be reevaluated once a more bioavailable preparation is available for human use (30).

A second phase II study is currently recruiting patients with multiple myeloma in order to test the efficacy and tolerability in this patient population.

Conclusions

2-Methoxyestradiol is a novel drug candidate with potential utility in many indications in the field of oncology. It acts directly on the tumor itself, both to inhibit tumor cell proliferation and to induce apoptosis in tumor cells, and it also acts on the tumor vasculature to indirectly inhibit tumor growth by reducing blood supply. Clinical data have shown 2-methoxyestradiol to be effective in maintaining disease status in patients with advanced breast and prostate cancer. Further results are expected soon from a phase II study in patients with multiple myeloma. 2-Methoxyestradiol is associated with very little toxicity. Commonly reported adverse events include fatigue, diarrhea, headache, hot flushes and myalgia. No grade 4 toxicities have yet been reported. Pharmacokinetic data have shown that the current preparation of 2-methoxyestradiol is lacking in bioavailability. A new formulation is currently going through preclinical trials, and will be available soon for use in a clinical context.

Source

EntreMed, Inc. (US); codeveloped with Aventis Pharma AG (CH).

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