Catechol-O-Methyltransferase Inhibitors and 2-Methoxyestradiol Suppress Leiomyoma Cell Proliferation: Potential Medical Therapy for Uterine Fibroids

Salama A. Salama and Ayman Al-Hendy

Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas

Ashbel Smith Building
"Old Red"
Built in 1891

ABSTRACT

Objective: Due to minimal success with nonsurgical treatment options for uterine leiomyomas, it is imperative that other compounds be tested for potential preventive/therapeutic use. Since estrogen plays a pivotal role in the development and progress of leiomyomas, it is rational to hypothesize that targeting estrogen metabolism, or using certain estrogen metabolites possessing antiestrogenic effects, might be a useful treatment of leiomyomas.

Methods: In the current study, we tested the effect of estradiol (E_2) and its metabolite, hydroxyestradiol (2-OHE_2), with or without catechol-O-methyl transferase inhibitor (COMT-I), on ELT-3 rat leiomyoma cell proliferation and the expression of adenovirus-estrogen response element-luciferase (AdERE-Luc) reporter vector. In addition, we tested the effect of 2-methoxyestradiol (2-$MeOHE_2$) on the growth of leiomyoma cells in culture.

Results: Our data indicated that COMT-I reversed the proliferative effects of E_2 on ELT-3 cells and reduced the E_2-induced upregulation of AdERE-Luc reporter. Furthermore, 2-OHE_2 antagonized the effect of E_2 on cell proliferation and AdERE-Luc activation. 2-$MeOHE_2$ exhibits a biphasic effect on leiomyoma cells. With relatively low concentration levels (10^{-10}–10^{-8} M), it is mitogenic, while at pharmacological concentrations (10^{-7}–10^{-6} M), it is antiproliferative. Our data also demonstrated that the antimitogenic effect of 2-$MeOHE_2$ is associated with downregulation of the vascular endothelial growth factor (VEGF), Cyclin D1, and bcl2.

Conclusion: The results of the current study suggest the potential usefulness of COMT-I, as well as the estrogen metabolite 2-$MeOHE_2$, as agents for nonsurgical management of uterine leiomyomas.

INTRODUCTION

Uterine leiomyomas (ULM) are benign smooth-muscle cell tumors of the uterus and are the most common pelvic tumor in women. These tumors occur primarily during the reproductive years, and are the most frequent indication for hysterectomy in the United States. Unfortunately, nonsurgical therapies are limited, hysterectomy or myomectomy being the treatment of choice in most cases. The discovery and development of medicinal therapies for ULM have been hampered by a lack of understanding regarding the etiology and molecular mechanisms underlying the development of these lesions. Early menarche, African-American ethnicity, nulliparity, and obesity are among the common risk factors for ULM. The impact of these factors has often been attributed to their effects upon estrogen and progesterone levels or metabolism, which may in part reflect aspects of a woman's hormonal milieu. Furthermore, another potential mechanism for development of ULM is a decrease in apoptosis. It has been reported that Bcl-2 protein, an apoptosis-inhibiting gene product, was abundantly expressed in leiomyomas relative to the normal myometrium. Developments of therapeutic agents which are capable of reversing ULM growth without serious side effects are urgently needed for the conservative treatment of ULM. Local estrogen metabolism and apoptosis pathways represent potential targets for treatment of ULM.

MATERIALS & METHODS

Culture of ELT-3 cells

ELT-3 cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotic. When experiments required the addition of exogenous compounds, cells were weaned by culturing for 48 h in phenol red-free medium containing 10% charcoal stripped fetal calf serum. For proliferation assays, cells were plated onto 12-well plates in DMEM at a density of 4×10^4 cells/well. The cells were allowed to attach overnight, and then treated with E_2 (10^{-6} –10^{-10} M) without COMT-I or with 10 uM of COMT-I; 2-OHE_2 (10^{-5}–10^{-10} M) without COMT-I, or with 10 uM COMT-I; or 2-$MeOHE_2$ (10^{-5}–10^{-10}M). Control cells were treated with medium containing 0.01% of absolute ethanol. After 48 h, the cells were harvested for the proliferation assays. For Western blot analysis, the cells were seeded in 100 mm plates at final density of 2×10^6 cells/plate. The cells were treated with 2-$MeOHE_2$ in the same way as for the proliferation assay. Cells were harvested 72 h after treatment and cell lysates were used for Western blotting.

Cell proliferation assay

A fluorometric assay, implementing Hoechst 33258 (bisbenzimide), was used for DNA quantitation. ELT-3 cells (5×10^3 cells/well) were plated (described above) and incubated with the treatments for 48 h, followed by cell lysis and DNA content determination using Hoechst dye. Fluorescence was measured (DyNA Quant 200; Hoefer Pharmacia Biotech) after excitation at 365 nm and fluorescence at 458 nm. Calf thymus DNA was used as a standard.

Western blots

Immunoreactive proteins corresponding to VEGF, Cyclin D1, bcl2, Bax, and –actin were identified from total protein by Western immunoblotting using specific monoclonal antibodies. After treatment of ELT-3 cells with 2-$MeOHE_2$ for 72 h, cells were lysed in lysis buffer containing protease inhibitors. Protein concentrations were quantified using bicinchoninic acid (BCA) Protein Assay Reagent. Ten µg of total cell lysates were resolved by SDS-PAGE. Primary antibodies recognizing VEGF, Cyclin D1, bcl2, or Bax were used. Secondary antibodies conjugated to horseradish peroxidase were used for hybridization. ECL western blotting detection reagent was used for visualization. ß-actin expression was used to assess consistent loading between samples. The expression level of target proteins was quantitated densitometrically and normalized using ß-actin expression.

Transfection of ELT-3 cells with AdERE-Luc reporter vector

For infection of ELT-3 leiomyoma cells with Ad ERE-Luc reporter vector, the cells were allowed to grow to 60% to 70% confluence, and then infected with Ad ERE-Luc vector at a multiplicity of infection (MOI) of 100 plaque-forming units (PFU)/cell for 4 h. ELT-3 cells transfected with AdERE-Luc were treated with different concentrations of E_2 (10^{-10}–10^{-5}M) in the presence or absence of COMT-I (10 uM); or 2-OHE_2 (10^{-10}–10^{-6} M) in the presence or absence of COMT-I (10 uM). After 48 h incubation with chemicals, cells were harvested with 100 µL of cell lysis buffer. The Luciferase activities were determined using a Luciferase Assay Kit.

RESULTS

COMT inhibitor diminished the mitogenic effect of estradiol on leiomyoma cell proliferation

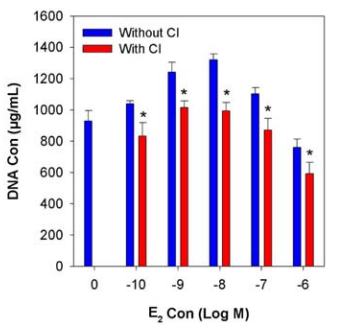

Figure 1. Effect of E_2 on the proliferation of ELT-3 cells in absence or presence of COMT-I. Cells were treated with different concentrations of E_2 (10^{-10}–10^{-6} M), alone or in combination with COMT-I (10^{-5}M). Cell proliferation was determined 48 h after treatment. Values are the mean ± SEM (N = 3 replicate cultures). The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

COMT inhibitor diminished E_2 induced ERE-Luc reporter transactivation in ELT-3 leiomyoma cells

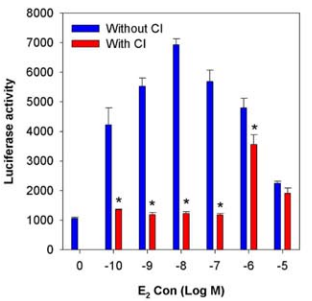

Figure 2. Effect of E_2 (10^{-10}–10^{-5} M) in the absence or presence of COMT-I (10^{-5} M) on the expression of AdERE-Luc reporter vector in ELT-3 cells. The cells were transfected with AdERE-Luc reporter vector at MOI of 100 PFU/cell. Twenty-four h after transfection, the cells were treated with different concentration of E_2, with or without COMT-I. 48 h after treatment the luciferase activity was determined using luciferase assey kit (Promega, Madison, Wis) according to manufacturer's instructions. The luciferase activity was normalized against protien concentration.. Values are the mean ± SEM (N = 3 replicates).The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

Effect of 2-OHE_2 and COMT Inhibitor on ELT3 cell proliferation

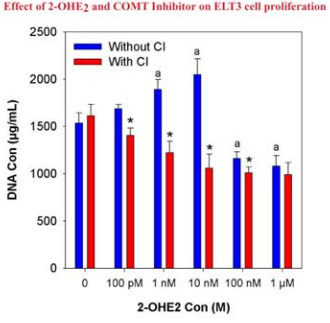

Figure 3. Effect of 2-OHE_2 on the proliferation of ELT-3 cells in the absence or presence of COMT-I . Cells were treated with different concentration of 2-OHE_2 (10^{-10}–10^{-6} M) alone or in combination with COMT-I (10^{-5}M). Cell proliferation was determined 48 h after treatment. Values are the mean ± SEM (N = 3 replicate cultures). The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

Effect of 2-OHE_2 and COMT-I on AdERE-Luc reporter transactivation in ELT-3 cells

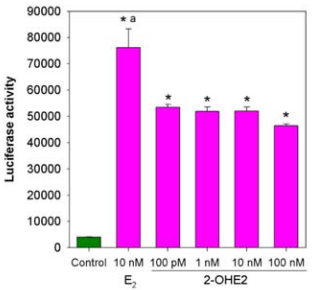

Figure 4. Effect of 2-OHE_2 (10^{-10}–10^{-7} M) on E_2 (10^{-8} M)-induced expression of AdERE-Luc reporter vector in ELT-3 cells. The cells were transfected with AdERE-Luc reporter vector at MOI of 100 PFU/cell. Twenty-four h after transfection, the cells were treated with E_2 (10^{-8} M) in the presence or absence of different concentration of 2-OHE_2. Luciferase activity was determined 48 h after treatment. Luciferase activity was normalized for protein concentration. Values are the mean ± SEM (N = 3 replicate cultures).The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

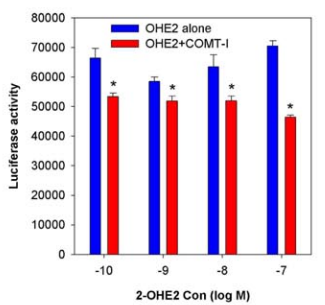

Figure 5. Effect of 2-OHE_2 (10^{-10}–10^{-7} M) alone or in combination with COMT-I (10^{-5} M) on E_2 (10^{-8} M)-induced expression of AdERE-Luc reporter vector in ELT-3 cells. The cells were transfected with AdERE-Luc reporter vector at MOI of 100 PFU/cell. The cells were maintained in media containing 10^{-8} M of E_2. Twenty-four h after transfection, the cells were treated with 2-OHE_2 (10^{-10}–10^{-7} M) in the presence or absence of different concentrations of COMT-I (10^{-5} M). Luciferase activity was determined 48 h after treatment. Luciferase activity was normalized for protein concentration. Values are the mean ± SEM (N = 3 replicate cultures).The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

Effect of 2-$MeOHE_2$ on the proliferation of leiomyoma cells

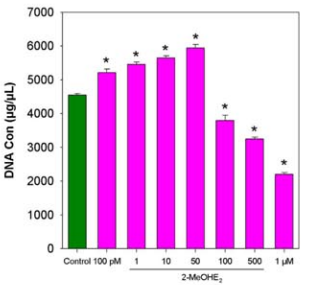

Figure 6. Effect of different concentrations of 2-$MeOHE_2$ on proliferation of ELT-3 cells. Cells were grown in media containing 10^{-8} M of E_2 and treated with different concentration of 2-$MeOHE_2$. Cell proliferation was determined 48 h after treatment. Values are the mean ± SEM (N = 3 replicate cultures).The data were analyzed using ANOVA and Fisher's least significant difference test. A value of $P \leq .05$ was considered statistically significant.

Effect of 2-$MeOHE_2$ on the expression of cell cycle and apoptosis regulating proteins in leiomyoma cells

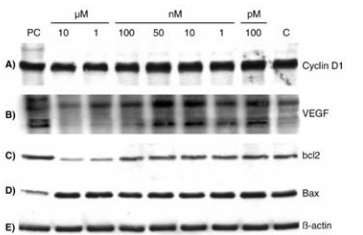

Figure 7. Western blot analysis of Cyclin D1, VEGF, Bcl2, and Bax protein in ELT-3 leiomyoma cells treated with different concentrations of 2-methoxyestradiol (10^{-10}–10^{-5} M) for 72 hours. Ten µg of total cell lysate were resolved by SDS-PAGE. MCF-7 breast cancer cell lysate was used as positive control. The corresponding monoclonal antibody was used for immunoblotting. ß-actin was used as loading control.

CONCLUSION

The results of this study demonstrated that COMT-I and 2$MeOHE_2$ exhibited antiproliferative effects against ELT-3 leiomyoma cells, suggesting their potential use as a medicinal treatment for uterine leiomyomas.