Retinoic Acid Upregulates Catechol-O-Methyltransferase Promoters: Potential Mechanism for the Mitogenic Effect of Retinoic Acid on Leiomyoma Cells

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ABSTRACT

Introduction: Catechol-O-methyltransferase (COMT) is a key enzyme in the metabolism of estrogens and, therefore, factors affecting COMT expression can indirectly affect the estrogenic milieu in situ. Retinoic acid has been proposed to promote the effect of estrogen in the guinea pig leiomyoma model. In addition, leiomyoma tissues express higher levels of all-*trans*-retinoic acid (ATRA) compared to adjacent normal myometrium. However, the mechanisms by which ATRA modulates estrogen activity and contributes to the pathophysiology of leiomyomas are not completely understood.

Methods: In the current study, we evaluate the effect of ATRA on the proliferation of cultured ELT-3 rat leiomyoma cells. In addition, we tested the hypothesis that the effect of ATRA on ELT-3 cells proliferation is mediated, in part, by altering the expression of COMT.

Results: Our data indicate that ATRA (10^{-8} and 10^{-7} M) significantly increased the proliferation of ELT-3 cells (120% and 130%, respectively) from the control ($P < .01$). Molecular mapping demonstrated that both COMT proximal (P1) and distal (P2) promoters are harboring retinoic acid response element (RARE). Western blot analysis indicates that treatment of ELT-3 cells with ATRA (10^{-8}, 10^{-7}, 10^{-6} M) resulted in a concentration-dependent increase in COMT protein expression. ATRA treatment (10^{-8}, 10^{-7}, or 10^{-6} M) of ELT-3 leiomyoma cells transfected with COMTP1-or COMTP2-luciferase (Luc) reporter resulted in significantly increased activity of both COMTP1-Luc (4-, 8- and 23-fold, respectively) and COMTP2-Luc (10-, 17-, and 21-fold, respectively) compared to the untreated control.

Conclusion: ATRA exerts a mitogenic effect on ELT-3 cells and this mitogenic effect may be attributed, at least in part, to upregulation of COMT expression in these cells. This is the first report that ATRA upregulates COMT expression. This finding has both therapeutic and toxicologic implications on the impact of ATRA on estrogen metabolism.

INTRODUCTION

Uterine leiomyomas are the most common tumors of the female genital tract. They account for 40% of hysterectomies in the United States. It is well-established that the growth of uterine leiomyomas is dependent on female sex hormones. Leiomyomas contain 4-fold higher levels of ATRA and nuclear receptor RXRα protein compared to the myometrium. In addition, the combination of troglitazone (a PPARγ ligand) with estradiol and ATRA in the guinea pig induces large uterine leiomyomas. This finding suggests that all-*trans*-retinoic acid plays a role in pathogenesis of leiomyoma. COMT expression regulates the local estrogenic milieu by metabolizing catechol estrogens. Recently, we found that COMT is highly expressed in leiomyomas compared to normal myometrium. COMT is highly regulated by nuclear receptors and it contains RARE consensus sequence. Thus, it is rational to propose that ATRA acid plays a role in the development of leiomyomas through the regulation of COMT expression.

AIM

The current work was undertaken to study the effect of all-*trans*-retinoic acid on COMT expression in ELT-3 Leiomyomas cells

MATERIALS & METHODS

Cells and cell culture

ELT-3 cells were maintained at 37ºC in 5% CO_2/air in Dulbecco's modified Eagle's medium (DMEM), with 10% fetal bovine serum. Cells (2×10^6) were seeded onto 100-mm culture dishes and incubated 24 hours. The media were removed, and the cells were re-incubated in fresh media with different concentrations of ATRA (10^{-8}, 10^{-7}, and 10^{-6} M) in 0.01% (v/v) ethanol. The cells were then harvested for protein assay.

Mammalian cell transfection with luciferase or ß-galactosidase plasmids

The activities of COMTP1-Luc and COMTP2-Luc constructs were determined in transiently transfected ELT-3 cells. Cells (60%–70% confluent) were cotransfected with luciferase reporter constructs (10 µg), and Promega pSV-ß-galactosidase control vector (1 µg) using calcium phosphate transfection method. After incubation for 5 hours at 37°C, the medium was removed, and the cells were incubated in fresh medium with the addition of different concentrations of ATRA (10^{-8}, 10^{-7}, and 10^{-6} M) in 0.01% (v/v) ethanol for 48 hours. Luciferase and pSV-ß-galactosidase activities were determined using ß-galactosidase and luciferase assay kits (Promega, Madison, Wis) according to the manufacturer's instructions. Protein concentrations were determined by the BCA protein assay kit (Pierce Co, Rockford, Ill). Luciferase activity is expressed after normalization against ß-galactosidase activity and protein concentration.

SDS-PAGE/Western blot analysis

Western blot analyses were performed using whole cell homogenate (50 µg per lane) prepared from control (ethanol only), or ATRA-treated cells according to the standard protocol. Purified COMT antibody (1:1500) raised in sheep was used to detect the COMT protein. Membranes were developed using HRP-conjugated sheep IgG with ECL Western Blotting Detecting Reagents (Amersham Biosciences, UK). The intensity of each band was determined using a scanning densitometer (Epson 4870, Epson America, Long Beach, Calif)

RESULTS

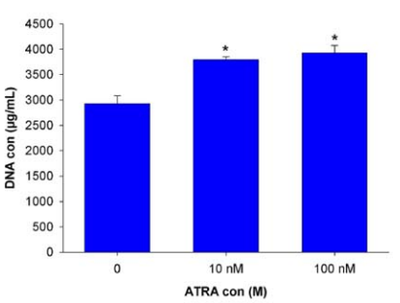

Figure 1. Effect of ATRA on the proliferation of ELT-3 cells were treated with ATRA (10^{-8}, 10^{-7} M) or vehicle (0.01% ethanl). The cell proliferation was determined 48 hours after treatment. Values are mean ± SEM (N = 3 replicates cutting) *Significantly different from vehicle-treated control ($P < .05$)

Regulation of COMT protein expression by ATRA in ELT-3 rat leiomyoma cells

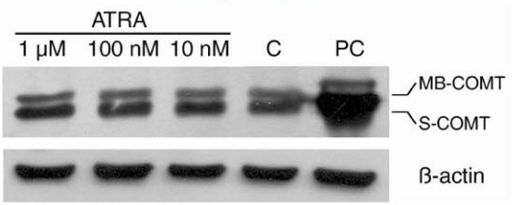

Figure 2. SDS-PAGE/Western blot analysis of MB-COMT and S-COMT protein levels in ELT-3 leiomyoma cells treated with different concentrations of ATRA (10^{-8}, 10^{-7}, or 10^{-6} M) for 72 hours. MCF-7 breast cancer cell lysate was used as a positive control. COMT polyclonal antibody was used at 1:1500. ß-actin was used as loading control.

ATRA upregulates COMT Promoter-Reporter Expression in ELT-3 cells

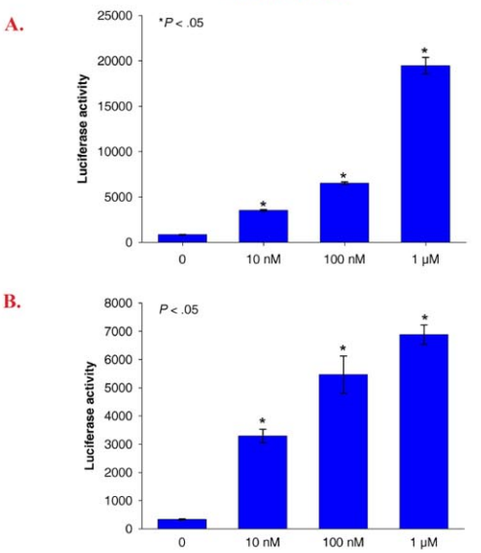

Figure 3. Effect of ATRA on luciferase activity in ELT-3 cells cotransfected by COMTP1-Luc (A) and COMTP2-Luc (B) constructs and pSV-ß-galactosidase. Constructs were cotransfected into ELT-3 cells and incubated with ATRA (10^{-8}, 10^{-7}, or 10^{-6} M). After 48 hours, the cells were harvested. Luciferase and ß-galactosidase activities were determined using ß-galactosidase and luciferase assay kits (Promega, Madison, Wis) according to the manufacturer's protocol. The results were expressed as a percentage of luciferase activity in ATRA-treated cells relative to vehicle-treated cells after correction for transfection efficiency using ß-galactosidase and protein concentration. The mean values ± SEM of at least three separate transfections are shown

CONCLUSION

ATRA upregulates COMT transcription and protein expression in ELT-3 rat leiomyoma cells. Upregulation of COMT by ATRA affects the local estrogen metabolism and may provide a possible explanation for the role of ARTA in the development of leiomyoma.