# Tranilast, an orally active antiallergic compound, inhibits extracellular matrix production in human uterine leiomyoma and myometrial cells

Md Soriful Islam, Ph.D.,[a,b] Olga Protic, M.Sc.,[a] Andrea Ciavattini, M.D.,[c] Stefano Raffaele Giannubilo, M.D.,[c] Andrea Luigi Tranquilli, M.D.,[c] William H. Catherino, M.D., Ph.D.,[d] Mario Castellucci, M.D., Ph.D.,[a] and Pasquapina Ciarmela, Ph.D.[a,e]

[a] Department of Experimental and Clinical Medicine, Faculty of Medicine, [c] Department of Clinical Science, and [e] Department of Information Engineering, Polytechnic University of Marche, Ancona, Italy; [b] Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh; and [d] Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, Maryland

**Objective:** To determine the effect of tranilast (an antiallergic drug known to suppress fibrosis or to stabilize mast cells) on extracellular matrix production in human leiomyoma and myometrial cells.
**Design:** Laboratory study.
**Setting:** University-affiliated laboratory.
**Patient(s):** Seven premenopausal women who were admitted to the hospital for myomectomy or hysterectomy.
**Intervention(s):** Cells were treated with tranilast (300 $\mu$M) for 48 hours to measure extracellular matrix and activin-A expression by real-time reverse-transcription polymerase chain reaction and/or immunocytochemistry.
**Main Outcome Measure(s):** The expression of fibronectin, collagen1A1, versican, and activin-A in myometrial and leiomyoma cells.
**Result(s):** Tranilast decreased fibronectin, collagen 1A1, and versican messenger RNA (mRNA) expression in human primary leiomyoma cell culture. Similar results were found in an immortalized human leiomyoma cell line. Tranilast also decreased the mRNA expression of fibronectin, collagen 1A1, and versican in human primary myometrial cells. The reduced expression of fibronectin and collagen 1 were observed by immunocytochemistry as well. Tranilast also reduced profibrotic growth factor, activin-A mRNA expression in primary myometrial and leiomyoma cells.
**Conclusion(s):** Our results indicate that tranilast reduced fibronectin, collagen 1A1, versican, and activin-A expression in leiomyoma and myometrial cells, demonstrating its potential as an antifibrotic therapy for human leiomyomas. (Fertil Steril® 2014;102:597–606. ©2014 by American Society for Reproductive Medicine.)
**Key Words:** Fibroid, tranilast, fibronectin, collagen 1A1, activin-A

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Reprint requests: Pasquapina Ciarmela, Ph.D., Department of Experimental and Clinical Medicine, Polytechnic University of Marche, Faculty of Medicine, Via Tronto 10/a, 60020 Ancona, Italy (E-mail: p.ciarmela@univpm.it).

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Uterine fibroids or leiomyomas are the most common female reproductive tract tumors (1, 2). They are highly prevalent, with 70%–80% of women burdened by the end of their reproductive years (3). Beside their high prevalence leiomyomas are associated with a variety of problems, such as menorrhagia, pelvic pain, and pelvic pressure, as well as infertility and

pregnancy complications (4). Hysterectomy is definitive treatment for this tumor, but loss of reproductive potential and significant morbidity and mortality are major limitations of this surgical intervention. Furthermore, surgical intervention for leiomyomas is associated with a substantial economic impact on health care systems that amounts to approximately $2.2 billion per year in the United States alone (5).

To avoid the risks of surgical intervention, many novel therapies are currently under investigation. The GnRH agonist leuprolide acetate is the only medical intervention approved by the US Food and Drug Administration for presurgical treatment of uterine leiomyomas (6). Unfortunately, because of the hypoestrogenic side effects associated with such therapy, long-term intervention is contraindicated. As a result, medical therapeutic options are quite limited for leiomyoma treatment.

Fibroids originate from the smooth muscle layer of the uterus and probably develop from a single transformed myometrial smooth muscle cell (7, 8). Tumor bulk results from a disorder of fibrosis (9–12). Leiomyomas produce large amounts of extracellular matrix (ECM) proteins, such as collagens, fibronectin, and proteoglycans (1, 11, 13–18). The majority of the tumor is made up of this disrupted matrix (19), and recent findings suggested that alterations in ECM can modify mechanical stress on cells, which leads to activation of internal mechanical signaling that may contribute to leiomyoma growth (20–22). Considering that leiomyoma has tumor and fibrotic characteristics, any effective therapy should regulate both leiomyoma cell proliferation and ECM production.

Tranilast (N-3, 4-dimethoxycinnamoyl anthranilic acid) is a synthetic drug of low toxicity that has been widely used clinically in Japan since the 1980s (23). This drug is taken orally and is effective against allergic diseases such as bronchial asthma, allergic rhinitis, atopic dermatitis, and allergic conjunctivitis (23, 24). Tranilast exhibits its therapeutic effect in these conditions by inhibiting the release of chemical mediators from mast cells and basophils (25, 26). Tranilast is also used to prevent keloid tumor (a fibrotic disorder that shares similar molecular and epidemiologic features with leiomyomas) (10, 27, 28) formation after skin injury by reducing collagen synthesis in keloid fibroblasts through interference with transforming growth factor (TGF)-$\beta$ effects (29). Tranilast has been reported to inhibit the TGF-$\beta$–induced transformation of fibroblasts to myofibroblasts and their contraction in vitro (30) and vascular endothelial growh factor–induced angiogenesis (31). Tranilast also inhibits the release of inflammatory and fibrotic mediators such as TGF-$\beta$1, interleukin (IL)-1$\beta$, prostaglandin $E_2$, IL-2, IL-8, and leukotriene C4 from human monocytes and macrophages (24, 32, 33). Furthermore, tranilast antagonizes angiotensin II (34), restores cytokine-induced nitric oxide production against platelet-derived growth factor (35), and inhibits calcium entry in smooth muscle cells (36). Subsequent studies have confirmed the ability of tranilast to inhibit cancer cell growth and proliferation in various tumor models, including breast, pancreatic, gastric, and prostate cancer, glioma, and other tumors (37).

Although tranilast exhibits multiple therapeutic effects in diverse pathologic conditions, limited work has been reported in uterine fibroid biology. In 2002, Shime et al. (38) reported that tranilast arrested the proliferation of uterine leiomyoma cells at the G0/G1 phase, through the suppression of cyclin-dependent kinase 2 activity via an induction of $p21^{waf1}$ and p53. Similar to a previous study, our group also found that tranilast inhibited the proliferation of human primary uterine leiomyoma cells (39). We also noted that tranilast inhibited the proliferation of normal myometrial cells (39). However, no study has addressed the effect of tranilast on ECM production in leiomyoma cells. Therefore, in the present study we hypothesized that tranilast could regulate ECM production in leiomyoma and myometrial cells.

## MATERIALS AND METHODS

### Drugs and Chemicals

Tranilast was purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide (DMSO) at 30 mM. Further, it was diluted with medium to reach 1, 10, 30, 100, 300, and 1,000 $\mu$M before treatment.

### Ethics Statement

This study was conducted according to the principles of the Declaration of Helsinki. It was approved by the internal institutional review and ethical board of the Department of Obstetrics and Gynaecology. All patients provided written, informed consent for the collection of samples and subsequent analysis.

### Tissue Collection

Seven premenopausal Caucasian women aged 41–49 years were included in this study, and they were not receiving any type of hormonal therapy. Fibroid and myometrial tissues were obtained by hysterectomy or laparotomic myomectomy from patients with symptomatic fibroid. The diagnosis of leiomyoma was confirmed by histologic examination of the specimens removed. After surgically removing fibroid and adjacent myometrial tissues, fresh tissue specimens 1.5 cm $\times$ 1.5 cm $\times$ 1.5 cm were collected from both submucosal and intramural leiomyomas. The size range was 3–10 cm in diameter.

### Primary Cell Cultures

Myometrial and leiomyoma samples were collected in Hanks' balanced salt solution (Euroclone) at the time of surgery. Samples were cut into small pieces with 0.1% collagenase type 8 (Serva Electrophoresis) solutions (serum-free Dulbecco's modified Eagle medium [DMEM; Sigma-Aldrich] containing 1% penicillin–streptomycin [EuroClone], 50 $\mu$g/L gentamicin [Lonza], and 1% amphotericin B [Lonza]). Tissues were incubated at 37°C for 5 to 6 hours in a water bath and shaken manually until complete digestion. Digested cell suspensions were then centrifuged at 1,200 rpm for 10 minutes and washed with regular media (DMEM containing 10% fetal bovine serum [Sigma-Aldrich], 1% penicillin–streptomycin [EuroClone], 50 mg/L gentamicin [Lonza], and 1% amphotericin B [Lonza]). Cells were plated in T75 plastic dishes and