Dysmenorrhea and its severity are associated with increased uterine contractility and overexpression of oxytocin receptor (OTR) in women with symptomatic adenomyosis

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Objective: To evaluate uterine contractility, oxytocin receptor (OTR) expression in myometrial smooth muscle cells (MSMCs) derived from uterine tissues from women with and without adenomyosis correlate OTR expression with uterine contractility and dysmenorrhea severity, and see whether trichostatin A (TSA) and andrographolide inhibit OTR expression.

Design: Laboratory study using human tissues.

Setting: Academic hospital.

Patient(s): Twenty patients (cases) with histologically confirmed adenomyosis and 10 (controls) with leiomyomas, cervical dysplasia, and cancer but no adenomyosis or endometriosis.

Intervention(s): Dysmenorrhea severity was scored by Visual Analog Scale. Uterine tissue samples were collected during hysterectomy. Myometrial smooth muscle cells derived from tissue samples were cultured and OTR protein levels were measured. The effect of TSA (0.5 or 1 μM) and andrographolide (15 or 30 μM) on OTR expression was evaluated.

Main Outcome Measure(s): Visual Analog Scale scores, and contractile amplitude and frequency. The OTR protein levels in MSMCs were quantified by Western blot analysis.

Result(s): The contractile amplitude and OTR expression levels were significantly higher in cases than in controls. Dysmenorrhea Visual Analog Scale scores correlated positively with contractile amplitude and OTR expression level. Both TSA and andrographolide dose-dependently inhibit OTR expression in MSMC.

Conclusion(s): Oxytocin receptor overexpression in MSMCs may be responsible for increased uterine contractility and adenomyosis-associated dysmenorrhea. Both histone deacetylase inhibitors and andrographolide are therapeutically promising.

Key Words: Adenomyosis, amplitude, dysmenorrhea, oxytocin receptor, severity, uterine contractility

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A denomyosis is a common gynecologic disorder with a poorly understood pathogenesis (1). Its presenting symptoms include menorrhagia, dysmenorrhea, and subfertility (2), with dysmenorrhea being the second most prevalent symptom after menorrhagia yet arguably the most debilitating (3). Currently, our knowledge of the mechanisms of adenomyosis-associated pain is woefully inadequate.
Considerable innervation has been reported in the endometrium and also in the myometrium in women with adenomyosis (4, 5). More specifically, the extent of innervation in the functional layer of endometrium appears to correlate with the presence of dysmenorrhea. In the myometrium, however, innervation is equally extensive regardless of dysmenorrhea or not or of the menstrual phase (5). These results seem to suggest that besides innervation, other factors may also be responsible for adenomyosis-associated pain. The finding that nerve fiber density in peritoneal endometriosis is independent of menstrual cycles (6) may add to the credence of this notion.

Nonpregnant uterus undergoes distinctive contractions throughout the menstrual cycle: pronounced contractility during menstruation and also in the periovulatory period, with relatively quiescent periods in the rest of the cycle (7). The increased contractility during menstruation is characterized by endometrial fundus-to-cervix wavelike movement, helping to expel the menstrual debris (7, 8). In contrast, the heightened contractility during the periovulatory period is featured by endometrial cervix-to-fundus wavelike movement, serving as a sperm transport mechanism (9). It is no coincidence that dysmenorrhea occurs in one of these two active periods of uterine contractility. Indeed, it was long recognized that dysmenorrhea is associated with an elevated basal intravaginal pressure tone and altered amplitude, duration, and frequency of uterine contraction (10). Uterine hyperperistalsis and dysperistalsis also has been proposed to be causally involved in the development of pelvic endometriosis and adenomyosis (11).

Although uterine contractility is a function of sex steroid hormones (12, 13), it is also regulated by prostaglandins, oxytocin, vasopressins, and neurotransmitters such as acetylcholine, all mediated through their respective receptors (14–17). Among them, oxytocin receptor (OTR) seems to be proximal and the most potent, at least in pregnant uteri, because the OTR expression level during labor can be 1,000-fold higher than its basal level. The facilitative effect of estrogen in promoting contractility is likely due to the up-regulation of OTR by estrogen (18–21). NF-κB activation may also induce OTR expression (22).

We have shown previously that OTR immunostaining, along with that of transient receptor potential vanilloid type 1, is significantly elevated in adenomyosis and its level is associated with the severity of dysmenorrhea (23). Oxytocin receptor is expressed in the myometrium from women with adenomyosis as well (24). We also have shown that NF-κB subunit p65 staining is increased in adenomyosis (25), indicating NF-κB activation. Thus, in light of increased local production of estrogen in adenomyosis (26), these data, taken together, strongly suggest that OTR is involved in adenomyosis and, in particular, adenomyosis-associated dysmenorrhea.

In this study, we evaluated the contractility of myometrial strips from women with and without adenomyosis. We also evaluated OTR expression in myometrial smooth muscle cells (MSMCs) derived from myometrial tissues. We correlated the OTR expression with uterine contractility and also with severity of dysmenorrhea in women with adenomyosis. After finding OTR overexpression in MSMC derived from women with adenomyosis and in light of the accumulating evidence that adenomyosis, as endometriosis, may be an epigenetic disease (27, 28) that can be treated with histone deacetylase inhibitors such as valproic acid (29–32), we investigated whether trichostatin A (TSA; Sigma Chemical Co.), a histone deacetylase inhibitor, can inhibit OTR expression. We also investigated whether andrographolide (Andro; Sigma), an NF-κB inhibitor (33) which has been shown to be promising in treating induced adenomyosis through inhibition of uterine contractile amplitude (32), can also inhibit OTR expression in MSMC.

**MATERIALS AND METHODS**

**Study Population and Study Design**

Similar to published studies by us (23) and others (34), this study was essentially a cross-sectional study of myometrial tissue samples harvested from women with and without adenomyosis, combined with laboratory studies OTR expression and the patients’ uterus size and their severity of dysmenorrhea. For the adenomyosis (i.e., case) group, the inclusion criteria were premenopausal, histologically confirmed adenomyosis (excluding endometriosis and/or leiomyomas) indicative for hysterectomy, and no hormonal treatment or the use of intrauterine device ≥6 months before the surgery. Premenopausal status was defined to have regular menses every 25–35 days. Twenty subjects at Renji Hospital met the inclusion criteria and were recruited for this study. Their diagnoses were made by transvaginal ultrasonography before surgery and then histologically confirmed postoperatively. The ultrasonographic diagnostic criteria followed published studies (35, 36) and were as follows: a globular and/or asymmetric uterus, asymmetrical thickening of the anterior or posterior wall, a poorly defined focus of abnormal myometrial echotexture, heterogeneous and distorted myometrial echotexture, myometrial linear striations, and myometrical cysts. It is noted that transvaginal ultrasonography is an accurate diagnostic test for adenomyosis (37), comparable to magnetic resonance imaging (38). In this group (case group), 11 (55%) and 9 (45%), respectively, were in the proliferative and secretory phase of their menstrual cycles at the time of hysterectomy based on Noyes dating criteria.

For controls, the inclusion criteria were premenopausal women undergoing hysterectomy, surgically confirmed to be free of endometriosis, histologically confirmed to be free of adenomyosis, and no hormonal treatment or the use of intrauterine device ≥6 months before the surgery. We recruited 10 patients with surgically diagnosed and histologically confirmed leiomyomas or cervical carcinoma in situ, but none had endometriosis or adenomyosis. The exclusion of adenomyosis and/or endometriosis was based on medical history, symptomology, gynecological and sonographic examination before the surgery, surgical examination, and histology after surgery. In this group, precisely half each were in the proliferative and secretory phases. The 5 controls with multiple leiomyomas received hysterectomy because of their age (all >45 years except one aged 42); enlarged uterus (equivalent to that of 3 months of gestation); heavy menstrual bleeding and prolonged menstruation; and no desire to have more
children. The selection of the controls was based on the inclusion criteria, menstrual phase and age that matched, roughly, in frequency with the case group.

For both groups, informed consent was sought before the surgery. The patients' uterine tissue samples were collected after hysterectomy. For all patients, information was collected through reading medical charts and interviewing patients on age at surgery, uterus size (estimated volume based on ultrasound measurements, as reported previously (23)), history of dysmenorrhea or not, severity of dysmenorrhea, and parity. The severity of the most recent dysmenorrhea was evaluated by a 10-cm Visual Analog Scale (VAS) before the surgery. This study was approved by the Ethics Committees of Shanghai Ob/Gyn Hospital and of Shanghai Renji Hospital.

**Myometrial Contraction Assay**

The assay was performed following Janicek’s method (39). Briefly, myometrial smooth muscle strips in the size of ~2 mm × 2 mm × 10 mm were collected from all women after hysterectomy and transported rapidly on ice to the lab. For all subjects, the myometrial strip was cut vertically lengthwise from the outer muscle layer at the center of the anterior uterine wall. The distance between the bottom of the strip and the anatomic internal os of the uterus was 1 cm. The uterine strip was washed first and then cut into 1-mm³ cubes. After digestion with 0.2% collagenase IV (v/v) myometrial tissue was washed first and then cut into 1-mm³ cubes. After digestion with 0.2% collagenase IV (v/v) for 4–6 hours, the cell suspension was filtered by using a 150-μm sieve. Then the isolated MSMCs were seeded in 10-cm² tissue culture plates and incubated at 37°C in a humidified 95% O₂ and 5% CO₂ atmosphere. The cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin–streptomycin. The medium was changed every 3 days. At 80% confluence, the cells were subcultured. All experiments used cells of the 1st to the 8th passage.

To confirm the identity of MSMC, routine immunocytochemistry analysis was performed, using the α-smooth muscle actin antibody (Abcam) as the primary antibody. Isotype-matched irrelevant IgGs were used as negative control.

**Western Blot and Drug Administration**

Protein were extracted following the protocol of the protein extraction kit (Roche Diagnostics) and their concentration was determined using bicinchoninic acid protein assay (Thermo Scientific). Thirty micrograms total proteins were loaded and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10% gel) and the electrophoresed proteins were transferred to a polyvinylidene fluoride membrane. After blocking with 2% bovine serum albumin, the membrane was incubated with a specific primary antibody against OTR or glyceraldehyde 3-phosphate dehydrogenase (CST), followed by a horseradish peroxidase conjugated secondary antibody. The amount of proteins was visualized by fluorography using an enhanced chemiluminescence system (Pierce). The bands were scanned and quantified by the Gel-Pro Analyzer software (Media Cybernetics) and normalized by that of glyceraldehyde-3-phosphate dehydrogenase.

In vitro experiments, the MSMC cells of the same passage were starved in fetal bovine serum and phenol red–free medium for 24 hours before administration of TSA (0.5 or 1 μM) or Andro (15 or 30 μM). Thereafter, the culture medium was replaced with that containing TSA, Andro, or, for controls, equal volume of vehicle (dimethyl sulfoxide).

**Statistical Analysis**

Wilcoxon test, Kruskal–Wallis tests, and Pearson’s correlation coefficient was used when appropriate. To evaluate possible effect of menstrual phase and of the tissue origin on VAS score, contractile amplitude, or OTR expression levels, a linear regression model was used. Probability values less than .05 were considered statistically significant. All computations were performed with R 2.14.1 (40).

**RESULTS**

The characteristics of the case and control groups are listed in Table 1. It can be seen that the two groups were comparable in age and menstrual phase. However, women with adenomyosis had significantly more cases with a history of dysmenorrhea, and the severity of dysmenorrhea, as measured by VAS, was higher in the case group than the control group (Table 1).

**Myometrial Contractility and Its Relationship with Dysmenorrhea Severity**

We found that there was no significant difference in the amplitude of myometrial contraction between proliferative and...
secretory phases (both $P$ values $>.26$ for case and control groups, $P=10$ if combined). The contractile amplitude in the case group was significantly higher than that of the control group ($P=0.008$), but contractile frequency was significantly lower ($P=0.01$; Fig. 1A and B). The contractile amplitude, but not the frequency, was significantly correlated with the uterus size ($r=0.90, P=7.4 \times 10^{-8}$ in the case group, but $r=0.56, P<.09$ in the control group; or $r=0.81, P=4.7 \times 10^{-8}$ if combined; Fig. 1C). A multiple linear regression analysis ($R^2=0.70$) using age, parity, menstrual phase, source of myometrial tissues (case or control), and uterus size as covariates revealed that the contractility amplitude was significantly associated with the source of myometrial tissues ($P=0.044$) and uterus size ($P=1.4 \times 10^{-7}$), with the case group having higher amplitude.

We found that the dysmenorrhea VAS score in patients with adenomyosis correlated significantly with the contractility amplitude ($r=0.83, P=8.3 \times 10^{-6}$, Fig. 1D) and uterus size ($r=0.70, P=0.0006$), but not quite with the contractility frequency ($r=-0.44, P=0.052$). A multiple linear regression analysis ($R^2=0.68$) using age, parity, contractility amplitude, frequency, and uterus size as covariates revealed that the contractile amplitude was the only variable that was associated with the dysmenorrhea VAS ($P=7.3 \times 10^{-6}$).

Primary Culture of MSMC and Its Identification

Under an inverted phase-contrast microscope, the morphology of MSMCs was examined after 24 hours of incubation and found to be characteristically flat and spindle-shaped—indistinguishable between the case and control groups (Fig. 2A and B). The purity of MSMCs was found to be $>95\%$, as judged by the positive cellular staining for $\alpha$-smooth muscle actin (Fig. 2D). The morphology of MSMCs within the first 10 passages in the primary culture was found to be quite consistent and homogenous.

OTR Expression in MSMC between Case and Control Groups and Its Correlation With Dysmenorrhea Severity

By Western blot assay, we found that glyceraldehyde 3-phosphate dehydrogenase-normalized OTR expression was significantly higher in the case group than that in the control group ($P=4.5 \times 10^{-6}$) (Fig. 3A and B). In the case group, OTR expression level correlated with the uterus size ($r=0.69, P=0.0007$), contractile amplitude ($r=0.79, P=4.1 \times 10^{-5}$), and contractile frequency ($r=-0.49, P=0.030$) but not parity or age (both $P$-values $>.28$). There was no difference in OTR protein levels between the two menstrual phases ($P=.60$). The results were similar when the control group also was included (data not shown).

We also found that the dysmenorrhea VAS score correlated positively with the OTR expression in MSMCs ($r=0.86, P=9.2 \times 10^{-7}$, for women with adenomyosis, and $r=0.91, P=4.3 \times 10^{-12}$, for both groups; Fig. 3C). A multiple linear regression analysis incorporating age, uterus size, parity, and OTR expression levels as covariates revealed that the OTR expression level was the only covariate that was associated with the VAS score ($P=9.2 \times 10^{-7}$, $R^2=0.75$). For both groups, a multiple linear regression analysis incorporating age, uterus size, parity, contraction amplitude, frequency, source of tissue samples (case or control), and OTR expression levels as covariates revealed that the OTR expression level was the only covariate that was associated with the VAS score ($P=4.3 \times 10^{-12}$, $R^2=0.83$; Fig. 3C).

TSA or Andro Treatment Reduces OTR Expression in MSMC

Treatment with TSA or Andro resulted in significantly reduced OTR expression in MSMCs in a dose-dependent fashion, especially for MSMC derived from the cases (Fig. 3D–3G). In fact, a multiple linear regression analysis indicated that, for

**TABLE 1**

Characteristics of the recruited patients with and without adenomyosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 10)</th>
<th>Adenomyosis (n = 20)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years; mean ± SD [range])</td>
<td>46.6 ± 5.1 (38–53)</td>
<td>46.3 ± 5.9 (32–56)</td>
<td>.86</td>
</tr>
<tr>
<td>Indications for hysterectomy</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Uterine leiomyomas</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CIN III</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cervical squamous cell carcinoma Ib1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Menstrual phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferative</td>
<td>5</td>
<td>9</td>
<td>1.0</td>
</tr>
<tr>
<td>Secretory</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>.54</td>
</tr>
<tr>
<td>≥1</td>
<td>10</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>History of dysmenorrhea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (8%)</td>
<td>2 (10%)</td>
<td>.0003</td>
</tr>
<tr>
<td>Yes</td>
<td>2 (20%)</td>
<td>18 (90%)</td>
<td></td>
</tr>
<tr>
<td>VAS score for dysmenorrhea (median [range])</td>
<td>0 (0–3)</td>
<td>5 (0–9)</td>
<td>.0002</td>
</tr>
<tr>
<td>Uterus size (in mm$^3$; mean ± SD [range])</td>
<td>195.2 ± 125.9 (62.8–460.4)</td>
<td>249.7 ± 148.0 (100.5–719.5)</td>
<td>.25</td>
</tr>
</tbody>
</table>

Note: NA = not applicable.

both TSA and Andro, the treatment was associated with the significant reduction in OTR expression in a dose-dependent manner ($P = .043$ for TSA and $P = .011$ for Andro). In addition, the treatment resulted in more reduction in OTR expression in MSMC derived from women with adenomyosis than from women without ($P = .0002$ for TSA and $P = 2.1 \times 10^{-6}$ for Andro; Fig. 3E and G).

**DISCUSSION**

It was long recognized that dysmenorrhea is associated with an elevated basal intrauterine pressure tone and altered amplitude, duration and frequency of uterine contraction (10). Our study provides evidence that adenomyosis-associated dysmenorrhea correlates with an elevated amplitude of uterine contractility. In fact, the contractile amplitude is found to be positively associated with the severity of dysmenorrhea in women with adenomyosis. In addition, we show that the elevated myometrial OTR expression is associated with, and likely attributable to, increased uterine contractility because uterine contractions of the junctional zone in the nonpregnant uterus are oxytocin-dependent (12). The elevated myometrial OTR expression, in fact, is found to be associated solely with the severity of dysmenorrhea in women with adenomyosis, eclipsing contractile amplitude and uterus size. Moreover, we show that treatment of myometrial smooth muscle cells derived from adenomyotic patients with either TSA or Andro can significantly reduce the expression of OTR, leading, possibly, to normalized uterine contractility and pain alleviation.

Our finding that women with adenomyosis had elevated amplitude of uterine contractility is consistent with our previous report that mice with induced adenomyosis have an increased uterine contractile amplitude concomitant with reduced response latency to noxious thermal stimulus as compared with those without (32). Our results, however, differ from our mouse study in that, instead of finding contractile frequency irrelevant to the presence of adenomyosis or not,
we found that women with adenomyosis had significantly lower contractile frequency than that in controls. Difference between humans and mice aside, it is likely that the difference may be attributable to the fact that we selected women with other benign and malignant diseases as controls. Although this uncertainty can be resolved using uterine tissues from healthy women, ethical concerns unfortunately preclude the use of uterine strips from women free from any disease. Although OTR is known to be involved in the pathogenesis of primary dysmenorrhea (41), its involvement in adenomyosis-associated dysmenorrhea has been implicated only recently (23, 24). This study further establishes that OTR overexpression in the myometrium is associated with increased uterine contractility in women with adenomyosis and also is associated with the dysmenorrhea severity in these women. The increased uterine contractility as a result of OTR overexpression in the myometrium, coupled with increased innervation in endometrium and myometrium in women with adenomyosis (5) and elevated expression of pain mediators/integrators such as transient receptor potential vanilloid type 1 (23), can conceivably make uterine contraction or cramping feel painful during menstruation. That is, uterine hyperactivity during menstruation, in conjunction with hypersensitivity or hyperalgesia in the endometrium and/or myometrium, can, in theory, cause dysmenorrhea. These findings immediately suggest that an ideal drug for treating adenomyosis, if exists, should desirably be both antidysperistaltic and antinociceptive, besides anti-inflammatory and antiproliferative against ectopic endometrial tissues. Oxytocin receptor expression has been recently shown to be increased in the epithelial, but not stromal, cells and in smooth muscle cells in endometriotic lesions (42). Although its overexpression signals its possible involvement in endometriosis, it is unclear whether it has any relationship with endometriosis-related dysmenorrhea and its severity. In this study, we found that OTR expression is increased in myometrial cells derived from women with adenomyosis. More interestingly, OTR expression was positively correlated with the severity of dysmenorrhea. Because deranged uterine contractility has been well documented in endometriosis (43–47), it is possible that aberrant OTR expression may also be responsible in endometriosis-associated dysmenorrhea. Besides causing uterine hyperactivity, OTR expression may also result in up-regulation of COX-2, leading to overproduction of PGE₂. This is because oxytocin, mediated by
OTR, is known to be involved in the release of PGF$_{2a}$ from endometrial cells (48, 49). The increased production of PGF$_{2a}$, which is known be to a coactivator of nociceptors and a pain mediator, can further increase PGE$_2$ and its own production in an autocrine/paracrine manner (50) and, together with PGE$_2$, causing dysmenorrhea in adenomyosis. Although the cause for OTR overexpression in adenomyosis is currently unclear, it has been shown that OTR expression increases in response to inflammatory cytokines and angiogenic factors (52, 53). It is also possible that increased local estrogen production (26) and constitutive activation of NF-$\kappa$B (54) may up-regulate OTR in adenomyotic lesions (21, 22).

Our finding that TSA can inhibit OTR in MSMCs is consistent with a previous report that valproic acid, TSA, and suberic bis hydroxamate—the three histone deacetylase inhibitors—can inhibit uterine contractility in pregnant uteri (55). Our study further observes that MSMCs derived from uteri of women with adenomyosis are more sensitive than

(A) Representative OTR protein expression levels in the case and control groups as determined by Western blot. (B) Box plot of OTR expression levels in the case and control groups. (C) The severity of dysmenorrhea, as measured by the VAS scores, as function of the OTR protein expression levels in women with adenomyosis. The dashed line indicates a linear regression fit of the data. (D) Representative Western blot results showing OTR protein levels in MSMCs derived from uterine tissues of women with and without adenomyosis when treated with different concentrations of TSA or vehicle. (E) Summary of OTR protein levels in MSMCs derived from uterine tissues of women with and without adenomyosis when treated with vehicle or different concentrations of TSA. (F) Representative OTR protein levels in MSMCs derived from uterine tissues of women with and without adenomyosis when treated with different concentrations of Andro or vehicle. (G) Summary of OTR protein levels in MSMCs derived from uterine tissues of women with and without adenomyosis when treated with vehicle or different concentrations of Andro. Veh = vehicle; TSA0.5 = TSA 0.5 $\mu$M; TSA1 = TSA 1 $\mu$M; Andro15 = Andro 15 $\mu$M; Andro30 = Andro 30 $\mu$M.

those from women without adenomyosis to TSA or Andro treatment. Therapeutically speaking, this increased sensitivity is certainly desirable.

Our finding that TSA can inhibit OTR expression provides further biological justification for its use to treat adenomyosis, which we found to be promising (29, 31). Our animal studies also support this notion (30, 32). Indeed, histone deacetylase inhibitors are shown to repress NF-κB DNA binding and suppress the expression of proinflammatory genes in human myometrial cells (56). Aside from having an excellent safety profile and besides being antiproliferative and anti-inflammatory (57–59), valproic acid or other histone deacetylase inhibitors may also be antisynderplastic and antinociceptive, a feature that perhaps no other compounds have as far as adenomyosis is concerned.

Andro is an active ingredient chemical extracted from Andrographis (Andrographis paniculate), which has been used as a medicinal herb in traditional Chinese medicine for alleviation of inflammatory disorders for thousands of years. Andro is known to be anti-inflammatory (60) and to interfere with NF-κB binding to DNA (61), and the underlying mechanism has been shown recently to result from suppression of NF-κB activation (33). Not surprisingly, it is known to exert a strong immunomodulatory effect (reviewed in Varma et al. (62)) and is reported to inhibit proinflammatory and angiogenic mediators such as COX–2 (61) and tissue factor (TF) (63), both of which are reportedly involved in endometriosis (64, 65). Andro has also been shown to be antinociceptive in animals (66, 67). Most remarkably, Andro, unlike many NF-κB inhibitors, is already commercially available with an excellent safety profile, and is a nonprescription medication in China indicative for upper respiratory tract infection. A recent clinical trial on the use of A. paniculate extract containing 30% total andrographolides to treat rheumatoid arthritis reported promising results (68). Our ongoing clinical study on its use to treat symptomatic adenomyosis appears to be promising (Liu et al., unpublished data).

Our study has limitations. First, we used ultrasound measurements to estimate uterus volume. This measurement may be less accurate than uterus weight. However, because the estimation was made uniformly for all subjects, it is unlikely that such a measurement would generate any particular bias in favor of or against our conclusions. Second, we used menstrual cycle pattern as the sole criterion to determine the menopausal status. This could be deficient because some women with leiomyomas might still have bleeding but do not have regular menstrual cycles. There are standardized criteria for defining the menopausal transition that includes menstrual pattern and FSH. In a cohort that may have abnormal bleeding and includes women in their 50s such as this, that would be a better definition. However, given the clear presence of regular menstrual cycles, it is unlikely that the recruited subjects were already menopausal. Indeed, rare may it be, a few women can reach their menopause at 61 years old (69). Lastly, we used uterine tissue samples taken from women with leiomyomas or cervical dysplasia or cancer because of ethical constraint on the use of tissue samples from apparently normal women. Although the uteri from these patients are unlikely to have reduced contractility—thus biasing our results, it is possible that they might have contractile irregularity, thus accounting for our negative finding. Alternatively, the myometrium might differ considerably between the groups and, given the moderate sample size, the difference could be masked. One possible solution would be the real-time measurement of uterine contractility, ideally by noninvasive means. Future studies are warranted to clarify this issue.

To summarize, we have found that uterine contractile amplitude is positively associated with the severity of dysmenorrhea in women with adenomyosis, and that the increased uterine contractility is associated with increased myometrial OTR expression. In addition, we have found that the myometrial OTR expression is associated with the severity of dysmenorrhea in women with adenomyosis. Moreover, we show that treatment of myometrial smooth muscle cells derived from adenomyotic patients with either TSA or Andro can significantly reduce the expression of OTR, leading, possibly, to normalized uterine contractility and pain alleviation. These results strongly suggest that OTR overexpression may be responsible for increased uterine contractility and for adenomyosis-associated dysmenorrhea. Both histone deacetylase inhibitors and Andro hold potential in treating adenomyosis.

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REFERENCES


