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Original article

A pilot study on the use of andrographolide to treat symptomatic adenomyosis

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ABSTRACT

Objective: To evaluate the efficacy of andrographolide in treating adenomyosis and to test the hypothesis that its efficacy may depend on the nuclear factor-kappa-light-chain enhancer of activated B cells (NF-κB) activation status in eutopic endometrium, which may be a proxy for the status in adenomyotic foci.

Materials and methods: Twenty-four patients with transvaginal ultrasound-confirmed adenomyosis (excluding ovarian endometriomas) were recruited for this study after informed consent. All patients had dysmenorrhea and/or heavy menstrual bleeding. All received andrographolide pill orally for 3 months and were followed up for an additional 3 months. The primary outcome measures included the severity of dysmenorrhea, as measured by the visual analog scale (VAS), and menstrual characteristics, such as the amount of menses, all measured before and 3 and 6 months after the drug treatment. In addition, the patients completed Clinical Global Impression rating scales at the end of the 6th month. Immunostaining of the phosphorylated NF-κB p65 (p-p65) subunit was also performed for eutopic endometrium.

Results: Andrographolide treatment appeared to be well tolerated by the patients. Six months after taking andrographolide, the average dysmenorrhea VAS score was decreased from the baseline level of 5.3 to 3.5. Twelve patients (50.0%) reported "marked" or "much" improvement, seven (29.2%) reported "minimal improvement" and five (20.8%) reported "unchanged or worse". The eutopic endometrial p-p65 staining levels were closely correlated with the satisfaction rating.

Conclusion: Andrographolide is effective in some patients with symptomatic adenomyosis, who have a higher endometrial expression of the activated form of the NF-κB p65 subunit. Future independent validation studies or randomized clinical trials may be needed to more precisely evaluate the efficacy of andrographolide.

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Introduction

Adenomyosis, characterized by the presence of heterotopic endometrial glands and stroma in the myometrium with adjacent smooth muscle hyperplasia, is a fairly common gynecologic disease responsible for menorrhagia, dysmenorrhea, and subfertility in women of reproductive age.[1] Although the disease is estrogen dependent, progestogenic agents are not very effective, and the use of gonadotropin-releasing hormone (GnRH) agonists is restricted by their short duration.[2] Worse, the symptoms quickly reappear after discontinuation of GnRH agonists therapy.[3] Although the levonorgestrel-releasing intrauterine system has been reported to have some efficacy,[4] side effects such as spotting were nonetheless seen in one third of the women, and oligomenorrhea was the most common complaint observed.[4] Therefore, the current arsenal for treatment of adenomyosis is limited. As such, the definitive treatment for symptomatic adenomyosis is hysterectomy,[1] even though the decision to remove the uterus, arguably an iconic symbol of womanhood, can be difficult and

Conflicts of interest: X.S.L. and S.-W.G. are applicants of a patent on the use of andrographolide for the treatment of endometriosis, submitted to the Patent Bureau of China.

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even agonizing to make. Unfortunately, there appears to be no new drug treatment currently on the horizon, as only 33 clinical trials on adenomyosis have been registered at **www.ClinicalTrials.gov** (accessed December 3, 2014), with only one Phase I trial that evaluated the novel nonhormonal compound, bromocriptine, a dopamine agonist.

Adenomyosis and endometriosis share uncannily many similarities in disease definition, estrogen dependency, and some molecular aberrations. In endometriosis, the pathophysiological role of nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) has long been suspected[5] and now well established.[6–8] Similarly, NF-κB seems to play a role in adenomyosis.[9]

Andrographolide (referred to as "Andro" from herein) is an active ingredient chemically extracted from andrographis (*Andrographis paniculata*), which has been used as a medicinal herb in traditional Chinese medicine for the alleviation of inflammatory disorders for thousands of years. Andro is known to be anti-inflammatory[10] and to interfere with NF-κB binding to DNA.[11] Its mechanism of action is shown to result from suppression of NF-κB activation through covalent modification of reduced cysteine 62 of the p50 subunit[12] and inhibition of p65 Ser^{536} phosphorylation.[13] Not surprisingly, it is known to exert strong immunomodulatory effects,[14] and is reported to inhibit proinflammatory and angiogenic mediators such as cyclooxygenase-2 (COX-2)[11] and tissue factor (TF),[5] both of which are reportedly involved in adenomyosis.[16,17] Andro has also been shown to be antinociceptive in animals.[18,19] It has been shown to suppress the expression of oxytocin receptor and modulate uterine contractility in adenomyosis,[20,21] and inhibit the expression of COX-2, vascular endothelial growth factor, and TF in adenomyosis.[22] Most remarkably, Andro, unlike many other NF-κB inhibitors, is already commercially available with an excellent safety profile, and is a nonprescription medication in China for the treatment of upper respiratory tract infections. A recent clinical trial on the use of *A. paniculata* extract containing 30% total andrographolides to treat rheumatoid arthritis reported encouraging results.[23]

We have previously reported the promising results on the off-label use of valproic acid (VPA) to treat adenomyosis.[24,25] Because VPA is approved for treating epilepsy and bipolar disorder, which are conspicuously labeled on the package, and, in the Chinese culture, are disorders of the nervous system that carry, perhaps unjustifiably, some negative connotation or even social stigma, many patients balk at the idea of taking the drug after reading the label, even though they are refractory to all existing therapeutics and the only option they have is hysterectomy. A strained patient–doctor relationship that is prevalent across China today and a health care system that is not conducive to promoting clinical trials certainly provides no help. In addition, there is concern that, being a global histone deacetylase inhibitor as well as a pregnancy category D drug, VPA may cause some unintended side effects. Therefore, we sought to identify novel therapeutics for adenomyosis.

Given the encouraging *in vitro* and *in vivo* results suggesting the therapeutic potential of Andro[20–22,26] and, equally important, its safety profile, we sought in this study to further evaluate the efficacy of Andro in patients with adenomyosis who presented with complaints of dysmenorrhea and/or menorrhagia and who also had an enlarged uterus. These patients were refractory to nonsurgical treatment and faced the only option of hysterectomy. The focus on patients with adenomyosis complaining of dysmenorrhea/menorrhagia also poses few, if any, ethical challenges given the safety profile and makes recruitment much easier. We hypothesized that, because the mode of action is mainly through suppression of NF-κB activation, the efficacy of Andro may depend on the NF-κB activation status in adenomyotic foci. Although it is difficult to evaluate the NF-κB activation status in adenomyotic foci without being invasive, we hypothesized that the NF-κB activation status in eutopic endometrium may be a proxy for the status in adenomyotic foci.

Materials and methods

Patients

Twenty-four patients with transvaginal ultrasound-confirmed adenomyosis (excluding ovarian endometriomas) who visited Shanghai Obstetrics and Gynecology Hospital, Fudan University Shanghai Medical College (Shanghai, China) from October 2009 to March 2010 were recruited for this study after obtaining informed consent (Table 1). The diagnosis of adenomyosis in all patients was made based on a combination of symptomatology, pelvic examination, and vaginal ultrasound evaluation by experienced physicians. All patients had either dysmenorrhea, as reflected by the visual analog scale (VAS) measurement and also based on gynecological examination, or heavy menstrual bleeding, as measured by the pictorial bleeding assessment chart (PBAC),[27] or both conditions (Table 1). All patients were premenopausal, with no history of hormone therapy or intrauterine device use for $\geq$ 6 months prior to recruitment. They were not pregnant or lactating, and had no intention to get pregnant. They all had normal hepatic and renal functions, as defined by serum transaminases $< 1.5 \times$ upper limit of normal (ULN), bilirubin $< 1.5 \times$ ULN, and creatinine $< 2.0 \times$ ULN. None of them had any psychiatric condition that would compromise the full compliance with the study, nor did they have any severe concomitant medical disorder, significant cardiovascular disease, major thromboembolic event in the last 6 months, or Grade 3 or 4 bleeding.

During the recruitment, all patients were offered the option of hysterectomy or the chance to try Andro. They all chose to try the drug and were given the full explanation of the experimental nature of this study and possible risks associated with it. Approval for this pilot study was obtained from the local Ethics Committee at Shanghai Obstetrics and Gynecology Hospital, Fudan University.

Treatment

All patients received Andro dripping pill (Tansly Pharmaceutics, Tianjin, China; Fig. 1) orally starting at the 5^{th} day of the menstrual cycle, with 600 mg (containing 150-mg pure Andro) t.i.d. for 3 months, when no serious adverse effect was reported. The patients stopped taking the pill when they had menstruation but resumed at the 5^{th} day of the menstrual cycle. For these patients, the treatment was terminated at the end of the 3^{rd} month after taking the drug. The patients were then followed up for an additional 3 months. During treatment, clinical signs and symptoms were monitored.

Outcome measures

The primary outcome measures were the severity of dysmenorrhea, as measured by a 10-cm VAS, and the uterine size by ultrasonographic measurement in length $\times$ width $\times$ height (in cm^3). The VAS and uterine size were measured before the drug treatment and 3 months and 6 months after the drug treatment, respectively. In addition, menstrual characteristics, such as duration of menstruation (in days), estimated amount of menses (by the PBAC method), and length of menstrual cycle (in days), were also measured before and 3 months and 6 months after the drug treatment.

Table 1
Characteristics of the 24 patients who were recruited to the study, along with the changes in primary and secondary outcome measures when available.

Variable name	Before Andro treatment	3 mo after treatment	6 mo after treatment	p[a]
Age (y)	40.2 (5.0) 40.0 (30–50)	N/A	N/A	N/A
Gravidity, *n* (%)				
0	5 (20.8)	N/A	N/A	N/A
1	2 (8.3)			
2	6 (25.0)			
2+	11 (45.8)			
Parity, *n* (%)				
0	5 (20.8)	N/A	N/A	N/A
1	14 (58.3)			
2	5 (20.8)			
Duration of menstruation (d)	6.6 (3.0) 7.0 (3–15)	6.0 (1.4) 6.3 (3–9)	5.7 (1.9) 5.8 (3–8) (n = 18 missing)	0.26[b]
Amount of menses (mL)	223.0 (168.5) 193.5 (24–900)	152.2 (68.4) 147.5 (24–320)	135.0 (48.5) 125.0 (90–200) (n = 18 missing)	0.009[b]
Length of menstrual cycle (d)	30.6 (10.0) 30 (15–70)	30.0 (5.5) 30 (20–50)	28.2 (3.2) 29 (25–30) (n = 18 missing)	0.79[b]
10-cm VAS score	5.3 (2.8) 5.5 (0–9)	3.4 (2.8) 3 (0–9)	3.5 (2.6) 3 (0–9)	0.0006[b] 0.0006[c]
Uterine size (cm^3)	150.9 (103.6) 123.7 (37.3–507.8)	186.9 (120.6) 146.9 (69.3–453.1) (n = 15 missing)	(all missing)	0.039[d]
CGI *Severity scale*	N/A	N/A	4.1 (0.9) 4 (3–6)[e]	N/A
CGI *Improvement scale*	N/A	N/A	2.6 (1.0) 2.5 (1–4)	N/A
CGI Efficacy index, *n* (%)	N/A	N/A	Marked improvement/no side effect: 3 (12.5) Moderate improvement/no side effect: 6 (25.0) Moderate improvement/no significant side effect: 3 (12.5) Minimal improvement/no side effect: 5 (20.8) Minimal improvement/no significant side effect: 3 (12.5) Unchanged or worse/no side effect: 5 (20.8)	N/A

Data are presented as mean (SD) or median (range), unless otherwise indicated.
CGI = Clinical Global Impression; N/A = not applicable; SD = standard deviation; VAS = visual analog scale.
[a] All tests were paired rank-sum test unless indicated otherwise.
[b] The p value is for 3 months after treatment versus baseline.
[c] The p value is for 6 months after treatment versus baseline.
[d] Paired test only for the nine patients who had data at both time points.
[e] Moderate dysmenorrhea.

We used the Clinical Global Impression (CGI) rating scales, which are the commonly used instrument to gauge a global severity of patients' symptoms, improvement after treatment, and the response to treatment.[28] Replacing mental illness with complaints of dysmenorrhea and menorrhagia and letting the patient instead of the physician do the rating, the CGI has three component scales: Severity Scale (CGI-S), Improvement Scale (CGI-I), and Efficacy Index. CGI-S is a 7-point scale that lets the patient rate the severity of her dysmenorrhea/menorrhagia at the time of assessment. A patient would rate her severity of dysmenorrhea/menorrhagia at the time of rating as follows: 1, normal, not at all; 2, borderline; 3, mild; 4, moderate; 5, marked; 6, severe; or 7, extreme.

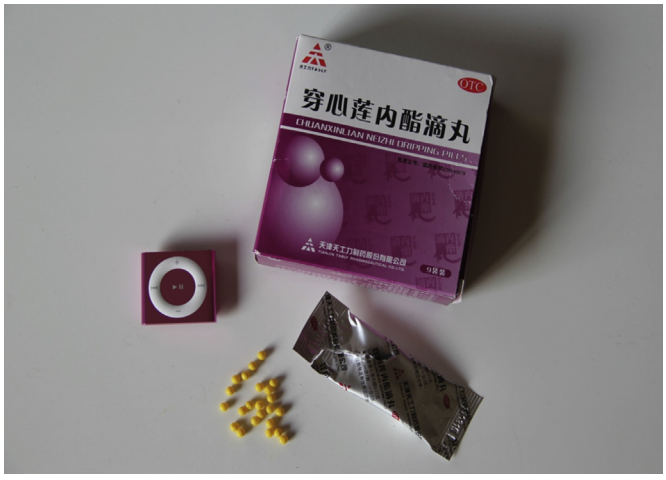

Fig. 1. Andrographolide dripping pills and the packaging box. An Apple iPod Shuffle (in purple) is also shown for scaling purpose.

The CGI-I is a 7-point scale that asks the patient to assess how much her dysmenorrhea/menorrhagia has improved or worsened relative to a baseline state at the beginning of the intervention. The rating scale was as follows: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse.

The Efficacy Index is a 4 point × 4 point rating scale that assesses the therapeutic effect of the treatment as 1, unchanged to worse; 2, minimal; 3, moderate; and 4, marked by side effects rated as none, do not significantly interfere with patient's functioning, significantly interferes with patient's functioning, and outweighs therapeutic effect.

Nine patients (37.5%) came to the hospital for clinical ultrasound examination after taking Andro for 3 months. Six patients (25%) completed the 6-month study and provided information about the length of menstrual cycle and the amount of menses. However, all

patients provided their VAS scores on dysmenorrhea and the CGI scores over the phone.

Immunohistochemistry of the phosphorylated p65 (pp65) subunit

With an exception for five patients, a small endometrial tissue sample was obtained by hysteroscopy for each patient after informed consent. The sample was then fixed with 10% formalin and paraffin embedded. Serial 4-μm sections were obtained from each block to confirm pathologic diagnosis, and to stain for the NF-κB p-p65 subunit, an activated form. Routine deparaffinization and rehydration procedures were performed. The mouse monoclonal antibody against phosphorylated NF-κB p-p65 (Ser 276; No. 3037, Cell Signaling, Santa Cruz, CA, USA), diluted to 1:50, was used as a primary antibody. For antigen retrieval, the slides were heated at 98°C in an ethylenediaminetetraacetic acid buffer (pH 9.0) for 30 minutes and cooled naturally at room temperature. Sections were then incubated overnight with the primary antibody at 4°C. After rinsing the slides, the biotinylated secondary antibody, Supervision TM Universal (antimouse/rabbit) detection reagent (horseradish peroxidase; GK500705, Shanghai Gene Tech Company, Shanghai, China), was incubated at room temperature for 30 minutes. The bound antibody complexes were stained for 3–5 minutes or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin and mounted. Immunoreactivity staining was characterized quantitatively by digital image analysis using the Image Pro-Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) without prior knowledge of any of the clinicopathological information.

Statistical analysis

The statistical significance of changes in continuous variables between before and after the treatment was made using the paired Wilcoxon rank sum test. If the number of missing values was considerably unequal, the unpaired Wilcoxon test was used. Pearson correlation coefficient was used while evaluating correlations between two variables when both variables are continuous. When at least one variable was ordinal, Spearman rank correlation coefficient was used instead. To see whether the p-p65 staining levels decrease with less improvement in treatment outcome, Jonckheere–Terpstra trend test was used.

A p value < 0.05 was considered statistically significant. All computations were made with R 3.1.0 (www.r-project.org).[29]

Results

The characteristics of the 24 patients who were recruited to and completed the study, along with the changes in the primary and secondary outcome measures are presented in Table 1. In these patients, the baseline (pretreatment) VAS score did not appear to correlate with the uterine size ($r = 0.20$, $p = 0.35$).

Treatment effect on primary outcome measures

Three months after taking Andro, 15 of 24 (62.5%) patients reported some relief of dysmenorrhea (change < 0), but eight (33.3%) of them reported no change in the VAS score, and one (4.2%) even reported the worsening, by 1 point, of dysmenorrhea (Fig. 2A). Compared with the baseline, the average VAS score on dysmenorrhea at the end of the 3-month Andro treatment was reduced from 5.3 to 3.4, a 35.8% reduction ($p = 0.0006$; Fig. 2B). Six months after taking Andro or 3 months after the discontinuation of the treatment, 16 patients (66.7%) reported some relief of pain, and the average VAS score was decreased from 5.3 to 3.5, a 34.0% reduction as compared with the baseline ($p = 0.0006$; Fig. 2B). However, there was no statistical difference in the VAS score between the two post-treatment evaluations ($p = 0.20$). In fact, compared with the VAS score evaluated at 3 months after treatment, the majority of patients ($n = 21$, or 87.5%) reported no further reduction in the VAS score evaluated at 6 months (Fig. 2B). Compared with the baseline, 16 (66.7%) patients reported reduced VAS scores while the remaining eight patients (33.3%) reported no change in or increased VAS score. Among those who had reduced VAS scores, 10 (41.7%) reported a reduction by two or more points.

For the nine patients (37.5%) who actually came to the hospital for post-treatment evaluation, the ultrasonographic examination

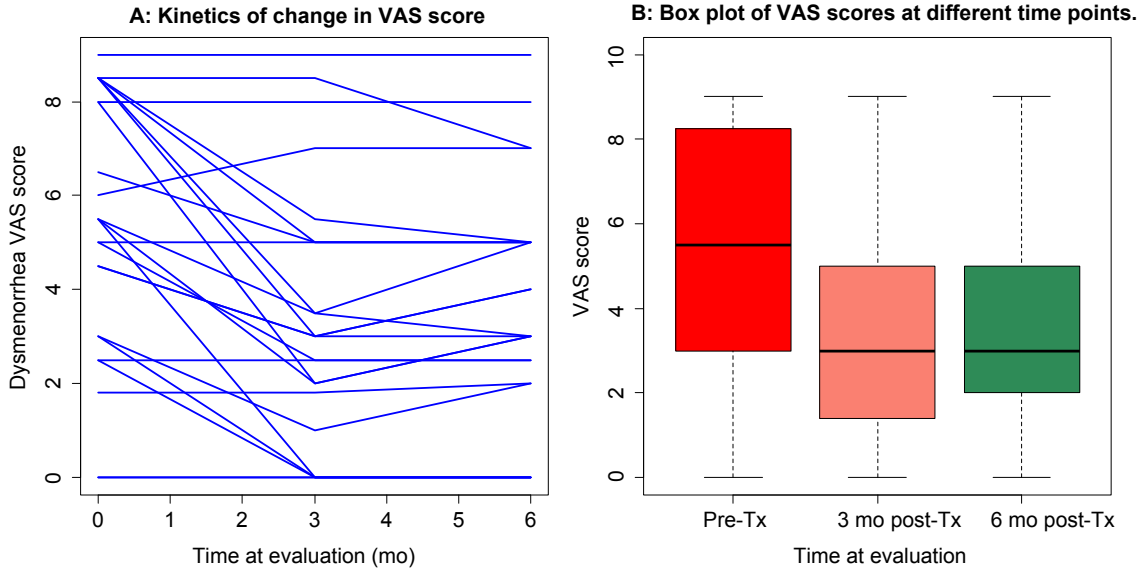

Fig. 2. (A) Kinetics of the visual analog scale (VAS) scores evaluated before and 3 months and 6 months after treatment with andrographolide and (B) box plot of the VAS scores before and 3 months and 6 months after taking the andrographolide dripping pills. Tx = treatment.

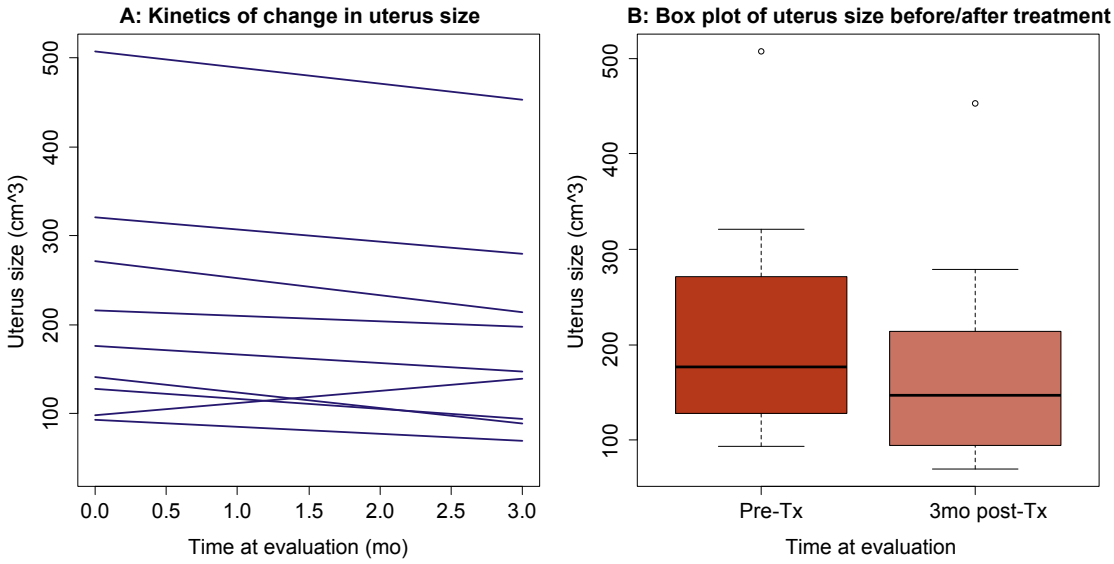

Fig. 3. (A) Kinetics of the uterus size evaluated before and 3 months after treatment with andrographolide and (B) box plot of the uterus size before and 3 months after taking the andrographolide dripping pills. Tx = treatment.

revealed that the average uterine size was decreased by 13.8% 3 months after taking Andro (Fig. 3A; $p = 0.039$).

The reduction in VAS score did not appear to correlate with the change in uterine size before and after the treatment ($r = 0.09$, $p = 0.81$ for 3 months after taking Andro vs. the baseline).

Menstrual characteristics and their changes

There was no change in the length of menstrual cycles 3 months after taking Andro [mean (standard deviation) = 30.6 (9.6) days vs. 29.9 (5.5) days, $p = 0.79$; Table 1 and Fig. 3]. Because only six patients reported this information, we did not perform any analysis on data at 6 months after the treatment.

Remarkably, the amount of menses 3 months after treatment was significantly reduced as a result of the Andro treatment

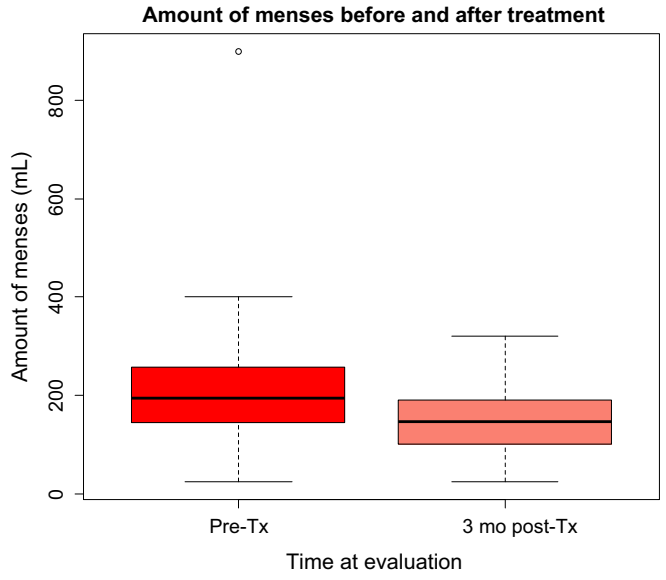

Fig. 4. Box plots of the amount of menses before and 3 months after taking Andro. Tx = treatment.

($p = 0.009$, Fig. 4), but the data at 6 months were too sparse to be meaningful ($n = 6$, or 25%). Although the duration of menstruation also appeared to be reduced, the change did not reach statistical significance ($p = 0.26$ for 3 months and 6 months after taking Andro).

Clinical Global Impression

The severity of dysmenorrhea as rated by the 24 patients ranged from 1 (no dysmenorrhea at all) to 6 (very severe, seriously interfering with their daily lives and work, without any relief from taking analgesia), with a median CGI-S score of 4, that is, moderately ill (dysmenorrhea/menorrhagia; Table 1). Six months after the treatment, three (12.5%) patients felt “marked improvement”, nine (37.5%) reported “moderate improvement”, seven (29.2%) reported “minimal improvement”, and five (20.8%) reported “unchanged or worse”, with a median score of 2.5, between 2 (minimal improvement) and 3 (unchanged or worse; Table 1). In all patients, no significant side effects were reported (Table 1).

The CGI-S scale correlated positively with the baseline VAS score on dysmenorrhea ($r = 0.89$, $p = 5.4 \times 10^{-9}$, Spearman's correlation) but only marginally with the baseline amount of menses ($r = 0.34$, $p = 0.10$). The CGI-I scale correlated positively with the VAS score on dysmenorrhea evaluated at the 6th month ($r = 0.50$, $p = 0.014$) but not with the amount of menses evaluated at the end of the 3rd month ($r = 0.14$, $p = 0.52$). However, the CGI-I scale correlated positively with both the change in the VAS score ($r = 0.77$, $p = 1.3 \times 10^{-5}$) and the change in the amount of menses ($r = 0.44$, $p = 0.033$).

Immunostaining of the phosphorylated NF-κB p65 subunit in the endometrium and its relationship with the treatment response

We performed an immunostaining on the activated NF-κB p65 subunit in the endometrium. We found no difference in p-p65 staining level between the proliferative and secretory phases ($p = 0.90$). We found that the p-p65 staining levels (square root transformed to improve normality) did not correlate with either the baseline VAS score ($r = -0.25$, $p = 0.29$), baseline uterus size ($r = -0.34$, $p = 0.16$), or the amount of menses ($r = -0.013$,

$p = 0.96$). However, the staining levels were negatively associated with the VAS scores evaluated at the 3rd month and 6th month ($r = -0.47$, $p = 0.042$, and $r = -0.50$, $p = 0.028$, respectively). We then wondered whether the change in dysmenorrhea VAS score at the end of the treatment as compared with its baseline level was associated with the immunostaining levels. We carried out a multiple linear regression analysis on the change in VAS score using the p-p65 immunostaining levels, the baseline VAS score, uterus size, menstrual phase, and age as covariates. We found that the baseline VAS score and the p-p65 staining levels were the two variables negatively associated with the change in the VAS score ($p = 0.015$ and $p = 0.032$, respectively; $R^2 = 0.39$). The scatter plots of the change in VAS score versus the baseline VAS score and the p-p65 staining levels are presented in Fig. 5A and B. With regard to the change in the amount of menses at the end of the 3rd month as compared with its baseline level, no relation with the p-p65 immunostaining levels was found ($r = -0.10$, $p = 0.70$). We then evaluated the p-p65 staining levels in patients with different rating of their impression of treatment response, and found that, after lumping the "Very much improved" rating with the "Much improved" rating (as there was only 1 patient who also had p-p65 data in the 1st rating), the rating was closely correlated with the p-p65 staining levels ($p = 0.011$ by Jonckheere–Terpstra test; Fig. 5C). Removing the patient who rated her impression as "Very much improved" gave a similarly significant result ($p = 0.025$).

Difference between patients who were lost to follow-up and those who were not

Given the 75% of patients who did not come for further evaluation, we wondered whether there was any difference between patients who were followed up and those who were lost to follow-up. It seemed that, compared with those who were lost to follow-up, those who were followed up appeared to be slightly "sicker" and their treatment outcome seemed to be slightly better, although the difference did not reach statistical significance (Table 2). It is unclear whether the lack of statistical significance is genuine or due to the lack of statistical power. However, there was a significant difference in p-p65 staining levels, with those who were followed up having higher staining levels (Table 2).

Adverse events

Andro appeared to be well tolerated by the patients. In all 24 patients who went through the 3-month period of treatment, no adverse event, major or minor, was reported.

Discussion

To the best of our knowledge, this is the first clinical use of an NF-κB inhibitor in treating adenomyosis, despite extensive studies demonstrating the role of NF-κB in the development of adenomyosis/endometriosis. In addition, the efficacy of Andro appears to be dependent on the extent of NF-κB activation in eutopic endometrium, which might be a proxy for the activation level in adenomyotic foci. Whereas Andro appears to be well tolerated, the results seem to be mixed. Judging by the dysmenorrhea VAS scores and the CGI scores, Andro seemed to work for about half of the patients with symptomatic adenomyosis, especially for some with more severe dysmenorrhea. However, for the other half of the patients who had seemingly lower canonical NF-κB activation level in eutopic endometrium, it did not seem to have much therapeutic effect. The somewhat high rate of lost to follow-up or of failure to show up for follow-up examination 6 months after treatment appears to support this view.

Although the quality of life in women with symptomatic adenomyosis may be reduced, adenomyosis is nonetheless a benign disease, and by no means life-threatening. As such, the desirable therapeutics for adenomyosis demands a stringent safety profile. Because the management of endometriosis, and likely adenomyosis as well, is going to be long term,[30] an ideal drug should also be inexpensive and affordable. Andro fits these two requirements.

Because even those who were lost to follow-up still provided their VAS and CGI scores over the phone, naturally one would

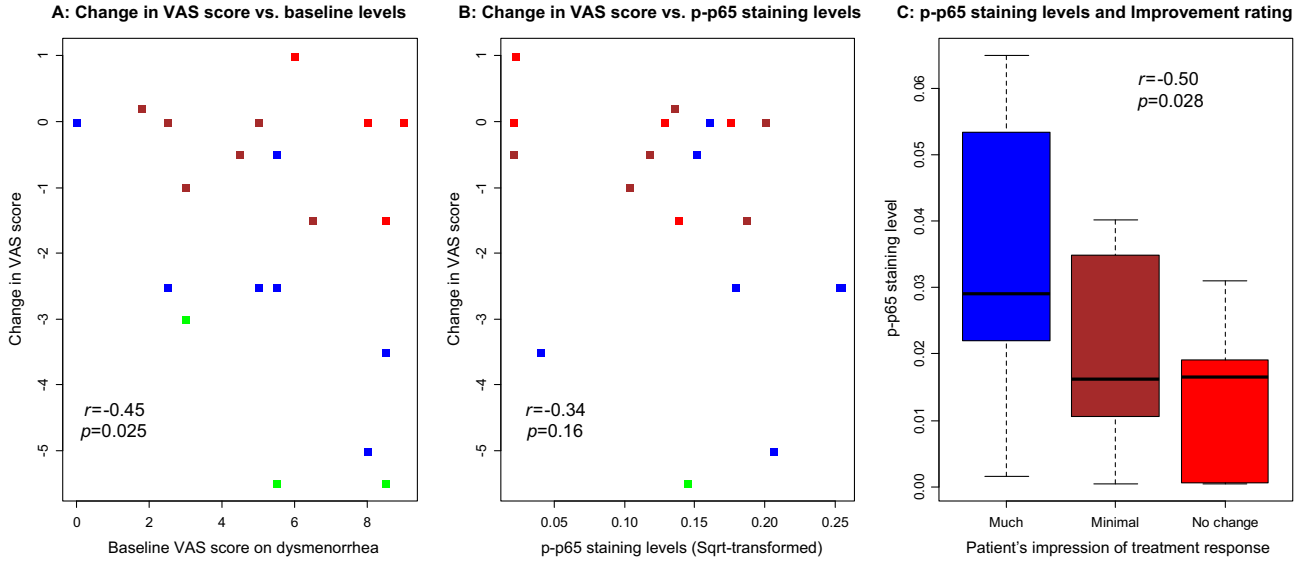

Fig. 5. (A) Scatter plot of the change in the visual analog scale (VAS) score and the baseline VAS score; (B) scatter plot of the change in the VAS score and the p-p65 staining levels in the endometrium; and (C) box plots of the p-p65 staining levels among patients who rated their treatment outcome as "Much improved" (blue), "Minimally improved" (brown) and "No change" (red). The Pearson correlation coefficient, along with its p value, is shown in the two scatter plots. In Panel C, the Spearman correlation coefficient, along with its p value, is shown. The green dots in Panels A and B are patients who rated their treatment outcome as "Very much improved". They were grouped into the "Much improved" group in Panel C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Characteristics of patients who were lost to follow-up and those who were not.

Variable	Patients who were lost to follow-up ($n = 18$)	Patients who completed the study ($n = 6$)	p
Age (y)	39.8 (5.4) 39.5 (30–50)	41.3 (4.0) 41.5 (34–45)	0.31
Amount of menses (mL)	213.8 (188.7) 188.5 (24–900)	250.7 (91.6) 216.0 (167–400)	0.22
10-cm VAS score	5.3 (2.7) 5.5 (0–9)	5.6 (3.2) 5.8 (2.5–8.5)	0.84
Uterine size (cm^3)	150.7 (115.0) 113.0 (37.3–507.8)	151.3 (66.0) 134.3 (93.2–271.1)	0.41
Change in VAS score at the end of the 6th mo	−1.4 (1.9) −0.5 (−5.5–1)	−3.0 (2.2) −2.8 (−5.5–0)	0.11
Change in the amount of menses at the end of the 3rd mo (mL)	−55.9 (181.1) −7.5 (−770–45)	−115.3 (127.6) −83.5 (−300–0)	0.15
CGI Severity scale	4.2 (0.9) 4 (3–6)	4.0 (0.9) 4 (3–5)	0.89
CGI Improvement scale	2.7 (0.9) 3 (1–4)	2.2 (1.2) 2.2 (1–4)	0.43
CGI Efficacy index, n (%)	Marked improvement/no side effect: 1 (5.6) Moderate improvement/no side effect: 5 (27.8) Moderate improvement/no significant side effect: 2 (11.1) Minimal improvement/no side effect: 4 (22.2) Minimal improvement/no significant side effect: 2 (11.1) Unchanged or worse/no side effect: 4 (22.2)	Marked improvement/no side effect: 2 (33.3) Moderate improvement/no side effect: 1 (16.7) Moderate improvement/no significant side effect: 1 (16.7) Minimal improvement/no side effect: 1 (16.7) Minimal improvement/no significant side effect: 0 (0.0) Unchanged or worse/no side effect: 1 (16.7)	0.68
Endometrial p-p65 staining levels	0.022 (0.018) ($n = 2$ missing)	0.035 (0.026) ($n = 2$ missing)	3.1×10^{-5}

Data are presented as mean (SD) and/or median (range), unless otherwise indicated.
CGI = Clinical Global Impression; SD = standard deviation; VAS = visual analog scale.

question as to whether the patients might have provided their evaluation better than they actually were simply because they wanted to please the interviewing physician.

In China's current health care system, all patients have the complete freedom to choose a hospital or even doctors to their liking, whether they have health insurance coverage or not. Many affluent patients in neighboring provinces outside Shanghai often travel to Shanghai in the hope to get the best medical care. For these patients, having a worse outcome or no change in their symptoms could likely prompt them to go to another hospital or other physicians, but they are also likely to come back again because of their initial trust in the hospital. Therefore, for patients who work and live outside Shanghai, loss of follow-up does not necessarily mean a worse outcome.

For patients residing in Shanghai, failure to show up at the second follow-up also could mean no change or worsened condition, and they voted their confidence by their feet. If they were genuinely unhappy about their care, however, it is likely that they would simply say so or refuse to provide more information regarding their condition. Because Shanghai has many tertiary hospitals and there are in fact many doctors even in our hospital, they could have done so with impunity. In other words, the high rate of lost to follow-up may not, again, necessarily be a sign of treatment failure.

In view of this analysis, the VAS and CGI scores provided over the phone can be viewed as fairly reliable. With that data, it seems that Andro is efficacious for about half of the patients. For the other half, it simply did not seem to work well.

Then, why a compound from a drug class that holds so much promise for treating adenomyosis/endometriosis is not even 70% effective? Should we sack Andro based on this pilot clinical study?

As is well-known in cancer therapy or even endometriosis treatment, no drug is 100% effective. Thus, Andro should not be an exception. We would argue that for a condition that is notoriously difficult to manage,[31] approximately 50% efficacy is rather encouraging, particularly so given the fact that Andro has an excellent side effect and safety profile and is very affordable. More clinical studies or clinical trials are perhaps warranted to further evaluate its efficacy.

There are in fact several reasons to explain why Andro treatment did not yield its expected efficacy for some patients. First, Andro may simply elicit no response from ectopic endometrial tissues because of the extensive presence of fibrosis. Our laboratory recently found extensive fibrosis in ectopic endometrial tissues harvested from patients who have undergone hysterectomy because of symptomatic adenomyosis (Shen et al, unpublished data). It is well-known that as of now there is no effective treatment for fibrosis.[32]

Second, Andro renders its action presumably by inhibiting the NF-κB p50 subunit.[33] As a family of structurally related dimeric transcription factors, NF-κB has five members, namely, p50 (NF-κB1), p52 (NF-κB2), p65 (Rel A), c-Rel, and RelB, which form various homo- and heterodimers when activated, with the p50–p65 dimers being the canonical activated forms.[33] Although many studies demonstrated constitutive activation of NF-κB in endometriosis/adenomyosis,[6–8] most, if not all, of them only examined the canonical activation form, that is, the p50–p65 dimers or simply p65 activation. However, if the adenomyotic lesion has a noncanonical activation form, then conceivably Andro treatment may not be effective. The finding that bigger reduction in the VAS scores was negatively associated with the p-p65 staining in the endometrium seems to support this notion, because the higher p-p65 staining and a better outcome appear to suggest that Andro was right on target when the p65–p50 heterodimers were canonically activated in the endometrium, and perhaps also in ectopic endometrium. If this proves to be true, then perhaps individualized treatment based on the specific NF-κB homo- and heterodimers activation may be a way to go.

Lastly, there is always the possibility that pathways other than NF-κB are involved in the development of adenomyosis, and therefore Andro treatment may not have any effect on these pathways.

Whatever the reason may be, future research is needed to clarify (1) whether the NF-κB activation status in eutopic endometrium can be used as a proxy for the status in ectopic endometrium in adenomyosis; (2) whether the extent of fibrosis in lesions affect treatment efficacy; and (3) which NF-κB subunits are involved in the development of adenomyosis aside from p50–p65.

Despite being the first in the clinical use of an NF-κB inhibitor in treating adenomyosis, and in the use of canonical NF-κB activation level in eutopic endometrium as a proxy for adenomyotic foci, this study has limitations. First, caution should be exercised when interpreting our findings because of a high rate of lost to follow-up. Second, because of lost to follow-up, we did not have any data for the long-term efficacy of Andro. However, even if the efficacy lasts for only 3 months after termination of the treatment, one could still argue that it is worthwhile, particularly so given the excellent safety profile and the cost profile of Andro.

In conclusion, we found that Andro is effective in some patients with symptomatic adenomyosis, who have a higher endometrial expression of the activated form of the NF-κB p65 subunit. Future independent validation studies or randomized clinical trials may be needed to more precisely evaluate the efficacy of Andro in treating adenomyosis.

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