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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.

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.