– Original Study –

Myomin: Natural Aromatase Inhibitor for Estrogen-related Conditions

by Tsu-Tsair Chi, NMD, PhD

Introduction

Aromatase inhibition is considered a novel methodology in the treatment of estrogen-responsive diseases. Aromatase inhibitor (AI) drugs have shown very promising results in clinical trials. In the Breast International Group 1-98 study, adjuvant treatment with the AI letrozole was found to reduce the risk of recurrent endocrine-responsive breast cancer in postmenopausal women.[1] This result was reinforced by the MA-17 trial, which found that extended adjuvant therapy with the same AI, up to 48 months, provides greater benefit.[2]

The success of the AIs on breast cancer and their potential effect on other estrogen-responsive diseases have led our team of researchers to explore possible alternative AIs in herbs. What we discovered was that the herbal combination of *Curcuma, Cyperus*, and *Astragalus* exhibits aromatase inhibition activity in rats, as presented in the following sections. This combination of herbs will be referred to as Myomin in this article. The succeeding in vitro, in vivo, and clinical studies will demonstrate Myomin's mechanism, functions, and applications.

MATERIALS AND METHODS

1. Culture of Primary Ovarian Granulosa Cells in Vitro

1.1 Obtaining and Culture of Ovarian Granulosa Cells

Bilateral ovaries were obtained aseptically from 21- to 23-day-old female SD rats that had each been injected with 0.5 mg diethylstilbestrol per rat for three days.[3] Excess fat and connective tissues were trimmed from the ovaries in surgery. Then the ovaries were punctured to release the granulosa cells in McCoy's 5A media. Through centrifugation for 7 minutes at 1000 rpm, cells were recovered and then eluted 3 times with culture media. The precipitate was then washed and suspended in the required McCoy's 5A media. After cell counting, the cells were inoculated in the 96 well plates to a final concentration of 3–6 × 104 cells per well and placed in the tissue culture incubator at 37 °C, in a 95% air/5% CO_2 atmosphere.

1.2 Effect of Myomin on the Survival Rate of the Ovarian Granulosa Cells

Twenty-four hours after inoculation, the cells were transferred to McCoy's 5A culture media containing 5, 10, 20, 40 and 80 µg/ml Myomin, respectively, and a McCoy's 5A culture medium containing 0.05% absolute alcohol.[4] Cells of 6 to 9 wells were treated as a group at the same dose. After 24, 48 and 72 hours' treatment, 10 µl Cell Count Kit (CCK-8) solution was added in every well. (CCK-8 is a sensitive nonradioactive colorimetric assay for determining the number of viable cells in cell proliferation and cytotoxicity assays. Since the CCK-8 solution is very stable and has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible). The plates were then placed in the tissue culture incubator at 37 °C, in a 95% air/5% CO_2 atmosphere for 1 to 4 hours. The absorbance of every well was measured with ELISA at 450 nm. This was repeated 3 times and the mean calculated. Cells of the control group were considered as 100%. Survival rate was calculated according to the following equation: survival rate = the mean absorbance of the experimental group/ the mean absorbance of the control group × 100%. Under microscopic examination, living cells had been stained yellow and dead cells appeared to have no stains with an increased volume.[5]

2. Effect of Myomin on the Expression of Aromatase in Ovarian Granulosa Cells

2.1 Expression of Aromatase in Ovarian Granulosa Cells

Twenty-four hours after the growth of cells on slides, the experimental cultures were transferred to McCoy's 5A culture media containing 0.01 IU/ml follicle stimulating hormone (FSH) and 0.5 µM androstenedione for a 72-hour culture. At the same time, the control cultures were transferred to a fresh McCoy's 5A culture medium for a 72-hour culture and fixed in a formic acid–glacial acetic acid solution (3:1 by volume).[6–8] These were incubated with 0.03% H_2O_2 in PBS for 10 minutes and washed with distilled water. These were incubated again in PBS for 5 minutes and blocked with normal goat blood serum for 10 minutes at room temperature, then incubated with the primary antibody (rabbit anti-rat P450 aromatase IgGs overnight at 4 °C). After incubation, these were washed with PBS 3 times, incubated with the secondary antibody (biotinylated anti-rabbit IgGs) for 10 minutes at room temperature, washed with PBS again, and colored with DAB. After being washed with water, the cultures were counterstained with hematoxylin and mounted. The PBS-treated cultures were considered as negative control.

2.2 Effect of Myomin on the Expression of Aromatase in Ovarian Granulosa Cells

Twenty-four hours after the growth of cells on slides, the experimental cultures were transferred to McCoy's 5A culture media containing 0.01 IU/ml FSH, 0.5 µM androstenedione, and 30 µg/ml Myomin for a 72-hour culture. At the same time, the control cultures were transferred to a fresh McCoy's 5A culture medium containing 0.01 IU/ml FSH and 0.5 µM androstenedione for a 72-hour culture and fixed in a solution of formic acid–glacial acetic acid (3:1 by volume). These were incubated with 0.03% H_2O_2 in PBS for 10 minutes and washed with distilled water. These were incubated in PBS again for 5 minutes and blocked with normal goat blood serum for 10 minutes at room temperature and incubated with the primary antibody (rabbit anti-rat P450 aromatase IgGs) overnight at 4 °C. After incubation, the cultures were washed with PBS, incubated with the secondary antibody (biotinylated anti-rabbit IgGs) for 10 minutes at room temperature, and colored with DAB. After washing with water, the cultures were counterstained with hematoxylin and mounted. PBS-treated cultures were considered as negative control. Positive results: particles

estrogen-mediated conditions. Increased aromatase expression promotes estrogen production, especially at the site of hormone-responsive tumors such as in breast, endometrial, and ovarian cancer.[10] The development of several AI drugs, primarily for breast cancer, is based on this premise. The success of AIs on this disease opens other potential applications. In fact, AIs are also being explored as possible treatment for estrogen-responsive conditions such as endometriosis and prostate cancer.[11,12]

Both in vitro and in vivo studies demonstrate that Myomin could be a potentially viable herbal AI. In the in vitro study in section 2.2, FSH was used to stimulate aromatase expression in cells. In the untreated positive control group, aromatase expression was signified by stained cells, which accounted for 94% of the total cells. After adding Myomin into the tissue culture, stained cells reduced to only 31% of the total cells, signifying a 67.02% reduction of aromatase expression.

The in vivo studies further reinforce the aromatase inhibition activity of Myomin (section 3). Aromatase expression was measured using grayscale values obtained through image analysis. In the ectopic endometrium and ovarian tissue positive control groups, the average aromatase expression was measured at 108.9 and 149.8, respectively. Aromatase expression is obviously much higher in these groups compared with that of the Myomin group, which has grayscale values of 34.7 for the ectopic endometrium and 47.0 for the ovarian tissue. This means that after administration of Myomin, aromatase expression in the ovarian tissue is almost comparable with that of the negative control group, while aromatase expression in the ectopic endometrium of the Myomin group is even much lower than the corresponding negative control group's value.

The clinical studies on Myomin demonstrate its efficacy in reducing estradiol and improving estrogen-responsive conditions such as fibrocystic breasts, uterine fibroids, ovarian cysts, and endometriosis (section 4). One particular case demonstrates how Myomin is beneficial for an estrogen-responsive condition like fibrous breasts. J. Blair, ND, of New Jersey has a 48-year-old female patient with a long history of dense breasts since she started menstruation more than 30 years ago. A mammogram was not adequate to test her breasts for growths; an ultrasound was always needed for further testing. The patient took Myomin for a year and a half. She reports that, for the first time in her life, a mammogram shows a clear picture: no more denseness at all. An ultrasound was no longer needed.

Myomin's estrogen-reducing effect is illustrated in a case report from J. Iannetta, DC, of Maine, of a 57-year-old postmenopausal female patient who initially had very high estradiol at 413 pg/ml. After taking Myomin for 14 months, her estradiol reduced to 43 pg/ml. In a similar case, J. Wright, MD, of Washington has a male patient in his 50s who had elevated estradiol. None of the supplements he tried seem to have any effect on it. After taking Myomin for 4 months, his estradiol has sharply dropped. This particular case shows that AIs like Myomin may have uses for estrogen-related conditions in men.

Indeed, as we learn more about the etiology of prostatic diseases, the

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Myomin

Figure 4. Effect Myomin on Estradiol Level of 60 Postmenopausal Women with Fibroids and Cysts

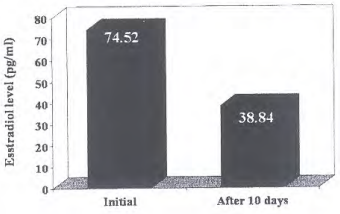


Figure 5. Myomin on 50 Patients with Fibrocystic Breasts (6 months)

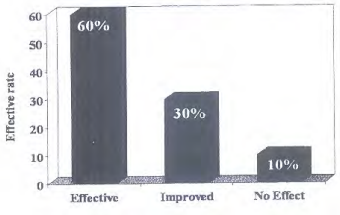


Figure 6. Myomin on 255 Patients with Ovarian Cysts and Endometriosis (5 months)

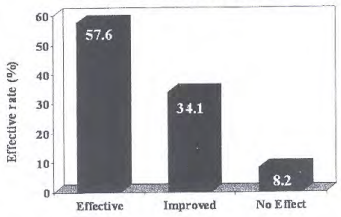


Figure 7. Myomin, Revivin and Angiostop on 60 Patients with Uterine Fibroids (6 months)

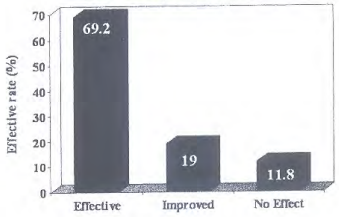
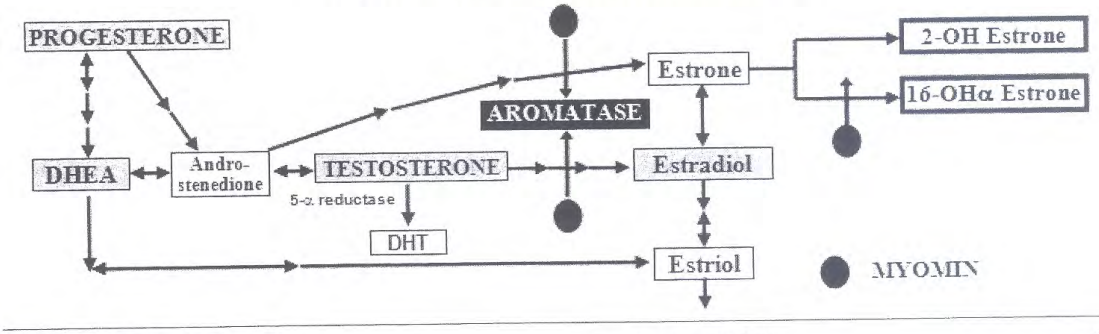


Figure 8. Biosynthesis of reproductive hormones



Myomin

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importance of AIs in men becomes increasingly clear. As men age, testosterone is more readily converted to estrogen through aromatase.[13] Some studies show that increased estrogen level is seen in problems such as benign prostatic hyperplasia (BPH),[14–16] prostate cancer,[17] prostatitis,[18,19] and even gynecomastia.[20–22] In fact, some studies report that estrogen promotes the overgrowth of prostate tissue in BPH.[14–16] Evidence also suggests that local aromatase expression in the prostate increases in malignancy. The level of aromatase expression in malignant prostate tissue is actually comparable with that in breast cancer tissue.[17] As a result, local estrogen levels are also promoted at the prostate tumor site, increasing growth. Aromatase inhibitors will help block the action of aromatase, thereby minimizing the depletion of testosterone and reducing estrogen levels.

One case showed that enlargement of the prostate may well be estrogen-mediated. L. Bazakos, DC, of New York has a 55-year-old male patient with BPH. He took Myomin and Prosta Chi for over a year. A test showed that his prostate has reduced in size and is soft, almost like that of a 19-year-old boy. In another case, a 16-year-old male gynecomastia patient of B. Sanborn, DC, of Michigan responded well to Myomin. He had elevated levels of estradiol and dihydrotestosterone (DHT). After taking Myomin for two months, his estradiol level reduced from 300 to 70, while his DHT level reduced from 1100 to 250. A noticeable difference in the size of his breasts was also observed during this time.

Myomin has also been found to increase the 2/16α hydroxyestrone ratio (Figure 8), according to a case study from E. Schlabach, DC, of Ohio. A higher 2/16α hydroxyestrone ratio is associated with a decreased risk for estrogen-related neoplasms. Dr. Schlabach reported that, according to many patient urine laboratory tests, Myomin not only can reduce estradiol but also can quickly increase the 2/16α hydroxyestrone ratio. After a month of taking Myomin, the ratio increased to over 2.0 to 6.0.

AIs like Myomin may also benefit those who are taking hormones such as progesterone, testosterone, androstenedione, or dehydroepiandrosterone (DHEA). These are precursors to estrone and estradiol (see Figure 8), both of which are potent forms of estrogen. Those taking hormone supplementation may accumulate estrone and estradiol if their production remains unchecked. AIs help reduce the accumulation of these hormones. By inhibiting aromatase, synthesis of estrone and estradiol from androstenedione and testosterone is also blocked (see Figure 8), while production of the weaker form of estrogen, estriol, goes unhindered. Studies have shown that estriol provides the benefits that estrone and estradiol do and may even have a better safety profile.[23,24] Those on bioidentical hormones may still have a risk for developing tumors or abnormal growths if they do not change their lifestyle. Exposure to xenoestrogens, which mimic the effect of estrogen, can increase their risk. Therefore, taking Myomin with the bioidentical hormones will provide a protective effect.

Aromatase inhibition may also play a role in reducing abdominal fat. Abdominal obesity has been associated with decreased testosterone levels and increased estradiol levels. Hypogonadism leads to the deposition of fat in the abdomen. As fat accumulates, aromatase activity increases at the site, causing more testosterone to be converted into estradiol.[25] An AI like Myomin blocks the action of aromatase, effectively preventing the testosterone from being converted into estradiol. This leads to more muscle mass and the loss of abdominal weight. Although more studies are needed to establish this concept, a case from E. Saugen, DC, of Minnesota demonstrates Myomin's effect on abdominal fat. A 48-year-old male patient was overweight, with much of his weight concentrated in the abdomen. After 6 months of taking Myomin, he lost 25 lbs. and 3 inches around the waist.

The studies presented here clearly demonstrate that Myomin inhibits aromatase expression in the in vivo ovary, in vivo ectopic endometrium, and in vitro cultured primary ovarian granulosa cells of rats, making it a potentially viable herbal AI. Clinical studies show that, through its aromatase inhibition function, Myomin is able to reduce estradiol and improve estrogen-responsive conditions like fibroids, fibrocystic breast, ovarian cysts, and endometriosis. While further investigation and more clinical studies are needed to identify its active ingredient and elucidate its complete mechanism, the studies and cases presented here provide compelling evidence that Myomin will be beneficial for estrogen-mediated conditions.

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Notes

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