Fertility & Sterility [Pending publication]

EFFECT OF MYOMIN ON THE EXPRESSION OF AROMATASE

T.C. Chi, Y.H. Yi

ABSTRACT:

Objective: To study the regulation effects of Myomin, a herbal formula, on the expression of aromatase in the *in vivo* ovary, ectopic endometrium and *in vitro* cultured primary ovarian granulosa cells of rats. **Methods:** The changes of aromatase expression in the *in vivo* ovary, ectopic endometrium and *in vitro* cultured primary ovarian granulosa cells of rats after treatment were evaluated in comparison to those of untreated rats. **Results:** Aromatase expression was inhibited in the *in vivo* ovary, ectopic endometrium and *in vitro* cultured primary ovarian granulosa cells of rats by adding Myomin into the culture media. **Conclusion:** Myomin inhibited the aromatase expressions in the *in vivo* ovary, ectopic endometrium and *in vitro* cultured primary ovarian granulosa cells of rats.

INTRODUCTION

In the last decade or so, the increasing incidence of hormone-related conditions, especially in women, has shifted the focus of researchers to other possible treatment methods besides surgery. Hormonal therapy has become quite popular in the last few years not only because it is a noninvasive procedure but also because it has shown very promising results. In particular, aromatase inhibitors (AIs) can effectively reduce levels of estrogen-- the primary cause of these hormone-related conditions-- by blocking the aromatase enzyme from converting androgens into estrogen. Currently, there are a number of different aromatase inhibitor drugs in the market; however, researchers are continually investigating other alternative therapies.

One such alternative therapy is Myomin, a combination of traditional Chinese herbs used historically for medicinal purposes. Since 1989, Myomin has been used in clinical settings and found to be effective for conditions such as ovarian cysts, endometriosis and fibrocystic breast. In one study from Chang Hai Hospital in Shanghai, China, 85 female patients with ovarian cysts and endometriosis were employed. The patients ranged in age from 27 to 64 years old, with more than 50% of them ages 34 to 45 years old. After 1-3 courses of Myomin, the cysts and symptoms of 15 patients completely cleared with no recurrence 3 months after the study. In 34 patients, the size of the cyst reduced by 50% and some of the associated symptoms were relieved.[1]

Another study from the same hospital employed 75 cases of fibrocystic breasts. The study involved 70 women ages 18 to 52 years old and 5 men ages 62 to 64 years old. Sixty five percent of the women were under 35 years old. Results of the study revealed that, in 14 cases, the fibrocystic breast completely cleared after 1 month of taking Myomin while, in 16 cases, the fibrocystic breast reduced by 50% in size. Among the 5 men in the study, the cysts cleared completely in three cases.[1]

Results such as these have led researchers to investigate into the mechanism by which Myomin is able to help in these conditions. Subsequent animal and clinical studies have revealed that Myomin is able to exert its effect on these conditions by reducing estrogen levels.[1]

Its estrogen-reducing property has led researchers to suspect that this might be due to a possible aromatase inhibition function. The aromatase P450 is the key enzyme that regulates estrogen formation. In the following studies, researchers first determine the appropriate dosage of Myomin to be used in tissue cultures that is safe for ovarian granulosa cell growth. After the correct dosage has been determined, researchers employed endometrial and ovarian tissues from rats to test the effect of Myomin on aromatase expression and its possible therapeutic effect on hormone-related conditions.

RESULTS

1. Effect of Myomin on the expression of aromatase in cultured rat primary ovarian granulosa cells

1.1 Cultured primary ovarian granulosa cells

The rat ovaries were stimulated after treatment with diethylstilbestrol (DES). About 4 x 10^5 cells were gained from every ovary. One day after inoculation, ovarian granulosa cells adhered well and spread out. Feelers presented confluence, with most conglomerately and a few singly. A lipid droplet was inside each cell. Cell appearance was just as the previous papers reported. The cells are able to survive in McCoy'5A media for at least seven days (Figure 1).

1.2 Effect of Myomin on the survival rate of the cells

Twenty four hours after subculturing, the cells were transferred to the McCoy'5A media containing different dosages of Myomin. The cells transferred to the McCoy'5A media containing 0.05% dehydrated alcohol served as control. Forty eight hours after the previous step, the cells were counted. It turned out that ≤ 20 μg/ml Myomin did not change the quantity of living cells while ≥ 40 μg/ml Myomin decreased the quantity of living cells significantly ($P<0.05$).

The concentration- percentage curve in the following graph shows the concentration of Myomin on the x-axis plotted against the percentage of living cells on the y-axis (Figure 2). The equation has been gained with the regression analysis of the curve, as follows: $Y= -0.0999X+0.8468$, $r^2=0.9376$

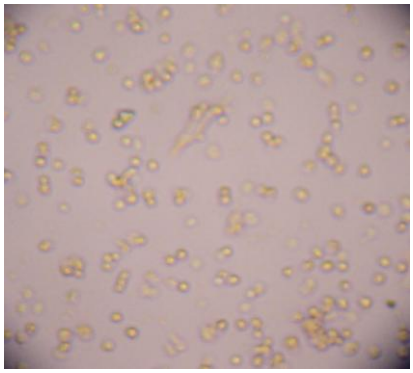


Figure 1 Ovarian granulosa cells adhered well, with most conglomerately and a few singly. Lipid droplet was found inside the cell.

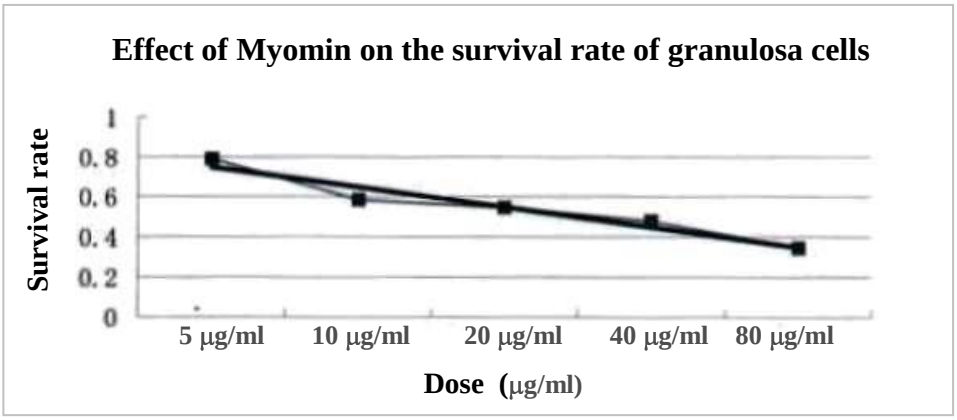


Figure 2 A dose vs. effect curve with the Myomin concentration

1.3 Effect of Myomin on the expression of aromatase in rat ovarian granulosa cells

1.3.1 Enhancement of FSH on aromatase expression

Twenty four hours after the growth of cells on the slides, the experimental cultures were transferred to McCoy' 5A culture media containing 0.01 *IU*/ml FSH and 0.5 μM androstenedione for a 72-hour culture. The cells were observed in situ. Immunohistochemical staining showed that endochylema of most cells treated with only media and media containing FSH had been stained yellow under microscopic examination. In the cells of the negative control group, no stains were employed.

1.3.2 Effect of Myomin on the enhancement of FSH on aromatase expression

Twenty four hours after the growth of cells on the slides, the experimental cultures were transferred to McCoy'5A culture media containing 0.01 *IU*/ml FSH, 0.5μM androstenedione and 30 μg/ml Myomin for a 72-hour culture. The cells were observed in situ. Under microscopic examination, in the cells of the negative control group, no stains had been employed. In the untreated control group, stained cells accounted for 94.0 ± 45.0 percent of the total cells. Stained cells decreased by adding Myomin into the culture media and accounted for 31.0±11.0 percent of the total cells. Using statistical t-analysis, a significant difference existed between the two groups ($P<0.05$).

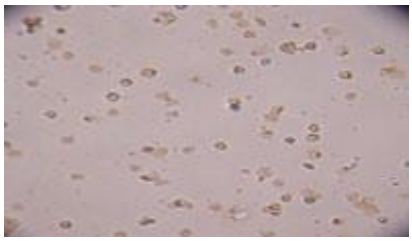


Figure 3.1 Negative control group stained with PBS: No stains

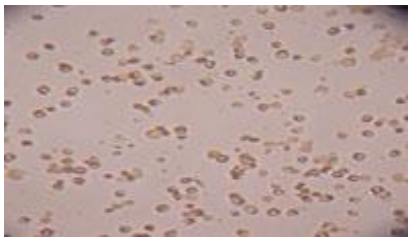


Figure 3.2 Model blank control group: Remarkable expression of aromatase

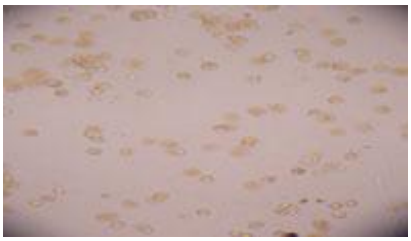


Figure 3.3 Myomin group: Aromatase expression significantly decreased

2. The effect of Myomin on the expression of aromatase in the rat ovary and rat ectopic endometrium in vivo

2.1. Aromatase was expressed markedly in the ectopic endometrium of the positive control group. Aromatase expression significantly decreased in the ectopic endometrium of the Myomin group 28 days after therapy in comparison with those in the ectopic endometrium of the positive control group (Figure 4). Using the image analysis system, the average grayscale value of aromatase expression in the positive control group was 108.9 ± 29.03 and that in the Myomin group was 34.7 ± 5.2 (see table below), with a significant difference between the two groups.

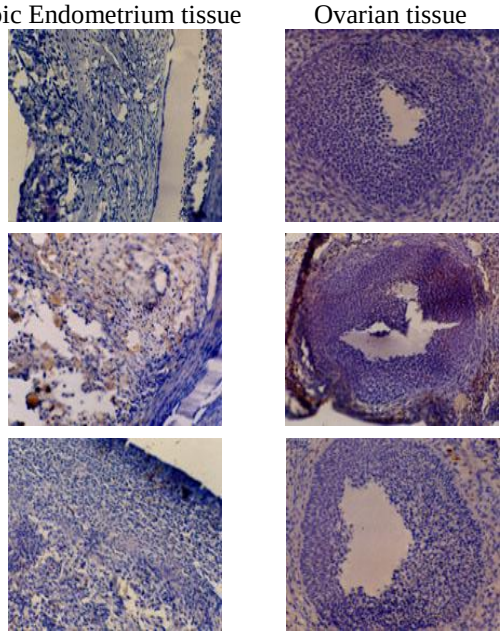
Comparison of grayscale values between ovary and ectopic endometrium ($p<0.05$)

Group	Ectopic endometrium **(X ± S)**	Ovary **(X ±S)**
PBS negative control group	48.0 ± 8.5	29.7 ± 6.4
Positive control group	108.9 ± 29.03	149.8 ± 33.86
Myomin group	34.7 ± 5.2**	47.0 ± 9.19**

**Significant difference compared with positive control group ($P<0.05$)

2.2. Aromatase was expressed markedly in ovaries of the blank control group. Aromatase expression significantly decreased 28 days after therapy in the ovaries of the Myomin group compared with those in the blank control group (Figure 4). Using the image analysis system, we obtained that the average grayscale value of aromatase expression in the ovaries of the blank control group was 149.8 ± 33.86, and that in the Myomin group was 47.0 ± 9.19 (see previous table), with significant difference between the two groups.

Figure 4 Aromatase expression in rat ovary and ectopic endometrium



DISCUSSION

The results of these studies are indeed very promising. Both in vitro and in vivo studies demonstrate that Myomin can inhibit the aromatase enzyme significantly with low toxicity. The in vitro study outlined in Section 1 aimed to find the optimum dosage of Myomin that is safe for the growth of ovarian granulosa cells. It was established that 20 μg/ml Myomin had no effect on the quantity of living granulosa cells while a dose of 40 μg/ml significantly reduced the number of living granulosa cells. Therefore, by extrapolation, 30 μg/ml Myomin was the ideal dosage to be used for the succeeding in vitro and in vivo studies.

Acute toxicity studies in rats have also found it to be safe with a LD_{50} of 10.15 g/kg. In human terms, a dosage of 3 capsules, 3 times daily at 400 mg/capsule would add up to 3,600 mg per day. Accounting for the average human weight of 60 kg (or 132 lbs), this would translate to 600 mg/kg, which is well below the LD_{50} of 10.15 g/kg, making Myomin a logically safe formula for human consumption. It is worth noting that in the Chang Hai Hospital studies, approximately 1-2% of the subjects taking Myomin experienced side effects such as nausea and bloating. However, these symptoms were abated after reduction of dosage.[1]

After the initial dosage determination study, the effect of Myomin on aromatase expression was observed and measured both in vitro and in vivo. In the in vitro study in Section 1.3, FSH was employed to stimulate aromatase expression in cells. In the untreated control group, aromatase expression was significant with stained cells accounting for 94% of the total cells. After adding Myomin into the tissue culture, stained cells accounted for only 31% of total cells, signifying the reduction of aromatase expression.

The in vivo studies further reinforce the aromatase inhibition activity of Myomin. 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 to that of the Myomin group which has grayscale values of 34.7 for the ectopic endometrium and 47.0 for the ovarian tissue. The Myomin group value for the ovarian tissue is almost comparable to 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 important role that the aromatase enzyme plays in the production of estrogen has made it a focus in the search for viable therapies for estrogen-related conditions. This search has given rise to the development of several aromatase inhibitor (AI) drugs for conditions such as breast, uterine and ovarian cancer and endometriosis. However, the AI drugs in the market today are indicated for postmenopausal women only.[7] This is very limiting especially since more and more premenopausal women are developing hormone-related conditions. It is for this reason that the results of these studies on Myomin are significant. As stated in an earlier section, Myomin has been used in clinical studies and found to be effective in both premenopausal and postmenopausal women with ovarian cysts, fibrocystic breast and endometriosis as well as in men with fibrocystic breast. It is able to produce these favorable results by inhibiting aromatase and consequently reducing estrogen levels.

Aromatase inhibition is important in men as well. As men age, testosterone is being more readily converted to estrogen through the aromatase enzyme, leading to problems such as prostate cancer, increased libido and, in some cases, fibrocystic breast. Therapies like AIs and Myomin will help block the action of aromatase, thereby minimizing the depletion of testosterone and reducing estrogen levels.

CONCLUSION

The studies presented here clearly illustrate that Myomin significantly inhibits aromatase expression in the in vivo ovary, ectopic endometrium and in vitro cultured primary ovarian granulosa cells of rats. This aromatase inhibition function explains why it has been able to effectively reduce estrogen levels in previous studies and has been clinically effective in conditions like ovarian cysts, endometriosis and fibrocystic breast. Myomin's aromatase inhibition function in vivo opens up many possibilities into its potential as a viable alternative aromatase inhibitor and its therapeutic effect on hormone-related conditions. However, while these results are very promising, they are still preliminary. Further investigation and clinical trials are warranted to clarify the entire mechanism by which Myomin affects hormone-related conditions.

References

1. 1989-1991 Chang Hai Hospital study on ovarian cyst patients. Shanghai, China.
2. Hillier SG, Zelexnik AJ, Ross GT, et al. Independence of steroidogenic capacity and luteinizing hormone receptor induction in developing granulosa cells. *Endocrinology*. 1978; 102(3):937-46.
3. Gregory FE, Richards JS, Ireland JJ, et al. Primary cultures of ovarian cells in serum-free medium as models of hormone-dependent differentiation. *Molecular and Cellular Endocrinology.* 1983; 29:21-49.
4. Fishen CJ, Gillett CE, Vojtesekb B, et al. Problems with P53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. *British Journal of Cancer.* 1994; 69(1):26-31.
5. Dorrington TH, et al.Estradiol-17 β biosynthesis in cultured granulosa cells from hypophysectomized immature rats: stimulation by follicle-stimulation hormone. *Endocrinology.* 1975; 97:1328-1342.
6. Yang Cui-bo, Li Yu-xia, Hu Zi-zheng. Effects of Prolactin and Dopamine on Steroidogenesis by Rat Granulosa Cells In Vitro. *Reproduction and Contraception.* 1992; 12(4):36-41.
7. Lake, Diana, MD and Clifford Hudis, MD. Aromatase Inhibitors in Breast Cancer: An Update. *Cancer Control Journal*. Nov/Dec 2002; 9(6):490-498.

Bibliography

Aromatase Inhibitors Show Promise for Early Breast Cancer. *National Cancer Institute*. 7 July 2003. <http://www.nci.nih.gov/clinicaltrials/results/aromatase-inhibitors0302> 12 Dec 2003.

Follingstad, Alvin MD. Estriol, the Forgotten Estrogen? [Commentary]. *Journal of the American Medical Association.* 1978; 239(1): 29-30.

Mouridsen, H, T. Palshof, J. Patterson, L. Battersby. Tamoxifen in advanced breast cancer. *Cancer Treatment Reviews.* 1978; 5:131-141.

Oktay, K. et al. Endocrine Function and Oocyte Retrieval After Autologous Transplantation of Ovarian Cortical Strips to the Forearm. *Journal of the American Medical Association*. 2001; 286: 1490-93.