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Combination Therapy of Methotrexate and Iguratimod for Female Patients with Moderate to Severe Active Rheumatoid Arthritis and its Impact on Ovarian Function

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ABSTRACT

Rheumatoid Arthritis (RA) is a common immune disease, with early symptoms of joint morning stiffness, pain, swelling, etc., and could develop into joint deformity as the disease progresses, resulting in the loss of normal joint function, seriously affecting the patient's daily work and life. Methotrexate (MTX) is the "anchor drug" for RA treatment, which has an immunosuppressive effect, and could effectively prevent the abnormal activation of B cell in vivo, inhibit abnormal generation of cytokine, and relieve related symptoms. However, some cases reflected that single treatment of MTX is not ideal and often needed to be combined with other drugs. Iguratimod (IGU), as a new Disease Modifying Anti-Rheumatic Drug (DMARDs), is a new small molecule drug with anti-inflammatory and immunomodulatory properties, and a derivative of 7-methanesulfonylamino-6-phenoxychromones and is a hormone with two amide groups. This study provided a medical basis for the clinical therapeutic options, by exploring the combined efficacy of MTX and IGU for female patients with moderate to severe RA and its impact on ovarian function.

Keywords: Rheumatoid Arthritis, Methotrexate, Iguratimod, Ovarian Function, Inflammation

INTRODUCTION

Study subjects

The 80 female patients diagnosed with moderate to severely active RA and enrolled in our department from January 2020 to January 2022 were selected as the study subjects.

Inclusion criteria:

- Meet the RA classification criteria proposed by the 2009 American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR).
- **Age:** 20-50 years old, with regular menstruation for nearly 3 months if under 40 years old (21-35 days as period, lasted for 2-7 days), and normal values of sex hormones in follicular phase tested on the 3rd day of period.
- Never received drug treatment within half a year.
- DAS28-ESR>3.2.

Exclusion criteria

- History of confirmed ovarian cyst, polycystic ovary syndrome, endometriosis, ovarian surgery or radiation therapy and sex hormone therapy for other reasons.
- History of contraceptives within half a year.
- Pregnancy and breastfeeding patients.
- Diagnosed with other basic diseases, such as diabetes and hypertension.
- History of malignant tumors.
- History of existing gynecological infectious diseases, such as pelvic inflammation.
- MTX, IGU, and meloxicam intolerance. The clinical trial was approved by the hospital ethics committee, and the subjects were all informed with consent.

Allocation

The 80 patients with moderate to severe active RA were randomly divided into MTX and MTX+IGU groups, with 40 patients in each group.

MATERIALS AND METHODS

Treatment

MTX group was treated with MTX+meloxicam 15 mg qd. MTX+IGU group was treated with MTX+IGU 25 mg bid+meloxicam 15 mg qd. The initial dose of MTX in both groups was 7.5 mg qw, which was gradually increased 1 week later with a maximum of 15 mg qw. Hydroxychloroquine was used as a remedy after 3 months [1].

Outcome measures

The Swollen Joint Count (SJC), Tender Joint Count (TJC), duration of morning stiffness, and menstrual cycle were recorded at week 0 and week 24, respectively. The patients Erythrocyte Sedimentation Rate (ESR), Rheumatoid Factor (RF), high sensitivity C-Reactive Protein (hsCRP), liver and kidney function, and cytokines (IL-6, TNF-α, etc.) were tested, and the DSA28-ESR score was calculated. On the 3rd day of period, ovarian reserve indicators (Anti-Mullerian hormone in serum (AMH), Follicle Stimulating Hormone (FSH), Luteotropic Hormone (LH) and Estradiol (E2)) were measured. The B ultrasound specialists measured Antral Follicle Counting (AFC) and Ovary Volume (OV) [2].

Statistical method

SPSS 22 was used to analyze data, with measurement data expressed as $x \pm s$ if normally distributed. Paired t-test was taken for comparison between before and after intervention. Those not conforming to normal distribution are expressed as median P50 (P25, P75), with Mann-Whitney U test for comparison between groups. $P<0.05$ was considered to be statistically significant [3-5].

RESULTS

General data

A total of 80 female patients with RA were included, with age of 20-50 years old and mean age of 41.90 ± 7.95 years. The average disease course was 12.15 ± 10.16 months. The average SJC was 2.53 ± 0.67, and the average TJC was 5.66 ± 3.25. The average duration of morning stiffness is 50.42 ± 42.45 min [6-8]. The average ESR was 59.10 ± 19.59 mm/Hr, with median RF of 154 (47.68, 356) iu/ml. The average DSA28 score was 5.17 ± 0.38. Patients randomized into 2 groups showed no statistical differences in age, disease course, STC, TJC, duration of morning stiffness, ESR, RF, and DSA28 scores before treatment [9,10].

Follow-up data

Three months after the treatment, due to poor efficacy, 4 patients in the MTX group were treated with extra hydroxychloroquine and 0 in the MTX+IGU group was taken extra.

Comparison of efficacy between the 2 groups after treatment

Comparison of clinical data: After treatment, the MTX+IGU group showed more obvious relief in SJC, TJC, ESR and DSA28-ESR score than MTX group [11,12]. There are no significant differences between the two groups regarding morning stiffness duration or RF (Figure 1 and Table 1).

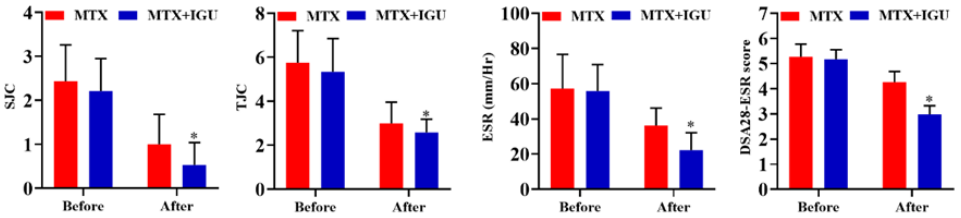

Figure 1. Comparison of clinical data

Table 1. Comparison of clinical data. SJC, TJC, morning stiffness duration (min), ESR (mm/Hr), RF (iu/ml), and DSA28-ESR score in the MTX and MTX+IGU groups before and after treatment. *P<0.05, MTX+IGU group *vs.* MTX group.

	Morning stiffness duration (min)		RF	(iu/ml)
	Before	**After**	**Before**	**After**
MTX	30 (10,105)	7.5 (5,30)	126 (43.68, 333	77(34.25,213.25)
MTX+IGU	30 (6.25,82.5)	10 (2.25,30)	103.4 (42.9,286.25)	53(25.75,107.25)
t	-0.334	-0.352	-0.656	-1.491
p	0.739	0.725	0.512	0.136

Comparison of cytokines: After the treatment, both groups showed decreased levels of IL-1β, IL-6, Il-17 and TNF-α compared to those before the treatment. The MTX+IGU group showed lower levels of IL-1β, IL-6, IL-17, and TNF-α after treatment as compared to the MTX group (Figure 2).

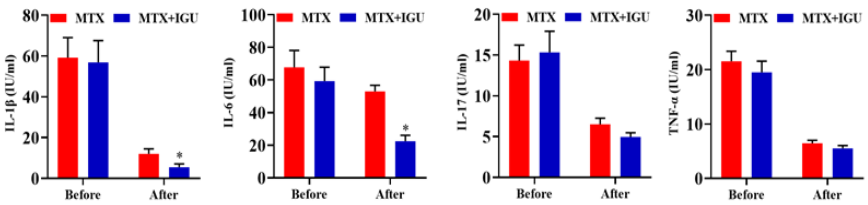

Figure 2. Comparison of cytokines IL-1β, IL-6, IL-17 and TNF concentrations in the MTX and MTX+IGU groups before and after treatment. *P<0.05, MTX+IGU group *vs.* MTX group.

Comparison of ovarian function between the 2 groups after treatment

Comparison of ovarian function between the 2 groups after treatment: There was no statistical difference in ovarian function between the 2 groups before treatment [13,14]. After treatment, the MTX+IGU group showed increased AMH level compared to that before. No statistical difference was observed in AMH level before and after treatment in the MTX group. The MTX+IGU group showed significantly higher level of AMH than the MTX group. However, no statistical differences were observed in FSH, LH, E2, AFC, and OV levels between the two groups either before or after treatment (Figure 3).

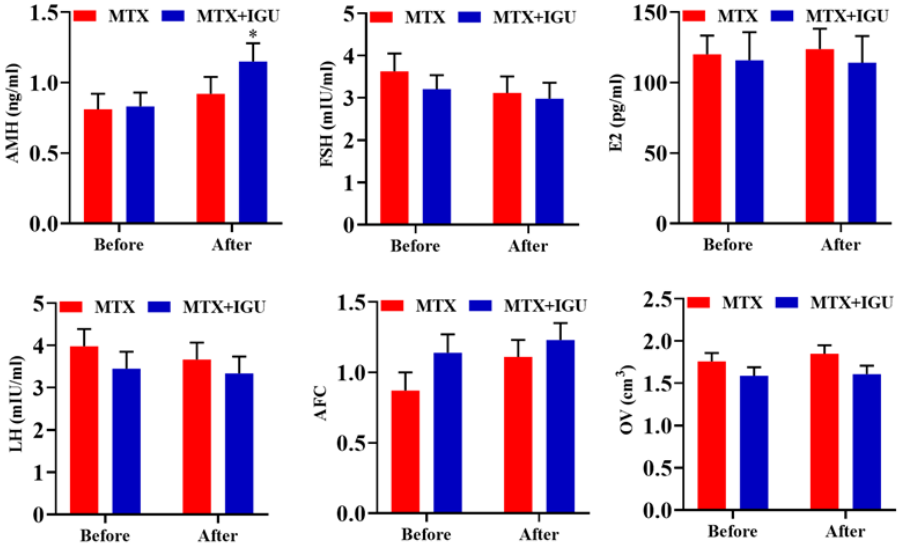

Figure 3. Comparison of ovarian function between the two groups. AMH (ng/ml), FSH (mIU/ml), E2 (pg/ml), LH (mIU/ml), AFC and OV (cm^3) in the MTX and MTX+IGU groups before and after treatment. $^*P<0.05$, MTX+IGU group *vs.* MTX group.

DISCUSSION

IGU is a novel slow acting anti-rheumatic drug, with both anti-inflammatory and immunomodulatory effects [15-18]. It functions by: Inhibiting Nuclear Factor-B (NF-B) activation *via* interfering with the NF-B translocation from the cytoplasm to the nucleus, without affecting the degradation of ikappaBalpa in LPS stimulated THP-1 inhibiting the production of immunoglobulin and multiple inflammatory cytokines in B cells *via* PKC/Egr1/BLIMP1 axis inhibiting cell immunity by up regulating treg cells to induce promoting bone formation, inhibiting bone resorption and preventing cartilage erosion, etc. Meanwhile, IGU is a derivative of nimesulide. It relieves the symptoms of RA patients by restraining the metabolism of prostaglandin E2, the metabolite of arachidonic acid, the release of bradykinin and the production of IL-1 and IL-6. Therefore, it is a new drug with both chronic anti-rheumatic function and NSAID (Nonsteroidal Anti-inflammatory Drugs) function. It was approved for treating RA in China and Japan in 2012 and was recommended as an effective option of intensive treatment for refractory RA in the RA guidelines of the 2014 Asia-Pacific League of Associations for Rheumatology (APLAR) conference. A large number of studies have shown that IGU has few adverse effects for RA when used for treatment. A double blind study which randomized 253 patients to IGU and placebo groups, found a ACR 20 response of 69.5% at week 24 in IGU group (30.7% in placebo group) and significant improvement in ACR 50 and ACR 70 responses, RF, Health Assessment Questionnaire Disability Index (HAQ-DI), and DAS28 scores after treatment, compared to those before. Another retrospective analysis of revealed that IGU has higher responder rate when used as treatment for elder RA patients than Salazosulfapyridine (SASP), with more significant decline in RF, and less adverse reaction. With 36 months of follow-up, the retention rates of IGU group and SASP group were 52.4% and 32.1% respectively, with the responder rates of 85.8% and 65.2% and cumulative rates of any adverse event of 16.7 and 46.7%. The extra treatment of IGU for RA patients without enough response to MTX could enhance the ACR 20 response from 30.7% at week 24 to 72.1% at week 52. The combination therapy of IFU and MTX was considered to be not likely to significantly increase the incidence rate of adverse drug event. A total of 106 RA patients with IGU treatment combined with/without MTX were retrospectively observed and divided into MTX+IGU and IGU groups. Both groups showed significantly reduced DAS28-CRP from baseline, suggesting that IGU treatment may be a useful treatment option for those who cannot be treated with MTX. Kosuke Ebina, et al. added IGU treatment to patients with inadequate responses to Tocilizumab (TCZ), which turned out that 64.5% of them, achieved a moderate response and 51.6% achieved ACR 20

at week 24. It suggested that IGU treatment for patients with inadequate responses to TCZ may be a promising and safe complementary treatment option. In this study, with female moderate to severe active RA patients as study subjects, the combination therapy of MTX and IGU significantly relieved the SJC, TJC, ESR and DSA28-ESR scores at week 24 than MTX alone, with more obvious decrease in IL-6 and TNF-α level of patients, suggesting the significant combined efficacy of MTX and IGU [19,20].

With the focus on the reproductive health of RA patients, female RA patients were found to have decreased fertility and premature ovarian failure performance. Clowse, et al. found that 578 women with RA had 1.5 times more infertility rates than the controls. A nationwide prospective cohort study of RA patients in the Netherlands showed that 42% women with RA have a Time to Pregnancy (TTP) of >12 months, significantly longer than the control group. Ovarian reserve function refers to the quantity and quality of follicles stored in oarium, which is necessary for the normal fertility function of women, and reflects the female fertility within a certain period of time. The decline of ovarian reserve function refers to the reduced ability to produce eggs and the quality of follicles, leading to weakened female fertility and sex hormone deficiency, and eventually developing to premature ovarian failure. AMH, a hormone reflecting the remaining follicle pool and secreted by granulosa cells at reproductive age, is used as a marker of ovarian reserve. Study demonstrated a significant reduction in AMH levels in premenopausal female RA patients, with lower levels in RA patients with positive CCP antibody, consistent with what described by Del Junco, et al. that the RA patients have earlier age of menopause. The reasons of the decreased fertility of female RA patients are considered to be related to RA itself, disease activity, age and drugs, with unknown specific mechanism. Sex hormones are involved in the pathogenesis and development process of RA. Estrogen promotes the onset of RA, whereas progesterone delays the onset of RA. The changes in sex hormone levels during the onset and development of RA affect hypothalamic pituitary ovarian axis through negative feedback, and on the other hand, affect local multifactor regulation system, either of which may impact the ovarian reserve function in RA patients. About 20% of the patients with premature ovarian failure have ever had autoimmune diseases, and some patients have shown positive autoantibodies, including anti-ovary antibody, antinuclear antibodies, RF, anti-cardiolipin antibody, anti-zona pellucida antibody, etc. Earlier studies have found that female RA patients with positive RF and CCP antibodies had an earlier menstrual arrest without cytotoxic drugs, suggesting that autoimmune factors might lead to the exhausted of early follicles. Using a Cox regression analysis, Brouwer, et al. concluded that age, labor absence, disease activity and pre-pregnancy use of NSAIDs and prednisone were independent risk factors for prolonged pregnancy in female RA patients. In this study, patients with moderate to severe active RA were studied, with higher AMH levels in both groups after treatment than those before, suggesting that disease activity is one of the reasons of decreased ovarian reserve function in RA patients.

Anti-rheumatic drugs are the important causes of the impaired female fertility, especially the impaired ovarian reserve function. A large number of studies showed that SLE patients treated with CTX have significantly lower AMH levels compared with SLE patients without CTX, and that the AMH value is related to age and the accumulation of CTX use. The cumulative dose of tripterygium glycosides also increases the probability of ovarian function impairment. By establishing a mouse animal model, Ma, et al. further confirmed that tripterygium glycosides damage the gonads by inducing a series of biochemical alterations in oxidative stress. In conclusion, CTX and tripterygium glycosides have confirmed damage on ovarian function. MTX is the anchor drug in RA therapy, and it is currently believed that MTX therapy for RA has little side effect on the female reproductive system. Brouwer J, et al. found no significant difference in AMH levels between RA patients treated with MTX at week 6 and those without. Brouwer also considered no correlation between previous MTX use and prolonged pregnancy of female RA patients. Reproductive physician found no difference in FSH or AFC before and after MTX treatment for eccyesis (both within and after 90 days of treatment) by comparing the ovarian reserve and ovarian reactivity during the subsequent IVF cycle. Although larger doses of gonadotropin were used in the cycles following MTX, there were no differences in the number of retrieved oocytes or the high quality embryos transplanted, arguing that MTX would not affect ovarian reserve, ovarian responsiveness, or IVF success in subsequent cycles.

CONCLUSION

There are no papers that report the effect of MTX treatment combined with IGU on the female reproductive system. This study is the first to find that the combination therapy of MTX and IGU for female patients with moderate to severe active RA does not increase the negative impact on ovarian function, compared to MTX alone. On the contrary, AMH levels in patients are significantly increased after treatment compared with before, which may be related to better disease activity control. In conclusion, the combination therapy of MTX and IGU has significantly better efficacy for female patients with moderate to severe active RA than MTX alone, with less impact on female ovarian function and even improves ovarian reserve function in female patients through more effective control of RA activity.

FINDING STATEMENT

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CONFLICTS of INTEREST

All authors confirm that there are no conflicts of interest in this study.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author under reasonable requests.

REFERENCES

1. Aikawa, Y., et al. An anti-rheumatic agent T-614 inhibits NF-kappa B activation in LPS- and TNF alpha stimulated THP-1 cells without interfering with IkappaBalpha degradation. *Inflamm Res*, **2002**.51:p. 188-194.
2. Boots, C.E., et al. Does methotrexate administration for ectopic pregnancy after *in vitro* fertilization impact ovarian reserve or ovarian responsiveness? *Fertil Steril*, **2013**.100:p. 1590-1593.
3. Brouwer, J., et al. Reduced ovarian functions in female rheumatoid arthritis patients trying to conceive. ACR *Open Rheumatol*, **2019**.1:p. 327-335.
4. Brouwer, J., et al. Fertility in women with rheumatoid arthritis: Influence of disease activity and medication. *Ann Rheum Dis*, **2015**.74:p. 1836-1841.
5. Brouwer, J., et al. Levels of serum anti-Mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis. *Arthritis Care Res*, **2013**.65:p. 1534-1538.
6. Clowse, M.E., et al. Effects of infertility, pregnancy loss, and patient concerns on family size of women with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Care Res*, **2012**.64:p. 668-674.
7. Del Junco, D.J., et al. The relationship between rheumatoid arthritis and reproductive function. *Br J Rheumatol,* **1989**.28(1):p. 33, 42-35.
8. Ebina, K., et al. The add on effectiveness and safety of iguratimod in patients with rheumatoid arthritis who showed an inadequate response to tocilizumab. *Mod Rheumatol*, **2019**.29:p. 581-588.
9. Fraenkel, L., et al. 2021 American college of rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol*, **2021**.73:p. 1108-1123.
10. Gan, K., et al. Iguratimod (T-614) suppresses RANKL induced osteoclast differentiation and migration in RAW264.7 cells *via* NF-κB and MAPK pathways. *Int Immunopharmacol*, **2016**.35: p. 294-300.
11. Hara, M., et al. Efficacy and safety of iguratimod compared with placebo and salazosulfapyridine in active rheumatoid arthritis: A controlled, multicenter, double blind, parallel group study. *Mod Rheumatol*, **2017**.17:p. 1-9.
12. Hara, M., et al. Safety and efficacy of combination therapy of iguratimod with methotrexate for patients with active rheumatoid arthritis with an inadequate response to methotrexate: An open label extension of a randomized, double blind, placebo controlled trial. *Mod Rheumatol*, **2014**.24:p. 410-418.
13. Henes, M., et al. Ovarian reserve alterations in premenopausal women with chronic inflammatory rheumatic diseases: Impact of rheumatoid arthritis, Behcet's disease and spondyloarthritis on anti-mullerian hormone levels. *Rheumatol*, **2015**.54:p. 1709-1712.
14. Hughes, G.C. et al. Modulation of autoimmune rheumatic diseases by oestrogen and progesterone. *Nat Rev Rheumatol,* **2014**.10:p. 740-751.
15. Inoue, A., et al. The effectiveness and retention rate of Iguratimod in Japanese rheumatoid arthritis patients with/without methotrexate in daily medical care. *Life (Basel),* **2020**. P. 10.

16. Ishiguro, N., et al. Concomitant iguratimod therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate: A randomized, double blind, placebo controlled trial. *Mod Rheumatol*, **2013**.23:p. 430-439.

17. Ma, M., et al. Biochemical changes of oxidative stress in premature ovarian insufficiency induced by tripterygium glycosides. *Int J Clin Exp Pathol*, **2014**.7:p. 8855-8861.

18. Manger, K., et al. Prevention of gonadal toxicity and preservation of gonadal function and fertility in young women with systemic lupus erythematosus treated by cyclophosphamide: The PREGO study. *Autoimmun Rev*, **2006**.5:p. 269-272.

19. Mok, C.C., et al. Anti-mullerian hormone and ovarian reserve in systemic lupus erythematosus. *Arthritis Rheum*, **2013**.65:p. 206-210.

20. Nozaki, Y. Iguratimod: Novel molecular insights and a new csDMARD for rheumatoid arthritis, from Japan to the world. *Life (Basel),* **2021**. p. 11.