RESEARCH

Methotrexate plus reduced or full-dose glucocorticoids for the treatment of active, moderate-to-severe Graves' orbitopathy

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Abstract

Objective: A combination of glucocorticoids with mycophenolate is recommended by current guidelines to boost response to Graves' orbitopathy (GO) therapy. This study was designed to evaluate the therapeutic effects and safety of methotrexate (MTX) plus reduced (3.0 g) or full-dose (4.5 g) i.v. methylprednisolone (MP) vs full-dose i.v. MP alone.
Design and methods: This was a prospective, randomized, observer-masked, single-center clinical trial conducted in a tertiary clinical center. Ninety-seven patients with active moderate-to-severe GO were screened and 90 patients underwent randomization between April 2018 and Oct 2019. All patients completed 12 weeks of treatment and received clinical assessment. The patients received either MP 4.5 g only, MP 4.5 g plus oral MTX, or MP 3.0 g plus oral MTX. The primary outcome was the CAS response at week 12. Secondary outcomes were adverse events and other individual ophthalmic parameters.
Results: At week 12, 53.3% of MP, 76.7% of reduced MP plus MTX, and 76.7% of MP plus MTX achieved a CAS response, although the difference was not significant ($P = 0.1$). The overall response rates of the MP group, the reduced MP plus MTX group, and the MP plus MTX group were 43.3%, 53.3%, and 60%, respectively ($P = 0.5$). Subgroup analysis found that smoking status interacted with marginal significance with treatment effect ($P = 0.048$). Importantly, adverse event incidence was significantly lower in the reduced MP + MTX group ($P = 0.017$).
Conclusions: Our study shows that reduced MP plus MTX therapy is effective and safer in treating active and moderate-to-severe GO patients than 4.5 g MP monotherapy.

Introduction

Graves' orbitopathy (GO) is the most common orbital disease in adults, which greatly affects patients' quality of life (1, 2, 3). Although bioagents such as teprotumumab showed a promising new paradigm in GO treatment (4, 5, 6), guidelines recommended that a 4.5 g weekly pulse i.v. methylprednisolone (ivMP) remains to be the more accessible and cost-effective choice for active and moderate-to-severe GO patients currently (7). In previous studies, the response rate of MP monotherapy was around 30–80% (8, 9, 10). Though efficacy increased with dosage, MP dosage should not exceed 8 g for the safety concerns (7, 8). The increased dose is accompanied by increased side effects including hypertension, infection, diabetes, osteoporosis, liver dysfunction, or electrolyte imbalance. Therefore, adding a second drug to reduce the dose of glucocorticoids is justified.

Methotrexate (MTX) has long been used as a steroid-sparing agent that elicits anti-inflammatory and immunomodulatory effects with chronic low-dose treatment in many autoimmune diseases. A study used MTX alone as second-line therapy in GO patients who failed to respond to MP and found that 29% achieved a VISA (vision loss, inflammation, strabismus, appearance) inflammatory score <3 within a mean of 3 months of treatment, indicating its direct anti-inflammatory effect in treating GO (11). A retrospective study reported a step-down approach using MTX as a steroid-sparing agent in combination with MP and achieved reduced MP total dosage to inactivate GO within an average of 13 months (12, 13, 14). MTX could upregulate GRα (glucocorticoid receptor α) expression (15), the active subtype of glucocorticoid receptor, which may theoretically enhance glucocorticoid effect. However, evidence from the prospective study has been lacking.

In this study, we conducted a 12-week prospective, randomized, observer-masked, single-center clinical trial to compare the combination therapy of reduced or 4.5 g MP plus MTX vs 4.5 g MP monotherapy in treatment efficacy of clinical activity score (CAS) response and analyze the safety profiles.

Methods

Trial design

This was a prospective, randomized, observer-masked, single-center clinical trial. The patients were consecutively enrolled between April 2018 and October 2019. Patients aged 18–65 years diagnosed as active moderate-to-severe GO in our clinical center were included in this study. The diagnosis was based on the EUGOGO consensus. Patients should not have received any immunosuppressive therapy or radiotherapy or decompression surgery in the previous 3 months. Patients with abnormal heart, liver, and kidney function, other known autoimmune diseases, or with malignancy were excluded from the study. The board of medical ethics of Ruijin Hospital approved the study, and all patients gave their written informed consent. This trial is registered with the Chinese Clinical Trial Registry, ChiCTR1800015912.

Patient randomization was generated in random block size with an allocation ratio of 1:1:1 for three groups: MP group (group 1), reduced MP plus MTX group (group 2), and MP plus MTX group (group 3).

MP was given as ivMP 0.5 g per week for 6 weeks followed by ivMP 0.25 g for 6 weeks. Reduced MP was given as ivMP 0.25 g per week for 12 weeks. MTX was given orally on the next day following ivMP as 10 mg per week for the first 2 weeks and then added to 12.5 mg per week for 10 weeks. Folic acid 5 mg was given orally once a week following the next day of MTX for 12 weeks (Fig. 1). The observer ophthalmologist (QJ) was blinded to the treatment groups. Patients should not inform the ophthalmologist about the medication they received.

Outcome evaluation

Primary outcome was the CAS response at the end of therapy (12th week). Secondary outcomes were the adverse events (AEs) and other individual ophthalmic parameters. We also examined the overall response at week 12 as a *post hoc* outcome.

All patients underwent ophthalmic, endocrine, and safety assessments at baseline, 6th week, and 12th week. Ophthalmic examinations were performed by a single ophthalmologist (QJ) who was blinded to the treatment. CAS, proptosis, eyelid width, Gorman diplopia score, intraocular pressure (IOP), and visual acuity were recorded by using the modified EUGOGO patient form. A Hertel exophthalmometer was used for the measurement of proptosis, and the upper limit of normal Chinese in our study was 18.6 mm. Active was defined when ≥3 of the 7-point CAS (spontaneous retrobulbar pain, pain on attempted eye movements, conjunctival hyperemia, eyelid redness, chemosis, swelling of the caruncle, and swelling of the eyelids) features were present. Improvement was defined as CAS decreased by at least 2 points, proptosis reduction by at
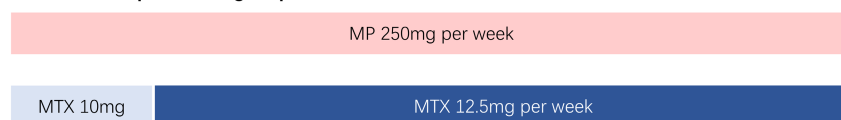
A

MP group

MP plus MTX group

Reduced MP plus MTX group

B

For each week

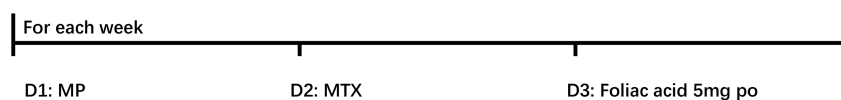D1: MP   D2: MTX   D3: Foliac acid 5mg po
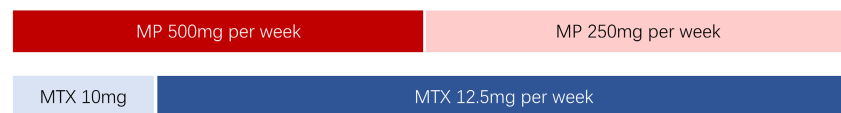
Figure 1
Study design. (A) showed three treatment protocols by week. Efficacy and safety profiles were assessed at baseline, week 6, and week 12. (B) showed combination treatment protocol by days of each week.

least 2 mm, reduction of lid width by at least 2 mm, degrade of Gorman score (from constant to inconstant, inconstant to intermittent, and intermittent to absent), degradation in any of the class 2 signs of NOSPECS by at least 2 grades, IOP reduced by at least 2 mmHg, and visual acuity improved by 1 Snellen line. Exacerbation was defined as increase by the same extent in the above parameters. Responsiveness was defined as improvement in at least one eye without exacerbation in the other eye. Deterioration was defined as exacerbation in at least one eye without improvement in the other eye. Conditions that fit in neither responsiveness nor deterioration were defined as unchanged. The overall response was defined as being responsive in at least two of the four ophthalmic parameters including CAS, proptosis, lid width, and Gorman score.

Whole blood count, body weight, blood pressure (BP), blood glucose, blood lipid, liver function, electrolytes, urea, urinalysis, and bone mineral density (BMD) were assessed to monitor treatment-associated agranulocytosis, weight gain, bone loss or osteoporosis, hypertension, diabetes, hyperlipidemia, impaired liver function, hypokalemia, hyperuricemia, and urinary tract infection. Any self-reported medical events were also counted. Common Terminology Criteria for Adverse Events (CTCAE) ver.5.0 was used to grade AE severity. Dual-energy X-ray absorptiometry (GE-Lunar Prodigy; GE Healthcare) was used to measure BMD (grams per square centimeter) in the lumbar spine, femoral neck, and hip of all subjects at baseline and week 12. AE that occurred in weeks 6 and 12 are evaluated below as follows: (i) body weight increased by more than 2.5%, 5% of baseline in the 12th week compared to baseline; since 5% increase was grade 1 in CTCAE, only 5% increase was counted into total AE; (ii) development of the following: diabetes mellitus is defined as fasting glucose ≥ 7 mmol/L or 2-h glucose ≥ 11.1 mmol/L after oral glucose tolerance test or glycated hemoglobin (HbA1C) $\geq 6.5\%$, with baseline fasting glucose <5.6 mmol/L and 2-h glucose <7.8 mmol/L and HbA1C <5.7%; (iii) development of hypertension is defined as systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg with normal BP at baseline; (iv) hypokalemia is defined as serum potassium < 3.5 mmol/L with normal potassium at baseline; (v) hyperuricemia is defined as serum uric acid >420 µmol/L (for man) or >360 µmol/L (for woman) with normal urea at baseline; (vi) liver function impairment is defined as alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than upper normal limits with normal ALT and AST at baseline; (vii) development of

hyperlipidemia as evaluated according to CTCAE criteria; and (viii) neutropenia is defined as neutrophil count <2 × 10^9/L and severity was graded by CTCAE criteria. BMD was assessed at baseline and week 12. BMD of L1–4, femoral neck, and total hip is represented by T score. Osteoporosis is defined as either one of the three T scores ≤−2.5. Bone loss is defined as –2.5 < T < −1.0 according to the World Health Organization criteria (1994).

The study prespecified outcomes did not involve quality of life (QoL), but most of the participants completed QoL assessment at outpatient follow-up. EUGOGO quality of life questionnaires (GO-QoL) were filled out by patients at baseline and week 12, which consists of two subscales: visual function and appearance (16). The questions were answered on a 3-point scale (1 = severely limited; 2 = a little limited; 3 = not limited at all). The points given to questions 1–8 and 9–16 was summed to yield 2 raw scores ranging from 8 to 24 points. The 2 raw scores are then transformed into 2 total scores from 0 to 100 by the formula: (total scores – #)/(2 × #) × 100, where # represents the number of completed items. For both scores, 0 indicated the worst possible health state and 100 indicated the best possible health state. QoL improvement was defined as increase by 6 points or more (17, 18).

Statistical methods

The sample size was calculated based on previous studies, assuming that add-on MTX could achieve 91% CAS response, compared with 60% for MP monotherapy (10, 11). The sample size of 29 patients per group was estimated to provide the trial with 80% power to detect such difference at a two-sided alpha level of 0.05.

Continuous variables are expressed as the mean ± s.D. or median and quartiles; categorical variables are expressed as frequency and percentage. Values of serum free tri-iodothyronine, free thyroxine, thyroid-stimulating hormone (TSH), TSH receptor autoantibodies (TRAb), thyroid peroxidase antibody, and thyroglobulin antibody are shown as median and quartiles of the original values and log10 normal transformed before statistical analysis. For each patient, the worst value of the two eyes was presented for each ophthalmological parameter. Student's *t*-test was used to compare continuous variables, and a Mann–Whitney *U*-test was used for comparing CAS. χ^2 test or Fisher's exact test was used for comparing proportions. *Post hoc* subgroup analyses were tested for interaction in logistic model of CAS response at week 12. Subgroup specific odds ratios and 95% CIs are presented as a forest plot. QoL was analyzed *post hoc* by both intention-to-treat

analysis, which analyzed the full data set, and per-protocol analysis, which analyzed patients with QoL questionnaires. *P*-values were two-sided and the significance level was set at 0.05. All analyses were performed with Statistical Analysis System (SAS) software for Windows, version 9.4 (SAS Institute Inc) and Prism 9 for Windows, version 9.0 (GraphPad Software Inc).

Results

Patient characteristics

A total of 97 consecutive patients were screened and 90 patients underwent randomization (Fig. 2). Each group was assigned 30 patients. Baseline clinical characteristics were not significantly different among the three groups (Table 1). Patients were mostly female (66.7–76.7%) with a median CAS of 4–4.5 and a median disease duration of 6–7.5 months. There were 26 patients who received glucocorticoids (i.v., oral, and orbital) at least 3 months prior to treatment and 1 patient who received strabismus surgery 7 months prior to the study in group 1 (4.5 g MP). No patient underwent orbital radiotherapy prior to the study. All patients completed 12 weeks of treatment and received clinical assessment at week 6 and week 12.

Ophthalmic assessment and outcome evaluation

CAS score decreased significantly in all three groups after treatment (Fig. 3A). There was no significant difference between groups at week 6 or 12. In group 1, the peak CAS response rate was 56.7% at week 6 and decreased to 53.3% at week 12 (Fig. 3B and Table 2). While in groups with MTX, CAS response rate continuously increased and reached 76.7% at week 12. At the 12th week, numerically more patients achieved CAS response in MTX groups (Fig. 3B), although the difference was not significant. The inactive (CAS <3) rates after treatment at week 12 were 63.3% in MP monotherapy, 66.7% in reduced MP plus MTX, and 70% in MP plus MTX (Supplementary Table 1, see section on supplementary materials given at the end of this article).

To further clarify whether stratification factors affect treatment efficacy, subgroup analysis of CAS response at week 12 was performed *post hoc* according to variables such as age, gender, duration, smoking status, baseline CAS, and TRAb level. Patients who were male, duration > 6 months, or smoking achieved a higher CAS response rate after reduced MP plus MTX treatment. Interaction analysis found that only smoking status was significant (OR: 9.64 (1.64,56.9), *P* = 0.048) between group 1 and
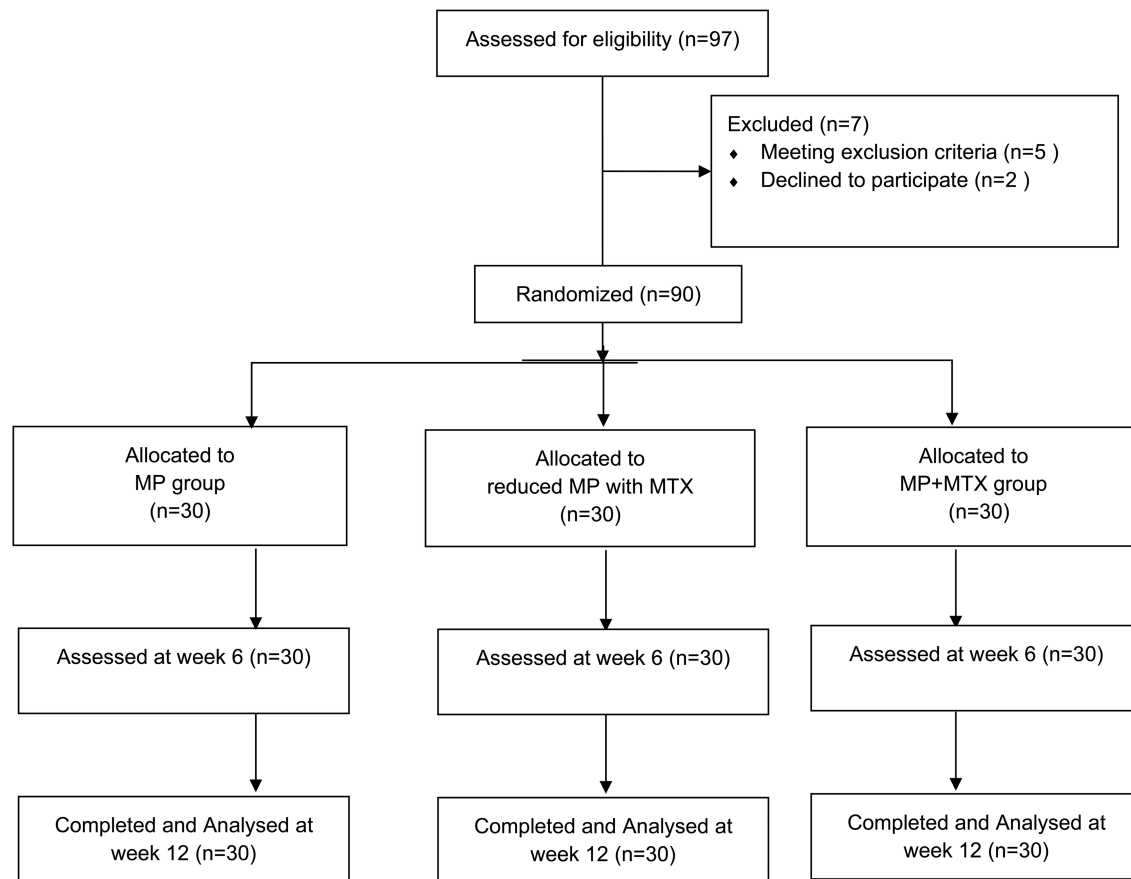
Figure 2
Flow chart of the study.

group 2. Smoking may interact with the treatment regimen to affect CAS response. Patients who had smoking exposure might be more likely to achieve CAS response with reduced MP plus MTX treatment. While comparing group 1 with group 3, no subgroup difference was found.

The response rate of other individual ophthalmic parameters did not show significant differences among groups (Table 2). Numerically group 3 achieved the highest overall response rate of 60%, followed by group 2 (53.3%) and group 1 (43.3%) (Table 2, $P = 0.5$).

Because CAS response in the MP group plateaued at week 6, we compared the TRAb difference among groups. TRAb was significantly suppressed in all three groups both at week 6 and week 12, while no difference was found among the three groups in regards to the suppression rate and differences, although groups with MTX had a slightly larger difference (Supplementary Fig. 1A and B).

QoL scores were analyzed *post hoc*. There were 22/30 patients, 21/30 patients, and 19/30 patients who fulfilled the QoL evaluation at baseline and week 12. QoL scores significantly increased in both visual function and

appearance after treatment in all three groups: 73–84% patients improved on the visual function scale, and 86–91% improved on the appearance scale. No significant difference was found among groups (Table 2).

There were 26/30 (86.7%) in group 1, 22/30 (73.3%) in group 2, and 23/30 (76.7%) in group 3 who visited the same ophthalmologist at week 24. One non-responsive patient from the MP+MTX group developed dysthyroid optic neuropathy (DON) at week 12 and received decompression surgery 2 weeks later. This patient initially improved at week 6 with increased visual acuity and degraded diplopia while DON occurred by week 12. CAS was not decreased throughout treatment. Relapse occurred in 1 responder (1/23, including 6 responders who lost follow-up) in the MP+MTX group at week 24. At week 36, further relapses occurred in 1 patient (1/16, including 3 patients who lost follow-up) in the MP group and 2 patients (2/23) in the MP+MTX group. Of the 20 CAS non-responders with CAS ≥3 at week 12, 10were from the MP group, 5 from reduced MP+MTX, and 5 from MP+MTX (including 1 lost follow-up). Among them, 3 patients in the MP group

Table 1 Baseline clinical characteristics.

|	MP (G1)	Reduced MP + MTX (G2)	MP + MTX (G3)	*P*-value (overall)/ G1 vs G2/G1 vs G3
N	30	30	30	
Age (year)	46.5 (35–52)	46 (37–53)	46.5 (39–53)	0.9/0.7.0.7
Gender (female %)	21 (70%)	23 (76.7%)	20 (66.7%)	0.8/0.8/1
Weight (kg)	62.5 ± 9.8	62.3 ± 9.8	65.6 ± 11.0	0.4/0.9/1
BMI	23.5 ± 3.1	23.6 ± 3.2	23.9 ± 2.5	0.8/0.9/0.3
Systolic blood pressure (mm Hg)	123.8 ± 17.6	126.5 ± 17.6	128.7 ± 18.2	0.6/0.6/0.3
Diastolic blood pressure (mm Hg)	72.9 ± 13.5	72.9 ± 8.5	76.9 ± 9.7	0.3/1/0.2
Hypertension (*n*, %)	6 (20%)	7 (23.3%)	8 (26.7%)	1/1/0.8
Diabetes (*n*, %)	0	1 (3.3%)	2 (6.7%)	0.8/1/0.5
Hyperlipidemia (*n*, %)	9 (30%)	8 (26.7%)	9 (30%)	1/1/1
Smoking history (*n*, %)				0.7/0.8/0.8
Current smoker	0	2 (6.7%)	0	
Passive smoker	13 (43.3%)	13 (43.3%)	10 (33.3%)	
Ex-smoker	3 (10%)	2 (6.7%)	3 (10%)	
Never-smoker	14 (46.7%)	13 (43.3%)	17 (56.7%)	
History of thyroid disease				
Graves' hyperthyroidism	29 (96.7%)	30 (100%)	30 (100%)	1/1/1
Primary hypothyroidism	0	0	0	-
TPOAb or TGAb positive	14 (46.7%)	21 (70%)	15 (50%)	0.2/0.1/1
Previous antithyroid treatments				
Anti-thyroid drugs	27 (90%)	28 (93.3%)	27 (90%)	1/1/1
Radioiodine	6 (20%)	8 (26.7%)	5 (16.7%)	0.7/0.8/1
Thyroidectomy	1 (3.3%)	0	1 (3.3%)	1/1/1
Current thyroid treatments				
None	2 (6.7%)	2 (6.7%)	2 (6.7%)	1/1/1
Levothyroxine only	6 (20%)	5 (16.7%)	2 (6.7%)	0.4/1/0.3
Tapzole only	5 (16.7%)	10 (33.3%)	7 (23.3%)	0.4/0.2/0.8
PTU only	1 (3.3%)	0	1 (3.3%)	1/1/1
Levothyroxine and tapzole	12 (40%)	12 (40%)	14 (46.7%)	0.9/1/0.8
Levothyroxine and PTU	4 (13.3%)	1 (3.3%)	4 (13.3%)	0.4/0.4/1
Euthyroid (*n*, %)	23 (76.7%)	25 (83.3%)	23 (76.7%)	0.9/0.8/1
Duration of eye symptoms (months)	6 (4–11)	7.5 (5–11)	6.5 (5–12)	0.3/0.2/0.7
Previous GO treatment (*n*, %)	9 (30%)	10 (33.3%)	8 (26.7%)	0.9/1/1
sTSH	0.3 (0.0–1.3)	0.1 (0.0–0.7)	0.3 (0.0–1.4)	0.7/0.4/0.6
FT3	4.7 (4.0–5.3)	4.5 (4.0–5.1)	4.4 (4.0–5.1)	1/0.9/0.8
FT4	12.6 (10.6–13.8)	12.6 (11.37–15.08)	12.8 (11.8–14.1)	0.1/0.7/0.2
TRAb	6.2 (2.2–20.5)	9.6 (3.23–21.92)	10.1 (3.2–26.8)	0.6/0.4/0.4
TPOAb	2.9 (0.4–363.6)	21.6 (1.13–162.45)	7.7 (0.6–322.4)	0.9/0.6/0.8
Lid erythema (*n*, %)	3 (10%)	3 (10%)	2 (6.7%)	1/1/1
Chemosis (*n*, %)	22 (73.3%)	21 (70%)	26 (86.7%)	0.3/1/0.3
Caruncle swelling (*n*, %)	20 (66.7%)	17 (56.7%)	18 (60%)	0.8/0.6/0.8
CAS	4.5 (3-5)	4 (3-5)	4 (4-5)	0.6/0.3/0.6

The worst value of the two eyes was presented for each ophthalmological parameter.

(3/10, 33.3%), and 2 patients in each of the 2 MTX groups (2/5, 40%) became CAS responders at week 24. Seven patients underwent second therapy in 6 months: 1 from group 1, 1 from group 2, and 4 from group 3.

Adverse event

All AEs were mild to moderate, and no patient discontinued treatment (Table 3). There were significantly fewer AEs in reduced MP plus MTX group (21 in group 2, 41 in group 1, 38 in group 3, *P*=0.017, Table 3).

The most prevalent AE was new-onset dyslipidemia, which was more frequently reported in group 1 and group 3 (50%) but with no significant difference among groups (Table 3). Compared to standard MP monotherapy, group 2 had significantly fewer new-onset hypokalemia at week 6 (*P*=0.02) and less weight gain at week 12 (*P*=0.04). New-onset hypertension occurred more frequently in group 1 compared to group 3 both at week 6 and week 12 (at week 6: *P*=0.047; at week 12: *P*=0.02). MTX-related AEs such as neutropenia were noted in 1 patient in group 2 (grade 2) and 3 patients in group 3 (grade 1).
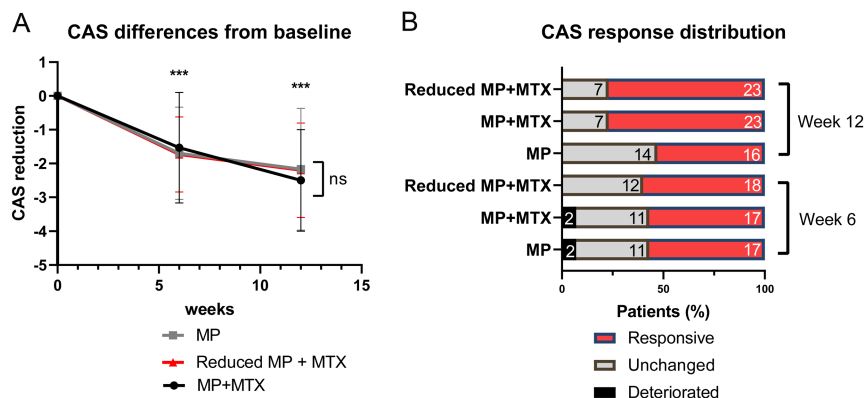
Figure 3
(A) showed CAS reduction compared to baseline. ****$P < 0.0001$. (B) showed CAS response distribution percentage after treatment in three groups. Numbers in the bar represent the number of patients.

Discussion

In this study, we provided the first randomized controlled trial (RCT) evidence to demonstrate that reduced MP (3.0 g) plus MTX therapy achieved a similar CAS response with fewer steroid-relevant AEs, compared to the standard 4.5 g MP regimen in active moderate-to-severe GO patients.

The combination of glucocorticoids with steroid-sparing agents has been explored in previous studies to boost response to GO therapy. Successful therapies such as the combination of ivMP and mycophenolate (19), the addition of azathioprine to oral prednisolone (20, 21), or the combination of oral glucocorticoids and cyclosporine (22, 23) all showed favorable response compared to monotherapy and are recommended by the current guideline (7), while the dose of steroid in those studies was not reduced. In a retrospective study, MTX was used concomitantly with ivMP in 23 patients for an average duration of 13 months and led to a reduced cumulative dose of ivMP (mean: 2.72 g) to inactive the disease, indicating that MTX could lower the dose of MP; however, the treatment took 13 months in average, and the study lacks ivMP monotherapy as control (12). This is the first prospective RCT study comparing reduced MP plus steroid-sparing agent to MP monotherapy with matched 12-week time course. Importantly, we found that reduced MP (3.0 g) plus MTX therapy achieved a similar CAS response as standard MP monotherapy.

The clinical course of GO was characterized by a self-limiting inflammatory stage accompanied by gradually prevailing fibrotic reconstruction. Therefore, treatment should be able to rapidly inactivate the inflammation to prevent reconstructive progression. When used in other noninfectious ocular diseases, the median time for MTX to achieve control of inflammation with corticosteroids tapered to 10 mg or less daily, ranging from 4.5 months to 9 months (24, 25). In this study, we investigated the MTX's steroid-sparing effect in a 12-week short period to match with the guideline-recommended 4.5 g MP therapy. Compared to 4.5 g MP therapy, responders to combined treatment continued to increase after week 6, indicating MTX's add-on efficacy in short-term treatment. Based on the current result and previous studies on combination therapy, extended use of MTX to 24 weeks might be beneficial to lift the response rate and prevent relapse at week 24.

MTX used in this study was 12.5 mg/week, which is the typical dose in the range of low-dose MTX (26, 27, 28). Low-dose MTX treatment inhibits purine synthesis in primary human T lymphocytes from rheumatoid arthritis (RA) patients, halting the proliferation of mitogen-stimulated T cells (29). MTX also leads to the increase of intracellular adenosine level, which is a potent anti-inflammatory mediator acting via interactions with a variety of immune-cell subtypes including neutrophils, macrophages, and T cells (30), resulting in a reduction of pro-inflammatory cytokines interleukin (IL)-1β, TNF-α, and IL-6 and reduced matrix metalloproteinases production (31). The effect of MTX on RA synovial fibroblasts/T cells cross-talk signals was suggested to be mediated by adenosine release and decreased cell adhesion (26, 32). In our study, we found that TRAb was suppressed in all three groups during treatment, while both the suppression rate and the differences between baseline and 12th week were slightly higher in MTX groups (Supplementary Fig. 1A and B).

Interestingly, our findings suggest that reduced MP plus MTX is a well-tolerated treatment option as this group showed a significantly lower AE incidence rate. AEs were mild to moderate and mostly steroid-related, such as new-onset dyslipidemia, new-onset hypokalemia, and weight gain were mostly seen in 4.5 g MP monotherapy, which could be explained by the larger dose of steroid used. We noted that new-onset dyslipidemia was the most common AE related to treatment in our study.

Table 2 Treatment responses.

Parameters	MP (G1) Baseline	Post-treatment	P-value	Reduced MP + MTX (G2) Baseline	Post-treatment	P-value	MP + MTX (G3) Baseline	Post-treatment	P-value	P-value overall/ G1 vs G2/ G1 vs G3
CAS score	4.5 (3–5)	2 (1–4)	<0.0001	4 (3–5)	2 (1–3)	<0.0001	4 (4–5)	2 (1–3)	<0.0001	0.1/0.1/0.1
Responsive		16 (53.3%)			23 (76.7%)			23 (76.7%)		
Unchanged		14 (46.7%)			7 (23.3%)			7 (23.3%)		
Deteriorated		0			0			0		
Lid edema			0.005			0.283			0.5	
None	0	1 (3.3%)		1 (3.3%)	0		1 (3.3%)	0		
Mild	11 (36.7%)	22 (73.3%)		16 (53.3%)	22 (73.3%)		14 (46.7%)	19 (63.3%)		
Moderate	17 (56.7%)	7 (23.3%)		12 (40%)	7 (23.3%)		13 (43.3%)	10 (30%)		
Severe	2 (6.7%)	0		1 (3.3%)	1 (3.3%)		2 (6.7%)	1 (3.3%)		
Responsive		2 (6.7%)			0			1 (3.3%)		0.8/0.5/1
Unchanged		28 (93.3%)			30 (100%)			29 (96.7%)		
Deteriorated		0			0			0		
Conjunctival hyperemia			0.05			0.0005			0.01	
None	0	4 (13.3%)		2 (6.7%)	11 (36.7%)		1 (3.3%)	7 (23.3%)		
Suspicious	0	1 (3.3%)		0	4 (13.3%)		1 (3.3%)	5 (16.7%)		
Hyperemia	30 (100%)	25 (83.3%)		28 (93.3%)	15 (50%)		28 (93.3%)	18 (60%)		
Responsive		5 (16.7%)			10 (33.3%)			7 (23.3%)		0.4/0.2/0.7
Unchanged		25 (83.3%)			20 (66.7%)			23 (76.7%)		
Deteriorated		0			1 (3.3%)			2 (6.7%)		
Proptosis (mm)	22.3 ± 2.3	21.4 ± 2.4	0.027	21.6 ± 2.1	20.8 ± 2.5	0.007	22.0 ± 2.7	21.6 ± 2.8	0.1	
Responsive		10 (33.3%)			10 (33.3%)			9 (30%)		0.9/1/0.9
Unchanged		16 (53.3%)			17 (56.7%)			15 (50%)		
Deteriorated		4 (13.3%)			3 (10%)			6 (20%)		
Lid width (mm)	11.8 ± 2.0	11.7 ± 2.3	0.7	12.0 ± 1.9	12.4 ± 1.6	0.1	11.6 ± 1.7	11.8 ± 1.9	0.8	
Responsive		7 (23.3%)			7 (23.3%)			7 (23.3%)		0.9/0.9/1
Unchanged		17 (56.7%)			15 (50%)			18 (60%)		
Deteriorated		6 (20%)			8 (26.7%)			5 (16.7%)		
Diplopia(Gorman)			0.2			0.9			0.9	
Absent	9 (30%)	15 (50%)		18 (60%)	20 (66.7%)		12 (40%)	14 (46.7%)		
Intermittent	3 (10%)	6 (20%)		4 (13.3%)	5 (16.7%)		5 (16.7%)	6 (20 %)		
Inconstant	7 (23.3%)	4 (13.3%)		3 (10%)	2 (6.7%)		3 (10%)	3 (10%)		
Constant	11 (36.7%)	5 (16.7%)		5 (16.7%)	3 (10%)		10 (33.3%)	7 (23.3%)		
With diplopia at baseline										
Responsive		12 (57.1%)			6 (50%)			8 (44.4%)		0.4/0.2/0.6
Unchanged		9 (42.9%)			4 (33.3%)			9 (50%)		
Deteriorated		0			2 (16.7%)			1 (5.6%)		
Without diplopia at baseline										
Responsive		0			0			0		0.3/0.5/0.2
Unchanged		9 (100%)			16 (88.9%)			9 (75%)		
Deteriorated		0			2 (11.1%)			3 (25%)		
Intraocular pressure (mmHg)	20.6 ± 4.6	19.8 ± 4.5	0.2	20.6 ± 4.5	20.1 ± 4.9	0.5	19.5 ± 3.4	19.9 ± 5.1	0.7	0.05/1/0.05
Responsive		14 (46.7%)			14 (46.7%)			10 (33.3%)		
Unchanged		7 (23.3%)			6 (20%)			16 (53.3%)		

(Continued)

Table 2 Continued.

Parameters	MP (G1) Baseline	MP (G1) Post-treatment	P-value	Reduced MP + MTX (G2) Baseline	Reduced MP + MTX (G2) Post-treatment	P-value	MP + MTX (G3) Baseline	MP + MTX (G3) Post-treatment	P-value	P-value overall/ G1 vs G2/ G1 vs G3
Deteriorated		9 (30%)			10 (33.3%)			4 (13.3%)		
Visual acuity	0.8 ± 0.4	0.9 ± 0.3	0.2	0.9 ± 0.3	1.0 ± 0.3	0.3	0.8 ± 0.4	0.9 ± 0.3	0.07	0.9/1/0.8
Responsive		15 (50%)			15 (50%)			12 (40%)		
Unchanged		7 (23.3%)			7 (23.3%)			9 (30%)		
Deteriorated		8 (26.7%)			8 (26.7%)			9 (30%)		
Overall response		13 (43.3%)			16 (53.3%)			18 (60%)			0.5/0.6/0.3
QoL										
Visual function	56.8 ± 28.3	69.3 ± 27.0	0.003	66.6 ± 26.2	88.2 ± 14.1	<0.0001	53.7 ± 24.4	74.8 ± 25.6	<0.0001	0.3/0.3/1
Appearance	52.5 ± 25.5	69.3 ± 21.0	<0.0001	54.2 ± 21.9	83.3 ± 17.4	<0.0001	51.9 ± 26.5	73.4 ± 21.9	<0.0001	0.2/0.3/1
Improved										
Visual function										
ITT (n = 30)		16 (53.3)			17 (56.7)			16 (53.3)		1/1/1
PP (n = 22,21,19)*		16 (72.7)			17 (81.0)			16 (84.2)		0.7/0.7/0.5
Appearance										
ITT (n = 30)		19 (63.3)			19 (63.3)			17 (56.7)		0.9/1/1
PP (n = 22,21,19)*		19 (86.4)			19 (90.5)			17 (89.5)		1/1/1

Ophthalmic parameters were analyzed by the worst eye. *(n = 22,21,19): n = 22 (MP, G1), n = 21 (reduced MP+MTX, G2) and n = 19 (MP+MTX, G3).
ITT, intention-to-treat, analyzed full data set; PP, per-protocol analysis, analyzed patients with QoL questionnaires.

Since accumulated evidence suggests that dyslipidemia is associated with GO development (33, 34, 35), cholesterol levels should be monitored in practice. The frequency of measuring BMD in our study (12-week interval) may have little long-term significance. However, the purpose of reassessing BMD was not to assess fracture risk, but to assess early BMD loss and its association with different treatment regimens. Therefore, in this study, we re-evaluated BMD at week 12. In groups with the same MP dosage, significantly fewer new-onset hypertension occurred in MP plus MTX group compared to MP monotherapy. This was in line with MTX usage in RA studies in which MTX treatment was associated with significantly lower clinic and 24-h peripheral and central BP compared to those who did not take MTX (36). The BP reducing effect was explained by MTX protection against stiffness-mediated BP increase (37). As for the screen of GC-induced diabetes (38, 39), the incidence of new-onset diabetes was low and without significant differences among groups in this study. MTX-related AE such as neutropenia was present in four patients and were ameliorated by leucogen tablets. Importantly, MTX use warrants monitoring infrequent but clinically serious AEs, particularly hepatic, pulmonary, and hematologic toxicity (40). In our study, patients with known risk factors that would lead to severe AE were excluded, such as liver or renal impairment, history of hepatitis or tuberculosis, and history of malignancy, and AEs were closely monitored every 6 weeks. We observed neutropenia in 4 patients (1/30 in reduced MP+MTX, 3/30 in MP+MTX group) and were all timely relieved by leucogen tablets. Therefore, under careful pretreatment inspection and monitoring, the reduced MP plus MTX regimen had fewer AEs compared to MP monotherapy.

Limitations of this study include the lack of prespecified QoL assessment and response evaluation at week 24. Those outcomes would likely yield significant differences among groups. Therefore, further prespecified studies of QoL and post-treatment follow-up are warranted to validate the current conclusion.

Previous GO treatment may contribute to reduced clinical response. One patient who received strabismus surgery 7 months prior to the study in goup 1 (4.5 g MP) was not responsive after treatment. There were 26 patients who received glucocorticoids (i.v., oral, orbital) at least 3 months prior to treatment. *Post hoc* analysis showed that the CAS response was 71.4% for naïve patients and 63% for patients with previous treatment. For naïve patients (n = 63), CAS response rate was 57.1% for group 1 (n = 21), 75% for group 2 (n = 20), and 81.8% for group 3 (n = 22).

Table 3 Adverse event.

Adverse event	MP (G1)	Reduced MP + MTX (G2)	MP + MTX (G3)	*P*-value overall/G1 vs G2/G1 vs G3
Any adverse event	25 (83.3%)	15 (50%)	22 (73.3%)	0.02/0.01/0.5
Weight gain, kg[a]	0.2 ± 2.3	−0.3 ± 2.0	−0.2 ± 2.3	0.8/1/1
≥2.5% increment				
6 weeks	2 (6.7%)	2 (6.7%)	2 (6.7%)	1/1/1
12 weeks	9 (30%)	2 (6.7%)	7 (23.3%)	0.06/0.04/0.8
≥5% increment (grade 1)[b]				
6 weeks	0	2 (6.7%)	1 (3.3%)	0.8/0.5/1
12 weeks	2 (6.7%)	2 (6.7%)	3 (10%)	1/1/1
Hypertension[ab]				
6 weeks	4 (21.1%)	2 (8.7%)	0	0.08/0.4/0.047
12 weeks	5 (26.3%)	2 (8.7%)	0	0.03/0.2/0.02
Hypokalemia[ab]				
6 weeks	4 (18.2%)	0	1 (4%)	0.02/0.038/0.2
12 weeks	5 (22.7%)	1 (3.9%)	1 (4%)	0.06/0.08/0.08
Diabetes[ab]				
6 weeks	0	1 (5%)	0	1/1/-
12 weeks	0	2 (10%)	0	0.3/0.5/-
Dyslipidemia[a]				
6 weeks	7 (35%) GR1	4 (18.2%) GR1	6 (28.6%) GR1, 2 (9.5%) GR2	0.3/0.3/0.6
12 weeks	10 (50%) GR1, 1(5%) GR2	6 (27.3%) GR1	10 (47.5%) GR1, 2 (9.5%) GR2	0.2/0.1/1
Osteoporosis[a] (12 weeks)	0	0	1 (4.6%)	-
Bone loss[a] (12 weeks)	1 (6.7%)	2 (10%)	2 (8.3%)	1/1/1
Neutropenia[a]				
6 weeks	0	1 (3.3%) GR2	2 (6.9%) GR1	0.3/1/0.5
12 weeks	0	1 (3.3%) GR2	3 (10.3%) GR1	0.1/1/0.2
Impairment of liver function[a]	1 (3.3%)	0	0	-
Leukocyturia (asymptomatic)[ab]	6 (21.4%)	4 (14.8%)	6 (20.7%)	0.8/0.7/1
Hyperuricemia[ab]				
6 weeks	0	0	2 (6.7%)	0.3/-/0.5
12 weeks	2 (6.7%)	0	3 (10%)	0.4/0.5/1
Other				
Cold	0	1 (6 weeks) GR1	1 (6 weeks) GR1	-
Facial paralysis	1 (6 weeks) GR1	0	0	-
Femoral head necrosis	0	0	1 (12 weeks) GR2	-
Insomnia	0	0	1 (12 weeks) GR1	-

[a]New onset; [b]the criteria of CTCAE grade 1 for weight gain is ≥5% of baseline body weight, which was close to 3 kg in this cohort.
Hypertension and diabetes were grade 2. Hypokalemia and hyperuricemia were grade 1. Asymptomatic leukocyturia was discovered by urinalysis without symptoms and did not require medical treatment. 12-week data are cumulative which includes AE that occurred at week 6.
GR1, grade 1; GR2, grade 2.

In summary, this is the first prospective RCT study to compare the efficacy and safety of reduced MP plus MTX vs MP monotherapy with matched 12-week time course. As most AEs were steroid dosage-dependent, we demonstrated that under careful pretreatment inspection and monitoring, reduced MP plus MTX had fewer AEs with similar efficacy. *Post hoc* subgroup analysis found that smoking interfered with treatment. Prespecified subgroup analysis should be conducted in future studies to test for subgroup superiority.

Conclusion

Reduced ivMP (3.0 g) plus MTX therapy was not significantly different in CAS response at week 12 but brought less steroid-related adverse effects compared with the standard 4.5 g ivMP regimen in active, moderate-to-severe GO. Therefore, we suggest reduced MP plus MTX combination therapy to lower steroid-related AEs as well as to achieve CAS response. Due to the potential side effects of MTX, clinical use should always be careful with pretreatment inspection and monitoring.

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