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A randomized clinical trial for the assessment of the efficacy and safety of guluronic acid (G2013) in patients with rheumatoid arthritis

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ABSTRACT
Objective: To evaluate the safety, efficacy and tolerability of guluronic acid (G2013) in order to treat the rheumatoid arthritis patients who had inadequate response to conventional drugs.
Methods: A randomized, 12-week clinical trial with two treatment arms: guluronic acid (G2013) and conventional treatment was performed. The diagnosed RA patients according to the ACR/European League against Rheumatism 2010 classification criteria, with an active disease at baseline that had inadequate response to conventional therapy were considered for the study. G2013 was administered orally twice a day with capsules of 500 mg during a period of 12 weeks and the patients were followed up for the safety and efficacy.
Results: Our data showed that, the mean changes in the G2013 and control groups were −7.54 and −2.5 for tender joint count; −7.59 and −3.59 for swollen joint count; −30 and −0.9 for physician global assessment; −23.18 and −1.81 for patient global assessment; −14.45 and −1.45 for erythrocyte sedimentation rate, respectively. Improvements seen with G2013 were significantly greater than those with conventional drugs. In total, in 15.3% of G2013-treated patients and 69.2% of conventional-treated patients adverse events (AEs) occurred in this study.
Conclusion: These data from routine rheumatology clinical practice highlight the effectiveness of G2013 in combination with conventional therapy with more desirable safety profile compared to the conventional-treated patients. Therefore, G2013 therapy could be an appropriate choice in order to manage the RA disease. (Clinical trial identifier: IRCT2016092813739N5).

Introduction
Rheumatoid arthritis (RA) is one of the most common inflammatory, autoimmune diseases with the prevalence of 1% in the world population. The etiology of disease remained unknown and frequently starts in middle age and is more common in women than in men. The impact of arthritis on pain, disability, and quality of life results in a considerable burden to the individual, health services, and society [1,2].

The main goals of RA management are to reduce disability and disease progression, minimize pain and swelling and eventually, prevent of joint damage and destruction [3].

Current treatments for RA include nonsteroidal anti-inflammatory drugs (NSAIDs), low-dose steroids, and disease-modifying anti-rheumatic drugs (DMARDs) [4].

Although, DMARDs are key therapeutic agents for patients with RA and they reduce synovitis and systemic inflammation and improve function; however, they have shown the lack of sustained respond, slow onsets and no analgesic activity [5].

The NSAIDs are used for the treatment of osteoarthritis and RA and provide effective relief in patient; however, the serious gastrointestinal (GI) complications like peptic and duodenal ulcers occur with their use that it even leads to death. There are between 2000 and 2500 deaths annually in the United Kingdom due to use of NSAIDs [1].

Although, glucocorticoids have more effect on the decrease of joint pain than NSAIDs they cause serious adverse effect (SAE) such as adrenal suppression, ulcers and osteoporosis [6].

The guluronic acid (G2013) with low molecular weight, patented (DE-102016113017.6), is one of the alginic acid comonomers that its safety, anti-aging feature with anti-inflammatory and immunosuppressive properties as well as its therapeutic effect have been demonstrated in the several experimental models of multiple sclerosis and TLR signaling pathway [7,8]. Moreover, the results of preclinical assessment of this drug on BALB/C mices during acute and subchronic study showed high safety when used orally [9].

The guluronic acid showed its anti-inflammatory effects by inducing SHIP1 and SOCS2, reducing TLR4, MyD88, NF-kB and COX1/COX2 inflammatory enzymes at the level of gene expression and decreasing the amount of IL-1B as a pro-inflammatory cytokine [10,11].
The guluronic acid is a safe agent without any toxicity on the GI tract and kidney function. Anti-inflammatory and immunosuppressive effect of this drug on TLR 2, 4 signaling pathway has been reported in CVID patients [7,9,12,13].

The previous study has shown that intraperitoneal (IP) injection of G2013 had beneficial effects on EAE with lower incidence, attenuation in the severity, and a delay in the onset of disease [7]. Also, G2013 reduces the number of inflammatory cells and plaques as well as the serum level of NO in G2013 dosed mice [7].

Based on these findings, G2013 could have a positive effect on the reduction of inflammation in inflammatory diseases like RA and the present study was aimed to assess the safety and the efficacy of -guluronic acid in Iranian RA patients.

**Patients and methods**

**Patients**

There were 52 patients with RA, taking conventional treatment and fulfilling the ACR/European League against Rheumatism 2010 classification criteria that had active disease defined based on having ≥6 swollen joints in 66-joint and ≥6 tender joints in 68-joint and taking conventional treatment for RA [14].

The other relevant eligibility criteria were an age of 18–80 years, at least six swollen joints and six tender joints and at least two of the following: A C-reactive protein (CRP) level of ≥10 mg/L, an erythrocyte sedimentation rate (ESR) of ≥20 mm/h, or morning stiffness lasting ≥30 min.

In the beginning of the study, in spite of the treatment with DMARDs, NSAIDs, steroids, the participants had active RA and inadequate response to treatment.

The eligible patients were included if they had been taking methotrexate (MTX) at a dosage of 15–20 mg/week, folic acid 1 mg/day, hydroxychloroquine (HCQ) 400 mg/day, prednisolone (PRD) 5–15 mg/day and NSAIDs for at least 6-month prior the study. Accordingly, the dosage had been stable at least 4 weeks without any change in patient’s treatment regimen before and during follow-up. Furthermore, in the G2013-treated group, the patients abstained from the use of NSAIDs at least 3–14 days in the time of washout period that depended on the half-life of NSAIDs prior the study and during follow-up. Patients were allowed to continue a stable dose of concurrent treatment with DMARDs, NSAIDs (with the exception of G2013 treated group).

The exclusion criteria were history of positive and latent tuberculosis, positive pregnancy test or lactation, vasculitis, pulmonary fibrosis, malignancy and acute or chronic infections.

**Study design and treatment protocol**

The study was designed for the comparison of the efficacy and safety of guluronic acid (G2013) with DMARDs over 12 weeks in the patients with active RA who had failed response to treatment by conventional drugs.

The protocols were reviewed and approved by regulatory authorities and the ethics committee of Tehran University of Medical Sciences (Tehran, Iran) and the study was conducted in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonization guidelines for Good Clinical Practice.

After confirmation that the patients fulfilled the defined criteria, they were randomly assigned at a 1:1 ratio. The guluronic acid (G2013) was given in the dose of two capsules of 500 mg per day orally after food over a period of 12 weeks.

 Afterwards, the patients were visited and examined for medical history, physical condition and clinical outcomes (achieving the ACR 20% improvement criteria) at baseline, week 4, and week 12 at the department of Rheumatology, Shariati Hospital (Tehran, Iran), Rheumatology Research Center and Iran Rheumatism Center during 12 weeks of G2013 therapy.

**Safety assessments**

To assess the safety, the samples were collected for hematology, clinical chemistry, routine urine analysis and fecal occult blood test at baseline, weeks 4 and 12. In addition, at each visit, patients were examined and questioned about adverse events (AEs) on the basis of self-reports questionnaire, the measurement of vital signs, physical examination. The safety endpoints included incidence and type of AEs, SAEs, infection and changes in clinical laboratory (hematological and biochemical) parameters from baseline to week 12. All parameters were recorded at all visits. Additionally, findings such as urinalysis, stool exam, blood pressure, heart rate, and weight were obtained at each visit.

Meanwhile, in order to assess the tolerability, the Patients Global Assessment of Tolerability to Therapy (PGATT) was evaluated based on a five-point scale with the following scale response categories including: Excellent, Good, Moderate, Poor, and Worst.

**Efficacy end point**

In order to recognize the improvements of the disease indexes at each visit with regard on the ACR core set of disease activity [15], the following efficacy end-point was evaluated: Disease Activity Score (DAS) in 28 joints, count of tender and swollen joints based on a 28-joint count, physician and patient assessment of global RA disease activity and pain intensity assessment on a visual analogs scale of 0 (no pain) to 100 (severe pain), duration of morning stiffness reported in minute, values of westergen ESR, CRP and rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP). Functional disability was measured with a health assessment questionnaire (HAQ) that rated the subject ability to perform daily activities from 0 (without difficulty) to 3 (unable to perform tasks).

The four primary efficacy end-points in this study were swollen and tender joint counts and global patient and physician assessment of disease activity. Overall patient clinical response to therapy was assessed by ACR criteria [16]. To be considered an ACR 20% responder, the following definition of improvement was selected: 20% improvement in tender and swollen joint counts and 20% improvement in three of the five remaining ACR core set measures: patient global
Statistical analysis

Data were represented as means and standard deviations (SD). Clinical data were analyzed based on per protocol subjects, completed case analysis; the subject completed the follow-up. So, ITT did not need to be applied.

In the baseline time, all of clinical parameters were not statistically different between intervention and nonintervention groups. Therefore, the analysis of covariance did not need to be applied, as independent t-test repeated measure was used after parametric assumptions were being established (e.g. normality and homogeneity of variance).

The ACR20 responses were analyzed between treatment groups by the chi-square test. The safety population included all patients who received a minimum of one dose of study treatment. Adverse events were analyzed descriptively. All statistical tests were two-sided, and a p value < .05 was considered to be statistically significant. The data were analyzed using SPSS software Version 23 (IBM Corporation, Armonk, NY).

Results

Baseline characteristics and disposition of the patients

Of the 52 randomized patients, 26 were assigned to guluronic acid, 26 to conventional group (Figure 1). Baseline demographic and disease characteristics were generally comparable between the treatment groups (Table 1), and there were no statistically meaningful differences between the treatment groups for any of the evaluated characteristics.
The majority of patients completed the study through week 12 (84.61% in the guluronic acid group, 84.61% in the conventional group). Before the starting of the study, all patients had high disease activity (DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity as well as DAS28 < 2.6 indicates the proportion of patients who were achieving disease remission). The majority of patients enrolled in this study were female (76.9%) and the mean age of the patients was 45.58 ± 12.68 years, and the mean disease duration was 5.76 ± 4.02 years.

Four patients in the G2013-treated group withdrew from the study; one patient withdrew consent for unknown reasons and three patients withdrew due to the loss of follow-up. Four patients in the conventional-treated group withdrew from the study; one patient withdrew consent for unknown reasons, one patient withdrew due to lack of efficacy and two patients withdrew due to AE.

### Efficacy profile

Changes in the four primary clinical efficacy end-points over the 12 weeks of treatment with G2013 and conventional drugs are shown in Table 2. Both treatments led to significant improvement in most primary efficacy end-points. However, the difference between baseline and end-point measurements of all efficacy end-points was significantly greater in G2013 than in conventional-treated subjects.

The changes in secondary clinical efficacy end-points after 12 weeks of treatment with G2013 and conventional drugs are shown in Table 3. There was statistically significant improvement from baseline in HAQ score, ESR, and CRP while morning stiffness, pain intensity, whereas RF had non-significant improvement after treatment with G2013 compared to conventional-treated patients.

The administration of G2013 at 500 mg twice daily (1000 mg/day) resulted in significantly greater ACR20 response rates compared to the conventional-treated patients after 12 weeks G2013 therapy (Figure 2). In addition, following treatment with G2013 a significant ACR20 improvement was observed at week 4, so that nine of 22 patients (40.9%) in the G2013-treated patients and one of 22 patients (4.5%) in the conventional-treated patients had achieved an ACR20 response at week 4 (p = .004). The efficacy was sustained throughout the study and 16 of 22 patients (72.7%) in the G2013-treated group and five of 22 patients (22.7%) in the conventional-treated patients had achieved a significant improvement from baseline in HAQ score, ESR, and CRP while morning stiffness, pain intensity, whereas RF had non-significant improvement after treatment with G2013 compared to conventional-treated patients.
ACR20 response after 12 weeks of treatment ($p=0.001$) (Figure 2).

Results of independent $t$-test repeated measure for DAS28 show a statistically significant improvement from baseline in mean change in DAS28 in G2013-treated patients ($p < 0.001$) compared to the conventional-treated patients after 12 weeks (Figure 3).

**Safety profile**

During the study, G2013 therapy showed an excellent tolerability without SAE. During the study, the incidences of AEs were higher in the conventional-treated patients (76.9%) compared with G2013-treated patients (15.3%). There were no patients in the G2013-treated patients that discontinued the study due to an AE, whereas, two patients withdrew the study because of AEs in the conventional-treated patients (Table 4). Furthermore, the most of patients reported significant satisfactory emotional changes after the consumption of G2013 and they declared that they have become more relaxed than before. Moreover, the hematological and biochemical findings in G2013-treated patients did not show any consistent treatment-related effects. There were no statistically significant differences in the means of hematological and biochemical values at weeks 0, 4, and 12 in G2013-treated patients (Tables 5 and 6). These parameters reached to the reference range level throughout the study. All of means and individual concentrations of the serum biochemical determinants in the G2013-treated patients became close to the reference range level throughout the study.

**Discussion**

This study was done for the first time in order to compare the safety and efficacy of guluronic acid (G2013) as a novel NSAIDs with immunomodulatory property with conventional treatment in active RA patient. The results of this study provided that guluronic acid with 500 mg two times per day during the 12-week period of treatment of RA has significantly a superior efficacy when compared with conventional treatment.

G2013 is an anti-inflammatory agent with therapeutic effects and the greatest tolerability in animal models of multiple sclerosis. It is a safe drug with a low molecular weight and no toxicity on GI tract and kidney function [9,18].

Mirshafiey et al. showed that the expression of antioxidant enzymes that play an important role against aging and age-related diseases like glutathione peroxidase (GPX1), superoxide dismutase 2 (SOD2), catalase (CAT), glutathione-S-transferase (GST) was increased in G2013-treated rats in comparison with the control group [19].

Its anti-inflammatory and immunosuppressive properties such as reducing the number of inflammatory cells and plaques, demyelination and serum nitric oxide (NO) levels in G2013-treated mice in comparison with control group have been demonstrated in several experimental models of EAE [7].

Taeb et al. showed that G2013 can normalize the gene expression of myeloperoxidase (MPO) and may reduce the pathologic effects age-related inflammatory diseases by adjusting the expression of GPX1, SOD2, GST, CAT, and iNOS [20].

The investigations in the field of molecular mechanism of G2013 have shown modulatory effects on TLR 2, 4 signaling pathway as well as anti-aging property on various oxidative stress determinants [12].

In this trial, we evaluated the safety and efficacy of G2013 in the patients of active RA that they respond inadequately to conventional treatment.

This study demonstrated that the oral use of G2013 in the dose of two capsules of 500 mg per day in combination to conventional treatment in the most patients (72.7%) led to a statistically significant increase in the ACR20 response rate after 12 weeks of G2013 therapy. Furthermore, the G2013-treated patients indicated a great significant reduction in inflammatory marker such as CRP and ESR after 12 weeks of G2013 therapy. Moreover, the G2013-treated patients showed a significantly superior efficacy along with a more favorable safety profile compared to the conventional-treated group.

In the present study, based on our previous preclinical assessment, the approved dosage of G2013 was considered with the amount of 25 mg/kg/d [21]. However, in this study we used from a minimum dose (18 mg/kg/day) of G2013. Therefore, it is possible that a more efficacy and a faster onset of action could be obtained with a higher/different dose of G2013.

Overall, G2013 showed a great tolerability and safety profile during the study and a well-tolerated without requirement for pharmacological intervene throughout the study and the majority of AEs were mild.

The most important concerns following the use of NSAIDs are the increased risk of GI, cardiovascular, and renal...
complications that could be fetal in some patients [22–24], whereas our findings provide evidence that G2013 as a derivative of alginic acid in addition to having therapeutic effects in RA patients; it could also reduce and diminish the GI and hematological complications. Therefore, it might be suggested that G2013 is suitable option for the long-term management of RA.

In conclusion, G2013 therapy led to a great significant improvement in the clinical response, patient-reported outcomes (PROs) such as tender joint count, swollen joint count, the PtGA, PGA scores that were associated with a statistically significant reduction in disease activity, the signs and the symptoms in the RA patients that had inadequate response to treatments and this effect was sustained throughout the study. Furthermore, G2013 therapy provided considerable tolerability and safety profile during the study and the incidences of AEs that occurred during the study were in an acceptable profile in the G2013-treated patients. This study showed that G2013 is effective, safe and generally well tolerated in patients with RA. However, it should be noted that running the phase III multicenter randomized clinical trial in RA patients by G2013 could provide further evaluation of the safety and the efficacy in RA patients.

Acknowledgements

We would appreciate the nurses and staff of Rheumatology Research Center, Shariati Hospital, Tehran University of Medical Sciences (TUMS).

Disclosure statement

The authors declare no conflict of interest.

Table 5. Hematological parameters in RA patients treated with guluronic acid, 500 mg two times per day.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 12</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3/µL), mean ± SD</td>
<td>7.68 ± 2.36</td>
<td>7.38 ± 2.15</td>
<td>7.29 ± 2.06</td>
<td>.250</td>
</tr>
<tr>
<td>RBC (10^9/µL), mean ± SD</td>
<td>4.71 ± 0.484</td>
<td>4.79 ± 0.645</td>
<td>4.85 ± 0.627</td>
<td>.194</td>
</tr>
<tr>
<td>HB (g/dL), mean ± SD</td>
<td>12.83 ± 1.43</td>
<td>12.95 ± 1.31</td>
<td>13.30 ± 1.35</td>
<td>.017</td>
</tr>
<tr>
<td>HCT (%), mean ± SD</td>
<td>39.60 ± 4.26</td>
<td>39.80 ± 4.03</td>
<td>40.47 ± 4.06</td>
<td>.159</td>
</tr>
<tr>
<td>Neutrophils (%), mean ± SD</td>
<td>67.76 ± 9.55</td>
<td>60.50 ± 6.37</td>
<td>60.54 ± 6.37</td>
<td>.779</td>
</tr>
<tr>
<td>Lymphocytes (%), mean ± SD</td>
<td>30.03 ± 9.30</td>
<td>32 ± 6.98</td>
<td>31.50 ± 5.76</td>
<td>.675</td>
</tr>
<tr>
<td>Platelet (10^9/µL), mean ± SD</td>
<td>281.57 ± 78.87</td>
<td>282.34 ± 79.68</td>
<td>278.77 ± 75.95</td>
<td>.422</td>
</tr>
</tbody>
</table>

WBC: white blood cell; RBC: red blood cell; HB: hemoglobin; HCT: hematocrit. Values are expressed as mean ± SD.

Table 6. Biochemical parameters in RA patients treated with guluronic acid, 500 mg two times per day.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 12</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL), mean ± SD</td>
<td>89.59 ± 9.41</td>
<td>86.72 ± 17.75</td>
<td>92.54 ± 7.17</td>
<td>.923</td>
</tr>
<tr>
<td>BUN (mg/dL), mean ± SD</td>
<td>17.18 ± 5.07</td>
<td>17.09 ± 4.37</td>
<td>16.63 ± 4.53</td>
<td>.914</td>
</tr>
<tr>
<td>Creatinine (mg/dL), mean ± SD</td>
<td>0.845 ± 0.167</td>
<td>0.879 ± 0.141</td>
<td>0.872 ± 0.158</td>
<td>.413</td>
</tr>
<tr>
<td>Uric Acid (mg/dL), mean ± SD</td>
<td>5.84 ± 1.15</td>
<td>5.89 ± 0.972</td>
<td>6.07 ± 1.17</td>
<td>.858</td>
</tr>
<tr>
<td>Calcium (mg/dL), mean ± SD</td>
<td>9.18 ± 0.494</td>
<td>8.77 ± 1.79</td>
<td>9.18 ± 0.411</td>
<td>.988</td>
</tr>
<tr>
<td>Phosphorous(mg/dL), mean ± SD</td>
<td>3.66 ± 0.587</td>
<td>3.53 ± 0.571</td>
<td>3.67 ± 0.570</td>
<td>.503</td>
</tr>
<tr>
<td>ALT (U/L), mean ± SD</td>
<td>31.13 ± 39.30</td>
<td>34.50 ± 41.05</td>
<td>30.36 ± 24.93</td>
<td>.719</td>
</tr>
<tr>
<td>AST (U/L), mean ± SD</td>
<td>23 ± 11.35</td>
<td>22.45 ± 14.57</td>
<td>22.45 ± 14.57</td>
<td>.898</td>
</tr>
<tr>
<td>ALP (U/L), mean ± SD</td>
<td>191.45 ± 74.54</td>
<td>191.86 ± 75.80</td>
<td>187.40 ± 62.49</td>
<td>.385</td>
</tr>
</tbody>
</table>

BUN: blood urea nitrogen; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase. Values are expressed as mean ± SD.

References


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