

Original Article

Combined Bushen Qiangdu Recipe and sulfasalazine treatment reduced cytokine levels and improved the symptoms of ankylosing spondylitis

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Abstract: Objective: Chinese medicine has a satisfactory performance in treating ankylosing spondylitis (AS). However, holistic research and literature is limited, especially combined research of Chinese and Western medicine. Herein, this study aimed to explore the safety and effectiveness of Bushen Qiangdu Recipe (BQR) combined with sulfasalazine for ankylosing spondylitis (AS). Methods: Sixty patients diagnosed with AS were randomly divided into a sulfasalazine group and a combined group. Patients underwent overall evaluation, evaluation of various kinds of pain (general pain, day/night pain, back pain, spinal pain), physical inspection, blood test, urinalysis, and measurement of erythrocyte sedimentation rate and C-reactive protein level during each visit. Furthermore, venous blood from patients with AS was evaluated for levels of collagen, interleukin-6 (IL-6), fibronectin (FN), and matrix metalloproteinase-3 (MMP-3). Results: A total of 57 patients completed the study: 29 in the combined group and 28 in the sulfasalazine group. Both BQR and sulfasalazine demonstrated minor, temporary, negative effects. With combination treatment and sulfasalazine treatment, the percentage of ASAS 20 reactors after 3 months was 82.75% (24 of 29) and 71.43% (20 of 28), respectively. Both combined medicine and sulfasalazine induced a considerable decline in the percentage of collagen, FN, IL-6, and MMP-3 expression after 3 months of treatment compared with baseline values ($P < 0.05$). Treatment outcomes were significantly better in the combined group compared with the sulfasalazine group. Conclusion: Combined BQR and sulfasalazine treatment reduced cytokine levels and improved the symptoms of AS, and it showed a better effect and safety than sulfasalazine alone.

Keywords: Ankylosing spondylitis, Bushen Qiangdu Recipe, sulfasalazine, cytokines

Introduction

Ankylosing spondylitis (AS) is an inflammatory, rheumatological chronic illness that mainly affects the spinal column and sacroiliac joints in humans. The pathogen of AS is still unclear [1]. It is a relatively common disease, found primarily in young people, and is characterized by marked stiffening of the spine. After onset of the disease, two-thirds of patients with AS may develop partial or complete stiffening of the spine within several years [2]. Clinically, sulfasalazine, methotrexate, and leflunomide have been used for the treatment of peripheral spondyloarthritis. However, these treatments were often unsuccessful and were associated with side effects such as liver and kidney damage and reduced fertility. Recent years have witnessed the emergence of some tumor necrosis

factor blockers such as Etanercept, Adalimumab, and Infliximab in clinical use, with satisfactory clinical performance [3]. However, these treatments place considerable financial pressure on patients, and may result in infections, lymphadenoma, or tumor. Producing a treatment with effective therapeutic action and reduced side effects is therefore very important.

Chinese medicine has a satisfactory performance in treating AS. However, holistic research and literature is limited, especially combined research of Chinese and Western medicine [4]. Bushen Qiangdu Recipe (BQR) is a treatment created by Professor Yan from China-Japan Friendship Hospital based on clinical experience. It has been used clinically for many years to effectively treat AS, with few side effects. In

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Table 1. General Traits of AS patients for each group

Group	Case	Female (case)	Male (case)	Age (year)	AS Duration (year)
Combined	29	6	23	31.68 ± 9.32	10.12 ± 4.07
Sulfasalazine	28	7	21	33.50 ± 10.82	12.68 ± 6.12
Normal	30	9	21	35.42 ± 7.46	-

this study, we aimed to examine the clinical safety and effectiveness of BQR combined with sulfasalazine, determine its effect on cytokines, and further evaluate the feasibility of its clinical application.

Methods

Diagnostic criteria

Sixty eligible patients, diagnosed with AS based on X-ray examination following the criteria of revised New York classification, with demonstrated sacroiliitis levels all below stage III, were included in the study [5].

Selection criteria

Inclusion criteria were as follows: (1) patients aged 16 to 69 years and of either sex; (2) above-mentioned AS diagnostic criteria were fulfilled; (3) patients diagnosed with spine stiffness without exposure to methotrexate, sulfasalazine, steroids, or tripterygium wilfordii vine for at least 30 days prior to our study; and (4) patients that voluntarily signed the consent form with review by the ethical oversight committee of Shaoxing Central Hospital (Approval Number: 20150016).

Exclusion criteria

Exclusion criteria were (1) other serum-negative spinal joint diseases that required treatment with adrenocortical hormones; (2) pregnancy or breast-feeding; (3) previous hypersensitivity responses to sulfasalazine or BQR; and (4) presence of other major illnesses including heart disease, liver problem, kidney disorder, or even psychiatric diseases.

Drop-out criteria

Drop-out criteria were (1) not meeting the selection requirements, and (2) not taking the test drugs according to schedule during the trial period.

Testing subjects

In total, 60 experimental cases were selected from patients of the Department of Rheumatology during May 2015 to March 2017 with confirmed AS diagnosed according to above-listed criteria. Patients were randomly assigned via a completely randomized number table to 2 groups with 30 subjects in each: the sulfasalazine-only group and the combined BQR-sulfasalazine group. Thirty healthy volunteers formed the control group.

Treatment

The combined group received BQR (consisting of rhizoma cibotii, drynariae baronii, malaytea scurfpea, epimedium, Himalaya teasel, prepared rhizome of Rehmannia, Ramulus cinnamomi, Antle, Eucommia bark, Joint fir, Radix Paeoniae Rubra, Radix paeoniae alba, and Divaricate Saposhnikovia) twice a day and sulfasalazine 2 grams daily. The sulfasalazine group received only sulfasalazine 2 grams daily. The treatment was given for 3 months.

Detection of cytokines

Levels of interleukin-6 (IL-6), fibronectin (FN), matrix metalloproteinase-3 (MMP-3), and collagen in the venous blood of AS patients were assessed at baseline and after 3 months of treatment. Collagen was measured by determination of hydroxyproline content. IL-6, FN, and MMP-3 levels were assessed via the ELISA method.

Efficacy assessment

For evaluation of AS patients, the Assessment in Ankylosing Spondylitis Response Criteria 20 (ASAS 20), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Metrology Index (BASMI) were used for scoring (on a scale of 1-10, with a higher score indicating a more severe illness stage), as well as general assessment and various kinds of pain measurements including general pain, night pain, back pain, spinal pain, etc. The definition of treatment response was a reduction of least 20% for 3 of the following 4 measurements: spinal pain, general assessment, BASFI, and BASMI (including the lasting period and morning ankylosis in the last two).

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Table 2. Change Index of AS patients relate to Base Value

Group	Case	Time	ASAS 20	BASDAI	BASFI	BASMI	Overall assessment	Spondylalgia	General pain	Night-hour pain
Combined	29	0-month		4.72 ± 1.28	1.35 ± 1.20	1.92 ± 1.23	5.12 ± 2.23	3.66 ± 2.65	3.59 ± 2.71	3.82 ± 2.01
		3-month	82.75%	2.01 ± 1.26** ^Δ	1.00 ± 0.72*	1.17 ± 1.02*	3.02 ± 1.78** ^Δ	2.41 ± 1.39** ^Δ	1.52 ± 1.56** ^Δ	1.03 ± 1.06** ^Δ
Sulfasalazine	28	0-month		4.80 ± 0.31	1.42 ± 1.01	2.02 ± 1.31	4.93 ± 1.84	4.12 ± 2.53	4.21 ± 2.13	4.01 ± 2.10
		3-month	71.43%	3.66 ± 1.22*	1.27 ± 0.63	1.91 ± 1.16	3.72 ± 1.70*	3.02 ± 2.50*	3.23 ± 2.03*	2.66 ± 2.04*

Note: *P<0.05, **P<0.01 relative to same group at initial stage (0-month); ^ΔP<0.05, relative to the sulfasalazine group at 3-month.

Table 3. Change of CRP and ESR levels Relate to Base Value

Group	Subject	Period	CRP (mg/dL)	ESR (mm/h)
Control group	20	0-month	2.54 ± 3.32	13.63 ± 8.90
Combined	29	0-month	18.30 ± 11.04	19.10 ± 12.21
		3-month	9.04 ± 5.32** ^Δ	10.03 ± 8.23** ^Δ
Sulfasalazine	28	0-month	17.28 ± 13.02	20.37 ± 12.33
		3-month	12.14 ± 13.21*	14.11 ± 13.51*

Note: *P<0.05, **P<0.01, relative to same group at initial stage (0-month); ^ΔP<0.05, relative to sulfasalazine group at 3-month.

Patients underwent general body examination, blood and urine tests, as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) measurement. The same sole examiner conducted all the above assessments.

Methods of measuring ESR

In the Westergren method, a fixed amount of blood is drawn into a vertical tube anticoagulated with sodium citrate. The blood is left to settle for 1 hour, after which the distance between the top of the blood column and the top layer of the red blood cells (RBCs) below is measured. The ESR is thus reported in millimeters/hour. Newer methods employ a special centrifuge and automated machines and can yield results in as quickly as 5 minutes [6, 7].

Methods of measuring CRP

CRP was originally measured via the Nephelometry, Serum (or heparinized plasma) was mixed with Intralipid 20% in Tris-calcium buffer (pH 7.5). After 12 minutes of incubation at 37°C, the CRP-phospholipid complexes were measured by nephelometry (840 nm) using a BN II nephelometer (Siemens).

Statistical analysis

All data are recorded in the format of mean ± standard deviation (mean ± SD), paired t-test was used for comparison between two groups, and analysis of variance of repeated measures was used for comparison among different time

points within group. P>0.05 was considered to be statistically significant. Data analysis was performed using SPSS (ver. 13.0).

Results

Experiment materials

Fifty-seven patients with AS completed the study. Differences in sex, age, and AS duration among the three groups were minimal (P>0.05, **Table 1**).

Assessment of efficacy

Twenty-nine and 28 subjects in the combined and sulfasalazine groups, respectively, completed the study. After treatment with the combined drug and sulfasalazine, the percentage of ASAS 20 responders by 3 months was 82.75% (24 of 29) and 71.43% (20 of 28), respectively. The rate of effective treatment was considerably higher in the combined group than in the sulfasalazine group (P<0.05). As shown in **Table 2**, compared with baseline values, all scores of BASDAI, BASFI, BASMI, overall assessment, spondylalgia, and night-time pain in the combined group greatly decreased, while in the sulfasalazine group, improvements were concentrated in BASDAI, overall assessment, spondylalgia, general pain, and night-time pain categories (P<0.05). Furthermore, regarding BASDAI, overall assessment, spondylalgia, general pain, and night-time pain, the scores in the combined group were much lower than those in the sulfasalazine group (P<0.05).

Laboratory examination

CRP and ESR values in the two groups were significantly reduced compared with the baseline values (**Table 3**). Furthermore, ESR and CRP levels in the combined group were much lower than those in the sulfasalazine group (P<0.05).

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Table 4. Detection of Collagen, FN, IL-6 and MMP-3 in AS patients' peripheral blood at 0-month

Group	Case	Time	Collagen (ug/ml)	FN (ng/ml)	IL-6 (pg/ml)	MMP-3 (ng/ml)
Combined	29	0-month	41.91 ± 13.03*	979.27 ± 210.30**	334.90 ± 131.60*	324.25 ± 127.25*
Sulfasalazine	28	0-month	42.36 ± 10.41*	967.06 ± 241.05**	327.16 ± 170.32*	316.40 ± 174.20*
Normal	30	0-month	30.57 ± 11.02	652.81 ± 170.14	236.26 ± 123.10	209.23 ± 191.51

Note: **P<0.01, *P<0.05, in comparison with control group at 0-month.

Table 5. Collagen, FN, IL-6 and MMP-3 Expression in Two Groups

Group	Case	Time	Collagen (ug/ml)	FN (ng/ml)	IL-6 (pg/ml)	MMP-3 (ng/ml)
Combined	29	0-month	41.91 ± 13.03	979.27 ± 210.30	334.90 ± 131.60	324.25 ± 127.25
		3-month	30.16 ± 11.79** ^Δ	805.20 ± 250.10*	299.63 ± 171.02*	254.04 ± 166.17** ^Δ
Sulfasalazine	28	0-month	42.36 ± 10.41	967.06 ± 241.05	327.16 ± 170.32	316.40 ± 174.20
		3-month	36.30 ± 12.72*	823.13 ± 270.23*	301.24 ± 122.14*	280.32 ± 172.02*

Note: *P<0.05, **P<0.01, relative to same group at initial stage; ^ΔP<0.05, relative to sulfasalazine one at 3-month.

Table 6. The adverse effect date in the two groups

Group	Case	Time	Digestive reaction case	Kin rash case	Liver enzymes case	Strong adverse case	Total percent
Combined	29	0-month					
		3-month	2	1			10.3%
Sulfasalazine	28	0-month					
		3-month	2	1	1	1	17.9%

cases in the combined group had side effects. Among these, 2 cases had a digestive reaction and 1 case developed a skin rash. All the above reactions subsided after another week of treatment, and no patient left the study due to side effects. In the sulfasalazine

group, 2 cases had digestive reaction and 1 case developed skin rash; one patient left the study due to strong adverse reactions, and another patient withdrew due to an increase in liver enzymes. The main adverse effects included skin rash, leukopenia, and liver functional impairment. Except for the case in which the patient withdrew because of an increase of strong adverse effect, all other side effects subsided after another week of treatment. The rate of side effects in the combined group was approximately 10%, which was considerably lower than 17.8% in the sulfasalazine group (P<0.05; **Table 6**).

Discussion

This study explored the efficacy and safety of a combined medicine in AS therapy. BQR is a Chinese patent medicine, which can facilitate nourishing of liver and kidney, dispel wind-cold evil, and reduce endogenous wetness, as well as stimulate the blood cycle to reduce stagnation, with few side effects. Sulfasalazine has been used to treat AS for over 20 years, and its adverse effects normally

Detection of collagen, FN, IL-6, and MMP-3 at 0 months

Levels of collagen, FN, IL-6, and MMP-3 in the venous blood of AS patients were much higher compared with those of normal subjects (P<0.05). There were no statistically significant differences between mixed and sulfasalazine groups in collagen, FN, IL-6, and MMP-3 at baseline (all P>0.05; **Table 4**).

Effects on expression of collagen, FN, IL-6, and MMP-3

Treatment with combined medicine and sulfasalazine both triggered considerable decline in the levels of collagen, FN, IL-6, and MMP-3 expression at 3 months compared with baseline and the collagen, FN, IL-6, and MMP-3 expression level were much lower than those in sulfasalazine group (P<0.05). (P<0.05; **Table 5**).

Adverse reactions

In the sulfasalazine group, one patient left the study due to strong adverse reactions. Three

include liver and kidney damage and sperm reduction [8]. Accordingly, we undertook the present research in an attempt to find a combined effect-enhancing and adverse event-decreasing method to treat patients with AS.

The study data demonstrated that after patients were administered the combination treatment, ASAS 20 and levels of BASDAI, BASFI, general pain, spinal pain, night pain, and global assessment were consistently largely reduced, which suggested the combined drugs' ability to attenuate symptoms of AS, thus improving patient quality of life. The effective treatment rate in the combined group was relatively higher than that in the sulfasalazine group. Both the combined group and sulfasalazine group showed improved levels of BASDAI, BASFI, BASMI, global assessment, and various kinds of pains including general pain, spinal pain, and night pain. However, compared with the sulfasalazine group, values of BASDAI, global assessment, general pain, spinal pain, and night pain in the combined group were considerably lower. CRP and ESR levels in the combined group and sulfasalazine group were significantly declined from baseline at the end of treatment, and CRP and ESR levels in the combined group were greatly lower than those in the sulfasalazine group. This indicates that the combined medicine was superior to sulfasalazine alone in alleviating symptoms and inflammation. Treatment efficacy was probably related to BQR with its function of nourishing the liver and kidney, dispelling wind-cold evil, and reducing endogenous wetness, because wind-cold evil, wetness, and deficiency of liver and kidney are main factors in the pathology of AS according to the Chinese medicine theoretical system. Moreover, the side effect rate in the combined group was considerably lower compared with that in sulfasalazine group.

Fibrosis is an important pathological change in AS and can lead to bone destruction and joint deformation [9]. Fibrosis has been found in the pulmonary apices [10], lumbar spine, paraspinal muscles [11], intervertebral discs [12], and retroperitoneal areas [13]. However, the mechanism of fibrosis in AS is unclear. Excessive collagen, especially type I collagen and FN, is a biological marker of fibrosis, and its expression is regulated by many kinds of cytokines [14].

Cytokines control inflammation, regulating both the activated immune and non-immune cells. They can accelerate and reinforce the inflammatory process. In contrast, they may also restrict the inflammatory process so as to accelerate healing. IL-6, a cytokine that is generally excreted by activated Th cells as well as mononuclear phagocytes, is a treatment target of AS with a large potential to impede disease progression [15]. Early studies discovered that increased IL-6 levels have a negative impact on young patients with juvenile idiopathic arthritis [16]. Apart from being an effective pro-inflammatory factor, IL-6 can also influence the progression of fibrosis. Its neutralization benefited graft-associated fibrosis in cases of cardiac allograft [17]. The impact of IL-6 on fibrosis was further consolidated through its proven capability of regulating transforming growth factor beta and transforming growth factor beta II mRNA and protein levels in mice skin cells [18], effectively inducing normal/keloid-derived fibroblasts as well as improving STAT-3 levels. The latter facilitated certain procedures such as increased collagen production [19]. IL-6 is elevated significantly in Crohn's disease, where it seems to induce the production of fibro-genetic mesenchymal cells [20]. Therefore, IL-6 plays an important regulatory role in the inflammatory and fibrosis pathologies of AS.

Increasing interest is being focused on MMPs, as they actively participate in many physiological and pathological processes. The MMP family is a group of zinc ion-dependent proteinases that exist among a wide variety of connective tissues. MMPs can degrade various extracellular matrix components such as collagen, proteoglycans, FN, and laminin [21]. MMP-3 is also known as stromelysin-1. The MMP-3 encoding gene is located on chromosome 11. MMP is secreted from chondrocytes and synovial cells as a 59/57 kDa zymogen that is proteolytically processed to the 45 to 28 kDa active forms. MMP can degrade the majority of collagens and matrix proteins such as proteoglycan and laminin, which contribute to damage articular cartilage. Importantly, it also activates other plasminogens and activates MMP-1, MMP-9, and MMP-13, thus producing a "waterfall-like" amplification effect and facilitating the destruction of cartilage [22]. Therefore, MMP-3 is an important regulatory factor in both inflammatory and fibrosis pathologies in AS. A study showed that

collagen, FN, IL-6, and MMP-3 in the AS group were higher than those in the normal group, indicating that excessive collagen and FN are biological markers of fibrosis [23]. Additionally, the IL-6 and MMP-3 levels in the AS group were greater than those in the control group with obvious statistical difference, indicating that excessive IL-6 and MMP-3 were associated with the activated state, and suggested that IL-6 and MMP-3 were related to AS. Our study showed a change in the levels of collagen, FN, IL-6, and MMP-3. After treatment by combined medicine and sulfasalazine, significant declines in the levels of collagen, FN, IL-6, and MMP-3 expression were noted relative to baseline values. The levels of collagen, FN, IL-6, and MMP-3 in the combined group at 3 months of treatment were much lower than those in the sulfasalazine group. This indicates the superiority of combined medicine over sulfasalazine alone in improving fibrosis and inflammation data. This likely is related to BQR and its function of activating blood and resolving stasis, because *drynariae baronii* in BQR had been identified with the function [24].

In conclusion, our study showed that BQR combined with sulfasalazine could improve clinical symptoms and boost treatment of AS with minimal adverse reactions. Furthermore, combined treatment decreased the levels of fibrosis and inflammation, which can be related to its inhibitory action on fibrosis and inflammatory cytokines. Combined treatment was more effective than treatment with sulfasalazine alone, and is a recommended method for treatment of AS.

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Disclosure of conflict of interest

None.

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