Chinese Journal of Integrative Medicine
Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
E-mail: cjim_en@cjim.cn

Original Article

Relation of Blood Arsenic Concentration with Effect and Safety of Arsenic-Containing Qinghuang Powder (青黄散) in Patients with Myelodysplastic Syndrome*

DENG Zhong-yang[1,2], ZHU Shi-rong[1,3], WANG Ming-jing[1,2], FANG Su[4], ZHAO Pan[1,2], ZHU Qian-zhe[1,2], WANG Hong-zhi[1], GUO Xiao-qing[1], XU Yong-gang[1], YI Bo-wen[1], SHANG Xiao-hong[1], MA Rou[1], and HU Xiao-mei[1]

ABSTRACT **Objective:** To investigate the relation of blood arsenic concentration (BAC) with clinical effect and safety of arsenic-containing Qinghuang Powder (青黄散, QHP) in patients with myelodysplastic syndrome (MDS). **Methods:** Totally 163 patients with MDS were orally treated with QHP for 2 courses of treatment, 3 months as 1 course. The BACs of patients were detected by atomic fluorescence spectrophotometry at 1, 3, and 6 months during the treatment, and the effective rate, hematological improvement and safety in patients after treatment with QHP were analyzed. **Results:** After 2 courses of treatment, the total effective rate was 89.6% (146/163), with 31.3% (51/163) of hematological improvement and 58.3% (95/163) of stable disease. The hemoglobin increased from 73.48±19.30 g/L to 80.39±26.56 g/L (P<0.05), the absolute neutrophil count increased from 0.81±0.48×10^9/L to 1.08±0.62×10^9/L (P<0.05), and no significant changes were observed in platelet counts (P>0.05). Among 46 patients previously depended on blood transfusion, 28.3% (13/46) completely got rid of blood transfusion and 21.7% (10/46) reduced the volume of blood transfusion by more than 50% after treatment. The BACs were significantly increased in patients treated for 1 month with 32.17±18.04 μg/L (P<0.05), 3 months with 33.56±15.28 μg/L (P<0.05), and 6 months with 36.78±11.92 μg/L (P<0.05), respectively, as compared with those before treatment (4.08±2.11 μg/L). There were no significant differences of BACs among the patients treated for 1, 3 and 6 months (P>0.05). The adverse reactions of digestive tract during the treatment were mild abdominal pain and diarrhea in 14 cases (8.6%), and no patients discontinued the treatment. The BACs of patients with gastrointestinal adverse reactions were significantly lower than those without gastrointestinal adverse reactions (22.39±10.38 *vs*. 37.89±11.84, μg/L, P<0.05). The BACs of patients with clinical effect were significantly higher than those failed to treatment (40.41±11.69 *vs*. 23.84±12.03, μg/L, P<0.05). **Conclusion:** QHP was effective and safe in the treatment of patients with MDS and the effect was associated with BACs of patients.

KEYWORDS myelodysplastic syndrome, realgar, arsenic, Qinghuang Powder, Chinese medicine

Myelodysplastic syndromes (MDS) is a clonal disorder of hematopoietic stem or progenitor cell, which is characterized by ineffective hematopoiesis of the bone marrow, long-term progressively refractory anemia and frequent development of leukemia.[1] Up to now, MDS is still a kind of refractory disease. The application of Chinese medicine Qinghuang Powder (青黄散, QHP) to treat MDS had achieved a good effect in Department of Hematology, Xiyuan Hospital, China Academy of Chinese Medical Sciences.[2-4] QHP was made up of *Indigo Naturalis* and *Realgar*. *Realgar* is an arsenic-containing mineral with toxicity (As_2S_2 > 90%). As a toxic Chinese medicine, its safety should be at priority. Our previous study has shown that the curative effect was related to the blood arsenic concentration (BAC) of patients after treatment with QHP.[5] We also noticed that in the course of clinical application of QHP, parts of patients had abdominal pain and diarrhea,

*Supported by Beijing Municiple Science and Technology Commission (No. Z141100006014003), National Natural Science Foundation of China (No. 81673821), and Special Research Foundation of Central Level Public Scientific Research Institutes (No. ZZ10-016)

1. Department of Hematology, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing (100091), China; 2. Graduate School of China Academy of Chinese Medical Sciences, Beijing (100700), China; 3. Graduate School of Beijing University of Chinese Medicine, Beijing (100000), China; 4. Zhengyuan Clinic of Nantong, Nantong (226000), Jiangsu Province, China
Correspondence to: Prof. HU Xiao-mei, Tel: 86-10-62835361, E-mail: huxiaomei_2@163.com

DOI: https://doi.org/10.1007/s11655-019-3070-2

Chin J Integr Med 2019 Jul;25(7):497-501

anorexia and nausea, etc.[2,3] These adverse reactions not only brought the abdominal pain to the patients, but also influenced the absorption of arsenic.[5] In this study, the association of BAC with the effect and safety of arsenic-containing QHP in patients with MDS was investigated.

METHODS

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) patients met the diagnosis and classification of MDS according to the diagnosis of MDS criteria in 2007,[6] the World Health Organization classification criteria in 2008,[7] and the prognosis standard of MDS adopted the revised international prognosis scoring system (IPSS-R);[8] (2) patients unused arsenic-containing drugs in the last 2 months before the enrollment; (3) the age of patients was between 18 and 75 years; (4) patients have signed the written informed consent forms.

Exclusion criteria were: (1) patients with severe organ dysfunctions: myocardial enzymes including lactate dehydrogenaseare (LDH), hydroxybutyrate dehydrogenase (HBDH), and creatine kinase isoenzyme (CK-MB) were greater than 490, 372 and 50 IU/L respectively; liver functions including alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamy transpeptidase (GGT) were greater than 100, 100 and 120 U/L, respectively; and kidney function evaluated by creatinine (Cr) was greater than 104 μmol/L; (2) women with pregnancy or lactation; (3) patients with severe mental illness; (4) patients in other trials.

Patients

Totally 200 eligible patients with MDS admitted to the Department of Hematology in Xiyuan Hospital from March 2015 to November 2016 were enrolled. The ages were between 18 and 72 years and the average ages were 56.5±15.5 years. This study was approved by the Clinical Research Ethics Committee of Xiyuan Hospital (Ethics approval No. 2014XL-070-2). Of the 200 patients treated with QHP, there were 37 cases dropped out, of which, 29 patients went to local hospitals for treatment and 8 cases did not take medication according to doctor's advice. Finally, 163 cases were evaluated.

Among 163 patients evaluated, there were 16 cases with refractory anemia (RA), 9 cases with RA with ringed sideroblasts (RARS), 96 cases with refractory cytopenia with multilineage dysplastic (RCMD), 31 cases with RA with excess blasts Ⅰ (RAEB-1), and 11 cases with RAEB-2.

The karyotypes were analyzed in 163 patients, including 92 cases with normal and 71 cases with abnormal karyotypes. In 71 cases with abnormal karyotypes, there were 37 cases with +8, 8 cases with 20q-, 5 cases with 5q-, 4 cases with -7, 7 cases with complex and 10 cases with other karyotypes.

Treatment

Patients were orally administered with arsenic-containing QHP (consisted of *Realgar* 0.1 g and *Indigo naturalis* 0.2 g), prepared by the Preparation Laboratory of Xiyuan Hospital. Among the 163 patients, 60 cases were additionally administered with formula granules including *Radix Paeoniae Alba* 0.96 g, *Atractylodes macrocephala koidz* 0.48 g, *Pericarpium Citri Reticulatae* 0.48 g, and *Radix Saposhnikoviae* 0.48 g; 52 cases were treated with combination of QHP and stanozolol (2–4 mg/d). Blood transfusion was used as the supportive therapy if necessary. Three months were considered as 1 course of treatment and the patients received 2 courses of treatment.

Assessment of BAC

The BAC was tested by atomic fluorescence spectrophotometry (HF-AFS, PyNN, USA, PAS 10.055 Excalibur) in patients treated for 1, 3 and 6 months, respectively. All patients continued to receive the QHP therapy after the end of clinical trials.

Effect Assessment

Effect was assessed according to International Working Group 2006 criteria of MDS,[9] with the main effective indicators as hematological improvement and stable disease. The hematological improvement was assessed by changes of peripheral blood cell counts and blood transfusion.

Safety Assessment

The safety assessment of QHP included symptoms of adverse reactions (including nausea, anorexia, abdominal pain, diarrhea, limb numbness, skin keratinization and swelling) and organ functions. Adverse reaction grading included: (1) light: patients could tolerate the adverse reaction and continue the study without other disposition, and there was no

significant damage of health; (2) moderate: patients' health was damaged and they could not tolerate the adverse reaction; (3) severe: the adverse reaction symptom jeopardized the life of patients who were disabled or died, and they needed to stop the treatment and disposed immediately.[10] The organ functions were analyzed by liver functions, myocardial enzymes, and renal functions.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism software version 6.0. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared with *t* test. Counting data were expressed as a percentage and compared with chi-square test. $P<0.05$ was considered as a statistical difference.

RESULTS

Clinical Effect

Total Effective Rate of QHP

The total effective rate was 89.6% (146/163), with hematological improvement in 51 cases (31.3%), stable disease in 95 cases (58.3%), and treatment failure in 17 cases (10.4%).

Changes of Peripheral Blood Cell Counts before and after QHP Treatment

After QHP treatment, the absolute neutrophil count (ANC) and hemoglobin (HB) counts significantly increased ($P<0.05$), while the counts of platelet (PLT) after treatment unchanged ($P>0.05$), as compared with those before treatment (Table 1).

Table 1. Comparison of Peripheral Blood Cell Counts before and after Treatment with QHP ($\bar{x} \pm s$)

Peripheral blood cell	Case	Before treatment	After treatment
ANC ($\times 10^9$/L)	79	0.81 ± 0.48	1.08 ± 0.62*
HB (g/L)	137	73.48 ± 19.30	80.39 ± 26.56*
PLT ($\times 10^9$/L)	108	44.58 ± 26.26	49.41 ± 34.22

Note: ANC: absolute neutrophil count; HB: hemoglobin; PLT: platelet. *$P<0.05$ *vs*. before treatment

QHP Reduced Blood Transfusion

Before treatment, 46 patients were dependent on blood transfusion. After 2 courses of treatment, 13 cases (28.3%) completely got rid of blood transfusion, and 10 cases (21.7%) reduced the volume of blood transfusion by more than 50%. The average transfusion amount of per patient was significantly decreased after treatment, as compared to before treatment (136.1 *vs*. 320.9, mL/month, $P<0.05$).

Effect of QHP on Patients with Different Karyotypes

Among the 163 patients, the hematologic improvement rates were 39.1% (36/92) in patients with normal karyotype and 32.4% (12/37) in patients with +8 karyotype, which were significantly higher than those with other karyotypes ($P<0.05$). There was no significant difference between patients with normal karyotype and patients with +8 karyotype ($P>0.05$, Figure 1).

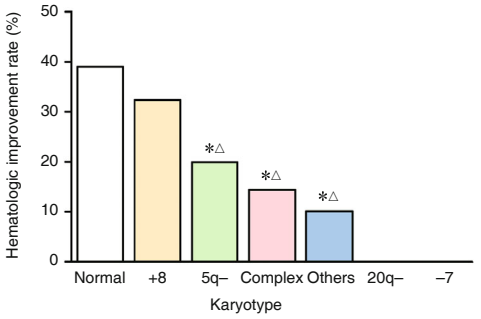

Figure 1. Comparison of Hematologic Improvement Rates in Patients Based on Cytogenetics

Notes: *$P<0.05$ *vs*. patients with normal karyotype; △$P<0.05$ *vs*. patients with +8 karyotype

Effect of QHP on Patients with Different Risks

Among the 163 patients, 52.9% (18/34) patients with very low/low risk achieved the hematologic improvement, which was significantly higher than those with intermediate risk (29/108, 26.9%) or those with high/very high risk (4/21, 19.0%, $P<0.05$, Figure 2).

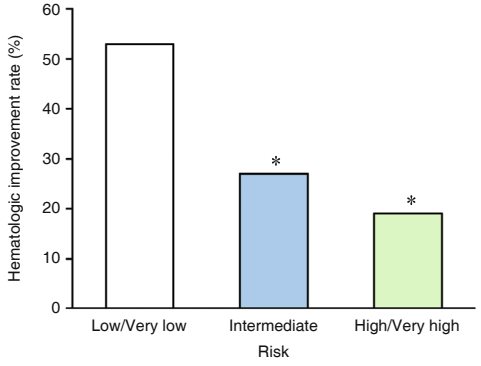

Figure 2. Comparison of Hematologic Improvement Rates in Patients Based on IPSS-R

Note: *$P<0.05$ *vs*. patients with very low/low risk

Changes of BAC before and after Treatment

After treatment for 1, 3 and 6 months, the BACs of patients were all significantly higher than those before treatment ($P<0.05$). There were no significant differences of BACs among patients treated for 1, 3 and 6 months ($P>0.05$, Figure 3).

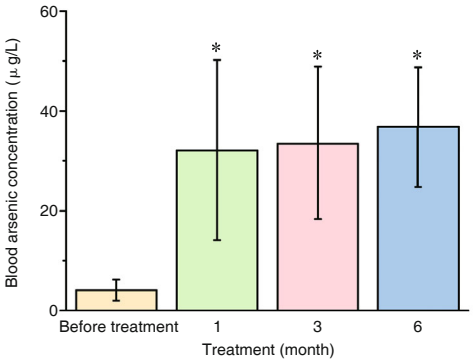

Figure 3. Changes of Blood Arsenic Concentrations in Patients before and after Treatment

Note: *P<0.05 *vs*. before treatment

Clinical Safety

Symptoms of Adverse Reactions

Clinical adverse reactions observed were mainly gastrointestinal symptoms. Out of 163 patients treated for 1 month, abdominal pain and diarrhea, anorexia and nausea, gastric malaise, and numbness were the main symptoms of adverse reactions. No cases with swelling or keratosis was observed. All the clinical adverse reactions were light and tolerated. No patients needed to stop the therapy. The incidence rates of adverse reactions in patients treated for 6 months showed no significant difference compared with those in patients treated for 1 month (P>0.05, Table 2).

Table 2. Symptoms of Adverse Reactions in 163 Patients Treated with QHP [Case (%)]

Symptom of adverse reaction	1-month treatment	6-month treatment
Abdominal pain and diarrhea	14 (8.6)	4 (2.5)
Anorexia and nausea	8 (4.9)	3 (1.8)
Gastric malaise	7 (4.3)	3 (1.8)
Numbness	3 (1.8)	2 (1.2)
Swelling	0	0
Keratosis	0	0

Organ Functions

Before treatment, there were 19 patients with liver dysfunctions including ALT at 67–96 U/L in 16 cases, AST at 46–96 U/L in 12 cases, and GGT at 63–90 U in 8 cases, because they had ever received stanozolol. After 2 courses of treatment, the abnormal index returned to normal in 9 patients, significantly reduced in 10 patients, and no new cases with liver dysfunction were observed.

Before treatment, there were 19 patients with increased myocardial enzymes including LDH at 251–400 IU/L in 19 cases, HBDH at 187–370 IU/L in 17 cases, and CK-MB at 31–48 IU/L in 8 cases because of long-term anemia. After treatment, the abnormal myocardial enzymes returned to normal in 3 cases, decreased obviously in 16 cases, and no new cases with abnormal myocardial enzymes were observed. No cases with renal dysfunction were observed before and after treatment.

Relation of BACs with Adverse Reactions

The BACs of patients with gastrointestinal adverse reactions were significantly lower than those without gastrointestinal adverse reactions (22.39 ± 10.38 *vs*. 37.89 ± 11.84 μg/L, P<0.05).

Relation of BACs with Effective Rate

The BACs of patients with effective rate were significantly higher than those failed to treatment (40.41 ± 11.69 *vs*. 23.84 ± 12.03 μg/L, P<0.05).

DISCUSSION

Our previous studies have indicated that QHP was effective in the treatment of MDS.[2-4] At the same time, the adverse reactions of QHP, especially the digestive reactions caused by *Realgar*, were also observed.[3,4] Our further studies showed that proper BAC was the key to obtain the curative effect in the treatment of MDS.[5] It was also found that diarrhea could reduce the absorption of arsenic-containing QHP in digestive tract and then reduced the effect because of insufficient BAC.[5]

The formula granules were added in the prescription of 60 patients, in addition to QHP, to prevent the gastrointestinal adverse reactions. Our previous data documented that the formula granules decreased the digestive adverse reactions and increased the effect in the treatment of patients with MDS.[11,12] In this work, the results confirmed that QHP was effective in the treatment of MDS, with 31.3% of hematological improvement, increased HB and ANC counts. It was noteworthy that there were approximately 50% of patients dependent on blood transfusion reduced or got rid of blood transfusion after treatment with QHP. Our previous *in vitro* studies showed that the active ingredient (As_2S_2) of *Realgar*, as the main drug contained in QHP, could induce the cell differentiation in acute myeloid leukemia cells progressed from MDS,[13-16] which might explain one of the mechanisms of QHP in the treatment of MDS.

The safety of *Realgar* has always been a concern because of containing arsenic. In this study,

the results showed that the incidence rate of tolerated adverse reactions, mainly gastrointestinal symptoms, was less than 9% in patients during the treatment with QHP and there were no cases needed to be stopped the therapy. The abnormal liver functions or cardiac enzymes in patients who had ever been treated with stanozolol or had long-term anemia, were improved after treatment. There were no new cases with abnormal liver functions, cardiac enzymes and renal functions after treatment. These data suggested that QHP is safe in the treatment of MDS, which was consistent with our previous studies.[3,4,11,12]

The relation of BAC with gastrointestinal adverse reactions and relation of BAC with clinical effect suggested that the gastrointestinal adverse reactions affected the absorption of arsenic and then decreased the BAC, which affected the effect. Our results even more revealed that patients with normal or +8 karyotype had a better response to QHP as compared with those with other karyotypes, based on cytogenetics. As well as, patients with very low/low risk could achieve a higher rate of hematologic improvement, according to IPSS-R.

Overall, QHP was effective and safe in the treatment of patients with MDS and its clinical effect was associated with BAC. It was also suggested that QHP is a good candidate for MDS patients with normal or +8 karyotype, or/and with very low/low risk.

Conflict of Interest

No conflict.

Author Contributions

Hu XM contributed to the study design; Deng ZY, Zhu SR, Wang MJ and Hu XM wrote the manuscript; Hu XM, Zhao P, Deng ZY, Fang S, and Zhu QZ performed the clinical research; Wang HZ, Guo XQ, Xu YG, and Shang XH performed the diagnosis; Ma R checked the diagnosis; Yi BW prepared the formula granules; Deng ZY, Wang MJ, Zhao P, and Zhu QZ performed the data processing and statistical analysis. All authors read and agreed on the final version of the manuscript.

REFERENCES

1. Garcia-Manero G. Myelodysplastic syndromes: 2015 Update on diagnosis, risk-stratification and management. Am J Hematol 2015;90:831-841.
2. Hu XM, Liu F, Ma R. Application and assessment of Chinese arsenic drugs in treating malignant hematopathy in China. Chin J Integr Med 2010;16:368-377.
3. Xu S, Ma R, Hu XM, Xu YG, Yang XH, Wang HZ, et al. Clinical observation of the treatment of myelodysplastic syndrome mainly with Qinghuang Powder. Chin J Integr Med 2011;17:834-839.
4. Sun SZ, Ma R, Hu XM, Yang XH, Xu YG, Wang HZ, et al. Karyotype and DNA-methylation responses in myelodysplastic syndromes following treatment with traditional Chinese formula containing arsenic. Evid Based Complement Alternat Med 2012;969476. doi: 10.1155/2012/969476.
5. Ma JL, Qu WW, Hu XM. Correlationship of clonal selection of treatment with arsenious compound formula Qinghuang Powder with *in vivo* effects of arsenic in patients with myelodysplastic syndrome. Chin J Inform Tradit Chin Med (Chin) 2013;20:8-10.
6. Valent P, Horny H, Bennett J M, Fonatsch C, Germing U, Greenberg P, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: consensus statements and report from a working conference. Leukemia Res 2007;31:727-736.
7. Swerdlow SH, Campo E, Harris NL, eds. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC;2008:88-93.
8. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 2012;120:2454-2465.
9. Tefferi A, Barosi G, Mesa RA, Cervantes F, Deeg HJ, Reilly JT, et al. International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia, for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). Blood 2006;108:1497-1503.
10. Zheng XY, ed. Guidelines for the clinical research of new traditional Chinese medicine (Trial). Beijing: China Medical Science and Technology Press; 2002:19-20.
11. Wang Y, Fang S, Song MM, Hu XM. Safety of Compound Qinghuang Powder in patients with myelodysplastic syndromes. Int J Tradit Chin Med (Chin) 2014;36:1074-1077.
12. Wang Y, Fang S, Deng ZY, Song MM, Ma JL, Yang XP, et al. Compound Qinghuang Powder in the treatment of patients with myelodysplastic syndromes. Int J Tradit Chin Med (Chin) 2015;37:1091-1095.
13. Hu XM, Tanaka S, Onda K, Yuan B, Toyoda H, Ma R, et al. Arsenic disulfide induced apoptosis and concurrently promoted erythroid differentiation in cytokine-dependent myelodysplastic syndrome-progressed leukemia cell line F-36p with complex karyotype including monosomy 7. Chin J Integr Med 2014;20:387-393.
14. Hu XM, Yuan B, Tanaka S, Song MM, Onda K, Tohyama K, et al. Arsenic disulfide-triggered apoptosis and erythroid differentiation in myelodysplastic syndrome and acute myeloid leukemia cell lines. Hematology 2014;19:352-360.
15. Hu XM, Yuan B, Song MM, Onda K, Tanaka S, Toyoda H, et al. Dose-dependent biphasic effects of arsenic disulfide on differentiation and apoptosis of HL-60 cells. Curr Topics Pharmacol 2014;17:13-25.
16. Hu XM, Yuan B, Tanaka S, Zhou QB, Onda K, Toyoda H, et al. Involvement of oxidative stress associated with glutathione depletion and p38 MAPK activation in arsenic disulfide-induced differentiation in HL-60 cells. Leuk Lymphom 2014;55:392-404.

(Accepted December 11, 2017)
Edited by YU Ming-zhu