Clinical Observations

Safety of Individual Medication of Ma Qian Zi (Semen Strychni) Based upon Assessment of Therapeutic Effects of Guo's Therapy Against Moderate Fluorosis of Bone

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Objective: To assess the safety of individual medication of Guo's Ma Qian Decoction on the basis of effective treatment of fluorosis of bone with Guo's therapy.

Methods: One hundred and fourteen cases of moderate fluorosis of bone were randomly divided into a treatment group (n=60) and a control group (n=54) between December 2007 and August 2009 by using the block randomized method and a central random system. At the same time of basic treatment, the patients in the treatment group were orally administrated with Guo's Ma Qian Decoction. The initial dose of Ma Qian Zi (Semen Strychni) was 0.4 g and increased by 0.05 g every two days, with the doses of other drugs unchanged, until the patient had "nux vomica response". For the patients with no "nux vomica response", the dosage was continued to increase and the maximum dosage was not more than 1.2 g/day. The control group was treated with decoction placebo. The changes of strychnine and brucine contents before and after processing and after decoction of Ma Qian Zi (Semen Strychni) were determined with reversed-phase high-performance liquid chromatography, which were controlled within ranges stipulated in the Pharmacopeia; Adverse events were analyzed; Blood strychnine and brucine contents in 10 cases who had taken the drugs were determined.

Results: 1) Strychnine (2.125%) and brucine (1.425%) contents before processing of Ma Qian Zi and 1.88% and 1.31% after processing all conformed with the standards of strychnine (1.2–2.2%) and brucine (no less than 0.8%) stipulated in the Pharmacopeia. When the maximum dosage of Ma Qian Zi was 1.2 g/day, strychnine in the decoction was 11.17 mg and brucine was 7.44 mg, which all conformed with the maximum limited amount (strychnine 13.32 and brucine no less than 4.8 mg) stipulated in the Pharmacopeia. 2) Eight cases had "nux vomica response" in the treatment group and one case in the control group, with a significant difference between the two groups (P<0.05). 3) Altogether 18 cases had adverse events, with an incidence rate of 15.38% (8 cases) in the treatment group and 18.52% (10 cases) in the control group, with no difference between the two groups (P>0.05); Among them, 10 cases (8.77%) with the adverse event were not related with therapeutic drugs, with an incidence rate of 6.67% (4 cases) in the treatment and 11.11% (6 cases) in the control group, with no significant difference between the two groups (P>0.05). Seven cases had suspicious relative adverse events, the risk in the treatment group was 0.658 times of the control group, with no significant difference (P>0.05), and one case had the toxic reaction of nux-vomica seed. 4) Strychnine and brucine were unable to be detected in the blood in all points of time in the 10 cases who had taken the drugs, indicating that plasma strychnine and brucine contents were lower than the minimum detectable amount (10 ng), and accumulation of strychnine and brucine were not found in blood of the patient during and after administration for 8 weeks.

Conclusion: The individual medication of Ma Qian Zi (Semen Strychni) in the Guo's therapy has a better safety.

Keywords: *safety; Ma Qian Zi (Semen Strychni); Guo's Ma Qian Decoction; Guo's therapy; individual medication*

"Guo's therapy" has been stated to improve the symptoms of moderate endemic fluorosis of bone.[1–4] Guo's Ma Qian Decoction is one of main therapies for this disease. In Guo's Ma Qian Decoction, prepared Ma Qian Zi (Semen Strychni) was used as the monarch drug. A dose of 0.4–1.2 g, regulated according to the reaction caused by the treatment, has a therapeutic effect. The clinical dosage of Ma Qian Zi (Semen Strychni)[5] has been strictly limited in the Chinese Pharmacopeia. In clinical studies using Ma Qian Zi, the dosages used were within the dose stipulated in the Chinese Pharmacopeia.[6–11] However, clinical reports focusing on the dosage of individual medications combined with safety assessment are lacking. In the present study, the

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safety of Ma Quan Zi individual medication in Guo's Ma Quan Decoction was studied based on the research of Guo's therapy for moderate endemic fluorosis of bone.

METHODS

Clinical Data and Grouping

One-hundred and twenty cases of moderate endemic fluorosis of bone confirmed by clinical means in the Xinzhou region of Shanxi Province from December 2007 to August 2009 were randomly divided into a treatment group and a control group. This was done using a block random method and a central random system (Clinical Research Data Collection and Management System of the Affiliated Hospital of Nanjing University of Chinese Medicine). At the end of the treatment, 6 cases were excluded from the study, leaving a study population of 114 cases (56 male and 58 female). In the treatment group, 26 cases were male and 34 cases were female (age range, 39–60 years; mean age, 51.68±4.98 years). In the control group, 30 cases were male and 24 cases were female (39–60 years; 52.15±4.68 years).

Diagnostic and Exclusion Criteria

The diagnostic criteria and scale criteria for endemic fluorine poisoning in "Medical Profession Criteria of The People's Republic of China (WS 192-2008)"[12] were used.

The exclusion criteria are referred to in the literature.[1] Twelve exclusion criteria were used: (i) subjects with osteoarthritis, rheumatic arthritis, ankylosing spondylitis, or rheumatoid arthritis; (ii) pregnant and breastfeeding women; (iii) subjects with mental disorders; (iv) patients with a history of allergic reactions to Ma Qian Zi (Semen Strychni); (v) individuals who had taken drugs related to skeletal or dental fluorosis within the previous month, such as non-steroidal anti-inflammatory drugs, cortical hormones or Chinese drugs containing Ma Qian Zi (Semen Strychni); (vi) subjects with severe disease of the heart, liver, lung, kidney; blood-based diseases, cancer or AIDS; (vii) individuals with abnormal renal function; (viii) subjects with alanine aminotransferase (ALT) levels twice the upper limit of normal values; (viii) abnormal and clinically significant electrocardiogram; (x) chest radiograph indicating infection, tuberculosis or tumor; (xi) subjects living in an area in which the fluorine content in water was > 3.9 mg/L; and (xii) individuals participating in clinical trials of other drugs.

Individual Treatment Methods

Simultaneously with basic treatment, patients in the treatment group were orally administrated Guo's Ma Qian Decoction[1] (Shanxi Xinzhou Pharmaceutical Group) twice (200 mL each time) for 8 consecutive weeks. The initial dose of Ma Qian Zi (Semen Strychni) was 0.4 g and was increased by 0.05 g every 2 days. The maximum dose was 1.2 g/day (the doses of other drugs were kept unchanged) until the patient had a "nux-vomica response": slight sweating, tongue rigidity, girdle sensation, and slight muscular twitch. At this time, the dose of Ma Qian Zi (Semen Strychni) in the decoction was left unchanged or reduced by 0.05 g until disappearance of a nux-vomica response: this was the treatment maintenance dose of Ma Qian Zi (Semen Strychni) in Guo's Ma Qian decoction. The next day, Guo's Ma Qian Decoction containing the treatment maintenance dose of Ma Qian Zi (Semen Strychni) started to be used. For patients in whom the nux-vomica response did not appear, the dose was increased day-by-day until 1.2 g/day according to the method described above. Patients in the control group were administered decoction placebo. After taking Guo's Ma Qian Decoction for 8 weeks, patients in the two groups were administered with Gu Kang Ning Jiao Nang (Capsules for Healthy Bone[1], Shanxi Province Heruida Pharmaceutical Co. Ltd., batch number JZ20070024) and a placebo capsule, respectively.

Observations

1. Strychnine contents before and after Ma Qian Zi (Semen Strychni) processing and in the decoction

Ma Qian Zi (Semen Strychni) was purchased once only and was supplied by the Shanxi Province Xinzhou City Special Hospital of Fluorosis (Xinzhou, Shanxi, China, batch number 070512). The brucine and strychnine contents in crude Ma Qian Zi (Semen Strychni), in prepared Ma Qian Zi, and in the Guo's Ma Qian Decoction, were determined according to the methods described in the Chinese Pharmacopeia.[13]

2. Nux-vomica response

Responses related to the treatment maintenance dose (slight sweating, tongue rigidity, girdle sensation, slight muscular twitch) were investigated.

3. Classification of adverse events

All adverse events (abnormal laboratory results, adverse clinical events, toxic reactions to Ma Qian Zi (Semen Strychni)) were recorded. The correlation between the drug and adverse events was based on five classifications: positive correlation; very possible correlation; possible correlation; suspicious correlation; and impossible correlation.

4. Blood contents of alkaloids of Ma Quan Zi (Semen Strychni)

High-performance liquid chromatography (HPLC) analyses showed a linear relationship (regression equation for strychnine: Y=4.8835+1.1708X, r=0.9962; brucine: Y=6.1819+1.4085X, r=0.9937). The rate of recovery (n=12) for strychnine was 91.0% (relative standard deviation (RSD) 4%), and for brucine was 91.63% (RSD 4.47%). The intra-day precision for strychnine was RSD 3.53% and for brucine was RSD 5.21%); The stability of strychnine was RSD 3.96%, and fro brucine was RSD 5.70%. To determine the detection

method of the blood content of Ma Qian Zi (Semen Strychni), the lowest detectable amount was 5.0 ng and the quantitative limitation was 10 ng.

The blood in 10 cases (5 males and 5 females) administered the decoction was taken before administration as well as 15 min after administration in the first, second, third, fourth and eighth weeks after administration. Samples (100 μL) were prepared according to the detection method for the blood content of Ma Qian Zi (Semen Strychni).

5. Statistical analyses

Descriptive statistical methods were adopted using SAS 6.12 software (SAS, Raleigh, NC, USA). Adverse events in the two groups were compared using the χ^2 test (two-sided). $P<0.05$ was considered significant. Evaluation of the relative risk (RR) of abnormal laboratory values after treatment was also carried out.

RESULTS

Contents of Alkaloids in Ma Qian Zi and Ma Qian Decoction

According to the China Pharmacopeia (2005 edition)[5], strychnine content in crude Ma Qian Zi was 1.20%–2.20%, and brucine content was not less than 0.80%. HPLC results indicated that strychnine content (2.125%) and brucine content (1.425%) in crude Ma Qian Zi were in accordance with the values in the Chinese Pharmacopeia (Table 1).

Table 1. Contents of alkaloids in crude Ma Qian Zi

Batch No.	No.	Strychnine (%)	Brucine (%)
070512	1	2.13	1.43
	2	2.12	1.42
Mean		2.125	1.425

After processing of Ma Qian Zi, strychnine content (1.88%) and brucine content (1.31%) detected by HPLC were in accordance with the values in the Chinese Pharmacopeia (Table 2).

When the prepared Ma Qian Zi in Guo's Ma Qian Decoction was increased by 0.4, 0.6, 0.8, 1.0, and 1.2 g/day dose in order, and at the maximum added amount of 1.2 g, the strychnine content was 11.17 mg and the brucine content was 7.44 mg. These values were in accordance with the maximum amount stipulated in the Chinese Pharmacopeia (after calculation, when the prepared Ma Qian Zi was 0.6 g, the amount of strychnine was 13.32 mg and that of brucine was not less than 4.8 mg, Table 2).

Table 2. Comparison of alkaloid contents in Guo's Ma Qian Decoction and single Ma Qian Zi

Prepared Ma Qian Zi (dosage/day)	Contents of alkaloids					
	Value stated in the Chinese Pharmacopeia (mg)		Detected value of prepared Ma Qian Zi (mg)		Guo's Ma Qian Decoction (mg)	
	Strychnine (upper limit, 2.2%)	Brucine (not less than 0.8%)	Strychnine (1.88%)	Brucine (1.31%)	Strychnine (corresponding to raw drug, %)	Brucine (Corresponding to raw drug, %)
0.4	8.80	3.20	7.52	5.24	3.82 (50.80)	2.02 (38.55)
0.6[1]	13.32[1]	4.80[1]	11.28	7.86	5.97 (52.93)	2.53 (32.19)
0.8	17.76	6.40	15.04	10.48	7.82 (51.99)	4.68 (44.66)
1.0	22.20	8.00	18.80	13.10	10.03 (53.35)	6.37 (48.63)
1.2[2]	26.64	9.60	22.56	15.72	11.17[2] (49.51)	7.44[2] (47.33)

Notes: 1. The theoretical value calculated according to the Chinese Pharmacopeia (2005 edition), when the upper limit was used (strychnine 2.2%; brucine was not less than 0.8%); 2. The contents of alkaloids when the maximum dosage (1.2 g/day) of prepared Ma Qian Zi was used.

Nux-vomica Response

The prevalence of the nux-vomica response was 13.33% (8 cases) in the treatment group and 1.85% (1 case) in the control group: this difference was significant ($P<0.05$) (Table 3).

Table 3. Comparison of the prevalence of a nux-vomica response between the two groups

Group	Response cases (*n*)		Total cases (*n*)	Prevalence (%)
	Yes	No		
Treatment	8	52	60	13.33
Control	1	53	54	1.85
Total	9	105	114	7.89

Notes: χ^2 value (continuity correction)=5.152, $P=0.023<0.05$.

Adverse Events

Of the 114 cases, 18 cases (15.38%) had clinically observed adverse events. The prevalence of adverse events was 15.38% (8 cases) in the treatment group and 18.52% (10 cases) in the control group: this difference was not significant ($P>0.05$, Table 4).

Of the 18 cases with adverse events, 10 cases (8.77%) were not related to treatment. Among them, 4 cases (6.67%) in the treatment group and 6 cases (11.11%) in the control group were not related to treatment; this difference was not significant ($P>0.05$, Table 5).

Table 4. Comparison of the prevalence of adverse events between the two groups

Group	Adverse events cases (*n*)		Total cases (*n*)	Prevalence (%)
	Yes	No		
Treatment	8	52	60	15.38
Control	10	44	54	18.52
Total	18	96	114	15.79

Notes: χ^2 value (Pearson chi-square)=0.575, $P=0.448>0.05$.

In the treatment of moderate fluorosis of bone with Guo's therapy, electrocardiography (ECG), blood, urine, as well as functions of the liver and kidney were examined. Most of these parameters had no significantly different abnormal changes in the two groups before and after treatment (Table 6). Only 7 cases had abnormal red blood cells (RBCs) in urine which, after causality analyses, was found to be weakly related to treatment. The risk of abnormal RBCs in urine in the treatment group was 0.658-times that in the control group (total RR 95% CI 0.140–3.082) with no significant difference between the two groups (P>0.05, Table 7).

Table 5. Comparison of the prevalence of adverse events between the two groups that was not related to treatment

Group	Adverse events cases (n)		Total cases (n)	Prevalence (%)
	Yes	No		
Treatment	4	56	60	6.67
Control	6	48	54	11.11
Total	10	104	114	8.77

Note: χ^2 value (Pearson chi-square) 0.702, P=0.402>0.05.

In the treatment group, one case related to adverse events of treatment was a toxic response to Ma Quan Zi: this response disappeared after dose reduction.

Table 6. Comparison of ECG and laboratory results after treatment between the two groups

Index	Result became abnormal after treatment (n)		Abnormal/normal (%)	
	Treatment group (n=60)	Control group (n=54)	Treatment group (n=60)	Control group (n=54)
ECG	0	0	0 (0/60)	0 (0/54)
WBC	0	0	0 (0/60)	0 (0/54)
RBC	0	0	0 (0/60)	0 (0/54)
HGB	0	0	0 (0/60)	0 (0/54)
PLT	0	0	0 (0/60)	0 (0/54)
RBCs in urine	3	4	5.00 (3/60)	7.41 (4/54)
Routine examination of stools	0	0	0 (0/60)	0 (0/54)
ALT	0	0	0 (0/60)	0 (0/54)
AST	0	0	0 (0/60)	0 (0/54)
AKP	0	0	0 (0/60)	0 (0/54)
BUN	0	0	0 (0/60)	0 (0/54)
Scr	0	0	0 (0/60)	0 (0/54)

Notes: ECG=electrocardiography, WBC=white blood cell, RBC=red blood cell, HGB=hemoglobin, PLT=platelet, ALT=alanine aminotransferase, AST=aspartate aminotransferase, AKP=alkline phosphatase, BUN= blood urea nitrogen, and Scr=serum creatinine.

Table 7. Comparison of the prevalence of normal RBCs in urine turning to abnormal RBCs between the two groups

Group	Normal turning to abnormal (n)		Total (n)	Prevalence (%)
	Yes	No		
Treatment	3	57	60	5.00
Control	4	50	54	7.41
Total	7	107	114	6.14

Notes: RR=0.658, χ^2 value (Pearson chi-square)=0.286, P=0.593>0.05.

Drug Contents in Blood

Strychnine and brucine could not be detected in the blood of the 10 patients who were randomly sampled each time. Blood samples possibly did not contain strychnine and brucine, or the concentrations of strychnine and brucine were below the lower limit of detection (100 ng/mL). Accumulation of strychnine and brucine in the blood of these patients was not found during administration and after administration for 8 weeks.

DISCUSSION

Significance of Ma Qian Zi Individual Medication

Ma Qian Zi (Semen Strychni) has been used clinically for ≈1000 years, so its therapeutic and adverse effects are known. It is regarded as a poisonous substance because the main components are strychnine and brucine. The median lethal dose (LD50) of strychnine and brucine as well as the kernel of nux vomica for intragastric administration in mice was found to be (in $mg \cdot kg^{-1}$) 3.27, 233 and 235, respectively.[14] The toxicity of strychnine is the largest. For an adult, the toxic amount is 5–10 mg, the lethal dose is 50–120 mg and, in human poisoning, the concentration of strychnine in the blood is 0.2 mg/mL.[15] For strychnine, the commonly used dose for adults (6 mg) is close to the toxic dose. Hence, Ma Qian Zi (Semen Strychni) is the most suitable for individual medication. Hence, the dosage is incrementally increased to attain the best therapeutic effect while ensuring that toxicity is kept to a minimum.

Contents of Alkaloids after Processing and Joint Decocting of Ma Qian Zi

Quality control of the processing of Ma Qian Zi (Semen Strychni) and joint decocting in the decoction as well as determination of the contents are key factors for ensuring the stability of alkaloid contents.

Firstly, the quality of crude Ma Qian Zi should conform to the criteria in the Chinese Pharmacopeia.[5] After single processing, the contents of strychnine and brucine also should conform to the criteria in the Chinese Pharmacopeia.[5] In the present study, strychnine and brucine contents before and after processing of Ma Qian Zi (Semen Strychni) and after joint decocting in the decoction conformed to these requirements.

For the quality control of decoction, standard rules of operation were established, and alkaloid contents in three batches of the decoctions added with 0.4, 0.6, 0.8, 1.0, and 1.2 g Ma Qian Zi (Semen Strychni) determined. The Chinese Pharmacopeia (2005 ed) stipulates that the upper limit of Ma Qian Zi in Wan (pills) and San (powder) is 0.6 g. In the present study, the highest dose of Ma Qian Zi (Semen Strychni) in the decoction was 1.2 g, with strychnine representing 11.17 mg and brucine 7.44 mg, which was 83.86% and 155% of the highest dose in the Pharmacopeia, respectively (when the prepared Ma Qian Zi (Semen Strychni) was 0.6 g, the upper limit of strychnine was 13.32 mg and that of brucine was not less than 4.8 mg), thereby conforming to the stipulation in the Chinese Pharmacopeia. Therefore, the dose of prepared Ma Qian Zi (Semen Strychni) in the decoction was beyond the upper limit given in the Chinese Pharmacopeia, but the contents of alkaloids were within the range stipulated. Hence, the dose was safe.

Nux-vomica Response

The treatment dose of Ma Qian Zi (Semen Strychni) is close to its toxic dose. According to the literature,[16–18] adverse reactions can be divided into mild, moderate and severe.[17] In this classification, mild reactions are represented by fever, headache, dizziness, palpitation, nausea and vomiting, stomach ache, tongue numbness, sweating, restlessness, blood-pressure increase, respiratory strengthening, mild muscular twitching, girdle sensation, and mild mental abnormality. Moderate adverse reactions include tonic spasm of entire muscles, and gaze shifting of both eyes. Severe adverse reactions include spasmodic contraction of respiratory muscles, convulsion, and dyspnea.

Clinical research[2,4] has shown that the highest dose of Ma Qian Zi results in the best therapeutic effect against the fluorosis of bone. A critical biochemical index for assessment of the improvement of fluorosis of bone is not available, so the nux-vomica response is used as basis of dose control of Ma Qian Zi. The present study demonstrated that dose regulation according to appearance or non-appearance of the nux-vomica response was safe. However, to ensure safety, the maximum dosage of Ma Qiann Zi (Semen Strychni) should be ≤1.2 g/day.

Of the 60 cases who took the drug, 52 subjects did not have a nux-vomica response during treatment. Eight cases had a nux-vomica response once during treatment; for doses of 0.4, 0.5, 0.55, 0.75, 1.1, and 1.2 g/day, one case for each dose was noted; for 1.15 g/day, 2 cases were noted; 12-h later, the response disappeared. The dose was regulated to 1.2 g/day according to the therapeutic program. Another case had dizziness, as well as numbness in the right side of the face and limbs that was not alleviated after 1 day when the dose of Ma Qian Zi (Semen Strychni) was 1.1 g/day. After the dose of Ma Qian Zi was reduced to 1.05 g/day, the symptoms disappeared, and this dose was maintained until the end of the therapeutic course. After analyses, this subject was regarded as having a toxic reaction to Ma Qian Zi (Semen Strychni).

Adverse Events

Eighteen cases with adverse drug events were found in the two groups. After analyses of causality, 10 cases had general adverse events with no relationship to the therapeutic drug: respiratory tract infection (8 cases), fracture (1 case) and appendicitis (1 case). In the treatment group, there were 7 cases with suspicious adverse events; all of them had occult blood in urine with abnormal laboratory results. The risk of an abnormal value in the treatment group was only 0.658-times that in the control group: this difference was not significantly different. Three months after suspension of drug administration, normality was restored. No abnormally was found at follow-up after 1–1.5 years. During administration of Guo's Ma Qian Decoction, ECG, blood parameters, stool parameters, as well as hepatic and renal functions, did not show abnormalities. Toxicity was found in 1 case but the reaction disappeared when the dose was reduced.

Determination of Drugs in Blood

The concentration of alkaloids of Ma Qia Zi (Semen Strychni) was determined in 10 cases taking Guo's Ma Qian Decoction to evaluate the concentrations of strychnine and brucine in blood so as to ensure safety of the medication. Concentrations of strychnine and brucine in the blood 1, 2, 3, 4, and 8 weeks after treatment were <100 ng/mL (the lowest detectable amount was 10 ng). This finding suggested that strychnine and brucine did not accumulate in blood during administration and after administration for 8 weeks. At the eighth week of administration, the dose of Ma Qian Zi (Semen Strychni) was 1.2 g/dose/day.

In the present study, the lowest detectable amount for the alkaloids of Ma Qian Zi was lower than those in other

reports.[19-21] Among those reports, the blood concentration in human poisoning was 2 μg/mL,[15] which is 20-times that of the lowest amount detectable. According to the literature report, the commonly used dose of strychnine for adults is 6 mg, and the toxic amount of single daily administration is 5–10 mg. Calculation revealed that when the maximum dosage of prepared Ma Qian Zi (Semen Strychni) was 1.2 g/day (more than one-fold the maximum dosage stipulated in the Chinese Pharmacopeia), and that the strychnine content in the decoction was 11.17 mg. Because the decoction was taken in two portions, it was relatively safety. Also, strychnine and brucine were not detected in the plasma of patients.

The key to Guo's therapy is individual medication of Ma Qian Zi (Semen Strychni). The program involves progressively small increases in the drug dose, quality control of the raw materials, quality control of the decoction, analyses of adverse events, and monitoring of drug concentrations in the blood. The present study demonstrated that, in the treatment of moderate endemic fluorosis of bone with Guo's therapy, individual regulation of Ma Quan Zi (Semen Strychni) dosage can ensure therapeutic effects[1] and increase the safety of Ma Quan Zi (Semen Strychni) in decoction application.

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