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Effect of Xingnaojing injection on rifampicin concentration in cerebrospinal fluid and prognosis of patients with severe tuberculous meningitis

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Abstract

Objective: To observe whether an Xingnaojing 醒脑静 injection could improve the prognosis of patients, by increasing rifampicin penetration through the blood-brain barrier. *Methods:* Patients with severe tuberculous meningitis were enrolled in this study. The concentrations of Xingnaojing in cerebrospinal fluid and blood in patients treated with Xingnaojing and control were determined by high performance liquid chromatography. The changes in cerebrospinal fluid and the improvement of clinical symptoms and signs, were evaluated two weeks after admission. The long-term prognosis of the patients in the two groups were evaluated by the Glasgow Outcome Scale (GOS). *Results:* The concentration of rifampicin in cerebrospinal fluid was significantly higher in the Xingnaojing group (1.77±0.17 μg/mL), than in the control group (1.27±0.16 μg/mL, $p<0.05$). The difference in concentration of rifampicin in the blood was not significant ($P>0.05$). The short-term effective rate of the Xingnaojing group was 92.5% (37/40), which was significantly higher than that of the control group (80%, 32/40, $p<0.05$). After 6 months, 75% (30/40) of the Xingnaojing group had good prognosis according to the GOS score, whereas that of the control group was 50% (20/40) showing significantly better long-term treatment effect of the Xingnaojing group compared to the control group ($P<0.05$).
Conclusion: Xingnaojing injection improved rifampicin penetration into the central nervous system. The increase in rifampicin concentration in cerebrospinal fluid improved outcomes in patients with severe tuberculous meningitis.

Keywords: Xingnaojing injection; rifampicin; severe tuberculous meningitis; high performance liquid chromatography

INTRODUCTION

Tuberculous meningitis (TBM) is a non-suppurative meningitis caused by *Mycobacterium tuberculosis*, and it is the most serious infection of *Mycobacterium tuberculosis*. Although TBM accounts for only approximately 1% of *Mycobacterium tuberculosis* infections, the mortality and long-term disability rates are very high.[1-3] Ensuring effective drug concentration in cerebrospinal fluid (CSF) has always been one of the main criteria for selecting anti-tuberculosis drugs. Among the current first-line drugs, isoniazid and rifampicin are the cornerstones of anti-tuberculosis treatment. Due to the existence of blood-brain barrier (BBB), rifampicin often cannot reach effective concentration in CSF at the recommended dose in current guidelines. Increasing the dose of drugs or combining drugs with better blood-brain barrier permeability are new treatment strategies based on the pharmacokinetics of these antibiotics.[4] Xingnaojing 醒脑静 injection was shown to temporarily improve the permeability of BBB and improve the therapeutic effect of central nervous system medications.[5] In the present study, 80 patients with severe TBM who were admitted to our hospital between May 2014 and August 2018 were enrolled to investigate the effect of Xingnaojing injection on rifampicin concentration in the CSF and prognosis of patients with severe TBM.

METHODS

A total of 80 patients who were diagnosed

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with severe TBM at Chongqing Sanxia Central Hospital, between May 2014 and August 2018 were enrolled into this study. Patients met the following two criteria: A. The diagnostic criteria for TBM[6]: 1. Patients met the diagnostic criteria of chronic meningitis, including headache, fever, meningeal irritation signs, and non-suppurative inflammation changes in CSF. 2. Cerebrospinal fluid Indian ink staining for cryptococcus was negative; patients with meningitis caused by cryptococcal meningitis, and other causes (viruses, bacteria, spirochetes, etc.) were excluded. 3. Patients with active pulmonary tuberculosis or extrapulmonary tuberculosis. 4. Imaging revealed meningeal enhancement of the skull base, hydrocephalus or intracranial tuberculoma. Patients met the above 1+2+3, 1+2+4, and met all four items were included in the study. In addition, they were actively treated with anti-tuberculous drugs. B. The severity of TBM was graded according to the modified MRC system: (I) Grade I, Glasgow Coma Scale (GCS) score of 15 with no focal neurology, (II) Grade II, GCS score of 11-14 or GCS score of 15 with focal neurological signs, (III) Grade III, GCS score of ≤10. Patients in grade II or grade III were classified as having severe TBM.[7,8] Exclusion criteria: Patients with severe diseases of the immune, cardiovascular and cerebrovascular and other systems that may have an impact on the results of the study. Patients who have experienced cerebrovascular events, or have had an infection other than tuberculosis. Patients who were allergic to the drugs or devices used in the study. After admission, gender, age, course of disease, GCS at admission, routine cerebrospinal fluid test, biochemistry, opening pressure, CSF sugar/blood sugar ratio and CSF cytological classification were recorded in all patients. The present study was approved by the Ethics Committee of our hospital. Patients and their families provided signed informed consent.

Therapeutic methods

Patients were randomized using random number table into two groups: control group and Xingnaojing group (n=40, each). Patients in the Xingnaojing group were given 20 ml of intravenous Xingnaojing injection daily, and intravenous rifampicin 30 minutes later. Xingnaojing was given for two weeks. Standardized and individualized anti-tuberculosis therapy was adopted according to the course of disease and the history of anti-tuberculosis medication.[9,10] The specific procedures were as follows: All patients were treated with quadruple anti-tuberculosis treatment with isoniazid, rifampicin, pyrazinamide and ethambutol; the anti-tuberculosis treatment regimen was 3 HRZE/9-15 HR (3 months of quadruple therapy, and 9-15 months of isoniazid and rifampicin.) The dosages were isoniazid (10 mg/kg), rifampicin (15 mg/kg), pyrazinamide (30 mg/kg) and ethambutol (20 mg/kg). Patients were given 20 mg/d of dexamethasone sodium phosphate (TaifiGroup Southwest Pharmaceutical Co., Ltd., National drug approval: No. H50021462, 1mL: 5mg) intravenously, the dose was reduced by 5 mg/d every two weeks, and eight weeks later, treatment changed to oral prednisone 30 mg/d; the dosage was gradually reduced until discontinuation, according to the patient's condition. The total course of treatment was approximately three months. Patients with high intracranial pressure (>300 mmHg) and high protein levels in CSF (>2 g/L) were treated with lumbar puncture once every two days, and intrathecal injection of 0.1 mg of isoniazid + 5 mg of dexamethasone sodium phosphate, and in some patients with high intracranial pressure, approximately 10-50 ml of CSF was removed. In patients with high protein levels in CSF, CSF could be replaced by normal saline (5-20 ml). Patients with high intracranial pressure were treated with dehydration therapy consisting of intravenous (within 15-30 min) mannitol 125-250 ml 2-6 times/d, and if the effect was inadequate, patients could be treated with alternately intravenous drip of the above drugs, combined with glycerol fructose or intravenous injection of furosemide. And if the effect was still inadequate, patients were treated with intravenous human serum albumin for adjuvant dehydration therapy. Patients with limb paralysis were started on rehabilitation as soon as possible to facilitate functional recovery.

Laboratory tests

After two hours of an intravenous infusion of rifampicin, 3 ml of blood and CSF were collected. Venous blood samples (4 ml, each) were placed in a heparin lithium anticoagulant glass tube, centrifuged at 4000 rpm for 12 minutes, the plasma was obtained, transferred to an EP tube, then stored in a refrigerator at -70 °C for later analysis. CSF samples were placed in sterile glass tubes and processed with the same procedure as blood samples, then stored in a refrigerator at -70 °C for later analysis. For chromatographic analysis, Shimadzu high performance liquid chromatograph HPLC-20A was used, and UV detection wavelength was 254 nm, the

chromatographic column was Symmetry C18 5 μm, the mobile phase was 0.02 mol/L KH_2PO_4 (pH=4.0)-acetonitrile (70:30, V/V), flow velocity was 1.0 ml/min, column temperature was room temperature. 0.2 ml of 70% $HClO_4$ and 0.4 ml of 20% triethylamine were added to every 500 ml of the mobile phase. Detection wavelength was 254 nm; and flow velocity was 1 ml/min.

Observation of short-term and long-term therapeutic effect

The short-term efficacy was comprehensively judged based on the changes in CSF, and the improvement of clinical symptoms and signs at two weeks of admission.

Evaluation criteria: Cure: Tuberculosis symptoms disappeared, without complications and sequelae, routine CSF test results were normal in two tests. Significant improvement: Tuberculosis symptoms and signs disappeared, without complications and sequelae, there was slight abnormality in CSF, WBC was (8-12) × 10^6/L, and protein was 0.45-0.6 g/L. Effective: the clinical signs improved markedly with mild sequelae, CSF improved significantly. Ineffective: after treatment, clinical symptoms and signs did not improve, or new clinical lesions appeared, there was no change in CSF test, or the patient died. We considered treatment was effective when the criteria met "cure", "significant improvement" and "effective" criteria.

Long-term prognosis of patients in these two groups was assessed by GOS at 6 months after discharged from the hospital.

Scoring criteria[11]: 5 points: the patient recovered well and returned to a normal life, with or without mild deficits. 4 points: mild disability, the patient is independent in activities of daily living; and could work with assistance. 3 points: severe disability, the patient had preserved consciousness and disability, and needed additional care and support in activities of daily living. 2 points: survival in vegetative state, the patient had only minimal responses (for example, with sleep/wakefulness cycles, the eyes could open). 1 point: death. Among these, a Glasgow Outcome Scale (GOS) score of 4 or 5 points was considered a good prognosis; a GOS score of 1-3 was considered a poor prognosis.

Statistical Analysis

The data was statistically analyzed using statistical software SPSS 22.0. Parametric data were expressed as mean ± SD, and compared using t-test. Non-parametric data were compared using Mann-Whitney rank sum test. $P<0.05$ was considered statistically significant.

RESULTS

Comparison of the demographic and cerebrospinal fluid data between two groups of patients: The differences between the two groups were not significant ($P>0.05$, Table 1).

Table 1: Basic characteristics of TBM in the two groups

	Xingnaojing group	Control group	P value
Age	48.33±16.21	43.78±15.62	0.205
Gender			
Male	24 (60%)	21 (52.5%)	
Female	16 (40%)	19 (47.5%)	
Duration of disease at admission (d)	7.13±4.21	7.80±3.03	0.622
CSF pressure (mmHg)	270.47±74.48	244.27±81.96	0.367
Protein content in CSF (g/L)	2.52±1.16	2.23±1.41	0.547
Ratio of CSF sugar to blood glucose	0.49±0.19	0.55±0.28	0.456
Chloride in CSF (mmol/L)	110.80±5.77	111.73±9.08	0.739
On admission Glasgow coma scale	6.47±1.19	6.00±1.36	0.326
Cytological classification of CSF			
Lymphocyte reaction is predominant	13(32.5%)	18(45.0%)	
Neutrophil reaction is predominant	27(67.5%)	22(55.0%)	
Leukocyte count in CSF	409.40±457.87	320.20±284.93	0.527

CSF: Cerebrospinal fluid

Table 2: Short-term therapeutic effect of two groups of patients (n, %)

	Cure	Significant improvement	Effective	Ineffective	P value
Xingnaojing group	15 (37.5)	16 (40)	6 (12.5)	3 (7.5)	
Control group	11 (27.5)	9 (22.5)	12 (30)	8 (20)	0.039

Comparison of the average concentration of rifampicin in the blood and CSF between the two groups: The concentration of rifampicin in the blood was 10.03 ± 1.61 μg/mL in the Xingnaojing group, and 9.48 ± 1.58 μg/mL in the control group; the difference was not statistically significant (P>0.05). The concentration of rifampicin in CSF was 1.77 ± 0.17 μg/mL in the Xingnaojing group, which was significantly higher than the control group of 1.27 ± 0.16 μg/mL (P<0.05).

Comparison of short-term therapeutic effects between the two groups: The effective rate was 92.5% (37/40) in the Xingnaojing group, and 80% (32%) in the control group, the short-term therapeutic effect was better in the Xingnaojing group than in the control group, the difference was statistically significant (P<0.05, Table 2).

Comparison of long-term therapeutic effects between the two groups: The good prognosis rate was 75% in the Xingnaojing group, and 50% in the control group. The mortality rate was 12.5% in the Xingnaojing group, and 27.5% in the control group. the long-term therapeutic effect was better in the Xingnaojing group than in the control group, the difference was statistically significant (P<0.05, Table 3).

DISCUSSION

Ensuring effective drug concentration in the CSF has always been one of the main criteria for selecting anti-tuberculosis drugs. Among the present first-line drugs, the permeabilities of isoniazid and pyrazinamide are very high, both up to 90%. The permeability of quinolones in CSF is also relatively high. The BBB permeabilities of rifampicin, ethambutol and streptomycin are not ideal, but during the acute phase, their permeabilities are increased due to meningeal inflammation; therefore, these drugs can still be used as the anti-tuberculosis drugs of choice in the intensive phase.[12,13] Increasing drug dosage or combining drugs with better permeability of BBB has become a new therapeutic strategy.[4,14-16]

Due to the existence of BBB, rifampicin, a first-line anti-tuberculosis drug, often cannot reach effective concentration in CSF at doses recommended in present guidelines. Therefore, increasing the dose of rifampicin was tested by some researchers. Heemskerk *et al*. conducted a multicenter, randomized, double-blind controlled trial in Vietnam[15] which revealed that increasing the rifampicin dose in the standard treatment regimen (3HRZE/6HR) and combining with levofloxacin did not increase the survival rate of adult TBM patients. After 9 months of follows-up, 113 and 114 patients died in the enhanced therapy and standard treatment groups, respectively; the differences in overall survival rate, disability rate and the proportion of patients with adverse reactions leading to discontinuation of treatment were not statistically different. In dose range studies, dosages of up to 1350 mg/d, equivalent to 30 mg/kg/d, had been found safe. In tuberculosis treatment trials, even dosages up to 35 mg/kg/d did not significantly increase the risk of adverse drug reactions. However, in view of the fact that Asians seem to be more prone to drug-induced liver injury[17], special vigilance should be taken.

The main components of Xingnaojing are musk and borneol. Borneol is absorbed, distributed and metabolized rapidly in human body. It can penetrate the BBB within five minutes, the peak

Table 3: Long-term therapeutic effect of two groups of patients (n, %)

	Good recovery	Mild disability	Severe disability	Vegetative state	Death	P value
Xingnaojing group	20(50)	10(25)	3(7.5)	2(5)	5(12.5)	0.036
Control group	13(32.5)	7(17.5)	6(15)	3(7.5)	11(27.5)	

time in serum and brain tissue is 21 minutes and 48 minutes, respectively. In the central nervous system tissues, the time required to accumulate at special sites is longer than that in other extracerebral tissues, and the accumulative volume is relatively high; the distribution-related half-life in the brain is three times as long as that in serum, and the concentration of borneol in the brain reaches 10 μg/g at five minutes after orally ingestion. Borneol loosens the tight junction between BBB cells, increases the cell volume and the number of intracellular ingestive vesicles, and the effect is reversible.[18] This may be one of the mechanisms that promote drug penetration through the BBB.[19] Wu *et al*'s[20] animal experiment revealed that, when combined with borneol, the CSF concentration of rifampicin was increased and adverse reactions such as drug-induced hepatitis caused by increasing rifampicin dosage was avoided.

The limitation of this study is the small-sample size, and it needs to be confirmed by more rigorous molecular and animal experiments as well as large-scale randomized double-blind clinical trials.

In summary, there are no current reports on Xingnaojing infusion as an adjuvant therapy for severe TBM. The present study showed that rifampicin concentration was increased in the CSF as determined by high performance liquid chromatography after intravenous infusion of Xingnaojing; the concentration of rifampicin in CSF was 1.77 ± 0.17 μg/mL, significantly higher than that in the control group (1.27 ± 0.16 μg/mL, $p<0.05$). The reason that Xingnaojing infusion increased the CSF concentration of rifampicin may be related to the effect of borneol, the main component of Xingnaojing. With the increase of rifampicin concentration in CSF, the short-term and long-term therapeutic effects of severe TBM were significantly better than those in the control group. The present study showed that Xingnaojing infusion may be used as an adjuvant therapy for severe tuberculosis in CSF.

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