Clinical Research of Bulbus Fritillariae Thunbergii Granule Adjuvant Chemotherapy Increased Therapeutic Effect in Refractory Acute Leukemia

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ABSTRACT Objective To observe bulbus fritillariae thunbergii granule increased therapeutic effect in refractory acute leukemia during the period of chemotherapy. **Methods** Taking randomized, double-blind and multicentre concurrent control clinical research project, divided the patients who were in line with diagnostic criteria, according to drug randomized method, into bulbus fritillariae thunbergii granule group and control group, respectively. All patients of two groups took observation drug or control drug in three days before chemotherapy, while evaluated therapeutic effect after one cycle of chemotherapy. **Results** According to clinical research project, there were 138 cases of patients who entered statistical treatment, of which bulbus fritillariae thunbergii granule group were 72 cases, and control group were 66 cases. The results showed that clinical complete remission rate (CR) of bulbus fritillariae thunbergiigranule group was 36.8%, and its total effective rate was 77.8%, while both in control group were 25.8% and 53.0% respectively ($P<0.05$). **Conclusion** Bulbus fritillariae thunbergii granule group could increase clinical remission rate in refractory acute leukemia during the period of chemotherapy.

Key words Bulbus fritillariae thunbergii granule; Clinical remission rate; Refractory acute leukemia

Refractory acute leukemia had the biology characteristic of bad reaction to treatment, such as low induced relieving rate, high recurrence rate, and short survival period. Therefore it really was a troubled problem in the treatment of refractory acute leukemia (RAL) [1]. Based on previous trials in vitro and pro-clinical trials, we took part in an important united key project of capital medical science and technology foundation (No: I-2) and scientific research foundation project of state traditional Chinese Medicine Administration (No: 02-03LP14). We also standardize clinical observation in bulbus fritillariae thunbergii granule adjuvant chemotherapy which increased refractory acute leukemia clinical therapeutic effect.

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CASES SELECTED CRITERIA

Diagnostic Criteria

According to acute leucemia diagnostic criteria drew up by Chinese Medical Association Hematopathy Association in 1978, and all clinical subtype diagnostic criteria of FAB typing standards from hematopathy diagnosis and therapeutic effect standards, chief edited by Zhang Zhinan, We selected cases and carried out all standards above mentioned but except M3 [2]. As for refractory acute leukemia which in accordance with the National Refractory Acute Leukemia 4th Seminar (Haikou meeting) about refractory acute leukemia diagnostic criteria revision, that is to say, consistent with any one term of 6 terms mentioned as follows: ①patients did not obtain complete remission after 2 cycles of standard chemotherapy. ② leukemia relapse after clinical complete remission with regular, strengthened therapy, and consolidating treatment (diagnose to leukemia relapse consistent with any one term of 3 terms mentioned as follows: marrow microleukoblast (Monoblast+ immature monocyte or primitive lymphocyte + immature lymphocyte) >5% , while ≤20%, through 1 cycle of valid anti-leukemia treatment without marrow complete re-

mission standard: marrow microleukoblast (Monoblast+ immature monocyte or primitive lymphocyte + immature lymphocyte)>20%; extrinsic marrow leukemic cell infiltration). ③ acute leukemia, turned from chronic leukemia or from myelodysplastic syndrome (MDS－RAEB or RAEB－type), without complete remission after 1 cycle of standard chemotherapy. ④heterozygosis (admixture)leukemia or combining 2 or more than 2 items of abnormal antigen presentation, without complete remission after 1 cycle of standard chemotherapy. ⑤peripheral blood leucocyte counts >100.0×109/L in preliminary diagnosis. ⑥high expression of one term or more than 1 item of drug resistance protein(Pgp, MRP, LRP).

Criteria of case selected

The criteria of selected case as follows: ①consistent with acute leukemia diagnostic criteria and refractory acute leukemia diagnostic criteria. ②voluntary entering trial, sign informed consent. ③years of age over 10 , while less than 75 years old. ④no entering other clinical trials(including use anyone but can increase curative effect traditional Chinese and western medicine).

Remove and reject criteria

Remove and reject criteria as follows: ①coincidence with severe heart, brain, live, kidney disease. ②Psychosis. ③ Glaucoma. ④ femme during pregnancy or lactation. ⑤known hypersensitiveness with the drug. ⑥ years of age less than 10 or over 75 years old. ⑦entering other clinical trials. ⑧did not take the drug following specify, therefore could not judge clinical therapeutic effect. ⑨deficiency of clinical data, therefore influence to appraisal therapeutic effect and safety evaluation. ⑩did not consist with entering criteria after being in clinical trials.

RESEARCH METHODS

Medicine preparation and blinded methods

According to free decoction granule decoction preparation technology prepared observation medicine and control medicine. Bulbus fritillariae thunbergii granule was extracted from single herb of bulbus fritillariae thunbergii, while using malt culms prepared control granule. Both observation medicine and control medicine should be in line with medicine blinding requisition, with the same color and overwrap, and paste blinded number according to corresponding random number.

Chemotherapy regimen choice

All research hospitals, according to China and overseas reference, could select corresponding chemotherapy regimen based on routing one. Chemotherapy regimen frequently used was mentioned as follows:①Acute lymphoblastic leukemia:VDCP (VCR +DNR +CTX + Pred), VAMP (VCR +MTX +6－TG +Pred), VDLP (VCR +DNR +L－ASP +Pred), and VDLD (VCR + DNR +L －ASP +DXM). ② Acute non －lymphocytic leukemia:DA (DNR +Ara－C), MEA (MIT +VP－16 + Ara－C), and FLAG(Flu+Ara－C+G－CSF).

Medication and course of treatment

Based on our clinical research before, we laid out clinical medication, that is to say, taking drug 3 days ahead of being standard chemotherapy regimen. Took 1 sack of bulbus fritillariae thunbergii granule each time (according to proportion combination,equivalently 10g crude drug, the same as follows), three times per day, or took 1 sack of control each time,three times per day, respectively. Medicine above－mentioned all were provide by Weiming Tianren Traditional Chinese Medicine Limited Company, and all patients should successively take medication for 14 days,in the end evaluated therapeutic effect after one cycle of standard chemotherapy regimen.

Data management and statistics

Experiment data was treated with Epidata 2.1a software to set up database. Setting twice unblinded, the first unblinded time was after locking of blind review data, which should be taken charge by scientific research administrators, unblinded, handed over corresponding letter of appoint group for statistical analysis. The second unblinded time was after statistical analysis and clinical research summarize rough draft completion to identify treatment group. All data was treated with

SPSS 11.0 statistical analysis software . Measurement data applied to T analysis, and enumeration data applied to $\chi 2$ analysis or rank−sum test, when $P<0.05$ was considered as to have statistical significance.

RESULTS

Clinical case

From Jan, 2004 to April, 2006, the case were received from Dong Zhimen Hospital Affiliated to Beijing University of Chinese Medicine, Beijing University of Traditional Chinese Medicine Subsidiary Dongfang Hospital, People´s Hospital of Peking University, people's Liberation Army Gen Hospital, General hospital of Beijing Military district, Xiyuan Hospital China Academy of Traditioal Chinese Medicine, Dalian 210th Hospital of People's Liberation Army, the First Affiliated Hospital of Zhengzhou University, Langfang Traditional Chinese Hospital Affiliated to Hebei Medicine Univercity, Guilin Medical School Attached Hospital, Shi Jiazhuang Ping−an Hospital, Shuguang Hospital Attached with Shanghai Chinese Medicine University. Ac-

Table 1 Comparison of age, sex, type of leukemia

Group	Cases	Age	Sex		Type of leukemia		
			Male	Female	AML	ALL	Others
Bulbus fritillariae thunbergiigranule	72	41.31±18.79	41	31	36	26	10
Control	66	37.94±18.55	39	27	36	15	15

Table 2 Comparison of clinical therapeutic effect

Group	Cases	Complete remission (CR)	Partial remission (PR)	Non−remission (NR)	Rank sum test	P
Bulbus fritillariae thunbergii granule	72	26(36.1%)	30(1.7%)	16(22.2%)	11.56	0.009
Control	66	17(25.8%)	18(27.3%)	31(47.0%)		

Table 3 Comparison of effectivity

Group	in effect	ineffective	$\chi 2$	P
Bulbus fritillariae thunbergii granule	56(77.8%)	16(22.2%)	15.210	0.002
Control	35(53.0%)	41(47.0%)		

cording to clinical trial project after rejecting disqualification ones, there were 138 cases could get into statistical treatment, of which 72 cases were bulbus fritillariae thunbergii granule, and 66 cases were control group.

Clinical data

Age, Sex, Type of patients with leukemia showed as table 1. From table 1, when compared with age, sex, and clinical classification, the diferences between two groups were no significance in statistics ($P>0.05$).

Clinical therapeutic effect

Comparison of clinical therapeutic effect, such as table 2−3. In table 2, when comparison of bulbus fritillariae thunbergii granule group with control group, there were obvious difference in therapeutic effect ($P<0.05$). In table 3, In effect=(CR+PR), there were significant difference between two groups ($P<0.05$). From table 2 and 3: When comparison of bulbus fritillariae thunbergii granule group with control group, it showed statistical significance, which meant bulbus fritillariae thunbergii granule had an advantage than that of control.

Safety index

Only fewer persons represented slightly abnormal in chemical examination results of urine and stool, while chemical examination results of liver and renal function (ALT, AST, Cr, and BUN) were even. After one cycle of chemotherapy treatment, all patients had no obviously abnormal in urine, and stool. After 2 weeks and 1 cycle of chemotherapy treatment, the patients who had normal safety index above mentioned of these three groups was 88%~96%, when compared both of these two groups, it did not show statistically significant ($P>0.05$), which meant bulbus fritillariae thunbergii group had the security.

DISCUSSION

Through clinical practice, we found that refractory acute leukemia had clinical manifestation of both sputum, such as sucutaneous nodule, scrofula, and blood stasis, such as ecchymosis, petechiae (hemorrhage). According to TCM differentiation of symptoms and signs theory, it should be phlegm−blood stasis blocking. Therefore we should take the therapeutical principle as dissipating phlegm and eliminating stagnation, while activating blood circulation to dissipate blood stasis in refractory acute leukemia. Bulbus fritillariae thunbergii granule concluded three different kinds of herbs: Bulbus fritillariae thunbergii, stephania tetrandra, and szechwan lovage rhizome. According to Chinese herbs efficiency analysis, bulbus fritillariae thunbergii had feature of bitter cold, distribution to lung meridian, heart meridian, and had the efficiency of eliminating phlegm by cooling, expelling stagnation and removing stasis. Therefore it could be use in refractory acute leukemia, which was suffering from phlegm−blood stasis blocking in TCM theories.

The clinical research had the main objective: apply basic research achievement to clinic, and evaluate bulbus fritillariae thunbergii granule increase clinical efficacy and its security in refractory acute leukemia. To enhance confidence and repeatability of the clinical research, we took random double−blind, and multiple hospital concurrent control research method. Research result displayed that clinical complete remission rate of bulbus fritillariae thunbergii granule, and control group was 36.8%, and 25.8%, and effective rate (CR+PR) was 77.8%, and 53.0%, respectively. The result of statistical analysis showed that the CR, CR+PR of two groups both had statistical significance, which showed that bulbus fritillariae thunbergii granule match chemotherapy could increase curative effect in refractory acute leukemia. At the same time we didn't find obviously adverse reaction in bulbus fritillariae thunbergii granule group through detected urine routine, stool routine, liver function, renal function, and electrocardiogram. Therefore bulbus fritillariae thunbergii granule had a favourable clinical medication security.

Based on potential mechanism of bulbus fritillariae thunbergii granule, and through reviewing on related reference and our protophase basic research, we suppose that bulbus fritillariae thunbergii granule increasing therapeutic effect might related to its could reverse acute leukemia multidrug resistance [3−7]. As for its exactly mechanism of bulbus fritillariae thunbergii granule in

how to increase refractory acute leukemia therapeutic effect, we should take further researches through practice national natural science fund project.

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