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Research Article
Pharmacodynamic Study of Redujing Oral Liquid on Epstein-Barr Virus (EBV) Associated Infectious Mononucleosis in Children

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Abstract

Background and Objective: The EBV-associated infectious mononucleosis is a common infectious disease in children. Some children may have multiple organ damages, which seriously affects their quality of life. At present, there is no effective antiviral drug for EBV. Traditional Chinese medicine has unique advantages in antiviral because of its multi-component, multi-pathway and multi-target action. This study aimed to assess the efficacy of Redujing oral liquid, a traditional Chinese medicinal preparation, on Epstein-Barr Virus (EBV)-associated infectious mononucleosis in children and the inhibition of EBV *in vitro*. **Materials and Methods:** The children hospitalized in the pediatric department of Beijing Friendship Hospital of Capital Medical University for EBV-associated infectious mononucleosis were randomly divided into control (acyclovir alone) and treatment (acyclovir combined with Redujing oral liquid) groups for clinical efficacy observation. The study followed a prospective randomized controlled approach. The treatment was administered for 7 days. Clinical symptoms were observed and scored before and after treatment. **Results:** After corresponding treatment, in the clinical study part, the improvement of clinical symptoms and EBV-DNA turned negative and were effective in both control and treatment groups. The EBV-DNA turned negative ($p = 0.002$) was significantly better, the antiviral effect was more significant and the time of fever remission was significantly better in the treatment group than that in the control group ($p = 0.045$). In addition, the treatment group had a significant recovery after liver enlargement. Moreover, the treatment group had a significant advantage in the recovery of liver enlargement ($p = 0.013$). The *in vitro* study of virus inhibition demonstrated that both Redujing oral liquid and acyclovir significantly inhibited viral DNA replication. The Redujing oral liquid drug-containing serum also had a good inhibitory effect on viral replication. **Conclusion:** Redujing oral liquid significantly reduces clinical symptoms and inhibits EBV replication *in vivo* and *in vitro* in children with EBV infection.

Key words: Clinical efficiency, EBV-associated infectious mononucleosis, *in vitro* virus suppression, redujing oral liquid, traditional Chinese medicinal preparation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious mononucleosis (IM) is a clinical syndrome with primary manifestations of fever, pharyngitis and enlarged lymph nodes in the neck. Most of the children with IM have a good prognosis, with approximately 1% of patients developing severe neurological, hematologic or hepatic complications[1]. The IM can occur owing to various pathogens, of which the most common causative agent is the Epstein-Barr Virus (EBV)[2]. The EBV belongs to the gamma herpes virus and is universally susceptible in the population, with an infection rate of approximately 80-90%[3] and causes IM in children. Malignant diseases can develop in some children, such as lymphoma and hemophagocytic lymphoid hyperplasia owing to EBV infection. The relationship between EBV and some immune-related diseases has been demonstrated[4], therefore, effective treatment of EBV has a critical role in the disease prognosis.

No acceptable specific therapeutic regimen exists for the treatment of EBV-associated IM, mainly antiviral therapy and immune regulation. At present, nucleoside analogues such as acyclovir and ganciclovir are widely used in clinical antiviral therapy. These drugs have certain antiviral effects *in vivo*, however, their clinical efficacy is ambiguous[5]. Other antiviral drugs, such as nucleotide analogues (cidofovir), pyrophosphate analogues (foscarnet) and EBV protein kinase BGLF4 inhibitors (maribavir), have been reported to be associated with EBV or CMV infection in adults, but no drug has been approved for the treatment of EBV infection[6]. Therefore, it is very important to develop antiviral drugs for EB virus. Traditional Chinese medicine has unique advantages in the treatment and prevention of viral infections and has been widely used in China. At present, studies have confirmed that traditional Chinese medicine can inhibit the entry of the virus by binding to the viral attachment protein on the cell surface, inhibit the replication of the virus in the cell, limit the packaging and assembly of the virus and regulate the immune system[7]. In this study, an in-hospital formulation of Redujing oral liquid was applied to treat children with EBV-associated IM at Beijing Friendship Hospital, Capital Medical University, to observe the efficacy and analysis and also observed the *in vitro* antiviral effect of Redujing oral liquid using *in vitro* cell culture.

MATERIALS AND METHODS

Clinical research

Research participants: Children admitted to the Department of Pediatrics, Beijing Friendship Hospital, Capital Medical University, for EBV-associated IM from November, 2015 to May, 2018.

Diagnostic criteria: All 93 patients with IM fulfilled the diagnostic criteria of the Zhu Fu Tang Textbook of Pediatrics (seventh edition)[8]. These included any three of the following symptoms: Fever, pharyngeal tonsillitis, enlarged cervical lymph nodes larger than 1 cm, liver enlargement in children under 4 years of age, subcostal more than 2 cm Liver enlargement in children aged 4 years and older with subcostal palpable and splenomegaly, subcostal palpable. Additionally, hemograms should demonstrate how lymphocyte percentage greater than 50% or total lymphocytes $>5.0\times10^9$/L, with atypical lymphocytes accounting for >10% of cells or having counts exceeding 1.0×10^9/L. Finally, EBV-specific antibodies and EBV-DNA detection have any of the following: Viral capsid antigen (VCA) IgM positive, double serum EBV-VCA-IgG antibody titer increased more than 4 times. The EBV-EA antibody transiently increased, EBV-VCA-IgG antibody positive and showed low affinity and/or EBV-VCA-IgG antibody positive ($\geq$1: 2560) showed high affinity, EB-NA antibody was positive in the later stage, EBV-DNA was positive.

Inclusion criteria: The diagnostic criteria for EBV-associated IM were met, at ages 6 months to 14 years and guardians signed an informed consent form. The participants were divided into treatment and control groups based on a random number table.

Exclusion criteria: (1) Combined severe hematologic damage such as phagocytic syndromes, lymphomas or malignant diseases such as central nervous system damage or connective tissue diseases, (2) congenital immunodeficiencies, congenital malformations or genetic metabolic diseases, (3) Previous treatment with hormones and immunosuppressive drugs and (4) Unable to cooperate with oral Chinese medicine treatment.

Shedding criteria: (1) The child was unable to comply, (2) Adverse events or special physiological changes were not appropriate to continue the experiment or (3) The child or guardian wished to withdraw.

Experimental method: Acyclovir injection (Hubei Hepu Pharmaceutical Co. Ltd., 10 mg kg^{-1} each time) was used once every 8 hrs in the control group. In the treatment group, along with acyclovir injection, an in-hospital preparation of Redujing oral liquid was added as follows: 5 and 10 mL/dose three times a day for children less than 6 months and over 6 months old, respectively, the observation cut-off point for both groups was 7 days of treatment.

Redujing oral liquid is a hospital preparation of Beijing Friendship Hospital affiliated with Capital Medical University. The approval number is (97) JWYZZ (009) No. F-464 (Batch number: 20030612). It comprises *Astragalus membranaceus*, Indigo naturalis, *Lithospermum*, *Cortex moutan*, *Scutellaria baicalensis* and Zedoary. The original drug concentration is 246 mg mL^{-1}.

Anti-infective therapy with an appropriate combination of antibiotics based on drug sensitivity tests was administered for children with combined bacterial or *Mycoplasma pneumoniae* infections. For children with fever oral antipyretic medications are administered when the child shows signs of discomfort owing to fever.

Observed indicators

Clinical signs and symptoms: Duration of fever, peak fever, time of disappearance of sore throat, changes in tonsils and cervical lymph nodes and hepatosplenomegaly.

The cervical ultrasound was carried out to observe the cervical lymph nodes and an abdominal ultrasound was employed to comprehend the liver, spleen and abdominal lymph nodes.

The atypical lymphocytes, immunoglobulins and lymphocyte subset changes were observed before and after treatment.

Number of EBV-DNA negative cases

Adverse reactions: Adverse reactions, such as the presence of diarrhea, vomiting, jaundice and allergic reactions, were recorded, the blood and urine levels and heart, liver and kidney functions were monitored.

Criteria for judging the efficacy: According to their clinical manifestations, the children were divided into normal, mildly abnormal, moderate abnormal and severe abnormal and scoring standards were drawn according to different grades as seen in Table 1.

***In vitro* virus inhibition study**

Cell line: The EBV-containing B95.8 cell line, provided by National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

***In vitro* experiments requiring supplies, reagents and instruments:** The T25 with filter membrane culture dish (Corning), 1640 medium (Hyclone), fetal bovine serum (Gibco), double antibodies (Hyclone), PBS (Hyclone), Vi-CELLTMXR cell viability analyzer (Beckman Coulter Ltd., USA), CO_2 incubator, OLYMPUS DP70 inverted microscope and ABI 7500 Real-Time PCR amplification instrument (USA), EBV nucleic acid amplification fluorescence quantification and detection kit provided by Zhongshan University Daan Gene Co.

Drug-containing serum preparation: Twenty Japanese large-eared white rabbits procured from Beijing Vital River Laboratory Animal Technology Co. Ltd., were used. The rabbits were of either sex, healthy, disease-free and weighed 1.0-1.5 kg. They were divided into the Redujing oral liquid (seven rabbits), acyclovir aqueous solution (seven rabbits) and saline groups (six rabbits). The conversion method of the body surface area of human rabbits was used to calculate the gavage dose and the drug was administered twice a day for 7 days. The rabbits fasted without water 12 hrs before the last dose. As 1 hr after the last dose, blood was collected by carotid artery isolation cannulation under intravenous anesthesia at the ear margin of 5% pentobarbital 0.6 mL kg^{-1}. The blood was centrifuged after resting for 2 hrs at room temperature at 3000 rpm min^{-1} for 15 min. The serum was separated, inactivated in a water bath at 56°C for 30 min, de-bacterized by 0.22 μm membrane extraction and stored at -80°C.

Drugs used in the experiment: Acyclovir for injection (250 mg pc^{-1}, Hubei Hope Pharmaceutical Co. Ltd., State Drug Quantifier H20065390), Redujing oral liquid (Beijing Friendship Hospital, Capital Medical University, batch number: 20030612), pre-prepared serum containing the drug.

Table 1: Clinical performance scoring form

Parameter	Normal (score: 0)	Mildly abnormal (score: 1)	Moderate abnormal (score: 2)	Severely abnormal (score: 3)
Body temperature peak (°C)	<37.4	37.4–37.9	38–38.9	≥39
Nasal obstruction	None	Occasionally, breathing with nose, does not affect sleep	Frequent nasal obstruction	Obvious nasal obstruction, affecting sleep
Tonsillar enlargement	None	I°	II°	III°
Inflammatory exudation	None	Minor	Moderate	Massive
Hepatosplenomegaly (subcostal, cm, 3 years old)	0	Accessible under ribs, 2	2-3.9	≥4
Hepatosplenomegaly (subcostal, cm, ≤3 years old)	2	2-3.9	≥4	

Scoring criteria for cervical lymph nodes: Score 0: 5 mm, Score 1:5-9 mm, Score 2:10-19 mm, Score 3: 20-29 mm, Score 4: 30-39 mm and Score 5: ≥40 mm

Methods: Six-well plates were used for culture and observation. The control group was B95.8 cells without drug treatment and the other groups were treated with drugs of different concentrations. Each hole was set with two double wells. The maximum non-toxic concentration of redujing and acyclovir on cells, according to the results of the previous experiment[9], high and low concentrations of 2.46 and 0.492 mg mL^{-1} were selected for the feverfew group, high and low concentrations of 0.1 and 0.05 g L^{-1} were selected for the acyclovir group. As 1:5 and 1:10 for a serum containing the drug were selected as high and low concentrations. The observation item comprised EBV-DNA copy number detection at different time points.

Statistical analysis: The Kolmogorov-Smirnov Test was used to determine whether the data exhibited a normal distribution. For normally distributed variables, If normally distributed with homogeneous variance, The one-way ANOVA Test was used for multiple group comparisons and t-test was used for intergroup comparisons. The data were expressed as means and standard deviations ($\bar{x}\pm s$), continuous variables were compared between the groups using the Mann-Whitney U Test for nonparametric data. The data were expressed as medians and interquartile ranges (IQRs) [M P25 P75]. The chi-squared test was used to compare the categorical variables. The p-values <0.05 were considered statistically significant. The SPSS Statistics, Version 25.0 (IBM Corp., USA), was used for statistical analyses.

RESULTS

Clinical research

Patients: Ninety-three children who met the inclusion criteria were randomly divided into treatment (46 cases) and control groups (47 cases). There were 25 boys, 19 girls and two abscissions in the treatment group. The age distribution ranged from 1.5 to 13.6 years. The control group comprised 16 men, 26 women and five abscissions. The age distribution ranged from 1.3 to 13.3 years as shown in Fig. 1.

Statistical analysis of general data of the two groups of patients: There was no statistical difference between the two groups regarding sex, age and symptom score before treatment as shown in Table 2.

Comparison of the improvement of symptom score between the two groups before and after treatment: The improvement of symptom scores between the two groups of children after treatment was statistically analyzed and demonstrated that the treatment group had a more significant role in improving the symptoms than the control group, in which the improvement of each symptom before and after treatment is more obvious. The treatment group is better than the control group in improving the fever time and liver shrinkage (Table 3). The improvement of nasal congestion, pharyngeal tonsils, cervical lymph nodes and spleen shrinkage was not statistically significant.

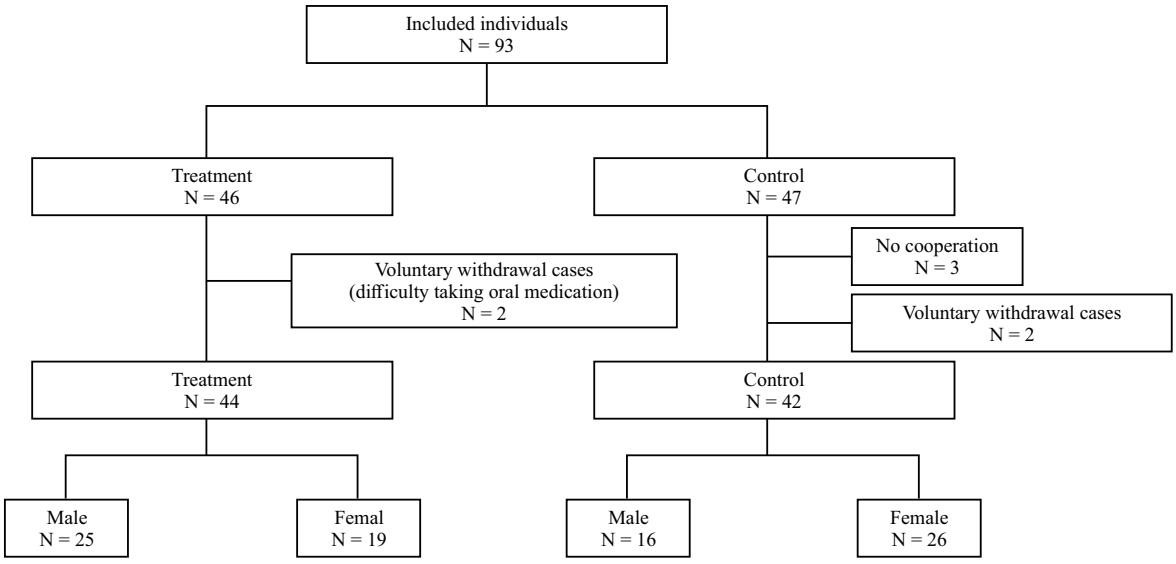

Fig. 1: Grouping of enrolled cases

Table 2: Baseline data of two groups before treatment

Parameter	Treatment	Control	p-value
Male/female (cases)	25/19	16/26	0.082
Age (years)	5.16±2.56	5.78±2.95	0.247
Symptom (score)	9.25±2.59	8.48±2.98	0.202

T-test used for two independent group comparisons, Data were expressed as means and standard deviations ($\bar{x}\pm s$), Chi-squared test was used to compare the categorical variables and Significance level is $p<0.05$

Table 3: Symptom improvement post-treatment in both groups

Parameter	Treatment	Control	p-value
Total symptom score improvement (score)	5(3,6)	4(2,5)	0.017
Duration of fever (days)	3(2,4)	4(3,5)	0.045
Degree of liver shrinkage (cm)	0(0,1.4)	0(0,0)	0.013

Significance level is $p<0.05$

Table 4: Epstein-Barr virus DNA turning negative after the treatment

Parameter	Epstein-barr virus DNA positive cases (cases)	Epstein-barr virus DNA negative cases (cases)
Treatment	6	30
Control	19	18
χ^2	9.478	
P	0.002	

Significance level is $p<0.05$

Comparison of improvements in experimental indicators between the two groups: There were changes in total leukocyte count, lymphocyte count, percentage of heterotypic lymphocytes, immunoglobulins and lymphocyte subpopulations before and after treatment in both groups as follows: The total leukocyte count, total lymphocyte count and percentage of heterotypic lymphocytes in both groups decreased significantly before and after treatment compared with those before treatment ($p < 0.05$), however, no significant difference was observed when the two groups were compared ($p = 0.729$). The immunoglobulins did not change significantly before and after treatment in both groups. The CD4, CD8, CD4/CD8 and CD19 were significantly changed before and after treatment in both groups, however, without a statistical difference ($p>0.05$). The CD3 and NK cell activity were not significantly changed before and after treatment in both groups.

Changes in biochemical indices (ALT, AST and ALP) before and after treatment in both groups: A significant improvement was observed in ALT, AST and ALP before and after treatment in both groups ($p<0.05$), however, no significant difference was observed between the two groups for comparison.

Changes in cervical lymph nodes before and after treatment in both groups: The cervical lymph nodes in both groups were significantly smaller after treatment than before treatment ($p = 0.001$ and 0.008 in the control and treatment groups, respectively). There was no statistical difference between the two groups regarding lymph node reduction ($p = 0.798$).

The number of EBV-DNA transitions before and after treatment in both groups: EBV-DNA transitions after treatment in both groups were compared and the treatment group showed good suppression of EBV compared to the control group, with significant differences as shown in Table 4.

Adverse reactions of the two groups of children before and after treatment: During treatment, no children experienced any adverse reactions such as allergy, vomiting, diarrhea or jaundice.

***In vitro* virus inhibition study:** The copy number of EBV-DNA in each group was measured at different time points. Redujing oral liquid and Redujing medicated serum effectively inhibited virus replication in the high-concentration intervention group were discovered. Figure 2 depicted the specific results. The effect of inhibiting virus replication was not evident in the low-concentration group and Fig. 3 depicted the specific results.

The number of DNA copies in each group was analyzed statistically. Except for the acyclovir-containing serum group, there was a statistical difference between the control group and the other drug and drug-containing serum treatment groups in the high-concentration treatment group ($p<0.05$), but no statistical difference between the drug groups and drug-containing serum groups ($p>0.05$). There was no statistical difference between the control group and the drug and drug-containing serum treatment groups in the low-concentration treatment group ($p>0.05$). The specific results were depicted in Table 5 and 6.

(a)

Time (hrs)	Control ($\times 10^9$ mL^{-1})	Acyclovir ($\times 10^9$ mL^{-1})	Redujing ($\times 10^9$ mL^{-1})	Normal saline serum ($\times 10^9$ mL^{-1})	Acyclovir serum ($\times 10^9$ mL^{-1})	Redujing serum ($\times 10^9$ mL^{-1})
0 hr	3.00	3.00	3.00	3.00	3.00	3.00
24 hrs	6.33	14.17	6.43	8.93	19.53	6.03
48 hrs	6.23	5.47	5.07	17.57	9.50	4.30
72 hrs	17.50	5.05	4.75	4.10	12.00	3.80
120 hrs	25.00	6.97	3.57	7.03	21.33	4.23
168 hrs	33.33	9.77	3.27	7.03	15.04	3.37

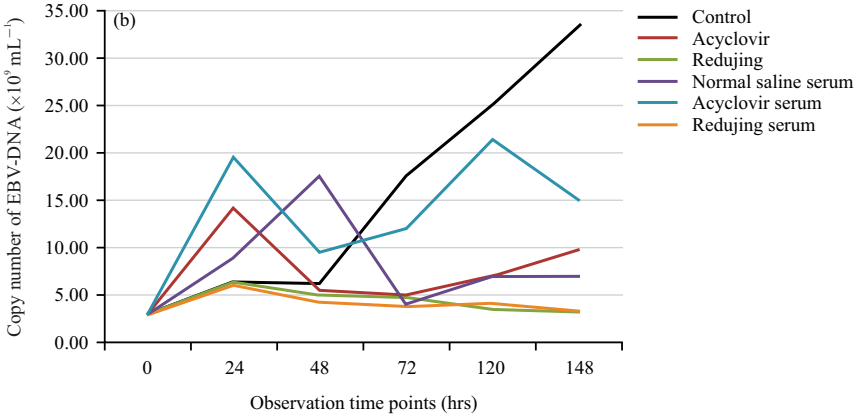

Fig. 2(a-b): EBV-DNA copies in high concentration treatment groups at different time points, (a) Results of DNA copy number of EBV at different time points in high concentration treatment groups and (b) Curve of EBV DNA changes at different time points in high concentration treatment groups

(a)

Time (hrs)	Control ($\times 10^9$ mL^{-1})	Acyclovir ($\times 10^9$ mL^{-1})	Redujing ($\times 10^9$ mL^{-1})	Normal saline serum ($\times 10^9$ mL^{-1})	Acyclovir serum ($\times 10^9$ mL^{-1})	Redujing serum ($\times 10^9$ mL^{-1})
0 hr	3.00	3.00	3.00	3.00	3.00	3.00
24 hrs	12.33	2.20	7.00	10.07	24.00	7.00
48 hrs	10.07	14.20	9.13	14.93	11.17	6.03
72 hrs	29.00	5.00	23.00	4.25	19.50	3.15
120 hrs	27.67	5.33	23.00	9.47	13.00	7.20
168 hrs	20.60	6.97	26.67	12.13	10.47	10.97

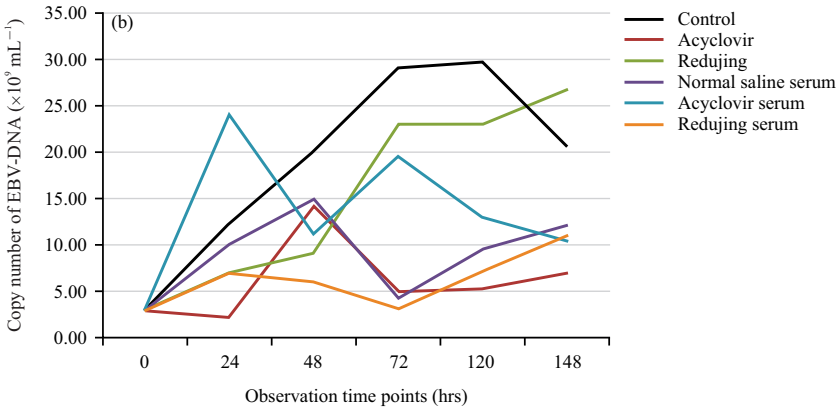

Fig. 3(a-b): EBV-DNA copies in low concentration treatment groups at different time points, (a) Results of DNA copy number of EBV at different time points in low concentration treatment groups and (b) Curve of EBV DNA changes at different time points in low concentration treatment groups

Table 5: Statistical analysis of EBV-DNA copy numbers in high concentration treatment groups

Group I	Group (J)	Mean difference (I-J)	SD	p-value
Control	Acyclovir	23.5667*	10.44311	0.043
	Redujing	30.0667*	10.44311	0.014
	Normal saline serum	26.3000*	10.44311	0.027
	Acyclovir serum	18.2900	10.44311	0.105
	Redujing serum	29.9600*	10.44311	0.014

Significance level is p<0.05

Table 6: Statistical analysis of EBV-DNA copy numbers in low-concentration treatment groups

Group I	Group (J)	Mean difference (I-J)	SD	p-value
Control	Acyclovir	13.6333	7.71410	0.103
	Redujing	-6.0667	7.71410	0.447
	Normal saline serum	8.4667	7.71410	0.294
	Acyclovir serum	10.1333	7.71410	0.214
	Redujing serum	9.6333	7.71410	0.236

Significance level is p<0.05

DISCUSSION

In the current study, on the one hand, adding redujing oral liquid significantly reduced the fever duration and promoted the recovery of liver enlargement in children with EBV-associated IM, on the other hand, the addition of redujing oral liquid promoted EBV-DNA negativity better than the use of acyclovir alone.

In vitro, pharmacodynamic experiments also confirmed that redujing oral liquid and redujing oral liquid drug-containing serum have the same antiviral effect as acyclovir.

Infectious mononucleosis (IM) is a common infectious disease. The global prevalence rate is approximately 90%[10]. Most IM cases have self-limitation and a favorable prognosis, with only a few patients experiencing spleen rupture, hepatitis or severe airway obstruction[11].

The main pathogen of infectious mononucleosis is Epstein Barr Virus (EBV), a double-stranded DNA virus that belongs to the herpesviridae family. The prevalence of EB virus infection rises year after year. Around 90% of people worldwide have been infected with the virus and will remain latent carriers for the rest of their lives[12]. Infection with the EB virus is a common infectious disease in children, with various clinical manifestations and degrees of severity. The most common is infectious mononucleosis caused by the EB virus. Currently, research has linked EB virus infection to malignant tumors such as Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma and post-transplant lymphoproliferative diseases[13]. Furthermore, it is thought that EBV is linked to several autoimmune diseases (Sjogren's syndrome, rheumatoid arthritis, thyroiditis)[14], so early treatment is essential.

At the moment, the treatment of EB virus infection is primarily antiviral, with acyclovir being the most commonly used antiviral in clinical practice. Acyclovir is an analog of a nucleoside. When the drug enters virus-infected cells, it can compete for viral adenosine kinase or cell kinase with deoxynucleoside. Phosphorylation can produce acyclovir monophosphate, which can then be converted into acyclovir diphosphate and acyclovir triphosphate by cellulase. Acyclovir triphosphate interferes with viral polymerase and combines with the growing DNA chain, causing the extension of the DNA chain to be interrupted. However, it was discovered during the clinical application process that acyclovir could reduce Epstein Barr virus shedding in the oropharynx, but there was no obvious clinical benefit[15]. Atypical T lymphocyte proliferation may occur in children with EB virus-associated infectious mononucleosis due to many cell-mediated immune responses against EB virus-infected B lymphocytes. Therefore, some people believe that antiviral and immunomodulatory drugs (such as corticosteroids used as a last resort) may be more beneficial for treatment. In a multicenter, double-blind, placebo-controlled trial, prednisone combined with acyclovir in treating infectious mononucleosis can inhibit oropharyngeal EBV replication but it does not affect the duration of clinical symptoms or EBV-specific cellular immunity[16].

Traditional Chinese medicine is a long-standing feature of Chinese culture. Previous research has discovered that polysaccharides found in traditional Chinese medicine have important biological activities such as anti-tumor, anti-oxidation, anti-diabetes, radiation protection, anti-virus, blood lipid reduction and immune regulation[17]. Traditional Chinese medicine has a unique role in virus prevention. Traditional Chinese medicine has also played an important

role in the anti-virus and anti-inflammatory treatment of COVID-19, which is popular worldwide[18-20]. Dialectically, infectious mononucleosis falls into the "epidemic febrile disease" category in Chinese medicine. Its etiology can be summed up as heat, toxin, phlegm and blood stasis, with heat and toxin being the primary pathogenic factors. The viscera of children are delicate, with insufficient qi and blood and they are sensitive to external evils. The mouth and nose allow heat, wind, dampness and the other six evils and pestilence toxins to enter. The first lung surface attack can result in lung qi loss, coughing and nasal congestion. The conflict between healthy and pathogenic qi causes fever. If the heat toxin is suppressed in the lung, the throat or tonsils will swell and become painful, the evil of wind heat invades the blood circulation and the heat breaks the blood circulation, causing a skin rash. The lung toxin obstructs the lung, clears and eliminates the loss of responsibility. The body fluid is applied abnormally, the burning fluid is phlegm, the phlegm heat is cemented, flows through the meridians and the nodes are in the neck, causing the lymph nodes in the neck to swell, the heat toxin does not heal because of prolonged heat. It deteriorates fluid and consumes gas. Blood circulation is poor, resulting in blood stasis and hepatosplenomegaly[21].

Redujing oral liquid is a hospital prepared independently by Beijing Friendship Hospital, affiliated with Capital Medical University. Among its main ingredients are *Astragalus*, *Scutellaria*, Indigo naturalis, purple grass, peony bark, zedoary and other traditional Chinese medicines. Its treatment consists of clearing heat and detoxifying, replenishing qi and nourishing blood, promoting blood circulation and removing blood stasis. It has been used successfully for decades to treat a variety of infectious diseases[22]. Polysaccharides, flavonoids, astragalosides, amino acids and trace elements are among the more than 100 compounds found in *Astragalus*. Its polysaccharide has immune regulation, anti-oxidation, anti-tumor, anti-virus, anti-inflammatory, liver protection and other functions[23]. *Astragalus* can inhibit the acute inflammatory response. *Astragalus* AMWP-1A can effectively protect the immune organs of tumor-bearing mice and promote macrophage pinocytosis[24]. Scutellaria can remove heat, dampness, fire and toxins. Scutellaria has antiviral properties against HIV, influenza, dengue, hepatitis B, type I human T-cell leukemia and herpes simplex virus[25]. Indigo naturalis is found in the leaves and stems of Assam indigo, false indigo, Ward and other plants, which has the effects of clearing heat and anti-inflammation[26]. Recent pharmacodynamic studies have confirmed that the main antiviral active ingredients of Indigo naturalis are indigo and indirubin. Indigo and indirubin have a good inhibition and killing effect on influenza virus and Japanese encephalitis virus. Further, indigo can inhibit the SARS virus's 3C like protease (3 CLpro)[27]. Shikonin, the main component of lithospermum, has anti-inflammatory, anti-HIV-1 and anti-vascular permeability effects[28]. Paeonol, the main active ingredient in Cortex Moutan, has anti-inflammatory and endothelial protective properties[29]. The traditional efficacy of zedoary turmeric is to expel qi, break blood, eliminate accumulation and relieve pain. Curcumol, curcuminol, thridone, curcuminol and curcumin in zedoary turmeric have been shown to have antiviral activity against influenza virus and grouper iris virus[30]. Furthermore, research on drugs for promoting blood circulation and removing blood stasis, such as alkannin and peach kernel, has discovered that such drugs can inhibit the extensive toxic effects caused by the body's T lymphocytes, reduce the immune inflammatory reaction, promote the body's non-specific immunity, improve local circulation, improve capillary activity, reduce inflammatory exudation, promote inflammatory absorption, antagonize viral infection and improve and regulate[31,32].

Redujing oral liquid has previously been shown to inhibit the expression of early antigens of EB virus, as well as the tumorigenicity of CNE2 cells in SCID mice[33,34] and a retrospective study confirmed that Redujing oral liquid has a good clinical therapeutic effect in the treatment of EB virus infection[35].

Redujing oral liquid inhibits viral DNA replication and improves clinical symptoms. The current study provides a new therapeutic option for the treatment of EBV and demonstrates the advantages of traditional Chinese medicinal preparations in antiviral therapy. Traditional Chinese medicine preparations not only have a direct antiviral effect, but also have immunomodulatory effects, thus giving the advantage of a combined dual effect in antiviral treatment.

This study only observed the clinical efficacy and antiviral effects of Redujing oral liquid in the treatment of IM caused by EB virus infection. The antiviral effect of Redujing oral liquid needs to be further explored in the future in terms of molecular mechanism.

CONCLUSION

In this investigation, Redujing oral liquid can improve the clinical symptoms of children with EBV-IM. Redujing oral liquid and its drug-containing serum have a good effect on inhibiting EBV replication. Therefore, redujing oral liquid has direct antiviral and immunomodulatory effects and can be used as an effective means of EBV treatment.

SIGNIFICANCE STATEMENT

This study aims to provide new methods for the treatment of EB virus. In this study, Redujing oral liquid played a very good antiviral role, especially in discovering the antiviral effect of redujing drug-containing serum. This also indicates that traditional Chinese medicine not only has a direct antiviral effect in antiviral treatment but also has an immune regulatory effect, which provides theoretical support to further understand the immune regulatory effect of traditional Chinese medicine.

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REFERENCE

1. Cohen, J.I., 2018. Vaccine Development for Epstein-Barr Virus. In: Human Herpesviruses, Kawaguchi, Y., Y. Mori and H. Kimura (Eds.), Springer, Singapore, ISBN: 978-981-10-7230-7, pp: 477-493.
2. Balfour Jr, H.H., S.K. Dunmire and K.A. Hogquist, 2015. Infectious mononucleosis. Clin. Transl. Immunol., Vol. 4. 10.1038/cti.2015.1.
3. Luzuriaga, K. and J.L. Sullivan, 2010. Infectious mononucleosis. N. Engl. J. Med., 362: 1993-2000.
4. Houen, G. and N.H. Trier, 2020. Epstein-barr virus and systemic autoimmune diseases. Front. Immunol., Vol. 11. 10.3389/fimmu.2020.587380.
5. Dunmire, S.K., K.A. Hogquist and H.H. Balfour, 2015. Infectious Mononucleosis. In: Epstein Barr Virus Volume 1, Münz, C. (Ed.), Springer, Cham, Switzerland, ISBN: 978-3-319-22822-8, pp: 211-240.
6. Andrei, G., E. Trompet and R. Snoeck, 2019. Novel therapeutics for epstein-barr virus. Molecules, Vol. 24. 10.3390/molecules24050997.
7. Li, T. and T. Peng, 2013. Traditional Chinese herbal medicine as a source of molecules with antiviral activity. Antiviral Res., 97: 1-9.
8. IDG, PB and CMA, 2016. Diagnosis and treatment principles for major non-neoplastic Epstein-Barr virus infection-related diseases in children. Chin. J. Pediatr., 54: 563-568.
9. Xiaohong, L., Y. Shuqing, Z. Ling, Z. Zhonghao and L. Lige *et al*., 2007. The contrasting experiment to the inhibition of Redujing and Acyclovir on EB virus antigen expression. Tianjin J. Tradit. Chin. Med., 24: 408-410.
10. Fugl, A. and C.L. Andersen, 2019. Epstein-barr virus and its association with disease-A review of relevance to general practice. BMC Fam. Pract., Vol. 20. 10.1186/s12875-019-0954-3.
11. Glynn, F.J., T. Mackle and J. Kinsella, 2007. Upper airway obstruction in infectious mononucleosis. Eur. J. Emerg. Med., 14: 41-42.
12. Cohen, J.I., 2000. Epstein-barr virus infection. N. Engl. J. Med., 343: 481-492.
13. Dunmire, S.K., P.S. Verghese and H.H. Balfour Jr., 2018. Primary epstein-barr virus infection. J. Clin. Virol., 102: 84-92.
14. Balandraud, N. and J. Roudier, 2018. Epstein-barr virus and rheumatoid arthritis. Joint Bone Spine, 85: 165-170.
15. Pagano, J., C.B. Whitehurst and G. Andrei, 2018. Antiviral drugs for EBV. Cancers, Vol. 10. 10.3390/cancers10060197.
16. Tynell, E., E. Aurelius, A. Brandell, I. Julander and M. Wood *et al*., 1996. Acyclovir and prednisolone treatment of acute infectious mononucleosis: A multicenter, double-blind, placebo-controlled study. J. Infect. Dis., 174: 324-331.
17. Zeng, P., J. Li, Y. Chen and L. Zhang, 2019. The Structures and Biological Functions of Polysaccharides from Traditional Chinese Herbs. In: Progress in Molecular Biology and Translational Science, Zhang, L. (Ed.), Elsevier Inc., ISBN: 978-0-12-817740-2, pp: 423-444.
18. Ren, W., P. Liang, Y. Ma, Q. Sun and Q. Pu *et al*., 2021. Research progress of traditional Chinese medicine against COVID-19. Biomed. Pharmacother., Vol. 137. 10.1016/j.biopha.2021.111310.
19. Tsai, K.C., Y.C. Huang, C.C. Liaw, C.I. Tsai and C.T. Chiou *et al*., 2021. A traditional Chinese medicine formula NRICM101 to target COVID-19 through multiple pathways: A bedside-to-bench study. Biomed. Pharmacother., Vol. 133. 10.1016/j.biopha.2020.111037.
20. Lee, D.Y.W., Q.Y. Li, J. Liu and T. Efferth, 2021. Traditional Chinese herbal medicine at the forefront battle against COVID-19: Clinical experience and scientific basis. Phytomedicine, Vol. 80. 10.1016/j.phymed.2020.153337.
21. Xuecheng, S., M. Yan, H. Runxuan, F. Jia, Y. Lin and Z. Chengpeng, 2020. Advances in research on differentiation and treatment of infectious mononucleosis by TCM. J. Liaoning Univ. Tradit. Chin. Med., 22: 136-139.
22. Deli, X., M. Hongqiu, H. Ancun, L. Jing and W. Tianli, 2004. Study on *in vitro* inhibitory test of different formulations of Redujing on *Mycoplasma pneumoniae* [In Chinese]. Chin. J. Exp. Tradit. Med. Formulae, 10: 43-45.
23. Jin, M., K. Zhao, Q. Huang and P. Shang, 2014. Structural features and biological activities of the polysaccharides from *Astragalus membranaceus*. Int. J. Biol. Macromol., 64: 257-266.
24. Liu, A.J., J. Yu, H.Y. Ji, H.C. Zhang, Y. Zhang and H.P. Liu, 2018. Extraction of a novel cold-water-soluble polysaccharide from *Astragalus membranaceus* and its antitumor and immunological activities. Molecules, Vol. 23. 10.3390/molecules23010062.

25. Wang, Z.L., S. Wang, Y. Kuang, Z.M. Hu, X. Qiao and M. Ye, 2018. A comprehensive review on phytochemistry, pharmacology, and flavonoid biosynthesis of *Scutellaria baicalensis*. Pharm. Biol., 56: 465-484.
26. Sugimoto, S., M. Naganuma and T. Kanai, 2016. Indole compounds may be promising medicines for ulcerative colitis. J. Gastroenterol., 51: 853-861.
27. Qi-Yue, Y., Z. Ting, H. Ya-Nan, H. Sheng-Jie, D. Xuan, H. Li and X. Chun-guang, 2020. From natural dye to herbal medicine: A systematic review of chemical constituents, pharmacological effects and clinical applications of indigo naturalis. Chin. Med., Vol. 15. 10.1186/s13020-020-00406-x.
28. Chen, X., L. Yang, J.J. Oppenheim and O.M.Z. Howard, 2002. Cellular pharmacology studies of shikonin derivatives. Phytother. Res., 16: 199-209.
29. Zhang, L., D.C. Li and L.F. Liu, 2019. Paeonol: Pharmacological effects and mechanisms of action. Int. Immunopharmacol., 72: 413-421.
30. Li, Z.Y., E.W. Hao, Z.C. Du, R. Cao and F. Chen *et al*., 2022. Research progress of *Curcuma kwangsiensis* root tubers and analysis of liver protection and anti-tumor mechanisms based on Q-markers. China J. Chin. Mater. Med., 47: 1739-1753.
31. Kourounakis, A.P., A.N. Assimopoulou, V.P. Papageorgiou, A. Gavalas and P.N. Kourounakis, 2002. Alkannin and shikonin: Effect on free radical processes and on inflammation-A preliminary pharmacochemical investigation. Arch. Pharm., 335: 262-266.
32. Liu, L., J.A. Duan, Y. Tang, J. Guo, N. Yang, H. Ma and X. Shi, 2012. Taoren-Honghua herb pair and its main components promoting blood circulation through influencing on hemorheology, plasma coagulation and platelet aggregation. J. Ethnopharmacol., 139: 381-387.
33. Xiaohong, L., Y. Shuqing, Z. Zhonghao, L. Lige and Y. Chunxia *et al*., 2007. Inhibitory effect of hot-plated crystals on the expression of epstein-barr virus antigen *in vitro* [In Chinese]. Chin. J. Exp. Tradit. Med. Formulae, 13: 62-63.
34. Shao-Bing, Z., Z. Ling, Y. Shu-Qing and L. Xiao-Hong, 2009. Inhibitory action of redujing to CNE2 cells. Chin. J. Exp. Tradit. Med. Formulae, 15: 41-43.
35. Yaoling, Z., D. Yingxue and C. Hong, 2017. Retrospective study of the clinical efficacy of redujing to treat children's infectious mononucleosis. Beijing Med. J., 39: 661-665.