Brief Report

Anti-virus effect of traditional Chinese medicine Yi-Fu-Qing granule on acute respiratory tract infections

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Summary **Yi-Fu-Qing granule is a traditional Chinese medicine for the treatment of acute respiratory tract infections. The present study sought to investigate the anti-virus effects of Yi-Fu-Qing granule on acute respiratory infections with respiratory syncytial virus (RSV) and human adenoviruses type 3 (Ad3). The cytotoxicity of Yi-Fu-Qing granule was evaluated by the neutral red assay on HeLa cells. The antiviral effect of Yi-Fu-Qing granule was tested by observing the cytopathogenic effect (CPE) with a compound mixture of Isatis leaf as the positive control drug. The results indicated that the highest non-toxicity concentration of Yi-Fu-Qing granule on Hela cells was 1:100. The CPE reduction assay showed that Yi-Fu-Qing granule inhibited RSV and Ad3 replication at a concentration of 1:100. Thus, Yi-Fu-Qing granule may have a significant antivirus effect on acute respiratory tract infections with RSV and Ad3 infections and this could prove useful for further antivirus research on acute respiratory tract infections.**

Keywords: Yi-Fu-Qing granule, acute respiratory tract infections, respiratory syncytial virus (RSV), adenoviruses type 3 (Ad3)

1. Introduction

Acute respiratory tract infections are the most common illnesses worldwide afflicting both adults and children. It can result in a surprisingly diverse range of disease from the mild common cold to severe life-threatening lower respiratory tract infections (*1*,*2*). These infections may result from invasion of the respiratory tract by bacteria, viruses, or other infectious agents, however, viruses are the most frequently identified pathogens. Of approximately 200 viral respiratory pathogens, those primarily associated with acute respiratory tract infections include: respiratory syncytial viruses (RSV), adenoviruses, influenza viruses, corona-viruses and so on (*3*). RSV disease spectrum includes a wide array of respiratory symptoms, from rhinitis to pneumonia and bronchiolitis, and it is reported that RSV is the main viral cause of bronchiolitis in infants (*4*). Adenoviruses (Ads) are a diverse group of double-stranded DNA viruses responsible for a wild variety of human ailments especially lower respiratory tract infections in infants and children (*5*). Among the 51 serotypes of human Ads identified to date, adenoviruses type 3 (Ad3) and adenoviruses type 7 (Ad7) can cause large disseminated outbreaks of severe respiratory tract infections and have been documented to co-circulate in a given geographic area, such as, China, Japan, USA, and South America (*6*).

The key step for treating acute viral respiratory tract infections is searching for effective antiviral agents. Researchers attempt to develop effective therapy for RSV, Ad3, and other virus infections have been ongoing for as long as the viruses have been recognized, however, apart from influenza, there are no effective antiviral chemotherapeutic agents and vaccines available (*7*). The emergence of severe acute

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Table 1. Composition and bioactive compounds of Yi-Fu-Qing granule

Composition	Main bioactive compounds	Amount used (g)
1. *Folium perillae* (*Perilla frutescens* (L.) Britt.)	perillaldehyde, perilloside, limonene, rosmarinic acid, ursolic acid, *et al.* (*13*)	7.5
2. *Scutellaria baicalensis* Georgi	baicalin, wogonoside, baicalein, wogonin, *et al.* (*14,15*)	5
3. *Bupleurum chinense* DC.	saikosaponin, saikogenin, *et al.* (*16*)	5
4. *Tinospora capillipes* Gagnep.	palmatine HCL, jatrorrhizine chloride, *et al.* (*17*)	5
5. *Semen armeniacae amarum* (*Prunus armeniaca* L. var. *ansu* Maxim)	amygdalin, emulsion, *et al.* (*18*)	4
6. *Glycyrrhixa uralensis* Fisch	glycyrrhizic acid, glycyrrhizin, liquiritin, isoliquiritin, isoliquiritigenin, *et al.* (*20,21*)	3

Italic numbers in parentheses denote the corresponding references.

respiratory syndrome (SARS) in late 2002, the recent outbreaks of avian influenza in Asia and especially the 2009 outbreaks of influenza A virus subtype H1N1 are timely reminders of the pandemic risks from respiratory viral disease (*8*). After the outbreak of SARS, traditional Chinese medicine has attracted more attention from researchers who endeavor to search for effective antiviral agents and it may be a good candidate with special characteristics for an antivirus.

Yi-Fu-Qing granule, a compound preparation of traditional Chinese medicine (for composition and main bioactive compounds see Table 1), jointly developed by Shandong Provincial Hospital and Shandong Qidu Medicine Co., Ltd., has been used as effective agents treating acute respiratory tract infections especially with the symptom of high fever for years in clinics in China. The clinical data documented that the total effective rate of Yi-Fu-Qing granule's antipyretic effect was 97.14%, while the total effective rate of ameliorating other symptoms (including headache, nasal obstruction, cough, malaise, and so on) was 95.71% (*9*). Although Yi-Fu-Qing granule has proven to be effective against acute respiratory tract infections according to the clinical data, there is no detailed experimental data on Yi-Fu-Qing granule. Thus, the current study investigated the antivirus effect of Yi-Fu-Qing granule on acute respiratory infections with respiratory syncytial virus (RSV) and human adenoviruses type 3 (Ad3), which may prove useful for further antivirus research on acute respiratory tract infections.

2. Materials and Methods

2.1. *Reagents*

Yi-Fu-Qing granule was produced by Shandong Qidu Medicine Co., Ltd., Zibo, China, batch number 20030201. Compound mixture of isatis leaf was purchased from Shandong Lunan pharmaceutical Co., Ltd., Linyi, China (10 mL/branch, batch number 20040611). Human cervical cancer cell line HeLa cells was provided by Shandong Province Medical Scientific Academy. The Long strain of RSV and Ad3 were provided by China Academy of Preventive Medicine. Fifty percent of tissue culture infectious dose ($TCID_{50}$) of RSV in HeLa cells was 1×10^{-7}/0.2 mL while the $TCID_{50}$ of Ad3 in HeLa cells was 1×10^{-5}/0.2 mL. In the current study, the infectious dose we used was 100 $TCID_{50}$/0.2 mL both for RSV and Ad3. Neutral red staining solution was purchased from Beyotime Institute of Biotechnology, Shanghai, China. Crystal violet solution was purchased from Sigma-Aldrich, St Louis, MO, USA. High glucose Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen, Carlsbad, CA, USA. Fetal calf serum (FCS) was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China.

2.2. *Toxicity detection of Yi-Fu-Qing granule*

HeLa cells (5×10^{-4} cells/mL) were plated in 96-well plates and incubated for 24 h at 37°C in a humidified atmosphere with 5% CO_2 in air. Then, five concentrations of Yi-Fu-Qing working solution (1:400, 1:200, 1:100, 1:50, and 1:25) were prepared by diluting the Yi-Fu-Qing granule with serum-free medium and added into each treated group of cells with an untreated group as a control (there were 6 repeat wells for each group). After that, the cells were incubated continually. Seventy-two hours later, 200 μL of Neutral red certified staining solution (working solution: 0.02%) was added in each well then incubated for another 2 h at 37°C, washed with phosphate buffered saline (PBS) two times, and 200 μL de-staining solution (0.1 mol/L NaH_2PO_4 and ethanol, v:v = 1:1, pH 4.5) was added in each well. Finally, after keeping 96-well plates in a dark place for 30 min, the optical densities (ODs) were monitored at a wavelength of 450 nm using an ELISA plate reader. Cell viability rate and the highest non-toxic concentration of Yi-Fu-Qing granule on HeLa cells were detected.

2.3. *Anti-RSV and anti-Ad3 effects detection of Yi-Fu-Qing granule*

HeLa cells (5×10^{-4} cells/mL) were plated in 96-well plates with four treated groups and a control group, and incubated for 24 h at 37°C in a humidified atmosphere with 5% CO_2 in air. Then the cells in each group were added into solutions of 100 $TCID_{50}$/0.2 mL RSV and Ad3, respectively. After being absorbed 90 min at 37°C with 5% CO_2 in air, the cells were given the following different treatments in each treated group (Yi-Fu-Qing working solution 1:400, 1:200, and 1:100; Compound mixture of Isatis leaf 1:100) and incubated for 72 h. After incubation, the infected cells were stained with 0.5% crystal violet solution for 30 min, and the CPE was observed under a light microscope. The CPE was graded as follows: 0 = 0% CPE, 1 = 0-25% CPE, 2 = 26-50% CPE, 3 = 51-75% CPE, and 4 = 76-100% CPE (*12*).

2.4. *Statistical analysis*

All experiments were performed in triplicate and the results were expressed as mean ± S.D. Statistical analysis was performed with the ANOVA method using SPSS.11.5 software.

3. Results and Discussion

Respiratory infections are recognized as the leading cause of acute morbidity in individuals of all ages especially children worldwide. After the emergence of SARS in 2002 and the current outbreak of novel influenza A (H1N1) virus, respiratory virus infections have caused high attention and even panic. Thus, searching for licensed vaccines and effective antiviral agents are the key step for treating respiratory infections. However, despite much activity during these years, there are no licensed vaccines available for the prevention of respiratory viral infections, other than influenza. Traditional Chinese medicine may be a great treasure for preventing respiratory viral infections. It is recorded on Treatise on Febrile Disease (Shang Han Lun) that a variety of herbal formulas have been used to treat patients with infectious diseases for over 1,800 years (*11*).

In the current study, we sought to investigate the anti-virus effect of Yi-Fu-Qing granule on acute respiratory infections with respiratory syncytial virus (RSV) and human adenoviruses type 3 (Ad3). According to the results of neutral red assay, it was indicated that the highest non-toxic concentration of Yi-Fu-Qing granule on HeLa cells was 1:100. Thus, we used concentrations of Yi-Fu-Qing granule of 1:400, 1:200, and 1:100 for the following detections. As shown in Table 2, the CPE of virus RSV and Ad3 both were decreased with increasing concentrations of Yi-Fu-Qing granule, compared with the untreated group. In addition, at the concentration of 1:100 for the group of Yi-Fu-Qing granule and compound mixture of Isatis leaf, there was no CPE for the virus RSV and Ad3. This means that the concentration of 1:100 Yi-Fu-Qing granule is best to treat RSA and Ad3 virus infections. The antivirus effects both of Yi-Fu-Qing granule and compound mixture of Isatis leaf, which is an extract of Isatis leaf and effective antivirus traditional Chinese medicine in clinical use in China for many years (*12*), are nearly the same. As shown in Figure 1, in untreated HeLa cells with RSV virus infections, the CPE was obvious with the cells swelling, aggregation, and fusion, and the CPE of untreated HeLa cells with Ad3 virus infections, was observed with the cells becoming rounded, with swelling, fusion and detachment, while with the Yi-Fu-Qing granule at a concentration of 1:100, there was no obvious CPE. According to these results, we can conclude that Yi-Fu-Qing granule may have a significant antivirus effect on acute respiratory tract infections with RSV and Ad3 infection.

Table 2. CPE of virus RSV and Ad3 for each group

Groups	The concentrations of drugs	CPE	
		RSV	Ad3
Un-treated group	0	4	4
	1:400	3	3
Yi-Fu-Qing granule	1:200	1	1
	1:100	0	0
Compound mixture of Isatis leaf	1:100	0	0

Yi-Fu-Qing granule is a compound preparation of traditional Chinese medicine consisting of 6 herbs including *Folium perillae*, *Scutellaria baicalensis* Georgi, *Bupleurum chinense* DC, and so on. Modern pharmacological research indicates that *Folium perillae* is the most important herb in the Yi-Fu-Qing granule. It has been used in China for centuries to treat various diseases including: cough, tumors, bacterial and fungal infections, allergy, intoxication, and its bioactive compounds such as rosmarinic acid, and ursolic acid also have anti-inflammatory and anti-tumour effects (*13*). *Scutellaria baicalensis* Georgi and its bioactive compounds have been shown to be effective in treating the common cold, hyperlipemia, atherosclerosis, cancer and inflammatory diseases such as atopic dermatitis (*14,15*). *Bupleurum chinense* DC, as a traditional Chinese medicine, has been wildly used for the treatment of analgesia, as an anti-inflammatory, an antitumor, an anti-biosis and an antivirus. Its bioactive compounds such as saikosaponin, and saikogenin are reported to effect the regulating of immunity (*16*). *Tinospora capillipes* Gagnep is mainly used to treat cough, swelling and throat pain, skin and breast inflammations and so on (*17*). *Semen armeniacae amarum*, the seed of *Prunus armeniaca* L.var *ansu*

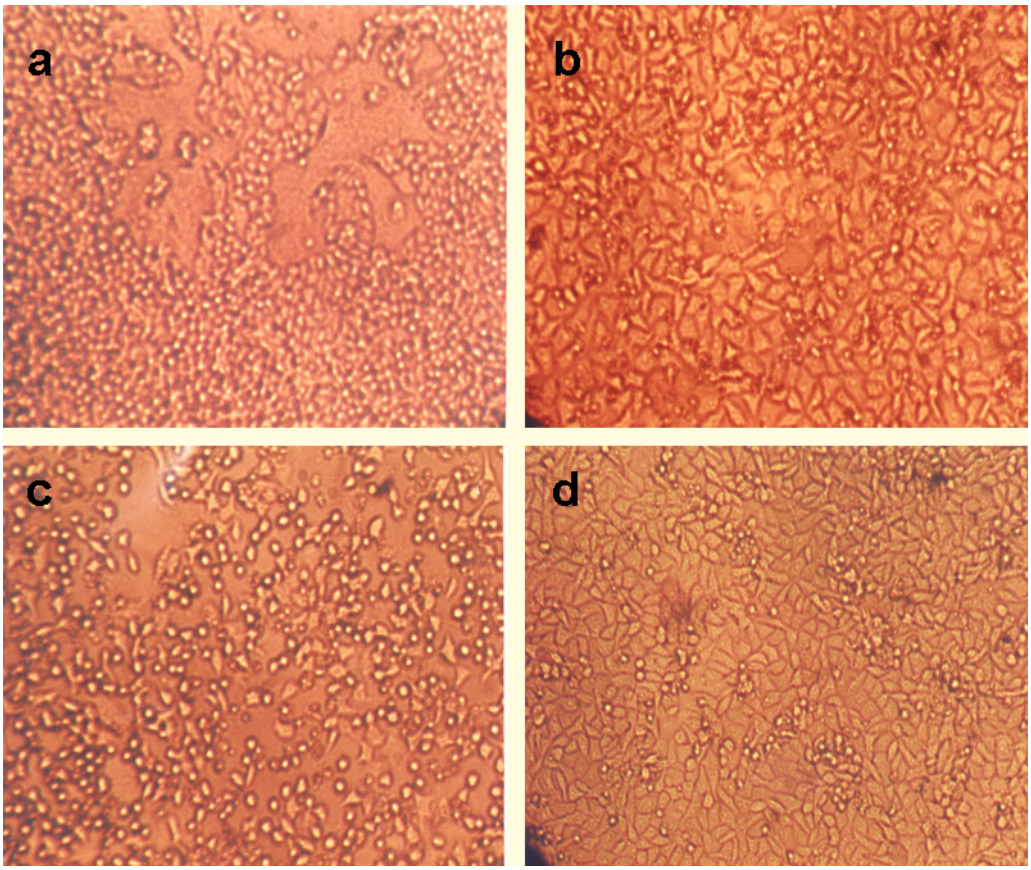

Figure 1. Cytopathogenic effect (CPE) observed on RSV and Ad3 virus infections in HeLa cells. (a) RSV virus infections without Yi-Fu-Qing granule treated; (b) RSV virus infections with Yi-Fu-Qing granule treated (1:100); (c) Ad3 virus infections without Yi-Fu-Qing granule treated; (d) Ad3 virus infections with Yi-Fu-Qing granule treated (1:100). Original magnification, 20×.

Maxim, is known to have many therapeutic effects such as relieving fever, stopping cough, quenching thirst and so on (*18*). The root of *Glycyrrhixa uralensis* Fisch has been used medicinally for over 2,000 years in China and it is generally prescribed by herbalists as a component in formulations (*19*). *Glycyrrhixa uralensis* Fisch and its bioactive compounds such as glycyrrhizic acid are also used as an expectorant in the treatment of bronchitis, catarrh, and coughs with antiviral, anti-inflammatory, antidotal, immune-modulating and other properties (*20*). According to the analysis of these 6 herbs, it is concluded that each herb has an antiviral and anti-inflammatory effect for treating respiratory infections.

In conclusion, the present study indicated that Yi-Fu-Qing granule may have a significant antivirus effect on acute respiratory tract infections with RSV and Ad3 infection, and this could prove useful for further antivirus research on acute respiratory tract infections. However, further study is needed to clarify its antivirus mechanism on virus RSV and Ad3, and its antivirus effects on other viruses.

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