Experiment study on Ma-Xing-Shi-Gan-Tang (MXSGT) and its decomposed recipes on anti-influenza virus A in vitro

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Abstract

Ma-Xing-Shi-Gan Tang (abb. MXSGT) is a famous complex prescription derived from the well-known clinical textbook《Treatise on Cold-induced Febride Diseases》which was compiled by the famous of Traditional Chinese physician Zhang Zhongjing. The MXSGT has been reported inhibitory effects on influenza A virus in vitro in recent years. In order to exploring prescription principle of MXSGT, We examined its decomposed recipes on anti-influenza virus A in vitro. Ma-Xing-Shi-Gan -Tang was disassembled into 15 recipes according to the design of composing prescriptions. Cell culture technique and MTT test were used to determine the effects of MXSGT and its decomposed recipes on MDCK cells infected by influenza virus A. The results show that decomposed recipe group of single medicine and two medicines didn't have inhibitory effects on influenza virus A. In the group of three medicines, all recipes have inhibitory effects except Xing Ren-Shi Gao-Gan Cao recipe. Interestingly, Ma Huang-Shi Gao-Gan Cao recipe has the same inhibitory effect as MXSGT at 200ug/ml. It conjectured that Ma Huang may play a very important role in complex prescription against influenza type A virus, and Shi Gao, though as a mineral medicine, also play an important role in complex prescription. The further research of MXSGT against influenza type A virus is under process.

Keywords：maxingshigan dectoctin，decomposed recipes，influenza virus A

Introduction

The using of Traditional Chinese Herbs in traditional medicine clinic based on unique principle---principal, assistant, complement and guide from the emergence of the earliest medicine guide book《Shen Nong Materia Medica》two thousand years ago to today. Ma Xing Shi Gan Tang is a most classical complex prescription derived from the well-known clinical textbook《Treatise on Cold-induced Febride Diseases》which was compiled by the most famous of Traditional Chinese physician Zhang Zhongjing. This complex prescription is composed of Chinese Ephedra Herb (Ma Huang), Bitter Almond (Xing Xen), Gypsum (Shi Gao) and Liquorice Root (Gan Cao) and act as principal, assistant, complement and guide correspondingly. The drug dose of four medicines is 6g, 9g, 24g and 6g recorded by tradition medicine book. It has the pharmacological action of relieving fever, anti-inflammatory, antivirus, relieving cough and so on, it is widely used in the clinic for thousands of years with an excellent safety record [1]. The MXSGT has been reported inhibitory effects on influenza A virus in vitro and its effect may interfering influenza virus A absorption and protecting the cells infected by influenza virus A[2-3]. With regard to the inhibitory on influenza A virus of decomposed recipes, thus far there has not been reported.

Materials and methods

Plant materials

Ma Huang (batch NO.080711), Xing Ren(batch NO.080623), Shi Gao(batch NO.080116) and Gan Cao(batch NO.080504) were bought in the Shanghai LeiYunShang pharmaceutical Co. LTD. in march of 2008. The collected samples were kindly identified by Professor Kong Deyun in the Department of Traditional Chinese Medicine, Shanghai Institute of Pharmaceutical Industry. All voucher specimens were deposited in department of traditional Chinese medicine, Shanghai Institute of Pharmaceutical Industry.

Decomposed formulas

Ma Xing Shi Gan Tang prescription was disassembled into 15 recipes according to the design of decomposed recipes (Tab 1) and every recipe was examined inhibitory effects on influenza A virus in vitro.

Extraction

According to the application of prescription, all the decoctions were prepared according to the list of decomposed recipe. All materials were cut into pieces and refluxed for 1.5 h with 75% EtOH three times. The extract was filtered and concentrated under reduced pressure. The samples saved in cryopreservation.

Cells and viruses

MDCK cells (Institute of Medicinal Biotechnology, Chinese Academy of Medical

Sciences). Influenza viruses (batch NO: A3/Beijing/30/95) (Shanghai Municipal Center for Disease Control & Prevention).

MDCK cells were used as host cells. They were grown in Eagle's minimum essential medium (EMEM) which contain 10% fetal bovine serum (FBS, Gibco), penicillin G (100 U/mL) and streptomycin (100μg/mL). The cells were maintained in a humidified atmosphere containing 5 % CO_2 at the temperature of 37 ℃.

Toxicity measurements

MDCK cells were seeded into 96-well culture plates at a density of 1×10^4 cells per well. Cells were incubated for 24 h until 90% confluency. Samples were dissolved in DMSO and then diluted to 400μg/mL, 200μg/mL, 100μg/mL, 50μg/mL and 25μg/mL, respectively. These dilutions were incubated with monolayer MDCK cells at the temperature of 37℃ in 5% CO_2 for 72 h. The growth of cells was detected by modified MTT assay. Cell viability was calculated according to the following formula:

Cell viability (%) = (OD Sample / OD normal control) ×100%

TC_0 is the concentration at which the cell viability of the sample >=90%

In vitro antiviral evaluation

The antiviral effect of the samples on influenza virus was measured by inhibition of viral cytopathic effect (CPE). MDCK cells were seeded into 96-well culture plates at a density of 1×10^4 cells per well and incubated for 24h until 90% confluency.

Monolayer MDCK cells were washed with serum-free EMEM culture medium twice. The MDCK cells were set to infect with influenza virus A3 at 100TCID50 (tissue culture infectious dose 50). After the incubation at 37℃ in 5 % CO_2 for 1.5 h, viruses were decanted from MDCK cells. Different samples were added into the 96-well plates containing MDCK cells and maintained in a humidified incubator with 5% CO_2 at 37℃ for another 72 h. Then the CPE was observed to evaluate the antiviral effect of the samples on influenza virus.

Results

In MDCK cells, all decomposed recipe groups except Ma Huang show no inhibitory effect on cell proliferation up to 400μg/ml, as analyzed by MTT assay (Tab 1). In vitro antiviral evaluation, the results show that decomposed recipe group of single medicine and two medicines didn't have inhibitory effects on influenza virus A. In the group of three medicines, all recipes have inhibitory effects on influenza virus A except Xing Ren-Shi Gao-Gan Cao recipe. Interestingly, the Ma Huang-Shi Gao-Gan Cao recipe has the same inhibition effect as MSXGT at 200μg/ml.

Discussion

Thus far, there is only a single report on the anti-influenza A virus activity of MXSGT decomposed recipe [4]. According to the results of toxicity experiments, it shows that complex prescription may imply low toxicity in view of the TC_0 of MXSGT is higher

two times than that Ma Huang recipe. In Tradition Chinese Medicine's opinion, the key principle of complex prescription is relationship of principal, assistant, complement and guide. It's known by all Tradition Chinese physicians that complex prescription not only improves the therapeutic effect but also reduce toxicity. Due to the group of one medicine and two medicines far beyond principle of complex prescription, the group from1 to 10 didn't show anti-influenza A virus activity.

In contrast, all the groups of three medicines have anti-influenza A virus activity when Ma Huang included in recipe. Once Ma Huang was excluded from recipe, the recipe shows no activity on influenza A virus. It conjectured that Ma Huang may play a very important role in complex prescription against influenza type A virus.

Interestingly, the anti-influenza A virus activity of group 13(Ma Huang-Shi Gao-Gan Cao) is markedly better than group12 (Ma Huang-Xing Ren-Gan Cao). In fact, the major chemical constituents of Shi-Gao is $CaSO_4 \bullet 2H_2O$ and has been used for quality evaluation of Radix Astragali (Pharmacopoeia of the People's Republic of China, 2005 edition). However, how and through what ways did Shi-Gao enhance anti-influenza A virus effect is a mystery and complicated research subject. The further research on decomposed recipes of MXSGT against influenza type A virus is under process.

Reference

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Table 1 The group of decomposed formulas of MXSGT

Group	Recipe	TC_0(μg/mL)	Inhibitory effect	
			Concentration (μg/mL)	Inhibition(%)
1	Ma Huang	200	200	---
			100	---
			50	---
2	Xing Ren	400	400	---
			200	---
			100	---
			50	---
3	Shi Gao	400	400	---
			200	---
			100	---
			50	---
4	Gan Cao	400	400	---
			200	---
			100	---
			50	---
5	Ma Huang+Xing Ren	400	400	---
			200	---
			100	---
			50	---
6	Ma Huang+Shi Gao	400	400	---
			200	---
			100	---
			50	---
7	Ma Huang+Gan Cao	400	400	---
			200	---
			100	---
			50	---
8	Xing Ren+Shi Gao	400	400	---
			200	---
			100	---
			50	---
9	Xing Ren+Gan Cao	400	400	---
			200	---
			100	---
			50	---
10	Shi Gao+Gan Cao	400	400	---
			200	---
			100	---
			50	---

11	Ma Huang+Xing Ren+Shi Gao	400	400	25%
			200	---
			100	---
			50	---
12	Ma Huang+Xing Ren+Gan Cao	400	400	25%
			200	---
			100	---
			50	---
13	Ma Huang+Shi Gao+Gan Cao	400	400	25-50%
			200	25%
			100	---
			50	---
14	XingRen+Shi Gao+Gan Cao	400	400	---
			200	---
			100	---
			50	---
15	Ma Huang+Xing Ren+Shi Gao+Gan Cao	400	200	25%
			100	---
			50	---
			25	---