Journal of Clinical Virology 31 (2004) 69–75

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Short communication

In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds

F. Chen [a], K.H. Chan [b], Y. Jiang [c], R.Y.T. Kao [b], H.T. Lu [a], K.W. Fan [a], V.C.C. Cheng [b], W.H.W. Tsui [b], I.F.N. Hung [b], T.S.W. Lee [b], Y. Guan [b], J.S.M. Peiris [b], K.Y. Yuen [b,*]

[a] *Centre for Research in Plant Drugs Development, Department of Botany, The University of Hong Kong, Pokfulam Road, Hong Kong*
[b] *Division of Infectious Diseases, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong*
[c] *Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong*

Received 17 November 2002; accepted 1 March 2004

Abstract

Effective antiviral agents are urgently needed to combat the possible return of severe acute respiratory syndrome (SARS). Commercial antiviral agents and pure chemical compounds extracted from traditional Chinese medicinal herbs were screened against 10 clinical isolates of SARS coronavirus by neutralisation tests with confirmation by plaque reduction assays. Interferon-beta-1a, leukocytic interferon-alpha, ribavirin, lopinavir, rimantadine, baicalin and glycyrrhizin showed antiviral activity. The two interferons were only active if the cell lines were pre-incubated with the drugs 16 h before viral inoculation. Results were confirmed by plaque reduction assays. Antiviral activity varied with the use of different cell lines. Checkerboard assays for synergy were performed showing combinations of interferon beta-1a or leukocytic interferon-alpha with ribavirin are synergistic. Since the clinical and toxicity profiles of these agents are well known, they should be considered either singly or in combination for prophylaxis or treatment of SARS in randomised placebo controlled trials in future epidemics.

Keywords: Severe acute respiratory syndrome; Antiviral compounds; Epidemics

1. Introduction

Although the SARS epidemic has been successfully contained with quarantine and infection control measures, the presence of this virus in wild game food animals (Guan et al., 2003), stocks in laboratories and possible seasonality of this disease suggest that recurrence of such an epidemic is not unlikely in the coming winters. Since all age groups are affected and a high fatality is noted in the elderly and those with co-morbidities (Donnelly et al., 2003), there is an urgent need to find a cure. Prospective clinical and viral load studies in nasopharyngeal secretions from SARS patients showed that viral replication peaked at the 10th day after the onset of symptoms with subsequent clinical deterioration in 30% of the cases despite a decreasing viral load (Peiris et al., 2003). Therefore the key facet of management should include respiratory support, immuno-modulation in selected cases and early institution of an effective antiviral agent. Such an antiviral agent, if given early, may decrease the peak viral load and the associated immuno-dysregulatory damage. At the moment, there are no commercially available antiviral agents tailored-made for SARS coronavirus. There is an urgent need to search for an agent with a known in use clinical and toxicity profile so that a randomised placebo control trial can be conducted if the epidemic recurs in one of the coming winters. We report in this study on the in vitro antiviral susceptibility of 10 isolates of SARS coronavirus to commercially available antiviral agents and pure chemical compounds including baicalin, glycyrrhizin, and chlorogenic acid extracted from traditional Chinese herbs.

2. Methods

Ten isolates of SARS coronavirus from 10 different SARS patients who satisfied the revised WHO criteria for SARS are listed in Table 1. The drugs used for antiviral susceptibility

* Corresponding author. Tel.: +852-28554892; fax: +852-28551241.
E-mail address: kyyuen@hkucc.hku.hk (K.Y. Yuen).

1386-6532/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2004.03.003

Table 1
Clinical data of 10 isolates of SARS coronavirus in 10 patients suffering from SARS

Patient number	Isolate number	Clinical specimen	Sex/age	Day of specimen after onset of symptoms	RT-PCR result	Day of seroconversion after onset of symptoms	Antibody titer by immunofluorescence staining	Hospital
1	M39849[a]	Lung tissue biopsy	M/54	9	Positive	9	1:160	KWH
2	M36871	NPA	F/41	6	Positive	12	1:1280	PMH
3	M65189	NPA	F/38	4	Positive	18	1:640	PYNEH
4	M67349	NPA	F/41	6	Positive	20	1:640	PYNEH
5	M70221	NPA	M/30	10	Positive	22	1:2560	PYNEH
6	M71749	NPA	M/48	11	Positive	24	1:160	PYNEH
7	M51776	NPA	F/39	6	Positive	10	1:640	TKOH
8	M61558	NPA	F/53	10	Positive	18	1:40	UCH
9	M61576	Urine	M/40	12	Positive	18	1:40	UCH
10	M61565	NPA	M/28	10	Positive	18	1:40	UCH

Note: KWH, Kwong Wah Hospital; NPA, Nasopharyngeal aspirates; PMH, Princess Margaret Hospital; PYNEH, Pamela Youde Nethersole Eastern Hospital; TKOH, Tseung Kwan O Hospital; UCH, United Christian Hospital.
[a] Prototype virus.

tests include acyclovir (Glaxo Wellcome, UK), ganciclovir (Sanofi Winthrop Pharmaceuticals McPherson, Kansas, USA), cidofovir (Pharmacia & Upjohn, Luxembourg), foscarnet (AstraZeneca, Australia), ribavirin (ICN, Switzerland), interferon-alpha-2a (Roferon-A, Roche, Switzerland), interferon-alpha-2b (Intron A, Schering-Plough, Ireland), interferon-beta-1a (Rebif, Serono, Italy), leukocytic interferon-alpha (Interferon Alfanative, BioNative AB, Umea, Sweden), amantadine (Unicorn), rimantadine (Forest, USA) zidovudine (Glaxo Wellcome, UK), stavudine (Bristol-Myers Squibb, USA), nevirapine (Boehringer Ingelheim, Germany), abacavir (Glaxo Wellcome, UK), ritonavir (Abbott, USA), and lopinavir (Abbott, USA).

The State Administration of Traditional Chinese Medicine of the People's Republic of China recently formed a panel of Chinese Medicine specialists to draw up a "technical scheme (tentative) for the prevention and treatment of severe acute respiratory syndrome (SARS) using traditional Chinese medicine". In the scheme, a recipe "Qing Fei Jie Du Tang" (soup for clearing the lung and detoxification) was recommended which consists of Huang Qi (*Astragalus membranaceus*) 15 gm, Chai Hu (*Bupleurum chinense*) 10 gm, Ma Huang (*Ephedra sinica*) 5 gm, Xing Ren (*Prunus armeniaca*) 10 gm, Sheng She Gao (Plaster Stone) 30 gm, Sheng Yi Ren (*Coix lacryma-jobi*) 15 gm, Gua Wei Pi (*Benincasa hispida*) 15 gm, Jie Geng (*Platycodon grandiflorum*) 9 gm, Bo He (*Mentha haplocalyx*) 6 gm, Huang Qin (*Scutellaria baicalensis*) 10 gm, Sheng Gan Cao (*Glycyrrhiza uralensis*) 5 gm, Jin Yin Hua (*Flos lonicerae*) 15 gm, and Qing Hao (*Artemisia apiacea*) 15 gm. Among them, only *Scutellaria baicalensis*, *Glycyrrhiza uralensis*, *Flos lonicerae* and *Artemisia apiacea* have their pure chemically defined ingredients being extracted, purified and documented to have antimicrobial activities. Consequently, the main bioactive compounds, namely, baicalin (derived from *Scutellaria baicalensis*), glycyrrhizin (from *Glycyrrhiza uralensis*), chlorogenic acid (from *Flos lonicerae*) and artesunate (from *Artemisia apiacea*) were investigated in the present study.

The pharmacological properties of baicalin, glycyrrhizin, and chlorogenic acid are summarized in Table 2. Artesunate is not included in this table since it is already well known as an anti-malarial drug in Western Medicine (Price, 2000). They were extracted as we have previously reported (Lu et al., 2003). The concentrations of baicalin, glycyrrhizin,

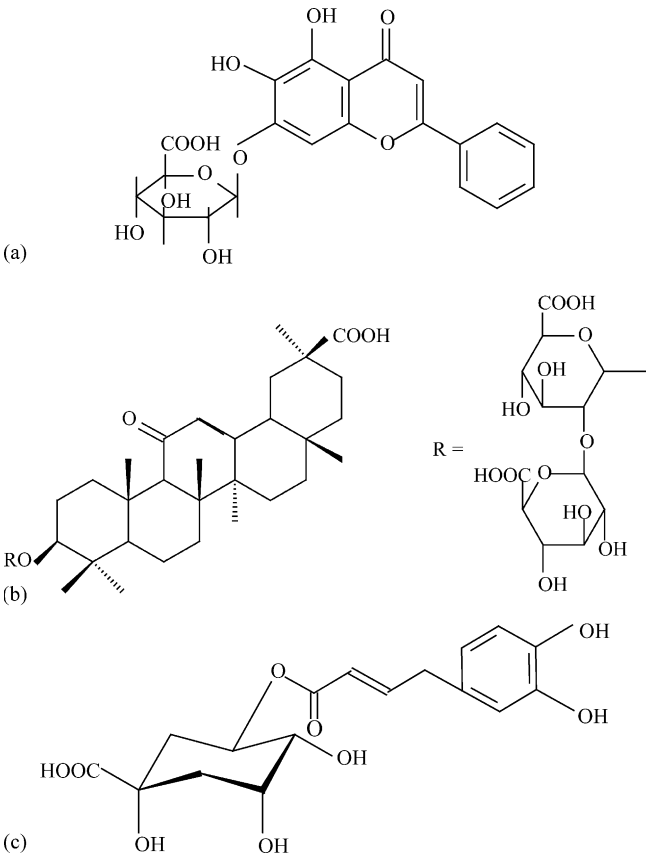

Fig. 1. (a) Chemical structure of baicalin (黄芩甙), (b) chemical structure of glycyrrhizin (甘草甜素), (c) chemical structure of chlorogenic acid (氯原酸).

Table 2
Summary on three natural compounds from traditional Chinese medicines

Compounds	Baicalin (黄芩甙)	Glycyrrhizin (甘草甜素)	Chlorogenic acid (氯原酸)
Name of herbs	*Scutellaria baicalensis* (Huang Qin)	*Glycyrrhiza uralensis* (Liquorice)	Flos Lonicerae (gold silver flower)
Chemical structure	Please refer to Fig. 1a	Please refer to Fig. 1b	Please refer to Fig. 1c
Chemical formulae	$C_{21}H_{18}O_{11}$	$C_{42}H_{62}O_{16}$	$C_{16}H_{18}O_9$
Molecular weight	446.38	822.93	354.3
Thermal stability	Stable at boiling temperature (a typical extraction method)	Stable at a temperature below 120 °C	Stable after long time boiling
Serum level (after oral administration)	C_{max} = 60 ng/ml (189.8 mg per person, in human). The amount (i.e., 189.8 mg per person) used for the experiment is very low	No glycyrrhizin in plasma is found after oral administration of 100 mg glycyrrhizin in healthy persons, presumably glycyrrhizin is metabolized to glycyrrhetinic acid by intestinal bacteria which contain β-D-glucuronidase or the amount consumed is too little	Only traces expected (1000 mg per person, in human); this may be due also to that the amount consumed is much lower than that for animals
Standard doses in oral administration in humans	∼1500 mg baicalin (as tablets); also can be up to ∼6000 mg baicalin (calculated from herb, assuming 30 g herb used; the herb may contain up to 20% as baicalin)	∼300 mg glycyrrhizin (as tablets) or ∼1700 mg glycyrrhizin (calculated from the herb assuming that the herb contains 5.65% glycyrrhizin)	∼2220 mg (calculated from the herb assuming that the herb contains 7.4% chlorogenic acid)
Serum level (after intravenous administration)	C_{max} = 74 μg/ml (360 mg per person)	C_{max} = 80 μg/ml (200 mg per person)	C_{max} = 34 μg/ml (24 mg per person)
Standard doses in intravenous administration in humans	∼600 mg baicalin	∼240 mg	>74 mg, when used together with baicalin in injection preparation; 180 mg for muscle injection
Half life (in humans)	∼3 h	∼10 h	>10 h
Antiviral effect	Inhibition of HIV-1	Inhibition of SARS-associated virus	Inhibition of various viruses

chlorogenic acid, and lopinavir in the cell culture system were also monitored by HPLC (Lu et al., 2003) whereas the concentration of others were monitored by neutralization assays with the Vesicular Stomatitis Virus Indiana strain and a laboratory strain of Influenza A H1N1.The procedures used for in vitro antiviral susceptibility testing are as follows. Initial screening of all compounds against the prototype SARS coronavirus strain no. 39849 was performed in 96-well microtitre plates seeded with foetal rhesus kidney-4 cells. Two-fold dilutions of antiviral agents starting from more than four times the peak serum concentration after the maximum therapeutic dose to less than one-quarter of the trough serum concentration were tested in quadruplicate against 100 $TCID_{50}$ of SARS coronavirus. A corresponding set of cell controls with drug but without virus inoculation was used as controls for drug toxicity. The cells were scored for the inhibition of the cytopathic effect (CPE) at 48 and 72 h. Those compounds with demonstrable in vitro inhibitory activity were re-assayed against the other nine strains of SARS coronavirus collected from different patients from different hospitals of the Hong Kong Special Administrative Region (HKSAR). Their antiviral activities were also compared in both foetal rhesus kidney-4 (fRHK-4) and Vero-E6 cell lines. Those likely to have clinically significant inhibitory activity were tested by the plaque reduction assay.

For selected agents with consistent activity in the plaque reduction assay, checkerboard assays for synergy were performed for combinations of interferons and ribavirin using the same neutralization test in 96 well microtiter plates seeded with Vero cell line. Cells were not incubated with the interferons before viral inoculation. Vero cells were used instead of Vero E6 and fRhK-4 cell lines because better antiviral effect can be demonstrated in Vero but less so in the other two cell lines for ribavirin and the interferons.

3. Results

Ten isolates of SARS coronavirus from 10 different patients with SARS admitted to different hospitals in HKSAR showing seroconversion towards the prototype virus infected fRhK-4 cell line were used in this study (Table 1). They were isolated from the lung tissue biopsy (prototype virus, M39849), urine, and nasopharyngeal aspirates. Initial screening of 20 commercially available antimicrobial agents against the prototype virus grown in fRhK-4 cell line did not reveal inhibitory activities for acyclovir, ganciclovir, cidofovir, foscarnet, interferon-alpha-2a, interferon-alpha-2b, amantadine, zidovudine, stavudine, nevirapine, abacavir, and ritonavir. Inhibitory activities were not detectable for glycyrrhizin, artesunate and chlorogenic acid in fRhK-4 cell line. Glycyrrhizin was still included for further testing because this was reported to be active in Vero-E6 cell lines (Cinatl et al., 2003a). Further testing by neutralization tests

Table 3
Comparison of antiviral activity of 10 compounds against 10 strains of SARS-CoV in fRhK4 cell line, against the prototype strains (39849) of SARS-CoV in fRhK4 and Vero-E6 cell lines by neutralization test

	fRhK4 cell line (against 10 strains of SARS-CoV)				fRhK4 cell line (against 39849)	Vero-E6 cell line (against 39849)
	EC_{50} (μg/ml) at 48 h	EC_{50} (μg/ml) at 72 h	CC_{50} (μg/ml)	SI = CC_{50}/EC_{50} at 48 h	EC_{50} (μg/ml) at 48 h	EC_{50} (μg/ml) at 48 h
Ribavirin	12.5 to 200	50 to 200	>1000	5 to >80	50 to 100	>200
Interferon alpha (natural multi-subtype) added at and after viral adsorption	5000 IU[a]	5000 IU[a]	>10,000 IU[a]	>2	5000 IU[a]	19.5 IU[a]
Interferon alpha (natural multi-subtype) pre-incubation for 16 h	39 to 625 IU[a]	10,000 IU[a]	>10,000 IU[a]	>16 to 250	39 IU[a]	19.5 IU[a]
Interferon beta 1a added at and after viral adsorption	2500 to 10,000 IU[a]	10,000 IU[a]	>10,000 IU[a]	>4	2000 IU[a]	106 IU[a]
Interferon beta 1a pre-incubation for 16 h	625 IU[a]	10,000 IU[a]	>10,000 IU[a]	>16	625 IU[a]	19.5 IU[a]
Rimantadine	8 to 16	32	64	4 to 8	16	8 to 16
Lopinavir	1 to 4	4 to 8	32	8 to 32	2 to 4	4 to 8
Baicalin	12.5 to 25	25 to 50	>100	>4 to 8	12.5	100
Glycyrrhizin	>400	>400	>400	NA	>400	100

Note: EC_{50}, effective concentration of compound required to inhibit the cytopathic effect to 50% of control value; CC_{50}, cytotoxic concentration of compound that reduced cell viability to 50%; NA, not applicable; SI, selectivity index.
[a] IU, international unit

with the other 9 isolates of SARS coronavirus against the active compounds confirmed detectable inhibitory activities for leukocytic interferon-alpha, interferon-beta-1a, ribavirin, lopinavir, rimantadine, and baicalin. The range of their effective concentration of compound required to reduce the plaque forming unit by 50% (EC_{50}) at 48 and 72 h, and their selectivity index are shown in Table 3.

When the same neutralization test on these compounds was run in Vero-E6 cell line, rimandatine, glycyrrhizin, leukocytic interferon-alpha and interferon-beta were more active especially at 72 h. Moreover, pre-incubation of the cell lines with these two interferons for 16 h before adding the virus markedly enhanced the inhibitory activity by three to over 100-fold. But ribavirin, lopinavir, and baicalin were less active in the Vero-E6 cell line (Table 3).

As for the plaque reduction assay, Vero cell lines were used instead of Vero E6 or fRhK-4 cell lines because antiviral activity can be demonstrated for most of the agents. Only interferon-beta-1a, leukocytic interferon-alpha, lopinavir, ribavirin, rimantadine, and baicalin were tested. The EC_{50} of interferon-beta-1a (8 U/ml), leukocytic interferon-alpha (30 U/ml), lopinavir (6 μg/ml), ribavirin (50 μg/ml), rimantadine (7 μg/ml), and baicalin (11 μg/ml) are comparable to the results obtained from CPE assays.

Tests for synergism between ribavirin and lopinavir have already been reported (Chu et al., 2004). No synergism could be demonstrated between rimantadine and ribavirin. The most active compounds are the interferons. Thus further checkerboard assays were performed with combinations of leukocytic interferon-alpha or interferon-beta-1a, and ribavirin. Marked synergism was seen at both 72 and 96 h. The combination of leukocytic interferon-alpha (78 μg/ml) and ribavirin (25 μg/ml) or interferon-beta-1a (312.5 μg/ml) and ribavirin (25 μg/ml) were shown to be active at 96 h of incubation (Table 4).

4. Discussion

Control of SARS may be achieved by epidemiological measures, antiviral prophylaxis or treatment, and vaccination. During the last pandemic of SARS, the only available means for control were public health measures such as isolation of suspected cases, quarantine of contacts, and personal protective infection control procedures for high-risk individuals such as health care workers. There is an urgent need to find effective antiviral agents with acceptable side effect profiles. In developing countries such as China, commercially available western antiviral medicine is unlikely to be affordable by most people. Moreover, the SARS mortality of Mainland China was only 7% comparing favourably with the 15% to 27% of other areas (WHO, 2003). China

Table 4
Checkerboard assay for synergism between interferons and ribavirin by neutralization test without pre-incubation

Interferon alpha	72 h										
	10000	5000	2500	1250	625	312.5	156	78	39	19.5	0
Ribavirin											
200	+	+	+	+	+	+	+	+	+	+	−
100	+	+	+	+	+	+	+	+	+	+	−
50	+	+	+	+	+	+	+	+	+	−	−
25	+	+	+	+	+	+	+	+	−	−	−
12.5	+	+	+	+	+	−	−	−	−	−	−
0	+	+	+	+	−	−	−	−	−	−	−
	96 h										
	10000	5000	2500	1250	625	312.5	156	78	39	19.5	0
Ribavirin											
200	+	+	+	+	+	+	+	+	+	−	−
100	+	+	+	+	+	+	+	+	−	−	−
50	+	+	+	+	+	+	+	+	−	−	−
25	+	+	+	+	+	+	+	+	−	−	−
12.5	+	+	+	+	+	−	−	−	−	−	−
0	−	−	−	−	−	−	−	−	−	−	−
Interferon beta 1a	72 h										
	10000	5000	2500	1250	625	312.5	156	78	39	19.5	0
Ribavirin											
200	+	+	+	+	+	+	+	+	+	+	−
100	+	+	+	+	+	+	+	+	+	+	−
50	+	+	+	+	+	+	+	+	+	−	−
25	+	+	+	+	+	+	+	−	−	−	−
12.5	+	+	+	+	+	+	+	−	−	−	−
0	+	+	+	+	+	+	+	−	−	−	−
	96 h										
	10000	5000	2500	1250	625	312.5	156	78	39	19.5	0
Ribavirin											
200	+	+	+	+	+	+	+	+	+	+	−
100	+	+	+	+	+	+	+	+	−	−	−
50	+	+	+	+	+	+	+	+	−	−	−
25	+	+	+	+	+	+	−	−	−	−	−
12.5	+	+	+	+	+	−	−	−	−	−	−
0	+	+	+	+	+	−	−	−	−	−	−

+: ≥50% inhibition; −: ≤50% inhibition.

is also the only place where traditional Chinese medicinal herbs were extensively used for treatment of SARS. The development of vaccine will take a much longer time. Therefore, we undertook these antiviral susceptibility tests for all commercially available antiviral agents in the HKSAR and pure chemicals purified from traditional Chinese herbs known to have antimicrobial activity. These chosen herbs were included in a standard formula used for the treatment of SARS in China and the HKSAR.

Only interferon-beta and glycyrrhizin were reported to have significant antiviral activity against SARS coronavirus (Cinatl et al., 2003a,b). Using the fRhK-4 cell line, we have shown that ribavirin, rimantadine, lopinavir, and baicalin also have detectable antiviral activities. However, like the interferons and glycyrrhizin, their activities tend to decrease with incubation beyond 48 h (Table 3). Judging from the achievable serum levels with standard oral or parenteral dosing, rimantadine, ribavirin, glycyrrhizin, and even the two interferons are unlikely to have clinically significant in vivo activities. Moreover, lopinavir, and rimantadine have a relatively inferior selectivity index of 4 to 32. Upon subsequent testing with Vero-E6 cell line, both leukocytic interferon-alpha and interferon-beta-1a were more active and especially after pre-incubation for 16 h before viral inoculation. The findings suggest that prophylaxis with the interferons should be considered. Though ribavirin was much less active in the Vero cell line, it is highly synergistic with either two interferons. Therefore, a combination of ribavirin with either of these two interferons should be considered for the treatment of SARS.

Interferon-gamma was reported not to possess antiviral activity against SARS coronavirus (Cinatl et al., 2003b), whereas interferon-beta was confirmed to be active in this study. What is interesting was the demonstration of activity of leukocytic interferon-alpha despite the lack of activity of the recombinant interferon-alpha-2a and interferon-alpha-2b. This was not unexpected because this preparation of leukocytic interferon-alpha is a multi-subtype natural interferon with predominantly interferon alpha-1 and alpha-2 in contrast to the other commercial preparation with a single subtype of recombinant interferon-alpha-2. In in vitro studies, different subtypes have been found to have different antiviral activities as well as immunological effects (Foster et al., 1996). It was also demonstrated that leukocytic interferon-alpha had a superior antiviral effect than that of recombinant interferon on Human immunodeficiency Virus infection (Fan et al., 1993).

It is important to know that in vitro findings may not correspond with clinical efficacy. Despite its in vitro activity, topical or systemic interferon-alpha did not produce a consistent reduction in symptoms or lesion duration of genital herpes (Eron et al., 1987; Lebwohl et al., 1992). And interferon-alpha was not effective in preventing CMV infections or treating CMV pneumonia in bone marrow transplant patients (Meyers et al., 1980). Despite its broad-spectrum antiviral activities against respiratory viruses in vitro, prophylactic intranasal interferon-alpha is only protective against rhinovirus-induced common cold under natural condition (Douglas et al., 1986). This was unexpected because intranasal leucocyte or recombinant interferon-alpha protect against experimental human infection by rhinovirus, coronavirus, and respiratory syncytial virus (Hayden and Gwaltney, 1984; Higgins et al., 1983; Higgins et al., 1990).

Besides the high cost of interferons, the high incidence of fever and flu syndrome of up to 98% during its initial phase of administration may pose confusions in terms of the response to treatment. The well-known side effect of pancytopenia may also be confused with markers of SARS activity such as a decrease in platelets and occasionally neutrophils (Raanani and Ben-Bassat, 2002). Although interstitial pneumonitis and bronchiolitis obliterans organising pneumonia are rare complications of prolonged use of interferons (Karim et al., 2001; Ogata et al., 1994), there is always a fear that their proinflammatory effect may worsen the viral pneumonitis caused by SARS.

As for the less expensive option, baicalin but not glycyrrhizin may be considered if traditional Chinese medicine is to be used for antiviral prophylaxis or treatment. The serum level after 100 mg of glycyrrhizin orally was not detectable. Even with a 200 mg dose of intravenous administration, the peak serum level is only 80 μg/ml which is still below the EC_{50} of glycyrrhizin. Although an oral dose of 1.5 gm of baicalin can only achieve a serum concentration of 0.47 μg/ml, intravenous administration of a 360 mg dose of baicalin in human can achieve a peak serum concentration of 74 μg/ml. Thus intravenous baicalin should be considered for treatment in randomised placebo control trials in developing countries where such formulations are available and affordable. Baicalin was shown to inhibit HIV-1 by two mechanisms (Kitamura et al., 1998; Li et al., 2000). At the level of cellular entry, baicalin can conjugate with selected chemokines such as MIP-1β and SDF-1α, and interfere with their capacity to activate cellular receptors CCR5 and CXCR4 respectively. These two co-recpetors are essential elements for HIV-1 infection and therefore baicalin can inhibit Env-protein mediated fusion of HIV with cells expressing CD4/CCR5 or CD4/CXCR4. Baicalin has also been known to inhibit HIV-1 reverse transcriptase probably by interfering with the binding of viral RNA to the RT molecule near the active site of the enzyme.

In terms of prophylaxis against SARS short of an effective vaccine, intranasal leukocytic interferon-alpha or interferon-beta-1a are likely to be effective. However the local side effect of nasal irritation can decrease compliance. It could also be considered for randomised placebo-control trials.

As for the antiviral treatment of symptomatic SARS, it is important to have a rapid and reliable diagnostic test since early institution of antiviral therapy is important to decrease the peak viral load (Poon et al., 2003). Interferon-beta-1a or leukocytic interferon-alpha plus ribavirin appear to be the most effective combination. Since interferons may not be effective in inducing an antiviral state in the uninfected host cells during the first 24 h, a combination with a short course of ribavirin appears to be reasonable. This will also reduce the side effects and fluid volume associated with a full course of ribavirin. Despite the superiority of interferons in the in vitro assays, there is little clinical data of its use in the treatment of acute viral respiratory infection in human. Thus the combination of ribavirin with lopinavir/ritonavir should still be considered since some positive clinical data has already been accumulated in a historical controlled treatment trial (Chu et al., 2004).

Acknowledgements

We acknowledge research funding from the Kai Cheong Tong SARS Research Fund, The University of Hong Kong.

References

Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 2003a;361:2045–6.

Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. Lancet 2003b;362:293–4.

Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, et al. The role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 2004;59:252–6.

Donnelly CA, Ghani AC, Leung GM, Hedley AJ, Fraser C, Riley S, et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. Lancet 2003;361:1761–6.

Douglas RM, Moore BW, Miles HB, Davies LM, Graham NM, Ryan P, et al. Prophylactic efficacy of intranasal alpha 2-interferon against rhinovirus infections in the family setting. N Engl J Med 1986;314:65–70.

Eron LJ, Toy C, Salsitz B, Scheer RR, Wood DL, Nadler PI. Therapy of genital herpes with topically applied interferon. Antimicrob Agents Chemother 1987;31:1137–9.

Fan SX, Skillman DR, Liao MJ, Testa D, Meltzer MS. Increased efficacy of human natural interferon alpha (IFN-alpha n3) versus human recombinant IFN-alpha 2 for inhibition of HIV-1 replication in primary human monocytes. AIDS Res Hum Retroviruses 1993;9:1115–22.

Foster GR, Rodrigues O, Ghouze F, Schulte-Frohlinde E, Testa D, Liao MJ, et al. Different relative activities of human cell-derived interferon-alpha subtypes: IFN-alpha 8 has very high antiviral potency. J Interferon Cytokine Res 1996;16:1027–33.

Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. Science 2003;302:276–8.

Hayden FG, Gwaltney Jr JM. Intranasal interferon-alpha 2 treatment of experimental rhinoviral colds. J Infect Dis 1984;150:174–80.

Higgins PG, Phillpotts RJ, Scott GM, Wallace J, Bernhardt LL, Tyrrell DA. Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrob Agents Chemother 1983;24:713–5.

Higgins PG, Barrow GI, Tyrrell DA, Isaacs D, Gauci CL. The efficacy of intranasal interferon alpha-2a in respiratory syncytial virus infection in volunteers. Antiviral Res 1990;14:3–10.

Karim A, Ahmed S, Khan A, Steinberg H, Mattana J. Interstitial pneumonitis in a patient treated with alpha-interferon and ribavirin for hepatitis C infection. Am J Med Sci 2001;322:233–5.

Kitamura K, Honda M, Yoshizaki H, Yamamoto S, Nakane H, Fukushima M, et al. Baicalin, an inhibitor of HIV-1 production in vitro. Antiviral Res 1998;37:131–40.

Lebwohl M, Sacks S, Conant M. Recombinant alpha-2 interferon gel treatment of recurrent herpes genitalis. Antiviral Res 1992;17:235–43.

Li BQ, Fu T, Dongyan Y, Mikovits JA, Ruscetti FW, Wang JM. Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. Biochem Biophys Res Commun 2000;276:534–8.

Lu HT, Jiang Y, Chen F. Application of high-speed counter-current chromatography to the preparative separation and purification of baicalin from the Chinese medicinal plant Scutellaria baicalensis. J Chromatogr A 2003;1017:117–23.

Meyers JD, McGuffin RW, Neiman PE, Singer JW, Thomas ED. Toxicity and efficacy of human leukocyte interferon for treatment of cytomegalovirus pneumonia after marrow transplantation. J Infect Dis 1980;141:555–62.

Ogata K, Koga T, Yagawa K. Interferon-related bronchiolitis obliterans organizing pneumonia. Chest 1994;106:612–3.

Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003;361:1767–72.

Poon LL, Chan KH, Wong OK, Yam WC, Yuen KY, Guan Y, et al. Early diagnosis of SARS Coronavirus infection by real time RT-PCR. J Clin Virol 2003;28:233–8.

Price RN. Artemisinin drugs: novel antimalarial agents. Expert Opin Invest Drugs 2000;9:1815–27.

Raanani P, Ben-Bassat I. Immune-mediated complications during interferon therapy in hematological patients. Acta Haematol 2002;107:133–44.

World Health Organization. Summary table of SARS cases by country, 1 November 2002–7 August 2003. Available at: http://www.who.int/csr/sars/country/table2003_09_23/en/. Accessed 29 October 2003.