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研究成果報告

Mental Health
精神健康

Pain
痛症

Respiratory Infectious Diseases
呼吸道感染疾病

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Dissemination reports are concise informative reports of health-related research supported by funds administered by the Food and Health Bureau, namely the *Research Fund for the Control of Infectious Diseases* (RFCID), the *Health and Health Services Research Fund* (HHSRF) and the *Health Services Research Fund* (HSRF). In this issue, 11 dissemination reports of funded projects related to mental health, pain and respiratory infectious diseases are presented. In particular, three projects are highlighted due to their potentially significant findings, impact on health care delivery and practice, and/or contribution to health policy formulation in Hong Kong.

Between 2000 and 2006, 166 suicidal attempts were reported in 26 public hospitals, in which 34 patients died. Frontline nurses have a crucial role to play in suicide prevention and management. Chan et al[1] evaluated the effects of an education programme (8.5 hours of learning activity) intended to enhance nurses' knowledge, attitude, and competence in dealing with such patients (who have attempted suicide or have suicidal ideation) and their family members. A total of 110 registered nurses from medical and surgical units were randomly assigned to the study and control groups. Although the participants found the programme useful and it enhanced their knowledge, there were no significant differences between the two groups for any of the outcome measures. The authors noted that nursing manpower, practical guidelines, interdisciplinary collaboration, and physical layout of the ward can prevent nurses from carrying out their roles and responsibilities, and need to be addressed. These are important considerations when planning further nurse-led programmes.

Neck pain is a common medical condition for which many causes have been identified. Psychosocial factors may play a significant role in its development. Of these, fear-avoidance beliefs have been identified as the most powerful cognitive variables in predicting disability and treatment outcomes of patients with low back pain. Chiu et al[2] adapted, translated and validated the Fear-Avoidance Beliefs Questionnaire (FABQ) for Chinese patients with neck pain. The validated FABQ was then tested for reliability and construct validity in 476 patients attending four physiotherapy out-patient departments in different regions of Hong Kong. The investigators found that fear-avoidance beliefs are an important psychosocial measure for predicting future disability and return to complete work capacity (immediately and 3 months after physiotherapy). The validated FABQ will facilitate further research on the effects of fear-avoidance behaviour in patients with neck pain and help provide a better service for and evaluation of them. It may also facilitate cross-cultural studies on this common problem between western and Chinese populations.

Acute upper respiratory tract infection (URTI) is the most common illness leading to consultation in primary care. In Hong Kong, Chinese herbal medicine is commonly used for treating URTI, but reports on its effectiveness and side-effects are scanty. Lam et al[3] conducted a prospective double-blind randomised placebo-controlled trial in 327 URTI patients on the use of two Chinese herbal remedies to determine if they could significantly enhance the resolution (reduce the duration and/or severity) of symptoms and quality of life. Despite being well tolerated, neither remedy was effective in this respect. The authors noted that randomised double-blind placebo-controlled trials are a suitable objective methodology to determine the effectiveness and side-effects of Chinese herbal medicines.

We hope you will enjoy this selection of research dissemination reports. Electronic copies can be downloaded from the Research Fund Secretariat website (**http://www.fhb.gov.hk/grants**). Researchers interested in the funds administered by the Food and Health Bureau may visit the website for detailed information about application procedures.

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3. Lam CL Wong W, Fong DY. Chinese herbal medicine in the treatment of acute upper respiratory tract infection: a randomised, double blind, placebo-controlled clinical trial. Hong Kong Med J 2009;15(Suppl 6):30-4.

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Provision and evaluation of a suicide prevention and management programme by frontline nurses in Hong Kong

Key Messages

1. An education programme to enhance the knowledge, attitudes and competence of nurses in patient suicide prevention in general hospitals was evaluated. There were no significant differences between the study and control groups for any of the outcome measures.
2. Nursing manpower, practical guidelines, interdisciplinary collaboration and physical structure in the ward, which can prevent nurses from carrying out their roles and responsibilities, need to be addressed.
3. Administrators have to bring about changes in nurses' existing knowledge, skills, and attitudes.
4. A continuous cycle of education is needed for new skills and knowledge to be internalised. Ongoing evaluation of the programme could facilitate improvements.

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Introduction

Health care professionals should increase awareness about patient suicides in hospitals. A retrospective study in Hong Kong from 2000 to 2002 reported 166 suicidal attempts in 26 public hospitals, in which 34 patients died.[1] Frontline nurses play a crucial role in suicide prevention and management; it is challenging for them to provide care for patients with suicidal ideation or after suicidal attempts. Nurses may feel frustrated, inadequate, and unsure whenever they fail to help these patients.[2] In England, education for nurses achieved positive results.[3] It is anticipated that an education programme can also enhance local nurses' knowledge, attitude, and competence about suicide prevention and management.

Aims and objectives

1. To evaluate an education programme for frontline nurses on patient suicide prevention and management.
2. To evaluate the effects of the education programme on nurses' knowledge, attitude, and competence for dealing with patients (who have attempted suicide or have suicidal ideation) and their family members.
3. To examine the strengths and weaknesses of the programme from the participants' perspectives.
4. To enhance nurses' knowledge and competence related to suicide prevention and management.

Methods

Study design

The study was conducted in two general hospitals from December 2004 to June 2006. We used an evaluative design that incorporated quantitative and qualitative methods to assess outcomes and processes. The content of the education programme was based on learning needs assessment and literature review.[3,4] The programme consisted of 8.5 hours of learning activity. Teaching and learning approaches were developed based on principles of reflective learning.[5]

Sample size

A total of 110 registered nurses from medical and surgical units were randomly assigned to the study (n=54) and control (n=56) groups. Their demographics are presented in Table 1. There were no dropouts from the study. A purposive sample was recruited for the focus group interviews: the process evaluation interviews (three focus groups with 24 participants) and the outcome evaluation interviews (three focus groups with 18 participants).

Outcome measures

Participants in both groups were assessed before (pre-test) and immediately (post-test 1), 3 months (post-test 2), 6 months (post-test 3) after intervention, using four instruments: the Suicide Opinion Questionnaire (SOQ), the test on knowledge of management of suicide, the nursing competency in suicidal prevention and management, and the nurses' stress and coping in caring for suicidal patients.

Table 1. Demographics of the participants

Demographics	Study group (n=54)	Control group (n=56)	P value
	No. (%) of participants		
Gender			χ^2=1.007, P=0.316
Male	6 (11.1)	10 (17.9)	
Female	48 (88.9)	46 (82.1)	
Age range (years)			χ^2=4.014, P=0.134
21-30	17 (31.5)	27 (48.2)	
31-40	28 (51.9)	19 (33.9)	
41-60	9 (16.7)	10 (17.9)	
Hospital			χ^2=0.00, P=1.00
Hospital A	27 (50.0)	28 (50.0)	
Hospital B	27 (50.0)	28 (50.0)	
Clinical specialty			χ^2=0.01, P=0.919
Medical	40 (74.10)	41 (73.20)	
Surgical	14 (25.90)	15 (26.80)	
Participation in any continuing education related to suicide prevention in past 2 years			χ^2=0.46, P=0.497
Yes	7 (13.0)	5 (8.90)	
No	47 (87.0)	51 (91.1)	
	Mean±SD (range)		
Years of experience	10.03±6.91 (0.25-28)	9.07±6.86 (0.25-29)	*t*=0.731, P=0.466, df=108
No. of suicidal patients cared in past 12 months	3.00±4.11 (0-20)	2.79±4.67 (0-30)	*t*=0.255, P=0.799, df=108
Duration (hours) of taking care of suicidal patients in past 12 months	16.42±32.79 (0-184)	24.22±49.72 (0-240)	*t*=-0.968, P=0.335, df=108

Focus group interviews

Process evaluation interviews were conducted immediately after the programme to identify its strengths and limitations from the perspectives of the participants. Outcome evaluation interviews were conducted 6 months after the programme to assess the participants' competence in caring for patients with suicidal intent, and to identify factors affecting the use of such knowledge in practice.

Results

Outcome measures

Table 2 shows the mean and standard deviation (SD) of all outcome measures for the two groups. Table 3 compares the four outcome measures between the two groups. There was no significant difference between the two groups at baseline. The interaction terms (between group × time) were not significant for any of the outcome measures. No treatment effect was detected for any of the outcome variables. However, significant time effect was found for the SOQ total scores (P=0.001) and subscales (social disintegration, P=0.009; personal defect, P=0.008; the competency checklist, P=0.014; and the stress and coping scale, P=0.045). Both groups showed improvement with respect to all post-test 1 scores, which then gradually declined in subsequent tests.

Process evaluation: evaluation form

The participants gave positive feedback about the programme. They agreed that its objectives were appropriate and achieved, and had enhanced their knowledge, attitudes and skills in caring for suicidal patients and their families, which included confidence and competency in practice. They also claimed that the programme helped increase their alertness with regard to suicide prevention. Topics related to assessment protocol, intervention, case studies, sharing of experience and information about suicide were considered the most useful. Many participants suggested that the programme be extended to a week and include more discussion, case sharing, and real-life examples. Some wanted more skills practice using role plays and videos.

Process evaluation: focus group interviews

The participants agreed that sessions on suicide theories, statistics, 'myths and facts' and assessment of suicide risks were useful, and a 'no suicide' contract was particularly interesting. Case sharing was more helpful than theory to change the mindset of general nurses, and helped their learning in the management of similar cases. Role plays were similarly useful and interesting. Questions posed in the research questionnaires reinforced positive values and concepts. The handouts, notes and community resources information were useful.

The participants agreed that the programme met their expectations, and regarded case sharing as helpful to change mindsets and attitudes towards their patients. Many participants mentioned that the programme had enhanced their knowledge of suicidal risk factors, and helped increase their awareness of patients with suicidal intent. General nurses play an important role in coordination among disciplines and a multidisciplinary approach is essential in the care of suicidal patients. All participants agreed that the duration of the programme should be longer, which concurred with written comments in the evaluation form. They also recommended continuous learning and updates on the topic and that suicide prevention education not be confined to just a one-off course.

Table 2. Means and SD of all outcome measures of the study and control groups

Outcome measures	Mean (SD)			
	Pre-test	Post-test 1	Post-test 2	Post-test 3
Knowledge on management of patients with suicidal risk				
Study	5.00 (1.57)	5.54 (1.79)	5.61 (1.47)	5.44 (1.73)
Control	5.34 (1.81)	5.27 (1.79)	5.41 (1.85)	5.16 (2.07)
Suicide Opinion Questionnaire				
Total score				
Study	155.5 (10.90)	161.5 (13.60)	159.0 (15.23)	159.1 (13.71)
Control	155.2 (12.26)	158.8 (16.85)	157.8 (16.75)	159.8 (16.10)
Acceptability				
Study	27.74 (5.42)	28.85 (6.03)	28.59 (6.03)	28.70 (5.53)
Control	27.98 (4.67)	28.73 (5.75)	28.55 (6.18)	28.77 (6.53)
Perfect factual knowledge				
Study	29.19 (3.31)	29.20 (3.33)	29.83 (4.11)	29.63 (3.61)
Control	28.73 (3.88)	29.96 (5.76)	29.46 (3.80)	29.91 (4.12)
Social disintegration				
Study	32.46 (3.97)	34.72 (3.52)	33.96 (4.26)	33.83 (4.40)
Control	32.48 (4.16)	33.09 (4.72)	33.07 (5.13)	33.63 (5.00)
Personal defect				
Study	37.37 (3.28)	38.93 (4.30)	37.57 (3.87)	37.85 (3.53)
Control	37.80 (3.83)	38.71 (4.89)	38.16 (3.65)	38.71 (3.07)
Emotional perturbation				
Study	28.72 (2.62)	29.78 (3.04)	29.04 (3.43)	29.04 (3.06)
Control	28.18 (3.24)	28.27 (3.28)	28.55 (5.24)	28.79 (4.18)
Checklist on nursing management of patient with suicidal precaution				
Study	27.49 (9.20)	31.03 (5.97)	26.64 (11.27)	24.38 (13.56)
Control	27.60 (10.95)	28.71 (10.22)	29.17 (9.19)	27.21 (11.09)
Nurse's stress and coping in caring for a suicidal patient				
Study	16.29 (3.33)	16.04 (3.35)	15.27 (3.19)	15.64 (3.22)
Control	16.07 (2.72)	15.27 (2.51)	15.32 (2.96)	15.32 (3.06)

Table 3. Comparison of the four outcome measures between the study and control groups

Outcome measures	Baseline scores	Repeated-measures ANOVA		
		Group	Time	Group x time
Knowledge on management of patients with suicidal risk	T=-1.049, df=108, P=0.296	F(1,108)=0.147, P=0.702	F(3,106)=1.378, P=0.254	F(3,106)=1.409, P=0.244
Suicide Opinion Questionnaire				
Total score	T=0.137, df=108, P=0.891	F(1,108)=0.134, P=0.715	F(3,106)=5.835, P=0.001	F(3,106)=0.861, P=0.464
Acceptability	T=-0.25, df=108, P=0.803	F(1,108)=0.002, P=0.969	F(3,106)=1.479, P=0.225	F(3,106)=0.048, P=0.986
Perfect factual knowledge	T=0.658, df=108, P=0.512	F(1,108)=0.008, P=0.928	F(3,106)=1.598, P=0.194	F(3,106)=1.022, P=0.386
Social disintegration	T=-0.025, df=108, P=0.98	F(1,108)=0.997, P=0.320	F(3,106)=4.101, P=0.009	F(3,106)=2.190, P=0.093
Personal defect	T=-0.637, df=108, P=0.526	F(1,108)=0.508, P=0.478	F(3,106)=4.118, P=0.008	F(3,106)=0.686, P=0.563
Emotional perturbation	T=0.967, df=108, P=0.336	F(1,108)=1.890, P=0.172	F(3,106)=1.211, P=0.309	F(3,106)=1.373, P=0.255
Checklist on nursing management of patient with suicidal precaution	T=0.273, df=101, P=0.786	F(1,79)=0.205, P=0.652	F(3,77)=3.765, P=0.014	F(3,77)=1.986, P=0.123
Nurse's stress and coping in caring for a suicidal patient	T=0.701, df=93, P=0.485	F(1,87)=0.319, P=0.573	F(3,85)=2.789, P=0.045	F(3,85)=1.092, P=0.357

Outcome evaluation

After the education programme, participants regarded themselves as more competent in assessing, communicating with, and helping suicidal patients. Subjectively they felt their assessment skills had improved and that they had put theory into practice. Because of enhanced knowledge, they had more confidence in caring for and communicating with suicidal patients. The programme helped expose myths they previously had about suicide, and led to changes in their attitudes.

Among the most frequent barriers to caring for suicidal patients were insufficient time and staff. All participants commented on nursing shortages in the hospitals, and expressed frustration that they did not have the time to assess and observe patients at risk. There was a lack of support from senior management in providing psychological care for this group of patients. The physical environment of wards made observation and care difficult. Protocols were useful to guide care.

Discussion

This study evaluated an education programme to enhance

the knowledge, attitudes and competence of nurses in patient suicide prevention. The participants had spent an average of 16.42 (SD, 32.79) hours taking care of patients at risk of suicide in the previous 12 months. Therefore, it was not uncommon to encounter such patients.

Contrary to our expectations, the results showed no significant differences between the study and control groups for any of the outcome measures. Both groups showed improvement in all outcome measures across time between the pre- and post-test 1, but the scores gradually declined thereafter. Several factors could have influenced the results. Previous studies used a qualitative or a one-group pre-post test design,[4] whereas the present study used a control group.

The duration of the education programme might have been too short to produce a statistically significant difference between the two groups. Furthermore, as the participants in both groups worked in the same venues, communication between them was inevitable. Although we monitored the control group to ensure that they did not participate in any formal learning on the topic, informal learning (reading articles or books related to suicide prevention and management) could not be controlled. The motivation of participants was high. The control group filled in four sets of questionnaires four times, indicating interest in the subject of the study. They might have already been aware of the problem of suicide and willing to learn more. The questionnaires might have stimulated them to think more about the issues, search for answers for the test or read more about the subject, thus leading to improvement in outcome measures.

Focus group interviews provided a better understanding of the intervention. Process evaluation interviews suggested that the programme content was essential and appropriate. The participants realised the need for continuous learning. They suggested lengthening the duration of the course and elaboration on topics such as handling aggression. These topics reflected their learning needs and concerns in clinical practice. The participants encountered more often patients with aggressive behaviour than in the past.

From outcome evaluation interviews, the participants considered that the education programme enhanced their knowledge, attitudes, confidence, and competence in the topics. The knowledge gained from the programme helped expose myths related to suicide, thus enabling the nurses to change attitudes towards the care of this patient group. With increased knowledge, they had more confidence in taking care of them.

The participants' verbal accounts revealed a change in attitudes towards suicide prevention and management. The findings of this study supported the importance of a positive attitude towards developing greater awareness of the problem of suicide, willingness to talk to patients, and improved assessment skills.

The reflective learning method used in the programme was appreciated, and was similar to a previous study showing that reflective discussion was an appropriate learning method for experienced nurses.[4] Adults learn by relating new knowledge to their personal experience and gain new perspectives from reflection.[5] Participants suggested that more discussions and role plays be included in future presentations of the programme.

The qualitative data revealed the particular concerns of nurses relating to the care of this patient group, which could be of relevance to future practice. Comments about support from senior staff members, nursing shortages, organisation of care and the physical environment reflected the difficulties they encountered when caring for patients at risk of suicide. Although suicide prevention and management is an important topic, nurses could not get support from the senior management in attending education programmes. In clinical areas, there was inadequate support for providing care to patients with suicidal intent. The social system and organisational factors were found to influence staff self-perceived ability to implement changes.

The physical structure of a general ward differs from that of a purpose-built mental health unit specially designed to take safety into account and enable observation of patients at high risk of suicide. This can pose problems of implementing common interventions such as the regular observation of patients for suicidal behaviour difficult. The crowded ward environment might also make it difficult to provide a place in which patients can privately express their feelings.

This study assessed only those who were willing to participate. The results might not be generalisable to those who refused to do so. We shortened the duration of the education programme, which may have influenced outcomes. This study measured only subjective attitudes and competency, not actual performance.

Implications

Future programmes could strengthen the content concerning watchfulness for potentially dangerous articles, communication and counselling for suicidal patients and their relatives and handling of their aggressive behaviour. Skills related to working and communicating in multidisciplinary teams in the care of suicidal patients could also be strengthened. Interactive learning methods in the form of role plays, practical sessions and case discussion are conducive to learning.

The duration of education needed to produce behavioural changes needs to be further studied. Continuous education is needed if new skills and knowledge are to be internalised, and changes made. Ongoing evaluation of the programme is needed to facilitate improvement.

There is a need to review the organisation of care and policies related to the care of suicidal patients in hospitals. Adequate staffing, improved communication with specialists in mental health services, support from senior colleagues and those in other disciplines, protocols to guide care and practice are all necessary. Furthermore, modification of care models and the physical environment are needed to facilitate appropriate care to this patient group from nurses.

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Effects of fear-avoidance beliefs on Chinese patients with neck pain

Key Messages

1. The Chinese version of the Fear-Avoidance Beliefs Questionnaire (FABQ) has good content validity, test-retest reliability, internal consistency, construct validity, responsiveness, and factor structure. Thus, fear-avoidance beliefs can be used in Chinese patients with neck pain.
2. The validated FABQ facilitates future research on the effects of fear-avoidance behaviour on patients with neck pain and hence a better service for and evaluation of patients with neck pain can be provided. It may also facilitate cross-cultural studies on this common problem between western and Chinese populations.
3. The construct of fear-avoidance beliefs can be applied to patients with neck pain.
4. The fear-avoidance beliefs are an important psychosocial measure in predicting future disability level and return to complete work capacity (immediately and 3 months after physiotherapy).

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Introduction

Neck pain is a common medical condition and of multifactorial origin. Psychosocial factors may contribute to its development.[1] Among them, the fear-avoidance beliefs are hypothesised as the most powerful cognitive variables in predicting disability and treatment outcomes of patients with low back pain.[2] There is no information about the effects of fear-avoidance beliefs in Chinese patients with neck pain. We hypothesised that the fear-avoidance beliefs may also affect disability and normal working in patients with neck pain.

Aims and objectives

1. To translate and adapt the Fear-Avoidance Beliefs Questionnaire (FABQ) into Chinese (Cantonese) and assess its content validity, test-retest reliability, construct validity, factor structure, and responsiveness.
2. To assess the correction between fear-avoidance beliefs and future disability and work capacity in patients with neck pain.

Methods

This study was conducted from February 2004 to January 2006.

Study design

In phase I, the English version of the FABQ[3] was adapted and translated into Chinese (Cantonese) and then validated by different panels involving physiotherapists, psychiatrists, neck pain patients, and secondary school students. The validated Chinese version of FABQ was then tested for reliability and construct validity in four physiotherapy out-patient departments in different regions of Hong Kong. In phase II, the role of fear-avoidance beliefs in predicting future disability and work capacity of patients with neck pain who had 6 weeks of physiotherapy was prospectively studied.

Subjects and sample size

Patients were recruited from physiotherapy out-patient departments of three public hospitals and one private clinic in Hong Kong. They were diagnosed with neck pain, with or without radiation symptoms, and were able to read and write Chinese. Patients who had other musculoskeletal problems, an infectious condition, previous brain surgery, congenital abnormality, or a history of malignancy or mental illness were excluded. A total of 476 patients were recruited for validation and 120 patients for 6 weeks of physiotherapy.

Outcome measures

In the validation study, patients completed the FABQ, the Northwick Park Neck Pain Questionnaire (NPQ),[4] the medical outcomes 36-item Short-Form Health Survey (SF-36),[5] and the 11-point Numerical Rating Scale (NRS) when they attended for physiotherapy at weeks 1, 3, 6, and upon discharge.

In phase II, the neck active range of motion (AROM) and isometric neck muscle strength were measured by the computerised multi-cervical rehabilitation unit before and after physiotherapy. The questionnaires and work status evaluation were completed again after physiotherapy. A telephone follow-up on their work status was carried out 3 months after physiotherapy.

Results

The content validity of FABQ as determined by the panels was satisfactory. The mean scores of the patient and student groups for each question ranged from 3.2 (good) to 3.6 (good). The mean scores of the expert panels for each question ranged from 3.5 (good) to 4.8 (very good). The mean interval for the test-retest reliability was 12.5±7.8 days and the mean time to complete the FABQ questionnaire was 5±3 minutes. The FABQ had very good test-retest reliability (ICC=0.81) and internal consistency (Cronbach's alpha=0.90). The correlation coefficients at entry into physiotherapy were 0.56 for the NPQ, 0.34 for NRS, -0.45 for SF-36 physical subscale, and -0.36 for SF-36 mental subscale. At discharge from physiotherapy the respective correlation coefficients were 0.53, 0.33, -0.64, and -0.43 (all P<0.001). The correlation between the change of the FABQ scores at weeks 3 and 6 of physiotherapy and the corresponding changes of the NPQ scores were fair (r_s=0.32 at week 3 and 0.38 at week 6) and highly significant. The correlations between changes in pain intensity scored by NRS (r_s=0.19 at week 3 and 0.18 at week 6) and changes in SF-36 scores (r_s=-0.18 at week 3 and -0.27 at week 6 for SF-36 physical subscale and r_s=-0.26 at week 3 and -0.24 at week 6 for SF-36 mental subscale) were weak (P=0.065-0.006). For the pre- and post-test comparison, the paired *t* test showed a significant difference between the FABQ scores before treatment (47.80±16.93) and upon discharge from physiotherapy (43.95±18.11, P<0.001). For responsiveness of the FABQ from the beginning to the end of physiotherapy, the standardised response mean and effect size were 0.38 and 0.32, respectively. Factor analysis showed that the three-factor solution produced a more reliable and interpretable solution with the total variance explained by the factors being 61.6% (Table 1). The three factors were labelled as prognosis work (FABQ_PW), work as a cause (FABQ_W), and physical activity (FABQ_PA).

Table 1. Factor analysis with the factor loadings of the 16 items related to the Fear-Avoidance Beliefs Questionnaire (FABQ) after Varimax Rotation

FABQ items*	Factor loadings		
	Prognosis work	Work as a cause	Physical activity
15	**0.84**	0.11	0.15
14	**0.84**	0.22	0.18
13	**0.82**	0.22	0.21
12	**0.77**	0.34	0.15
16	**0.75**	0.14	0.03
8	**0.46**	0.34	0.21
7	0.23	**0.81**	0.07
10	0.30	**0.77**	0.18
11	0.30	**0.76**	0.07
6	0.06	**0.75**	0.09
9	0.24	**0.71**	0.13
1	0.05	0.42	**0.40**
4	0.07	-0.03	**0.77**
3	0.11	0.25	**0.70**
5	0.30	-0.02	**0.69**
2	0.13	0.30	**0.68**

* 1 denotes pain caused by physical activity, 2 physical activity worsens pain, 3 physical activity might harm, 4 should not do physical activity, 5 cannot do physical activity, 6 pain caused by work, 7 work aggravated pain, 8 claim for compensation, 9 work too heavy, 10 work makes pain worse, 11 work might harm, 12 should not do work, 13 cannot do work, 14 wait until pain is treated, 15 no return to work within 3 months, and 16 never return to work

For phase II, linear regression analysis of the correlation between initial neck disability score (dependant variable) and fear-avoidance beliefs score showed that none of the added FABQ subscale scores improved the fit of the model after controlling for pain intensity, physical impairment (neck AROM and strength), and general health measures. For the 6-week disability score, the change in the R^2 with the addition of the treatment group was not significant in the second step, but did attain significance with the addition of the FABQ_W and FABQ_PW in the third step (Table 2). The R^2 value reflects the goodness of fit of the linear model adjusted for the number of independent variables in the equation.

After 6 weeks of physiotherapy, 73 (61%) of the subjects had complete return of work capacity and 47 (39%) remained to have incomplete work capacity. Hierarchical logistic regression analysis of return to complete work capacity showed that, after controlling for the pain intensity, physical impairments, general health status, and initial neck disability level, adding a treatment group in the second step significantly improved the fit of the model, and adding the FABQ_W and FABQ_PW in the third step further improved the fit significantly.

Three months after the physiotherapy, telephone follow-up on their work status showed that 88 (82%) of the subjects had complete work capacity and 20 (19%) remained to have incomplete work capacity. Twelve (10%) of the subjects could not be contacted. For those with complete return to work capacity at 18 weeks, after controlling for the pain intensity, physical impairments, general health status, and initial neck disability level, adding a treatment group in the second step significantly improved the fit of the model and adding the FABQ_W and FABQ_PW in the third step further improved the fit significantly (Table 3).

Discussion

This is the first study to adapt, translate and validate the FABQ questionnaire for Chinese patients with neck pain. The Chinese version of the FABQ is practical and shows good reliability, validity, and consistent factor structures compared to the original version. The responsiveness of the FABQ assessed by standardised response mean and effect size is low (0.38 and 0.32 respectively) compared to that of the NPQ (0.73 and 0.62 respectively) and pain measured by the NRS (0.83 and 1.0 respectively). The low responsiveness of the FABQ may be because the follow-up period was not long enough to allow adequate detectable change in the effects of fear-avoidance beliefs in patients with neck pain. However, the standardised response mean and effect size of

Table 2. Hierarchical linear regression analysis (n=120) of the correlation* between 6-week Northwick Park Neck Pain Questionnaire (NPQ) score (dependent variable) and fear-avoidance beliefs about physical activity, work as a cause, and prognosis work after controlling for pain, active range of motion (AROM), strength, SF-36 score (physical and mental component scores, PCS and MCS), initial NPQ score and treatment group

Variables of fear-avoidance beliefs		Adjusted R^2	Significance of R^2 change	Standardised beta coefficient (final model)	Significance of beta coefficient
Physical activity					
Step 1	Pain rating			-0.059	0.516
	AROM index			-0.049	0.582
	Strength index			0.004	0.967
	PCS			-0.008	0.935
	MCS			-0.007	0.927
	Initial NPQ	0.408	<0.001	0.634	<0.001
Step 2	Treatment group	0.403	0.993	-0.003	0.965
Step 3	FABQ physical activity	0.409	0.154	0.116	0.154
Work as a cause					
Step 1	Pain rating			-0.100	0.273
	AROM index			-0.029	0.736
	Strength index			-0.014	0.874
	PCS			0.005	0.953
	MCS			0.049	0.521
	Initial NPQ	0.408	<0.001	0.624	<0.001
Step 2	Treatment group	0.403	0.993	-0.041	0.571
Step 3	FABQ work as a cause	0.431	0.012	0.224	0.012
Prognosis work					
Step 1	Pain rating			-0.072	0.424
	AROM index			-0.046	0.601
	Strength index			<0.001	0.996
	PCS			0.013	0.883
	MCS			0.027	0.717
	Initial NPQ	0.408	<0.001	0.617	<0.001
Step 2	Treatment group	0.403	0.993	-0.031	0.670
Step 3	FABQ prognosis work	0.424	0.027	0.194	0.027

* Interaction between treatment and fear-avoidance beliefs was not significant

Table 3. Hierarchical logistic regression analysis (n=108) of the correlation* between return to work capacity after 18 weeks and fear-avoidance beliefs about physical activity, work as a cause, and prognosis work

Variables of fear-avoidance beliefs†		Step Chi-square	Nagelkerke's R^2	Odds ratio (95% CI)
Physical activity				
Step 1	Pain rating			0.825 (0.553, 1.231)
	AROM index			0.969 (0.905, 1.037)
	Strength index			1.264 (1.034, 1.546)
	PCS			0.949 (0.877, 1.026)
	MCS			0.923 (0.864, 0.986)
	Initial NPQ	χ^2=23.769, df=6, P=0.001	0.320	1.045 (0.985, 1.109)
Step 2	Treatment group	χ^2=3.106, df=1, P=0.078	0.357	2.984 (0.831, 10.720)
Step 3	FABQ physical activity	χ^2=3.517, df=1, P=0.061	0.398	1.115 (0.989, 1.258)
Work as a cause				
Step 1	Pain rating			0.756 (0.496, 1.153)
	AROM index			0.980 (0.917, 1.047)
	Strength index			1.242 (1.003, 1.538)
	PCS			0.946 (0.873, 1.024)
	MCS			0.938 (0.875, 1.006)
	Initial NPQ	χ^2=23.769, df=6, P=0.001	0.320	1.041 (0.982, 1.104)
Step 2	Treatment group	χ^2=3.106, df=1, P=0.078	0.357	1.999 (0.543, 7.355)
Step 3	FABQ work as a cause	χ^2=5.831, df=1, P=0.016	0.424	1.198 (1.025, 1.399)
Prognosis work				
Step 1	Pain rating			0.785 (0.502, 1.228)
	AROM index			0.960 (0.891, 1.034)
	Strength index			1.332 (1.039, 1.708)
	PCS			0.966 (0.885, 1.055)
	MCS			0.938 (0.866, 1.016)
	Initial NPQ	χ^2=23.769, df=6, P=0.001	0.320	1.031 (0.969, 1.097)
Step 2	Treatment group	χ^2=3.106, df=1, P=0.078	0.357	2.377 (0.563, 10.038)
Step 3	FABQ prognosis work	χ^2=15.778, df=1, P<0.001	0.529	1.191 (1.077, 1.317)

* Interaction between treatment and fear-avoidance beliefs was not significant

† AROM denotes active range of motion, PCS physical component score, MCS mental component score, NPQ Northwick Park Neck Pain Questionnaire, FABQ Fear-Avoidance Beliefs Questionnaire

our study were similar to those of the French version of the FABQ (0.31 and 0.30 respectively). Furthermore, the three-factor structure solution resulting from the factor analysis is consistent with the German version of the FABQ in patients with low back pain. It provided evidence that the construct of the fear-avoidance beliefs could apply to the patients with neck pain.

Linear regression analysis of phase II also showed that in patients with neck pain, fear-avoidance beliefs play an important role even after controlling for factors related to pain intensity, physical impairments, general health measures, initial disability level, and type of treatment in affecting disability and normal working capacity. The level of future disability and, more importantly, the likelihood of return to complete work capacity (immediately and 3 months after physiotherapy) could be predicted by the FABQ at the earlier phase of physiotherapy. Therefore, the validated FABQ facilitates future research on the effects of fear-avoidance behaviour on patients with neck pain, and hence a better service for and evaluation of patients with neck pain.

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Effect of mindfulness-based stress reduction programme on pain and quality of life in chronic pain patients: a randomised controlled clinical trial

Key Message

A mindfulness-based stress reduction programme may not be superior to an education programme in terms of improving disability and pain in patients with a moderate degree of chronic pain.

Introduction

Chronic pain is a prevalent health problem and a frequent cause of disability and suffering. It is also associated with significant health care costs. Although psychological interventions are alternatives to traditional medical approaches, many individuals with chronic pain do not benefit from these treatments.

Mindfulness-based stress reduction (MBSR) is a clinical programme to increase self-acceptance coping and reduce suffering in patients with medical illness using mindfulness meditation as a self-regulated approach for stress reduction and emotion management. Preliminary evidence demonstrated that MBSR may reduce pain and improve mood symptoms. However, no definitive conclusion could be drawn as no randomised controlled trial with an active control group had been carried out. The objective of this trial was to compare the effectiveness of MBSR with an education programme in terms of reduction of pain and improvement in quality of life for chronic pain patients.

Methods

This study was conducted from October 2006 to September 2007. A total of 100 participants were recruited from primary care, geriatric and pain clinics in the community and hospitals that most chronic pain patients attended.[1] Patients included were aged 18 to 65 years, with any chronic pain for at least 3 months. The pain had to be moderate to severe (scoring at least 4 out of 10 in an 11-point Numeric Rating Scale) verified by a trained research assistant and confirmed by a family physician. The patients had to agree not to receive other new treatments (including topical, over-the-counter, and non-pharmacological medication) during the intervention. Patients were excluded if they (1) received concurrent treatment other than medications for pain or psychological symptoms, (2) had a concurrent diagnostic and statistical manual of mental disorders axis-I diagnosis, (3) participated in an MBSR group, engaged in current or prior practice of meditation or relaxation techniques including MBSR, or (4) were illiterate and unable to complete the meditation diary. All participants gave written informed consent, and the study was performed according to the Good Clinical Practice guideline. This trial was also registered with the Centre for Clinical Trials of the Chinese University of Hong Kong, and was approved by the ethics committee of the university.

Study instruments

Outcome measures were collected at baseline, 8 weeks (end of intervention), 3 and 6 months after the intervention. Primary outcome measures were self-reported pain intensity measured by the 11-point Numeric Rating Scale[2] and the Dual Visual Analogue Sensation of Pain and Distress Scales.[3] Both scales have been demonstrated to be reliable and sensitive measurements of pain. Secondary outcome measures were mood status and symptoms assessed using the Profile of Mood States, the validated Chinese version of the Centre for Epidemiological Studies-Depression Scale, and the State Trait Anxiety Inventory. Health-related quality of life was measured by the validated Chinese version of the Short-Form

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Health Survey (SF-12).

Results

Before intervention, patients in both the MBSR and education programme groups did not differ with regard to demographics, pain intensity, mood symptoms, health-related quality of life scores, the amount of sick leave taken, or the use of services and analgesics. After intervention, patients in both groups had significant improvements in pain intensity, which was sustained until 6 months post-intervention.

There were no significant differences in the SF-12 scores between the two groups at baseline, 8 weeks, and 3 and 6 months post-intervention. At 3 months after intervention, physical and mental component scores of SF-12 improved significantly in both groups. Nonetheless, only the physical component score improved further at 6 months post-intervention. There was a significant difference in the Profile of Mood States activity subscale between the two groups at the end of intervention, but the difference was not sustained thereafter.

The mean anxiety state scores of both groups improved 2.4 (95% confidence interval [CI]=0.3-4.5, P=0.027) at 3 months and 3.1 (95% CI=1.9-4.3, P=0.005) at 6 months post-intervention, compared with baseline scores. There were no significant differences between the two groups at baseline, 8 week, and 3 and 6 months post-intervention. Depressive symptoms (according to the Centre for Epidemiological Studies-Depression Scale) were not significantly different between the two groups and did not change over time.

Discussion

The randomised clinical trial design was used to study the effects of MBSR on chronic pain intensity with an active control group that could be adjusted for the confounding effects of group attention and therapist time. The effects of MBSR on chronic pain in a non-Caucasian population were also studied. The MBSR programme was not superior to multidisciplinary education programme based on the principles for management of chronic pain. We could not show that MBSR was not effective per se for improving quality of life or some of the mood symptoms, as we observed significant improvement in both groups.

There were several limitations to this study. First, the unexpectedly high dropout rate in the MBSR group and the low proportion of subjects who completed all 10 sessions might have contributed to the negative results of this intervention. As a result, the study could have had a type-II error. Second, for the MBSR group, only a proportion of subjects practiced daily for the recommended amount of time. Thus, MBSR might not be effective for those who attended the class only. If all those who attended the class also practised daily at home as instructed by the therapist, the results could have been different.

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Determination of the functions of the putative metal-binding domain of the SCV helicase

Key Messages

1. The inhibition of SARS coronavirus (SCV) helicase by bismuth compounds is mediated via the abilities of bismuth ions to displace (essentially required) zinc ions that are bound to cysteine residues located within the metal-binding domain.
2. Several novel bismuth compounds targeting SCV helicase have been synthesised. These compounds can inhibit SCV helicase activity and viral growth and potentially target other viruses.

Introduction

Severe acute respiratory syndrome (SARS) was first recognised in late 2002 and was shown to be caused by the SARS coronavirus (SCV).[1,2] We have reported the purification and characterisation of the SCV helicase,[3] and identified a number of lead compounds able to inhibit SCV helicase activities and viral growth.[4,5] One of these contained bismuth—ranitidine bismuth citrate (RBC). To reveal the mechanisms underlying RBC action, we investigated the interaction between zinc, bismuth, and the helicase protein. Mutational analysis was used to pinpoint the key amino acid residues necessary for the helicase function. Several novel bismuth compounds with SCV inhibiting activity were synthesised. This study was conducted from February 2005 to January 2007.

Characterisation of the Zn^{2+}- SARS coronavirus helicase interaction

The N-terminal of the SCV helicase consisted of a cysteine rich domain: the metal-binding domain (MBD). Sequence alignment suggested that this entailed Zn^{2+} binding. To determine how helicase binds to Zn^{2+} ions, we used 4-(2-pyridylazo) resorcinol / *p*-hydroxymercuriphenylsulfonic acid assay to demonstrate that zinc did indeed bind to the MBD of SCV helicase via the cysteine residues (Fig 1).

To study the effect on the structure of MBD upon zinc binding, we measured the circular dichroism of MBD (Fig 2). The zinc-bound form of MBD showed the characteristics expected of high helix content. However, addition of excess ethylenediaminetetraacetic acid to remove the zinc ions led to a decrease in ultraviolet light absorption, suggesting that the protein changed to a random structure in the absence of Zn^{2+}.

Mutational analysis of the SARS coronavirus helicase metal-binding domain

To define the precise role of individual amino acids within the SCV helicase MBD, we individually mutated several key cysteine residues within the MBD. The mutant proteins were then studied by measuring their ATPase and unwinding activities. All the mutant proteins lost their activities.

Determination of the mechanism of ranitidine bismuth citrate–mediated inhibition of SARS coronavirus helicase activity

Bi^{3+} is known to have a high affinity for thiolate sulphur.[6] We therefore measured the ultraviolet light absorbance spectrum of the SCV helicase, both in the absence and presence of RBC. The cysteine-rich MBD was the target of the bismuth ion. Addition of Bi^{3+} to the Zn^{2+}-bound form of MBD led to an increase in ultraviolet light absorbance, indicative of replacement of Zn^{2+} by Bi^{3+}.[7]

Synthesis of bismuth complexes against SARS coronavirus

Bismuth compounds effectively inhibited SCV growth in cell culture. The

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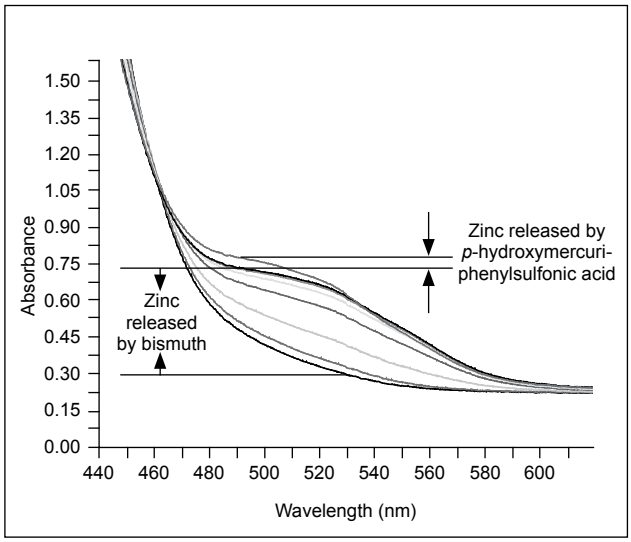

Fig 1. Zinc that binds to the metal-binding domain can be replaced by bismuth

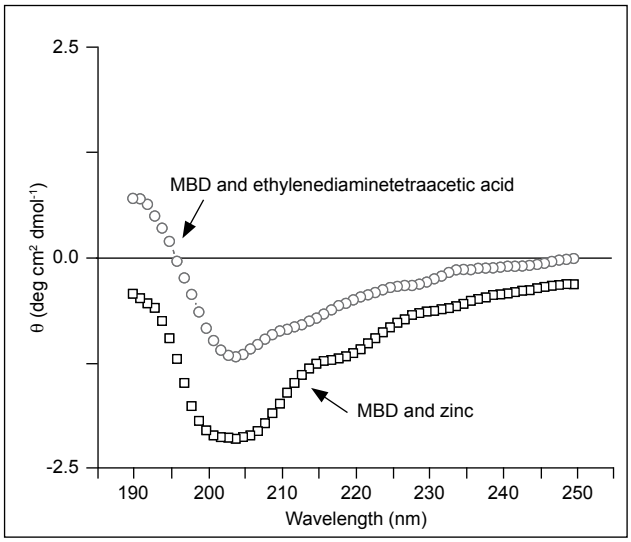

Fig 2. Circular dichroism of metal-binding domain (MBD) with and without zinc binding

mechanism was through replacement of zinc bound to the MBD. We then designed and synthesised a series of bismuth complexes, including complexes of: bismuth porphyrin, bismuth macrocyclin, bismuth 12-crown-4, bismuth bipyridine, bismuth phenanthroline, bismuth nitrilotriacetate, bismuth ethylenediaminetetraacetic acid, and bismuth acetohydroxamate.[3] The newly synthesised compounds were tested for their activity against SARS helicase. The two bismuth porphyrin complexes and RBC exhibited the the most significant inhibition activities as revealed by in vitro experiments.[8]

Conclusion

We determined the interaction between zinc, bismuth, and the helicase protein, enabling us to construct mechanistic models for bismuth-mediated inhibition of the SCV helicase functions. The zinc ions bound to the MBD of SCV helicase were replaced by bismuth ions upon addition of RBC and other bismuth compounds, resulting in dysfunction of the helicase. These compounds may also be used to inhibit other viruses.

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A pan-Asian survey of risk perception, attitudes and practices associated with live animal markets

Key Messages

1. Causal attributions for highly pathogenic avian influenza (HPAI) of the H5N1 virus among live poultry, consumers, retailers and breeders in Vietnam, Thailand, Guangzhou and Hong Kong were studied.
2. Three main themes embodying lay explanation for the causes of H5N1 HPAI emerged: viruses, husbandry-related factors, and vulnerability factors.
3. A deeper understanding of the perceptions of risks, biases, causal attributions, and both the facilitators and barriers to change is needed for planning effective changes in health-related behaviour.

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Introduction

Highly pathogenic avian influenza (HPAI) of the H5N1 virus is endemic in many countries.[1,2] Asia generates 81% of egg production of all developing countries.[3] Day-old broiler seed chick exports, the adult bird trade, and wild birds may all facilitate HPAI distribution.[4] Slaughter/consumption of infected poultry accounts for most human HPAI infections,[1] and live poultry retail and domestic husbandry makes documenting related human behaviour and perceptions important.[4] Preventive practices require population adherence to be effective. Beliefs about why outbreaks happen (causal attributions) and their control (control beliefs) can modulate adherence.[5] Studies of individual attributions for diseases often differ from pathophysiological causes of those diseases. When this happens, treatment adherence declines.[6,7] No reports we have seen describing causal attributions for H5N1 HPAI, though several 'knowledge, attitude, practice' studies exist.[8–13] Most of these have failed to address lay explanation of HPAI. We describe causal attributions for HPAI among live poultry, consumers, retailers and breeders in Vietnam, Thailand, Guangzhou and Hong Kong, regions historically affected by H5N1 HPAI.

Methods

In Vietnam, two communes within Chuong My province, Chuc Son (urban) and Dai Yen (rural) formed the sample frame. Purposive sampling criteria were formerly epidemic/non-epidemic area; rural/urban residence; gender, age and chicken farmer/backyard raiser/retailer/consumer/non-consumer. Individual households were identified randomly from commune residence lists and approached for interview. Trained local health bureau interviewers completed one face-to-face interview per household between mid-February and mid-March 2006.

In Thailand, five districts in Suphanburi province formed the sample frame: Nongyasai (rural), Doembangnanbuat (rural), Songpinong (rural), Uthong (rural) and Muangsuphanburi (rural-urban). Within districts, stratified cluster sampling selected interviewees from two large and two smaller poultry farms, two retailers and two consumers. The Institute for Health research staff, Chulalongkorn University, conducted interviews from October to December 2006.

In Guangzhou, two-tiered stratified cluster sampling of metropolitan households (2 723 288) were used according to residential and occupational criteria from records of the occupational health unit, No. 12 Peoples' Hospital, Guangzhou. Kish-grid-derived face-to-face interviews were performed between March and May 2007.

In Hong Kong, a pre-existing random sample (n=1760) was used. Purposive sampling by gender, age, educational level and perceived risk from live poultry sales identified adults aged above 17 years and selected by Kish-grid to complete contract telephone interviews between mid-December 2005 and mid-February 2006.

None of the regions had H5N1 HPAI outbreaks within 6 months before

or during the study. Because qualitative interviewing inexperience affects data quality, in Thailand we adopted a semi-structured interview approach using a set of questions to minimise interviewer variation. Pre-specified criteria-guided questions were used to initiate data collection. During a longer interview, respondents were asked about the causes of the HPAI epidemic.

Results

Of 123 interviews performed, there were 38 Vietnamese (11 poultry buyers, 22 domestic or commercial poultry keepers, and five poultry sellers), 20 Hong Kong Chinese poultry consumers aged 18 to 73 years, 40 Thai commercial or domestic poultry breeders, retailers, consumers or non-consumers, and 25 Mainland Chinese Guangzhou residents of various backgrounds. Three main themes embodying lay explanation for the causes of H5N1 HPAI emerged: viruses, husbandry-related factors and vulnerability factors.

Viruses

The viruses theme comprised two main components: old diseases and new diseases. In old diseases, many rural respondents believed that H5N1 HPAI was nothing new, another periodic disease affecting animals as occurred from time to time and of little concern. Traditional zoonotic epidemics were ordinary problems and were often undifferentiated from each other. "*I think last time my chickens got infected with cholera. In my opinion, bird flu and cholera are the same disease. Do you think the bird flu is a new disease in Thailand? No*." (T9)

Several respondents felt that the re-interpretation or discovery by science of these old diseases did nothing to alter the fact that this was part of farming life. "*I don't know the reason why the bird flu epidemic has occurred. As far as I know, the bird flu epidemic has existed for a long time but people haven't detected it until recently. In the past, it was less serious and people did not know that the flu can be transmitted from poultry to humans*." (V27)

With respect to new diseases, few respondents identified H5N1 HPAI as novel, although some alluded to mutations. However, while these views corresponded to contemporary scientific opinion, they were not widespread. "*I heard that there is a shuffling effect from time to time, I mean, made it to be a combination of H5N1*." (H16)

Husbandry practices

Poor husbandry was highly culpable, involving overcrowding, antibiotic resistance, cross infection, and keeper and commercial behaviour. Many interviewees thought poultry breeds, commercial feed and additives were core components of H5N1 HPAI emergence. These views were widespread among rural and urban respondents. Keeper and commercial behaviour encapsulated poor husbandry, sanitation and lax scrutiny of imported poultry strains. "*I think that there were some sources of infection such as unsafe breeding facilities, outside impacts, illegal selling, people's lack of awareness, insects, and birds. The most important source is people's lack of awareness. It is the breeders who are responsible for poultry becoming infected. They should take preventive measures when the environment is unsafe*." (V15)

Commercial practices of husbandry were also implicated. In particular, both rural and urban dwellers often raise the issue of maximising profit by intensively raising fast-growing birds fed on poor-quality feedstuffs, enhanced by growth-promoting antibiotics and sometimes fluid-retaining hormones as well. "*I think those people want to earn quick money too much…and I also think there are some illegal merchants…I think they really want to earn quick money, and the opportunists are really crucial*." (H1)

Overcrowding within the coops was considered to be important and often linked to poor ventilation and poor sanitation—as being risk factors for H5N1 HPAI outbreaks. "*I think it is too stuffy...the quality of the air is bad...and the sanitary conditions of the whole farm are not so good…. Thus the virus is born*." (H10)

Cross-infection was widely commented on by younger, more educated farmers. "*There was no problem in the second epidemic, but this area was designated yellow. The third time this area became red zone, some of the village farmers raised baby ducks in the field...only people in this area who raise poultry...didn't have much experience. What happened? A lot of rain then the duck pen got wet, the owner of the ducks brought them to the dirty area, so the baby ducks got diarrhoea, unfortunately. Is that so? After that the baby ducks died near the rice field. The owner didn't see some chickens [were there]. Some ducks died in the water, then the owner brought the ducks again, after that they got infected*." (T29)

Traditional varieties of poultry were generally viewed as hardier than contemporary commercial broiler breeds. Poultry weakness was exacerbated by both poor housing and feeding practices. "*Because of mass production breeding, it is very hard to choose portly ducks as in the past. Nowadays, ducks are mainly raised by combination of free roaming and mass production breeding. This model of raising is called semi-mass production*." (V38)

Many respondents considered the use of industrial mash feed in husbandry and retail outlets weaken chickens, but such feed was popular because of its convenience and low costs. Most small-scale farmers however rejected this feed, believing it to increase vulnerability to disease, and preferring to use rice, paddy (unhusked rice) or other grain instead. "*The poultry were fed with natural feedstuff before, but the feedstuff now has added catalyst...they don't use the correct ways to feed, but use some chemical ways…. So the chickens change; they do not grow normally, and are not healthy.*" (H3)

Vulnerability and environmental factors

Factors increasing vulnerability to H5N1 HPAI included: the weather, pollution, and wild birds. This category featured external causal agents of transmission and spread, not under the control of man. Weather was widely cited as a causal factor—most respondents mentioning weather cited change of season as the most likely time for H5N1 HPAI outbreaks. "*It is easy for the epidemic to occur, especially in the transitional time from one season to another, for example, from summer to autumn or from autumn to winter. In recent years, chickens are easily infected in this transitional time. I don't know whether it was due to bird flu virus H5N1 or not*." (V27)

Pollution, in contrast to weather, was cited as a component that included industrial pollutants and agricultural practices, including pesticide use. "*In my opinion, there are a lot of reasons why it occurs. Maybe it's because of the industrialisation process and climate changes. The process of industrialisation results in more dust and pollution*." (V38)

Wild birds were often implicated, but more often they too were victims. Several respondents mentioned how wild birds died off suddenly, whereas others cited migratory behaviour as being responsible. "*Pigeons died before other birds. In my village, there are a lot of pigeons. There is a house (in the village); the owner lives in Bangkok and doesn't come here. Many pigeons live in that. One day they dropped dead, but there are still lots of them. They breed very fast*." (T18)

Not surprisingly, public knowledge of H5N1 HPAI and the impact of health education on poultry practices remains modest.[8-13] Simply providing information takes no account of a population's causal attributions, perceived risks,[4,14,15] perceptual bias[16] or structural determinants of behaviour, and is unlikely to result in significant and sustained change. For this reason, a deeper understanding of the perceptions of risk, biases, causal attributions, and both the facilitators and barriers to change is needed for planning effective change of health-related behaviour.

Discussion

Many rural dwellers view H5N1 HPAI as yet another periodic but natural zoonosis, many of which have occurred in the past and life still went on largely unaffected. Poultry died, people ate the poultry, most were unaffected. There was a view that rural dwellers felt the urban dwellers had suddenly 'discovered' these diseases and felt threatened. Because of this, wide-scale culling became the response common to outbreaks. However, within many rural villages, residents are not motivated to change their husbandry and other relevant practices to protect themselves where they do not see any threat. Health education efforts that fail to take heed of prevailing views are likely to remain ineffectual.

Many respondents denied threats from these 'natural' zoonoses and this often removed any justification for precautionary practices such as using personal protective equipment. These perceptions remain common in rural areas of South East Asia, and pose a significant barrier to the adoption of hygienic practices, particularly among small-scale farmers with small investment.

The lay explanations people hold for why H5N1 HPAI has occurred are unlikely to change only by providing information, unless active epidemics increase the threat, which then has to be avoided. Both non-congruent information (contradicts or challenges beliefs) and congruent information (agrees with beliefs) are received and recalled differently, with the former being perceived as erroneous and subsequently poorly recalled.[16] Frequency of exposure can enhance acceptability of messages. More frequently encountered messages,[17,18] especially those presented by known and popular individuals (hence media and sports personalities), or those perceived to be competent[16] can enhance message acceptability. The more cognitive processing a message receives, the more likely it will be effective if prior conditions are in place.[16] Anxiety generation can be helpful in changing behaviour. However, if no anxiety is aroused, the message may be dismissed as irrelevant.[16,19] This seems to be happening among a large segment of rural respondents. Despite efforts to optimise health messages, they remain of limited effectiveness. However, recent evidence suggests that perceived risk enhancement is associated with changed risk-taking behaviour towards live poultry.[15] This approach is consistent with earlier work, and suggests that addressing and then redirecting perceptions of H5N1 HPAI away from it being just another manifestation of an old problem is critical. Emphasising the potential harm may be an important strategy to help reduce risky behaviour around live poultry.

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The macrophage in the pathogenesis of severe acute respiratory syndrome coronavirus infection

Key Messages

1. Severe acute respiratory syndrome (SARS) coronavirus (SCoV) initiates virus replication in macrophages with the production of negative sense viral ribonucleic acid and viral protein, but such viral replication is abortive and no infectious virus is produced.
2. In contrast to influenza A virus, infection with SCoV fails to induce type-1 interferons (eg IFN-β), which are key mediators of innate immune defence, but does lead to a strong induction of proinflammatory chemokines (eg IP10).
3. Sub-neutralising antibody to SCoV does not lead to the mediation of antibody-dependent enhancement of SCoV viral replication in macrophages.
4. Microarray analysis of SCoV infected cells identifies novel pathways including cytokines and chemokines and apoptotic pathways that are differentially activated by SCoV, which may be important in the pathogenesis of SARS, and deserve further studies.

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Introduction

A novel coronavirus was identified as the causative agent of severe acute respiratory syndrome (SARS).[1] Compared with common respiratory viral infections, SARS is unusually severe, with an overall fatality rate of about 10%. The SARS coronavirus (SCoV) causes a range of disease from flu-like symptoms and viral pneumonia to acute respiratory distress syndrome and death. The previously known human coronaviruses 229E (HCoV-229E) and OC43 have only been linked with the common cold. However, several animal coronaviruses have resulted in severe animals diseases of the respiratory or gastrointestinal tract or disseminated infections.

Macrophages are key cells for host defence and are abundant within all tissues of the body, including the respiratory system. They are potent producers of cytokines that are crucial components of innate immunity and potential mediators of immunopathology. Genetic resistance to strains of the coronavirus mouse hepatitis virus is associated with the ability of the virus to replicate in macrophages.[2] In contrast, feline infectious peritonitis is a disease caused by a coronavirus in which prior immunity or passive antibodies increase the severity of the disease.[3] In this disease, macrophages are the main target cells for virus replication, and antiviral antibodies enhance the replication of the virus in macrophage cultures in vitro. This has led to concerns about whether antibody-mediated enhancement of disease may be relevant to the pathogenesis of SARS.

Aims and objectives

1. Establish an in vitro model of SCoV infection of human primary macrophages;
2. Define the gene expression profile of SCoV-infected macrophages and compare it with human coronavirus 229E and influenza A (H1N1); and
3. Define the effect of antibody on neutralisation or enhancement of virus entry and replication.

Methods

This study was conducted from June 2005 to November 2006.

In vitro model of SCoV infection in macrophages and gene expression profiling

Using microarray gene expression profiling, we compared host response of primary human macrophages to infection with SCoV (strain HK39849), HCoV-229E, and influenza A virus (A/HK/54/98). The study was performed using macrophages derived from peripheral blood mononuclear cells of three different donors. The cells of each donor were subjected to microarray analysis after infection with each virus for 1, 3, and 6 hours. Ribonucleic acid (RNA) extracted from each macrophage preparation was examined for human genome-wide gene expression with a GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, USA) by the use of oligonucleotide probe sets, which spread across the full length of each gene in order to interrogate 28 869 genes (Genome Research Centre, The University of Hong Kong). Microarray data was normalised using

an ExonRMA summarisation algorithm on probe sets and baseline transform to a medium of all samples using the GeneSpring GX 9.0.5 software. By performing principal components analysis looking for outlier samples falling distal to the dataset at large and using filters on flags, quality control of sample levels was attained. Statistical analysis entailed a 2-way ANOVA test with a P-value cut-off of <0.05. Differential expression of genes to corresponding mock entities was selected with fold change of ≥1.5. Genes of individual pathways of interest were further studied using quantitative real-time polymerase chain reaction (RT-PCR) methods. Protein levels of key mediators were confirmed using enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. Culture supernatants were ultraviolet-irradiated for 20 min to inactivate infectious viruses prior to assay in a biosafety level-3 facility. Previous experiments have confirmed that cytokine levels are not affected by the dose of ultraviolet radiation used.[4]

Immune enhancement assay of SCoV replication in macrophages

We investigated the effect a human monoclonal (CR3014, a gift from Crucell Holland BV, Leiden, The Netherlands) and polyclonal (convalescent SARS serum) antibody to SCoV on the entry and replication of SCoV in human macrophages. Serial dilutions of the respective antibody or relevant control serum were mixed with a fixed dose of SCoV and infected onto human macrophages. Samples of the culture supernatants were collected at days 0, 1, 2, 3, 5, and 7 post-infection and titrated for virus infectivity. Ribonucleic acid was isolated from infected macrophages at 6 and 24 hours post-infection using the RNeasy Mini kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. Quantification of positive- and negative-strand viral RNA was performed by quantitative RT-PCR targeting the ORF1b gene, as described previously.[5] The SCoV RNA levels were normalised for the levels of β-actin mRNA.

Results

SCoV infection of macrophages

After infection of macrophages with SCoV, there was an increase in the copy numbers of both the positive and negative RNA strands of the SCoV ORF-1b and nucleocapsid genes over the first few hours after infection. Viral RNA levels in macrophages peaked at modest levels at about 6 h post-infection, but in FRhK-4 cells they continued to increase, reaching much higher absolute levels. A mouse monoclonal antibody (4D11) was used to demonstrate nucleoprotein expression in SCoV-infected macrophages. More than 90% of macrophages showed nucleoprotein expression when infected at a multiplicity of infection of one to two. However, no infectious virus was detected in the supernatant of virus-infected macrophages for up to 7 days post-infection, indicating that virus infection of these cells was abortive. In contrast, virus-infected FRhK-4 cells produced infectious virus titres up to 10^5 in 50% tissue culture infective doses/ml (data not shown), peaking at about 2 to 3 days post-infection.

Microarray analysis

In order to identify host genes that are affected by SCoV and thus account for its virulence, we compared the gene expression profile of SCoV-infected macrophages with that of low pathogenic viruses (influenza H1N1 and HCoV 229E) at various time points post-infection. Figure 1 illustrates the number of genes differentially affected by SCoV infection in comparison with influenza A or HCoV 229E. The proportion of genes uniquely affected by SCoV in comparison to H1N1 or HCoV 229E or both remained consistent at 77% at 1 and 3 h post-infection, but was down to 35% at 6 h post-infection. Figure 2 summarises selected microarray data presented as fold-change of gene expression in comparison to mock infected cells for innate immune markers at 1, 3 and 6 hours post-infection with SCoV, HCoV229E, and influenza A H1N1. Notably, IFN-β and -α-1 induction appears delayed or absent in SCoV- or HCoV 229E-infected macrophages. IP-10 induction is strongly induced at 1 and 3 hours by SCoV, while IL-8 is differentially down-regulated by SCoV (Fig 3). Apoptotic and anti-apoptotic pathways were also differentially activated in SCoV infected macrophages (data not shown).

Microarray analysis also suggests that other proinflammatory cytokines such as TNF, CCL2/MCP-1, CXCL10/IP-10 were strongly induced. Quantitative RT-PCR analysis confirmed an early induction of several chemokines, such as CXCL10/IP-10 and CCL2/MCP-1, in SCoV-infected macrophages. The ELISAs for CXCL10/IP-10 and CCL2/MCP-1 in macrophage culture supernatants

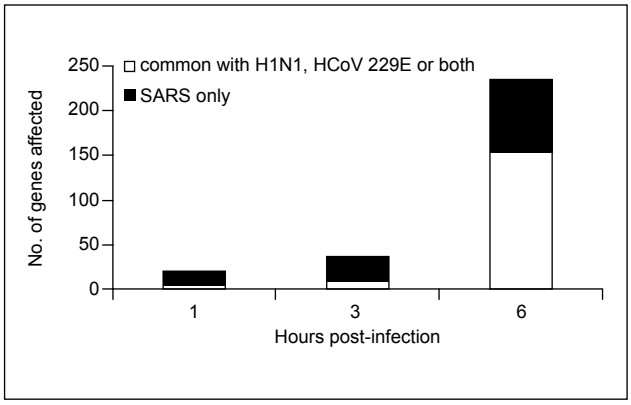

Fig 1. Microarray analysis of host genes affected by SCoV-infected macrophages

Gene expression profile of SCoV-infected, primary monocyte–derived macrophages is compared with that of H1N1 and HCoV 229E infections at 1, 3, and 6 hours post-infection. Genes with level change of 1.5 folds are regarded as affected. Genes that are affected by SCoV infection as well as by H1N1, HCoV 229E, or both are shown (white bar), with the proportion of the genes that are affected by SCoV indicated (black bar)

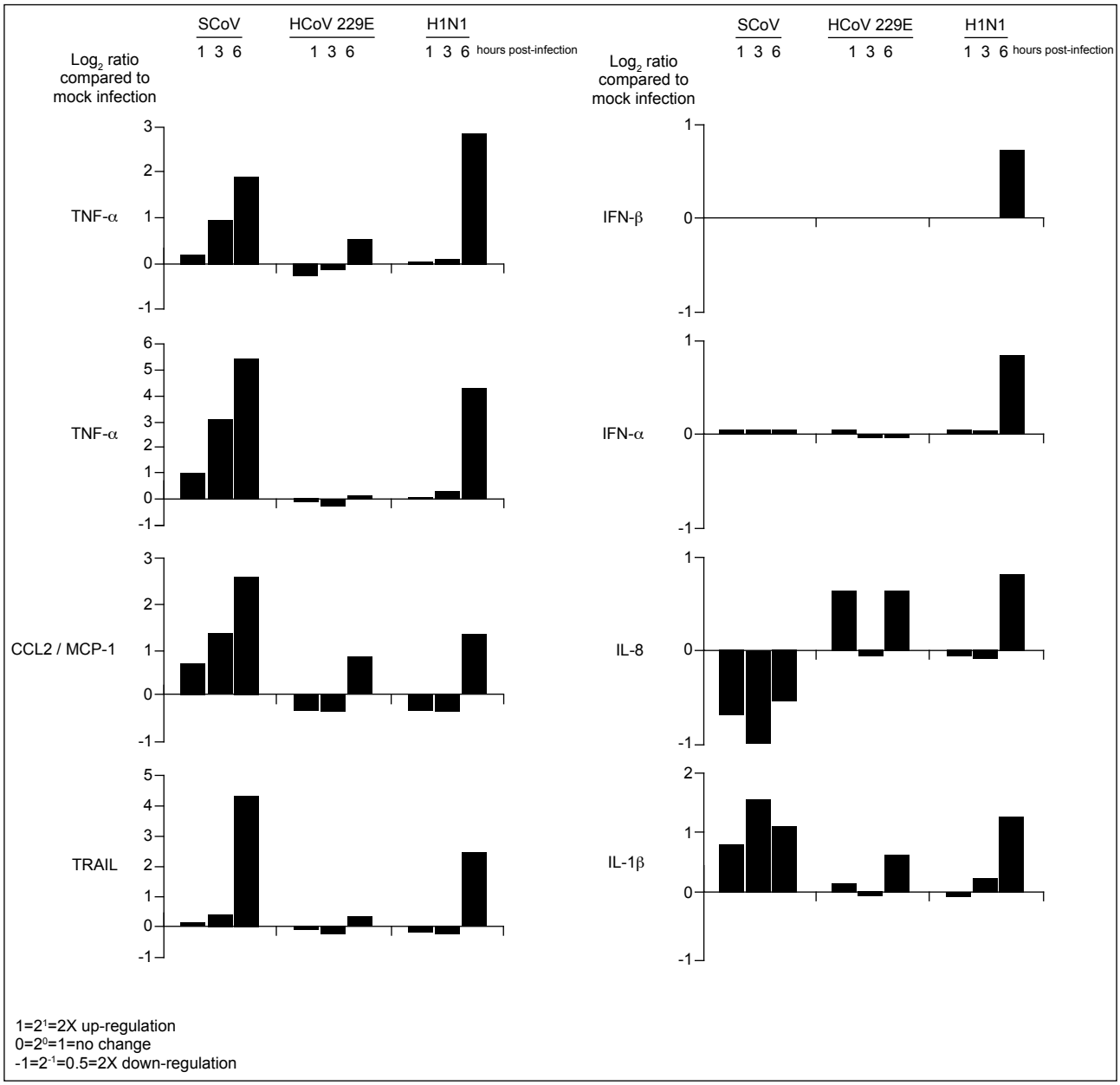

Fig 2. Microarray data showing fold change in gene expression of selected innate immune response genes differentially affected by SCoV-infected macrophages

Gene expression profile of SCoV-infected, primary monocyte–derived macrophages is compared with that of H1N1 and HCoV 229E infections at 1, 3, and 6 hours post-infection. Composite data from three different donors is presented. Gene expression is denoted with reference to that in mock infected cells

confirmed that SCoV induced CXCL10/IP-10 and CCL2/MCP-1 secretion in macrophages in the first few hours after infection.

In collaboration with a research group showing that SCoV Orf3a has apoptotic activity, we investigated the function of SCoV3a in SCoV-infected cells. The SCoV3a was localised to the Golgi region and interacted with caveolin 1 (according to yeast two hybrid analysis). There was evidence that caveolin 1 may be found in SCoV particles (data not shown). This is relevant to the microarray data where SCoV differentially activated a number of pro- and anti-apoptotic genes.

SCoV does not replicate in human macrophages in the presence of mAb CR3014 or convalescent serum

The SCoV did not replicate in primary human macrophages to produce infectious virus at measurable titres and the addition of serial dilutions of a human monoclonal antibody to SARS CoV spike (mAb CR3014). Serum from a convalescent SARS patient did not convert this abortive infection to a productive one. Productive virus replication was assayed by cell culture titration and by strand-specific RT-PCR assays to detect the negative sense (replication

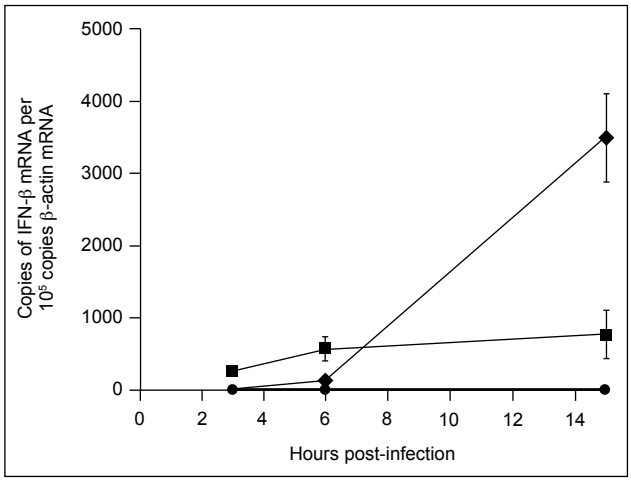

Fig 3 (a). Lack of induction of IFN-ß gene expression in SCoV-infected macrophages

Levels of IFN-ß mRNA are determined by quantitative real-time polymerase chain reaction. Macrophages are infected with SCoV (●), HCoV-229E (◆), and influenza A (H1N1) virus (■) at a multiplicity of infection of one to two, and RNA is extracted at 3, 6, and 15 h post-infection. The SCoV-infected macrophages does not induce IFN-ß at any of the three time points, in contrast to infections with influenza A (H1N1) and HCoV-299E viruses

intermediate) and positive sense viral RNA. With or without monoclonal or polyclonal antibody, macrophages took up SCoV, but this uptake did not lead to the productive virus replication and release of infectious virus.

Discussion

This study confirmed that virus gene transcription and translation were initiated in infected macrophages and that the block in productive virus replication occurred subsequently. Although double-stranded RNAs (which are potent inducers of type-1 interferon) and viral protein were expressed in SCoV-infected macrophages, there was no detectable IFN-β response in these cells. Others have reported similar findings in other cell types (eg epithelial).[6] The SCoV also failed to induce IL-28 and -29, which are two other recently discovered interferon-like cytokines with antiviral activities.[7] In contrast, both HCoV-229E and influenza A virus induced IFN-β as well as IL-28 and IL-29 in macrophages, although such induction was delayed in HCoV 229E in comparison with influenza A. This lack of innate immune defences may explain the progressive

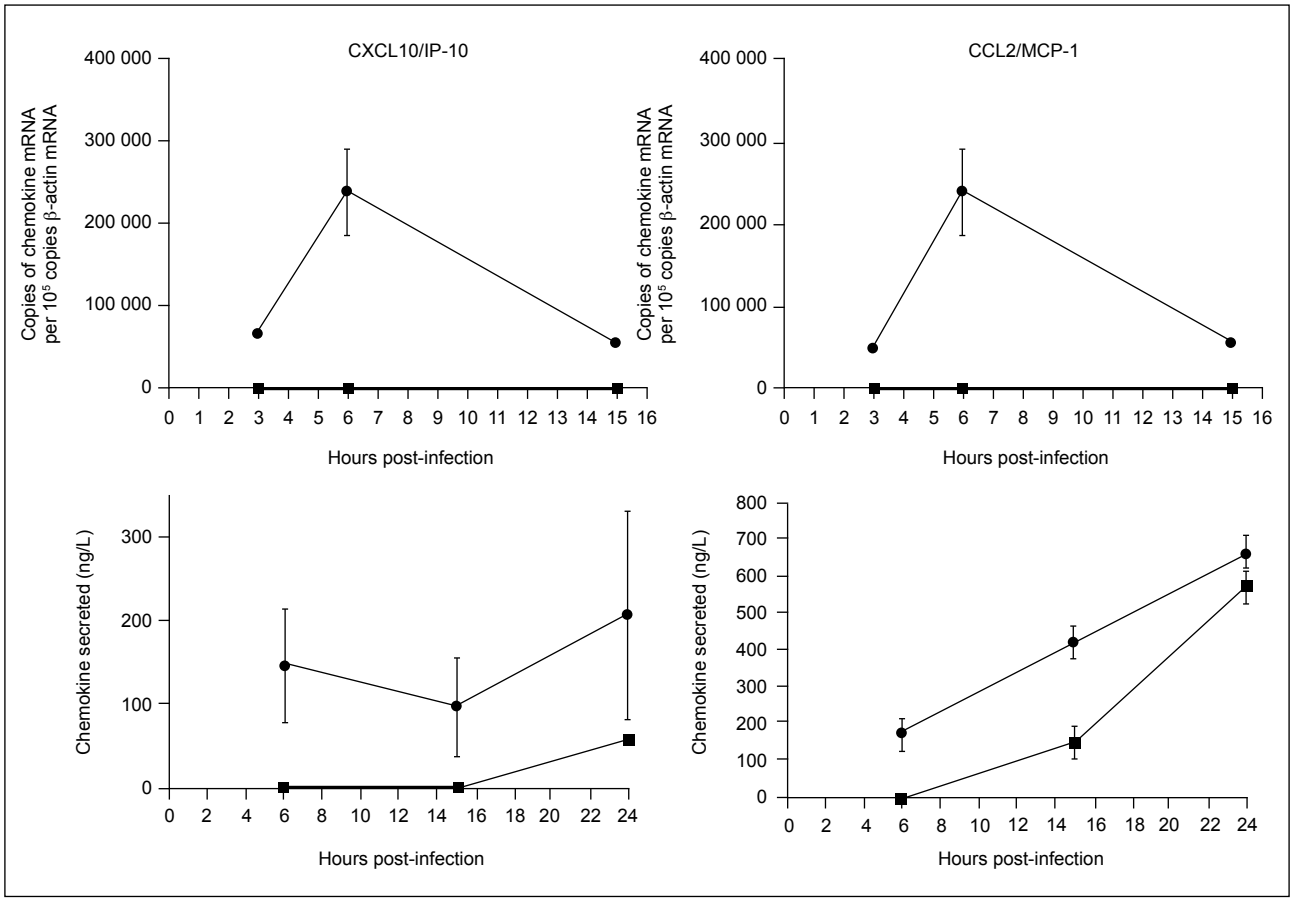

Fig 3 (b). Levels of CXCL10/IP-10 and CCL2/MCP-1 are elevated in SCoV-infected macrophages

Macrophages are infected with SCoV at a multiplicity of infection of one to two. Ribonucleic acid is extracted at 3, 6, and 15 h post-infection, and the levels of mRNA for CXCL10/IP-10 and CCL2/MCP-1 are determined by quantitative real-time polymerase chain reaction (top). Aliquots of the culture supernatant are taken at 6, 15, and 24 h post-infection, and the levels of secreted CXCL10/IP-10 and CCL2/MCP-1 are determined by specific enzyme-linked immunosorbent assays (bottom). The means±standard deviations of duplicate cultures from the same donor are representative of three independent experiments with similar results. SCoV infection (●) of macrophages induces higher levels of gene expression and secretion of CXCL10/IP-10 and CCL2/MCP-1 than does mock infection (■)

increase of viral load in the nasopharyngeal secretions up to week 2 of SARS infection, in contrast to other respiratory infections such as HCoV-229E and influenza A virus.[8]

The SCoV protein nsp1 has been identified as a putative interferon antagonist but its signalling pathways are unclear.

Chemokines such as CXCL10/IP-10 and CCL2/MCP-1 were up-regulated in macrophages by SCoV. CXCL10/IP-10 and CCL2/MCP-1 are chemotactic for monocytes/macrophages, which are the predominant inflammatory cell type in the lungs of SARS patients. We (unpublished data) and others[9] have found significantly elevated blood levels of CXCL10/IP-10 and CCL2/MCP-1 in SARS patients and that both chemokines were significantly elevated during the early stage of the illness. The chemokines CCL3/macrophage inflammatory protein 1, CCL7/MCP-3, and CCL8/MCP-2 were induced by SCoV according to the microarray analysis, and their biological effects were similar to CCL2/MCP-1. Therefore, the members of the monocyte chemotactic protein and macrophage inflammatory protein can synergistically induce a cycle of monocyte/macrophage recruitment and, potentially, monocyte/macrophage-induced immunopathology.

In this study, we also addressed the potential problem of antibody-dependent enhancement (ADE), which is a well-recognised phenomenon observed in infections with other coronavirus—feline infectious peritonitis virus. Given that ADE in feline infectious peritonitis virus infection is mediated by increased macrophage uptake of virus in the presence of neutralising antibody, we performed human macrophage infectivity assays in the presence of serial dilutions of CR3014 and human convalescent serum. The addition of varying concentrations of CR3014 or convalescent SARS serum to SCoV did not convert the abortive infection into a productive one. This reduced the likelihood that ADE in macrophages will be observed in vivo after passive immunisation in a manner analogous to that with feline infectious peritonitis.

Conclusions

The lack of a type-1 interferon response despite a strong induction of macrophage tropic chemokines may explain aspects of the pathogenesis of SARS. Although putative viral proteins such as the nsp1 have been implicated as interferon antagonists in SCoV, the signalling mechanisms that underlie this suppression of interferon remain unknown and deserve further research. The apparent inability of SCoV to trigger interferon responses may provide support for the use of interferon treatment for SARS.

Acknowledgements

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Functional role of ICAM-3 polymorphism in genetic susceptibility to SARS infection

Key Messages

1. Severe acute respiratory syndrome (SARS) patients who are homozygous for intercellular adhesion molecule-3 (ICAM-3) Gly143 showed significant association with higher lactate dehydrogenase levels and lower total white blood cell counts on admission.
2. In vitro functional studies demonstrated low level binding of ICAM-3 to DC-SIGN and a wide variation in T-cell response of the wild-type ICAM-3 genotype.

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Introduction

Genetic polymorphisms are associated with vulnerability to a variety of human infections. Association between susceptibility to severe acute respiratory syndrome (SARS) and major histocompatibility complex (MHC) class I has been reported.[1] Clinical outcome of SARS patients vary; only a small proportion needed intubation or intensive care. Moreover, immunopathological damage rather than uncontrolled viral replication may correlate with clinical progression of the disease beyond the first week.

Dendritic cells (DCs) are crucial in the defence against pathogens. The dendritic cell–specific intercellular adhesion molecule-3 (ICAM-3) grabbing non-integrin (DC-SIGN) is a mannose binding lectin expressed on the surface of DCs. The ICAM-3 is the natural ligand for DC-SIGN. Expressed constitutively on T-cells, ICAM-3 is a potent signalling molecule and major ligand in the initiation of T-cell–mediated immune response.[2] The interaction of adhesion molecules between antigen-presenting cells and T-cells is critical for activating antigen-specific T-cells. The binding between ICAM-3 and its ligands provides transient engagement of naive T-cells with DCs, which then allow the T-cells to sample large numbers of MHC molecules for the presence of specific peptides.[3] This initial cell-to-cell engagement step is critical for induction of T-cell responses, which play a central role in the immuno-regulation of infectious diseases. We hypothesised that polymorphisms of ICAM-3 may also influence susceptibility to SARS infection.

Aims

1. To determine whether ICAM-3 Asp143Gly polymorphism associates with susceptibility of SARS infection and its relation to clinicopathological outcome of SARS patients.
2. To investigate the role of ICAM-3 polymorphism influencing DC-SIGN-ICAM-3 interaction and immune response in SARS infection using functional studies.

Methods

This study was conducted from January 2006 to December 2007. A case-control genetic association study was performed to examine the contribution of ICAM-3 Asp143Gly polymorphism to SARS infection and/or any association with the clinicopathological outcome of SARS patients. A total of 817 SARS patients confirmed by serology and/or real-time polymerase chain reaction were recruited from follow-up outpatient clinics of the Pamela Youde Nethersole, Princess Margaret, United Christian, Queen Mary, Alice Ho Miu Ling Nethersole, and Prince of Wales hospitals. The controls included (1) 260 patients from the general outpatient clinics seen at least 2 months after the SARS outbreak, with no clinical history, signs or symptoms of inflammation/infection; (2) 307 health care workers who had worked in SARS wards but were disease-free and sero-negative for SARS; (3) 309 household contacts of SARS patients that remained unaffected and sero-negative for SARS. To prevent genotype and allele frequency distribution bias, family members of the same household who were genetically related were taken into consideration in the statistical analysis of genotypes. All control subjects were Hong Kong Chinese.

Main outcome measures

The clinical data of the SARS patients were retrospectively obtained from Hospital Authority, with the permission of all attending clinicians. Data collected include: age, sex, length of hospital stay, treatment in intensive care unit, and whether patients received assisted ventilation, steroid treatment, pulse steroids or intravenous immunoglobulin, and final outcome of patients (survival and death). Results of haematological and biochemical laboratory investigations on admission included the haemoglobin level, absolute lymphocyte count, platelet count, white blood cell (WBC) count, and biochemical indices (serum/plasma alanine aminotranseferase, albumin, globulin, creatinine kinase, lactate dehydrogenase [LDH], urea, sodium, potassium and serum creatinine).

Study instruments

Genotyping of the ICAM-3 polymorphism

Genotyping of the initial SARS patients and health care workers was performed using Sequenom MassARRAY (Sequenom Inc, San Diego, CA, USA). Genotyping of the additional SARS patients, household contacts and outpatient controls for the ICAM-3 Gly143 SNP was performed by Allelic Discrimination TaqMan Assay (Applied Biosystems Inc, Foster City, CA, USA). Appropriate controls and replicates were included for quality control.

Production of wild-type and polymorphic variant ICAM-3 protein

The coding sequences of ICAM-3 containing Asp143 and Gly143 allele were separately cloned and then stably transfected into 293 cell lines. The stable transfectants thus expressed a fusion protein containing the ICAM-3 of known genotype. The secreted soluble protein was purified and concentrated for in vitro binding studies.

Culturing monocyte-derived dendritic cells

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coat samples obtained from the Hong Kong Red Cross Blood Transfusion Service. $CD14^+$ monocytes were isolated from PBMCs, from which DCs were induced, using MACS separation (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). These $CD14^+$ cells were cultured in the presence of 50 ng/ml GM-CSF and 10 ng/ml IL-4. The cells were harvested at the 6th day and analysed by flow cytometry.

ICAM-3 binding assay

To investigate the binding affinity of soluble wild-type ICAM-3 (Asp143) with DC-SIGN transfectants, a stable DC-SIGN transfectant was used whereas parental 293 cell line was used as negative control. These cells were treated with (1) recombinant human ICAM-3 protein (R&D Systems Inc, MN, USA, Cat No 715-IC), (2) purified ICAM-3 protein containing Asp143, or (3) purified ICAM-3 protein containing Gly143. Cells not treated with ICAM-3 protein were used as reference. After incubation at 4ºC for 40 min, the treated cells were washed twice to remove unbound ICAM-3 proteins, and then stained by FITC-labelled anti-ICAM-3 antibody for analysis by BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). Cells not treated with ICAM-3 but stained by anti-ICAM-3 antibody were used as reference. To investigate the binding affinity of soluble wild-type ICAM-3 with DC-SIGN expressed on monocyte-derived DCs, similar procedures were performed, except that male human serum of AB blood type was included in the binding buffer to reduce non-specific binding by blocking the Fc receptors known to be present on DCs.

T-cell response

Convalescent SARS patients with the homozygous wild-type, heterozygous, and homozygous variant genotypes were identified. Those who were HLA-A2 positive were re-contacted to donate further blood samples for study. Isolated PBMCs were used for T-cell response analysis using ELISPOT assay.

Statistical analysis

For the risk association analysis, genotype distributions of the patient and control groups were assessed by χ^2 test. Odds ratio (OR) and 95% confidence intervals (CI) were used to measure the strength of association. Genotyping results were checked for Hardy-Weinburg equilibrium. As a significant proportion of the household contacts recruited were genetically related with each other, logistic regression with the cluster and robust methods (STATA program, College Station, TX, USA) was used to factor in genetic relations. For in vitro studies, statistical significance was calculated using Student's *t*-test. For analysis for association with clinical outcome, the χ^2 test was used to test for possible association with nominal clinical outcome measures. For analysis of numerical variables, each parameter was first analysed by Student's *t* test. Parameters that were significantly different were further studied using the χ^2 test.

RESULTS

Genetic association study

The demographics of the SARS patients, control groups, and their clinical features are summarised in Table 1. The genotype and allele frequencies of ICAM-3 Asp143Gly SNP of the 817 SARS patients, 260 outpatient controls, and 309 household contacts were in Hardy-Weinberg equilibrium. As the genotype distribution of the health care workers was not in Hardy-Weinberg equilibrium, this group was excluded from risk association analysis. No significant difference in genotype or allele frequency distribution was found when comparing these 2 control groups with SARS patients (data not shown). This lack of risk association was confirmed even with blood relationship taken into account, either by excluding patients who were blood relatives or by using logistic regression model analysis. Among clinical outcome measures, Student's *t* test showed significant association for LDH levels on admission (P=0.036) and for WBC counts (P=0.036) when comparing homozygous wild-type Asp versus homozygous Gly (Fig). The LDH levels and WBC counts were divided into high- and low-level groups, and the χ^2 test performed. For LDH levels, the overall genotype yielded a P value of 0.015; homozygous Gly versus

Table 1a. Demographics of severe acute respiratory syndrome (SARS) patients and controls

Demographics	SARS patients (n=817)	Controls		
		Health care workers (n=307)	Household contacts (n=309)	Out-patients (n=260)
Mean±SD, median (range) age (years)	40±14, 38 (5-88)	35±10, 33 (21-60)	42±14, 43 (18-80)	50±20, 47 (4-95)
Male:female	2:3	2:6.6	2:2.4	2:4
No. (%) of female	505 (61.8)	235 (76.5)	168 (54.4)	173 (66.5)

Table 1b. Characteristics of severe acute respiratory syndrome (SARS) patients

Characteristics	No. (%) of SARS patients
Treated in intensive care unit (ICU)	136 (16.6)
Received assisted ventilation	76 (9.3)
Received steroid treatment	795 (97.3)
Received pulse steroid/intravenous immunoglobulin	517 (63.3)
Mean±SD, median (range) length of hospital stay (days)	28.2±17.9, 23 (4-235)
Death	12 (1.5)
Mean±SD, Median (range) of length of ICU stay (days)	2.7±10.8, 0 (0-139)

homozygous Asp (P=0.007, OR=4.31, 95% CI=1.37-13.56). Allelic association was observed (P=0.0093, OR=1.75, 95% CI=1.14-2.67, Table 2a). Association for WBC counts was also demonstrated for homozygous Gly versus homozygous Asp (P=0.022, OR=0.30, 95% CI=0.10-0.89, Table 2b). Notably, the homozygous Gly genotype associated with higher LDH levels and lower WBC counts. No significant association was found for nominal clinical outcome measures.

Functional studies

Using DC-SIGN transfectants, binding experiments showed that ICAM-3 protein did bind to DC-SIGN but at a low level; at most binding was 15%. Similar binding was also shown in the soluble ICAM-3 produced and purified in our laboratory, expressing either wild-type Asp143 genotype or variant ICAM-3 Gly143 genotype (data not shown). Binding experiments using DC-SIGN expressed on DCs did not demonstrate ICAM-3 binding to DC-SIGN. Blood samples from a total of 15 convalescent SARS-infected individuals with different ICAM-3 genotypes and HLA-A2 positive were retrieved. T-cells harvested from these samples were incubated overnight with IL-2 before stimulation by SARS peptide and measured for IFN-γ. Results showed a wide range of response for the wild-type genotype, but with no significant difference in T-cell responses between the wild-type and variant genotypes (data not shown).

Discussion

In this large genetic association study for SARS susceptibility, no significant risk association was found for SARS infection for the ICAM-3 Asp143Gly SNP. Nonetheless, genotype analysis of our 817 SARS patients showed significant association of higher LDH levels and lower WBC counts in SARS patients on admission with the homozygous Gly143 genotype of ICAM-3, which supports the role of ICAM-3 in the immunopathogenesis of SARS. These findings are in keeping with the role of ICAM-3 in T-cell activation and the immune response. As the SARS patients were from six different hospitals throughout Hong Kong, there may have been confounding factors, such as differences in management preferences pertaining to length of hospital stay, level of intensive care, and decision to initiate assisted ventilation or administer steroids. This may have contributed to non-identification of significant associations with other clinical parameters. Laboratory parameters on the other hand were more standardised.

Although the LDH level is a relatively non-specific reflection of tissue destruction, the association with higher

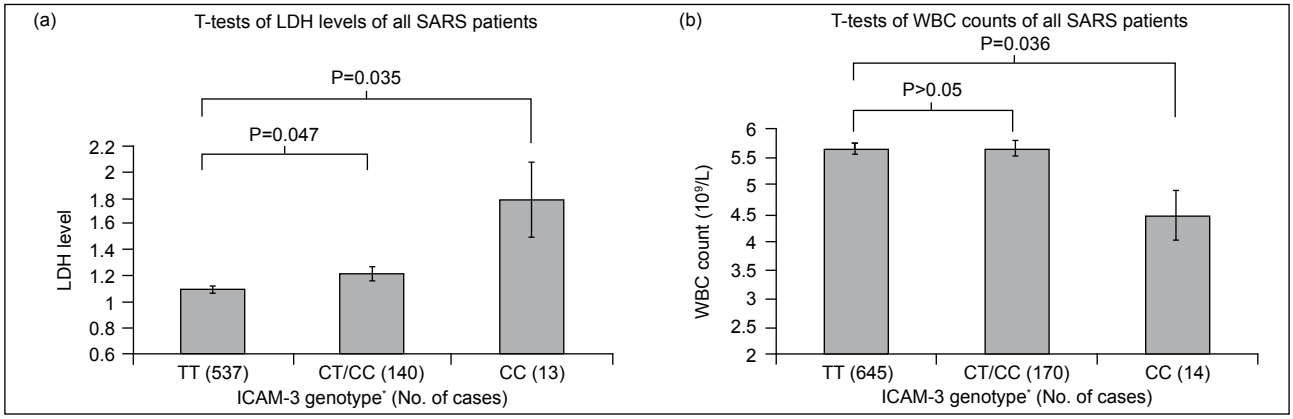

Fig. Genotype frequencies of intercellular adhesion molecule-3 (ICAM-3) of all severe acute respiratory syndrome (SARS) patients: (a) normalised lactate dehydrogenase (LDH) levels on admission (as ratio to the upper limit of the normal reference range) (b) white blood cell (WBC) counts on admission (copyright 2007 *Journal of Infectious Diseases*)

* TT denotes homozygous Asp143, CT heterozygous Gly/Asp, and CC homozygous Gly143

Table 2a. Genotype and allele analysis of the intercellular adhesion molecule-3 (ICAM-3) Asp143Gly, with lactate dehydrogenase (LDH) levels on admission of all severe acute respiratory syndrome (SARS) patients

ICAM-3	No. (%) of SARS patients		Odds ratio (95% CI)	P value†
	Lower LDH levels*	Higher LDH levels*		
Genotype	n=582	n=95		
TT (Asp)	469 (80.6)	68 (71.6)	Reference	0.015
CT (GlyAsp)	105 (18)	22 (23.2)	-	0.168
CC (Gly)	8 (1.4)	5 (5.2)	4.31 (1.37-13.56)	0.007
C-carrier (Gly-carrier) [CT and CC]	113 (19.4)	27 (28.4)	1.65 (1.009-2.69)	0.044
Allele	n=1164	n=190		
T (Asp)	1043 (89.6)	158 (83.2)	Reference	-
C (Gly)	121 (10.4)	32 (16.8)	1.75 (1.14-2.67)	0.009

* The cutoff value of normalised LDH levels on admission is 1.6
† χ^2 test for overall genotype

Table 2b. Genotype and allele analysis of the intercellular adhesion molecule-3 (ICAM-3) Asp143Gly, with white blood cell (WBC) counts of all severe acute respiratory syndrome (SARS) patients

ICAM-3	No. (%) of SARS patients		Odds ratio (95% CI)	P value†
	Lower WBC counts*	Higher WBC counts*		
Genotype	n=246	n=569		
TT (Asp)	186 (75.6)	459 (80.7)	Reference	0.047
CT (GlyAsp)	52 (21.1)	104 (18.3)	-	0.270
CC (Gly)	8 (3.3)	6 (1.0)	0.30 (0.10-0.89)	0.022
C-carrier (Gly-carrier) [CT and CC]	60 (24.4)	110 (19.3)	-	0.102
Allele	n=492	n=1138		
T (Asp)	424 (86.2)	1022 (89.8)	Reference	-
C (Gly)	68 (13.8)	116 (10.2)	0.71 (0.51-0.98)	0.034

* The cutoff value of WBC counts on admission is 4.5
† χ^2 test for overall genotype

LDH levels and lower WBC counts in SARS patients suggests immune response–associated leukocyte destruction. Indeed high peak LDH levels have been reported to be independent predictors of adverse outcome.[4] Thus SARS patients who are homozygous for Gly 143 genotype of the ICAM-3 Asp143Gly SNP have a four-fold chance of higher LDH levels on admission and a poorer prognosis. This may have implications for other infectious diseases in which viral-induced cell death and/or immune responses contribute significantly to outcome. Thus, by knowing the genotype of the patient, we might be able to predict clinical outcome and offer suitable treatment in advance.

Using DC-SIGN transfectants, in vitro ICAM-3 binding experiments demonstrated low levels of soluble ICAM-3 binding to DC-SIGN, in keeping with previous reports suggesting binding between ICAM-3 with DC-SIGN might be transient, allowing T-cells to sample large numbers of MHC molecules for the presence of specific peptides. Our method of detection was thus not sensitive enough to demonstrate significant differences in binding affinity between the two different genotypes. Although binding levels may be low, in vivo they may be sufficient for transient interactions between DCs and T-cells.

A wide variation in T-cell response suggested that at least for wild-type ICAM-3, other factors also affect the response. The DCs might transfer SARS coronavirus to other cells through DC-SIGN, with interactions between ICAM-3 and DC-SIGN contributing to trans-infection from DCs to T cells. Thus the higher LDH levels and lower WBC counts associated with homozygous Gly143 genotype could also be attributed in part by viral-induced cell death.

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Chinese herbal medicine in the treatment of acute upper respiratory tract infection: a randomised, double blind, placebo-controlled clinical trial

Key Messages

1. Neither the duration nor severity of symptoms of upper respiratory tract infection was reduced after Traditional Chinese medicine treatment with either *Jing Fan Bai Du san* or *Ying Qiao san*.
2. For patients with wind-cold syndrome, *Jing Fan Bai Du san* might be able to improve general health more than placebo.
3. Both *Jing Fan Bai Du san* and *Ying Qiao san* were well tolerated, with no excess in the incidence of side effects compared to placebo.
4. Randomised double-blind placebo-controlled trials are objective methodology to determine the effectiveness and side effects of Chinese herbal medicines.

Introduction

Acute upper respiratory tract infection (URTI) is the most common type of illness leading to consultation in primary care.[1] There is no established cure for this ailment in western medicine. In Hong Kong, Chinese herbal medicine is commonly used for treating URTI, but research on its effectiveness or side effects is meagre.

The aim of this study was to determine whether treatment with *Jing Fan Bai Du san* and *Ying Qiao san* based on traditional Chinese medicine (TCM) diagnoses would significantly enhance the resolution (reduce the duration and/or severity of symptoms) and improve quality of life in patients with URTIs in primary care.

Methods and subjects

This prospective randomised double-blind placebo-controlled trial was conducted from January 2006 to January 2007. It entailed 327 patients diagnosed with URTI in two government outpatient clinics in Hong Kong. Eligible patients were diagnosed by a registered Chinese medicine practitioner based on TCM and classified into wind-cold syndrome (n=162) and wind-heat syndrome (n=165). Patients in each group were randomised to receive concentrated TCM granules (*Jing Fan Bai Du san* for wind-cold syndrome and *Ying Qiao san* for wind-heat syndrome) or placebo for as long as the URTI symptoms persisted but up to a maximum of 10 days.

Patients recorded their symptoms and possible side effects in a diary for 21 days, and were also followed up by telephone on days 0, 1, 4, 10, 14 and 20. On day 7, patients were assessed by the Chinese medicine practitioner for the URTI symptoms, side effects, and health-related quality of life (HRQOL) measured by the SF-36 health survey and Chinese Quality of Life (ChQoL) instrument. The diary and unused TCM were returned after 21 days, using a pre-paid postal envelope. Randomisation and follow-up rates of the subjects are shown in Fig 1. The drop out rate was low, ranging from 3% to 9%.

The TCM and placebo groups were similar in terms of age, sex and other socio-demographics (Table 1), as were the mean numbers of URTI contracted in previous year and the smoking history. The baseline HRQOL scores of TCM and placebo groups were not significantly different, except for general health scores of wind-cold syndrome group (44.86 vs 53.58).

Outcome measures and data analysis

The primary outcome measure was the proportion of patients with resolution of all URTI symptoms on days 4 and 7. Each symptom was scored 0 (if absent) or 1 (if present). Secondary outcome measures included the number of days to symptom resolution, change in total symptom score, the area under the curve of the total symptoms score, change in the SF36 and ChQoL scores,

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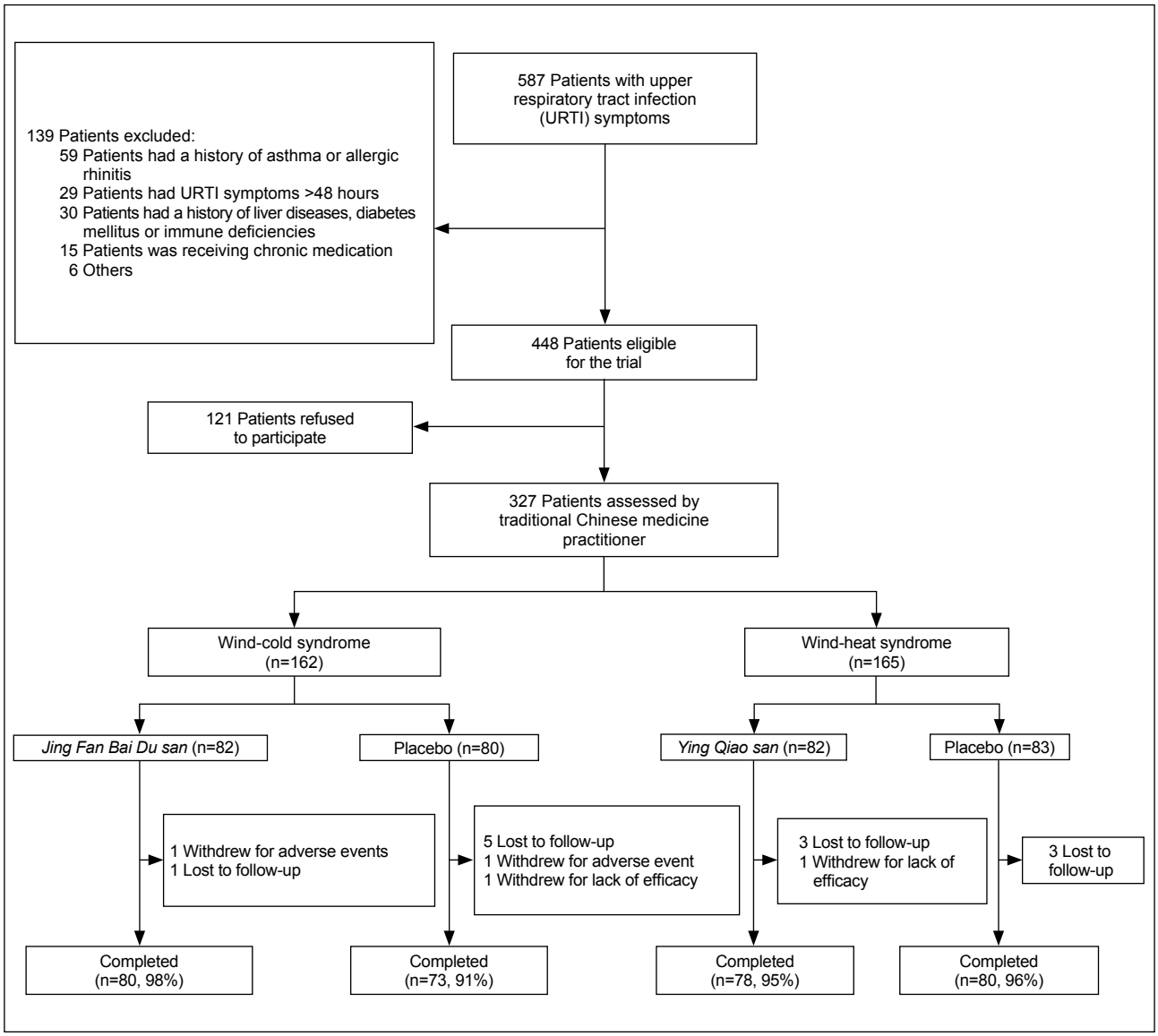

Fig 1. Patient randomisation and follow-up flow chart

number of sick-leave days, and the incidence of adverse effects. The difference in the proportion of patients with resolution of all URTI symptoms was tested separately in the wind-cold and wind-heat syndrome subgroups using Fisher's exact test. The difference in proportion on day 7 between the TCM and placebo groups was compared using logistic regression with adjustment for the use of western medicine. Sensitivity analyses were also performed. The area under the curve of the total symptom score over the 21 days between the TCM and placebo groups were compared using regression analysis with and without adjustment for the use of western medicine. The change in individual and total symptom scores over time was compared between the two groups by the sign-rank tests. The incidence of side effects in the two groups were compared by Fisher's exact test. All analyses were performed based on an intention-to-treat basis. Missing values were replaced by the last observed value.

Results

Symptoms resolution

The proportion of patients with symptom resolution was not significantly different between the TCM and placebo groups on days 1, 4, 7, 10, 14, 20 and 21, after adjusting for baseline values (Table 2). Around 50% of patients had symptoms resolved by day 7. More than 40% of patients had taken western medications (paracetamol, anti-histamines, soothing lozenges, and/or nasal decongestants), but the duration or severity of symptoms was not changed. No significant difference was found in the area under the curve of the total or individual symptom score in both the wind-cold and wind-heat syndrome subgroups (Figs 2 and 3).

Change in symptoms and health-related quality of life scores

There was no difference in the change in total or individual

Table 1. Baseline characteristics, symptoms, and health-related quality of life scores of patients

Parameters	All patients		Wind-cold syndrome		Wind-heat syndrome	
	Traditional Chinese medicine	Placebo	*Jing Fan Bai Du san*	Placebo	*Ying Qiao san*	Placebo
No. of patients	164	163	82	80	82	83
Mean±SD (range) age (years)	44.34±11.02 (20-77)	43.20±11.48 (18-74)	44.41±11.67 (22-77)	42.04±11.46 (20-73)	44.27±10.40 (20-71)	44.33±11.45 (18-74)
Female:male (%)	49:51	54:46	51:49	56:44	46:54	52:48
Mean (SD) symptom score						
Total	26.90 (13.14)	27.29 (13.60)	26.63 (14.49)	27.14 (14.06)	27.18 (11.71)	27.43 (13.26)
Chills	0.76 (1.52)	0.83 (1.57)	0.72 (1.32)	0.96 (1.78)	0.81 (1.70)	0.71 (1.34)
Fever	1.17 (1.99)	0.82 (1.59)	1.04 (2.02)	0.65 (1.20)	1.31 (1.97)	0.97 (1.87)
Cough	2.74 (2.34)	2.71 (2.12)	2.91 (2.54)	2.58 (2.08)	2.58 (2.13)	2.82 (2.16)
Headache	2.31 (2.27)	2.81 (2.52)	2.23 (2.39)	2.73 (2.59)	2.38 (2.17)	2.87 (2.48)
Hoarseness	3.12 (2.45)	3.27 (2.41)	2.94 (2.56)	2.92 (2.27)	3.31 (2.33)	3.59 (2.50)
Muscle-ache	3.67 (2.64)	3.40 (2.50)	3.79 (2.55)	3.52 (2.56)	3.55 (2.73)	3.29 (2.47)
Running nose	2.50 (2.53)	2.66 (2.51)	2.63 (2.57)	3.18 (2.63)	2.37 (2.49)	2.19 (2.31)
Nasal obstruction	2.03 (2.30)	1.90 (2.39)	2.01 (2.42)	2.03 (2.54)	2.05 (2.19)	1.78 (2.25)
Itchy throat	3.34 (2.42)	3.35 (2.70)	3.59 (2.50)	3.39 (2.64)	3.09 (2.32)	3.30 (2.77)
Sore throat	3.76 (2.61)	3.76 (2.69)	3.09 (2.84)	3.01 (2.73)	4.42 (2.19)	4.43 (2.49)
Sneezing	1.49 (1.97)	1.79 (2.04)	1.68 (2.07)	2.17 (2.18)	1.31 (1.85)	1.46 (1.85)
Mean (SD) SF-36 health-related quality of life score*						
PCS	40.37 (8.18)	39.73 (9.23)	39.88 (7.69)	40.20 (9.73)	40.86 (8.66)	39.31 (8.79)
MCS	45.01 (11.41)	46.57 (10.26)	44.97 (11.97)	47.57 (11.66)	45.05 (10.90)	45.67 (8.80)
PF	87.21 (12.64)	85.60 (14.20)	88.40 (11.21)	85.00 (14.34)	86.03 (13.90)	86.14 (14.14)
RP	37.98 (35.07)	40.50 (36.48)	34.62 (33.28)	43.31 (35.34)	41.35 (36.67)	37.97 (37.52)
BP	57.97 (21.41)	56.84 (22.24)	57.10 (22.26)	58.73 (23.26)	58.83 (20.64)	55.14 (21.28)
GH	48.23 (19.20)	50.59 (19.40)	44.86 (19.58)†	53.58 (19.13)†	51.60 (18.32)	47.90 (19.37)
VT	43.33 (20.39)	43.67 (21.93)	43.40 (20.94)	46.83 (23.73)	43.27 (19.95)	40.82 (19.91)
SF	75.72 (25.37)	76.58 (23.09)	76.12 (26.13)	76.23 (25.89)	75.32 (24.75)	76.90 (20.42)
RE	49.57 (41.39)	51.78 (39.49)	48.29 (40.07)	54.46 (39.13)	50.85 (42.88)	49.37 (39.89)
MH	67.18 (19.22)	70.29 (17.12)	67.33 (20.85)	71.89 (18.13)	67.03 (17.57)	68.86 (16.14)
Mean (SD) Chinese quality of life score						
Physical	56.10 (12.27)	56.66 (12.85)	55.94 (11.44)	57.45 (13.74)	56.26 (13.13)	55.95 (12.02)
Vitality and spirit	54.36 (15.04)	54.03 (14.33)	55.30 (15.41)	53.96 (14.87)	53.41 (14.71)	54.09 (13.92)
Emotion	78.51 (13.14)	80.47 (11.52)	78.89 (13.72)	80.96 (12.84)	78.13 (12.61)	80.03 (10.25)
Overall	62.99 (11.21)	63.72 (10.22)	63.38 (11.39)	64.13 (11.10)	62.60 (11.09)	63.36 (9.41)

* PCS denotes physical component summary score, MCS mental component summary score, PF physical functioning, RP role limitation due to physical problems, BP bodily pain, GH general health, VT vitality, SF social functioning, RE role limitation due to emotional problems, and MH mental health

† P<0.05 by Monte Carlo exact test for likelihood-ratio Chi-square

Table 2. Proportion of patients with resolution of symptoms

Day	Wind-cold syndrome					Wind-heat syndrome				
	Jing Fan Bai Du san (n=82)	Placebo (n=80)	P value*	Risk difference	95% CI	*Ying Qiao san* (n=82)	Placebo (n=83)	P value*	Risk difference	95% CI
1	8.5%	6.3%	0.77	0.02	-0.06-0.10	3.7%	7.2%	0.50	-0.04	-0.11-0.03
4	35.4%	40.3%	0.62	-0.05	-0.50-0.10	26.6%	36.1%	0.24	-0.10	-0.24-0.05
7	51.2%	56.0%	0.63	-0.05	-0.20-0.11	49.4%	51.3%	0.87	-0.02	-0.17-0.14
10	67.9%	70.3%	0.86	-0.02	-0.17-0.12	67.9%	70.0%	0.86	-0.02	-0.17-0.12
14	82.5%	81.1%	0.84	0.01	-0.14-0.11	73.4%	82.5%	0.19	-0.09	-0.04-0.22
20	92.5%	83.8%	0.13	0.09	-0.19-0.02	89.7%	90.0%	1.00	0.00	-0.09-0.10
21	91.3%	91.8%	1.00	-0.01	-0.08-0.09	87.2%	83.8%	0.65	0.03	-0.14-0.08

* By Fisher's exact test

symptom score between TCM and placebo groups at day 4, 7 or 21. After the adjustment for baseline values and western medicine use, patients receiving *Jing Fan Bai Du san* (in the wind-cold syndrome subgroup) had significantly greater improvement in the SF-36 general health scores at day 7 than those receiving placebo (Table 3). No statistical significant difference was found in SF-36 or ChQoL scores.

Adverse effects

One or more adverse effects were reported by 11% (placebo) and 11.25% (*Jing Fan Bai Du san*) patients in the wind-cold syndrome subgroup, and 20% (placebo) and 20.5% (*Ying Qiao san*) patients in the wind-heat syndrome subgroup. No significant difference was noted between the TCM and placebo groups in the incidence of adverse effects and the number of sick-leave days.

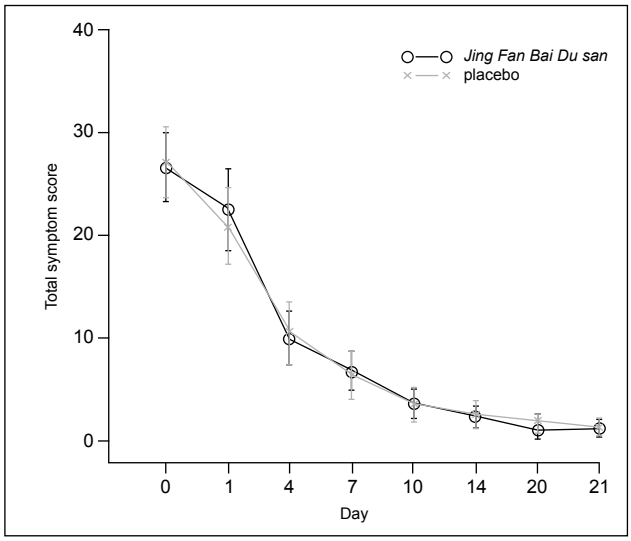

Fig 2. Comparison of total symptom scores in patients with wind-cold syndrome receiving *Jing Fan Bai Du san* or placebo

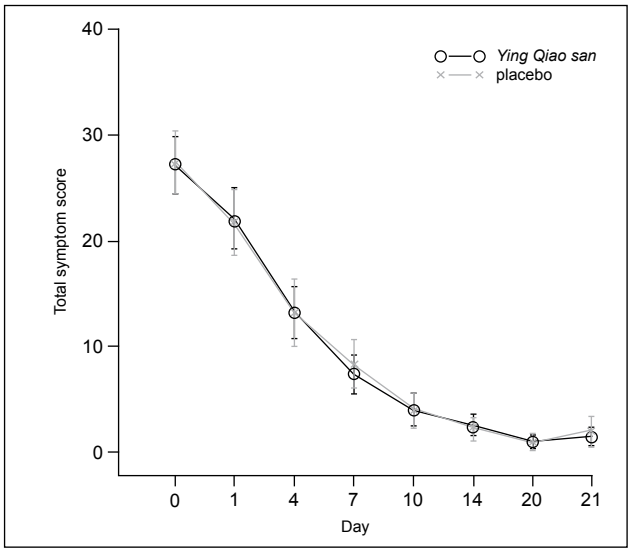

Fig 3. Comparison of total symptom scores in patients with wind-heat syndrome receiving *Ying Qiao san* or placebo

Table 3. Change in symptom and quality of life scores of patients on day 7

Parameters	All patients		Wind-cold syndrome		Wind-heat syndrome	
	Traditional Chinese medicine	Placebo	*Jing Fan Bai Du san*	Placebo	*Ying Qiao san*	Placebo
No. of patients	164	163	82	80	82	83
Mean (SD) change in symptom score						
Total	2.32 (1.15)	2.35 (1.28)	2.30 (1.28)	2.36 (1.34)	2.34 (1.01)	2.34 (1.23)
Chills	0.73 (1.49)	0.82 (1.61)	0.68 (1.28)	0.96 (1.77)	0.79 (1.68)	0.69 (1.45)
Fever	1.13 (1.92)	0.78 (1.58)	1.05 (2.01)	0.63 (1.18)	1.22 (1.84)	0.91 (1.86)
Cough	2.49 (2.31)	2.41 (2.14)	2.74 (2.42)	2.38 (2.17)	2.24 (2.17)	2.43 (2.12)
Headache	2.13 (2.20)	2.67 (2.61)	2.09 (2.31)	2.63 (2.76)	2.18 (2.09)	2.71 (2.47)
Hoarseness	3.01 (2.43)	3.15 (2.42)	2.80 (2.54)	2.81 (2.31)	3.22 (2.31)	3.46 (2.48)
Muscle-ache	3.46 (2.56)	3.24 (2.44)	3.50 (2.48)	3.32 (2.45)	3.41 (2.66)	3.16 (2.44)
Running nose	2.32 (2.47)	2.52 (2.57)	2.46 (2.54)	3.01 (2.68)	2.17 (2.39)	2.08 (2.39)
Nasal obstruction	1.92 (2.27)	1.79 (2.45)	1.91 (2.41)	1.89 (2.56)	1.94 (2.13)	1.70 (2.36)
Itchy throat	3.18 (2.36)	3.20 (2.71)	3.41 (2.46)	3.32 (2.65)	2.95 (2.24)	3.09 (2.79)
Sore throat	3.68 (2.58)	3.58 (2.72)	3.01 (2.79)	2.95 (2.77)	4.37 (2.17)	4.16 (2.55)
Sneezing	1.43 (1.93)	1.67 (2.05)	1.61 (2.05)	2.04 (2.20)	1.24 (1.78)	1.33 (1.85)
Mean (SD) change in SF-36 health-related quality of life score*						
PCS	13.32 (8.44)	13.30 (9.45)	13.95 (7.85)	12.87 (9.57)	12.70 (8.99)	13.69 (9.39)
MCS	10.77 (11.91)	10.80 (10.35)	11.35 (12.77)	9.83 (11.80)	10.19 (11.04)	11.68 (8.81)
PF	11.19 (12.63)	11.69 (13.18)	10.58 (10.96)	12.29 (12.70)	11.79 (14.14)	11.14 (13.66)
RP	56.41 (37.12)	53.81 (39.55)	62.18 (35.75)	49.65 (40.13)	50.64 (37.79)	57.59 (38.89)
BP	32.11 (25.82)	34.23 (25.17)	31.60 (26.68)	32.79 (26.30)	32.62 (25.09)	35.53 (24.20)
GH	19.96 (18.39)	17.72 (19.10)	23.42 (19.53)†	16.08 (15.87)†	16.50 (16.60)	19.20 (21.63)
VT	30.67 (25.16)	31.03 (23.78)	32.24 (24.36)	27.78 (24.46)	29.10 (26.01)	33.99 (22.89)
SF	19.87 (26.02)	21.11 (23.68)	20.19 (28.08)	21.01 (26.02)	19.55 (23.97)	21.20 (21.50)
RE	41.45 (43.73)	45.03 (40.41)	44.02 (43.48)	43.52 (39.42)	38.89 (44.11)	46.41 (41.49)
MH	16.41 (19.01)	14.49 (16.65)	16.97 (20.14)	12.78 (18.17)	15.85 (17.93)	16.05 (15.07)
Mean (SD) change in Chinese quality of life score						
Physical	6.69 (7.45)	7.88 (7.61)	6.43 (7.57)	7.98 (8.07)	6.96 (7.36)	7.79 (7.20)
Vitality and spirit	28.13 (18.04)	28.35 (17.56)	28.18 (19.20)	28.39 (16.87)	28.07 (16.93)	18.32 (18.27)
Emotion	-0.45 (7.96)	0.97 (6.63)	-0.53 (7.75)	0.56 (6.45)	-0.37 (8.22)	1.35 (6.81)
Overall	9.49 (6.85)	10.52 (6.88)	9.38 (6.94)	10.40 (6.49)	9.60 (6.80)	10.63 (7.26)

* PCS denotes physical component summary score, MCS mental component summary score, PF physical functioning, RP role limitation due to physical problems, BP bodily pain, GH general health, VT vitality, SF social functioning, RE role limitation due to emotional problems, and MH mental health

† P<0.05 by sign-rank test

Discussion

This study adhered to guidelines of the consolidated standards for reporting trials. The numbers of patients in each subgroup were similar. Double-blind clinical trials based on TCM diagnoses were feasible. Over 90% of the

patients completed their treatment courses, suggesting good acceptance to clinical trials with TCM.

This study did not reveal any significant benefit in terms of reducing the duration or severity of URTI symptoms after the treatment of *Jing Fan Bai Du san* (for wind-cold syndrome) or *Ying Qiao san* (for wind-heat syndrome). Other placebo-controlled trials have shown a benefit from these two TCMs in treating URTI.[2-4] However, it is difficult to judge the validity of these trials because of methodological flaws such as problems with medication standardisation, randomisation, blinding, and analytic methods. A recently published randomised controlled trial also did not find any difference between *Yin Qiao san* and western medicine in resolving URTI symptoms.[5]

Jing Fan Bai Du san was associated with a significantly greater improvement in general health than placebo in the wind-cold syndrome subgroup. It might be able to improve general well-being although it could not alleviate specific URTI symptoms. An important objective of TCM is to improve the general well-being. This suggests that HRQOL might be an important outcome measure for TCM-treated patients.

Both TCM formulae were well tolerated with no serious adverse event reported by the patients. Although a higher percentage of wind-heat syndrome patients reported adverse effects, the events were mild and no different from those encountered on placebo treatment. Some of these adverse events might have been related to the URTI itself or to the western medicine use.

Several factors could have affected the effectiveness of TCM. The TCM should be initiated at the onset of URTI symptoms, but most patients had already endured symptoms for more than 24 hours. We applied a more stringent criterion for the diagnosis of URTI, compared to trials that showed positive results included all patients with any subjective symptoms. The TCM formulae were given as minimum doses. Further studies with larger dosages should be performed.

Conclusions

No effect on URTI symptom resolution or reduction was demonstrated after treatment with *Jing Fan Bai Du san* or *Ying Qiao san*. Nonetheless, in patients with wind-cold syndrome. *Jing Fan Bai Du san* might be able to improve general health more than placebo. Both TCM formulae were well tolerated, and there was no difference in the incidence of side effects compared to placebo.

Randomised double-blind placebo-controlled trials can be used to determine the effectiveness and side effects of TCM treatments. It is the recommended research methodology to obtain objective evidence on the usefulness of TCM treatments.

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Influenza-like illness in residential care homes: a study of the incidence, aetiological agents, natural history, and health resource utilisation

Key Messages

1. Assuming vaccine reduces influenza A infection, it is likely that only a small proportion of influenza-like illness (ILI) can be avoided. Pneumococcal and other viral vaccines should be considered.
2. Influenza-like illness does not equate with influenza. Nursing home–acquired pneumonia might be a better term, and less misleading.
3. Patients with ILI should not automatically be regarded as infectious and quarantined, as many are secondary to Gram-negative infections.
4. Hospital admissions owing to ILI are unlikely to be prevented by outreach health services, surveillance, or influenza vaccination, because frail elderly people with infection have a deteriorating general condition and need oxygen, intravenous drugs, and support from various health professionals. Policies requiring report of death to the police and infection control requirements also encourage hospital admission.

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Introduction

Outbreak of influenza-like illness (ILI) in residential care homes for the elderly (RCHE) is a public health concern. Such illness is a common cause of admission to hospitals or accident and emergency departments and may result in mortality. It is difficult to identify whether the ILI is caused by infection of SARS, H5N1, influenza or respiratory syncytial viruses (RSV). Influenza vaccination is effective in reducing hospital admissions during an epidemic, particularly in institutional settings,[1] and may reduce the death rate by half in residential institutions.[2] Nonetheless, vaccination is effective only if the ILI is caused by influenza virus. In old age homes, respiratory infections are mostly caused by pathogens other than influenza virus.[3] The disease burden of RSV infection is similar to that of non-pandemic influenza A in a population with high prevalence of vaccination (eg in the elderly or high risk adults).[4] This vaccination policy assumes that influenza causes most of ILI and appears to be empirical rather than evidence-based. It consumes considerable resources from the Hong Kong Centre for Health Protection (CHP) and the Hospital Authority (community geriatric outreach teams and visiting medical officers), which are the two health care organisations supporting RCHEs.

Methods

This study was conducted from March 2006 to April 2007. Four RCHEs supported by the Hospital Authority's New Territories East Cluster Community Geriatric Outreach Teams (CGAT) in the Shatin district were studied. Two were subvented—Sage and Caritas—with 204 and 256 residents respectively; two were private—Shui On and Cambridge—with 221 and 91 residents respectively.

Research staff liaised with the nurse of the CGAT or the visiting medical officer responsible for the RCHEs to identify residents with ILI on a daily basis. An ILI is defined as a temperature of ≥37.8ºC measured by a digital aural thermometer, or an acute deterioration in physical or mental ability, plus new onset of respiratory symptoms or an acute worsening of a chronic respiratory condition.

Patients with ILI underwent the following microbiological investigations: (1) a sputum sample for routine bacterial culture (covering *Streptococus pneumoniae, Pseudomonas sp, Haemophilus influenzae, Moraxella catarrhalis* and other likely pathogens) and for mycobacterial culture of tuberculosis (restricted to those with prolonged respiratory symptoms eg coughing for ≥2 weeks, systemic symptoms associated with weight loss or hospital admission); (2) multiplex-nested polymerase chain reaction for respiratory pathogens; (3) a paired serum sample for atypical pneumonia serology (including influenza A, influenza B, parainfluenza 1, 2, 3; RSV, adenovirus, mycoplasma, *Legionella*, *Coxiella*, and psittacosis); (4) a urine sample for *Legionella* antigen and Pneumococcal antigen (for hospitalised cases).

The aetiology of ILI was determined based on positive culture from sputum,

blood or nasopharyngeal aspirate, positive urinary antigen for *S pneumoniae* or *Legionella*, positive polymerase chain reaction identification, serology of 4-fold difference or single titre of 80 or above, and positive IgM antibody titres.

Patients with ILI were cared for in the usual way by the visiting medical officer or a doctor from the CGAT. Management included prescription of drugs eg antipyretics, antibiotics, or antiviral drugs (depending on the clinical presentation), as well as attention to hydration and nutrition. The decision on admission to hospital was made by the attending doctor or RCHE staff, who might have difficulty in providing additional care or in monitoring of the patient. For example, physical signs requiring further investigation (such as chest radiography), failure to eat or drink, or the need for oxygen, usually resulted in further care in the hospital setting. Any RCHE residents admitted to hospital were investigated routinely.

Statistical analysis

Approximately 10% of RCHE residents had an ILI episode over a one-year period. Assuming a standard deviation of 4%, a total of 217 ILI episodes would be needed. Late summer and winter were the two peaks for ILI episodes, in addition to low-level background occurrences. Assuming an incidence of 10%, the number of episodes covering the two peaks over a one-year period would be 2x0.1x1000=200. Additional episodes of 80 during the other periods could be expected. Assuming 10% were missed, the remaining episodes that could be studied were expected to be 0.9x280=252.

Results

A total of 259 episodes of ILI occurred in 194 patients; 77.8% had one episode, 4.4% had two, 4.6% had three, 2.6% had four, and one had five episodes. An infectious agent was identified in 61.4% of these ILI episodes (Table). Bacterial infections were the commonest, accounting for 53.3%, while viruses were identified in 46.7%; 16.2% of the patients had 2 or more organisms identified. The top five causes in order of frequency were *S pneumoniae*, RSV, *P aeruginosa*, Parainfluenza virus type 1 and Metapneumovirus. Seasonal variations in the organisms were observed: Metapneumovirus and Parainfluenza virus type 3 from April to June, RSV and *P aeruginosa* from July to September, *Chlamydia sp*, Parainfluenza virus type l and *S pneumoniae* from October to December, and *S pneumoniae*, Influenza virus type A, RSV, and human coronaviruses from January to March. For the latter period, RSV, coronavirus and influenza A infections were equally prevalent. Viral infections showed more seasonal variations than did bacterial infections. Among the bacterial infections, *S pneumoniae* was the commonest during the autumn and winter months, and *P aeruginosa* in the summer months, and *Staphylococcus aureus* including methicillin-resistant *S aureus* (MRSA) were among the five commonest bacterial agents. Atypical organisms (*Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*) were detected using molecular methods. The prevalences of organisms in the four RCHEs were similar.

The mean age of the patients was 85 years, and there were slightly more women than men. Presentation with 'decrease in general condition' in terms of cognition, activities of daily living and/or mobility was noted in 71%. Approximately 90% had received influenza vaccination (half within 6 months and half between 6 and 12 months), indicating good compliance with the policy of 100% vaccination rate in RCHEs. Decline in physical function occurred in about 66% of patients as a result of the illness, while cognitive decline was also observed. There were 87% of the ILI episodes resulting in hospital admission.

No particular clinical features identified any particular organism. Mortality was 9.7%. Factors associated with mortality were withholding of food (odds ratio, 3.37; 95% CI, 1.22-9.27), decrease in body mass index, decrease in activities of daily living score, and infections attributed to MRSA and *Klebsiella*.

Management included intravenous antibiotics (in 87% of patients), oxygen and intravenous fluids (in over 60% of patients), and extensive involvement of allied health staff. Owing to concerns about possible aspiration or that aspiration might have precipitated the ILI episode, food was withheld in 57% of patients, who were more likely to have fever, shortness of breath, decline in general condition, be admitted to hospital, have longer hospital stays, have crepitations on auscultation and consolidation on chest radiographs. They also had a three-fold higher mortality. The difference between the three age groups (<80, 80-89, and ≥90) was insignificant, except that older age groups had more female, and had lower BMIs and MMSE scores.

No significant differences were observed in any of the clinical characteristics of ILIs due to different aetiologies. Thus, it would be difficult to determine the underlying cause of an ILI episode based on clinical features alone.

Discussion

In Hong Kong, only 4% of the ILI were caused by influenza A virus over the study period, but influenza A infection tends to equate with ILI. This is misleading in terms of clinical consequences. A broader term—such as nursing home–acquired pneumonia rather than community-acquired pneumonia—might be more appropriate.

Previous studies on the community-dwelling elderly (aged ≥65 years) showed that RSV and human metapneumovirus were each responsible for as many hospitalised cases of respiratory infection as influenza,[5] and that metapneumovirus was a common and ubiquitous human pathogen in children and the elderly.[6] The RSV has

Table . Attributed underlying aetiology over the study period

Aetiology	2006				2006				2006				2007				Total
	Apr	May	Jun	No. (%)	Jul	Aug	Sep	No. (%)	Oct	Nov	Dec	No. (%)	Jan	Feb	Mar	No. (%)	No. (%)
Bacteria																	
Streptococcus pneumoniae	1	2	2	5 (9.4)	1	3	1	5 (10.9)	4	2	1	7 (13.0)	2	3	9	14 (18.9)	31 (13.7)
Pseudomonas aeruginosa	1	1	-	2 (3.8)	1	4	3	8 (17.4)	-	1	2	3 (5.6)	1	1	1	3 (4.1)	16 (7.0)
Chlamydophila species	-	1	-	1 (1.9)	1	-	-	1 (2.2)	7	2	2	11 (20.4)	1	-	-	1 (1.4)	14 (6.2)
Haemophilus influenzae	3	-	1	4 (7.5)	-	1	1	2 (4.3)	1	2	-	3 (5.6)	2	-	2	4 (5.4)	13 (5.7)
Methicillin-resistant *Staphylococcus aureus*	-	-	2	2 (3.8)	-	1	1	2 (4.3)	-	1	-	1 (1.9)	2	-	2	4 (5.4)	9 (4.0)
Mycoplasma pneumoniae	-	1	1	2 (3.8)	1	-	-	1 (2.2)	1	1	1	3 (5.6)	1	1	-	2 (2.7)	8 (3.5)
Escherichia coli	-	-	1	1 (1.9)	-	1	-	1 (2.2)	1	-	-	1 (1.9)	1	-	2	3 (4.1)	6 (2.6)
Moraxella catarrhalis	1	-	-	1 (1.9)	-	-	-	-	1	-	-	1 (1.9)	1	1	1	3 (4.1)	5 (2.2)
Klebsiella species	-	-	-	-	1	2	-	3 (6.5)	1	-	-	1 (1.9)	1	-	-	1 (1.4)	5 (2.2)
Mycobacterium avium complex	1	-	1	2 (3.8)	-	-	-	-	-	-	-	-	-	1	-	1 (1.4)	3 (1.3)
Serratia species	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	2 (2.7)	2 (0.9)
Acinetobacter species	-	-	-	-	-	-	-	-	1	-	-	1 (1.9)	-	1	-	1 (1.4)	2 (0.9)
Enterobacter species	-	-	1	1 (1.9)	-	-	-	-	-	-	-	-	-	-	1	1 (1.4)	2 (0.9)
Proteus mirabilis	-	-	-	-	1	-	-	1 (2.2)	-	1	-	1 (1.9)	-	-	-	-	2 (0.9)
Mycobacterium tuberculosis complex	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1 (1.4)	1 (0.4)
Morganella morganii	-	-	-	-	-	-	-	-	-	1	-	1 (1.9)	-	-	-	-	1 (0.4)
Coxiella burnetii	1	-	-	1 (1.9)	-	-	-	-	-	-	-	-	-	-	-	-	1 (0.4)
Virus																	
Respiratory syncytial virus	2	1	2	5 (9.4)	4	5	1	10 (21.7)	-	-	-	-	-	-	6	6 (8.1)	21 (9.3)
Parainfluenza virus type 1	-	-	-	-	1	-	-	1 (2.2)	5	2	3	10 (18.5)	-	-	4	4 (5.4)	15 (6.6)
Metapneumovirus	6	7	-	13 (24.5)	-	-	-	-	1	-	-	1 (1.9)	-	1	-	1 (1.4)	15 (6.6)
Parainfluenza virus type 3	2	1	3	6 (11.3)	1	1	-	2 (4.3)	1	-	-	1 (1.9)	-	-	4	4 (5.4)	13 (5.7)
Influenza virus type A	-	-	-	-	4	-	-	4 (8.7)	1	-	-	1 (1.9)	-	-	6	6 (8.1)	11 (4.8)
Human coronavirus 229E/OC43	-	-	1	1 (1.9)	-	-	-	-	1	-	-	1 (1.9)	4	1	1	6 (8.1)	8 (3.5)
Rhinovirus	1	1	-	2 (3.8)	-	2	2	4 (8.7)	-	-	-	-	-	1	-	1 (1.4)	7 (3.1)
Influenza virus type B	-	-	-	-	-	-	-	-	-	1	-	1 (1.9)	1	-	4	5 (6.8)	6 (2.6)
Enterovirus	2	-	-	2 (3.8)	-	-	-	-	-	-	2	2 (3.7)	-	-	-	-	4 (1.8)
Parainfluenza virus type 2	-	-	-	-	1	-	-	1 (2.2)	-	2	-	2 (3.7)	-	-	-	-	3 (1.3)
Parainfluenza virus type 4	1	-	1	2 (3.8)	-	-	-	-	-	1	-	1 (1.9)	-	-	-	-	3 (1.3)
Total No. of organisms	22	15	16	53 (23.3)	17	20	9	46 (20.3)	26	17	11	54 (23.8)	18	12	44	74 (32.6)	227 (100)
No organism	12	12	11	35 (35)	14	4	11	29 (29)	7	6	4	17 (17)	2	3	14	19 (19)	100 (38.6)
1 organism	9	8	14	31 (26.5)	7	9	5	21 (17.9)	9	7	5	21 (17.9)	9	12	23	44 (37.6)	117 (45.2)
2 organisms	2	0	1	3 (13)	1	1	2	4 (17.4)	5	3	0	8 (34.8)	3	0	5	8 (34.8)	23 (8.9)
≥3 organisms	3	2	0	5 (26.3)	2	3	0	5 (26.3)	2	1	2	5 (26.3)	1	0	3	4 (21.1)	19 (7.3)
Total No. of patients	26	22	26	74 (28.6)	24	17	18	59 (22.8)	23	17	11	51 (19.7)	15	15	45	75 (29.0)	259 (100)

been suggested as giving rise to a disease burden similar to that of influenza A; RSV vaccination should therefore be considered.[4]

The high admission rate to hospital, despite on-site medical and nursing support, suggests that deployment of outreach Hospital Authority staff to RCHEs may not have any impact on reducing hospital admissions for ILI episodes. Because a decrease in general condition may result from many conditions, of which infection is only one, hospital admission is preferred. Furthermore, deaths in RCHEs must be reported to the police, and concerns with meeting government infection control policies may also have predisposed to hospital admissions.

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Identification of human cell line model of persistent SARS coronavirus infection and studies of the response to cytokines and chemokines

Key Messages

1. The human intestinal cell line LOVO, human lung epithelial cell lines NCI-H1650 and NCI-H1563, and normal nasopharyngeal epithelial cell lines NP69 and NP460 were permissive to SARS coronavirus (SARS-CoV) infection.
2. These human cell lines could be useful for future in vitro investigations of SARS.
3. The cytokine profiles induced following SARS-CoV infection varied among different cell lines, suggesting that the cellular response to SARS-CoV infection was tissue specific and may also correlate with the degree of permissiveness.
4. The results provide important insights into possible pathogenic mechanisms and potential novel therapeutic targets such as CXCL10/IP-10 in SARS.

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Introduction

Severe acute respiratory syndrome (SARS) is typified by its high infectivity, morbidity and mortality caused by a new SARS coronavirus (SARS-CoV).[1] The pathogenesis of SARS remains unclear, and therefore the current choices for specific, effective treatments remains limited.

Clinically, SARS gave rise to a predominantly lower respiratory tract illness. Diarrhoea and other organ dysfunction were also common. Based on our virological, ultrastructural, and in situ hybridisation (ISH) studies in human tissues, we identified persistent SARS-CoV infection in human lung pneumocytes and intestinal enterocytes, but not in other organs or cell types,[2] indicating both lung and intestinal epithelial cells in the pathogenesis of SARS. For practical reasons, animal studies were not feasible in most laboratories and had limitations and may not be relevant to the human situation. Identification of relevant human cell line models of SARS-CoV infection greatly facilitates further laboratory investigations into the virology of SARS-CoV, pathogenesis and various therapeutic approaches. We identified persistent SARS-CoV infection in human lung pneumocytes and intestinal enterocytes, and a human intestine cell line that can maintain persistent SARS-CoV infection. It was not clear whether other cell types, in particular in the human upper respiratory tract, could be infected by SARS-CoV. We investigated the susceptibility of human lung, intestinal, and nasopharyngeal epithelial cell lines to SARS-CoV infection, in order to identify in vitro experimental models for SARS studies.

Altered inflammatory or immune responses have been implicated in the pathogenesis of SARS and in its various manifestations. Thus, the cellular expression profiles of cytokines/chemokines induced by SARS-CoV were investigated in the selected permissive cell line models. These findings may enhance our understanding of the pathogenic mechanisms of this new human infectious disease and provide insights into the identification of potential therapeutic targets. We aimed to (1) identify human lung and intestinal epithelial cell lines that can maintain persistent SARS-CoV infection, and (2) study the expression profiles of cytokines and chemokines in cell lines infected with SARS-CoV.

Methods

This study was conducted from January 2005 to January 2006.

The study samples were human cell lines. The tested cell lines included: (1) lung epithelial cells (including lines derived from normal epithelial cells and lung adenocarcinoma): HBE4-E6/E7, NCI-H23, NCI-H522, NCI-H1563, NCI-H1650, NCI-H526, NCI-H520, NCI-H292; (2) intestine cell lines (including lines derived from intestinal adenocarcinoma): LS-180, SW-480, SW-620, HT-29, DLD-1, LOVO, HCT-116; (3) nasopharyngeal epithelial cell lines (including immortalised normal nasopharyngeal epithelial cell lines and nasopharyngeal carcinoma cell lines): C666-1, HONE1, HK1, NP69, NP460. NP69 and NP460

were kindly provided by Dr SW Tsao, Department of Anatomy, University of Hong Kong.

Viral preparation

The CUHK-W1 strain of SARS-CoV (GenBank accession no AY278554) was grown in Vero cells and the third passage at a concentration of $5x10^6$ 50% tissue culture infective dosed ($TCID_{50}$)/mL was kept at -70°C for experiments.

Inoculation with SARS-CoV

Cell lines were maintained in recommended media and incubated in a 37ºC, humidified, 5% CO_2 incubator. Cells at 60 to 70% confluence in 25-cm^2 flasks were inoculated with 300 μL of virus suspension to provide a multiplicity of infection (MOI) of 10. Inoculated cells were maintained at 37ºC for 7 days. A mock infection was performed in parallel for each cell line. The cells were examined daily for cytopathic effects (CPE). Cells were harvested after 7 days of incubation for virus detection. All laboratory investigations related to viral culture were performed in a class-III viral laboratory.

Indirect immunofluorescence assay

Intracellular viral antigens were detected by standard indirect immunofluorescence staining, using hybridoma fusion mouse-anti-SARS antibody as described previously.[3]

In situ hybridisation

In situ hybridisation was developed for the detection of SARS-CoV as described previously.[2]

Electron microscopy

Cell pellets harvested after 7 days of SARS-CoV incubation were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), and processed for examination by transmission electron microscopy.

Quantitative real-time detection of SARS-CoV by reverse transcriptase–polymerase chain reaction

Cells at 60 to 70% confluence were inoculated with SARS-CoV at 10, 1 and 0.1 MOI, respectively. The concentration of virus in cell culture supernatant was monitored by quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) as described previously.[3]

Expression profile of cytokines and chemokines in permissive cell lines

The expression profile of the cytokines, chemokines and their receptors was investigated in selected cell lines confirmed to be SARS-CoV permissive. Cells were harvested at different time points (0, 6 and 24 hours after SARS-CoV inoculation) and total RNA was prepared using Qiagen RNeasy RNA isolation kit (QIAGEN). cDNA synthesis was carried out using Superscript III reverse transcriptase (Invitrogen). For quantitative real-time RT-PCR analysis, either the Taqman PCR Master Mix or the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) was used. The reaction was performed using specific primers for CXCL13/BCA-1, CCL2/MCP-1, CCR2, CXCL14/BRAK, CXCR5, IFN-γ, IL-10, IL-12α, IL-12b, IL-18, IL-α, IL-1b, IL-6, CXCL8/IL-8, IL-8Rα, IL-8Rβ, CXCL10/IP-10, CXCL9/MIG, CXCL12/SDF-1, TGF-β1, TGF-β2, TNFα, CCL5/RANTES, CCL20/MIP-3α, CXCR4 and run on an ABI 7900 Sequence Detection System. All reactions were performed in triplicate. Relative levels of expression were normalised, using GAPDH as an internal reference control, and calculated using the 2[-ΔΔC(T)] method for comparing the mRNA amount of each sample to that of time zero.

Results

Susceptibility of human intestinal, lung and nasopharyngeal epithelial cell lines to SARS-CoV infection

The susceptibility of the tested cell lines is summarised in Table 1. Of the seven human intestinal cell lines tested (LS-180, SW-480, SW-620, HT-29, DLD-1, LOVO, and HCT-116), only LOVO showed permissiveness to SARS-CoV infection. None of the infected intestinal cell lines showed CPE during the 7 days of incubation. Indirect immunofluorescence staining indicated the presence of viral antigens in the cytoplasm of 75% of infected LOVO cells. In situ hybridisation also revealed the presence of SARS-CoV RNA in LOVO cells, whereas all other cell lines were virus-free after incubation (Fig). Under electron microscopy, the infected LOVO cells contained cytoplasmic vesicles, consistent with dilated endoplasmic reticulum and Golgi apparatus, which were packed with numerous spherical viral cores at different stages of maturation; mature virus particles sizes ranged from 80 to 120 nm. In situ hybridisation, indirect immunofluorescence, and ultrastructural studies confirmed the permissiveness of LOVO cells to SARS-CoV.

Out of eight cell lines from human lung tissue (HBE4-

Table 1. Susceptibility of cell lines to SARS-CoV inoculation

Cell line	Organ	Cytopathic effects (day 7)	% of infected cells
Vero	Kidney	++	70
NCI-H1563	Lung	None	50
NCI-H1650	Lung	None	80
NCI-H23	Lung	None	5
NCI-H292	Lung	None	5
NCI-H520	Lung	None	5
NCI-H522	Lung	None	5
NCI-H526	Lung	None	5
HBE4-E6/E7	Lung	None	5
C666-1	Nasopharynx	None	5
Hone-1	Nasopharynx	None	5
NP69	Nasopharynx	+++	60
NP460	Nasopharynx	++	5
HK-1	Nasopharynx	None	0
SW620	Colon	None	5
SW480	Colon	None	15
LOVO	Colon	None	75
HCT-116	Colon	None	0
DLD-1	Colon	None	0
LS180	Colon	None	5
HT-29	Colon	None	0

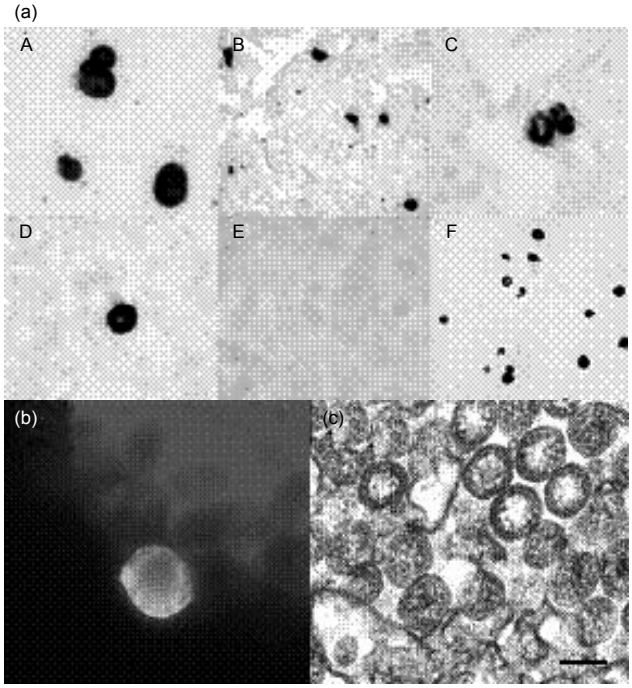

Fig. (a) In situ hybridisation analysis of cells inoculated with SARS-CoV. Presence of SARS-CoV RNA is shown by dark spots. A: NCI-H1650, B: LOVO, C: NP69, D: NP460, E: negative control, F: Vero (positive control). (b) Indirect immunofluorescence staining: SARS-CoV infected cells (LOVO) indicating presence of SARS-CoV antigens is shown as a light spot. (c) Viral particles in dilated vesicles are seen in ultra structural examination (LOVO cell). The cross bar represents 100 nm

E6/E7, NCI-H23, NCI-H522, NCI-H1563, NCI-H1650, NCI-H526, NCI-H520, NCI-H292), none showed CPE after 7 days of SARS-CoV infection at a MOI of 10. Indirect immunofluorescence staining at day 7 indicated the presence of viral antigens in over 80% of SARS-CoV–infected NCI-H1650 cells and 50% of NCI-H1563 cells. Viral RNA was also detected by ISH in infected NCI-H1650 cells (Fig). The other six cell lines were negative for SARS-CoV by immunofluorescence and ISH.

Inoculation of SARS-CoV at a MOI of 10 produced CPE in two immortalised normal nasopharyngeal epithelial cell lines (NP69 and NP460) at day 7. Whereas no CPE was observed in all three nasopharyngeal carcinoma cell lines: C666-1, HK-1 and HONE-1. After 7 days of inoculation, indirect immunofluorescence staining was performed to confirmed viral replication in NP69 and NP460 cells. Greater than 60% of NP69 and around 5% NP460 cells showed positive immunofluorescence for SARS-CoV antigen. In situ hybridisation demonstrated that SARS-CoV infected NP69 and NP460 cells contained detectable viral RNA (Fig).

Expression profiles of selected cytokines, chemokines, and their receptors in SARS-CoV infected by LOVO, NCI-H1650, NP69, and NP460 cells

Four cell lines demonstrated permissive infection of SARS-CoV, namely LOVO, NCI-H1650, NP69 and NP460 cells. They were selected for expression profiling of selected cytokines, chemokines, and their receptors. The selected significant findings were summarised in Table 2.

α-Chemokine CXCL10/IP-10 and CXCL9/MIG are potent chemoattractants for activating T and NK cells. Up-regulation of IP-10 and MIG mRNA expression was detected in all four cell lines. The up-regulation was detected at 6 hours after SARS-CoV infection, and remained at a high level at 24 hours. Notably, in cell lines more susceptible to SARS-CoV infection, higher expression of CXCL10/IP-10 and CXCL9/MIG was detected. The expression level of CXCL10/IP-10 and CXCL9/MIG at 24-hour post-inoculation correlated with the proportion of infected cells determined by indirect immunofluorescence assay (CXCL10/IP-10, P=0.02, CXCL9/MIG, P=0.001). The ubiquitous up-regulation of IP-10 and MIG after SARS-CoV inoculation independent of cell types suggested that it might be a general response to viral infection in susceptible cells.

In NCI-H1650 cells, the CXCL8/IL-8 and CCL20/MIP-3α level were 70-fold and 112-fold higher, respectively, at 6-hour post-inoculation, and remained at a higher level (13-fold and 9-fold, respectively) at 24-hour post-inoculation. A transient surge of CXCL8/IL-8 and CCL20/MIP-3α was also observed 6 hours after SARS-CoV inoculation

Table 2. Cytokines/chemokines gene expression levels in SARS-CoV–infected human epithelial cells by quantitative reverse transcriptase–polymerase chain reaction

	NCI-H1650		LOVO		NP460		NP69	
	6 hours	24 hours	6 hours	24 hours	6 hours	24 hours	6 hours	24 hours
CXCL10/IP-10	82.28 (6.26)	156.74 (11.42)	2.77 (0.78)	7.07 (4.67)	4.69 (2.21)	5.37 (2.69)	2.29 (0.71)	1.88 (0.30)
CXCL9/MIG	2.85 (0.29)	9.11 (0.39)	4.03 (1.37)	3.47 (0.84)	11.31 (3.51)	2.31 (0.32)	2.08 (1.70)	2.06 (0.46)
CXCL8/IL-8	70.2 (2.24)	13.49 (0.45)	1.36 (0.52)	1.6 (0.32)	3.44 (0.99)	0.24 (0.16)	4.37 (3.13)	0.97 (0.67)
CCL20/MIP-3α	112.61 (6.28)	9.21 (0.18)	0.3 (0.04)	2.21 (0.49)	3.11 (0.11)	0.64 (0.05)	2.58 (0.32)	0.66 (0.03)
IL-6	14.29 (0.65)	5.65 (0.61)	0.73 (0.031)	14.07 (15.09)	0.6 (0.05)	0.16 (0.05)	0.56 (0.09)	0.66 (0.06)
TNF-α	30.89 (5.02)	13.69 (3.86)	2.39 (0.47)	23.2 (17.50)	0.03 (0.01)	0.16 (0.06)	0.12 (0.04)	0.05 (0.01)
IL-18	385.67 (260.51)	2088.44 (1302.49)	38 (14.03)	0.69 (0.24)	0.01 (0.01)	0.67 (0.57)	3.18 (1.89)	4.79 (1.81)
IFN-γ	0.81 (1.06)	11.51 (6.26)	1.92 (0.77)	29.94 (37.69)	0.01 (0.01)	0.19 (0.04)	0.03 (0.01)	0.01 (0.01)
IL-10	0.5 (0.07)	4.79 (0.45)	0.64 (0.17)	7.75 (10.32)	0.02 (0.004)	0.14 (0.02)	0.11 (0.01)	0.04 (0.005)
IL-12β	0.73 (0.11)	2.4 (0.76)	0.74 (0.19)	16.84 (12.19)	0.05 (0.01)	0.18 (0.02)	0.11 (0.01)	0.13 (0.03)

in NP69 and NP460 cells, and then decreased by 24-hour post-inoculation. In LOVO cells, infection with SRAS-CoV slightly induced the expression of CXCL8/IL-8 and CCL20/MIP-3α at 24-hour post-inoculation, while no significant effect above background level was detected in these genes at the 6-hour time point. The expression level of CXCL8/IL-8 and CCL20/MIP-3α in NCI-H1650, NP69, NP460 and LOVO also correlated with the proportion of infected cells, indicated by immunofluorescence of viral antigen (P<0001). Significant up-regulation of CXCL8/IL8 receptors IL8Rα and IL8Rβ was observed at 24-hour post-inoculation in NCI-H1650 and LOVO cells, but not in NP69 and NP460.

In NCI-H1650 and LOVO cells, proinflammatory cytokines IL-6, TNF-α, IL-18 were strongly induced by SARS-CoV at 6-hour post-inoculation. The early induction of IL-6, TNF-α, and IL-18 was seen at 6-hour post-inoculation in NCI-H1650 cells and sustained a high expression level till 24-hour post-inoculation. Induction of TNF-α was detected at 6 hours and continued to increase in LOVO cells, reaching a much higher level (23-fold) at 24 hours. On the contrary, expression of IL-18 peaked at 6 hours (38-fold) and decreased to background levels at 24 hours in LOVO cells. Marked elevation of IFN-γ and to a lesser extends IL-10, and IL-12-β expression in these cell lines was seen at 24-hour post-inoculation.

Discussion

Confirming our earlier observations, intestinal cell line LOVO can maintain a permissive infection of SARS-CoV. Four more cell lines were also found to be permissive to SARS-CoV, including human lung adenocarcinoma cell line NCI-H1650, NCI-H1563, immortalised normal nasopharyngeal epithelial cell lines NP69 and NP460. In American green monkey kidney cells Vero, typical CPE was observed several hours post-inoculation. However CPE was not observed in LOVO, NCI-H1650 and NCI-H1563 cells after 7 days inoculation of SARS-CoV. In contrast, CPE was observed in SARS-CoV–inoculated nasopharyngeal epithelial cells NP69 and NP460. The basis of the differences in cellular response is unclear. Although SARS-CoV was detected in the lungs and intestine specimens from SARS patients, severe cellular damage was characteristically observed in the lungs, while no morphological change was detected in the intestine. The discrepancies in clinical manifestations and in vitro experiments highlight the importance of host cell responses in SARS infection. The mechanisms underlying SARS-CoV–mediated pathogenesis remain largely unexplained. Our findings of persistent infection in human intestinal, lung, and nasopharyngeal epithelial cells provide useful in vitro models for SARS-CoV–related studies.

In NCI-H1650 and LOVO cells, SARS infection significantly induced the expression of α-chemokines CXCL10/IP-10 and CXCL9/MIG, cytokines IL-6, IL-10, TNF-α, IL-18, IFN-γ, IL-10, and IL-12-β. Infection with SARS-CoV did not induce the expression of α-chemokines CXCL13/BCA-1, CXCL14/BRAK, receptor CXCR5, cytokines TGF-β1, TGF-β2, IL-1α, IL-1β and IL-12α in NCI-H1650 and LOVO cells. Nonetheless, cell type–dependent and/or time-dependent differences between NCI-H1650 and LOVO cells were noted. CCL20/MIP-3α and CXCL8/IL-8 markedly elevated in NCI-H1650 but not in LOVO.

NP69 and NP460 cells were less inducible in comparison to NCI-H1650 and LOVO. Induction of chemokines and inhibition of proinflammatory and anti-viral cytokines has been reported in different cell types after in vitro exposure to SARS-CoV. CCL2/MCP-1 and CXCL8/IL-8 were induced in lung adenocarcinoma cell line A549. SARS-CoV strongly induced IP-10 and IL-18 but suppressed anti-viral system such as IFN-α, IFN-β CCL5/RANTES, and IL-6 in colon carcinoma cell line Caco-2. CXCL10/IP-10 and CCL2/MCP-1 but not IFN-β was induced in macrophage. In monocytes-derived human dendritic cells, SARS-CoV induced CCL3/MIP1α, CCL5/RANTES, CXCL10/IP-10 and CCL2/MCP-1. Unlike the usual response of dendritic cells to viral infection, anti-viral cytokines such as IFN-α, IFN-β, IFN-γ and IL-12β, proinflammatory cytokines TNF-α and IL-6 were not activated. Significant up-regulation of chemokines in our cell line models was in concordance with previous reports. Proinflammatory and antiviral cytokines IFN-γ, TNF-α and IL-6 were also markedly up-regulated in our highly permissive models LOVO and NCI-H1650. These two cell lines are the only in vitro models in which successful induction of proinflammatory cytokines could be demonstrated. The cellular mechanisms underlying the differential proinflammatory cytokine induction remain poorly understood. The difference in cell type and degree of permissiveness to SARS-CoV infection might play a role.

After SARS-CoV infection, the circulating concentrations of most of the cytokines showed only transient and short-lived activation in patients. In contrast, circulating CXCL9/MIG, CXCL10/IP-10 and CCL2/MCP-1 were markedly increased in SARS patients. We have previously demonstrated that high levels CXCL10/IP-10 mRNA were present in autopsy lung specimens from SARS victims and that serum level of CXCL10/IP was an independent indicator of poor prognosis.[4] In the lungs of SARS patients, CXCL10/IP-10 was expressed in the pneumocytes, CD3+ T lymphocytes and monocytes/macrophages and was a potent chemoattractant for activated T cells, natural killer cells, leading to immune and inflammatory reactions. In our lung cell line model NCI-H1650, marked elevation of CXCL10/IP-10 (82-fold) was detected at 6-hour post-inoculation and reached 156-fold after 24 hours, whereas up-regulation of IFN-γ was not detected until 24-hour post-inoculation. The initial induction of CXCL10/IP-10 occurred without the presence of IFN-γ, suggesting that SARS-CoV induced expression of CXCL10/IP-10 ensues in an IFN-γ-independent

manner. These in vivo data were consistent to our in vitro observations. These chemokines appear to be important elements in the pathogenesis of SARS. CXCL10/IP-10 has been used as a therapeutic target in a viral model of multiple sclerosis and also in autoimmune and inflammatory renal disease.[5] Thus, our findings provide information for rational therapeutic approaches, such as targeting CXCL10/IP-10 for anti-SARS treatment.

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Cost-effectiveness of influenza vaccination for elderly people living in the community

Key Messages

1. Influenza vaccination of elderly people living in the community was cost-effective from a societal perspective but did not cut publicly funded medical costs or total medical costs.
2. For the oldest group (≥75 years) living in the community, influenza vaccination can cut publicly funded medical costs if the total vaccination cost per head is HK$39.6 or less.
3. Influenza vaccination is cost-effective if the value of increasing an elderly person's lifespan for a year at most is measured as HK$68 047 or more.

Introduction

Influenza is one of the leading causes of respiratory infection. It is a highly contagious viral infection that affects 10 to 20% of the population every year.[1] Influenza generates both medical expenses, from resource use, and societal costs, due to lost productivity and mortality.

Older people are particularly vulnerable to influenza infection and its complications can result in serious illness and death. Some countries recommend an annual influenza vaccination for older and high-risk groups because it is the most effective means of preventing influenza and reducing the impact of epidemics.[2]

Since 1998, the Department of Health in Hong Kong has been providing free annual influenza vaccination to older people living in residential care homes.[3] In addition, mainly since the outbreak of severe acute respiratory syndrome (SARS) in 2003, the Department of Health and non-governmental organisations have also provided free or low-cost annual influenza vaccinations to older people.

Studies from other countries have shown that influenza vaccination in older people is cost-effective and can be cost saving for the health care provider.[4] These studies are not directly applicable to Hong Kong with its different influenza seasonal pattern, health care cost structure, and relatively healthy older people.[5] The only study on the cost-effectiveness of influenza vaccination in Hong Kong published to date concluded that influenza vaccination was not cost-effective in any age-group.[6] That study was based on a limited survey that found no additional risk of hospitalisation from the complications of influenza in older people and no influenza-related mortality in older people in Hong Kong. Subsequent analyses of influenza-related hospitalisation and mortality suggest that neither of these assumptions is appropriate.[7] Thus, there is a need to conduct a local evaluation of the cost-effectiveness of influenza vaccination among older people in the Hong Kong community, taking into account the latest information on the impact of influenza in Hong Kong.

Aim

We evaluated the cost-effectiveness of influenza vaccination in Hong Kong for people aged 65 years and over living in the community. We also considered the following subsidiary questions:

1. Would a publicly funded influenza vaccination programme be cost saving?
2. What is the maximum cost per head of influenza vaccination at which a publicly funded influenza vaccination programme in older people would become cost saving?
3. What is the value of a life at which a publicly funded influenza vaccination programme in older people would break even?
4. Are there differences in cost-effectiveness or cost saving of medical costs in those aged 65 to 74 years and those 75 years and over?

Methods

This study was conducted from August 2004 to July 2005. We evaluated the

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Table 1. Vaccination cost, uptake, and effectiveness

	Age-group (years)	
	65-74	≥75
Vaccination cost		
Influenza vaccination	HK$25	
Staff costs	HK$25	
Administration	HK$6.5	
Refund system for opportunistic programme only	HK$6.5	
Lost productivity	HK$3.6	N/A
Travel cost for comprehensive programme only	HK$5.6	HK$4.6
Vaccine uptake		
Comprehensive programme	90%	
Opportunistic programme	62%	76%
Vaccine effectiveness in preventing		
Self-care cases	5%	
Doctor consultations	5%	
Hospitalisations*	22-27%	
Mortality†	47%	

* 22% for respiratory diseases, 24% for cardiac diseases, and 27% for pneumonia and influenza

† 47% for all-cause mortality

Table 2. Annual mortality and morbidity associated with influenza in older people and unit costs

	Age-group (years)	
	65-74	≥75
Deaths		
Value of life	HK$10 million	
Annual number due to 'flu'	272	628
Hospital use		
Cost per bed-day	HK$3132	
Annual admissions due to 'flu'	759	1321
Length of stay*	Various	Various
Doctor visits		
Unit cost (public)	HK$251	
Unit cost (private)	HK$164	
Travel cost	HK$5.6	HK$4.6
No. of visits due to 'flu'	14 340	6464
Self-care		
Western medicine cost	HK$134	HK$61
Chinese medicine cost	HK$79	HK$41
No. using self-care for 'flu'	34 901	19 656
Lost productivity		
Value of work-time lost per worker	HK$912	N/A

* The length of stay varies depending on the type of disease

cost-effectiveness of an influenza vaccination programme by comparing the dollar value of the benefits obtained with the cost of a vaccination programme over 1 year, as influenza vaccinations are repeated annually. We considered (1) a comprehensive vaccination programme, where vaccination was centrally provided in the autumn, and (2) an opportunistic programme, where older people visiting a doctor for a reason other than cold, 'flu', or fever between October and December were offered an influenza vaccination. These programmes have different costs and uptakes.

Given concerns over avian influenza and SARS we assumed a high uptake rate of 90% for a centrally provided vaccination, but travel costs for the older people concerned. An opportunistic programme generates no additional travel costs for the older people vaccinated, but there are additional costs involved in refunding the private general practitioners who administered the vaccine on an opportunistic basis to 90% of those who made an appropriate doctor visit in the relevant months.

The effectiveness of influenza vaccination as a means of preventing influenza was estimated from the most recent meta-analysis.[8] Table 1 summarises the information used to cost an influenza vaccination programme and to estimate its benefits.

Excess mortality, admissions to hospital, visits to a general practitioner, and number of cases where self-care was used for influenza-related illness were estimated from published studies and local survey data.[7] Unit costs were obtained from routine data and local surveys. Table 2 summarises the information used to cost influenza-related illness and shows the number of deaths, hospitalisations, doctor consultations, and self-care episodes estimated to be associated with influenza annually. The risk of influenza-related mortality or hospitalisation is higher in the older age-group.

We took three perspectives on costs and benefits (1) societal (2) personal or individual and (3) government or publicly funded health care. Societal costs and benefits included mortality, lost productivity, public and private use of medical resources, and personal expenses, such as travel costs.

We carried out a probabilistic sensitivity analysis to identify the maximum vaccination cost at which influenza vaccination would be cost saving. We carried out our analysis for all older people in Hong Kong who live in the community, separated into two age-groups. Of the 729 200 elderly people in Hong Kong, we estimated that 447 190 aged 65 to 74 years and 237 222 aged 75 years and above lived in the community.[9] Costs relate to the year 2000 and are in Hong Kong dollars (HK$).

Results

Cost of influenza

We estimated the total annual cost of influenza in older people living in the community as HK$9068 million, of which HK$60.7 million is publicly funded medical costs (Table 3). This is equivalent to HK$13 250 per head, and HK$88.6 per head in public health care costs.

Cost of vaccination

In a comprehensive programme reaching 90% of older people in the community, the total vaccination cost would be HK$40 million (Table 4). This is equivalent to a cost per person vaccinated of HK$64, including HK$57 in health care costs, HK$2 in lost productivity and HK$5 in personal

Table 3. Cost of influenza in older people living in the community

	Perspective			Total
	Personal	Public	Other societal	Societal
Deaths (million)			HK$9000	HK$9000
Hospitalisation (million)	HK$0.1	HK$60.3	HK$0.2	HK$60.7
Out-patient (million)	HK$3.3	HK$0.3	HK$1.2	HK$4.9
Self-care (million)	HK$1.7		HK$1.4	HK$3.1
Total (million)	HK$5.1	HK$60.7	HK$9002	HK$9068
Cost per head	HK$7.5	HK$88.6	HK$13 154.1	HK$13 250

Table 4. Monetary benefits and costs of influenza vaccination for all people aged 65 years and over living in the community: from personal, governmental, and societal perspectives

Perspective (HK$)	Comprehensive programme (90% uptake)				Opportunistic programme (62% and 76% uptake, respectively of those aged 65-74 and ≥75 years)			
	Personal	Government	Indirect costs	Societal	Personal	Government	Indirect costs	Societal
Benefits								
Deaths				3 810 000 000				3 030 000 000
Hospitalisations	32 358	13 129 999	43 025	13 205 382	25 486	10 360 305	29 640	10 415 431
Out-patient	149 030	14 471	55 798	219 299	109 865	10 625	38 438	158 928
Self-care	75 437	-	62 470	137 907	53 706		43 035	96 741
Total benefits	256 825	13 144 470	161 293	3 823 562 588	189 057	10 370 930	111 113	3 040 671 100
Benefits per person vaccinated	0.4	21.3	0.3	6207.4	0.4	22.7	0.2	6645.6
Costs	3 235 938	34 802 371	1 450 080	39 488 389		28 825 450		28 825 450
Cost per person vaccinated	5.3	56.5	2.4	64.1		63.0		63.0
Net benefits	-2 979 113	-21 657 901		3 784 074 199	189 057	-18 454 520		3 011 845 650
Per person net benefits	-4.9	-35.2		6143.3	0.4	-40.3		6582.6

costs. In an opportunistic programme in which 62% of those aged 65 to 74 years and 76% of those aged 75 years and over are vaccinated, the total vaccination cost would be HK$29 million or HK$63 per person vaccinated and this involves health care costs only.

Monetary benefits from influenza vaccination

A comprehensive vaccination programme would yield benefits of HK$3824 million, or HK$6207 per person vaccinated (Table 4). Most of this is from avoided mortality. The savings in public health care costs are HK$13 million or HK$21 per person vaccinated. An opportunistic programme yields savings in public health care of HK$10 million or HK$23 per person vaccinated.

Cost-effectiveness of influenza vaccination

The net benefits for both types of programme are negative from a governmental perspective but positive from a societal perspective. Vaccination would, on average, cut medical costs (from the government's perspective) in the 75 years and over age-group if the vaccination cost per person was HK$40 or less. For the 65 to 74 years age-group, vaccination would not reduce public health care costs even if the vaccination cost per person were as low as HK$15.

Breakeven value of life

For vaccination to be cost-effective, the value placed on saving a life would have to be at least HK$16 947 for those aged 75 years and older and at least HK$186 243 for those aged 65 to 74 years. If we put the age-groups together, the value of saving the life of anyone aged 65 years or older living in the community would have to be at least HK$68 047.

Discussion

Vaccinating people living in the community against influenza is cost-effective from a societal perspective. Most of the cost reductions from a vaccination programme come from mortality reductions. Vaccination does not save health care costs at the estimated cost of vaccination used in the analysis. These findings are consistent with some overseas studies.[10] The results are not surprising because hospitalisation rates for influenza-related diseases in older people in Hong Kong appear to be lower than those observed elsewhere, making potential benefits from vaccination also lower. Local people also have lower mortality rates from cardiovascular disease and lower lifetime rates of smoking, which could contribute to them being less vulnerable to the cardiorespiratory complications of influenza.[11]

We used a value of life of HK$10 million in this study. The analysis shows us that vaccination is still cost-effective from a societal perspective at values of life much lower than this.

Conclusion

Vaccinating elderly people living in the community against influenza was found to be cost-effective from a societal perspective but not from a governmental perspective at the values of costs and benefits used in this analysis. Nonetheless, influenza vaccination in older people (≥75

years) in the community would be cost saving from the governmental (public health care) perspective if the cost of vaccinating each person, including the cost of the vaccine and its administration, was HK$39.6 or less. Furthermore, vaccination would be cost-effective from a societal perspective if the value of a life of a person aged over 65 years were put at HK$68 047 or more.

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