Effect of Rorrico, extracted from group of Chinese medicines, on influenza A and H1N1 infections

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**Abstract**

**OBJECTIVE:** To investigate the therapeutic and preventive effects of Rorrico on influenza, especially influenza A viral infection, including swine flu (H1N1) in humans.

**METHODS:** Eighty-nine subjects were recruited in Hong Kong and Macau, and divided into treatment group (TG) and prevention group (PG) based on their influenza A and swine flu symptoms. All subjects were prescribed Rorrico or placebo, and monitored by a Chinese medicine practitioner. Blood samples were collected before and after 7-day Rorrico or placebo treatment for laboratory investigations.

**RESULTS:** After treatment, there were some full recoveries and obvious relief of onset symptoms in the TG. Blood test results showed that Rorrico produced (a) no adverse effects on subjects' renal and liver functions, muscle enzyme and hematological status, (b) no up-regulation of pro-inflammatory cytokines tumour necrosis factor-a and interleukin-18 in both TG and PG, (c) mild yet statistically significant elevation of plasma mannose-binding lectin (MBL) in PG.

**CONCLUSION:** Rorrico has no up-regulating effect on the participants’ immune response, or, equally likely, the immuno-modulatory effects of Rorrico do not non-specifically or unnecessarily promote inflammation when not required. It is possible that oral administration of Rorrico can promote hepatic synthesis of MBL in healthy PG subjects, thereby conferring increased protection against infection.

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**Key words:** Rorrico; Influenza A Virus, H1N1 Sub-type; Tumor necrosis factor-alpha; Interleukin-18

**INTRODUCTION**

Influenza (flu) is an acute illness of the respiratory tract caused by influenza viruses. Influenza A viruses belong to the orthomyxoviridae family, members of which are classified into 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) according to their antigenicity of hemagglutinin (HA) and neuraminidase (NA) molecules.1 Influenza viruses can cause recurrent epidemics and pandemics globally. The Spanish flu pandemic caused by H1N1 in 1918 killed as many as 50 million people worldwide.1 The outbreak of highly pathogenic avian H5N1 virus in 2003 spread from Hong Kong to Europe and Africa resulting in 261 deaths.1 An outbreak of swine-origin H1N1 virus started in Mexico and United States in 2009 and emerged to cause illness
in humans globally. It resulted in a pandemic in June 2009. According to the US Center for Disease Control (CDC), influenza-positive tests have been reported from all 50 states, the District of Columbia, and Puerto Rico since September 2013 and 97% were influenza A viruses. The 2009 H1N1 virus has predominated during the 2013-14 season. Two FDA-approved antiviral drugs, oseltamivir (Tamiflu®) and zanamivir (Relenza®), have been recommended for treating influenza A infection. Clinical and observational data have shown that early antiviral treatment can shorten the duration of fever and illness symptoms, lessen the risk of complications from influenza, and reduce the duration of hospitalization. However, these drugs can cause serious side effects and must be prescribed by doctors.

In terms of theory of Traditional Chinese Medicine (TCM), cold, or catching cold, is caused by exogenous factors, namely evil wind and other pathogenic factors invading the body. It is manifested as blocked or running nose, sneeze, cough, headache, aversion to cold, fever, general discomfort, superficial pulse, etc. Cold that presents with symptoms of more serious nature, and that transmits more widely and rapidly, is known as influenza. Drug therapy focuses on heat elimination and detoxification and is efficient to treat the disease of cold. Rorrico, an extract prepared with a TCM prescription comprising 21 Chinese medicines, has been formulated for treating influenza including human H1N1 swine flu. The antiviral efficacy of Rorrico on influenza A/H1N1 virus in vitro has been studied and reported in 2009 that it inhibited viral replication. The objective of this study was to investigate the therapeutic and preventive effects of Rorrico on influenza, especially influenza A viral infection, including swine flu (H1N1) in humans.

MATERIALS AND METHODS

Subjects

This study was conducted from March 2010 to November 2011. It was approved by the Clinical Research Ethics Committee of the Macau University of Science and Technology, Macau. Eighty nine subjects were recruited in Hong Kong and Macau, of whom 28 with flu symptoms (treatment group, TG) were prescribed Rorrico upon recruitment. Sixty one subjects without flu symptoms (prevention group, PG) were further divided randomly into test group (n = 31) and placebo group (n = 30) on a double-blinded and placebo-controlled allocation.

The recruitment criteria were as follows: TG subjects: subjects with at least two of the following symptoms of flu infection: (a) fever; (b) running nose; (c) sore throat; (d) cough; (e) nausea, vomiting/diarrhea. PG subjects: subjects easily infected by flu viruses (more than 4-5 times per year), but were asymptomatic at the time of recruitment. Patients having the following conditions were excluded: (a) serious cardiac, respiratory, renal and hepatic diseases, and diabetic mellitus; (b) HIV infection (by history only, laboratory confirmation not required); (c) drug abuse; (d) had received any Chinese medicine or western medicine treatment within one month prior to the start of the study; (e) had vaccination for flu in the last 9 months; (f) pregnancy; (g) psychiatric disease.

All subjects were interviewed by a registered Chinese medicine practitioner (CMP) before and after completion of the Rorrico or placebo treatment. They signed a written informed consent form prior to enrolment into the study.

A flowchart summarizing the study process is shown in Figure 1.

Rorrico

Rorrico is a patented proprietary product in China (Patent number: CN101584836A). It was prepared with 21 Chinese medicines.

In a Chinese medicinal preparation, various drugs are combined to form a balanced formulation using the four fundamental classes of drugs: Emperor drugs are the main therapeutic ingredient producing the leading therapeutic effects in treating the cause of the main symptoms of a disease/syndrome. In

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Figure 1 Study flow chart

Recruited subjects were divided into treatment group (TG) and prevention group (PG) according to the inclusion and exclusion criteria of the study

28 subjects were allocated into TG and received Rorrico treatment

61 subjects were allocated into PG and they were randomised

26 subjects completed 7 days treatment and 2 subjects discontinued intervention due to fluctuations of symptoms

61 subjects received allocated intervention (Rorrico or placebo) and completed the study

Results of 87 subjects were analyzed
Rorrico, Huanglian (Capitidis Rhizome) and Baizhu (Atractylodis Macrocephalae Rhizoma) are the Emperor drugs for clearing away heat evil of the heart and strengthening the spleen function (equivalent to the immune system).

Minister drugs: ministers support and augment the emperor drugs and produce the leading effect in the treatment of the accompanying symptoms. In Rorrico, Xiakucuo (Prunellae Spicae), Jinyinhu (Lonicerae Japonicae Flo), Banlangen (Iatidis Radix), Qinghao (Artemisiae Annuae Herba), Pugongying (Taraxaci Herba) and Chuaxinliang (Andrographis Herba) work together to clear away the external infectious evils (including viruses and other infectious agents). Dahuang (Rhei Radix Et Rhizome), Xuanshen (Scrophulariae Radix), Yinchen (Artemisiae Scopariae Herba) jointly eliminate the toxic effects of the fire, heat and dampness evils in the internal organs. Huzhang (Polygoni Cuspidati Rhizoma Et Radix), Chuaxinliang (Andrographis Herba), Shegan (Belamcandae Rhizoma) and Chaihu (Bupleuri Radix) release the exterior and lung heat. Yinchen (Artemisiae Scopariae Herba) and Qinghao (Artemisiae Annuae Herba) clear the damp heat of the liver and gall bladder. All these herbs are Minister drugs.

Assistant drugs: contribute to several aspects: (a) assist the Emperor and Minister drugs to intensify their therapeutic effects; (b) treat less important symptoms; (c) prevent or neutralize the toxic and side effects of the Emperor and Minister drugs.

In Rorrico, Sharen (Amomi Fructus) and Shanzha (Cotaeqi Fructus) tonify and protect the spleen and stomach. Huangqi (Astragali Radix), Baizhu (Atractylodis Macrocephalae Rhizoma), Dangshen (Codonopis Radix), Sanqi (Notoginseng Radix Et Rhizoma) and Danshen (Salviae Miltiorrhizae Radix Et Rhizoma) are the Assistant drugs being used to regulate Qi, replenish the vital Qi or nourish Yin.

Servant drugs help direct or deliver the effect of the medication to a particular part of the body and coordinate the effects of various ingredients in the prescription. In Rorrico, Baishao (Paeoniae Radix Alba) and Chaihu (Bupleuri Radix) are the Servant drugs to direct all herbs to the exterior and internal organs as well as other parts of the body to deliver their therapeutic effects.

The method for the preparation of Rorrico is outlined as follows. Firstly, Jinyinhu (Lonicerae Japonicae Flo), Sharen (Amomi Fructus), Chuaxinliang (Andrographis Herb), Chaihu (Bupleuri Radix), Baishao (Paeoniae Radix Alba), Baizhu (Atractylodis Macrocephalae Rhizoma), Dangshen (Codonopis Radix), Shanzha (Cotaeqi Fructus) and Huangqi (Astragali Radix) were dissolved in 50%-90% ethanol. The mixture was subjected to reflux extraction under vacuum condition at 30-40 °C for 0.5-3 h. Ethanol was then removed by desiccation and Extract A was acquired. Secondly, Banlangen (Iatidis Radix), Dahuang (Rhei Radix Et Rhizome), Xuanshen (Scrophulariae Radix), Pugongying (Taraxaci Herba), Danshen (Salviae Miltiorrhizae Radix Et Rhizoma), Sanqi (Notoginseng Radix Et Rhizoma), Shegan (Belamcandae Rhizoma), Huzhang (Polygoni Cuspidati Rhizome Et Radix), Qinghao (Artemisiae Annuae Herba), Xiakucuo (Prunellae Spicae), Huanglian (Capitidis Rhizome) and Yinchen (Artemisiae Scopariae Herba) were dissolved in water. The mixture was extracted at 100 °C for 0.5-3 h by reflux extraction. The extracted solution was cooled down to room temperature and ethanol was added in 1-3 times until the final ethanol concentration reached 60%-80%. This solution then underwent sedimentation. The sediment was removed and the clear portion was condensed and dried to obtain Extract B. Thirdly, equal portions of Extract A and Extract B were mixed thoroughly and lyophilized. The final product in powder form was packaged at 2.5 g per packet.

**Administration of rorrico**

TG subjects were prescribed Rorrico 8 packets daily (4 times each day) for 7 days. PG Test Group subjects were prescribed 3 packets daily (3 times each day) for 7 days, while PG Placebo Group subjects took placebo 3 packets daily (3 times each day) for 7 days. Each packet contained 2.5 g of Rorrico or placebo.

Placebo was made of water soluble starch, food coloring substances, flavor agent and solvent containing no medicine. It was prepared through a process involving mixing, drying, smashing, sieving and granulation. The final product in powder form was packaged at 2.5 g per packet.

**Clinical evaluation**

At their interview with the CMP, all subjects had their personal particulars, relevant medical history and flu A symptoms (TG subjects only) recorded. Any changes in symptoms during and/or on completion of the treatment course were also noted for assessment of treatment efficacy.

**Laboratory investigation for influenza A and swine flu infection**

Initially, all TG subjects were tested for influenza A and influenza B infections with throat swabs using a flu A/B rapid test kit (ESPLINE Influenza A&B-N, Fujirebio Inc., Tokyo, Japan). The test was performed by Diagnostix Medical Centre (DMC), a medical laboratory accredited by the National Association of Testing Authorities (NATA), Australia. Subjects with positive results were further tested for H1N1 infection with throat swab samples using a molecular biology test (Influenza A virus RNA) which was performed by a local laboratory in Hong Kong with an in-house developed PCR method. PG subjects did not go through these tests.

**Analysis of blood samples**

Ten mL blood samples were collected from all subjects...
before and after completion of the Rorrico course, and analysed for complete blood picture (CBP) and serum alanine transaminase (ALT), creatinine, creatine kinase (CK), tumour necrosis factor-α (TNF-α), interleukin 18 (IL-18) and mannose binding lectin (MBL). ALT, creatinine and CK were measured on the Dimension RXL analyzer (Siemens Dimension RXL, Siemens Healthcare Diagnostics Inc, Newark, USA). CBP analysis was performed using the Beckman Coulter LH750 cell counter (Beckman Coulter Inc, Fullerton, CA, USA). TNF-α, IL-18 and MBL were assayed by automated enzyme-linked immunosorbent assay (ELISA) using reagent kits of R and D Systems Inc, Minneapolis, MN, USA; Biological Laboratories Co., Ltd., Naka-ku Nagoya, Japan; and Hycult Biotech, Uden, the Netherlands, respectively. All tests were performed by DMC.

**Statistical and analysis**

For data analysis, TNF-α and IL-18 were analyzed by paired t-test and MBL was analyzed by Mann-Whitney rank sum test. Paired t-test and Mann-Whitney rank sum test were performed using SPSS V12.0 software (IBM Corporation, Armonk, NY, 10504-1722, USA). A probability value ($P < 0.05$) was considered significant.

**RESULTS**

Totally eighty nine subjects were recruited and eighty seven of them completed the 7-day treatment. Two TG subjects dropped out because of fluctuations of symptoms. Throat swabs from 2 TG subjects were positive for influenza A with the influanza A rapid test, and one was additionally positive for the H1N1 test. Most of the TG subjects had flu symptoms including cough, running nose, sore throat, fever, diarrhea and nausea. After the 7-day Rorrico treatment, 12 TG subjects recovered fully and 14 TG subjects showed obvious relief of onset symptoms (Table 1).

<table>
<thead>
<tr>
<th>Flu A symptom</th>
<th>Baseline</th>
<th>After Rorrico treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Running nose</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Sore throat</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Fever</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes: TG subjects were prescribed Rorrico 8 packets daily (4 times each day) for 7 days. Subjects might have improved symptoms after the period and the improvements were not due to Rorrico treatment. TG: treatment group.

As for the PG subjects, no onset of influenza was observed during and shortly after the study period. Post-treatment throat swab from the H1N1-infected subject was tested negative for both the Flu A rapid test and the H1N1 test. Blood test results showed that oral administration of Rorrico or placebo produced no adverse effects on liver function, renal function, muscle enzyme and haematological status (Table 2).

There was no up-regulation of pro-inflammatory cytokines TNF-α and IL-18 in both TG and PG (all $P > 0.05$, paired t-test), and concentrations of these two cytokines at baseline and 7 days after Rorrico therapy were all within their normal ranges (Table 3).

**DISCUSSION**

The modern literature on Chinese herbal pharmacology has confirmed the regulatory effects of wind-damp herbs on many aspects of immune function, including the interleukins, interferons, and TNF. TNF-α is a pro-inflammatory cytokine which enhances T lymphocytes and natural killer cell maturation, as well as the production of cytokines, chemokines and cell adhesion molecules. The above results may imply (a) both TG and PG subjects had no active influenza A, or other acute or chronic infections to result in elevated TNF-a and IL-18 levels; (b) Rorrico has no up-regulating effect on the participants’ immune response, or equally likely; (c) the immunomodulatory effects of Rorrico do not non-specifically or unnecessarily promote inflammation when not required. This last point has been advocated as the optimal regulatory effects of Traditional Chinese Medicine being fine-tuning but not over-reacting or exaggerating.

However, mild yet statistically significant ($P < 0.05$, Mann-Whitney rank sum test) elevation of MBL was observed in PG. MBL is a serum lectin synthesized by liver and is one of the important molecules of the innate immune system. Serum MBL level is relatively constant in an individual and, unlike other lectin proteins which increase drastically, MBL increases only 2-3 fold upon infection and inflammatory challenges. Since MBL can enhance the destruction of microorganisms via complement activation, it is possible that oral administration of Rorrico can promote hepatic synthesis of MBL in healthy PG subjects, thereby conferring increased protection against infection.

In conclusion, Rorrico has no up-regulating effect on the participants’ immune response, or, equally likely, the immune-modulatory effects of Rorrico do not non-specifically or unnecessarily promote inflammation when not required. It is possible that oral administration of Rorrico can promote hepatic synthesis of MBL in healthy subjects, thereby conferring increased protection against infection. In addition to further evaluate the efficacy of Rorrico, a larger scale study is warranted.
Table 2 Changes in biochemical parameters of the combined cohort on Rorrico and the placebo subjects with reference intervals in brackets (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (IU/L)</th>
<th>Creatinine (μmol/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG+PG (n = 57)</td>
<td>Baseline</td>
<td>36±16</td>
<td>76±16</td>
</tr>
<tr>
<td></td>
<td>7 days post Rorrico</td>
<td>32±12</td>
<td>76±16</td>
</tr>
<tr>
<td>Placebo (n = 30)</td>
<td>Baseline</td>
<td>37±18</td>
<td>72±20</td>
</tr>
<tr>
<td></td>
<td>7 days post Rorrico</td>
<td>37±20</td>
<td>74±17</td>
</tr>
</tbody>
</table>

Notes: TG subjects were prescribed Rorrico 8 packets daily (4 times each day) for 7 days. PG test group subjects were prescribed 3 packets daily (3 times each day) for 7 days, while PG placebo group subjects took placebo 3 packets daily (3 times each day) for 7 days. Each packet contained 2.5 g of Rorrico or placebo. ALT: alanine transaminase; CK: creatine kinase; TG: treatment group; PG: prevention group.

Table 3 Changes in immunological parameters of the combined cohort on Rorrico and the placebo subjects with reference intervals in brackets (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (ng/L)</th>
<th>IL-18 (ng/L)</th>
<th>MBL (PG only) (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG+PG (n = 57)</td>
<td>1.8±3.8</td>
<td>193.8±83.7</td>
<td>289.0±139.0</td>
</tr>
<tr>
<td></td>
<td>1.8±4.3</td>
<td>193.5±70.8</td>
<td>301.0±149.0’</td>
</tr>
<tr>
<td>Placebo (n = 30)</td>
<td>1.6±2.9</td>
<td>205.1±106.6</td>
<td>294.0±126.0</td>
</tr>
<tr>
<td></td>
<td>1.6±3.3</td>
<td>210.0±121.2</td>
<td>317.0±134.0’</td>
</tr>
</tbody>
</table>

Notes: TG subjects were prescribed Rorrico 8 packets daily (4 times each day) for 7 days. PG test group subjects were prescribed 3 packets daily (3 times each day) for 7 days, while PG placebo group subjects took placebo 3 packets daily (3 times each day) for 7 days. Each packet contained 2.5 g of rorrico or placebo. TNF-α: tumour necrosis factor-α; IL-18: interleukin 18; MBL: mannose binding lectin; TG: treatment group; PG: prevention group. *P < 0.05 when compared with the baseline level.

REFERENCES