Xiao Chai Hu Tang inhibits CVB₁ virus infection of CCFS-1 cells through the induction of Type I interferon expression

Pei-Wen Cheng^a, Lean-Teik Ng^b, Chun-Ching Lin^{a,*}

^a Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, No. 100, Shin-Chuan 1st Road, Kaohsiung 807, Taiwan

^b Department of Biotechnology, Tajen University, No. 20 Weishin Road, Yanpu Shiang, Pingtung, Taiwan

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Abstract

Coxsackie B virus type 1 (CVB₁) infection is known to cause high morbidity and mortality in children, however, there is no effective drug for treating this disease. The present study aimed to examine the antiviral activity of *Xiao Chai Hu Tang* (XCHT), a popular herbal drug for treating viral and bacterial infections, against CVB₁ infection and its mechanisms of action. Our data showed that XCHT neutralized the CVB₁-induced cytopathic effect in human neonatal foreskin fibroblast cell line (CCFS-1/KMC), with IC₅₀ (virus-induced cytopathic effect by 50%) and EC₅₀ (concentration of 50% effectiveness) values around 12.39 and 50.93 µg/ml, respectively. Its CC₅₀ (concentration of 50% cellular cytotoxicity) and SI (selective index) values were 945.75 µg/ml and 18.92, respectively. These results suggest that XCHT possessed anti-CVB₁ activity, and showed no effect on CCFS-1 cell viability and growth at concentration 250 µg/ml. The time-of-addition studies showed that XCHT (50, 100 and 200 µg/ml) added at various time of preinfection (–1 to –3 h), coinfection (0 h) and postinfection (1–3 h) could inhibit CVB₁ infection. Interestingly, XCHT also showed an inhibition on viral replication through the induction of IFN-α/β expression. In conclusion, XCHT possessed antiviral activity against CVB₁ infection. It interfered the early stage of viral replication (prophylactic effect) and viral replication after infection (therapeutic effect) through the induction of Type I interferon expression.

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1. Introduction

Coxsackieviruses (CVs) are important human pathogens, which are known to cause a variety of diseases, such as common colds, myocarditis, cardiomyopathy, diabetes, neurological disorders and inflammation [1–

4]. CV group A (CVA) and CV group B (CVB) together with echoviruses and polioviruses are enteroviruses of the family *Picornaviridae*. CVB₁ has been implicated in human diseases of pleurodynia, aseptic meningitis, meningoencephalitis, and myocarditis [5]. It has been experimentally used to induce diabetes mellitus [5], myocarditis, hepatitis, pancreatitis and encephalitis in various strains of mice [6].

The interferons (IFNs) are a large family of multifunctional cytokines involve in antiviral defense, cell growth regulation and immune modulation. They have been demonstrated to have antiviral effects through

* Corresponding author. Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, No. 100, Shin-Chuan 1st Road, Kaohsiung 807, Taiwan. Tel.: +886 7 312 1101x2122; fax: +886 7 313 5215.

E-mail address: aalin@ms24.hinet.net (C.-C. Lin).

binding to their respective receptors (IFN- α/β or IFN- γ receptor), followed by activating the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathways [7]. Type I interferons (IFN- α and IFN- β) possess several important functions, such as antiviral, antiproliferative and immunomodulatory effects. They are expressed and secreted as an early response to various stimuli, most prominently to viral infections. Picornaviruses are sensitive to IFNs [8,9]. IFNs are known to play a central role in the innate immune antiviral response. Infected cells produce α/β IFNs, which induce a number of genes called IFN-stimulated genes (ISGs) that confer an antiviral state [9,10]. The antiviral potential of IFN- β against coxsackieviruses has been demonstrated in vitro [11] and in animal models [12].

In Japan, certain traditional herbal medicines (called Kampo medicines), which have been used for a long time, are now being manufactured as approved ethical drugs with standardized quality and quantities of ingredients. One such medicine, known in Japanese as *Sho-saiko-to* or Chinese as *Xiao Chai Hu Tang* (XCHT), has been used for treating various chronic liver diseases [13,14], including chronic hepatitis, liver cirrhosis [15] and fibrosis [16,17]. XCHT has been shown to be effective in various experimental and clinical studies, including a double-blinded multicenter clinical trial for liver injury in patients with chronic hepatitis C virus (HCV) infection [18]. Recent in vivo and in vitro studies have demonstrated that XCHT functions as a biological response modifier in the chemoprevention [19].

Studies have shown that XCHT possessed antioxidant [20,21], immunomodulating [15,22–24] and antiproliferative [25,26] activities. It ameliorates chronic viral infected liver diseases [25,26], enhances production of IFN [15,27,28] and antibodies in patients with chronic hepatitis [29]. Furthermore, it could also inhibit the reverse transcriptase activities in murine leukemia virus and human immunodeficiency virus [30].

In the present study, our aim was to examine the antiviral activity of XCHT, a well known traditional Chinese medicine for treating viral and bacterial infection, against CVB₁ infection. It was found to possess appreciable activity against CVB₁ replication through the induction of IFN- α/β expression.

2. Materials and methods

2.1. Materials

Ribavirin, dimethylsulfoxide (DMSO), cell culture medium RPMI-1640 and interferon- α /beta inhibitors (monoclonal

antibody) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). XTT (2, 3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)-carbonyl-2H-tetrazolium hydroxide]) kits were purchased from Roche Diagnostics GmbH (Germany). The positive control (ribavirin) was dissolved in DMSO and diluted with cell culture medium to yield the final DMSO concentration at 0.1%.

2.2. Preparation of plant extracts

Xiao Chai Hu Tang (XCHT) comprised 32% *Bupleurum chinense* D.C., 20% *Pinellia ternate* Breitenach, 12% *Scutellaria baicalensis* Georgi, 12% *Panax ginseng* C.A. Meyer, 12% *Zizyphus vulgaris* Lam., 8% *Glycyrrhiza glabra* L. and 4% *Zingiber officinale* Roscoe. The authenticity of these materials was confirmed by Professor C.C. Lin, Kaohsiung Medical University (Taiwan).

Aqueous extract of XCHT was prepared as reported previously [31]. Briefly, 100 g of XCHT was shade-dried and then decocted for an hour with 1 l of boiling reverse-osmotic water. After filtering and collecting the filtrate, the residue was subjected to the similar extraction twice. The decoctions collected from three separate extractions were mixed, filtered, concentrated and then lyophilized. The dried XCHT extract was collected and stored at 4°C until use. The yield of XCHT was about 31.3%.

2.3. Virus and cells

Human neonatal foreskin fibroblast cell line (CCFS-1/KMC) was used as target cells for viral infection [32]. It was grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin G, 100 μ g/ml streptomycin and 0.25 μ g/ml amphotericin B. In the antiviral assay, the basal medium was supplemented with 2% FCS and the above mentioned antibiotics.

The strain of coxsackievirus B1 (CVB₁ strain Conn-5) used in this study was obtained from the American Type Culture Collection (ATCC VR-28; Rockville, USA) which was propagated in CCFS-1/KMC cells. Its titer was determined by cytopathic effect in CCFS-1/KMC cells and was expressed as 50% tissue culture infective dose (TCID₅₀) per milliliter. The virus was stored at –70°C until use.

2.4. Cytotoxicity

The CCFS-1/KMC cells were seeded onto a 96-well plate at a concentration of 1.0×10^5 cells per ml and a volume of 90 μ l per well. Different concentrations of XCHT were applied to culture wells in triplicate. DMSO and ribavirin were used as negative control and positive control, respectively. After incubation at 37°C with 5% CO₂ for 2 days, a 96-well plate was washed with PBS twice, a mixture of 0.1 ml phenazine methosulfate (PMS: electron-coupling reagent) and 1 mg/ml XTT was added to each well. The trays were further incubated for 3 h to allow XTT formazan production. The absorbance

was determined with an ELISA reader (Multiskan EX, Labsystems, Helsinki, Finland) at a test wavelength of 492nm and a reference wavelength of 690nm. Data were calculated as percentage of inhibition using the following formula: $\text{Inhibition\%} = [100 - (\text{At}/\text{As}) \times 100]\%$. At and As refer to the absorbance of the test substance and the solvent control, respectively. The concentration of 50% cellular cytotoxicity (CC_{50}) of test substance was calculated according to Chiang et al. [33].

2.5. Neutralization test

The antiviral efficacy of XCHT was conducted by measuring the inhibition of cytopathic effect induced by CVB₁ on CCFS-1/KMC cells. The 96-well tissue culture plates were seeded with 100 μl of CCFS-1/KMC cells at a concentration of 1×10^5 cells per ml in RPMI-1640 with 10% FCS. The plates were incubated for 24h at 37°C and used at about 90% confluence. The virus (100 TCID_{50}) was mixed with different concentrations of XCHT (0, 10, 50 and 100 $\mu\text{g}/\text{ml}$) and then incubated for 1h before adding to the plates. After incubation at 37°C with 5% CO_2 for 2 days, the XTT test was carried out as previously described. The percent of cyto-protection was calculated as $(\text{Atv} - \text{Acv}) / (\text{Acd} - \text{Acv}) \times 100\%$. Atv indicates the absorbance of the test substance with virus infected cells. Acv and Acd indicate the absorbance of the virus control and the absorbance of the cell control, respectively. The concentration required for XCHT to reduce the virus-induced cytopathic effect by 50% relative to the virus control was expressed as IC_{50} .

2.6. Antiviral assay using XTT method

The antiviral activity of test compounds against CVB₁ was evaluated by the XTT method [33]. CCFS-1/KMC cells, treated with trypsin, were seeded onto 96-well plates with a concentration of 1.0×10^5 cells per ml and a volume of 70 μl per well. After incubation at 37°C with 5% CO_2 overnight, 20 μl test virus was added and incubated for another 2h. Different concentrations of test substances were then added to the culture wells. DMSO concentration at 0.1% was used as a negative control and ribavirin was used as a positive control for CVB₁. After incubation at 37°C with 5% CO_2 for 2 days, the XTT test was carried out as previously described. The antiviral concentration of 50% effectiveness (EC_{50}) was defined as the concentration which achieved 50% inhibition of virus-induced cytopathic effects. The number of virus used in each experiment was based on infection target cells by 100 TCID_{50} of CVB₁ virus to produce 50% XTT formazan products as in the uninfected control cells.

In time-of-addition study, 20 μl of 100 TCID_{50} CVB₁ per well was absorbed onto confluent monolayers of CCFS-1/KMC cells for 2h. Different concentrations of XCHT were added to culture cells at various times of preinfection (–3 to –1 h), coinfection (0h) or postinfection (1–3h) of the virus to CCFS/KMC cells at 37°C. After 2 days, XTT test and antiviral activity were done as previously described.

2.7. Enzyme-linked immunosorbent assay (ELISA) of IFN- α/β expression

An enzyme-linked immunosorbent assay (ELISA) was used to quantify IFN- α/β expression in culture supernatants collected from virus infected cells after treating with different concentrations of XCHT (50, 100 and 200 $\mu\text{g}/\text{ml}$) at 12 and 48h postinfection. Secreted IFN- α/β was measured with the human IFN- α and IFN- β (HuIFN- α/β) ELISA kit (PBL Biomedical Laboratories, NJ, USA) according to the manufacturer's protocol. Briefly, different dilutions of collected cell supernatant, as well as human IFN- α/β standard, were added to wells on a microplate that were coated with an anti-HuIFN- α/β antibody. After binding and washing, a second horseradish peroxidase-labeled monoclonal antibody against HuIFN- α/β was added to the wells. A color-developing reagent was then used to generate a product that can be quantified by spectrophotometric measurement. A calibration curve of the standard HuIFN- α/β absorbances at 450nm versus the concentrations of HuIFN- α/β in the standard was used to find the concentration of HuIFN- α/β in each sample.

2.8. Analysis of CVB₁ replication in IFN inhibitor-treated cells

CCFS-1/KMC cells were cultured in 96-well plates at a density of 7×10^3 cells per well for 24h, and then different concentrations of IFN- α/β inhibitor (0, 0.1, 1 or 10U/ml) were added to the culture wells for 1h prior CVB₁ infection. After CCFS-1/KMC cells were infected with 100 TCID_{50} CVB₁ for 2h and then treated with different concentrations of XCHT (50, 100, 200 $\mu\text{g}/\text{ml}$) for 48h, the XTT test was carried out as previously described. The percent cyto-protection was calculated as $(\text{Atv} - \text{Acv}) / (\text{Acd} - \text{Acv}) \times 100\%$. Atv indicates the absorbance of the test substance with viral infected cells. Acv

Fig. 1. Effects of XCHT on cell viability. The cell viability of human neonatal foreskin fibroblasts (CCFS-1/KMC) cell line was determined by XTT assay. Cells were treated with various concentrations of XCHT (0, 50, 250, 500 and 1000 $\mu\text{g}/\text{ml}$) for 12 and 48h. The data represents means $\pm$ S.D. of three independent experiments with each treatment in triplicate. The asterisk (* $P < 0.05$) indicates significant difference between treatment and control group as analyzed by Scheffe post hoc test.

and Acd denote the absorbance of the virus control and the absorbance of the cell control, respectively.

2.9. Statistical analysis

Data were presented as the mean $\pm$ S.D. from at least three separate experiments. The statistically different effects of XCHT on the inhibition of viral replication were compared with the control group by one-way ANOVA, followed by the Scheffe post hoc test. A $P < 0.05$ was considered as statistically significant.

3. Result

3.1. Cytotoxicity

We first evaluated the cytotoxicity of XCHT against the target cells for antiviral assay by XTT reduction method. The results of confluent CCFS-1/KMC cell monolayers treated for 12 and 48 h with XCHT at 250 μ g/ml showed no effect on cell viability (Fig. 1). Furthermore, no significant changes in cell morphology and cell density were observed at XCHT concentrations 500 μ g/ml and below. The CC_{50} and SI values

Fig. 2. Inhibition of CVB₁-induced cytopathic effect on target cells. Human neonatal foreskin fibroblast cell line (CCFS-1/KMC) was cultured in 96-well plates to reach 90% confluence. Cells were then infected with virus (100 TCID₅₀) for 48 h. XCHT was added at the stage of viral adsorption. The cytopathic effect was observed under microscopy ($\times 100$). (A) Mock infection. (B) Infection without XCHT. (C) Mock infection plus 50 μ g/ml of XCHT. (D) Infection plus 50 μ g/ml of XCHT. (E) Mock infection plus 100 μ g/ml of XCHT. (F) Infection plus 100 μ g/ml of XCHT.

were 945.75 $\mu\text{g}/\text{ml}$ and 18.92, respectively. However, ribavirin (a commercial drug) at concentration 25 $\mu\text{g}/\text{ml}$ treated group showed a significant ($P<0.05$) reduction in cell viability at 12 and 48 h.

3.2. Neutralization of CVB₁-induced cytopathic effect

The inhibition of CVB₁-infected cytopathic effect is shown in Fig. 2. CVB₁-infected CCFS-1/KMC cells exhibited a typical tear-like cytopathic effect (Fig. 2B). This cytopathic effect was completely neutralized when 50 or 100 $\mu\text{g}/\text{ml}$ of XCHT were added (Fig. 2D and F). There was no obvious change in cell morphology when the cell culture medium contained the same concentration of XCHT (Fig. 2C and E). This study showed that XCHT could neutralize the infection of CVB₁ in a dose-dependent manner at 48 h (Fig. 3), with IC_{50} value 12.39 $\mu\text{g}/\text{ml}$.

3.3. Effect of XCHT on CVB₁ infection

Fig. 4 shows the antiviral activity of XCHT. Results demonstrated that XCHT significantly affected the CVB₁ infection at concentrations 200 $\mu\text{g}/\text{ml}$ and below. It also significantly inhibited the infection of CVB₁ in a dose-dependent manner at 12 and 48 h, with EC_{50} value $50.93 \pm 0.65 \mu\text{g}/\text{ml}$. Since XCHT at concentrations $\leq 200 \mu\text{g}/\text{ml}$ showed a significant antiviral activity, therefore these concentrations were used to investigate the drug effects on virus infection and to characterize the mechanism of action.

3.4. Time-of-addition studies of XCHT on viral replication

In order to investigate the mechanism on how XCHT inhibits the infection of CVB₁, we studied the time-course effect of viral infection by various concentrations of XCHT. Results showed that XCHT at concentrations 50, 100 and 200 $\mu\text{g}/\text{ml}$ exhibited a significant antiviral activity against CVB₁ infection from 3 h before and 0–3 h after the viral

Fig. 3. Antiviral activity of XCHT against CVB₁ infection. Viruses were treated with various concentrations of XCHT (15, 50 and 100 $\mu\text{g}/\text{ml}$) for 48 h and were determined by XTT assay. The data represents means $\pm$ S.D. of three independent experiments with each treatment in triplicate. The asterisk ($*P<0.05$) indicates significant difference between treatment and control group as analyzed by Scheffé post hoc test.

Fig. 4. Antiviral activity of XCHT against CVB₁ replication. Viruses were treated with various concentrations of XCHT (50, 100 and 200 $\mu\text{g}/\text{ml}$) and ribavirin (25 $\mu\text{g}/\text{ml}$) for 12 h and 48 h and were determined by XTT assay. The data represents means $\pm$ S.D. of three independent experiments with each treatment in triplicate. The asterisk ($*P<0.05$) indicates significant difference between treatment and control group as analyzed by Scheffé post hoc test.

infection. XCHT was also shown to decrease the infection of CVB₁ in a dose- and time-dependent manner (Fig. 5).

3.5. Effect of XCHT on Type 1 interferon (IFN- α and IFN- β) expression in CVB₁-infected cells

In order to examine the effect of XCHT on induction of Type I interferon in CVB₁ infected cells, we measured the expression of IFN- α and IFN- β proteins in virus infected cells after treating with XCHT (50, 100 and 200 $\mu\text{g}/\text{ml}$) for 12 and 48 h. Results showed that XCHT could induce IFN- α and IFN- β protein expression in a dose-dependent manner at 12 and 48 h (Fig. 6). A significant effect on the induction of IFN- α and IFN- β protein expression in CVB₁ infected cells was observed.

3.6. Effect of CVB₁ infection by IFN- α and IFN- β inhibitors

In order to determine the mechanism on how XCHT-induced Type 1 interferon expression affects the CVB₁ replication, we conducted a study on the effect of IFN- α and IFN- β inhibitors on viral multiplication in CCFS-1/KMC cells. Results showed that different concentrations of IFN- α and IFN- β inhibitors significantly blocked 50 $\mu\text{g}/\text{ml}$ of XCHT-mediated inhibition of CVB₁ multiplication at 48 h in CCFS-1/KMC cells. Besides, different concentrations of IFN- α and IFN- β inhibitors (0, 0.1, 1 and 10 U/ml) also neutralized the inhibitory rate of multiplication of CVB₁ after treatment with 100 $\mu\text{g}/\text{ml}$ of XCHT (Fig. 7). These results suggest that the XCHT-mediated inhibition of viral multiplication after CVB₁ infection is dependent of IFN- α and IFN- β .

4. Discussion

Xiao Chai Hu Tang (XCHT) is one of the most commonly prescribed drugs in Japan to outpatients for treating chronic hepatitis, liver cirrhosis and digestive

Fig. 5. Time-course effect of XCHT on CVB₁. Different concentrations of XCHT (50, 100 and 200 µg/ml) were added at various times of preinfection (–3 ~ –1 h), coinfection (0 h) or postinfection (1 ~ 3 h) of CVB₁ to CCFS-1/KMC cells at 37 °C for 2 days. The percentage of inhibition was evaluated by XTT method. Each time point represents the mean ± S.D. of three independent experiments with each treatment in triplicate. The asterisk (**P* < 0.05) indicates significant difference between treatment and control group as analyzed by Scheffe post hoc test.

discomforts [18,34,35]. It has been shown to promote production of IFN-γ [28], TNF-α [27,35], IL-1 [15,27], IL-6 [15,28], IL-10 [23,27], IL-12 [27,36], G-CSF [15,23,27] and GM-CSF [15,37], as well as enhancing the natural killer (NK) 1 activity [15,38,39]. It also promoted the clearance of HBeAg in children with chronic HBV infection [14]. These immunomodulatory activities of XCHT could have contributed to the prevention of viral infection. Saikosaponins, the main bioactive components of *B. chinense*, were also shown to possess anticancer [40,41] and antiviral [40,42] activities. Polysaccharides isolated from XCHT showed anti-ulcer, anti-complement and augmentation of NK cell activities [43].

Detailed chemical studies showed that XCHT contains fifteen major low molecular compounds (i.e. baicalin, wogonin-7-*O*-glucuronide, liquiritin, their three aglycons, liquiritin apioside, glycyrrhizin, saikosaponin b1, saikosaponin b2, ginsenoside Rg1, ginsenoside Rb1, 6-gingerol, 6-shogaol and arginine) [43]. These compounds have various pharmacological actions, and are assumed to be responsible, at least partly, for the pharmacological effects of XCHT. For example, wogonin-7-*O*-glucuronoside and wogonin (main bioactive components of *Scutellariae Radix*) were found to increase CD4/CD8 ratio via a decrease of CD8+ T-cell counts but have no effect on CD4+ T-cell counts [44]. Liquiritigenin, liquiritin, and genistein (main bioactive components of *Glycyrrhizae Radix*) are active against influenza virus [45]. Glycyrrhizin and glycyrrhizic acid showed hepatoprotective [46], anti-inflammatory [47,48], anti-ulcer [49], and anti-HIV [50] activities. Baicalin and baicalein exhibited anti-inflammatory [46,51,52], anti-cancer [53] and anti-HIV [54]

activities, as well as stimulating IL-1β production [55]. Betulinic acid, active compound of *Z. vulgaris*, showed anti-cancer activity [56]. 6-gingerol, active compound of *Z. officinale*, was active against angiogenesis [57], inflammation [58] and cancer [59,60]. Ginsenoside Rb1 and ginsenoside Rg1, active components of *P. ginseng*, showed hepatoprotective [61], neuroprotection [62,63], anti-amnesic [63], anti-aging [63] and immunomodulatory [64] activities.

The present study showed that CCFS-1/KMC cell monolayers treated with XCHT at 250 µg/ml for 12 and 48 h showed no effect on cell viability. No significant changes in cell morphology and cell density were also observed at concentrations below 500 µg/ml of XCHT. Its CC₅₀ and SI values were 945.75 µg/ml and 18.92, respectively. In neutralization test, the results suggested that CVB₁-induced cytopathic effect in CCFS-1/KMC cells was neutralized by XCHT. The time course study revealed that the anti-CVB₁ efficacy of XCHT was significant when it was added before the virus entered the host cells and after the virus had infected the host cells. These results suggest that XCHT interfered the early stage of viral infection, which could be at viral adsorption and penetration, and viral replication after infection. This antiviral activity with the expression of IFN-α/β induced by XCHT could be neutralized by IFN-α/β inhibitors. Our study also showed that there was no difference in the levels of IFN-α/β between XCHT with and without polymixin B treatment (data not shown). This suggests that the expression of IFN-α/β induced by XCHT was not due to the presence of LPS.

There are two distinct types of IFNs; Type I IFNs consist IFN-α and IFN-β, which are produced in direct response to viral infection. The IFN-α multigene family

Fig. 6. Effects of XCHT on IFN- α and IFN- β protein expression on CVB₁-infected cells. ELISA method was carried out to determine IFN- α and IFN- β proteins in culture supernatants collected from infected cells treated with different concentrations of XCHT (0, 50, 100 and 200 μ g/ml) at 12 and 48 h postinfection. The data represents the mean $\pm$ S.D. of three independent experiments with each treatment in triplicate. The asterisk (* P < 0.05) indicates significant difference between treatment and control group as analyzed by Scheffé post hoc test.

is predominantly synthesized by leukocytes, whereas IFN- β gene is synthesized by most cell types but mainly by fibroblasts [39,40]. The Type II IFN consists the IFN- γ gene and is induced directly by virus infection. It is synthesized in response to the recognition of infected cells by activated T lymphocytes and natural killer (NK) cells [65,66].

Type I interferons (IFN- α and IFN- β) belong to a family of cytokines whose actions include antiviral, antiproliferative and immunomodulatory effects. They are expressed and secreted as an early response to various stimuli, most prominently to viral infections. The antiviral potential of IFN- β against coxsackieviruses has been demonstrated in vitro [11] and in animal models [12]. IFN- β could also protect against coxsackievirus B3 infection [67]. IFN system was also reported to be the major factor that inhibits poliovirus

replication [68]. Our study was the first to show that XCHT could significantly decrease CVB₁ survival after treating the cells 3 h preinfection or postinfection, suggesting that XCHT may act on all stages of viral life cycle. It is likely that the effect contributing to CVB₁ inhibition in the preinfection case is due to XCHT interfering the early stage of viral infection, possibly at viral adsorption and penetration. The inhibitory effects observed in postinfection may likely due to XCHT affecting viral replication. These results suggest that XCHT could prevent CVB₁ growth in both preinfection and postinfection and that the antiviral cytokine IFN- α / β was induced upon XCHT treatment. By inducing IFN- α / β in uninfected cells it can help the host cells to become resistant against viral infection as ISGs are being upregulated. Thus, we propose that XCHT treatment possibly limits CVB₁ spread by preventing

Fig. 7. Effects on CVB₁ infection by IFN- α and IFN- β inhibitors with XCHT treatment in virus infected cells. CCFS-1/KMC cells were cultured in 96-well plates at a density of 7×10^3 cells per well for 24h, then different concentrations of IFN- α and IFN- β inhibitors (0, 0.1, 1 or 10 U/ml) were added to the cultured cells medium for 1 h before virus infection. Subsequently, CCFS-1/KMC cells were infected with 100 TCID₅₀ CVB₁ for 2 h, and were treated with different concentrations of XCHT (50, 100 and 200 μ g/ml) for 48 h, followed by evaluating with the XTT method. The data represents the mean $\pm$ S.D. of three independent experiments with each treatment in triplicate. The asterisk (* $P < 0.05$) indicates significant difference between treatment and control group as analyzed by Scheffe post hoc test.

viral entry and also by arming both infected and uninfected cells to counter viral replication or infection through induction of IFN- α/β expression.

In conclusion, the present study conclude that: (i) XCHT reduces viral infectivity at non-cytotoxic concentrations; (ii) the 50% inhibitory concentration of XCHT for neutralizing the CVB₁-induced cytopathic effect was about 12.38 μ g/ml in CCFS-1/KMC cells; (iii) it may interfere the early stage of viral infection through viral absorption and penetration; (iv) the inhibition of CVB₁ replication after virus infection was through the induction of IFN- α/β expression.

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