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VIRAsol™ Anti-Viral Extract

Ionophoric Zn™ Anti-Viral Extract

Anti-Viral 2-Part Strategy
Information Sheet

Virasol™ "Anti-Viral Extract" combined with **Ionophoric Zn™ "Anti-Viral Extract"** form the basis of a 2-part strategy designed stop viruses from high-jacking your cells.
1) The first part of the strategy is to prevent the viruses from docking and entering the cell when a person becomes infected.
2) The second part of the strategy is to neutralize any viruses that may have entered the cell by preventing them from replicating.

The principal action of **Virasol™** is to prevent the virus from docking with and entering your cells. It does this through the protease inhibiting effect of **Isatis indigotica.** Protease inhibitors prevent viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors that are necessary for the production of infectious viral particles.**,** and ***Pterocarpus soyauxii,*** (contains Savinin) which suppresses TNF-a, a pro-inflammatory cytokine mainly produced by monocytes and macrophages. **Polygonum multiflorum** (contains Emodin), a compound that blocks the virus spike S protein and angiotensin-converting enzyme 2 (ACE2) interaction, preventing the virus from docking with the cell. (see supporting documentation below)

The principal action of **Ionophoric Zn** is to stop the coronavirus from replicating once it has entered the cell. It does this by introducing zinc (found in ***Elitaria cardamomum***) through the cell membrane via the ionophoric action of **Camellia sinensis**, **Cinchona officinalis** and **Taraxacum officinale**. The mechanism for viral infection is one of ingress through the cell membrane into the cell's cytoplasm, where the virus RNA replicase hi-jacks the cells protein production process to reproduce its own RNA. It has been demonstrated that RNA-dependent RNA polymerase (RdRp), a replicase, can be stopped by the introduction of Zn2+. However, Zn2+ cannot freely penetrate the cell membrane on its own. Subsequently, it was discovered that ionophores will bind with Zn2+ and escort the zinc through the cell membrane where it can stop the virus replicase from reproducing. (see supporting documentation below)

Virasol™contains **Phytotherapeutic** Extracts of*:*
Sambucus nigra, prevents or shortens the duration of herpes outbreaks, and inflammation, and reduces symptoms of upper respiratory infections. The elderberry plant is one of the most

powerful for preventing and treating colds and influenza. Elderberry has been used to treat influenza, colds, and sinusitis, and has been reported to have antiviral activity against influenza and herpes simplex.

Tabebuia impetiginosa is rich in naphthoquinones, plant-based compounds that exert antibacterial, antiviral, and antifungal effects, treats anemia, asthma, bronchitis, and influenza.

Isatis indigotica, the Traditional Chinese Medicine (板藍根 Bǎn Lán Gēn) is used for high fever, headache, infections, scarlet fever, pharyngitis, cold and flu, and pneumonia. It is antibacterial and antiviral. It contains inhibitors against the virus enzymes, such as the nsP13 helicase and 3CL protease.

Pterocarpus soyauxii , inhibited the production of NO and PGE_2, and the expression of iNOS and COX-2 proteins in LPS-stimulated BV2 microglial cells. In addition, this compound significantly suppressed the release of IL-1β and TNF-α at the protein and mRNA levels.

Polygonum multiflorum contains Emodin, an anthraquinone compound that significantly blocked the S protein and ACE2 interaction.

Ionophoric Zn™ contains **Phytotherapeutic** Extracts of:

Elitaria cardamomum, the highest source of plant-based zinc.

Curcuma longa a powerful anti-oxidant and has anti-inflammatory properties.

Camellia sinensis, Source of Epigallocatechin 3-gallate (EGCG) an Ionophoric molecule.

Cinchona officinalis, Source of Quinine- an ionophoric molecule.

Taraxacum officinale, Source of Quercitin (QCT)- an ionophoric molecule.

Cinnamomum verum. Contains antioxidants, including polyphenols, phenolic acid and flavonoids.

Quercitin (QCT), epigallocatechin3-gallate (EGCG) and Chloroquine (Cq) are ionophores and can efficiently transport Zn2+ through the cell membrane where it can impair the replication of a variety of RNA viruses, including Sars-CoV-2 virus. Increasing the intracellular Zinc concentration with zinc-ionophores has been attributed to interference with viral polyprotein replication.

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PLoS PATHOGENS

Zn^{2+} Inhibits Coronavirus and Arterivirus RNA Polymerase Activity *In Vitro* and Zinc Ionophores Block the Replication of These Viruses in Cell Culture

Aartjan J. W. te Velthuis[1], Sjoerd H. E. van den Worm[1], Amy C. Sims[2], Ralph S. Baric[2], Eric J. Snijder[1]*, Martijn J. van Hemert[1]*

1 Molecular Virology Laboratory, Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands, **2** Departments of Epidemiology and Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America

Abstract

Increasing the intracellular Zn^{2+} concentration with zinc-ionophores like pyrithione (PT) can efficiently impair the replication of a variety of RNA viruses, including poliovirus and influenza virus. For some viruses this effect has been attributed to interference with viral polyprotein processing. In this study we demonstrate that the combination of Zn^{2+} and PT at low concentrations (2 μM Zn^{2+} and 2 μM PT) inhibits the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture. The RNA synthesis of these two distantly related nidoviruses is catalyzed by an RNA-dependent RNA polymerase (RdRp), which is the core enzyme of their multiprotein replication and transcription complex (RTC). Using an activity assay for RTCs isolated from cells infected with SARS-CoV or EAV—thus eliminating the need for PT to transport Zn^{2+} across the plasma membrane—we show that Zn^{2+} efficiently inhibits the RNA-synthesizing activity of the RTCs of both viruses. Enzymatic studies using recombinant RdRps (SARS-CoV nsp12 and EAV nsp9) purified from *E. coli* subsequently revealed that Zn^{2+} directly inhibited the *in vitro* activity of both nidovirus polymerases. More specifically, Zn^{2+} was found to block the initiation step of EAV RNA synthesis, whereas in the case of the SARS-CoV RdRp elongation was inhibited and template binding reduced. By chelating Zn^{2+} with MgEDTA, the inhibitory effect of the divalent cation could be reversed, which provides a novel experimental tool for *in vitro* studies of the molecular details of nidovirus replication and transcription.

Citation: te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, et al. (2010) Zn^{2+} Inhibits Coronavirus and Arterivirus RNA Polymerase Activity *In Vitro* and Zinc Ionophores Block the Replication of These Viruses in Cell Culture. PLoS Pathog 6(11): e1001176. doi:10.1371/journal.ppat.1001176

Editor: Raul Andino, University of California San Francisco, United States of America

Received May 17, 2010; **Accepted** October 1, 2010; **Published** November 4, 2010

Funding: This work was supported by the Netherlands Organization for Scientific Research (NWO) with grants from the Council for Chemical Sciences (NWO-CW grant 700.55.002 and 700.57.301) and an NWO Toptalent grant (021.001.037). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: e.j.snijder@lumc.nl (ES); m.j.van_hemert@lumc.nl (MJvH)

Introduction

Zinc ions are involved in many different cellular processes and have proven crucial for the proper folding and activity of various cellular enzymes and transcription factors. Zn^{2+} is probably an important cofactor for numerous viral proteins as well. Nevertheless, the intracellular concentration of free Zn^{2+} is maintained at a relatively low level by metallothioneins, likely due to the fact that Zn^{2+} can serve as intracellular second messenger and may trigger apoptosis or a decrease in protein synthesis at elevated concentrations [1,2,3]. Interestingly, in cell culture studies, high Zn^{2+} concentrations and the addition of compounds that stimulate cellular import of Zn^{2+}, such as hinokitol (HK), pyrrolidine dithiocarbamate (PDTC) and pyrithione (PT), were found to inhibit the replication of various RNA viruses, including influenza virus [4], respiratory syncytial virus [5] and several picornaviruses [6,7,8,9,10,11]. Although these previous studies provided limited mechanistic information, this suggests that intracellular Zn^{2+} levels affect a common step in the replicative cycle of these viruses.

In cell culture, PT stimulates Zn^{2+} uptake within minutes and inhibits RNA virus replication through a mechanism that has only been studied in reasonable detail for picornaviruses [11,12]. *In vitro* studies with purified rhinovirus and poliovirus 3C proteases revealed that protease activity was inhibited by Zn^{2+} [13,14], which is in line with the inhibition of polyprotein processing by zinc ions that was observed in cells infected with human rhinovirus and coxsackievirus B3 [11]. The replication of segmented negative-strand RNA viruses such as influenza virus, however, does not depend on polyprotein processing and the effect of PDTC-mediated Zn^{2+} import was therefore hypothesized to result from inhibition of the viral RNA-dependent RNA polymerase (RdRp) and cellular cofactors [4]. Moreover, an inhibitory effect of Zn^{2+} on the activity of purified RdRps from rhinoviruses and hepatitis C virus was noted, but not investigated in any detail [15,16].

Details on the effect of zinc ions are currently largely unknown for nidoviruses. This large group of positive-strand RNA (+RNA) viruses includes major pathogens of humans and livestock, such as severe acute respiratory syndrome coronavirus (SARS-CoV), other human coronaviruses, the arteriviruses equine arteritis virus (EAV), and porcine reproductive and respiratory syndrome virus (PRRSV) [17,18]. The common ancestry of nidoviruses is reflected in their similar genome organization and expression strategy, and in the conservation of a number of key enzymatic functions in their

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Zinc Ionophore Activity of Quercetin and Epigallocatechin-gallate: From Hepa 1-6 Cells to a Liposome Model

Husam Dabbagh-Bazarbachi,[†,⊥] Gael Clergeaud,[‡,⊥] Isabel M. Quesada,[§] Mayreli Ortiz,[‡] Ciara K. O'Sullivan,*[,‡,∥] and Juan B. Fernández-Larrea*[,†]

[†]Nutrigenomics Research Group, Department of Biochemistry and Biotechnology, and [‡]Nanobiotechnology & Bioanalysis Group, Department of Chemical Engineering, Universitat Rovira i Virgili, 43007 Tarragona, Spain

[§]Vascular Biology Laboratory, IMBECU-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, 5500 Mendoza, Argentina

[∥]Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain

ABSTRACT: Labile zinc, a tiny fraction of total intracellular zinc that is loosely bound to proteins and easily interchangeable, modulates the activity of numerous signaling and metabolic pathways. Dietary plant polyphenols such as the flavonoids quercetin (QCT) and epigallocatechin-gallate act as antioxidants and as signaling molecules. Remarkably, the activities of numerous enzymes that are targeted by polyphenols are dependent on zinc. We have previously shown that these polyphenols chelate zinc cations and hypothesized that these flavonoids might be also acting as zinc ionophores, transporting zinc cations through the plasma membrane. To prove this hypothesis, herein, we have demonstrated the capacity of QCT and epigallocatechin-gallate to rapidly increase labile zinc in mouse hepatocarcinoma Hepa 1-6 cells as well as, for the first time, in liposomes. In order to confirm that the polyphenols transport zinc cations across the plasma membrane independently of plasma membrane zinc transporters, QCT, epigallocatechin-gallate, or clioquinol (CQ), alone and combined with zinc, were added to unilamellar dipalmitoylphosphocholine/cholesterol liposomes loaded with membrane-impermeant FluoZin-3. Only the combinations of the chelators with zinc triggered a rapid increase of FluoZin-3 fluorescence within the liposomes, thus demonstrating the ionophore action of QCT, epigallocatechin-gallate, and CQ on lipid membrane systems. The ionophore activity of dietary polyphenols may underlay the raising of labile zinc levels triggered in cells by polyphenols and thus many of their biological actions.

KEYWORDS: *clioquinol, epigallocatechin-gallate, flavonoids, liposomes, quercetin, zinc ionophores*

1. INTRODUCTION

Quercetin (QCT), a water-insoluble flavonoid present in onions, nuts, and many other vegetables, and epigallocatechin-3-gallate (EGCG), a water-soluble flavonoid present in green tea, are among the most consumed and most studied polyphenols present in the human diet.[1] Flavonoids are considered bioactive micronutrients whose regular consumption, either as food components, or as dietary supplements and nutraceuticals,[2] entails benefits for human health, including prevention and amelioration of cancers,[3] diabetes, and cardiovascular[4] and neurodegenerative[5] diseases. Many of the health benefits of flavonoids have historically been ascribed to their antioxidant activity, which they exert directly by scavenging reactive oxygen species (ROS) and by chelating the redox-active transition metals iron and copper, which may act as ROS generators in biological systems.[6] Flavonoids also act as antioxidants indirectly by inhibiting redox-sensitive transcription factors and pro-oxidant enzymes as well as through induction of phase II and antioxidant enzymes.[7] However, it is currently believed that the levels of polyphenols achieved through ingestion are not enough to justify their wide array of biological actions. Beyond their antioxidant actions, flavonoids are also known to act as signaling molecules that, either directly or indirectly, interact with proteins and nucleic acids, thus modulating multiple cell signaling pathways, gene transcription, metabolic fluxes, and cell fate including apoptosis.[8,9]

Diverse polyphenols have been shown able to form complexes with the redox-inactive transition metal zinc.[10] Zinc is an essential micronutrient for humans, the deficiency of which causes multiple dysfunctions, including alterations of glucidic and lipidic metabolisms.[11] Within cells, the vast majority of zinc cations (in concentrations usually ranging from 100 to 300 μM for most cells) are tightly bound to proteins, functioning as a catalytic or structural component of an estimated 3000 mammalian proteins involved in virtually all cellular processes.[12] A minor fraction of intracellular zinc, termed labile zinc, exists in its free ionic form (picomolar concentrations) or loosely bound to proteins (in nanomolar concentrations). This pool of zinc is detectable by specific fluorophores with very high affinities for zinc cations at neutral pH such as Zinquin and FluoZin-3. Zinc ionophores such as pyrithione and clioquinol (CQ) have been used to increment labile zinc within cells and determine the fundamental roles that this zinc pool plays in cellular biology. Thus, free and labile zinc acts as second messenger molecule, which modulates the

Received: April 1, 2014
Revised: July 22, 2014
Accepted: July 22, 2014
Published: July 22, 2014

OPEN ACCESS Freely available online

PLOS ONE

Chloroquine Is a Zinc Ionophore

Jing Xue[1,2], Amanda Moyer[1], Bing Peng[1,3], Jinchang Wu[2], Bethany N. Hannafon[1], Wei-Qun Ding[1]*

1 Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, United States of America, **2** Department of Radio-Oncology, Nanjing Medical University Affiliated Suzhou Hospital, Suzhou, China, **3** Department of Pharmacology, School of Pharmacy, Xuzhou Medical College, Xuzhou, China

Abstract

Chloroquine is an established antimalarial agent that has been recently tested in clinical trials for its anticancer activity. The favorable effect of chloroquine appears to be due to its ability to sensitize cancerous cells to chemotherapy, radiation therapy, and induce apoptosis. The present study investigated the interaction of zinc ions with chloroquine in a human ovarian cancer cell line (A2780). Chloroquine enhanced zinc uptake by A2780 cells in a concentration-dependent manner, as assayed using a fluorescent zinc probe. This enhancement was attenuated by TPEN, a high affinity metal-binding compound, indicating the specificity of the zinc uptake. Furthermore, addition of copper or iron ions had no effect on chloroquine-induced zinc uptake. Fluorescent microscopic examination of intracellular zinc distribution demonstrated that free zinc ions are more concentrated in the lysosomes after addition of chloroquine, which is consistent with previous reports showing that chloroquine inhibits lysosome function. The combination of chloroquine with zinc enhanced chloroquine's cytotoxicity and induced apoptosis in A2780 cells. Thus chloroquine is a zinc ionophore, a property that may contribute to chloroquine's anticancer activity.

Citation: Xue J, Moyer A, Peng B, Wu J, Hannafon BN, et al. (2014) Chloroquine Is a Zinc Ionophore. PLoS ONE 9(10): e109180. doi:10.1371/journal.pone.0109180

Editor: Yuan-Soon Ho, Taipei Medical University, Taiwan

Received June 25, 2014; **Accepted** September 10, 2014; **Published** October 1, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Funding: This work was supported in part by grants from the American Cancer Society (CNE-117557); the Susan G. Komen for the Cure Foundation (KG081083); the NIH OK-INBRE program (3P20RR016478-09S2); and the Oklahoma Center for the Advancement of Science and Technology (HR09-025). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: weiqun-ding@ouhsc.edu

Introduction

Chloroquine is an antimalarial drug that has been used in humans for many years [1]. In recent years, Chloroquine has been shown to inhibit autophagy and induce apoptosis in malignant cells and thus has been tested in various experimental model systems [2] and in human clinical trials [3,4]. Studies have demonstrated that chloroquine sensitizes tumor cells to radiotherapy [5] or chemotherapy [6–8]. Therefore, chloroquine may potentially be an effective anticancer drug in clinical oncology. It has also been demonstrated that chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy inhibition [9], and the potential side effects of chloroquine therapy have also been cautiously discussed [10,11]. This indicates that a more detailed understanding of chloroquine's anticancer mechanism is required in order to further develop this compound into an effective anticancer agent.

Chloroquine exerts a pleiotropic effect in eukaryotic cells, including an elevation of vacuolar pH when trapped in acidic organelles, such as lysosomes. This increase in pH disrupts lysosomal acidification leading to the impairment of autophagosome fusion and autophagic degradation [12,13]. At the molecular level, chloroquine has been shown to act synergistically with an Akt inhibitor to induce tumor cell death [14]. However, our understanding of chloroquines' action at the cellular and molecular level in cancer cells is quite limited.

We have previously reported that zinc ions exhibit anticancer activity by altering lysosome membrane permeability [15] and via gene expression regulation [16]. Zinc binding compounds, especially zinc ionophores, are a new group of potential anticancer agents that target zinc to the lysosomes and induce lysosome-mediated apoptosis of cancer cells [17]. In addition, the role of zinc in regulating autophagy has been recently realized [18]. Whereas previous studies have found that metal containing chloroquine complexes may lead to enhanced antimalarial activity [19], its interaction with zinc ions has never been investigated in any biological system. Given the reported anticancer activity of zinc ions and chloroquine and their involvement in lysosomal functions, we sought to investigate whether zinc ions interact with chloroquine and whether this interaction alters chloroquine's anticancer activity. We report that chloroquine is a zinc ionophore, which targets zinc to the lysosomes, and that the combination of zinc and chloroquine enhances their cytotoxicity and induces apoptosis in a human cancer cell model system.

Materials and Methods

Materials

The antibody for caspase-3 was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The LC3B-II antibody was from Stressgen (Ann Arbor, MI). The PARP antibody was from Cell Signaling Technology (Danvers, MA). The FluoZin-3 AM probe and LysoTracker probe were purchased from Life Technologies Co. (Carlsbad, CA). CellTiter 96 Aqueous Solution (MTS assay) was from Promega (Madison, WI). Chloroquine diphosphate, zinc chloride, cupric chloride, iron chloride, N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), Ca-EDTA, the β-actin antibody and other chemical agents were analytic grade and purchased from Sigma-Aldrich (St. Louis, MO).

Virology Journal

BioMed Central

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Chloroquine is a potent inhibitor of SARS coronavirus infection and spread

Martin J Vincent[1], Eric Bergeron[2], Suzanne Benjannet[2], Bobbie R Erickson[1], Pierre E Rollin[1], Thomas G Ksiazek[1], Nabil G Seidah[2] and Stuart T Nichol*[1]

Address: [1]Special Pathogens Brach, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, Georgia, 30333, USA and [2]Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, 110 Pine Ave West, Montreal, QCH2W1R7, Canada

Email: Martin J Vincent - mvincent@cdc.gov; Eric Bergeron - bergere@ircm.qc.ca; Suzanne Benjannet - benjans@ircm.qc.ca; Bobbie R Erickson - BErickson1@cdc.gov; Pierre E Rollin - PRollin@cdc.gov; Thomas G Ksiazek - TKsiazek@cdc.gov; Nabil G Seidah - seidahn@ircm.qc.ca; Stuart T Nichol* - SNichol@cdc.gov

* Corresponding author

Published: 22 August 2005

Virology Journal 2005, **2**:69 doi:10.1186/1743-422X-2-69

Received: 12 July 2005
Accepted: 22 August 2005

This article is available from: http://www.virologyj.com/content/2/1/69

severe acute respiratory syndrome coronavirus chloroquine inhibition therapy

Abstract

Background: Severe acute respiratory syndrome (SARS) is caused by a newly discovered coronavirus (SARS-CoV). No effective prophylactic or post-exposure therapy is currently available.

Results: We report, however, that chloroquine has strong antiviral effects on SARS-CoV infection of primate cells. These inhibitory effects are observed when the cells are treated with the drug either before or after exposure to the virus, suggesting both prophylactic and therapeutic advantage. In addition to the well-known functions of chloroquine such as elevations of endosomal pH, the drug appears to interfere with terminal glycosylation of the cellular receptor, angiotensin-converting enzyme 2. This may negatively influence the virus-receptor binding and abrogate the infection, with further ramifications by the elevation of vesicular pH, resulting in the inhibition of infection and spread of SARS CoV at clinically admissible concentrations.

Conclusion: Chloroquine is effective in preventing the spread of SARS CoV in cell culture. Favorable inhibition of virus spread was observed when the cells were either treated with chloroquine prior to or after SARS CoV infection. In addition, the indirect immunofluorescence assay described herein represents a simple and rapid method for screening SARS-CoV antiviral compounds.

Background

Severe acute respiratory syndrome (SARS) is an emerging disease that was first reported in Guangdong Province, China, in late 2002. The disease rapidly spread to at least 30 countries within months of its first appearance, and concerted worldwide efforts led to the identification of the etiological agent as SARS coronavirus (SARS-CoV), a novel member of the family *Coronaviridae* [1]. Complete genome sequencing of SARS-CoV [2,3] confirmed that this pathogen is not closely related to any of the

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Antiviral Research 74 (2007) 92–101

Antiviral Research
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Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction

Tin-Yun Ho [a], Shih-Lu Wu [b], Jaw-Chyun Chen [c], Chia-Cheng Li [d], Chien-Yun Hsiang [d,*]

[a] *Molecular Biology Laboratory, Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan*
[b] *Department of Biochemistry, China Medical University, Taichung, Taiwan*
[c] *Graduate Institute of Chinese Pharmaceutical, China Medical University, Taichung, Taiwan*
[d] *Department of Microbiology, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan*

Received 9 January 2006; accepted 11 April 2006

Abstract

Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel coronavirus (SARS-CoV). SARS-CoV spike (S) protein, a type I membrane-bound protein, is essential for the viral attachment to the host cell receptor angiotensin-converting enzyme 2 (ACE2). By screening 312 controlled Chinese medicinal herbs supervised by Committee on Chinese Medicine and Pharmacy at Taiwan, we identified that three widely used Chinese medicinal herbs of the family *Polygonaceae* inhibited the interaction of SARS-CoV S protein and ACE2. The IC_{50} values for Radix et Rhizoma Rhei (the root tubers of *Rheum officinale* Baill.), Radix Polygoni multiflori (the root tubers of *Polygonum multiflorum* Thunb.), and Caulis Polygoni multiflori (the vines of *P. multiflorum* Thunb.) ranged from 1 to 10 μg/ml. Emodin, an anthraquinone compound derived from genus *Rheum* and *Polygonum*, significantly blocked the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of SARS.

Keywords: SARS coronavirus; Spike protein; Angiotensin-converting enzyme 2; Emodin

1. Introduction

Severe acute respiratory syndrome (SARS) is a new human disease that results in progressive respiratory failure and death in close to 10% of infected individuals (Ksiazek et al., 2003; Peiris et al., 2003). The etiological agent, SARS coronavirus (SARS-CoV) (Drosten et al., 2003; Fouchier et al., 2003) contains a single-stranded plus-sense RNA genome about 30 kb in length that has a 5′-cap structure and a 3′-polyadenylation tract (Marra et al., 2003; Rota et al., 2003). The genomic organization is typical of coronaviruses, having 14 potential major open reading frames that encode replicase, spike (S), envelope, membrane, and nucleocapsid proteins in the same order as those of other coronaviruses (Tan et al., 2005).

SARS-CoV S protein is a large type I membrane glycoprotein projection from viral envelope (Bosch et al., 2003). SARS-CoV S protein is responsible for binding to cellular receptors and for mediating the fusion of viral and host membranes (Simmons et al., 2004; Tripet et al., 2004). It also contains important virus-neutralizing epitopes that elicit neutralizing antibody in the host species (Hofmann et al., 2004a; Sui et al., 2004). Furthermore, mutations in this gene dramatically affect the virulence, pathogenesis, and host cell tropism (Petit et al., 2005; Yi et al., 2005). Angiotensin-converting enzyme 2 (ACE2) has been identified as a functional receptor for SARS-CoV (Li et al., 2003). Soluble S fragment or ACE2 is able to block S protein-mediated infection (Hofmann et al., 2004b; Moore et al., 2004). Monoclonal antibodies against S protein efficiently neutralize SARS-CoV *in vitro* and *in vivo* (Greenough et al., 2005; Sui et al., 2004). Moreover, vaccines that express the S protein induce T cell and neutralizing antibody responses, and protect animals from SARS-CoV infection (Chen et al., 2005; Yang et

Abbreviations: SARS, severe acute respiratory syndrome; SARS-CoV, SARS coronavirus; S, spike; ACE2, angiotensin-converting enzyme 2; HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; *E. coli*, *Escherichia coli*; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; IFA, immunofluorescence assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HSV, herpes simplex virus

* Corresponding author. Tel.: +886 4 22053366x8503; fax: +886 4 22053764.
E-mail address: cyhsiang@mail.cmu.edu.tw (C.-Y. Hsiang).

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.04.014