Enhancement of the anti-herpetic effect of trichosanthin by acyclovir and interferon

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Abstract Trichosanthin (TCS) is a type I ribosome-inactivating protein that has a wide range of pharmacological activities. The present study investigated the effectiveness of TCS on herpes simplex virus (HSV-1). The anti-viral activity and toxicity of TCS on Vero cells were measured. Results showed that the ED_{50}, TD_{50} and the therapeutic indices were 38.5, 416.5 and 10.9 μg/ml, respectively. Anti-viral activity of TCS was substantially potentiated when it was used in conjunction with other anti-viral agents. The ED_{50} of TCS was reduced 125-fold by acyclovir at a concentration of 0.001 μg/ml, which was practically devoid of significant anti-viral activity. Similarly, the ED_{50} of TCS was reduced 100-fold by interferon-α2a at a concentration of 100 IU/ml. In conclusion, TCS is effective against HSV-1 and other anti-viral agents such as acyclovir or interferon can potentiate its action substantially.

Key words: Trichosanthin; Ribosome-inactivating protein; Anti-viral; Acyclovir; Interferon; Herpes simplex virus

1. Introduction

Trichosanthin (TCS) is a type I ribosome-inactivating protein (RIP) purified from the root tubers of *Trichosanthes kirilowii* [1]. In the crude form, it was used in ancient China as an abortifacient agent. After TCS was purified and identified to be the active ingredient, more biological activities of this compound were unveiled [2]. A major finding was that TCS inhibited the replication of human immunodeficiency virus (HIV) in both acutely infected T-lymphoblastoid cells and chronically infected macrophage [3]. This generated great enthusiasm leading to phase I/II clinical trials. It was shown that TCS lowered serum p24 antigen level and increased $CD4^+$ T cells in HIV-infected patients [4,5]. However, antigenicity and other side effects precluded further investigation of its potential use as a therapeutic agent. There have been efforts to reduce the antigenicity of TCS by molecular manipulation and coupling to PEG [6].

Type 1 (HSV-1) and type 2 (HSV-2) herpes simplex virus are responsible for a broad spectrum of human infectious diseases. They range in severity from herpes labialis, genital herpes, herpes keratitis, to the life-threatening herpes encephalitis [7]. HSV infections were also recognized as a risk factor for HIV infection and can be lethal to AIDS patients [8]. There is only a limited choice of drugs currently available for the management of HSV infection. The list includes interferons (INFs), acyclovir (ACV), vidarabine, ganciclovir and foscarnet. All these drugs have undesirable complications and may induce resistance [7]. It is therefore necessary to expand the scope of anti-HSV agents. Combination of different anti-viral drugs working via different mechanisms proved to be effective. It appears that TCS, being an RIP, has a unique mechanism compared to the conventional ones. It can play an attractive role in combination therapy. In this study, the effect of TCS on HSV-1 was investigated. The potentiation effect when used in conjunction with other anti-viral drugs was also explored.

2. Materials and methods

2.1. Reagents

TCS was obtained from Professor Y.B. Ke of the Shanghai Institute of Cell Biology, the Chinese Academy of Sciences. Recombinant human INF-α2a was generously provided by Changsheng Gene Pharm Co. Ltd. (China).

2.2. Cells and virus

Vero cell (African green monkey kidney cell, ATCC CCL 81) and HSV-1 (F strain, ATCC VR733) were obtained from the American Type Culture Collection (Rockville, MD, USA). Vero cells were maintained in culture flasks in complete RPMI medium. Subculture was done every 2–3 days after it had formed a confluent monolayer. During subculture, cells that attached to the culture flasks were trypsinized (0.25% trypsin containing 0.01% EDTA) for 2–5 min at 37°C and then stopped by the addition of complete medium to the cell suspension. The cells were washed once again with complete medium. About 10^5 viable cells were then re-suspended in complete medium. HSV-1 was propagated in Vero cells and the viral titers were determined by plaque formation. The infectious titer of stock solution was 10^6 $TCID_{50}$/ml (50% tissue culture infectious doses).

2.3. Cytotoxicity assay

Cytotoxicity was measured by the XTT method using the Cell Proliferation Kit II (Boehringer-Mannheim). Briefly, 100 μl of Vero cells (1×10^5/ml) was seeded onto a microtiter plate and allowed to attach to the well bottom. Various concentrations of anti-viral agents were added and incubated for 48 h. XTT reagent was added to a final concentration of 0.3 mg/ml. Absorbance of the color complex was read at 490 nm with the reference wavelength set at 690 nm. TD_{50} was defined as the concentration of the anti-viral agent at which absorbance was reduced by 50%.

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Abbreviations: TCS, trichosanthin; HSV, herpes simplex virus; HIV, human immunodeficiency virus; INF, interferon; ACV, acyclovir; RIP, ribosome-inactivating protein; TI, therapeutic index

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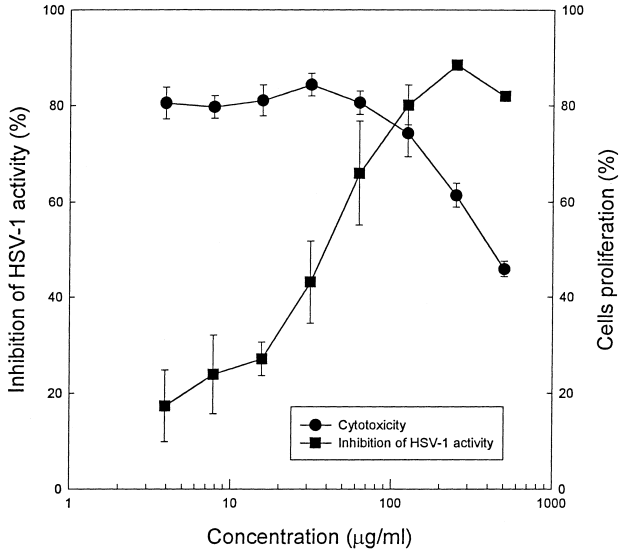

Fig. 1. Anti-herpetic activity and cytotoxicity of TCS. Data are expressed as means ± S.E.M. of triplicate measurements ($n = 7$).

2.4. Anti-herpetic activity assay

Vero cells (1×10^4 cells/well) were seeded onto the microtiter plate and allowed to attach to the well bottom. The cells were pre-incubated with a single or combination of the anti-viral agents for 2 h. HSV-1 containing 10 $TCID_{50}$ per well was added and incubated for 48 h [9]. Replication of HSV-1 was determined by in situ HSV antigen ELISA assay using peroxidase-conjugated rabbit anti-HSV-1 antibody [10,11]. The concentration of an anti-viral agent reducing HSV-1 replication by 50% (ED_{50}) was determined from the dose–response curve. The therapeutic index (TI) was calculated from the ratio of TD_{50}/ED_{50}.

3. Results

3.1. Cytotoxicity and anti-herpetic activity of TCS, ACV and INF

TCS, ACV and INF exhibited a dose-dependent inhibitory effect on HSV-1 replication (Figs. 1 and 2). The ED_{50}, TD_{50} and TI are summarized in Table 1. On a molar basis, the potency of TCS is similar to ACV (ED_{50} 1.4 μM and 1.2 μM, respectively), whereas the toxicity of TCS is much higher.

3.2. ACV and INF enhanced anti-herpetic activity of TCS

The anti-viral effects of TCS used in conjunction with ACV or INF are summarized in Figs. 3 and 4. Both demonstrate that it was substantially potentiated. ACV at a concentration of 0.001 μg/ml was practically inactive (Figs. 2A and 3A). The ED_{50} of TCS determined in the presence of this amount of ACV was significantly decreased by 125-fold from 64.7 ± 16.2 to 0.52 ± 0.18 μg/ml (Fig. 4A). A similar finding was observed when TCS was used in conjunction with INF. The ED_{50} was reduced by 100-fold from 64.7 ± 16.2 to 0.62 ± 0.06 μg/ml (Fig. 4B). The concentration of INF used together with TCS (100 IU/ml) contained minimal anti-viral activity (Figs. 2B and 3B).

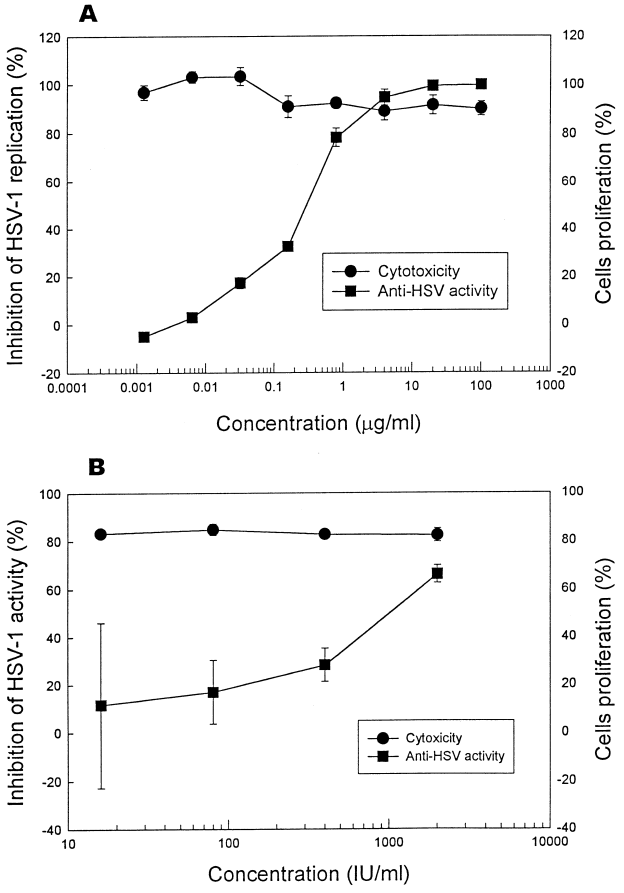

Fig. 2. Anti-herpetic activity and cytotoxicity of ACV (A) and INF-α2a (B). Data are expressed as means ± S.E.M. of triplicate measurements ($n = 4$).

4. Discussion

It was found that TCS inhibited replication of HIV, but its action on other viruses is not known. The present investigation examines the efficacy of TCS on HSV as well as its combined effect with other anti-viral agents. It was clearly demonstrated that TCS is active against HSV and its efficacy is similar to ACV on a molar basis. TCS is more toxic than ACV and therefore the calculated TI is smaller. Toxicity of TCS varies between cell types, for example, choriocarcinoma is much more sensitive to TCS than hepatoma [6]. Vero cells were used as hosts in this study and this cell line is not particularly sensitive to TCS.

Combination therapy using more than one anti-viral agent has been demonstrated to be effective against some viral diseases [12,13]. In this study, the synergistic effect of TCS with two other anti-viral agents was investigated. The first is ACV, which is commonly used for treatment of HSV infection. It targets viral DNA polymerase after being phosphorylated by HSV-specific thymidine kinase [7]. It is therefore active only in

Table 1
Summary of the cytotoxicity and anti-viral activity of TCS, ACV and INF-α2a

Compound	ED_{50}	TD_{50}	TI
TCS	38.4 ± 17.5 μg/ml	416.5 ± 34.5 μg/ml	10.8
ACV	0.27 ± 0.02 μg/ml	> 500 μg/ml	> 1850
IFN-α2a	1007 ± 69.5 IU/ml	> 5000 IU/ml	> 4.9

infected cells. The other anti-viral agent is INF. Its action depends on the kind of INF and the type of cells [14]. Vero cells are not particularly sensitive to INF-α2a used in this study. The anti-viral mechanism of INF is not well established. It is known that treatment of HSV-infected cells resulted in a decrease of ribonucleotide reductase leading to a fall in the size of deoxyribonucleotide pools [15,16]. In this study, it was clearly demonstrated that ACV or INF enhanced the anti-viral action of TCS. In the presence of a fixed low dosage of either ACV or INF that has no significant anti-viral effect, the potency of TCS was enhanced by over 100-fold.

How RIP inhibits viral replication is not clear. Intuitively, it may be attributed to ribosome inactivation. However, not all RIPs are anti-viral. There is evidence to suggest that the anti-viral mechanism of some RIPs does not involve ribosome inactivation [17]. The anti-viral action of pokeweed anti-viral protein is due to selective inhibition of DNA synthesis [18]. Momordica anti-viral protein 30 and Gelonium anti-viral protein 31 are believed to inhibit viral integrase activity [19]. Although some biological activities of TCS can be explained by ribosome inactivation, the exact mechanism of anti-viral activities remains to be determined. There may not be enough information from this study to suggest any plausible mechanism for TCS action. But it can be speculated that the anti-viral mechanism of TCS should not be the same as that of ACV or INF. Alternatively, if the mechanisms were the same, the synergistic effect would have been summative rather than 100-fold potentiated as found in this study.

In conclusion, this study shows that TCS is active against HSV and this action can be potentiated by ACV or INF.

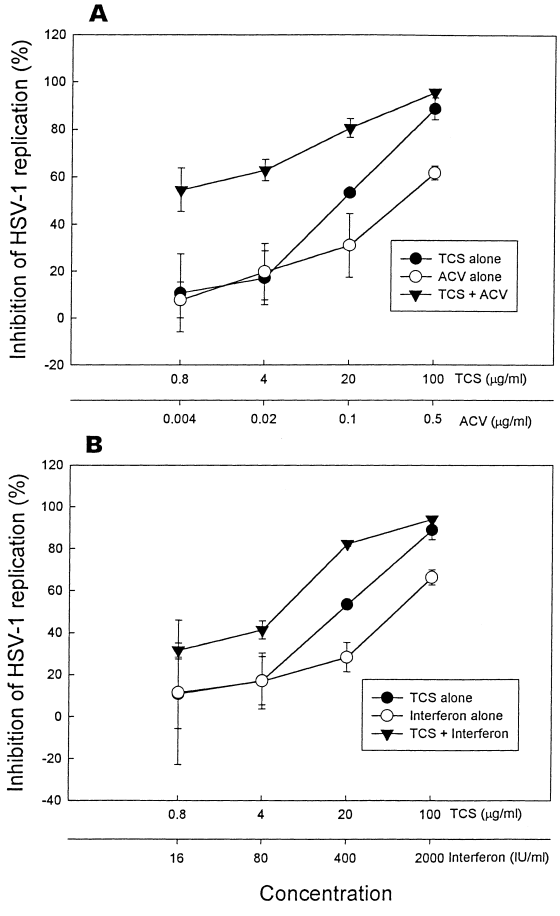

Fig. 3. Anti-viral effect of TCS when used in conjunction with various doses of ACV (A) or INF-α2a (B). Data are expressed as mean ± S.E.M. of quadruplicate measurements ($n = 4$).

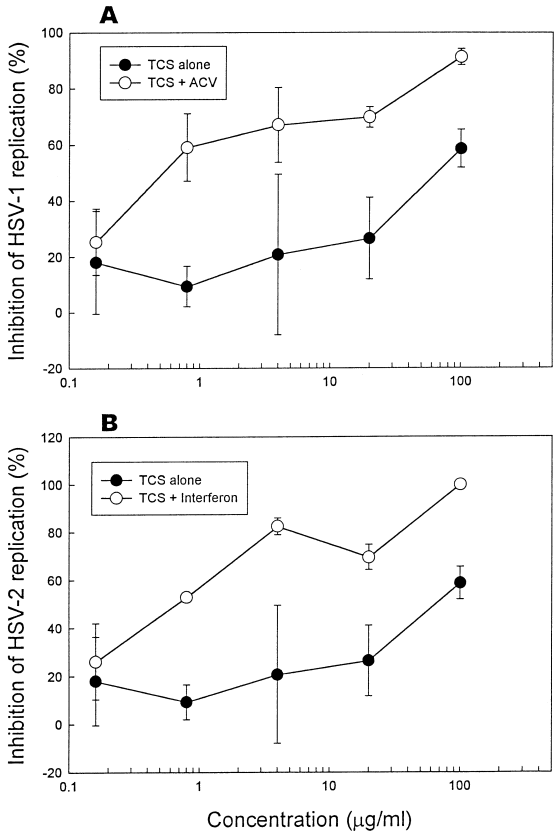

Fig. 4. Anti-viral effect of TCS when used in conjunction with a fixed dosage of ACV (A) or INF-α2a (B). The concentration of ACV was 0.001 μg/ml and INF-α2a was 100 IU/ml. The ED_{50} of TCS was reduced from 64.7 ± 16.2 μg/ml to 0.52 ± 0.18 μg/ml by ACV and to 0.62 ± 0.06 μg/ml by INF-α2a. Data are expressed as means ± S.E.M. of quadruplicate measurements.

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