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Research article

Evaluating the therapeutic efficacy of triptolide and (S)-10-hydroxycamptothecin on cutaneous and ocular Herpes Simplex Virus type-1 infections in mice

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ABSTRACT

Objective: The emergence of Acyclovir-Resistant Herpes Simplex Virus type-1, which is the result of clinical over usage calls for the urgent need of a novel anti-HSV agent. Hence, the activity of Triptolide (TP) and (S)-10-Hydroxycamptothecin (10-HCPT) were investigated as natural products in two infection models of HSV-1.

Methods: The antiviral efficacy of TP and 10-HCPT was evaluated in mice ocular and cutaneous infection models of HSV. Groups of 10 mice were infected with HSV-1. Both compounds were administered topically on corneal and skin. The disease severity, viral titer (plaque reduction assay), and histopathology were evaluated in the ocular and cutaneous models of HSV-1 infection on days 3, 5, 7, 9, and 12 post infection, as well as genome loads on days 3 and 12.

Results: Topical treatment of corneal with TP, 10-HCPT, and ACV was effective in reducing stromal disease (after day 3, $P = 0.001$), plus TP and ACV on vascularization (after day 7, $P = 0.001$). The virus titer decreased significantly in the infected treated groups after day 3 ($P < 0.05$). Also, on day 12 post-infection, the virus genome volume in the TP and ACV groups was significantly reduced. With respect to virus titers and the DNA yield, significant difference was observed, merely in the ACV group in comparison to the control ($P = 0.013$). Immunohistochemistry analysis showed that corneal epithelium healing was partially visible in the 10-HCPT group, which gradually increased in TP, and was the highest in the ACV group. The skin epithelium healing was only observed in TP and ACV groups, and was superior in the ACV group.

Conclusions: This study revealed the virologic and clinical potential of TP *in-vivo* to treat ocular mouse model.

1. Introduction

Herpes Simplex Virus-type 1 (HSV-1) can cause ailments, especially in neonates or immunocompromised hosts. HSV is a double-stranded DNA virus with a large genome of about 150 kb [1, 2]. Approximately, the virus genome encodes 80 proteins [3]. After a primary infection, HSV usually develops into a latent infection in the trigeminal ganglia of the neuronal cells until it is re-stimulated, which initiate the productive cycle [4]. This latent infection can be reactivated under certain condition, which is generally as a result of recurrent diseases [5, 6]. Viral infection is associated with its degree of manifestation, ranging from mild to severe. Furthermore, it can cause life-threatening illnesses, such as gingivitis, kerato-conjunctivitis, and herpes encephalitis, with recurrence in an immunocompromised transplant recipient or HIV-infected person [7, 8, 9]. Worldwide, the prevalence of HSV-1 and HSV-2 infections in adult is more than 80% and 20%, respectively [10, 11]. For treatment, Acyclovir (ACV) and other nucleoside analogues, such as valaciclovir, famciclovir, ganciclovir, and penciclovir are prescribed for the management of herpes infections [12, 13, 14]. Unfortunately, HSV ACV resistance rate has increased significantly, from 5% to 14% in bone marrow transplant recipients [15, 16]. Lately, the surge in ACV-resistant strains has led to long-term and worrying treatment of this viral infection with expected recurrences and failure in the treatment process [17, 18]. Therefore, the transmission of resistant strains to susceptible individuals has to be

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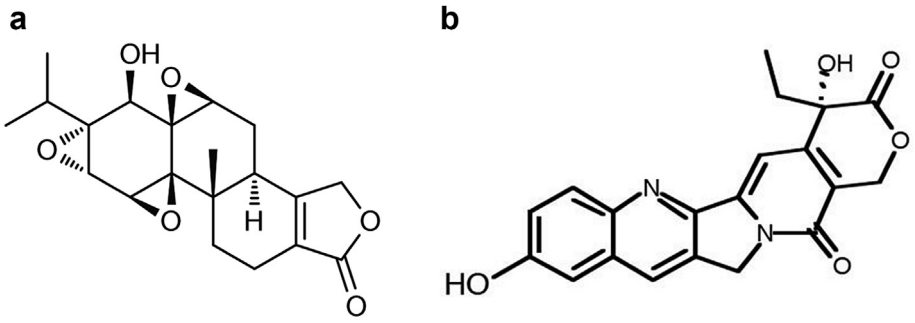

Figure 1. Chemical structure of Triptolide (a) and (S)-10-Hydroxycamptothecin (b). Determination of the structure was described in the reference [24, 26].

considered. The rise in acyclovir-resistant strains has become an obstacle, when treating immunocompromised patients, confirming the necessity for a new, effective and safe complementary or alternative antiviral drug. Natural products, particularly traditional medicines are known to be an important source of anti-HSV agents, even though their mechanisms of action and targets are still unknown. They are important sources of molecules that can be purified and act as active components, such as plant components or biological derivatives of the marine life [19, 20, 21]. For instance, ent-epiafzelechin-(4a→8)-epiafzelechin extracted from *Cassia javanica* can inhibit HSV-2 replication, hydrolysable tannins chebulagic acid and punicalagin as a glycosami-noglycan (GAG) competitors that has the ability to inhibit HSV-1 entry or cell-to-cell spread. Houttuynoids A–E are flavonoids isolated from *Houttuynia cordata* with potent anti–HSV-1 activity. [15, 22]. Recently, the aforementioned components have been considered as an important source of antiviral agents, despite the fact that their mechanism of action is not yet understood.

TPs are active natural products, extracted from the medicinal plant *Tripterygium wilfordii* Hook F., which exhibits a combination of medicinal properties, such as anti-cancer, anti-inflammatory, anti-obesity, anti-diabetic activities as well as antimicrobial properties [23]. Diterpene triepoxide comprises of three epoxy groups of a C-14-hydroxyl group and a lactone ring (Figure 1a) [24]. Due to its potent anti-inflammatory activity, this natural product has attracted considerable attention from the scientific community [23, 25]. The 10-HCPT (Figure 1b), a Camptothecin (CPT), can specifically target the topoisomerases DNA [26, 27]. To the best of our knowledge, its antiviral activity has not been reported in any previous study. Hence, the aim of this study was to assess the anti-HSV efficacy of the two mentioned compounds on mouse models of keratitis and cutaneous infections.

2. Materials and Methods

2.1. Mice

In total, 100 female BALB/c (18–20 g) mice in the age range of 4–6 weeks were acquired from the Centre for Comparative and Experimental Medicine in Iran. The study protocol was approved by the local ethics committee of the Clinical Microbiology Research Centre, Shiraz University of Medical Sciences (IR. SUMS.REC. 1397.901).

2.2. Cell and virus

The employed HSV in this study was characterized in another study [18], which was isolated from the orolabial region of a 56 year old male patient, who had referred to the Prof. Alborzi Clinical Microbiology Research Centre, Shiraz, Iran. Subsequent to the isolation, and 2–3 passages on vero cells, the virus was identified by type-specific primers and probes [28], using real-time PCR (Applied Biosystem, CA, USA) as well as direct Immunofluorescence staining, using type-specific monoclonal antibody agent HSV-1/HSV-2 (Catalog number: K610611-2, Therm Scientific™). The isolate's sensitivity to Acyclovir was evaluated, using the plaque reduction assay standard method [29]. Genotypic assay was done on Thymidine kinase and DNA polymerase genes of the HSV. The prototype HSV-1 was assigned as the sensitive laboratory strain HSV-1AN95. It should be worthy of note, that the vero cells were purchased from the Pasteur Institute of Iran. Finally, cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY), 1% Penicillin-Streptomycin (Gibco™, 10000 U/mL), supplemented with 10% fetal bovine serum (FBS) (Gibco), at 37 °C in 5% CO_2.

2.3. Compounds

ACV was purchased from Sigma-Aldrich (St. Louis, MO, USA). TP and 10-HCPT were obtained in dry powder from Selleckchem Company (Selleckchem Natural Product Library, Catalog No. L1400) and then solved in DMSO.

2.3.1. In vivo evaluation for toxicity

In the pilot phase, the *in vivo* toxicity of TP and 10-HCPT was assessed by treating mice with the compounds for cutaneous and ocular routs as follows: Mice were randomly divided into 6 groups (5 per group) as follows; Group 1, 2 and 3 consisted of uninfected mice, topically treated with 50 μl TP (containing 0.02 mg/kg of TP in DMEM), 50 μl 10-HCPT (containing 0.6 mg/kg of 10-HCPT in DMEM) and 50 μl ddw, respectively. Group 4, 5 and 6 were treated with 10 μl TP (containing 0.02 mg/kg of TP in DMEM), 10 μl 10-HCPT (containing 0.6 mg/kg of 10-HCPT in DMEM) and 10 μl ddw in the form of eye drop, respectively. The systemic toxicity and the signals toxicity in cornea were assessed at the sites of administration for all of the topical application models during 12 days (OLYMPUS SZ40 Microscope, Japan).

2.4. Herpes corneal infection

Under IM injection of 5 mg ketamine hydrochloride and 10 mg of xylazine anesthesia, one cornea was scratched two to three times vertically and then two to three times horizontally, using a 30 gauge sterile needle. Mice were infected by inoculating 10 μl of DMEM containing 3% serum and 200 PFU of HSV-1 in the form of eye drop to the wound cornea.

In this phase, there were 5 groups (10 mice each). Group 1 included the uninfected mice that were treated topically with 10 μl of TP (containing 0.02 mg/kg of TP in DMEM) and 10-HCPT (containing 0.6 mg/kg of 10-HCPT in DMEM) solution in the eye drop form (negative control). Group 2 comprised of infected mice and the untreated as positive control. Group 3 was treated topically with 10 μl of TP (containing 0.02 mg/kg of TP in DMEM) solved in DMEM, containing 3% serum on the scarified cornea (eye drop). Group 4 received topical10 μl of 10-HCPT (containing 0.6 mg/kg of 10-HCPT in DMEM), which was solved in DMEM, containing 3% serum on the scarified cornea (eye drop). Finally, group 5 received the topical treatment containing ACV solution (comprising of 0.1 mg/kg of ACV in DMEM), similar to groups 3 and 4. Treatment was

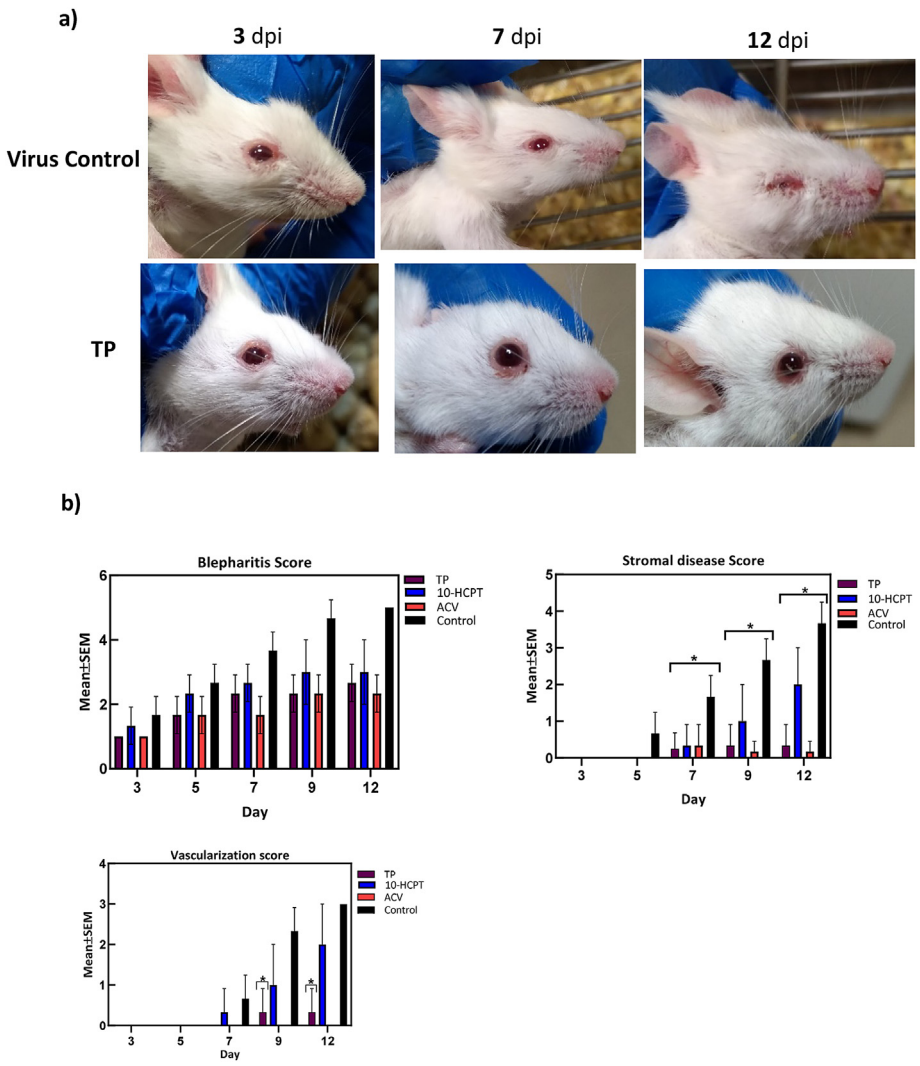

Figure 2. (a) Visual inspection of HSV-1 infection of ocular from infected and treated with TP and untreated mice on a specified day. (b) Efficacy of TP and 10-HCPT topical treatment on HSV-1 mean ocular disease scores. Mice (n = 10) had their one cornea infected and treated as follows: group 1, uninfected, treated; group 2, infected, untreated as (positive control); group 3, treated topically with 50 μl of TP solution (containing 0.02 mg/kg of TP in DMEM) for 12 days (3 times per day); group 4, received 50 μl of 10-HCPT solution topically on the wound area (containing 0.6 mg/kg of 10-HCPT in DMEM) for 12 days (3 times per day); Group 5, treated with topical treatment with ACV solution (containing 0.1 mg/kg of ACV in DMEM) for 12 days (3 times per day). The disease scores were determined as described in Materials and Methods. Values are expressed as means ± SEM from 10 mice in each group. Statistical analysis (one-way ANOVA followed by the Student Newman-Keuls test) demonstrated significant differences among the groups; *, $P < 0.05$.

commenced within 24 h after afflicting the infections and was continued for the next 12 days (3 times a day).

2.5. Herpes cutaneous infection

For this phase, mice were anesthetized via intramuscular (IM) injection of 5 mg of ketamine hydrochloride, in addition to 10 mg of xylazine. Then, the back of the mice was shaved and scratched several times vertically and horizontally with a sterile 27-gauge needle. Infected mice were prepared by the topical application of 50 μl of DMEM, 3% FBS (Gibco) containing 1.0×10^5 PFU of HSV-1 strain to the scratched area.

The mice were randomly divided into 5 groups (10 mice in each) as follows; Group 1 consisted of uninfected mice treated with 50 μl of TP (containing 0.02 mg/kg of TP in DMEM) (5 mice) and 10-HCPT solution (containing 0.6 mg/kg of 10-HCPT in DMEM) (5 mice) as the negative control. Group 2 included the infected mice and untreated as the positive control. Group 3 treated topically with 50 μl of TP solution (containing 0.02 mg/kg of TP in DMEM) on the wound area. Group 4 received 50 μl of 10-HCPT solution topically on the wound area (containing 0.6 mg/kg of 10-HCPT in DMEM). Group 5 received the topical treatment with ACV solution (containing 0.1 mg/kg of ACV in DMEM), as explained for the groups 3 and 4. Treatment was commenced within 24 h after inducing the infection and continued for the next 12 days (3 times per day).

2.6. In vivo antiviral efficacy of TP and 10-HCPT on HSV-1

In vivo antiviral efficacy was evaluated, as described in the following sections:

2.6.1. Clinical scoring

2.6.1.1. Ocular disease scoring. The severity of eye injury was examined by microscope (OLYMPUS SZ40 Microscope, Japan), using a scoring system [30]. Blepharitis scoring system is as follows: puffy eyelids 1+; puffy eyelids with some crusting 2+; eye swollen shut with severe crusting 3+; and eye completely swollen shut and crusted over 4+. To determine the severity of vascularization, we used the vascularization score, as: 1+ means less than 25% of the cornea involvement; 2+, 25%–50% corneal involved; and 3+, more than 50% corneal involvement. Stromal disease score includes 4 grades of cloudiness, some iris detail visible 1+; iris detail obscured 2+; cornea totally opaque, 3+; and corneal perforation 4+.

2.6.1.2. Cutaneous disease scoring. The skin HSV-1 infection scoring was calculated, based on the Park et al. method [31]. In brief, 0, no visible infection; 1, a visible area with yellowish swelling (prelesions), or healing ulcers; 2, ulcers at inoculation site only with swelling, crust formation, and skin erythema, or healing ulcers; 3, spreading ulceration with some clear ulcers, 4, zosteriform rash; 5, rash confluent but without

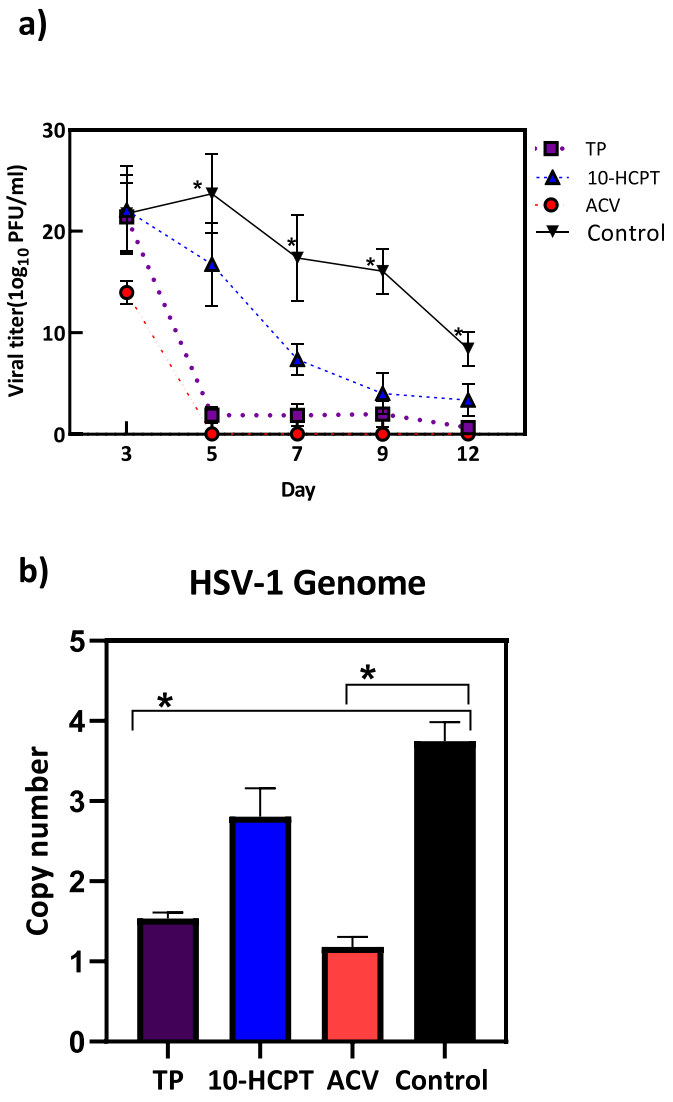

Figure 3. Efficacy of TP and 10-HCPT topical treatment on ocular viral DNA and titers. Virus titer (a) and HSV-1 DNA (b) in the tear samples were assessed by plaque reduction assay on days 1, 3, 5, 7, 9 and 12 and qPCR on the day 12, respectively, as described in Materials and Methods. Significance of difference between the 5 groups was determined by one-way ANOVA followed by Dunnett's multiple comparisons tests, on day 12th and Sampling days post-infection; *, $P < 0.05$.

necrosis or ulceration; 6, complete rash with necrosis or ulceration, hind limb paralysis, bloating, or death.

2.6.2. Virus titer assay

2.6.2.1. Ocular viral titers assay. Samples were taken on days 1, 3, 5, 7, 9, and 12 from the scratched eye. To do so, the subjects were first anesthetized, using 5 mg ketamine hydrochloride, combined with 10 mg of xylazine, and then the infected cornea was flushed with 10 μ l of DMEM (3% serum). Next, 190 μl from DMEM (3% serum) was added to 10 μl of the rinsing solution. Samples were stored at -80 °C for further assessment. 10-fold serial dilutions were quantified on vero cells, using the standard plaque method [30].

2.6.2.2. Skin viral titer assay. To determine viral shedding before TP (containing 0.02 mg/kg of TP in DMEM) and 10-HCPT (containing 0.6 mg/kg of 10-HCPT in DMEM) and ACV (containing 0.1 mg/kg of ACV in DMEM) were administration to the skin samples on days 1, 3, 5, 7, 9, and 12 post-infection and then collected, using DMEM-wet swab. Each swab was placed in 200 μl of DMEM containing 3% serum, and stored at -80 °C for further evaluations. As previously explained, serial dilution of the samples was quantified, using the plaque method on vero cells [30].

2.6.2.3. Quantitative polymerase chain reaction (qPCR). The viral genomes were extracted from the swab samples, using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Basel, Switzerland) and quantified via real-time PCR, using an Applied Biosystem step one plus real-time PCR machine (Applied Biosystem, CA, USA). For HSV-1, the oligonucleotide primers and probes were used, as described in another study (Forward 5′- CCGTCAGCACCTTCATCGA-3′; Reverse 5′-CGCTGGACCTCCGTGTAGTC-3′ and probe 5′-CCACGAGATCAAGGACAGCGGCC-3′) [20].

2.6.2.4. Histopathology of skin and ocular tissues. The subjects were sacrificed on days 3, 5, 7, 9 and 12. The skin and ocular tissues were excised and fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Slides were examined for surface ulceration, leukocyte infiltration, and surface re-epithelialization in the cornea and skin.

2.7. Statistical analysis

The data were analyzed, using Graph Pad Prism version 5.01. Score differences between groups on the specified days were analyzed via One Way ANOVA (ANOVA), followed by Student-Newman-Keuls multiple-comparison test. Dunn's Multiple Comparison test and ANOVA were used to compare viral titers. Furthermore, Ocular diseases scores analysis was carried out, using one-way ANOVA Dunnett test.

3. Results

3.1. In vivo toxicity evaluation

During the pilot phase, the substance toxicity on the skin and cornea of the non-infected mice was investigated. None of the models exhibited any symptom like edema, erythema, and corneal opacity, or swelling at the site of topical application.

3.2. Antiviral activity effect on HSV-1 ocular disease in mice

This was done to determine, if TP or 10-HCPT could reduce herpes virus infection when applied topically to the cornea. To evaluate the ocular infection, the ocular HSV-1 infection of the infected mice treated with TP and the untreated group were followed visually on specific days. The virus-untreated eye showed signs of ocular infection at day 3 after infection, then symptoms worsened by days 7 and 12 (Figure 2a). In contrast, the signs of recovery were visible in treated group (Figure 2a). However, Blepharitis Score analysis did not reveal any significant reduction with respect to the severity of blepharitis amongst the treated groups. As for the severity of vascularization score assessment, there was a significant difference between the ACV and TP, when compared with the control group on days 9 and 12. Also, stromal disease score analysis revealed a significant difference between the infected treated groups on days 7, 9, and 12, when compared with the infected untreated group (Figure 2b). On days 5, 7, 9, and 12, the infected treated groups had significantly lower viral titers, when compared with the control group ($P = 0.0001$). however, the viral titers between the infected treated groups were not significantly different on day 7 (Figure 3a), and the titers between ACV and TP groups were not significantly different on days 3, 5, and 12, suggesting a similar antiviral activity of TP and ACV that begin much faster than 10-HCPT and lasted much longer. The virus DNA was examined, and the results showed that on day 3 there was no significant difference between the infected, the treated and the virus control groups. Nonetheless, on day

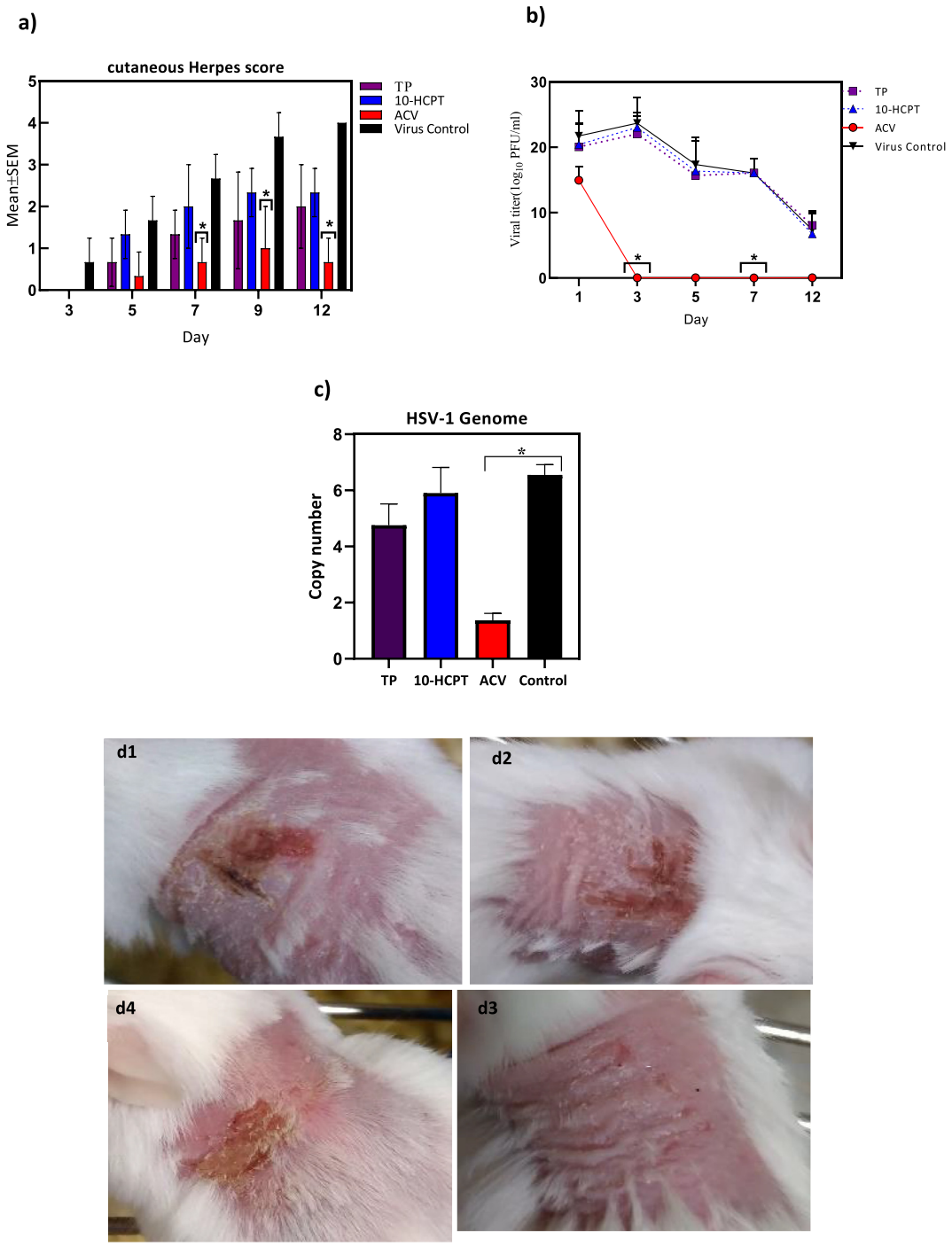

Figure 4. Antiviral Efficacy of TP and 10-HCPT topical treatment on HSV-1 cutaneous disease scores (a), virus titers (plaque reduction assay) (b) and HSV-1 DNA (qPCR on the day 12) (c). Mice ($n = 10$) had their corneas infected and treated as follows: Group 1, uninfected and treated with TP and HCPT solution as the negative control. Group 2, infected and untreated as the positive control. Group 3 treated topically with TP solution (containing 0.02 mg/kg of TP in DMEM), Group; 4, treated with 10-HCPT solution topically (containing 0.6 mg/kg of HCPT in DMEM). Group 5; treated with ACV solution (containing 0.1 mg/kg of ACV in DMEM), for 12 days (3 times per day). Statistical analysis (one-way ANOVA followed by the Student Newman-Keuls test) demonstrated significant differences among groups; *, $P < 0.05$. (d) Representative examples of cutaneous infection at day 8. (d1) Zosteriform lesion, group 4, score 4; (d2) zosteriform healing lesion, group 3, score 3; (d3) spreading ulceration, group 5, score 3; (d4) healing ulcers, group 5, score 2.

12, DNA synthesis was significantly lower in the TP and ACV groups in comparison to 10-HCPT and virus control groups (Figure 3b). Hence, TP exhibited better antiviral properties than 10-HCPT at the tested concentration against HSV-1 ocular infection.

3.3. Anti-HSV-1 activity on cutaneous herpes infection

The activity of TP and 10-HCPT against HSV was evaluated by scratching the subjects. However, there was no significance difference in

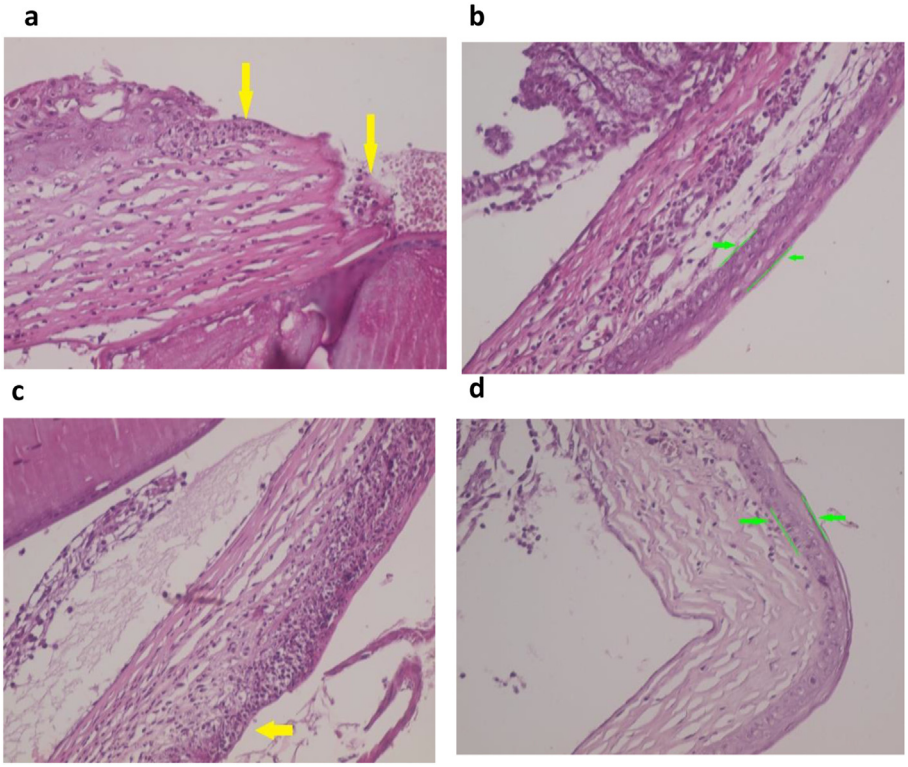

Figure 5. Representative photomicrography of corneal tissue sections. Group 2 (infected, untreated) showing denuded epithelium (a) (yellow arrow); (b,c,d) treatment groups showing healing of corneal surface (green arrowhead). Slides were stained with H&E; (magnification ×400). a; virus control, b; TP, c; 10-HCPT, d; ACV.

disease scores between the treated groups and the control during the study days (Figure 4a). The differences were merely significant in ACV groups on days 7, 9 and 12. A swab sampling was performed to measure the viral titer and genome load of the infection site on days 3, 5, 7, 9, and 12. A significant difference in virus titers (Figure 4b) and DNA yield (Figure 4c) was observed only in ACV group in comparison to the virus controls.

3.4. Histopathology results

3.4.1. Anti-HSV activity of TP and 10-HCPT in cutaneous and corneal tissue of HSV-1 infected mice

Sections from the virus control tissue (group 2) revealed keratitis with crust formation as well as inflammatory cell infiltration consisting of neutrophils and mononuclear cells. In the mice treated with 10-HCPT, healing of corneal epithelium was partially detectable, which gradually increased in the TP treated group. Re-epithelialization was observed in the ACV treated subjects. The results are shown in Figure 5.

Figure 6, depicts the skin tissue of an infected untreated animal (group 2), exhibiting surface ulceration with crust formation, in addition to inflammatory cell infiltration, comprising of neutrophils and mononuclear cells along with multinucleated giant cells. In the infected mice that were treated with 10-HCPT, skin epithelium healing was not observed. On the contrary, in TP and ACV treated groups, re-epithelialization was clearly better in ACV subjects.

4. Discussion

In the present study, the anti-HSV effect from two natural products (TP and 10-HCPT) on ocular and cutaneous HSV-1 infections in a mouse model was investigated by assessing the clinical severity scores, histopathology, viral titer, and genome load. Hence, novel approaches might be able to pave the way in dealing with the rise of drug-resistant strains of HSV [32, 33]. Increase in the number of immunodeficient patients as well as long-term treatment can lead to surge of such drug-resistant strains within the human population [17]. Resistance to acyclovir, which is the standard treatment for HSV infections was reported in 5–14 % of BMT patients [34]. Finding new anti-HSV agents that have different mechanisms with lower rate of resistance and side effects is vital to improve the clinical management of HSV infections [20]. A previous study on topical application of a natural product called MI-S (A. brasiliensis mycelium, chemically modified by sulfation) could not reach a favorable results on the ocular and cutaneous model of mice, which was assessed, using the clinical diseases severity score and viral titers [35]. Both the Tp and 10-HCPT had acceptable anti-HSV effects *in vitro*, which was assessed by genome load and viral titer. They mainly acted in the first stage of virus replication ([36] and Unpublished data of our previous study); consequently, these two agents have different mechanisms of action from ACV. Thus, it seem to serve as proper agents for ocular and cutaneous infections in animal models.

In comparison with anti HSV-1 effect of acyclovir, TP and 10-HCPT exhibited similar effects on HSV-1 ocular infection by improving stromal disease score while reducing the viral titer. Topical application of 10-HCPT in the cornea post infection failed to reduce the vascularization disease severity, and genome load, but improved histopathological changes. The lower anti HSV effect of 10-HCPT compared to TP can be explained by lower absorption and distribution; however, both had similar anti-HSV effects *in vitro* [36]. Brandt, C.R., et al., examined the effect of retro-cyclin (RC)-2, a synthetic ɵ-defensin, on an ocular mouse model, and reported that by incubating the virus with RC-2 or applying the peptide in 2% methylcellulose to the cornea before viral infection can significantly reduce the severity of ocular disease [30]. Such measures were effective in activating the compound against the virus. Further formulation and pharmaco-kinetic studies in animal models can lead to a more promising results for HCPT in ocular infection [35]. In the present study, TP usage led to the reduction of vascularization and stromal

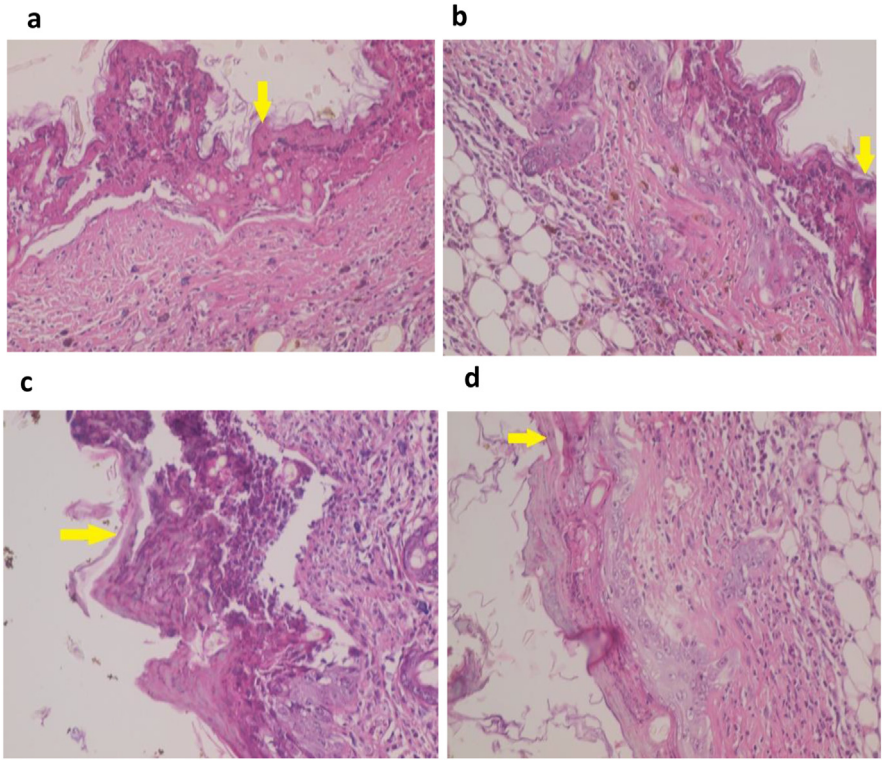

Figure 6. Representative photomicrography of skin tissue sections. In HSV infected group complete surface ulceration was present in group 2 (a); the re-epithelialization of skin was gradually observed in treatment groups (b,c and d). The better results belonged to ACV treated group. Slides were stained with H&E; (magnification ×400). yellow arrow; area of surface ulceration, a; control, b; TP, c; 10-HCPT, d; ACV.

disease severity, which is similar to ACV after one week post-infection, suggesting that topical administration of TP can accelerate the healing process of a lesion. The viral titers in ACV and TP groups revealed that the effect of TP as a viral inhibitor can lead to better clearance of the virus from the eye.

In the cutaneous model, TP and 10-HCPT did not exhibit antiviral effects in reducing the severity of clinical symptoms during the post-infection days; in addition to no evident histopathological and virological changes. The promising anti-HSV effect of TP *in vitro* and on the ocular infections of mice, lack of anti-HSV-1 effect on the mice skin model can be attributed to lower absorption and distribution of these compounds in the skin in comparison to the cornea [35]. If a virus is appropriately incubated with compounds (pre-infection), or if another route of administration (for example *per os*) is applied, it might yield better antiviral effects. Brandt, C.R., et al., observed better anti-HSV effect of retro-cyclin (RC)-2, a synthetic ɵ-defensin, on an ocular mouse model after incubating the virus with RC-2 or applying the peptide in 2% methylcellulose to the cornea before viral infection [30]. Cardozo, F.T., et al., reported no anti-HSV effect of MI-S on the skin lesions of infected mice, but reported that the disease was significantly reduced after its oral administration [35]. Hence, TP and 10-HCPT require further investigation by incubating the virus with these compounds pre-infection or applying them orally in the animal models.

5. Conclusions

We revealed a promising anti-HSV-1 effect of TP and 10-HCPT *in vivo* for the treatment of ocular in mouse model, which was supported by histopathological and virological findings. In contrast, the anti-HSV effects of TP and 10-HCPT were not supported by the clinical, virologic and histopathologic evidence. Hence, further studies are warranted to confirm the promising effects of TP and 10-HCPT on ocular models while assessing the effect of other measures, such as oral administration to improve bioavailability of the compound on the site of infection.

Declarations

Author contribution statement

Nasrin Aliabadi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marzieh Jamalidoust: Conceived and designed the experiments; Analyzed and interpreted the data.

Gholamreza Pouladfar: Conceived and designed the experiments; Wrote the paper.

Negar Azarpira; Atoosa Ziyaeyan: Analyzed and interpreted the data.

Mazyar Ziyaeyan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

The datasets used in the study are not publicly available due to protecting the participants' anonymity but are available on reasonable request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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