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SHORT COMMUNICATION

Anti-herpes activity of polysaccharide fractions from *Stevia rebaudiana* leaves

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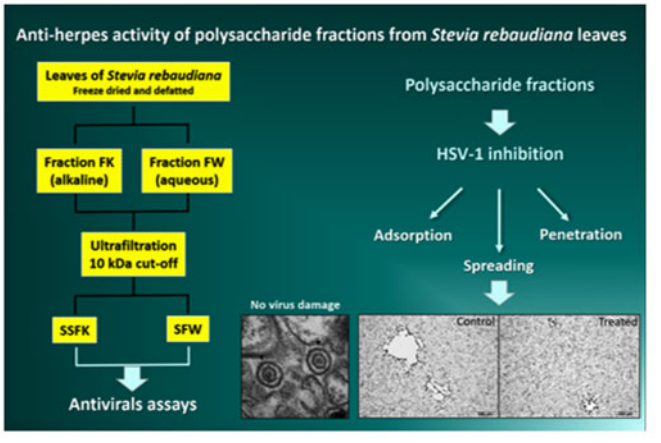
ABSTRACT

The antiviral potential of natural polysaccharide compounds has been demonstrated, especially against enveloped viruses and members of the *Herpesviridae* family. Two polysaccharide fractions obtained from *Stevia rebaudiana* (Bertoni) leaves, that were active against Herpes simplex virus type 1 (HSV-1) were studied to investigate their mode of action. Both polysaccharides - SFW (crude faction) and SSFK (homogeneous alkaline fraction) - exerted antiviral effects on the initial stages of HSV-1 infection by inhibiting viral adsorption and penetration. When added after virus internalization, both fractions decreased plaque size. The effect of the fractions was confirmed by investigating viral glycoprotein expression. Based on the mode of action of the polysaccharides demonstrated in the present work and on their selectivity index, the polysaccharides obtained from *S. rebaudiana* could be an alternative treatment of infections caused by HSV-1.



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1. Introduction

Herpes simplex virus type 1 (HSV-1) is a double-strand DNA-enveloped virus that belongs to the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family. The symptomatic disease that is caused by HSV-1 is typically limited to oral lesions and keratitis in the eyes (Shukla and Spear 2001). More than 80% of adults have latent infection (Akhtar and Shukla 2009), and although inhibitors of the DNA polymerase of HSV (p. e. acyclovir) are the drugs of choice for treatment of the infection, the infection are not eliminated. Among the natural antiviral agents that have been studied, polysaccharides have shown potent activity against several enveloped viruses, including members of the *Herpesviridae* family, by interfering with the early stages of virus infection, particularly adsorption. Many studies have indicated that anionic polysaccharides that are derived from plants can inhibit HSV by competing with virus-cellular receptor interactions, interacting with positive charges of the virus or interacting with the cell surface, thereby preventing penetration of the virus into the host cell (Witvrouw and De Clercq 1997; Damonte et al. 2004). Such anionic polysaccharides can also promote beneficial effects after viral penetration (Martinez et al. 2005).

Stevia rebaudiana is a perennial herbaceous plant of the *Asteraceae* family, (Soejarto et al. 1983). A previous study reported the anti-HSV-1 activity of two polysaccharide fractions that were isolated from *S. rebaudiana*: homogeneous alkaline fraction (SSFK) and crude fraction (SFW) (de Oliveira et al. 2013). The present study investigated the ways in which these fractions influence HSV-1 interactions with host cells *in vitro*.

2. Results and discussion

As described in previous work which afforded the polysaccharides used in this work (de Oliveira et al. 2013), the SSFK and SFW fractions inhibited HSV-1 infection in Vero cells *in vitro*, with EC_{50} values of 18.8 μg/ml (selectivity index [SI] > 53) and 0.3 μg/ml (SI = 917), respectively. Low toxicity of these polysaccharides was also observed, in which the cytotoxic concentrations (CC_{50}) of the SSFK and SFW fractions were >1000 μg/ml and 275 μg/ml, respectively. The present study further investigated the ways in which the fractions exert their actions during the life cycle of HSV-1.

The plaque reduction assays showed that the polysaccharide fractions inhibited the initial stages (adsorption and penetration) of viral infection (Figure S1). The best EC_{50} values were obtained with treatment during the adsorption phase: 6.2 μg/ml (SI >161) for the SSFK fraction and 11.5 μg/ml (SI = 23.9) for the SFW fraction. Treatment during the adsorption and penetration phases was also effective, with EC_{50} values of 11.1 μg/ml (SI >90) for the SSFK fraction and 31.3 μg/ml (SI = 8.8) for the SFW fraction. The kinetics penetration assay was performed to confirm the effect of the polysaccharides on the penetration phase. In this assay, the polysaccharides were tested at a concentration of 100 μg/ml, and both inhibited penetration by ~70% within 30 min of incubation. After 60 and 90 min, inhibition reached ~100% (data not shown). Both polysaccharides exerted virucidal effects at a higher concentration than in the adsorption and penetration assays, with EC_{50} values of 56.0 and 60.0 μg/ml for the SSFK and SFW fractions, respectively, indicating that the polysaccharides blocked infection.

The polysaccharides did not have any effects when they were added to Vero cells 24 h before HSV-1 infection.

Polysaccharides are mimetic to heparan sulfate, a cellular receptor, which is required for virus adsorption and penetration into the cell (Aguilar et al. 1999; Ekblad et al. 2010). In the presence of the polysaccharides, the virus appeared to bind to these molecules rather than the cellular receptors. Therefore, the polysaccharides had competitive actions (Cheshenko and Herold 2002).

Treatment with the SSFK fraction after entry of the virus into the cell (i.e., treatment after infection) did not inhibit lysis plaque formation, but the size of the plaques that were formed was visually smaller than the virus control. This reduction of plaque size also appeared with treatment with the SFW fraction. An additional assay for evaluation of viral spread was performed. As a result, a reduction of the size of plaques and even the absence of plaques were observed. Both polysaccharides of *S. rebaudiana* inhibited viral spread. The SFW fraction had the best results, with an approximately 70% reduction of the size of lysis plaques. The SSFK fraction decreased the size of lysis plaques by ~42% at a concentration of 50 μg/ml. Treatment with heparin, SSFK and SFW at 100 μg/ml completely inhibited viral spread (Figure S2). Low-molecular-weight polysaccharides appear to inhibit the lateral spread of infection more efficiently (Ghosh et al. 2009) . This may explain the better results for the SFW fraction, which had a lower molecular weight, compared with the SSFK fraction (de Oliveira et al. 2013).

Anionic compounds are able to interact with viral glycoproteins. These glycoproteins are positively charged and are involved in attachment of the virus particles to negatively charged cell membrane constituents, such as heparan sulfate proteoglycans (Neyts et al. 1992). *S. rebaudiana* polysaccharides are negatively charged, similar to heparin although non-sulfated. This suggests that the SSFK and SFW fractions influenced the interaction between HSV-1 and the heparan sulfate cellular receptor.

The Western blot analysis of viral surface glycoproteins showed that treatment with 100 μg/ml of the SSFK and SFW fractions during the adsorption phase (Figure S3(a)) or after infection (Figure S3(b)) reduced the expression of gB, gC, and gD. Treatment throughout infection, covering both the entry and post-infection phases, completely inhibited the expression of viral surface glycoproteins (Figure S3(c)). When comparing the results of the viral glycoproteins expression during the adsorption and post-infection phases (although the SFW fraction inhibited viral adsorption – Figure S1), the best results were seen with post-infection treatment. This suggests that the continued presence of the polysaccharide, covering all stages subsequent to virus entry into the cells, was more effective than the initial treatment only. The smaller number of PFU in the post-infection assay (Figure S1) and inhibition of the lateral spread of cell-to-cell infection (Figure S2) reinforce this possibility.

To investigate the effect of the polysaccharides on virus-cell interactions and virus particles themselves, the samples were analyzed by TEM (Figure S4). We observed no morphological alterations of the virus particles after treatment with 100 μg/ml of the SSFK fraction, in which the virus particles had structural aspects that were similar to the untreated negative control. Unlike the work done with the cholestanol-conjugated sulfated oligosaccharide against HSV-2, in which the treatment resulted in disruption of the HSV-2 particles (Said et al. 2016).

In summary, both polysaccharide fractions had anti HSV-1 activity, which was more pronounced for the SFW fraction. The inhibition of viral adsorption, penetration, and lateral spread and the virucidal effect suggest that this activity was directly related to interactions between Stevia-derived polysaccharides and viral glycoproteins and not cellular receptors. The good SIs that were obtained in ours assays demonstrated that polysaccharides should be further investigated as treatment for infections that are caused by HSV-1. Even if such treatment does not eliminate latent HSV-1, it may prevent viral spread as soon as the first symptoms appear.

Dislcosure statement

No potential conflict of interest was reported by the authors.

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