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Full paper

A novel rare sugar inhibitor of murine herpes simplex keratitis

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ABSTRACT

Purpose: To determine the therapeutic efficacy of a novel rare sugar, L-psicose, for the treatment of HSV-1 induced herpetic stromal keratitis (HSK) in a mouse eye model.
Methods: One rare sugar L-psicose was assayed for HSV-1 inhibition of *in vitro* virus adsorption. The IC_{50} and IC_{90} values of L-psicose were determined using plaque reduction assay (PRA) in CV-1 cell. Female Balb/c mice were corneally infected with HSV-1, strain KOS-GFP; A topical eye drop treatment of L-psicose was started 24 h after infection and continued four times daily for ten consecutive days. The severity of HSK was monitored by slit lamp examination in a masked fashion and Infectious HSV-1 shedding was determined by PRA.
Results: L-psicose was found to have anti-viral activity *in vitro* at an IC_{50} dose of 99.5 mM and an IC_{90} dose of 160 mM. Topical eye drop treatment with 200 mM L-psicose in PBS solution significantly reduced the severity of HSK compared to the mock treatment group. The *in vivo* mouse ocular model results of L-psicose therapy correlated with accelerated clearance of virus from eye swabs.
Conclusion: The results suggest that topical treatment with rare sugar L-psicose has efficacy against HSK through inhibition of HSV-1.

1. Introduction

Monosaccharides are essential component of cell surface receptors and play a very important role in cell signaling pathways (1). As a result, carbohydrates that can disrupt binding of molecules to the cell surface receptors or interrupt cell-signaling pathways may have important therapeutic potentials (2).

On the basis of availability we can classify monosaccharide into two groups, natural and rare. According to International Society of Rare Sugars (ISRS) rare sugars are monosaccharides and their derivatives that rarely exist in the nature. The practical application or usefulness of rare carbohydrates has not been well investigated because of high costs and unavailability. However, because of the recent introduction of "Izumoring" schemes (3) it is now possible to produce many of these rare monosaccharides in large quantities by using microbial and enzymatic transformations. As a result, functional studies of rare sugars have gained momentum in recent years. Utilization of rare sugars for various purposes still awaits extensive exploration. Despite their costs and low availability, these rare carbohydrates are very important since they have the potential for use in many areas such as food additives, diet sugars, antioxidants, antiviral agents, nucleoside analogs, glycosidase inhibitors, and anticancer agents (4–11). In a previous study Muniruzzaman et al found that some simple rare ketoses (L-Xylulose and L-Fructose) were specific inhibitors of alpha-glucosidase and glucosidase *in vitro* and *in vivo* (4). This study also reported that some rare sugars inhibited the removal of glucoses from N-linked oligosaccharides of the influenza viral hemagglutinin in the cell culture systems. Activities of glucosidases are required for the right processing of glycoproteins so that correct folding and sorting can be maintained (12). Our knowledge about the physiological effects of rare carbohydrates is very limited. In a previous study, since some rare keto-sugars appear to be inhibitory to glycoprotein processing enzymes (4), it was of

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interest to screen ketohexoses to determine whether any of them would inhibit the entry of HSV1 to cell. Among the eight ketohexoses D-tagatose, L-tagatose, D-psicose, L-psicose, D-sorbose and L-fructose are considered to be rare as they are found in nature very low quantities. We screened all these ketohexoses (Fig. 1) *in vitro* for their anti-HSV-1 activity and found L-psicose to have relatively strong activity. In the present study, we show that L-psicose is effective in inhibiting HSV-1 *in vitro*. The mechanism of HSV-1 inhibition by L-psicose is mediated through blocking of viral adsorption to cell surface. We also show that *in vivo*, L-psicose is effective in inhibiting HSV-1 induced corneal opacity and ocular virus shedding in mouse eye model.

2. Materials and methods

2.1. Antiviral compounds

All ketohexoses used in this study was produced in Dr. Izomuri's lab. Anti-viral peptide apoEdp was commercially synthesized (GeneMed, Texas, US) and used as described before (13). ApoEdp is a short 18 aa peptide derived from receptor binding region of human apolipoprotein E effectively inhibits HSV-1 adsorption *in vitro* and herpetic stromal keratitis (HSK) *in vivo* (13).

2.2. Cells, viruses and treatment

CV-1 cells (African green monkey kidney cells) were purchased from American Type Culture Collection, Manassas, VA) were propagated in Eagle's minimum essential medium (EMEM) containing 0.15% Na_2CO_3 supplemented with 10% fetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100 mg/ml). HSV-1 strain KOS-GFP (gift from Timothy P Foster, LSU Health Sciences Center, New Orleans, LA, USA) was used and titrated in CV-1 cells.

2.3. Virus adsorption assay

CV-1 cells prepared with a final density of 4×10^6 cells/mL added with HSV-1 KOS-GFP at 1 PFU (Plaque forming Units)/cell and varying concentrations of L-psicose was incubated at 4 °C for 1 h to allow adsorption but to prevent penetration. Following adsorption, unbound virus and sugar was removed by washing three times with cold PBS (4 °C). Cell pellets were serially diluted and plated on 24-well monolayer of CV-1 cells with an overlay of 0.5% methylcellulose. Following 48 h of incubation at 37 °C, cells were then fixed with 5% buffered formalin (5% formalin in PBS) and stained with 0.05% crystal violet. Positive control well did contain apoEdp peptide and performed in parallel. The number of HSV-1 infected plaques was counted for each concentration of L-psicose or peptide and for no drug controls. The inhibitory effect of the L-psicose on plaque reduction was calculated by following formula. Percentage inhibition (number) = (1 − plaque number in antiviral well/plaque number in no drug control well) × 100. The 50% and 90% dose of inhibitory concentration was hand calculated from the dose response curves generated from the data (14). For each experiment, three independent replicates were performed.

2.4. Mice

For the animal experiment, Xavier University of Louisiana Institutional Animal Care and Use Committee approved procedure was followed. We used female C57BL/6 (Taconic Farms, NY) from 5 to 6 weeks of age.

2.5. Ocular infection

Mice were anesthetized using a mixture of Xylazine (6.6 mg/kg of body weight) and Ketamine (100 mg/kg) through intraperitoneal route of injection. Scarification of eyes was performed in a 2X2

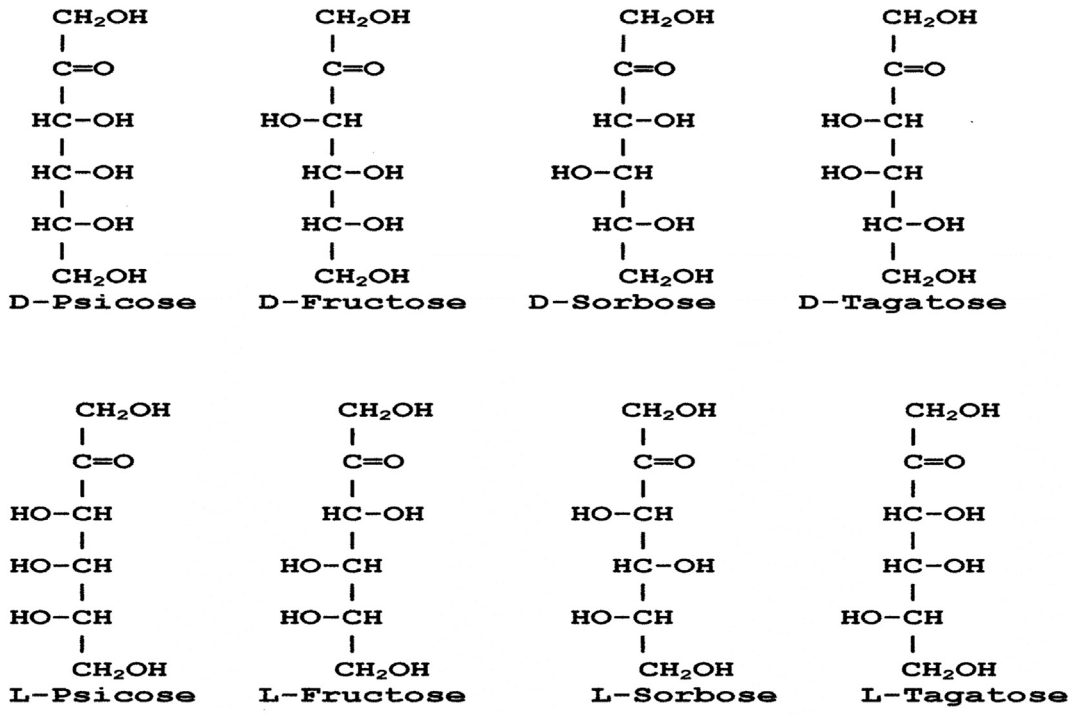

Fig. 1. Structure of eight ketohexoses screened in this study for anti-HSV-1 activity.