Modified cyclodextrins as broad-spectrum antivirals

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Abstract

Viral infections kill millions of people and new antivirals are needed. Nontoxic drugs that irreversibly inhibit viruses (virucidal) are postulated to be ideal. Unfortunately, all virucidal molecules described to date are cytotoxic. We recently developed nontoxic, broad-spectrum virucidal gold nanoparticles. Here, we develop further the concept and describe cyclodextrins, modified with mercaptoundecane sulfonic acids, to mimic heparan sulfates and to provide the key nontoxic virucidal action. We show that the resulting macromolecules are broad-spectrum, biocompatible, and virucidal at micromolar concentrations in vitro against many viruses [including herpes simplex virus (HSV), respiratory syncytial virus (RSV), dengue virus, and Zika virus]. They are effective ex vivo against both laboratory and clinical strains of RSV and HSV-2 in respiratory and vaginal tissue culture models, respectively. Additionally, they are effective when administrated in mice before intravaginal HSV-2 inoculation. Lastly, they pass a mutation resistance test that the currently available anti-HSV drug (acyclovir) fails.

Reference


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Section S1. Synthesis, characterization, and assays

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S.1.1 Mass Spectroscopy.

Mass spectrometry analyses were performed on a LTQ Orbitrap Elite FTMS instrument (Thermo Scientific, Bremen, Germany) operated in the negative mode coupled to a modified HESI-II probe in an Ion Max ion Source able to perform Cold-spray ionization (CSI). Cold-spray ionization is an ESI-MS modification operated at very low temperature that can be used to prevent decomposition of labile molecules that are difficult to observe by conventional MS techniques. The experimental conditions for the ionization voltage were -1.2kV. The temperature of ion transfer capillary was 60°C, tube voltages. Sheath and auxiliary gases were 35 and 10 respectively to obtain a spray temperature around +5°C. The infusion rate was 5 µl/min. FTMS spectra were obtained in the 80-1000 m/z range in the reduce profile mode with a resolution set to 120,000 and automatic gain control (AGC) value set at 10^6. A total of 100 scans each consisting in 10 µscans were acquired in reduced profile mode and averaged.

S.1.2 CE-UV.

Synthesised compounds were analysed by capillary electrophoresis (CE) with UV detection (CE-UV) following the protocol described by the United States Pharmacopeia (USP) and the National Formulary (NF) with minor modifications (Betadex Sulfobutyl Ether Sodium USP NF 33, Official Monograph, Betadex 6546-6550). CE-UV experiments were carried out using a 7100 A CE apparatus (Agilent, Waldbronn, Germany) and untreated fused silica separation capillary (BGB analytik AG, Böckten, Switzerland) of 50 µm i.d., 40.5 and 49 cm of effective and total length, respectively. Background electrolyte (BGE) was 30 mM benzoic acid with pH 8 adjusted by addition of 100 mM Tris solution. Prior to sample analysis each new capillary was conditioned by flushing for 10 min with 1 M NaOH, followed by 5 min flushing with 0.1 M NaOH and then 5 min with milliQ H₂O. CE-UV analysis program consisted of the following steps: a) flushing with 0.1 M NaOH for 2 min; b) flushing with milliQ H₂O for 2 min; c) flushing with BGE for 2 min; d) sample injection for 5 sec with 40 mbar pressure; e) application of 30 kV separation voltage for 10 min. UV signal was monitored at 230.0 nm wavelength. Peak assignment was performed with captisol as a reference compound taking into account capillary-to-capillary migration time variation.

S.1.3 Molecular dynamics simulations.

Atomistic molecular dynamics (MD) simulations were performed of cyclodextrin (CD) interacting with Herpes Simplex Virus (HSV) Glycoprotein B (gB). Three different βCDs were simulated (CD1, CD2 and CD3). The structure of gB was based on the structure with pdb ID
5FZ2, each protein unit of the tetramer contained residues 142 to 476, and the missing atoms in the structure were added with the VMD plugin psfgen.

In the simulations, 10 CD molecules were initially placed near the fusion loops of gB and solvated in a 0.15 M NaCl solution. The key amino acid residues of gB fusion loop that are responsible for the cell fusion activity are known (Trp 174, Phe 175, Gly 176, Tyr 179, Ala 261, His 263, Arg 264). Unit cells of the simulated systems, containing CDs and gB in aqueous solution, had in total between 400,000 and 500,000 atoms.

The CD ligands were described with the CHARMM general force field and proteins were described with the CHARMM36 protein force field. The simulations were performed with NAMD2.12. The particle-mesh Ewald (PME) method was used for the evaluation of long-range Coulomb interactions. The time step was set to 2.0 fs, and long-range interactions were evaluated every 1 (van der Waals) and 2 timesteps (Coulombic). After 2,000 steps of minimization, ions and water molecules were equilibrated for 2 ns around gB and CDs, which were restrained using harmonic forces with a spring constant of 1 kcal/(mol Å²). For all the systems, the last frames of restrained equilibration were used to start simulations of partially restrained proteins (restraints were placed on the atoms of the bottom part of the red domains in Figure 1) and free CDs. All the simulations were performed for 50 ns in the NPT ensemble (pressure p = 1 bar and temperature T = 300 K), using the Langevin dynamics (γLang = 1 ps-1).

S.1.4 Cholesterol replenishment.

Vero cells were seeded in 24-well plates. The following day mixtures of CD1 (100 µg/ml) or methyl-beta-CD (3 mg/ml) and HSV-2 were incubated for 1h at 37 °C and subsequently subjected to cholesterol replenishment. 1 mM of cholesterol or the equal volume of ethanol were added for 30 minutes at 37 °C. After they were added for 2 hours at 37 °C on Vero cells. The inocula were subsequently removed from the wells, and the cells were washed with medium twice and overlaid with a medium containing 1.2% methylcellulose. After 24 hours cells were stained and plaques were counted in order to determine % of infection.

S.1.5 Lactate dehydrogenase assay (LDH).

LDH release in basal medium was measured with the Cytotoxicity Detection Kit (Roche 04744926001).

S.1.6 ELISA.

Interleukin-6 (IL-6), CXC motif chemokine 10 (CXCL10 or IP-10), CC motif chemokine5 (CCL5 or RANTES), interleukin-8 (IL-8 or CXCL-8) and interferon lambda (IL-29 /IL-28B) were measured in the basal and apical medium by ELISA (R&D DY206-05, DY266-05, DY278-05, DY208-05 and DY1598B-05) following everyday treatment with different concentrations of CD 1.
Fig. S1. Reaction scheme overview of the synthesized modified CDs.
Fig. S2. Characterization of CD1. a) HRMS Cold-Spray Ionisation spectrum of CD1 in EtOH/H2O (50:50), b) HRMS Summary of CD1 in EtOH/H2O (50:50). Values correspond to the monoisotopic mass and c) CE-UV electropherogram of CD1 at 230 nm with assigned CD species showing 31% of 6 and 69% of 7 sulfonates per CD.
Fig. S3. Characterization of CD2. a) HRMS Cold-Spray Ionisation spectrum of CD2 in EtOH/H2O (50:50), b) HRMS Summary of CD2 in EtOH/H2O (50:50). Values correspond to the monoisotopic mass and c) CE-UV electropherogram of CD2 at 230 nm with assigned CD species showing 6% of 3, 32.7% of 5 and 61.3% of 7 sulfonates per CD.
Fig. S4. Characterization of CD3. a) HRMS Cold-Spray Ionisation spectrum of CD3 in EtOH/H2O (50:50), b) HRMS Summary of CD3 in EtOH/H2O (50:50). Values correspond to the monoisotopic mass and c) CE-UV electropherogram of CD3 at 230 nm with assigned CD species. Integration was not possible due to low intensity of peaks.
**Fig. S5. Cholesterol replenishment.** CD1 or methyl-beta-CD were incubated for 1 h at 37 °C with HSV-2. Following this incubation 1 mM of cholesterol or the corresponding volume of ethanol were added to the samples for 30 min. The mixtures was then added on cells and the infectivity evaluated through plaque assay. Results are mean and SEM of 2 independent experiments.

**Fig. S6. Viral yield reduction assay of DENV-2.** BHK-21 were infected with DENV-2 at a MOI of 0.1, 2 hpi the virus was washed out and serial dilutions of CD1 were added. Supernatants were collected 48 hpi and titrated by TCID50. Results are mean and SEM of 2 independent experiments. UT, untreated. **** p<0.0001 *** p<0.0005
Fig. S7. Virucidal activity and ex vivo inhibition against ZIKV. a) Virucidal activity of CD1 against ZIKV: ZIKV was incubated with CD1 (30 µg) for 1h and then serially diluted on Vero cells and b) ZIKV inhibition in vaginal tissues: Tissues were infected in presence or absence of 100 µg/ml of CD1. After 4 h the inocula were removed and tissues were washed extensively. Every 24 h after that, 200 µl of culture medium was applied apically for 30 min at 37°C for collection of apically released viruses. RNA copies were evaluated with qPCR. Results are mean and SEM of 2 independent experiments.

Fig. S8. Viral inhibition in the presence or absence of serum. a) HSV-2 and b) RSV-A inhibition assays were conducted in presence of 2.5% FBS as in all the other experiments presented in the paper or in presence of serum-free medium. Results are mean and SEM of 2 independent experiments.
**Fig. S9. Simulations of CDs with gB fusion loops.** a) Fusion loops of gB protein (circled green) with inter-loop distances (d1, d2 and d3) identified. b) Averaged binding energy of CD molecules to gB fusion loop (energy values are reported per single CD molecule; smoothing of data was performed by adjacent averaging method).
Fig. S10. Toxicity of CD1 in respiratory tissues. a-d) Cytokine/chemokine levels by ELISA assays a) in basal medium, 2 days post a single administration of the indicated concentration of CD1 b) in basal medium four days after daily treatment with the indicated concentration of CD1 c) in apical sample collected 2 days post a single administration of the indicated concentration of CD1, d) in apical sample collected four days after daily treatment with the indicated concentration of CD1. e) Transepithelial electrical resistance (TEER) of tissues measured at the indicated time post daily treatment with the indicated concentration of CD1. f) LDH release every 24 h after treatment.