The Human NEU1-Selective Sialidase Inhibitor, GSC-649, Blocks NEU1-Mediated Bioactivities in Human Airway Epithelia and Lung Microvascular Endothelia

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ABSTRACT

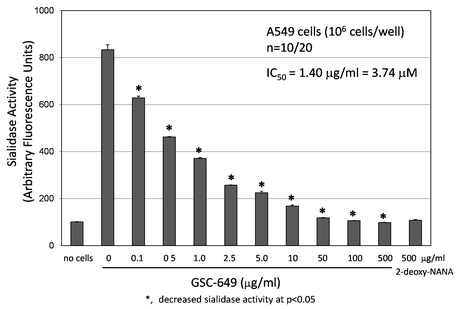
We have previously reported that NEU1 is the predominant sialidase expressed in both human airway epithelia (E.P. Lillehoj *et al*; *J. Biol. Chem.* 287:8214-8231, 2012) and human lung microvascular endothelia (A.S. Cross *et al*; *J. Biol. Chem.* 287:15966-15980, 2012). In airway epithelia, we found that *Pseudomonas aeruginosa* (Pa)–derived flagellin engages the MUC1-ectodomain (ED) to increase NEU1 association with and desialylation of MUC1-ED. NEU1-mediated MUC1-ED desialylation increased both its adhesiveness for flagellin-expressing Pa and its shedding from the airway epithelial surface. In postconfluent human lung microvascular endothelia, we found that NEU1 was recruited to and desialylated CD31 (C. Lee *et al*; *J. Biol. Chem.* 289:9121-9135, 2014). NEU1 restrained endothelial cell migration into a wound and disrupted CD31-driven capillary-like tube formation, i.e. *in vitro* angiogenesis. We have designed and synthesized the only reported selective inhibitor of human NEU1 sialidase (S. Magesh *et al*; *Biorg. Med. Chem. Lett*. 18:532-537, 2008). We asked whether this inhibitor, GSC-649, might inhibit one or more established NEU1-mediated bioactivities in human lung cells. In human airway A549 cells, we established the IC_{50} for GSC-649 for total sialidase activity to be 3.74 μM. We found that GSC-649 dose-dependently inhibited flagellin-induced NEU1-mediated MUC1-ED desialylation, increases in Pa adhesion, and MUC1-ED shedding. In human lung microvascular endothelia, we established the IC_{50} for GSC-649 for total sialidase activity to be 13.0 μM. We found that GSC-649 completely reversed NEU1-mediated restraint of endothelial cell migration into a wound and counter-acted NEU1-driven disruption of capillary-like tube formation. Our combined data indicate that the NEU1-selective sialidase inhibitor, GSC-649, inhibits multiple NEU1-mediated bioactivities in both human airway epithelia and human lung microvascular endothelia.

METHODS

- Synthesis of NEU1-Selective Sialidase Inhibitor, GSC-649
- Human Airway Epithelial Cell and Lung Microvascular Endothelial Cell Cultures
- Fluorometric Assay for Sialidase Activity
- Purification of *P. aeruginosa* Flagellin
- Peanut Agglutinin (PNA) Lectin Blotting of Desialylated MUC1-ED
- Bacterial Cultures
- Bacterial Adhesion
- ELISA for MUC1-ED
- Migration in a Wounding Assay
- *In Vitro* Endothelial Cell Capillary-like Tube Formation Assay

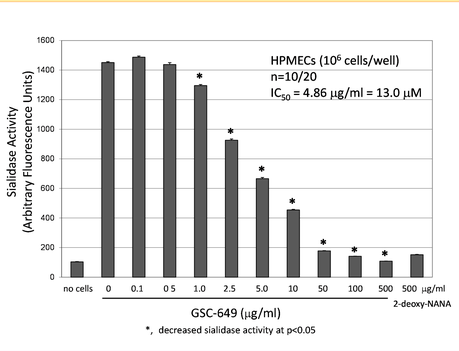
RESULTS

Fig. 1. GSC-649 Inhibits Sialidase Activity in Human Airway Epithelia.



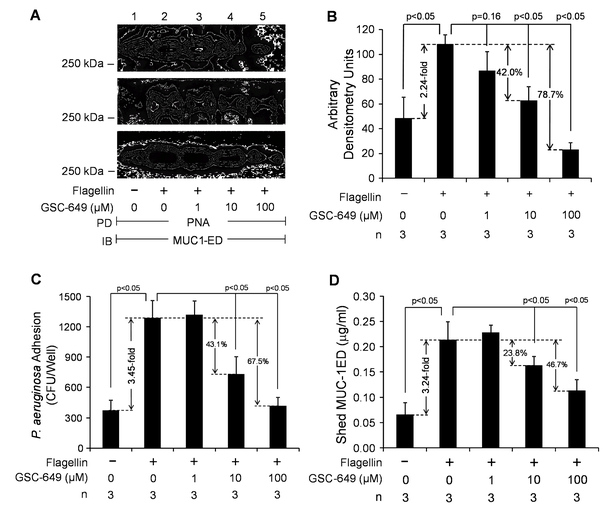
A fixed number of A549 cells were assayed for sialidase activity for the fluorogenic substrate, 4-MU-NANA, in the presence of increasing concentrations of the NEU1-selective sialidase inhibitor, GSC-649. Vertical bars represent the mean (± SE) sialidase activity expressed as arbitrary fluorescence units. The n for each experimental and control group is 10/20. * significantly decreased compared with sialidase activity in total A549 cell lysates in the absence of GSC-649 at p<0.05.

Fig. 2. GSC-649 Inhibits Sialidase Activity in Human Lung Microvasular Endothelia.



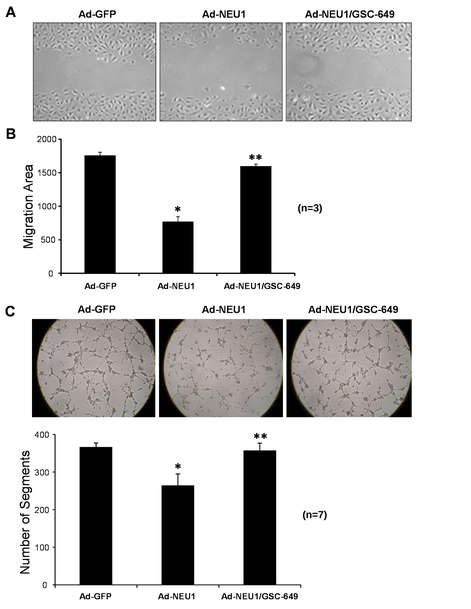
A fixed number of HPMECs were assayed for sialidase activity for the flurogenic substrate, 4-MU-NANA, in the presence of increasing concentrations of the NEU1-selective sialidase inhibitor, GSC-649. Vertical bars represent the mean (±SE) sialidase activity expressed as arbitrary fluorescence units. The n for each experimental and control group is 10/20. *, significantly decreased compared with sialidase activity in total HPMEC lysates in the absence of GSC-649 at p<0.05.

Fig. 3. GSC-649 Inhibits Sialidase Activity in Human Lung Microvasular Endothelia.



(A-D) A549 cells were incubated for 30 min with 10 ng/ml of Pa flagellin or medium alone in the presence of increasing concentrations of GSC-649. (A) The cells were lysed and the lysates were incubated with PNA-agarose and the PNA-binding proteins processed for MUC1-ED immunoblotting. MW in KDa in indicted on the left. (B) Densitometric analyses of the blots in (A). Vertical bars represent mean (±SE) PNA-bound MUC1-ED signal (n=3). *, increased MUC1-ED desialylation in flagellin-treated *vs* untreated cells at p<0.05. **, decreased MUC1-ED desialylation in GSC-649 treated vs untreated cells at p<0.05. (C) The cells were fixed, washed, and incubated for 30 min with Pa (MOI=100). Nonadherent Pa were removed by washing, and CFU's of cell-bound Pa quantified. Vertical bars represent mean (±SE) Pa CFU's/well (n=9). *, increased Pa adhesion to flagellin-treated *vs* untreated cells at p<0.05. **, decreased Pa adhesion to GSC-649 treated *vs* untreated cells at p<0.05. (D) The cells were cultured for 24h after which MUC1-ED levels in supernates were quantified by ELISA and normalized to total cellular protein. Vertical bars represent mean (±SE) shed MUC1-ED normalized to total cellular protein (n=9). *, increased shed MUC1-ED in supernates of flagellin-treated *vs* untreated cells at p<0.05. **, decreased shed MUC1-ED in supernates in GSC-649 treated *vs* untreated cells at p<0.05. Each result is representative of ≥ 3 independent experiments.

Fig. 4. GSC-649 Counteracts NEU1-mediated Inhibition of HPMEC Migration and Capillary-Like Tube Formation.



(A-C) HPMECs infected with Ad-NEU1 or Ad-GFP were cultured for 48h. (A) and (B) A single wound was made across the diameter of each confluent monolayer, after which the wounded monolayers were incubated for 24h in the presence or absence of GSC-649 (50 μg/ml) and photomicrographs taken. (B) HPMEC area was calculated using ImageJ software for comparison with that observed in the same wounded monolayer at 0h, as a measure of migration into the wound. Each vertical bar represents mean (±SE) migration into the wound (n=3). *, decreased migration in Ad-NEU1 infected *vs* Ad-GFP infected cells at p<0.05. **, increased migration in GSC-649 (50 μg/ml) treated *vs* untreated cells at p<0.05. (C) HPMECs were seeded on Matrigel-coated wells at 7×10^3 cells/well, and after 6h, tubular structures photographed and segments of capillary-like tubes per high power field counted. Vertical bars represent mean (±SE) segments/HPF (n=7). *, decreased tube formation in Ad-NEU1 infected *vs* Ad-GFP infected cells at p<0.05. **, increased tube formation in GSC-649 treated *vs* untreated cells at p<0.05.

CONCLUSIONS

- The NEU1-Selective Sialidase Inhibitor, GSC-649, inhibits total sialidase activity for the 4-MU-NANA substrate in both human airway epithelia (IC_{50} = 3.74 μM) and human lung microvascular endothelia (IC_{50} = 13.0 μM).
- In human airway epithelial cells, GSC-649 dose-dependently inhibited *P. aeruginosa* flagellin-induced, NEU1-mediated
  - a) MUC1-ED desialylation,
  - b) *P. aeruginosa* adhesion to MUC1-ED expressing cells, and
  - c) MUC1-ED shedding.
- In human lung microvascular endothelial cells, GSC-649 counter-acted NEU1-mediated
  - a) Restraint of endothelial cell migration into a wound, and
  - b) Disruption of endothelial cell capillary-like tube formation on Matrigel, i.e. *in vitro* angiogenesis.