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DAS181 Blocks Respiratory Syncytia Virus Infection of Hep-2 Cells

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Abstract

Respiratory syncytia virus (RSV) infection is a common respiratory infection in infants and children for which there is a need for new therapies. Viral attachment appears to be mediated by interaction of the G protein with negatively charged carbohydrates such as glycosaminoglycans (GAGs) on the cell surface. DAS181 is a bifunctional fusion protein comprised of a sialidase linked to a polycationic sequence derived from human amphiregulin (hAR) that allows the molecule to target the sialidase enzymatic activity to GAGs. Based on the ability of DAS181 to bind to cell-surface GAGs we investigated whether DAS181 might interrupt RSV infection of Hep-2 cells by virtue of its unique hAR sequence. We report here that pre-exposure of Hep-2 cells to DAS181 appears to block infectivity of RSV of Hep-2 cells with an estimated IC50 value of 13.40 μM (p=0.009). In comparison, ribavirin exhibited an IC50 of 44.55 μM (p=0.004) under the same conditions. These data suggest that the expression of the hAR sequence may be sufficient to block RSV infection of Hep-2 cells and thus may provide a novel approach to target RSV infection.

Keywords Syncytia virus; Hep-2 Cells; Infection; Sialidase

Introduction

RSV is the major cause of respiratory infections in infants and children as well as infections in elderly and immunocompromised subjects [1-5]. The only approved treatment is ribavirin, a nucleoside inhibitor [5]. Recent studies have suggested that the humanized mAb palivizumab, which targets the fusion protein of RSV, may be effective for prophylaxis of RSV in high risk infants [6] to prevent hospitalization. However, a need still exists for new agents to prevent and treat RSV infection.

The first step in RSV infection is the binding of RSV (G) glycoprotein to cellular glycosaminoglycans (GAGs) particularly heparin sulfate proteoglycans (HSPG) on the target cell [7,8]. Interrupting this initial step in viral infection could provide a viable target to treat RSV infection. HSPG's are present on normal human bronchial epithelial cells [9,10] and basal cells [11] but not on cultured human airway epithelial cells (HAEs). Soluble heparin can inhibit the initial culture passage of primary isolates of RSV presumably through the biding of the anionic heparin to the G protein of RSV [12]. This ability to bind heparin has also been reported for the fusion protein of RSV [13]. Unfortunately, targeting the virus G protein with heparin based compounds is not feasible due to the potential side effects of heparin and its derivatives.

DAS181 is a 44kD novel fusion protein comprised of a sialidase catalytic domain fused to a GAG-binding domain from human amphiregulin (hAR), which localizes and retains the fusion protein to the respiratory epithelium [3]. Amphiregulin (AR) is composed of a C-terminal EGF-like domain and an N-terminal GAG-binding domain [14]. DAS181 expresses only a portion of the N-terminal GAG-binding sequence of amphiregulin, which enables DAS181 to bind to heparin-like polysaccharides but does not confer the EGF-like activity of the full length amphiregulin.

Sialic acids have not been reported to play a role in RSV infectivity. Recently it has been reported that cationic sequences derived from chemokines have the ability to block RSV infection, via competition with cell surface GAGs [15]. Based on its ability to bind to cell-surface GAGs we investigated whether constructs expressing the hAR GAG binding sequence with (DAS181) or without (DAS185) sialidase activity could inhibit infection of Hep-2 cells. These results were compared to the activity of ribavirin under the same experimental conditions. Our results suggest that these hAR expressing compounds may inhibit RSV infection of Hep-2 cells at concentrations superior to ribavirin.

Materials and Methods

DAS180, DAS181, DAS185

Test compounds (DAS181, DAS185 and DAS180) were kindly provided by Ansun Biopharma. DAS181 consists of a GAG binding domain derived from amphiregulin (amino acid residues 125 to 145 in GenBank entry AAH09799) fused via its N terminus to the catalytic domain of A. viscosus sialidase (AvCD, amino acid residues 274 to 667 in GenBank entry X62276) [4]. DAS185 is a mutated (Y348F) sialidase expressing construct that has the identical GAG binding amphiregulin tag but exhibits 400 fold reduced sialidase activity compared to DAS181. DAS180 is a sialidase expressing construct without the GAG binding amphiregulin tag. The drugs were supplied as a dry powder and solubilized in sterile PBS to a stock concentration of 50 mg/ml.

Cell and virus

Hep-2 cells were maintained at 37°C, 5% CO_2 in MEM+10% FBS. The RSV strain A2 was amplified in Hep-2 cells at 37°C, 5% CO_2 in Opti-MEM+2% FBS. The viral stock was titrated with methylcellulose 0.8% in 24-well plates and diluted to 40 PFU/ml (MOI 0.00016) in order to perform the assay.

Binding of DAS181 and 185 to human airway epithelial cells

To detect levels of cell surface binding of DAS180, DAS181and DAS185 HAE monolayers were treated with increasing concentrations (μg/mL) of each of the constructs at 37°C for 2 h. Binding was revealed by staining the cells with sialidase specific affinity purified sheep anti-DAS sialidase sequence specific antibody followed by an HRP conjugated mouse anti-sheep antibody.

Determination of IC50 of compounds on Hep-2 cells

Hep-2 cells were seeded in 24-well plates to 90% confluence. DAS180, DAS181 and DAS185 compounds were diluted in MEM+10% FBS at concentrations ranging from 20 μM to 0.3125 μM and added to the plates. Ribavirin was used as a positive control drug and tested from 100 μM to 1 μM as described above. Assays for the three test compounds and the positive control were performed in triplicate. Plates were incubated at 37°C, 5% CO_2 for 24 hours, washed with PBS and then 500 μl of RSV containing 40 PFU/ml in Opti-MEM + 2% FBS were added to the cells for 1.5 hr at 37°C, 5% CO_2. The medium was removed and cells were washed with PBS. An overlay of Opti-MEM 2x +methylcellulose 1.6% 1:1 containing the same concentrations of DAS compounds as in the pre-treatment step was added to the wells. The plates were incubated at 37°C, 5% CO_2 for 5 days. After the incubation period, cells were fixed with 7% formalin for 1 h at room temperature. RSV titers were determined using Goat anti-RSV Cederlane MD-05-0391 antibody at 1/1000 followed by donkey anti-goat Cederlane PA1-86326 antibody at 1/1000 and developed with KPL True Blue to reveal infected cells. The 50% inhibitory concentration (IC50) value was determined from the dose-response curve using the program Excel. Statistical significance of the plaque reduction at the highest concentration of DAS181, DAS185 and DAS180 relative to no drug was determined by paired T test.

Results

As shown in Figure 1, both DAS181 and DAS185 bound to human airway epithelium cells, while the amphiregulin derived sequence deficient molecule DAS180 did not.

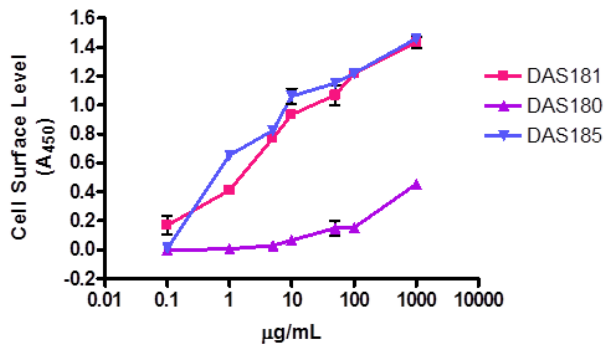

Figure 1: DAS181 (hAR tag+), DAS185 (hAR tag+) and DAS180 (hAR tag-) binding to HAE cells.

These results demonstrate that expression of the hAR tag sequence allows for binding of the DAS181 and DAS 185 molecules to the target cell. To determine if these any of constructs could inhibit RSV infection of Hep-2 cells, the test compounds were pre-incubated with Hep-2 cells at descending concentrations starting at 20 uM. Hep-2 cells were exposed to ribavirin in a similar fashion starting at 100uM as a positive control for inhibition of RSV infection of Hep-2 cells. As seen in Figure 2 both DAS181 (2A) and DAS185 (2B) inhibited RSV in a dose-dependent manner with an IC50 value of 13.40 μM (p=0.009) and 12.74 μM (p=0.001) respectively. Suppression of RSV was not observed with DAS180 (p=0.13), which expresses sialidase activity but lacks the GAG binding domain (Figure 2C). The positive control ribavirin exhibited an IC50 of 44.55 μM (p=0.004) under the same conditions (Figure 3). No detectable cytotoxicity of DAS181, DAS185 or DAS180 on the Hep-2 cell line was observed in these studies. Based on these results, we conclude that both DAS181 and DAS185 are slightly more potent than ribavirin for inhibition of RSV infection of Hep-2 cells.

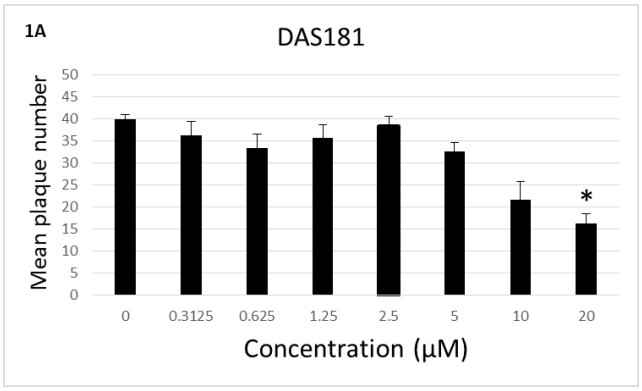

Figure 2 (A): The effect of DAS181 on RSV infection of Hep-2 cells. Cells were infected and scored for PFUs according to Materials and Methods. Bars show mean number of plaques+/- standard deviation over the range of drug concentrations. * Denotes p<0.05 paired T test. ** Denotes p>0.05 paired T test.

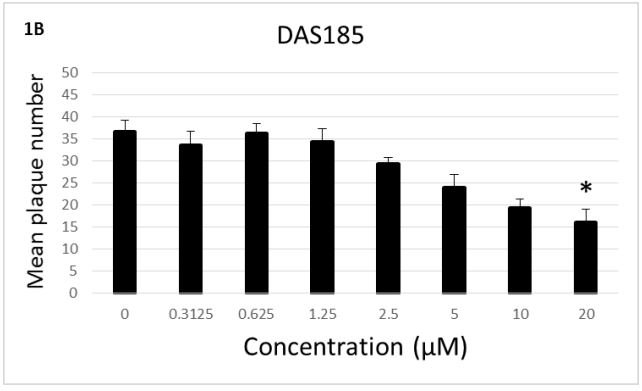

Figure 2 (B): The effect of DAS185 on RSV infection of Hep-2 cells. Cells were infected and scored for PFUs according to Materials and Methods. Bars show mean number of plaques+/- standard deviation over the range of drug concentrations. * Denotes p<0.05 paired T test. ** Denotes p>0.05 paired T test.

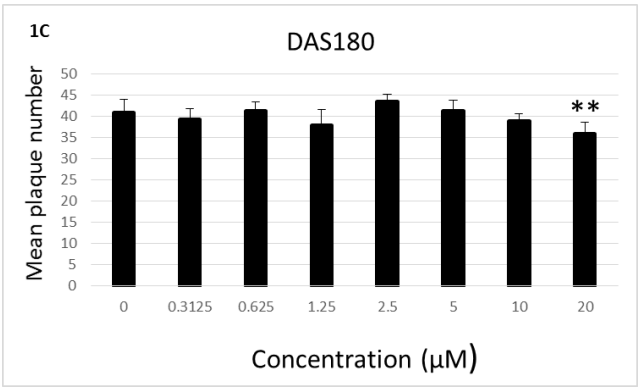

Figure 2 (C): The effect of DAS180 on RSV infection of Hep-2 cells. Cells were infected and scored for PFUs according to Materials and Methods. Bars show mean number of plaques+/- standard deviation over the range of drug concentrations. * Denotes p<0.05 paired T test. ** Denotes p>0.05 paired T test.

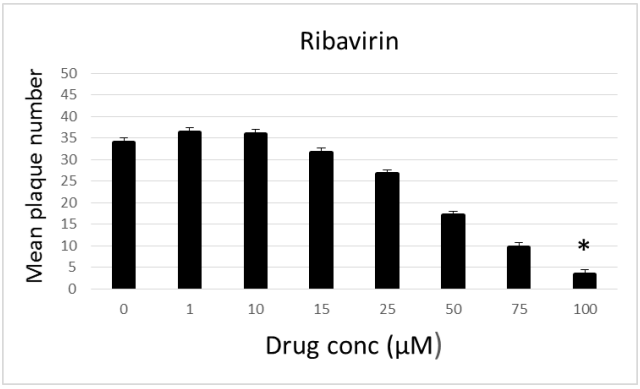

Figure 3: The effect of ribavirin on RSV infection of Hep-2 cells. Cells were infected and scored for PFUs according to Materials and Methods. Bars show mean number of plaques+/- standard deviation over the range of drug concentrations. * Denotes p<0.05 paired T test.

Discussion

We show here that DAS181 is capable of blocking RSV infection of Hep-2 cells by virtue of the hAR GAG binding portion of the molecule. Similar activity was observed with DAS185, a control construct with a mutated sialidase but a functional hAR tag. Previous reports have shown a lack of sensitivity to sialic acid cleavage for Hep-2 cell infection [16,17]. As predicted, DAS180, an analogue which contains an active sialidase without the hAR GAG binding domain showed no activity in this system. Based on the results presented here we conclude that the inhibition of infection is due to the blocking of cell-surface GAGs by DAS181 thereby prohibiting the binding of RSV to the Hep-2 cell membrane. RSV includes two subtypes (A and B) that are distinguishable serologically due to differences in the structure of the G protein [18]. This study only evaluated the activity of DAS181 on subtype A. While it is possible that differences in the relative affinity for GAGs could exist between A and B subtypes for DAS181 inhibition, DAS181 should be equally potent on both subtypes based on the proposed mechanism of action described here. Studies with clinical isolates can be undertaken in the future to examine the breadth of activity against circulating strains of RSV. Finally, although the possibility exists that the hAR tag could also bind to an as yet unidentified viral-associated GAGs, to date no report of this moiety has been reported on RSV particles.

One of the confounding factors with respiratory virus infection is the identification of the causative pathogen (s). DAS181 has shown in-vitro and in-vivo activity against influenza [19,20], parainfluenza [21] and EV68 [22]. DAS181 has been examined in clinical trials for influenza and parainfluenza viruses [23,24] with encouraging results. These viruses all use sialic acid as the primary receptor. Negatively charged glycosaminoglycans (GAGs), such as the sulfated polysaccharides including heparin sulfate or keratin sulfate, are ubiquitously present on the surface of respiratory epithelium and function as a receptor for a number of viruses [12]. Thus, DAS181 may also be useful in treating viruses that target GAGs for entry.

The advantages of using a host directed therapy are important to note. Since DAS181 targets the host-cell respiratory epithelium and not the virus [3], the chance for the generation of resistant viruses is markedly lower. In addition, the ability of DAS181 to target binding, the initial step in viral replication, allows for the potential combination of DAS181 with other replication-specific drugs. These data presented suggest that DAS181, an investigational host-directed antiviral, may also be active against RSV. Additional studies are underway to further test this hypothesis.

Conflicts

RT and RBM are employees of Ansun Biopharma. XB, MEH, and GB received a grant from Ansun Biopharma for this work.

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