Antiviral Research 123 (2015) 114–119

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Phase 1 clinical trials of DAS181, an inhaled sialidase, in healthy adults

Jonathan M. Zenilman [a,*], Edward J. Fuchs [b], Craig W. Hendrix [b], Christine Radebaugh [b], Robert Jurao [a], Seema U. Nayak [a], Robert G. Hamilton [c], J. McLeod Griffiss [d]

[a] Division of Infectious Diseases, Department of Medicine, Johns Hopkins University, Baltimore, MD, United States
[b] Clinical Pharmacology, Department of Medicine, Johns Hopkins University, Baltimore, MD, United States
[c] Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University, Baltimore, MD, United States
[d] Clinical RM, Inc., Hinckley, OH, United States

ARTICLE INFO

Article history:
Received 10 July 2015
Revised 13 September 2015
Accepted 15 September 2015
Available online 25 September 2015

Keywords:
Influenza
Parainfluenza
Sialidase
DAS-181

ABSTRACT

DAS181, (study drug, Fludase®) was developed for treatment of influenza and parainfluenza infections. Delivered by inhalation, DAS181 cleaves sialic acid receptors from respiratory epithelial cells. Treatment of influenza for three days with DAS181 reduced viral shedding. To increase deposition in the upper airways and decrease systemic absorption, the particle size was increased to 10 μm. We conducted two Phase I trials with three cohorts, randomized 2:1, active drug to placebo. The initial cohort got a single 20 mg dose of DAS181, or placebo; the second, 20 mg DAS181 or placebo for 10 days, and the third got 20 mg of DAS181 or placebo for 3 days. Formulations differed slightly in their excipients. Subjects in the 1- and 3-day cohorts completed dosing without serious adverse events. Two subjects in the 10-day cohort stopped at Day 9 after developing respiratory and systemic symptoms, and a third experienced a decrease in FEV_1 (Forced Expiratory Volume in 1 s) after the 9th dose and a further decline after the 10th dose. Plasma DAS181, in the 10-day cohort, peaked and began falling before the last dose. Antibodies, predominately IgG with neutralizing activity, were detected in 15/18 subjects by Day 30. The highest IgG concentrations were in the 10-day cohort. The respiratory adverse events occurring after seven days and rapid drug clearance during continued dosing are consistent with the induction of DAS181 antibodies. This could preclude use of this medication for longer than seven days or for repeated courses. (These studies have been registered at ClinicalTrials.gov under registration Nos. NCT 00527865 and NCT 01651494.)

1. Introduction

Influenza vaccines have limited effectiveness (Osterholm et al., 2012); this and the increasing resistance to influenza antivirals emphasizes the need to develop alternative approaches (Osterholm et al., 2012; Garg et al., 2013). DAS181 is such an alternative; it targets the sialic acid adornments on respiratory epithelial cells to which influenza and parainfluenza viruses bind and has the potential for preventing and treating infections caused by both viruses.

DAS181 is a recombinant sialidase, derived from *Actinomyces viscosus*, fused to an anchoring domain from the binding sequence of human amphiregulin (Belser et al., 2007). Administered by inhalation, DAS181 removes sialic acid from the respiratory epithelium (Triana-Baltzer et al., 2010) and thereby prevents the binding of influenza (Malakhov et al., 2006; Chan et al., 2009; Triana-Baltzer et al., 2009) and parainfluenza (Moscona et al., 2010) viruses. This potentially could provide prophylaxis and treatment for all strains of influenza, including those that are resistant to neuraminidase inhibitors (Triana-Baltzer et al., 2009).

Phase I trials of inhaled DAS181, conducted during its development, evaluated various doses, formulations and particle sizes (Moss, 2010). The primary adverse event noted during these trials was elevation of serum alkaline phosphatase (ALP) which was thought to result from the systemic absorption of the drug and de-sialylation of circulating glycoproteins (Moss, 2010). In addition, circulating, and neutralizing, antibodies were induced after a single dose of DAS181. Increasing the particle size from ~3.5 μ (DAS181-F01) to ~6 μ (DAS181-F02) reduced systemic absorption by depositing the drug higher in the respiratory tract, but did not eliminate elevation of ALP or antibody induction. DAS181-F02 was used in a randomized, double-blind, Phase II trial to determine the safety and tolerability of 10 mg of DAS181, inhaled once or over

* Corresponding author at: Division of Infectious Diseases, Johns Hopkins University, Johns Hopkins Bayview Medical Center, 5200 Eastern Ave, MFL Center Tower, Suite 381, Baltimore, MD 21224, United States.
E-mail address: jzenilma@jhmi.edu (J.M. Zenilman).

http://dx.doi.org/10.1016/j.antiviral.2015.09.008
0166-3542/

three days, in otherwise healthy adults with laboratory confirmed influenza (Moss et al., 2012). As compared with placebo, DAS181 reduced the influenza viral load in pharyngeal washes, but the reduction after a single dose was no longer significant after 48 h. After three daily doses, reduction in viral load remained significant for 48 h, or to Day 5. No significant differences were noted in time to resolution of clinical symptoms, and ALP elevations were noted in 19% of the multi-dose recipients.

The results of the Phase II trial lead to the formulation of DAS181-F03 which has a particle size of 10 μ, designed to reduce deep lung deposition and the potential for systemic absorption, and DAS181-F04 which differs from DAS181-F03 only in the addition of $MgSO_4$ as a counter ion excipient (Table 1). In hopes of increasing the effectiveness and duration of its antiviral effect, the dose of DAS181 was increased to 20 mg in the clinical trials reported here, and a duration of ten daily doses was assessed. In the first trial, DAS181-F03 was administered once (1 Day Cohort) or daily for ten days (10 Day Cohort); in the second trial DAS181-F04 was administered daily for three days (3 Day cohort). We assessed the toxicity, systemic absorption and immunogenicity of the two formulations dosed at 20 mg/d. The results of the two trials are combined in this report.

2. Materials and methods

We conducted three randomized, double-blind, placebo-controlled trials, each consisting of nine healthy adult volunteers who received DAS181 or placebo at a ratio of 2:1. The first cohort received a single dose of 20 mg DAS181-F03, or placebo. The second was to receive 20 mg DAS181-F03, or placebo, daily for 10 days. The third was administered DAS181-F04, 20 mg per day, or placebo, for 3 days. The DAS181 formulations were provided by Ansun Biopharma, Inc., San Diego, CA.

The studies were approved by the Johns Hopkins University Institutional Review Board, and written informed consent was obtained from all participants prior to screening.

2.1. Subjects

The studies enrolled 18 healthy adults, who were, non-smokers with no recent respiratory illness and no lactose intolerance, and who had normal complete blood counts, chemistries, coagulation profiles, complement tests, urinalyses, drug abuse screens, HIV, hepatitis B and hepatitis C serologies, chest radiographs (CXR), and electrocardiograms (ECG). Women had negative pregnancy tests. Subject pulmonary function tests (PFTs) had peak expiratory flow rates, and FEV_1s >80% predicted. Subjects were trained to use the Cyclohaler® (N.V. Medicopharma, NL) device and achieve an inspiratory flow rate of >60 L/min, as measured by In-Check DIAL low-range inspiratory flow meter (Alliance Tech Medical, Inc., Grandbury, TX).

Table 1
Composition of DAS181-F03 and DAS181-F04 dry powder.

Component wt/wt (%)	DAS181-F03	DAS181-F04	Function
DAS181	70.08	65.06	Active pharmaceutical ingredient
Histidine	10.11	10.09	Prevent oligomerization
Trehalose	9.23	8.50	Moisture binding
$MgSO_4$	0.00	6.16	Counter ion
Citric acid	2.53	2.13	Counter ion
Sodium acetate	0.04	0.03	Maintaining pH
Acetic acid	0.03	0.01	Maintaining pH
Water	8.00	8.00	N/A
Total	100.00	100.00	

2.2. Study design

Subjects inhaled 20 mg of DAS181 powder, or lactose monohydrate as placebo, once daily, for one (DAS181-F03), three (DAS181-F04) or ten (DAS181-F03) days. Dosing was done under direct supervision. An independent Safety Monitoring Committee reviewed clinical and safety data from each cohort.

Safety laboratory tests were obtained at pre-determined time. PFTs were performed at screening, on admission (Day-1), Day 0 before dosing, and 1, 2, 4, and 8 h after the first dose, for all three cohorts. The 1-day cohort also had PFTs at 24 and 48 h after the dose, and again at Day 7. The 3-day cohort had additional PFTs before the second and third doses, one hour after those doses, and on days 3, 4 and 9 after the initial dose. The 10-day cohort had PFTs gotten before the second and fourth doses, on Day 7, pre-dose and 1 h post-dose on Day 9, and on Days 10 and 17. CXRs were performed one week after the last dose.

Serum samples for the 10-day cohort were collected before each of the 10 doses; 4, 8, and 24 h after the 1st dose; 4, 8, and 24 h after the 10th dose, and on Days 11, 13, 15, 17, 20, 22, 24 and 27. Drug concentrations were measured by a biological sialidase assay (Moss et al., 2012). Sera for antibody measurements were obtained before the first dose and 30 and 90 days after the first dose.

2.3. Immunogenicity

Enzyme-linked immunoassays were used to quantify antibodies (Hamilton, 2013). DAS181 was adsorbed onto microtiter plates at 20 μg/mL in phosphate-buffered saline (PBS). After 16–18 h at 4 °C, wells were washed with PBS 0.05% Tween 20 and blocked with bovine serum albumin. Serum (100 μL) was added at 1:100, 1:25 and 1:2 dilutions for IgG, IgA and IgE assays, respectively. After 16–18 h at 4 °C, plates were washed, horseradish peroxidase conjugated monoclonal anti-human IgG Fc- (clone HP6043-HRP) or monoclonal anti-IgA Fd (clone HP6123-HRP, Hybridoma Reagent Laboratory, Baltimore, MD) were pipetted into the wells of the IgG or IgA antibody plates (1 μg/mL, 0.1 mL/well). After 1 h, plates were washed and. substrate (ABTS + H_2O_2) was added. Color development was stopped with 100 μL of 1 M NaN_3. For the IgE assay, after the serum incubation, biotin-conjugated monoclonal anti-human IgE Fc (clone HP6029B) was added. After 1 h and a buffer wash, streptavidin HRP (1 μg/mL) was added and allowed to bind to the bound biotin-anti-IgE. The plates were developed with substrate.

IgG in a 1:100 dilution of a high-titered reference serum was arbitrarily assigned a value of 1000 U, and an 11 point calibration curve constructed. Concentrations of Ig in the samples were determined from the calibration curve by heterologous interpolation and reported as arbitrary units (AU) of antibody relative to the 1:100 dilution of the reference serum. The lower limit of detection for IgG was 200 AU; that for IgA was 100 AU. The specificity of antibody responses was verified by pre-incubation of serum with or without soluble DAS181 (1 mg/mL).

2.4. Neutralizing antibody

A competitive assay was used to determine whether DAS181 antibodies neutralized the enzymatic activity of the drug. Sera were diluted in acid citrate dextrose. Sialidase activity was determined using 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MuNaNa), VWR (Biosynth) Cat # 101369-938 (M-5507), a fluorogenic substrate. DAS181 cleaves the sialic acid from the substrate, and the assay detects free sialic acid. The samples were incubated with DAS181 and MuNaNa substrate solution. After 20 min, the reaction was stopped with 0.5 M Glycine, pH 10.2,