Post exposure efficacy of AVI-7100 against influenza A in mouse and ferret infection models

P. L. Iversen[1] , F. Schnell[1], S. Crumley[1], D. Mourich[1], and T. Voss[2]
[1]AVI BioPharma, Bothell, WA, [2]Tulane Univ. Sch. of Med., New Orleans, LA.

Purpose

The objective of these studies was to evaluate the therapeutic utility of AVI-7100 administered as a single intranasal dose up to one day post infection with influenza A.

Conclusions

- AVI-7100 is effective against influenza A (H1N1 and H3N2) in both mouse and ferrets when administered after viral challenge.
- Post-exposure efficacy data indicate robust antiviral and symptom benefit can be provided by AVI-7100 as a single intranasal dose.
- Infection does not significantly alter plasma pharmacokinetics relative to uninfected ferrets.

Abstract

AVI-7100 is a phosphorodiamidate morpholino oligomer containing three modified linkages (PMO*plus*) that is designed to interfere with expression of the M1 and M2 genes of influenza A virus.

Methods. A single 0.1mg intranasal (i.n.) dose of AVI-7100 was administered to female BALB/c mice (n=10/group) either 4 hours prior to or 4 hours after viral challenge with 5 X 10^5 pfu of A/Port Chalmers/1/73 (H3N2). Lung viral load was determined on day 6 post infection. In a similar efficacy study in outbred ferrets (*Mustela putorius furo*; n=7/group) , AVI-7100 was administered as a single i.n. dose 4 hours prior to or 1 day post insufflation viral challenge with 5 X 10^5 pfu H1N1 A/Hong Kong/2369/09 per ferret. Negative control groups were treated with saline and positive controls were administered oseltamivir at 10 mg/kg p.o. every other day beginning 7 days prior to infection. A plasma pharmacokinetic study with 16 ferrets (4 groups of 4 ferrets/group) in which a 10 mg/kg or 30 mg/kg i.v. dose was evaluated prior to and three days post viral challenge with H1N1 strain A/Mexico/4108/09 or H5N1 strain A/Vietnam/1203/04 .

Results. A single intranasal dose of AVI-7100 (0.1mg/mouse) administered either 4 hours prior to or 4 hours after infection with A/Port Chalmers/1/73 (H3N2) significantly ($p<0.05$) reduced lung viral titers in each group compared to vehicle controls and oseltamivir treated mice. In the ferret, a single i.n. dose of AVI-7100 administered 4 hours prior to exposure or 1 day after exposure with A/Hong Kong/2369/09 (an oseltamivir resistant pH1N1) significantly ($p<0.05$) reduced cumulative viral load in nasal wash and in lung bronchiolar lavage compared to saline controls and oseltamivir treated ferrets. A plasma pharmacokinetic study revealed no differences between infected and uninfected ferrets.

Conclusions. AVI-7100 is effective against influenza A (H1N1 and H3N2) and in both mouse and ferrets when administered as a single intranasal dose for greater than one day post viral exposure. Post-exposure efficacy data indicate robust antiviral and symptom benefit can be provided by AVI-7100. Infection does not significantly alter plasma pharmacokinetics relative to uninfected ferrets. These data provide a rationale for a therapeutic use of AVI-7100 following influenza exposure.

Introduction

A triple-reassortant influenza A (H1) virus has been circulating since 1998 with segments from pigs (HA, NP, NA, M and NS), humans (PB1), and birds (PB2 and PA). The newly described and novel swine-origin influenza A (H1N1) virus is a triple reassortant virus that includes genetic elements of this preexisting virus that have reassorted with the neuraminidase (NA) and matrix (M) segments of a Eurasian swine virus. The previous influenza A triple-reassortant virus was occasionally transmitted to humans but not spread efficiently from human-to-human but the new H1N1 is very efficient in human-to-human transmission.

Early studies targeting multiple viral segments identified PB1-AUG as a highly effective target (Ge et al., 2006). The sequence provided a >3 log reduction in viral titer at 10uM and >3 log reduction at 20uM against H1N1 PR/8/34 (MOI of 0.05) in Vero cells 48 hours after treatment. The sequence also provided for concentration-dependent reduction in viral titer against WSN/33 in MDCK cells 24 hours after infection (MOI of 0.001) of 0.2 log at 5uM, 0.7 log at 10uM and 1.5 log at 20uM. A highly conserved region was selected for targeting and efficacy was confirmed by Lupfer et al., (2008) and Gabriel et al., (2008). In addition to reduction in viral titer, reduced body weight loss, enhanced survival and improved histopathology endpoints were observed in mice challenged with H1N1, H3N8, and H7N7. Recent studies employing the 3-PMO*plus* linkages revealed greater antiviral activity and improved clinical outcome for a sequence targeting M1/M2 indicating this is a superior target.

M1/M2-AUGplus (AVI-7100) was designed to inhibit the translation of both the matrix protein and the M2-ion channel. These two protein products are splice variants of the same segment and both share the same translation initiation start site which is targeted by this oligomer. Loss of these protein products may interfere with viral uncoating, nuclear export of viral transcripts, viral assembly, and viral budding.

AVI BioPharma has conducted studies that encompass evaluation of our candidate agents in cell culture and in both mouse and ferret models of influenza infection leading to selection of AVI-7100 as the lead candidate. The cytotoxic concentration in cell culture studies utilizing both murine and human cell lines is CC_{50} = 333 uM. No inhibition of the human cytochrome P450 enzymes was observed below 30 uM and no inhibition of the multidrug efflux pumps has been observed. Most nucleic acid based therapeutics interact with Toll-Like Receptors (TLR) resulting in immune activation or immune suppression through short sequences referred to as CpG motifs. PMO*plus* oligomers containing CpG motifs were evaluated and do not result in immune activation or immune suppression. The mechanism of action of AVI-7100 involves reduction of target viral protein expression, reduction in viral mRNA and viral titer. Finally, studies were conducted in which the concentration of AVI-7100 in individual cells was compared to expression of the M2 protein in individual cells with an effective concentration (EC_{50}) of less than 50 nM. Comparing the CC_{50} with the EC_{50} suggests a broad therapeutic index.

Literature Cited:

Ge Q, Pastey M, Kobasa D, Puthavathana P, Lupfer C, Bestwick RK, Iversen PL, Chen J and Stein DA (2006) Inhibition of multiple subtypes of influenza a virus in cell cultures with morpholino oligomers. *Antimicrob Agents Chemother.* **50(11):** 3724-3733.

Gabriel G, Nordman A, Stein DA, Iversen PL and Klend H-D (2008) Morpholino Oligomers Targeting the PB1 and NP Genes Enhance Survival of Mice Infected with highly Pathogenic Influenza A H7N7 Virus. *J Gen Virology* **89:** 939-948.

Lupfer C, Stein DA, Mourich DV, Tepper SE, Iversen PL and Pastey M (2008) Inhibition of influenza A H3N8 virus infections in mice by morpholino oligomers. *Arch Virol.* **153:** 929-937.

Support

This study was conducted under contract with the Department of Defense Joint Project Manager Transformational Medical Technologies.

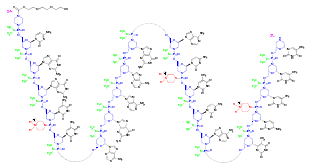
Background

AVI-7100 Targets a Highly Conserved Viral Sequence in Influenza A (subscripts indicate percent conservation at that sequence position):

5'-$A_{99.6}A_{100}A_{100}G_{99.6}A_{99.7}T_{99.9}G_{99.9}A_{99.9}G_{99.9}T_{99.9}C_{99.9}T_{99.}{}_9T_{99.6}C_{100}T_{100}A_{99.}{}_9A_{100}C_{100}C_{100}G_{100}$-3'

NCBI Influenza Virus Resource (11/10/2009)
(database utilized for sequence analysis):
H1N1 – 845 seqs from humans
H1N1 (S-OIV) – 775 seqs from humans
H5N1 – 947 seqs from all species
H3N2 – 835 from humans in North America
H9N2 – 348seqs from all species
H2N2 – 107seqs from all species
H7N7 – 35seqs from all species

AVI-7100

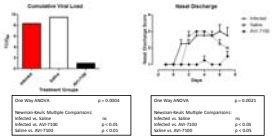


AVI-7100 Efficacy Studies Summary:

1. Effective in mouse and ferret after single i.n. administration.
2. Effective in mouse and ferret after daily (4-6) i.p. doses .
3. Effective against neuraminidase resistant pH1N1 (A/Hong Kong/2369/09) in ferret model.
4. Effective in immune suppressed (CD4 depleted) and autoimmune (EAE) mouse models.
5. Effective in blocking viral transmission (see figure below)

AVI-7100 Prevents Transmission of Oseltamivir Resistant pH1N1 from Infected to Naive Ferrets

Group	Treatment	Dose	Route	Schedule	Number
1	AVI-7100	3 mg/kg	i.n.	-1D, 0D, 1D, 2D, 3D, 4D, 5D	6; 2 infected, 4 naive
2	Saline	-	i.n.	-1D, 0D, 1D, 2D, 3D, 4D, 5D	6; 2 infected, 4 naive



One Way ANOVA	p < 0.0002
Newman-Keuls Multiple Comparisons:	
Infected vs. Saline	ns
Infected vs. AVI-7100	p < 0.01
Saline vs. AVI-7100	p < 0.01

One Way ANOVA	p < 0.0021
Newman-Keuls Multiple Comparisons:	
Infected vs. Saline	ns
Infected vs. AVI-7100	p < 0.05
Saline vs. AVI-7100	p < 0.05

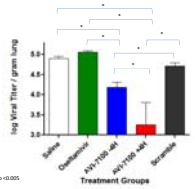
Post Exposure Efficacy

Mouse

Table 1. Study Design for Balb/c Female Mice infected with A/Port Chalmers/1/73 (H3N2) via the intranasal route

Group	Treatment	Number	Dose	Route	Schedule
1	Saline	10	-	i.n.	-7D, -3D, -4H
2	Oseltamivir	10	10 mg/kg[1]	p.o.	-7D, -3D, -4H
3	AVI-7100	10	5 mg/kg[2]	i.n.	-4 H
4	AVI-7100	10	5 mg/kg	i.n.	+4 H
5	Scramble	10	5 mg/kg	i.n.	-7D, -3D, -4H

[1] dose volume 200 µl/dose
[2] dose volume 50 µl/dose



* p <0.005

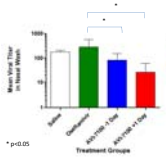
Figure 1: AVI-7100 reduced lung viral titer in female Balb/c mice when administered 4 hours prior to infection (-4H) or 4 hours post infection (+4 H).

Ferret

Table 2. Study Design for male *Mustela putorius furo* infected with A/Hong Kong/2369/09(H1N1) via the intranasal route[3]

Group	Treatment	Number	Dose	Route	Schedule
1	Saline	7	-	i.n.	-7D, -5D, -3D, -1D
2	Oseltamivir	7	10 mg/kg[2]	p.o.	-7D, -5D, -3D, -1D
3	AVI-7100	7	3 mg/kg[1]	i.n.	-1 Day
4	AVI-7100	7	3 mg/kg	i.n.	+1 Day

[1] 5 X 10^5 pfu/ferret in 200 µl
[2] dose volume 200 µl/dose
[3] dose volume 200 µl/dose



* p<0.05

Figure 2: AVI-7100 reduced nasal wash viral titer in male ferrets when administered 1 day prior to infection (-1D) or 1 day post infection (+1D).

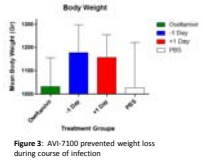
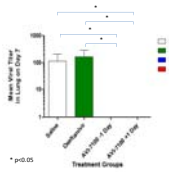


Figure 3: AVI-7100 prevented weight loss during course of infection



* p<0.05

Figure 4: AVI-7100 reduced lung viral titer in male ferrets when administered 1 day prior to infection (-1D) or 1 day post infection (+1D).

Infection Does Not Alter Plasma Pharmacokinetics

Table 4: Study Design [conducted at Battelle]

Group	Number	Dose mg/kg	Schedule Dose Day	Challenge Strain	Viral Titer	Plasma PK
1	4	10	0,1,2,3	A/Mexico/4108/09	-4,1,2,3	0,3
2	4	30	0,1,2,3	A/Mexico/4108/09	-4,1,2,3	0,3

Table 5: Plasma Pharmacokinetic Data for AVI-7100 administered intravenously at two doses in ferrets before and after infection with A/Mexico/4108/09 (pH1N1)

Group	AUC	Peak (ng/mL)	Kel dist	Kel elim
10mg/kg pre	15,155	25,378	-0.96±0.05	-0.52±0.01
10mg/kg post	17,469	34,761	-1.19±0.05	-0.07±0.02
30mg/kg pre	44,306	48,749	-0.96±0.07	-0.20±0.07
30 mg/kg post	61,430	49,017	-1.15±0.04	-0.09±0.03

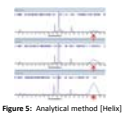


Figure 5: Analytical method [Helix]

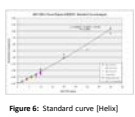


Figure 6: Standard curve [Helix]

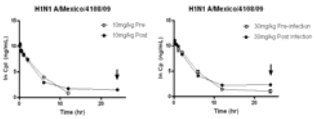


Figure 7: Semi-log Plot of mean (+SD) Plasma Concentrations of AVI-7100 versus Elapsed Time. Infection did not alter the plasma concentration profile.