

Plasma and Pulmonary Pharmacokinetics of Intravenous Zanamivir in the Cynomolgus Macaque

Alison Churchill³, Koert Stittelaar¹, Mark Shelton³, James Simon¹, Frank Pistor¹, Mark Lovern³, Margaret Tisdale¹, and Albert Osterhaus²
¹ViroClinics BV, ²Erasmus Medical Centre and ³GlaxoSmithKline

Introduction

Relenza (zanamivir for inhalation) is approved for prophylaxis of influenza and treatment of influenza A and B. In mice, zanamivir has shown efficacy against lethal H5N1 infections [Gubareva et al., 1998]. In humans, intravenous (IV) zanamivir 600mg twice daily (median half-life 1.5-2.1 hours) prevented experimental infection with influenza A (A/Texas, H1N1) [Caffee et al., 1999]. Due to the ~15 minute half-life of IV zanamivir, small animal models (mouse, rat) are not appropriate for the evaluation of H5N1 activity. Preliminary pharmacokinetic studies using IV zanamivir at 10 mg/kg demonstrated that the plasma half-life in macaques ranged from 1.2-3.8 hours, which was comparable to that reported in humans (approximately 2 hours) [Cass LMR., 1999]. However, there is a macaque model in which H5N1 infection manifests primarily as influenza pneumonia [Rimmelzwaan et al., 2001].

To evaluate this macaque model for treatment/prophylaxis studies of IV zanamivir, we studied plasma and pulmonary pharmacokinetics (epithelial lining fluid and alveolar macrophage concentrations) following IV zanamivir (20mg/kg) in 6 male cynomolgus macaques. Pharmacokinetic (PK) data from the macaque will be compared to data from similar IV zanamivir PK studies in humans. These PK results will be used to aid dose selection for future studies in humans to investigate the efficacy of zanamivir in severe seasonal influenza and H5N1 infections.

Methods

Study Conduct

- Six male healthy cynomolgus macaques (*Macaca fascicularis*: age ca. 36 to 54 months, bodyweights 2.66 – 4.35 kg on the day of dosing)
- Animals were housed as a group for the duration of the study in caging appropriate to the species under controlled conditions.
- Clinical signs were monitored at regular intervals throughout the study in order to assess any reaction to treatment.
- Zanamivir was dissolved in 0.8% w/v Sodium Chloride in Water (target dose concentrations of 16 mg/mL).
- Before dose administration all animals were anaesthetised with ketamin (10 mg/kg) and domitor (0.1 mg/kg). Two intravenous zanamivir doses (20mg free moiety/kg, separated by 12 hours) were administered to each animal by injection as a bolus into a vein at a target dose volume of 1.25 mL/kg.
- Pharmacokinetic samples were collected after the second zanamivir dose:
 - Plasma: pre-dose 5, 10, 30 minutes, 1, 2, 4, 6, 8 and 24 hours after dosing.
 - Bronchoalveolar lavage (BAL): 4 hours after dosing, two consecutive BAL samples (BAL1 and BAL2) were collected by instillation of 10 mL of 0.9% saline into the lungs using a laryngoscope.
- After recording the volume recovered by aspiration, BAL samples were collected, stored on ice, and centrifuged within 2 hours in order to obtain BAL fluid (supernatant) and cells (cell pellet).

Bioanalytical/Pharmacokinetic

- Plasma was analysed for zanamivir using a validated analytical method based upon protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) was 10 ng/mL using 50 µL aliquot of plasma with a higher limit of quantification (HLQ) of 10,000 ng/mL.
- BAL samples (supernatant) were analysed for zanamivir using a validated analytical method based on solid phase extraction, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) was 0.2 ng/mL using a 500 µL aliquot of BAL sample with a higher limit of quantification (HLQ) of 100 ng/mL.
- Macrophage cell pellet samples were treated with 100 µL of methanol and shaken vigorously to completely lyse the cells and made up to 2 mL (reconstitution volume) with 0.85% saline and then treated as a BAL sample.
- The intravenous plasma pharmacokinetics were determined using the standard algorithms of the non-compartmental data analysis program WinNonLin Enterprise, Version 4.1.
- Urea in plasma and BAL supernatant was analyzed using the Bayer Diagnostics Urea kit (catalogue number 03040257, Lot number 071805, Expiry May 2007) intended for use on the Advia 1650 Clinical Chemistry Analyser (LLQ was 0.05 md/dL).

Pulmonary Pharmacokinetic Calculations:

$$UREA_{BAL} \text{ (mg)} = \text{Concentration}_{UREA-BAL} \text{ (mg/dL)} \times (V_{BAL} \text{ (mL)} / 100)$$

$$V_{ELF} \text{ (mL)} = (UREA_{BAL} \text{ (mg)} / (UREA_{blood} \text{ (mg/dL)})) / 100$$

$$ZAN_{ELF} \text{ (ng/mL)} = (ZAN_{BAL} \text{ (ng/mL)} \times V_{BAL} \text{ (mL)}) / V_{ELF} \text{ (mL)}$$

$$V_{AC} \text{ (mL)} = (85\%) \times WBC_{BAL} \text{ (cells/mL)} / 10^6 \times (V_{BAL} \text{ (mL)}) \times 0.00242 \text{ (mL/10}^6 \text{ cells)}$$

$$ZAN_{AM} \text{ (ng/mL)} = (ZAN_{pellet} \text{ (ng/mL)} \times \text{reconstitution volume (mL)}) / V_{AC} \text{ (mL)},$$

where Concentration_{UREA-BAL} is the concentration of urea in the BAL supernatant in mg/dL, V_{BAL} is the volume of the BAL solution recovered from the BAL procedure in mL, UREA_{blood} is the concentration of urea in the blood in units of mg/dL, 85% is the presumed percentage of white blood cells expected to be macrophages in the BAL supernatant, and WBC_{BAL} is the white blood cell count in the BAL supernatant in cells/mL. The 0.00242 mL/10⁶ cells is a published estimate of the mean volume of pulmonary macrophages [Baldwin 1992]. ZAN_{BAL} is the concentration of zanamivir measured in the BAL supernatant in ng/mL, and ZAN_{pellet} is the concentration of zanamivir in the reconstituted BAL cell pellet in ng/mL (2mL for all samples in this study).

The derived variables ZAN_{ELF} and ZAN_{AM} are the concentrations of zanamivir in the epithelial lining fluid (ELF) and alveolar macrophages (AM), respectively. The derived variables V_{AC} and V_{ELF} are the volume of alveolar cells in the cell suspension and the volume of ELF in BAL fluid, respectively.

Results

- No adverse clinical signs were observed during the study.
- Plasma levels of zanamivir showed a monophasic elimination (Figure 1). The terminal half-life of zanamivir was calculated to be approximately 2.7 hours and a MRT to be approximately 1.7 hours (Table 1). The mean plasma clearance was low (3.4 mL/min/kg) and the apparent volume of distribution was approximately 0.3 L/kg.
- Cytospin preparations of the BAL samples were generated and stained with Giemsa. In all samples erythrocytes were absent or were only sporadically observed suggesting that tissues were not damaged by the BAL procedure. As expected macrophages and ciliated epithelial cells were both identified in the BAL samples.
- Median epithelial lining fluid concentrations were 13,200 ng/mL for BAL1 and 4,220 ng/mL for BAL2 (Tables 2 and 3). Median plasma concentration at the corresponding timepoint (4 hours after dosing) was 3,380 ng/mL. Although supernatant concentrations of zanamivir appeared to be comparable between BAL1 and BAL2, urea concentrations were approximately 3-fold higher for BAL2 compared to BAL1. BAL2 concentrations appeared to be comparable to those in plasma at the same timepoint.
- Median alveolar macrophage concentrations were 10900 ng/mL for BAL1 and 8060 ng/mL for BAL2 (Table 4). White blood cell counts and concentrations of zanamivir in the cell pellets appeared to be higher for BAL 2 compared to BAL 1, but the alveolar macrophage concentrations appeared to be comparable between BAL1 and BAL2. Alveolar macrophage concentrations for BAL1 and BAL2 appeared to be approximately 3-fold higher compared to those in plasma and BAL2 epithelial lining fluid at the same timepoint.
- Epithelial lining fluid concentrations 12 hours after dosing (trough) were estimated via linear regression (Figure 2).

Pharmacokinetics

Table 1. Intravenous pharmacokinetics of Zanamivir in Male Cynomolgus Macaque Monkeys Following Intravenous Administration of Zanamivir 20 mg/kg

| Monkey ID | C _{max} (µg/mL) | AUC ₀₋₁ (µg.h/mL) | MRT ₀₋₁ (h) | Cl _{P0-1} (mL/min/kg) | Vd ₀₋₁ (mL/kg) | t _{1/2} (h) |
|-----------|--------------------------|------------------------------|------------------------|--------------------------------|---------------------------|----------------------|
| 2323 | 97.0 | 92.0 | 1.68 | 3.62 | 369 | 3.10 |
| 4049 | 91.6 | 113 | 2.04 | 2.95 | 364 | 2.81 |
| 9067 | 134 | 116 | 1.79 | 2.87 | 312 | 2.29 |
| 9081 | 108 | 79.4 | 1.19 | 4.18 | 310 | 1.21 |
| 10029 | 127 | 111 | 1.56 | 3.00 | 284 | 2.87 |
| 12001 | 93.5 | 92.9 | 1.70 | 3.59 | 371 | 3.08 |
| Mean | 109 | 101 | 1.66 | 3.37 | 335 | 2.66 |
| SD | 18.3 | 14.7 | 0.28 | 0.52 | 37.7 | 0.72 |

Table 2. BAL1 epithelial lining fluid concentrations of Zanamivir in Male Cynomolgus Macaque Monkeys at 4 Hours After Intravenous Administration of Zanamivir 20 mg/kg

| Monkey ID | BAL volume (mL) | BAL urea (mg/dL) | BAL urea (mg) | Plasma urea (mg/dL) | Volume ELF (mL) | Supernatant Concentration (ng/mL) | Epithelial Lining Fluid Concentration (ng/mL) | Plasma Concentration (ng/mL) |
|-----------|-----------------|------------------|---------------|---------------------|-----------------|-----------------------------------|-----------------------------------------------|------------------------------|
| 2323 | 6.5 | 0.134 | 0.00874 | 18.2 | 0.0482 | 164 | 22100 | 2980 |
| 4049 | 3.5 | 0.608 | 0.0213 | 17.4 | 0.123 | 99.6 | 2840 | 5620 |
| 9067 | 7.5 | 0.115 | 0.00861 | 16.0 | 0.0540 | 132 | 18300 | 4640 |
| 9081 | 6 | 0.0812 | 0.00487 | 14.8 | 0.0330 | 207 | 37700 | 2260 |
| 10029 | 3.5 | 0.0896 | 0.00314 | 16.2 | 0.0193 | 44.3 | 8020 | 3520 |
| 12001 | 6 | 0.140 | 0.00840 | 17.4 | 0.0483 | 48.7 | 6050 | 3230 |
| Mean | 5.5 | 0.195 | 0.00917 | 16.6 | 0.0542 | 116 | 15800 | 3710 |
| SD | 1.64 | 0.204 | 0.00636 | 1.2 | 0.0358 | 64.5 | 13000 | 1220 |
| Median | 6 | 0.125 | 0.00851 | 16.8 | 0.0482 | 116 | 13200 | 3380 |

Table 3. BAL2 epithelial lining fluid concentrations of Zanamivir in Male Cynomolgus Macaque Monkeys at 4 Hours After Intravenous Administration of Zanamivir 20 mg/kg

| Monkey ID | BAL volume (mL) | BAL urea (mg/dL) | BAL urea (mg) | Plasma urea (mg/dL) | Volume ELF (mL) | Supernatant Concentration (ng/mL) | Epithelial Lining Fluid Concentration (ng/mL) | Plasma Concentration (ng/mL) |
|-----------|-----------------|------------------|---------------|---------------------|-----------------|-----------------------------------|-----------------------------------------------|------------------------------|
| 2323 | 7.5 | 0.490 | 0.0368 | 18.2 | 0.203 | 105 | 3890 | 2980 |
| 4049 | 5 | 0.406 | 0.0203 | 17.4 | 0.117 | 106 | 4550 | 5620 |
| 9067 | 8 | 0.300 | 0.0240 | 16.0 | 0.150 | 167 | 8920 | 4640 |
| 9081 | 10 | 0.154 | 0.0154 | 14.8 | 0.104 | 48.5 | 4650 | 2260 |
| 10029 | 7 | 0.137255 | 0.00961 | 16.2 | 0.0592 | 13.5 | 1590 | 3520 |
| 12001 | 10 | 0.482 | 0.0482 | 17.4 | 0.277 | 52.7 | 1900 | 3230 |
| Mean | 7.92 | 0.328 | 0.0257 | 16.6 | 0.152 | 82.1 | 4250 | 3710 |
| SD | 1.91 | 0.157 | 0.0143 | 1.2 | 0.0778 | 54.8 | 2640 | 1220 |
| Median | 7.75 | 0.353 | 0.0221 | 16.8 | 0.134 | 78.9 | 4220 | 3380 |

Table 4. Zanamivir Alveolar Macrophage Concentrations in Male Cynomolgus Macaque Monkeys at 4 Hours After Intravenous Administration of Zanamivir 20 mg/kg (median values)

| Matrix | BAL white blood cell count (cells/mL) | VAC (mL) | Concentration in cell pellet (ng/mL) | Concentration in alveolar macrophages (ng/mL) |
|--------|---------------------------------------|----------|--------------------------------------|-----------------------------------------------|
| BAL1 | 17500 | 0.000216 | 1.50 | 10900 |
| BAL2 | 55000 | 0.00072 | 2.68 | 8060 |

Figure 1. Plasma Concentrations of Zanamivir in Male Cynomolgus Macaque Monkeys Following Intravenous Administration of Zanamivir 20 mg/kg

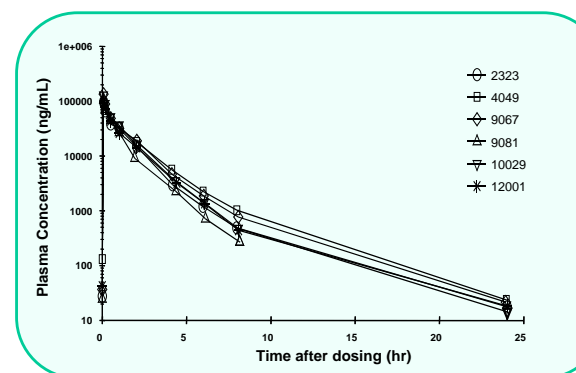
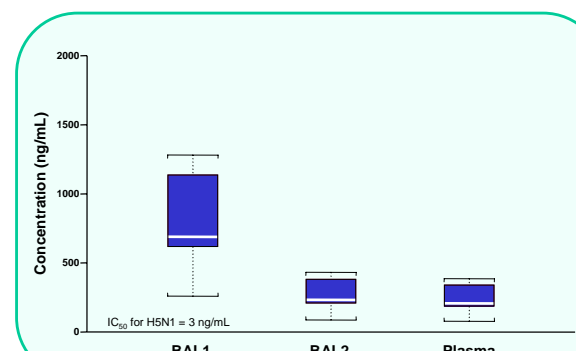


Figure 2. Estimated Epithelial Lining Fluid and Plasma Concentrations of Zanamivir in Male Cynomolgus Macaque Monkeys 12 hours Following Intravenous Administration of Zanamivir 20 mg/kg



Discussion

- Establishment of a pharmacokinetic link between macaques and humans based upon both systemic and pulmonary pharmacokinetics may facilitate dose-ranging and dose-selection activities for influenza.
- IV zanamivir 20mg/kg was selected for this study because it provided systemic exposure in macaques (mean AUC₀₋₁ of 101 µg.h/mL) that roughly corresponded to the highest dose that can be supported in humans. IV zanamivir 600mg provided a median AUC₀₋₁ of 73140.6 µg/L.h in humans after 5 days of dosing [Cass LMR, 1999].
- This study confirmed that the elimination half-life in macaques was comparable to the approximately 2 hour half-life reported in humans [Cass LMR, 1999].
- Epithelial lining fluid is considered to be an important site of infection in pneumonia, whereas, alveolar macrophage concentrations are important for intracellular infection [Baldwin DR, 1992]. Hence, epithelial lining fluid is likely to be the most clinically relevant matrix for pulmonary pharmacokinetics.
- Given the potential for contamination of BAL1 with cellular contents, BAL2 will be considered the primary matrix for characterization of epithelial lining fluid.
- The comparable concentrations of zanamivir in plasma and epithelial lining fluid following administration of zanamivir 20 mg/kg to macaques indicates good distribution of zanamivir into the pulmonary compartment.
- Estimated median pulmonary concentrations at 12 hours post-dose (trough) were estimated to be approximately 230 ng/mL, representing 77x the *in vitro* IC₅₀ for H5N1 neuraminidase (3ng/mL).

Conclusions

- Intravenous zanamivir 20mg/kg provided systemic exposure in macaques that roughly corresponded to the highest dose that can be supported in humans. Plasma half-life in macaques was similar to that reported in humans [Cass LMR, 1999].
- Both plasma and pulmonary concentrations exceeded the IC₅₀ for H5N1 throughout the 12-hour dosing interval.
- Plasma and pulmonary pharmacokinetics of intravenous zanamivir are conducive to the evaluation of antiviral activity against H5N1 virus infection in macaques.

References

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