Formulation, Stability, Pharmacokinetic and Modeling Studies for Tests of Synergistic Combinations of Orally Available Approved Drugs Against Ebola Virus In Vivo

Finch et al. 2021

Supplemental Tables (see end of document for References to Supplemental Tables)

|  |  |
| --- | --- |
| ***Tk0*** (day) | 0.0873 |
| ***V*** | 25.2 |
| ***k12*** (per day) | 6.71 |
| ***k21*** (per day) | 1.61 |
| ***k*** (per day) | 0.348 |

**Supplemental Table 1. PK parameters for bepridil**

|  |  |
| --- | --- |
| ***Tlag*** (day) | 0.0976 |
| ***ka*** (per day) | 25.3 |
| ***V*** | 909 |
| ***vm*** (per day) | 2.61×104 |
| ***km*** (per day) | 32.2 |

**Supplemental Table 2. PK parameters for sertraline**

|  |  |
| --- | --- |
| ***ka*** (per day) | 16.6 |
| ***V*** | 354 |
| ***k12*** (per day) | 4.28 |
| ***k21*** (per day) | 0.992 |
| ***k*** (per day) | 0.000198 |

**Supplemental Table 3. PK parameters for toremifene**

|  |  |  |
| --- | --- | --- |
| **Drug** | **IC50 (µM)** | **Hill coefficient** |
| **Bepridil** | 5.86 | 4.859375 |
| **Sertraline** | 3.79 | 1.628 |
| **Toremifene** | 3.64 | 3.057 |

**Supplemental Table 4. In vitro PD parameters for single drugs.** Data are from Ref.[1]

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Drug** | **IC50**  **(Huh7)**  **(μM)** | **IC50**  **(HepG2)**  **(μM)** | **IC50**  **(average)**  **(μM)** | **Oral Dose 1**  **(mg)** | **Cmax**  **(PO)**  **(μM)** | **Cmax/**  **IC50** | **Reference**  **PK Data** |
| apilimod | 0.17 | nd | 0.17 | 70 | 0.26 | 1.56 | [2] |
| aripiprazole | 7.8 | 3.76 | 5.78 | 30 | 1.01 2 | 0.17 | [3] |
| azithromycin | 10.75 | nd | 10.75 | 500 | 0.64 | 0.06 | FDA |
| bepridil | 5.86 | 3.21 | 4.53 | 400 | 2.43 | 0.54 | [4] |
| clomiphene | 1.96 | 0.76 | 1.36 | 50 | 0.05 | 0.04 | [5] |
| piperacetazine | 7.58 | 3.30 | 5.44 | na | na | na | na |
| sertraline | 3.79 | 1.44 | 2.62 | 200 | 0.54 3 | 0.21 | [6] |
| toremifene | 3.64 | 0.03 | 1.83 | 60 | 1.97 4 | 1.08 | [7] |

**Supplemental Table 5. Predicted drug exposures (Cmax) in humans following PO administration compared to drug efficacy (IC50) against EBOV in cultured liver cells**. Values in column 1 and 2 are from Dyall ([1]; Dataset 1, all at moi 0.21; all from Tab 4 except clomiphene and sertraline, which are from Tab 2) and Johansen [8], Table 1), respectively. Values in column 3 are the averages of the values in columns 1 and 2. 1Cmax after a single oral dose, unless specified. 2Cmax after 14 days of daily dosing. 3Cmax after 30 days of daily dosing to females (18-45 years of age); the 200 mg dose was reached after 3 days of dose increases starting at 50 mg. 4Cmax after multiple days of daily dosing to healthy adults (see Figure 6 in [7]). FDA refers to the FDA package insert for azithromycin. Abbreviations: na, not available (piperacetazine is only authorized for veterinary use in the USA); nd, not done; PO, Per Os (by mouth).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Drug** | **Dose**  **(mg/kg)** | **Regimen** | **Virus** | **Survival**  **(%)** | **Reference** |
| Apilimod | 44 | IP, SID | EBOV | 0 | \* |
| Aripiprazole | 20 | IP, SID | EBOV | 10 1 | [8] |
| Azithromycin | 100 | IP, SID | EBOV | 10, 30, 60 2 | [9] |
| Bepridil | 12 | IP, BID | EBOV | 100 3 | [8] |
|  | 12 | IP, BID | MARV | 90 4 | [10] |
| Clomiphene | 60 | IP, QOD | EBOV | 90 5 | [11] |
|  | 60 | IP, BID | EBOV | 10 6 | [9] |
| Piperacetazine | nd | nd | nd | nd | nd |
| Sertraline | 10 | IP, BID | EBOV | 70 | [8] |
| Toremifene | 60 | IP, QOD | EBOV | 50 | [11] |

**Supplementary Table 6. Prior studies of drug efficacies as single agents in mouse models of lethal EBOV infection**. For the studies described in \*(Supplementary Figure 5, this study) and in References 8, 10 and 11, mice were treated on day 0 (d0) and then on d1-9 as indicated (either SID, BID, or QOD) and observed for a total of 28 days. For the study described in Reference 9, mice were treated on d0 and then on d1-7 and observed for a total of 14 days. 1Mice became extremely somnolent, thwarting eating and drinking, which likely contributed to low survival. 220% survival was seen in the control group in the study yielding 60% survival with azithromycin. 390-100% survival has been seen dosing with 12 mg/kg, IP, SID (unpublished data). 4In the same report, 80% mice were protected if treated with 12 mg/kg bepridil, IP, SID. 5In the same report, a study comparing male and female mice (with fewer mice per group) yielded 60% and 40% protection of female and male mice, respectively (dosed with 12 mg/kg, IP, BID). 6In the same report, no mice were protected if treated with 21 mg/kg clomiphene, PO, SID. Abbreviations: BID, twice daily dosing; EBOV, Ebola virus; IP, intraperitoneal; MARV, Marburgvirus; nd, not done; QOD, dosing on d0, 1, 3, 5, 7, 9. SID, once daily dosing.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vehicle #** | **Bepridil**  **(solubility)** | **Sertraline**  **(solubility)** | **Toremifene**  **(solubility)** |
| 1 | Suspension | Suspension | Suspension |
| 2 | Slightly hazy | Clear solution | Clear solution |
| 3 | Clear solution | Clear solution | Suspension |
| 4 | Clear solution | Clear solution | Non-homogeneous |
| 5 | Clear solution | Clear solution | Suspension |
| 6 | Clear solution | Clear solution | Suspension |
| 7 | Clear solution | Clear solution | Clear solution |
| 8 | Clear solution | Clear solution | Clear solution |
| 9 | Clear solution | Clear solution | Clear solution |
| 10 | Clear solution | Clear solution | Insoluble |

**Supplementary Table 7. Solubility of bepridil, sertraline and toremifene in ten test vehicles.** The vehicle formulations were: #1, Aqueous Suspension Vehicle; #2, 20% Captisol in water; #3, 37.5% PEG 400/ 37.5% Tween 20/25% Capmul MCM NF; #4, Vegetable Oil; #5, PEG 400; #6, 10% Solutol/90% PEG 400; #7, 5% NMP/95% PEG 300; #8, 80% PEG 400/ 20% of 0.1% Tween-20 in Water; #9, 3% NMP/45% PEG300/12% ethanol/40% sterile water; #10, 40% Propylene Glycol/30% Solutol HS 15/30% Sterile Water. Drug concentrations were: Bepridil, 25 mg/ml; Sertraline, 3 mg/ml; toremifene, 8.4 mg/ml. See the Supplementary Document for details on sample preparation.

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| --- | --- | --- | --- |
| **Drug 1** | **Drug 2** | **MacSynery**  **(Av. LogV)** | **n** |
| Aripiprazole | Piperacetazine | 15.0 | 2 1 |
| Aripiprazole | Amodiaquine | 3.2 | 3 |
| Aripiprazole | Bepridil | 7.5 | 2 |
| Favipiravir | Bepridil | 0.0 | 3 |
| Favipiravir | Amodiaquine | 7.6 | 3 |
| Favipiravir | Aripiprazole | 2.0 | 3 |
| Favipiravir | Ribavarin | 2.8 | 3 |
| Favipiravir | Sertraline | 0.9 | 2 |
| Favipiravir | Toremifene | 0.1 | 2 |
| Favipiravir | Azithromycin | 1.4 | 2 |
| Favipiravir | Clomiphene | 1.3 | 2 |
| Favipiravir | Favipiravir | 0.0 | 2 |

**Supplementary Table 8. Additional *in vitro* drug synergy tests.** All synergy tests were performed in Huh7 cells with Ebov/Mak at moi 0.21 and analyzed using MacSynergy software as described in [1]. Data are presented at the 99.9% confidence level. 1In a third test using higher top doses of both drugs, the LogV was 71.95, which may be erroneous (https://www.uab.edu/images/pediatrics/ID/MacSynergy.pdf). Abbreviations: Av. LogV, average log volume; n, number of replicate experiments; each experiment performed in triplicate plates.

References to Supplemental Tables, Finch et al, 2021

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