CTSI COVID-19 Rapid-Response Research Project
Developing innovative therapeutic strategies to combating Sars-CoV-2

October 2020

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https://ptr.pharmacy.ufl.edu/research/areas-of-research/bulitta-research-lab/

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Open postdoc position
Outer membrane permeability in KPC-*Klebsiella pneumoniae*

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Imipenem penetrated >100 fold faster than non-carbapenem β-lactams.

<table>
<thead>
<tr>
<th>Beta-lactam</th>
<th>Permeability coeff. (nm/s)</th>
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<tbody>
<tr>
<td>Imipenem</td>
<td>2,042 ± 338</td>
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<tr>
<td>Meropenem</td>
<td>83.3 ± 9.17</td>
</tr>
<tr>
<td>Cefepime</td>
<td>13.6 ± 9.26</td>
</tr>
<tr>
<td>Azetronam</td>
<td>8.64 ± 8.46</td>
</tr>
<tr>
<td>Ceftadizime</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

Results not affected by efflux pump inhibition.

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R01 AI136803  R01 AI130185

Where we started from:
Translational anti-microbial pharmacology

NIH/NIAID R01 AI136803
Bulitta JB (PI) $5.7M 2018-23
“Combating resistant super-bugs by understanding the molecular determinants of target site penetration and binding”
Intracellular FAV and FAV triphosphate

Sciex 6500+ with Waters UPLC
Identify and Optimize Antiviral Therapy

- Determine the minimal **dose** of a drug and **how often** to give that drug that will maximize viral suppression.
- Focus on Nucleoside/Nucleotide Polymerase Inhibitors

![SARS-CoV-2 RNA dependent RNA polymerase](image)

- **Galidesivir**
- **Favipiravir**
- **Remdesivir**
LC-MS/MS development for Favipiravir-triphosphate

1. A polar analyte. Precipitation in MeOH or ACN
2. No retention on reserve phase column
3. Endogenous interference identified

Intra-cellular concentrations of favipiravir and its active triphosphate metabolite in three different cell lines (LNCAP, HELEA and HUH-7)
Mechanism-based pharmacokinetic / pharmacodynamic model for favipiravir, ribavirin and interferon against ZIKV: Describing efficacy, cytotoxicity, synergy and antagonism
Favipiravir and its triphosphate

→ Perfectly linear relationship
→ No saturable phosphorylation

Dr. Yinzhi Lang
Dr. Xun Tao
Mr. Jieqiang Zhou

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Chromatographic separation between Favipiravir-triphosphate and the cell matrix derived interference

EIC of compounds on new developed RPC-MS (on AB Sciex 6500+ system)
Lower carryover as well as a good linear
8 minutes was optimized to 2 minutes
Working range: 20 ng/mL ~ 4000 ng/mL
LLOQ: From 500 ng/mL to 20 ng/mL

EIC of compounds on previously developed PGC-MS (on older Agilent 6460)
Working range: 500 ng/mL ~ 20000 ng/mL
Excellent lower limit of quantification (LLOQ) for all 3 compounds

Galidesivir  LLOQ: 1 ng/mL in Caco2 cells
Remdesivir   LLOQ: 1 ng/mL in Caco2 cells
Favipiravir  LLOQ: 1 ng/mL in Caco2 cells

Other two triphosphate metabolites
UPLC-MS/MS analysis of FAV on A549 cells

Michaelis-Menten kinetics do not exist for FAV in A549 cells!!

Near steady-state is achieved by 48 h, as there is little difference between the 24 h and 48 h sampling times
UPLC-MS/MS analysis of FAV on A549 cells

+ FAV

24 h

Remove FAV

Harvest Cells at various times post-FAV washout

Extracellular FAV

Intracellular FAV

Intracellular FAV-TP

FAV out-diffusion occurs in 2 phases:
1st phase $t_{1/2} = 0.09$ h
2nd phase $t_{1/2} = 2.64$ h
UPLC-MS/MS analysis of FAV on A549 cells

Loss of FAV-TP is a zero order process. Over the 4 hour observation period half is lost in about 3 hours.

This loss is a maximal rate as all external FAV was removed at time zero.
FAV against SARS-CoV-2 on ACE2-A549 cells in the HF system: Simulated PK profiles

**Influenza Regimen**
(1800 mg @ 0 & 12 h, then 800 mg Q12h)

**JIKI/Ebola Regimen**
(2400 mg @ 0 & 8 h, then 1200 mg Q12h starting at 16 h)

**Continuous Infusion**
(66.7 mg/h)

**Continuous Infusion w/ Loading dose**
(66.7 mg/h)
**UPLC-MS/MS analysis of FAV on A549 cells**

- **JIKI/Ebola**
  - Regimen: 2400 mg @ 0 & 8 h, then 1200 mg Q12h starting at 16 h

- **Influenza**
  - Regimen: 1800 mg @ 0 & 12 h, then 800 mg Q12h

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Comparison of the phosphorylation of FAV in ACE2 A549 and normal A549 cells (both uninfected cells)

As expected, the ACE2 receptor did not affect the extent of FAV phosphorylation in uninfected ACE2 cells.

The jury is out there for infected ACE2 cells. Experiments are keenly ongoing.

<table>
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<tr>
<th>Comparison</th>
<th>Intracellular FAVTP</th>
<th>Extracellular FAV</th>
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<tbody>
<tr>
<td>ACE2 A549 / normal A549</td>
<td>88%</td>
<td>102%</td>
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</table>
Nucleos(t)ide Polymerase Inhibitors “Nucs”

Galidesivir

SARS-CoV-2
RNA-dependent RNA polymerase

Remdesivir

MK-4482
(Formerly EIDD-2801)

UPLC-MS/MS available for multiple cell lines with intracellular concentrations of:
- All 4 parent compounds
- Active intracellular triphosphate metabolites available for FAV and GAL.
- TP-compounds incoming for MK-4482 (remdesivir pending)

Favipiravir
Translational pharmacology to combat SARS-CoV-2

1. We extensively upgraded and refined our assays for intracellular drug concentration quantification, including cell volume determinations.

2. We made substantial progress in defining the reasons for suitable and less suitable cell lines. This is mission critical for SARS-CoV-2.

3. Which dose is safe and effective?

4. Optimal / feasible dosing interval?

5. Design safe and effective dosage regimens via Monte Carlo simulations.

6. Future research on antiviral combination therapies and accounting for host responses.

7. **Platforms are ready for collaborations inside and outside of UF!**