THE ROLE OF OATP1B1 IN GRAZOPREVIR DRUG-DRUG INTERACTIONS AND THE ELDERLY

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Background

Grazoprevir (GZR) is a hepatitis C (HCV) NS3/4A protease inhibitor that can be combined with antiretroviral drugs to treat HIV/HCV co-infected patients. Coadministration with ritonavir (RTV)-boosted atazanavir (ATV/r) and darunavir (DRV/r) is contraindicated as it increases GZR area under the curve (AUC) by 10.6-fold and 7.5-fold, and C_{max} by 6.2-fold and 5.3-fold, respectively **[1]**. Although assumed to be caused by OATP1B1/3 and CYP3A4 inhibition, the mechanism of these drug-drug interactions (DDI) has not been fully elucidated.

Moreover, administering GZR elicits a 20% increase in GZR AUC in elderly (≥65 years) patients **[2]**. Differences between drug exposure in the young and elderly are suggested to be caused by changes in hepatic blood flow and liver mass **[3]**; although existing literature regarding the abundance of OATP1B1 in the elderly is contradictory, stating both a weak correlation **[4]** and no correlation between age and OATP1B1 abundance **[5]**.

Objectives

To validate a pooled suspended primary human hepatocyte *in vitro* assay using the well characterised OATP1B1/3 substrate and inhibitor, pitavastatin (PIT) and rifampicin (RIF).

To quantify OATP1B1/3-mediated transport of GZR when co-incubated with either ATV, DRV or RTV using the validated pooled suspended primary human hepatocyte *in vitro* assay.

To evaluate the abundance of OATP1B1 in primary human hepatocytes from both young (30-62 years) and elderly (74-80 years) donors *in vitro* using a validated sandwich ELISA method.

Method

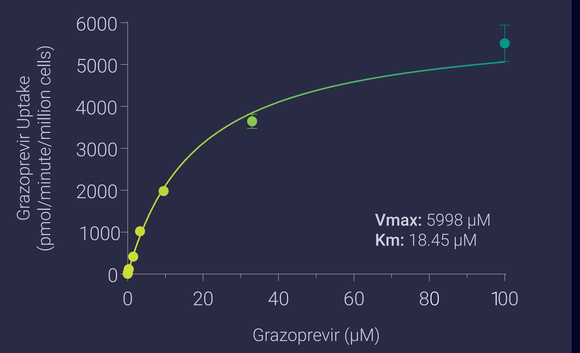
Pooled cryopreserved primary human hepatocytes were suspended in Krebs-Henseleit buffer at $1x10^5$ cells per well in 24-well cell culture plates. For assay validation, cells were incubated for 2 minutes at 37°C with PIT alone (0.1-300 µM) and PIT (0.1 µM) with RIF (0.1-300 µM). Similarly, test compounds GZR (0.1-100 µM) and GZR (0.1 µM) together with either ATV (0.0033-10 µM), DRV (0.01-33 µM) or RTV (0.01-33 µM) were incubated for 2 minutes at 37°C. Transporter uptake was terminated using ice-cold phosphate buffered saline (PBS) followed by immediate centrifugation, washing with ice-cold PBS and a single freeze-thaw cycle (-80°C to room temperature; 18-25°C) to lyse the cells. PIT and GZR concentrations were quantified using a validated LC-MS/MS assay and V_{max}, Km and IC_{50} were calculated.

The abundance of OATP1B1 in pooled cryopreserved primary human hepatocytes aged 30-62 years old and three individual elderly donors aged 74-80 years old were quantified using a validated sandwich ELISA method. Statistical significance was assessed using an unpaired t-test.

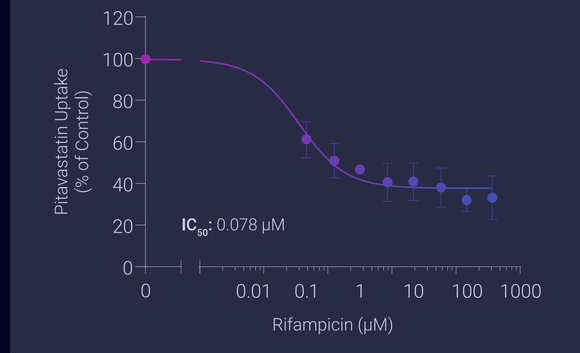
References

[1] FDA. (2016); Zepatier (Elbasvir/Grazoprevir) Tablet Summary Review [online] **[2]** FDA. (2016); Zepatier (Elbasvir/Grazoprevir) Tablet Clinical Pharmacology and Biopharmaceutics Review [online] **[3]** Stader et al. (2018); Clin Pharmacokinet [Epub ahead of print] **[4]** Badée et al. (2015); Drug Metab Dispos 43(4):424-32 **[5]** Burt et al. (2016); Drug Metab Dispos 44(10):1550–61 **[6]** Prueksaritanont et al. (2014); Br J Clin Pharmacol 78(3):587-98 **[7]** Kiang (2018); Eur J Drug Metab Pharmacokinet 43(5):509-31

Grazoprevir — Figure 1

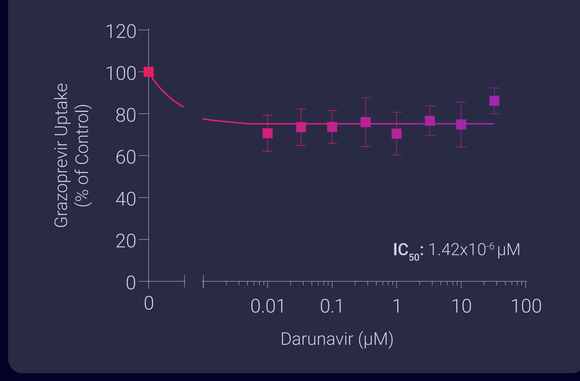


Rifampicin — Figure 2

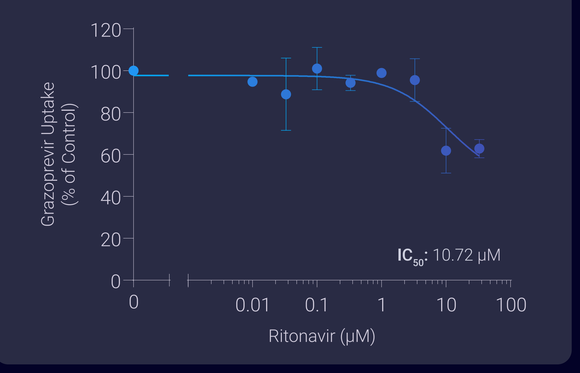


Atazanavir — Figure 3

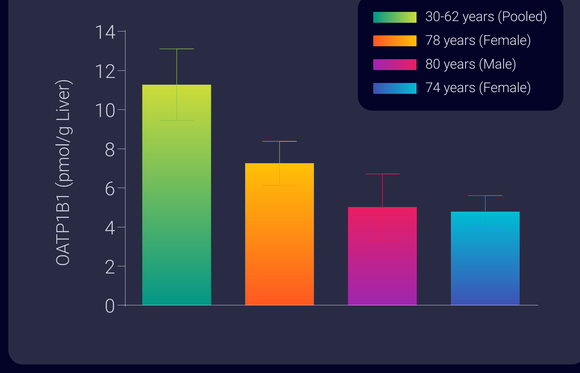
Darunavir — Figure 4



Ritonavir — Figure 5



OATP1B1 — Figure 6



Results

In pooled primary human hepatocytes, PIT and GZR uptake was mediated by OATP1B1/3 with a calculated V_{max} of 5490 and 5998 pmol/minute/10^6 cells, and Km of 55.41 and 18.45 µM, respectively **(Fig. 1)**. RIF reduced OATP1B1/3-mediated uptake of PIT by 68% (maximal inhibition at 100 µM; IC_{50} of 0.078 µM) **(Fig. 2)**. ATV reduced OATP1B1/3-mediated uptake of GZR by 16% (maximal inhibition at 10 µM; IC_{50} of 0.001 µM) **(Fig. 3)** whilst DRV reduced GZR uptake by 29% (maximal inhibition at 1 µM; IC_{50} of $1.42x10^{-6}$ µM) **(Fig. 4)**. RTV reduced OATP1B1/3-mediated uptake of GZR by 38% (maximal inhibition at 10 µM; IC_{50} of 10.72 µM) **(Fig. 5)**.

When compared to pooled hepatocytes from donors aged 30-62 years old, the abundance of OATP1B1 in the three individual elderly donors aged 74, 78 and 80 years old were, on average, 50% lower with a P-value of 0.013 **(Fig. 6)**.

Discussion

Using the well characterised OATP1B1/3 substrate and inhibitor, PIT and RIF, the *in vitro* assay demonstrated its validity with the inhibition of PIT by RIF being comparable to the literature **(Fig. 2)** **[6]**. The inhibition of OATP1B1/3-mediated uptake of GZR *in vitro* **(Fig. 3-5)** was not as strong as the observed clinical GZR exposure when coadministered with ATV/r and DRV/r **[1]**. This data suggests that OATP1B1/3 inhibition may not play as significant a role in GZR DDIs as previous *in vitro* studies have suggested **[2]**. Unlike this study, previous GZR OATP1B1/3 *in vitro* studies utilised concentrations of GZR that are not physiologically relevant in MDCKII cell lines transfected with OATP1B1/3 **[7]**. Further studies are required to fully understand the differences between the described *in vitro* techniques and their extrapolation to in vivo GZR data as well as the role of RTV in the ATV/r and DRV/r DDIs with GZR *in vivo*.

In contrast to the literature, our data suggests a significant correlation between age and OATP1B1 abundance **(Fig. 6)**. However, sample size in this study is much smaller than that of the meta-analysis literature and must be taken into consideration when interpreting this data **[5]**. Additional studies are required to elucidate the discrepancies between the correlation of age and OATP1B1 in this study and the current literature.

Conclusion

The *in vitro* model of drug uptake used here suggests that RTV does not inhibit OATP1B1/3-mediated transport in the range of physiologically relevant concentrations. DRV produced a moderate inhibition of OATP1B1/3-mediated transport whilst ATV produced surprisingly a minor inhibition of OATP1B1/3-mediated transport in the range of physiologically relevant concentrations. Our experimental approach represents an effective strategy to characterize the role of transporters in DDIs and may be useful to identify other clinically relevant DDIs.

Furthermore, the lower expression of OATP1B1 in hepatocytes taken from elderly donors provides one plausible mechanistic basis for the increased GZR AUC reported in this sub-population and justifies further investigation.