THE METABOLISM OF LUFOTRELVIR, A PRODRUG FOR THE TREATMENT OF SARS-COV2, IN HUMANS FOLLOWING INTRAVENEOUS ADMINISTRATION

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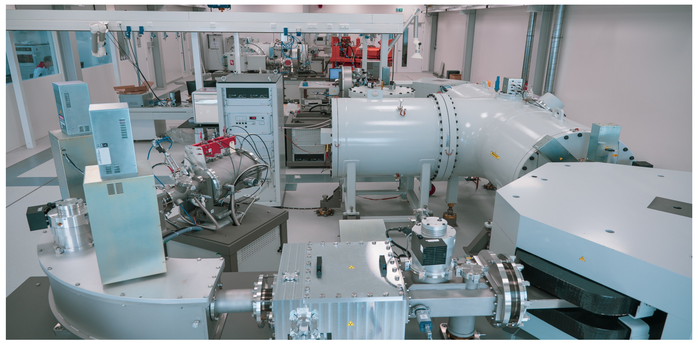
Introduction

Microdosing and microtracer approaches in combination with accelerator mass spectrometry (AMS) allow the generation of human ADME (absorption, distribution, metabolism and excretion) data early during drug development. With these data the overall drug development program can be optimized and shortened.

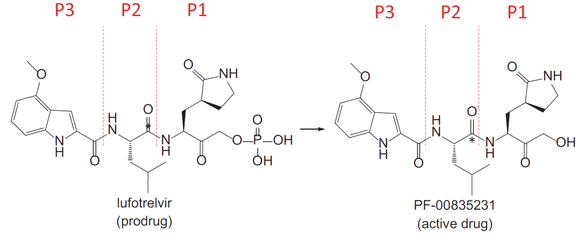
Here, an intraveneous human microtracer study was used for the clinical evaluation for lufotrelvir. Lufotrelvir is a novel phosphate prodrug of PF-00835231 used for the treatment of COVID-19. The pandemic emphasized the importance of this accelerated approach because of the potential global need for this drug.



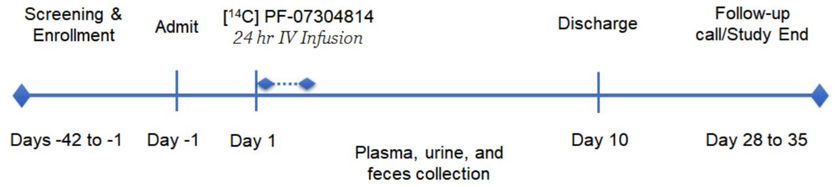
Biomedical AMS facility: 3 AMS instruments (2 x 1 MV, 200 kV), 9 UPLC systems, 3 HRMS instruments

Methods

Five healthy participants received a single 24-hour i.v. infusion of 500 mg lufotrelvir containing ~420 nCi [14C]-lufotrelvir. Blood, urine and fecal samples were collected and analyzed for total ^{14}C by AMS on a 1MV multi-element instrument (HVE. Model 4110; The Netherlands). For urine and feces one overall pool (across time and subjects) was used for quantitative metabolite profiling. Hamilton pools for plasma were prepared for 0–24 hours (during infusion) and 24–216 hours (post infusion).



Methods

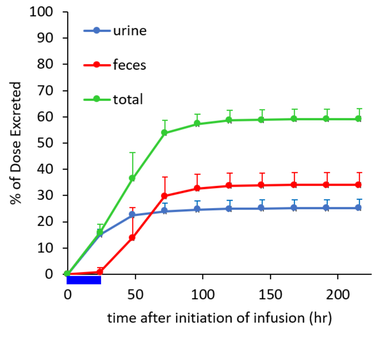


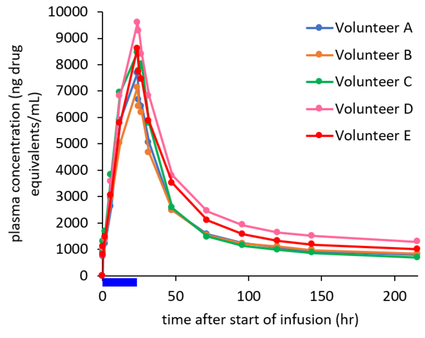
Plasma and fecal homogenates were extracted with acetonitrile. Urine was processed by adding acetonitrile and formic acid. The samples were analyzed on an Acquity UPLC system (Waters) coupled to a Q-Exactive mass spectrometer (Thermo). The column effluent was split to the fraction collector and mass spectrometer. AMS analysis was done off-line on the fractions of the eluent.

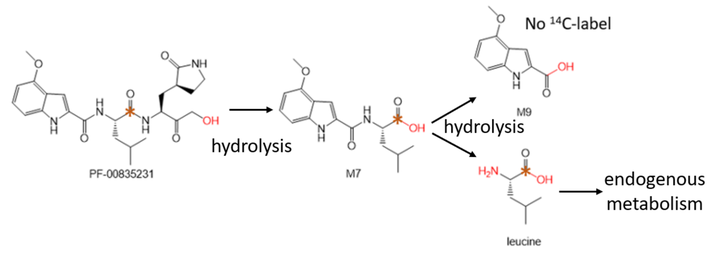
The pellet remaining after plasma and fecal homogenate extraction was pronase digested. Subsequently the amino acids were derivatized using an AccQ-Tag Ultra derivatization kit (Waters). The processed samples were analyzed by UHPLC-HRMS with fraction collection for off-line AMS analysis.

Results

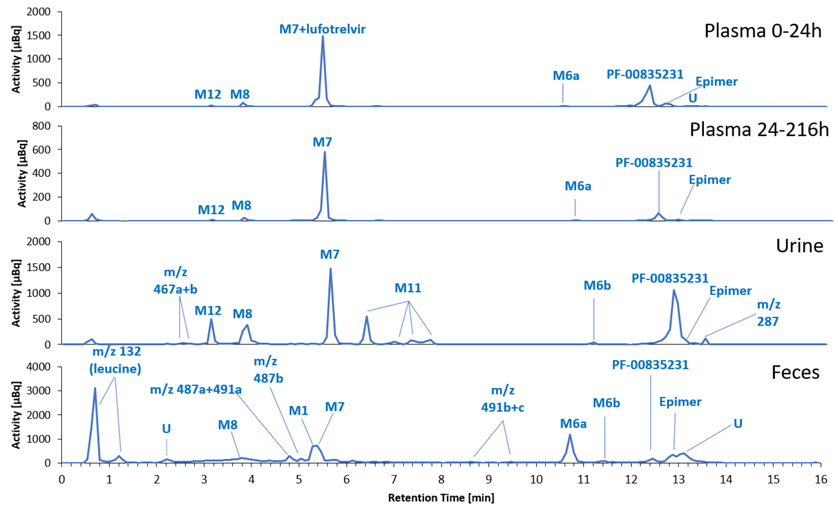
On average only 63% of the activity administered was recovered in urine and feces after 10 days. Plasma total ^{14}C analysis indicated a terminal phase half-life >200 hours.



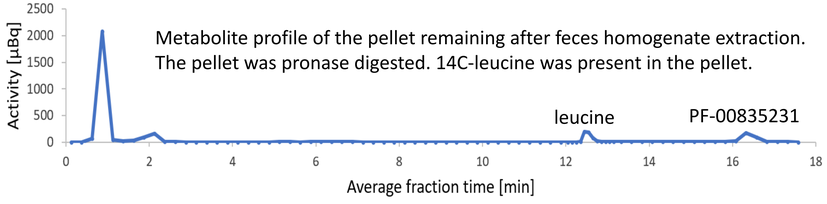




Results continued



The metabolite profiles showed complete conversion of lufotrelvir into the active drug PF-00835231 which was subsequently cleared by hydrolysis, hydroxylation, ketoreduction, epimerization, renal clearance, and secretion into the feces. Also a significant amount of radioactivity was not extractable from plasma and feces and remained in the pellet.



Conclusion

After plasma and feces extraction the majority of the activity remained in the pellet. The ^{14}C-label in lufotrelvir was at the leucine carbonyl position. With hydrolysis as an important metabolizing route, it was hypothesized that ^{14}C-leucine was released and subsequently incorporated into macromolecules. This was confirmed after investigation of the pellets using pronase digestion in combination with amino acid derivatisation. UHPLC-HRMS and AMS. The presence of ^{14}C-leucine in the pellet was shown, also explaining the low mass balance recovery. Using a modern AMS approach, human mass balance and metabolite profiling data can be available as early as Phase 1. In this particular study AMS accommodated the very tight timelines for development.