DOI: 10.1002/hsr2.1144

RESEARCH LETTER

Health Science Reports Open Access WILEY

Transplacental transfer of Remdesivir and GS-441524: An ex vivo perfusion study

1 | INTRODUCTION

Remdesivir (Rmd) is a prodrug of GS-441524 (GS) originally developed against Ebola, proven to reduce in vitro SARS-Cov-2 replication.[1,2] Pharmacokinetics (PK) data are few and show that Rmd is rapidely metabolized into GS.[3] Maximal Remdesivir concentrations (C_{max}) and different PK exposure parameters have been reported according to different dosing regimen.[3] In healthy volunteers, a C_{max} higher than 4000 ng/mL was found after an IV administration of 225 mg of Rmd. However, pregnant women were not included in this report. Several reviews reported the use of Rmd in pregnant women,[4–7] but did not analyse Rmd or GS concentration neither in mothers nor in cord blood. A physiological based PK model has been done to predict mother concentrations but without fetal concentrations.[8] Gilead's report suggested some reproductive toxicity in rats,[3] but no data regarding Rmd and its metabolite transfer through the placental was available. Being a large spectrum antiretroviral drug, Rmd can be given in any emerging RNA virus infection, and the lack of information regarding pregnant women may be a concern in drug dosing. Among the different methods to study the transplacental transfer of drugs, the closest to human physiology is the perfusion of the human cotyledon.[9] In the present study, we aimed to evaluate the transfer of Rmd and GS-441524 in an ex vivo cotyledon perfusion model.

2 | METHOD

Placentas were obtained from women who delivered at Port Royal maternity, Paris, France. All patients received an information note and gave a written consent for this study, approved by the ethic board of Nord Ouest IV committee, and registered in clinicaltrial.gov (NCT04400084). We included women aged over 18 years, with social insurance, with a single pregnancy between 37 and 41 weeks of gestation, from April to June 2020. Patients with a chronic therapy or HIV, HBV or HCV infection, or with a pregnancy disorder, which likely induces modifications of the placenta vascularization, or with fetal growth delay, were not included.

Cotyledons were perfused within the hour following the delivery in double closed circuit, for 3 h (see supplemental data).[10] Antipyrine (Sigma, Acros Organics Society) and Rmd (purchased from Gilead in march 2020) were added at the beginning of the perfusion into the maternal compartment, at 20 mg/L[11] and 4000 ng/mL, respectively.[3] No GS was added. Samples from the maternal and fetal compartments were collected throughout the 3 h of perfusion and stored at −30°C until analysis. Antipyrine, our control molecule, was first quantified by liquid chromatography, and the standard validation criteria were calculated: fetal to mother concentration ratio and a fetal transfer rate (FTR), which is a ratio of drug quantity transferred to the fetus to the total quantity (from both sides) at the end of the experiment (see supplemental data).[11] A perfusion is validated when the ratio is ≥75% and the FTR ≥ 20%. Then Rmd and GS of the validated perfusions were quantified by a mass spectrometry detection (Xevo TQD, Waters®) (see supplemental data). A FTR at 3 h was calculated using the same formula as for antipyrine. Categorical data were summarized and presented as frequencies and percentages. Continuous data were presented as median (Inter quartile range [IQR]) (range). Statistical descriptive analysis was performed on R software (http://cran.r-project.org/).

3 | RESULTS

From the 19 women included, 10 placentas were successfully perfused during 3 h. Only eight perfusions were validated with antipyrine our control molecule, from women aged between 29 and 39 years, who gave birth to four girls and four boys born by vaginal delivery. Population characteristics are presented in the Supporting Information: Table S1.

As shown in Figure 1, Rmd decreased rapidly in the maternal compartment, from around 4000 ng/mL to a mean concentration of 867 ng/mL after 3 h of perfusion (Table 1). But the fetal Rmd concentrations rose up only to a median of 170 ng/mL at 3 h. Fetal to maternal concentrations ratios and FTR were weak (0.22 and 8.15%, respectively) for Rmd at the end of the perfusion. The FTR obtained from girls' placentas were higher than those obtained from boys' ones (see Table 1).

Rmd is degradated into GS by the placental tissues. Maternal GS concentrations reached a median of 74 ng/mL (higher for girls' placentas with medians of 99 vs. 42.4 ng/mL for boys' ones). In the

fetal compartment, GS reached the low limit of detection (10 ng/mL) after 2 h of perfusion. The median fetal/maternal ratios of concentrations at 3 h and the median FTR were respectively 0.16 and 9.9%.

4 | DISCUSSION

We report for the first time that the Rmd and the GS cross the placental barrier in an ex vivo perfusion model. The Rmd concentration in the maternal compartment decreased rapidly but did not increase in the fetal compartment as fast as it decreased in the maternal one. This suggests a high degradation as it has been already described, with a short half-life of less than 1 h,[12] eliminated by different esterase enzymes released by cells in the plasma or metabolized in tissues.[13] Our findings suggested that Rmd transfer was low in comparison to the high initial drug concentration but unneglectable. The ratio and quantity of drug transferred in the fetal compartment were higher in girls' cotyledons than in boys' ones. Furthermore, we showed that the GS was generated in the maternal side of the cotyledon in the same range of concentrations as in healthy volunters[12] and crossed the placenta after 3 h but in a much lesser extent than Rmd did. After 2 h, the GS was detected in fetus'

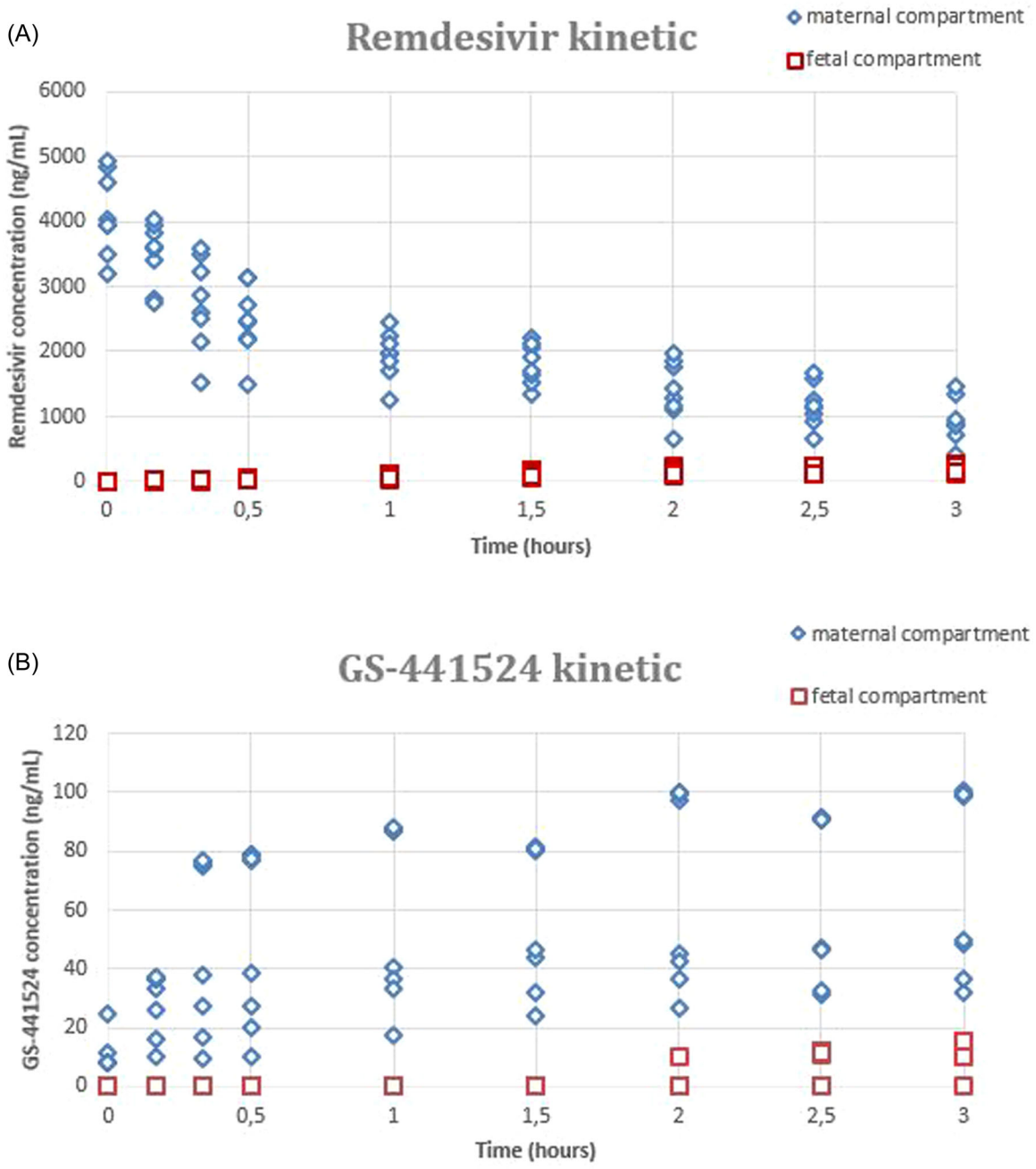

FIGURE 1 Remdesivir and GS-441524 transfer.

TABLE 1 Results.

Median [iqr] (range)	Total	Girls (n = 4)	Boys (n = 4)
$[Rmd]_m$ at 3 h (ng/mL)	867	825	882
	[822; 1047]	[634; 1046]	[867; 1036]
	(423–1457)	(423–946)	(861–1457)
$[Rmd]_f$ at 3 h (ng/mL)	170	240	152
	[128; 243]	[207; 243]	[127; 192]
	(105–260)	(109–251)	(105–260)
F/M ratio of Rmd	0.22	0.26	0.16
	[0.16; 0.27]	[0.24; 0.28]	[0.11; 0.22]
	(0.09–0.36)	(0.18–0.36)	(0.09–0.3)
Rmd FTR (%)	8.15	16.4	7.6
	[7.7; 15.7]	[13.38-18.2]	[6.38; 7.9]
	(3.3–19.8)	(8.2–19.8)	(3.35; 8.15)
$[GS]_m$ at 3 h (ng/mL)	74	99.1	42.4
	[39.3; 99.2]	[61.9; 100]	[33; 86]
	(31.9–100)	(49.6–100)	(31.9–98.4)
$[GS]_f$ at 3 h (ng/mL)	12.5	15.2	11.8
	[10.2; 15.42]	[11.4; 15.4]	[2.5; 12.3]
	(<10–15.4)	(10.2–15.4)	(<10–12.5)
F/M ratio of GS	0.16	0.16	0.2
GS FTR (%)	9.9	10.19	7.3
	[5–12.6]	[7.7–12.6]	[1.4–17.2]
	(0–19.5)	(13.18–7.2)	(<10–19.52)

Abbreviations: $[Rmd]_m$, concentration of Remdevisir in the maternal compartment; $[Rmd]_f$, concentration of Remdevisir in the fetal compartment; $[GS]_f$, concentration of GS in the fetal compartment; $[GS]_m$, concentration of GS in the maternal compartmen; FTR, fetal transfer rate; F/M ratio, fetal to maternal concentration ratio; iqr, interquartile range.

compartment. As GS half-live is between 20 and 30 h,[12] GS transfer in vivo might be higher than in our experiments. It might be interesting to perfuse the GS to better evaluate its transfer.

Our data suggest that Rmd transfer and GS generation may be influenced by the gender of the fetus. It is known that girls and boys placentas may be different,[14] but no difference depending on gender have been reported for transporters expression or for the size of the cotyledon, on which the exchange surface may depend. Moreover, Rmd is substrate of the P-glycoprotein (P-gp) and of the organic anion-transporting polypeptide 1B1 both known to be expressed on the syncytiotrophoblast.[3] P-gp expression has been described to be influenced by a polymorphism.[15] It might be interesting to investigate this point in future studies.

The main limit of our study is that no protein was added in the medium. Rmd is considered as a moderate binding protein, 88%–93.6% bound.[3] In the organism, the transfer of Rmd may be lesser as only the free fraction crosses the placenta, but this needs further investigations.[16,17] For the GS, as it exhibits very low protein binding,[3] the absence of protein might not influence the transfer.

This study has to be consolidated with a higher sample size, particularly to confirm the correlation between fetus' gender and drug transfer and metabolism, never described before. These results have been obtained with third trimester placentas and cannot be extrapolated to the other trimesters, as the placenta composition changes all along the pregnancy.

Even if close to physiological conditions, these results need an in vivo confirmation based on cord blood concentrations. The impact of fetus' gender on transplacental transfer remains to be explored as well as fetal toxicity issues.

AUTHOR CONTRIBUTIONS

Margaux Louchet: Conceptualization; data curation; formal analysis. **Mégane Ribot**: formal analysis; methodology. **Naïm Bouazza**: Methodology; supervision; validation; writing—review and editing. **Frantz Foissac**: Methodology; supervision; validation; writing—review and editing. **Léo Froelicher**: Methodology; writing—review and editing. **Victoria Buth**: Project administration; resources. **Sihem Benaboud**: Conceptualization; data curation; formal analysis; methodology; writing—review and editing. **Jean-Marc Treluyer**: Methodology; supervision; writing—review and editing. **Gabrielle Lui**: Conceptualization; formal analysis; supervision; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

The authors thank the URC Necker-Cochin and CIC Port Royal for recruiting mothers and their involvement in the management of the study. All authors have read and approved the final version of the manuscript. Gabrielle Lui, had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

TRANSPARENCY STATEMENT

The lead author Gabrielle Lui affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

KEYWORDS

ex vivo cotyledon perfusion, GS-441524, placenta, Remdesivir, transplacental transfer

Margaux Louchet[1]
Mégane Ribot[1]
Naïm Bouazza[2]
Frantz Foissac[2]
Léo Froelicher[1]
Victoria Buth[3]
Sihem Benaboud[1]
Jean-Marc Treluyer[1,2,3]
Gabrielle Lui[1,3]

[1]*Service de Pharmacologie, Hôpital Cochin, Paris, France*
[2]*Unité de Recherche Clinique, Assistance Publique Hôpitaux de Paris, Hôpital Tarnier, Paris, France*
[3]*CIC-1419 Mère-enfant Cochin-Necker, Paris, France*

Correspondence
Gabrielle Lui, Service de Pharmacologie, Hôpital Cochin, 27 rue du Faubourg Saint Jacques, Paris 75014, France.
Email: gabrielle.lui@aphp.fr

ORCID

Gabrielle Lui http://orcid.org/0000-0002-6529-0024

REFERENCES

1. Brown AJ, Won JJ, Graham RL, et al. Broad spectrum antiviral Remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res*. 2019;169:104541.
2. Agostini ML, Andres EL, Sims AC, et al. Coronavirus susceptibility to the antiviral Remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *mBio*. 2018;9(2):1.
3. Gilead R. *Summary on Compassionate Use*. European Medicines Agency.
4. Westhoff WJ, Smith LH, Wyszynski DF, Hernandez-Diaz S. COVID-19 pharmacotherapy utilization patterns during pregnancy: international registry of coronavirus exposure in pregnancy. *Pharmacoepidemiol Drug Saf*. 2022;31(7):804-809.
5. Marzban-Rad S, Ghafarzadeh M, Bahmani S, Kazemi A. The use of Remdesivir among pregnant women and associated clinical outcomes in mother and the child. *Ann Med Surg*. 2022;77:103681.
6. Ray A, Samra T, Mahajan V, et al. Characteristics and outcomes of parturients with COVID-19, admitted to a critical care unit: a single-center retrospective observational study. *J Family Med Prim Care*. 2022;11(10):6478-6486.
7. Burwick RM, Yawetz S, Stephenson KE, et al. Compassionate use of Remdesivir in pregnant women with severe coronavirus disease 2019. *Clin Infect Dis*. 2021;73(11):e3996-e4004.
8. Liu XI, Dallmann A, Brooks K, et al. Physiologically-based pharmacokinetic modeling of Remdesivir and its metabolites in pregnant women with COVID-19. *CPT Pharmacomet Syst Pharmacol*. 2022;12(2):148-153.
9. Bouazza N, Foissac F, Hirt D, et al. Methodological approaches to evaluate fetal drug exposure. *Curr Pharm Des*. 2019;25(5):496-504.
10. De Sousa Mendes M, Hirt D, Vinot C, et al. Prediction of human fetal pharmacokinetics using ex vivo human placenta perfusion studies and physiologically based models. *Br J Clin Pharmacol*. 2016;81(4):646-657.
11. Schneider H, Panigel M, Dancis J. Transfer across the perfused human placenta of antipyrine, sodium and leucine. *Am J Obstet Gynecol*. 1972;114(6):822-828.
12. Humeniuk R, Mathias A, Cao H, et al. Safety, tolerability, and pharmacokinetics of Remdesivir, an antiviral for treatment of COVID-19, in healthy subjects. *Clin Transl Sci*. 2020;13(5):cts.12840.
13. Deb S, Reeves AA, Hopefl R, Bejusca R. ADME and pharmacokinetic properties of Remdesivir: its drug interaction potential. *Pharmaceuticals*. 2021;14(7):655.
14. Buckberry S, Bianco-Miotto T, Bent SJ, Dekker GA, Roberts CT. Integrative transcriptome meta-analysis reveals widespread sex-biased gene expression at the human fetal–maternal interface. *Mol Hum Reprod*. 2014;20(8):810-819.
15. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA*. 2000;97(7):3473-3478.
16. Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. *Clin Pharm Ther*. 2011;90(1):67-76.
17. Gavard L, Gil S, Peytavin G, et al. Placental transfer of lopinavir/ritonavir in the ex vivo human cotyledon perfusion model. *Am J Obstet Gynecol*. 2006;195(1):296-301.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Louchet M, Ribot M, Bouazza N, et al. Transplacental transfer of Remdesivir and GS-441524: an ex vivo perfusion study. *Health Sci Rep*. 2023;6:e1144. doi:10.1002/hsr2.1144