Comparison of anti-SARS-CoV-2 activity and intracellular metabolism of remdesivir and its parent nucleoside

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ABSTRACT

Remdesivir, a monophosphate prodrug of nucleoside analog GS-441524, is widely used for the treatment of moderate to severe COVID-19. It has been suggested to use GS-441524 instead of remdesivir in the clinic and in new inhalation formulations. Thus, we compared the anti-SARS-CoV-2 activity of remdesivir and GS-441524 in Vero E6, Vero CCL-81, Calu-3, Caco-2 cells, and anti-HCoV-OC43 activity in Huh-7 cells. We also compared the cellular pharmacology of these two compounds in Vero E6, Vero CCL-81, Calu-3, Caco-2, Huh-7, 293T, BHK-21, 3T3 and human airway epithelial (HAE) cells. Overall, remdesivir exhibited greater potency and superior intracellular metabolism than GS-441524 except in Vero E6 and Vero CCL-81 cells.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (Cucinotta and Vanelli, 2020). Ever since the initial outbreak in December 2019 in Wuhan, China, the global scientific community has been actively developing and testing therapeutic agents and vaccines against SARS-CoV-2 (Ciotti et al., 2020; Nitulescu et al., 2020; Asselah et al., 2020). Remdesivir was initially developed by Gilead Sciences as an anti-Ebola agent (Warren et al., 2016). In record time, remdesivir was found to have anti-SARS-CoV-2 efficacy in vitro (Wang et al., 2020a), and clinical trials were initiated and conducted to evaluate its safety and efficacy in COVID-19 patients (Eastman et al., 2020). On May 1, 2020, remdesivir was issued FDA Emergency Use Authorization for COVID-19 patients hospitalized with severe disease for its potential to shorten the time to recovery (FDA, 2020a) and on October 22, 2020, FDA approved remdesivir for intravenous administration in adult and pediatric patients (>12 years old, > 40 kg) for treatment of COVID-19 requiring hospitalization (FDA, 2020b). The drug does not appear to reduce mortality in patients with severe symptoms (Dyer, 2020; Beigel et al., 2020) and there is some controversy regarding its value for the treatment of COVID-19 by the WHO (Dyer, 2020). It is worth noting that, unlike the Adaptive COVID-19 Treatment Trial-1 (ACTT-1) study which showed significant reduction in the duration of patient hospitalization, the WHO study was not performed under rigorous placebo controlled double-blind study conditions.

As a McGuigan-type adenosine monophosphate prodrug, remdesivir is hydrolyzed to the corresponding monophosphate (GS-441524-MP) and then intracellularly phosphorylated to its active metabolite GS-441524-triphosphate (GS-443902) (Warren et al., 2016; Li et al., 2021a,b) (Fig. 1). It has been reported that remdesivir exhibits a short plasma half-life of about 0.4 h in monkeys (Warren et al., 2016), 1 h in humans (Humeniuk et al., 2020), and that GS-441524 is the main metabolite. Interestingly, GS-441524 showed comparable efficacy to remdesivir against SARS-CoV-2 in Vero E6 African green monkey kidney cells and...
Calu3 2B4 human lung epithelial cells (Pruijssers et al., 2020). However, as GS-441524 has an easier synthetic route, potentially better safety profile, and may not require intravenous administration (Warren et al., 2016), some researchers in the field are advocating for the alternate use of GS-441524 for patients with COVID-19 (Yan and Muller, 2020). Thus, a side-by-side evaluation of these two compounds was warranted to determine advantages and differences between remdesivir and GS-441524.

In this study, we compared the anti-coronavirus activity of remdesivir and GS-441524 in Vero E6, Vero CCL-81, Calu-3, Caco-2 and Huh-7 cells. Furthermore, we compared the cellular pharmacology of these two compounds in Vero E6, Vero CCL-81, Calu-3, Caco-2, Huh-7, 293T, BHK-21, 3T3 and human airway epithelial (HAE) cells. Our results in cellular models provide a reference set of data for a comparison between remdesivir and GS-441524.

2. Material and methods

2.1. Compounds and reagents

Remdesivir and GS-441524 were synthesized in house with a purity higher than 98%. GS-443902 was purchased from MedChem Express (Monmouth Junction, NJ). All other reagents were the highest quality available from Thermo Fisher Scientific (Waltham, MA).

2.2. Cells and virus

The African monkey kidney Vero E6 (ATCC® CRL-1586™) and Vero (ATCC® CCL-81™) cells, human lung epithelial cells Calu-3 (ATCC® HTB-55™), human epithelial colorectal adenocarcinoma cells Caco-2 (ATCC® HTB-37™), human kidney epithelial cells 293T/17 (ATCC® CRL-11268™), baby golden hamster kidney fibroblasts BHK-21 (ATCC® CCL-10™) and mouse embryonic fibroblasts 3T3 (ATCC® CRL-1658™) were purchased from ATCC (Manassas, VA). Human hepatocellular carcinoma cells Huh-7 (JCRB0403) was purchased from JCRB Cell Bank (Japan). Primary human airway epithelium cells (HAE) from healthy donors were obtained from Lonza Biosciences (CC-2540s) and cultured in air-liquid interface by Candela Manfredi in Dr. Eric Sorscher’s group at Emory University. SARS-CoV-2 was provided by BEI Resources (NR-52281: USA-WA1/2020) and HCoV-OC43 was obtained from ATCC (Manassas, VA).

2.3. Antiviral activity and toxicity assays

To determine the antiviral activity of remdesivir and GS-441524 against SARS-CoV-2 in Vero E6, Vero CCL-81, Calu-3, and Caco-2 cells and against HCoV-OC43 in Huh-7 cells, confluent monolayers of cells in a 96-well cell culture microplate were treated with a range of concentrations of each compound in triplicate, followed by inoculation with virus MOI of 0.1 for Vero E6, Vero CCL-81, and Huh-7 or 0.01 for Calu-3 and Caco-2 cells, respectively. The production yield of progeny virus was assessed in cell supernatants collected optimally at 48 h (Vero) or 72 h (Calu-3 and Caco-2) post-infection using a specific q-RT PCR for SARS-CoV-2 and 72 h post-infection using a specific q-RT PCR for HCoV-OC43 (Zandi et al., 2020).

SARS-CoV-2 infectious clone virus containing mNeonGreen reporter (icSARS-CoV-2-mNG) inhibition assay (Xie et al., 2020) was further conducted in Caco-2 cells. Briefly, cells were treated with each compound in a range of concentrations in triplicate for 1 h prior to infection at 0.01 MOI. After 48 h, cells were fixed with 4% paraformaldehyde for 0.5–1 h, washed with PBS, permeabilized with 0.1% NP-40 in PBS and the nuclei stained with Hoechst 33342 (1:10,000 dilution in PBS) to facilitate autofocusing and imaging using a Cytation 5 cell imaging multi-mode reader (Biotek, Winooski, VT). Infection was quantified by counting mNeonGreen-expressing cells using Gen5 software (Biotek, Winooski, VT).

Cytotoxicity was evaluated using MTS method in Vero CCL-81, Calu-3, Caco-2, and Huh-7 cells as previously described. (Zandi et al., 2020).

2.4. Cellular pharmacology studies

Vero E6, Vero CCL-81, Calu-3, Caco-2, Huh-7, 293T, BHK-21, 3T3 and HAE cells were seeded at 1 × 10^4 cells per well in 12-well plates or 0.15 × 10^4 cells per well in 24-well plates (HAE only) and incubated with 10 μM of each compound at 37 °C with 5% CO₂. After 4 h, cells were washed twice with ice-cold PBS and resuspended in 70% ice-cold methanol overnight at −20 °C. The supernatants were then dried and reconstituted by HPLC mobile phase and then subjected to LC-MS analysis (Tao et al., 2019). Levels of the phosphorylated metabolites 5’-mono-, di-, and triphosphate (GS-441524-MP, GS-441524-DP, and GS-443902) were measured. GS-441524-MP and GS-441524-DP were semi-quantified using the calibration curve for GS-443902.

3. Results and discussion

3.1. Anti-coronavirus activity and cytotoxicity profile of remdesivir and GS-441524

Vero E6 and Vero CCL-81 cells are interferon deficient and do not secrete interferon alpha or beta when infected by viruses (Desmyter et al., 1968). Hence, these two cell lines are commonly used as in vitro models for virus research. As they are also highly susceptible to SARS-CoV-2 infection (Pruijssers et al., 2020), they have been used broadly to evaluate anti-SARS-CoV-2 compounds in vitro (Pruijssers et al., 2020; Zandi
Hoechst 33342-stained cells (blue) were analyzed in Gen5 software to control (bottom) was shown next to the respective montage image. Cell count per well. A representative image of cell control (top) or virus (bottom) was shown next to the respective montage image. Routinely predict liver toxicity (Zandi et al., 2020). No relevant toxicity was reported in all cell lines for GS-441524 and GS-443902 nucleotides, the GS-443902 nucleotide was the most abundant intracellular metabolite following treatment with either compound.

We further validated the higher potency of remdesivir versus GS-441524 in Caco-2 cells using icSARS-CoV-2-mNG inhibition assay (Fig. 2) (Xie et al., 2020). Resultant mean EC50 of remdesivir from three independent experiments was 0.018 μM while EC50 of GS-441524 was 1.3 μM, 70-fold lower compared to remdesivir. These data independently confirmed the antiviral results in Caco-2 cells determined by q-RT PCR assay (Table 1).

It has been established that SARS-CoV-2 infects the liver and directly contributes to the liver injury in patients with COVID-19 (Wang et al., 2020b). Therefore, we also used the human hepatocarcinoma Huh-7 cells to evaluate compounds against closely related virus HCoV-1 strain OC43. Remdesivir was active against this virus with an EC50 of 0.01 μM while GS-441524 was markedly less active with an EC50 of 4.1 μM. Of note, Huh-7 cells have lower susceptibility to SARS-CoV-2 infection than Vero E6 cells, and therefore are not always selected as an in vitro model for anti-SARS-CoV-2 assays (Pruijssers et al., 2020).

Cytotoxicity was evaluated using MTS method in Vero CCL-81, Calu-3, and Caco-2 cells, and additionally Huh-7 cells, a cell line we use to routinely predict liver toxicity (Zandi et al., 2020). No relevant toxicity was detected for either compound in Vero CCL-81, Calu-3 and Caco-2 cells. Interestingly, remdesivir was quite toxic in Huh-7 cells (Table 1). Notably, liver injury has been reported in remdesivir-treated COVID-19 patients (Zampino et al., 2020). Fortunately, remdesivir is only given for 10 days or less.

(A) Inhibition assay was performed in a 96-well format. Hoechst 33342-stained cells were imaged using a Cytation 5 cell imaging reader (A) and positively correlates with the higher level of GS-443902 formed in these cells from remdesivir. In Caco-2 cells, the levels of GS-443902 formed from remdesivir were 67-fold higher (p < 0.001, t-test) than that from GS-441524 (Fig. 3). Anti-SARS-CoV-2 activity of remdesivir in Caco-2 cells is usually measured by analyzing cytopathic effect (EC50 ranging from 0.1 to 0.8 μM (Meyer et al., 2020; Ellinger et al., 2021; Bojkova et al., 2020)) or by assessing virus expression using RT-qPCR (EC50 = 46 μM) (Krüger et al., 2021). In our hands, we observed a correlation between the higher potency of remdesivir vs GS-441524 (EC50 of 1 nM vs 80 nM, respectively, or 0.018 μM vs 1.3 μM by icSARS-CoV-2-mNG inhibition assay) in Caco-2 cells and the higher levels of active NTP (308 vs 4.6 pmol/million cells, respectively) formed in this cell line. Interestingly, the levels of active NTP formed with GS-441524 only marginally fluctuate from cell line to cell line, which seemed to correlate with the relatively similar antiviral activity in Vero E6, Calu-3, and Caco-2 cells (Table 1). On the other hand, remdesivir produced significantly more GS-443902 in Calu-3 and Caco-2 cells than in Vero E6 cells (6- and 37-fold more, p < 0.001, t-test), a trend that follows the potency observed for that compound in the three different cell lines. We hypothesize that this difference is most likely due to a variation in the level of enzymes required to cleave remdesivir’s produg.

**Table 1**

| Compound       | EC50 (μM) | Cytotoxicity: MTS CC50 (μM) |
|----------------|----------|----------------------------|
|                | Vero E6  | Vero CCL-81 | Calu-3 | Caco-2       | Vero CCL-81 | Calu-3 | Caco-2 | Hub-7 |
| Remdesivir     | 1.0/3.1  | 0.7/1.7     | 0.11/0.49 | 0.001/0.022 | >100        | 72.8   | >100   | 2.1   |
| GS-441524      | 1.1/3.9  | 0.8/1.6     | 0.25/2.35 | 0.08/1.42   | >100        | >100   | >100   | >100   |

*Antiviral assays conducted in triplicate at least three times.*
moiety from one cell line to the other.

This difference in anti-HCoV-OC43 potency between remdesivir and GS-441524 (0.01 μM vs 4.1 μM) correlated with the levels of active NTP formed, since incubation of Huh-7 cells with 10 μM remdesivir for 4 h generated more than 120-fold higher level of metabolite GS-443902 than incubation with 10 μM GS-441524. Notably, remdesivir was quite toxic in Huh-7 cells (Table 1), probably due to the very high intracellular levels (about 1160 pmol/million cells) of active NTP formed (Fig. 3).

In addition to Vero E6, Vero CCL-81, Calu-3, Caco-2 and Huh-7 cells, we also evaluated the intracellular metabolism of remdesivir and GS-441524 in 293T, BHK-21, 3T3 and HAE cells. 293T cell line is one of the most commonly used cell lines for in vitro SARS-CoV-2 studies because of its high expression of ACE2 and TMPRSS2 (Hoffmann et al., 2020b; Kumar et al., 2021). BHK-21 and 3T3 cells are also essential cell lines used for vaccine production (Kumar et al., 2021). Besides, BHK-21 cells support the replication of HCoV-OC43 (Shen et al., 2016). 3T3 cells had previously been modified for SARS-CoV-2 studies by transfection with a SARS-CoV-2 spike and ACE2 glycoprotein expression vector (Nunes-Santos et al., 2021). Recently, 293T and BHK-21 cell lines have been used by our group for anti-SARS-CoV-2 studies (unpublished data). The phosphorylated metabolites profiles of remdesivir and GS-441524 were very similar in 293T, BHK-21 and 3T3 cells, with the most abundant metabolite GS-443902 formed from remdesivir over 100-fold more (p < 0.001, t-test) than that from GS-441524. (Fig. 3). Finally, primary HAE cells have been used widely by researchers to study the SARS-CoV-2 (Sheahan et al., 2020; Pizzorno et al., 2020; Hattori et al., 2021; Mulay et al., 2021; Do et al., 2021). Researchers compared the anti-SARS-CoV-2 activity of remdesivir and GS-441524 in HAE models and showed that remdesivir was more potent than GS-441524 in human tracheal airway cultures (Do et al., 2021). In HAE cells at 4 h, remdesivir generated about 20-fold more (p < 0.001, t-test) GS-443902 than the parent nucleoside GS-441524 (Fig. 3), and these levels positively correlated with the higher potency. It has been reported that inhalation formulations are being developed for pulmonary administration (Sahakijpijarn et al., 2020; Sahakijpijarn et al., 2021) and clinical trials underway. With the higher potency against SARS-CoV-2 in HAE cells and superior cellular metabolism, remdesivir could be a better option than GS-441524 in the development of the inhalable formulation.

Overall, the large differences in active NTP formation after incubation with remdesivir or GS-441524 in different cell lines probably results from the fact that remdesivir is a nucleoside phosphoramidate prodrug that can be directly metabolized intracellularly to GS-441524 monophosphate, therefore bypassing the often-limiting monophosphorylation step (Varga et al., 2016). It is also worth noting that, if only passive permeation is considered, remdesivir, with an estimated LogP of 3.2 (ChemDraw Professional 16.0 - PerkinElmer), is more likely to effectively cross the hydrophobic phospholipid bilayer than the parent nucleoside GS-441524 (estimated LogP = −1.65) and therefore display higher potency.

It is worth noting that during the preparation of this manuscript, Li et al. reported that GS-441524 effectively inhibits SARS-CoV-2 infection in a mouse model (Li et al., 2021a). Their reported in vitro data seems consistent with ours except that we observed a far greater difference in EC_{50} between GS-441524 and remdesivir in Caco-2 cells (80-fold vs 6-fold). It is not surprising to see some in vivo efficacy in a mouse model (transduced BALB/c mice with adenovirus associated virus vector expressing hACE2) with dosing at 25 mg/kg per day (ip for 8 days) started one day before infection. Based on that study, and without a side-by-side mouse experiment with remdesivir, no definitive conclusion can be made regarding a potential advantage of GS-441524 over remdesivir in mice.

4. Conclusion

In summary, we systematically evaluated the anti-coronavirus activity of remdesivir and its parent nucleoside GS-441524 in Vero E6, Vero CCL-81, Calu-3, Caco-2, and Huh-7 cells, and intracellular pharmacology of these two compounds in Vero E6, Vero CCL-81, Calu-3, Caco-2, Huh-7, 293T, BHK-21, 3T3 and HAE cells. Except for Vero E6 and Vero CCL-81 cells, remdesivir demonstrated greater antiviral potency and
markedly higher phosphorylation efficiency than GS-441524 in all cells. These results show that the relative potency of the nucleoside prodrug and the parent nucleoside is cell-type dependent, emphasizing the fact that cell model selection is highly important for evaluation of antiviral activity, especially with nucleoside analogs (Mumtaz et al., 2017). Ideally, the best nucleoside analogs are those that are active in most susceptible relevant cell culture systems. Because of the broader phosphorylation profile and higher potency of remdesivir compared to GS-441524 in the several cell systems we studied, we anticipate that remdesivir will likely continue to be a better clinical option than GS-441524.

CRediT authorship contribution statement

Sijia Tao: Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. Keivan Zandi: Methodology, Investigation, Data curation, Writing – review & editing. Leda Bassit: Methodology, Investigation, Data curation, Writing – original draft. Kiran Verma: Investigation. Peng Liu: Investigation. Jessica A. Downsbowen: Investigation. Tamara McBrayer: Investigation. Julia C. LeCher: Investigation, Writing – review & editing. James J. Kohler: Writing – review & editing. Phillip R. Tedbury: Writing – review & editing. Baek Kim: Funding acquisition. Franck Amblard: Writing – review & editing. Stefan G. Sarafianos: Writing – review & editing, Funding acquisition. Raymond F. Schinazi: Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Asselah, T., Durantel, D., Pasmant, E., Lau, G., Schinazi, R.F., 2020. COVID-19: discovery, diagnostics and drug development. J. Hepatol. 74 (1), 168–184. https://doi.org/10.1016/j.jhep.2020.09.031.

Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., Zingman, B.S., Kalil, A.C., Holmman, E., Chu, H.Y., Luetkemeyer, A., Kline, S., Lopez de Castilla, D., Finberg, R.W., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T.F., Paredes, R., Sweeney, D.A., Short, W.R., Touloumi, G., Lye, D.C., Ohmagari, N., Oh, M.D., Ruiz-Palacios, G.M., Benfield, T., Falkenhauer, G., Kortepeter, M.G., Atmar, R.L., Creech, C.B., Lundgren, J., Babiker, A.G., Pett, S., Neaton, J.D., Burgess, T.H.,
Bonnet, T., Green, M., Makowskii, O., Osaini, A., Nayak, S., Lane, H.C., ACTT-1 Study Group Members, 2020. Remdesivir for the treatment of Covid-19 - final report. J. Med. 383 (19), 1813-1826. https://doi.org/10.1056/NEJMoa2009754.

Bokova, D., McGreig, J.E., McLaughlin, K.M., Masterson, S.G., Widera, M., Krahlng, V., Ciesek, S., Wass, M.N., Meach, M., Cinati, J., 2021. SARS-CoV-2 and SARS-CoV differ in their tropism and drug sensitivity profiles. bioRxiv. https://doi.org/10.1101/2021.04.25.441524.

Ciotti, M., Cocozzi, M., Terrironi, A., Jiang, W.C., Wang, C.B., Bernardi, S., 2020. The SARS-CoV-2 cytopathicity dataset generated by high-content screening of a large drug reprofiling collection. Sci. Data 8 (1), 70. https://doi.org/10.1038/s41597-020-00848-4.

Desmyter, J., Melnick, J.L., Rawls, W.E., 1968. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). J. Virol. 2 (10), 955-961. https://doi.org/10.1128/jvi.2.10.955-961.1968.

Do, T.N.D., Donckers, K., Vangeel, L., Chatterjee, A.K., Gallya, P.A., Bobardt, M.D., Billelo, J.P., Giph, J., Torge, S.D., Neys, J., Jochnam, D., 2021. A robust SARS-CoV-2 replication model in primary human epithelial cells at the air liquid interface to assess antiviral agents. Antiviral Res. 192, 105122. https://doi.org/10.1016/j.antiviral.2021.105122.

Dyer, O., 2020. Covid-19: remdesivir has little or no impact on survival, WHO trial shows. Science 368 (6490), 905. https://doi.org/10.1126/science.aaz5562.

Eastman, R.T., Roth, J.S., Brimacombe, K.R., Simeonov, A., Shen, M., Patnaik, S., Humeniuk, R., Mathias, A., Cao, H., Osinusi, A., Shen, G., Chng, E., Ling, J., Vu, A., Dyer, O., 2020. Covid-19: remdesivir has little or no impact on survival, WHO trial shows. J. Clin. Infect. Dis. 77, 9-10. https://doi.org/10.1093/clinid/mcaa086.

Muly, A. Konda, B., Garcia Jr., G., Yao, C., Beil, S., Villalba, J.M., Kozol, C., Sen, C., Purkayastha, A., Kolls, J.K., Pociask, D.A., Perussia, P., de Aja, J.S., Garcia-de-Alba, C., C., Gomperts, B., Hamamura, V., Stripp, B.R., 2021. SARS-CoV-2 infection of primary human lung epithelium for COVID-19 modeling of drug discovery. Cell Rep. 35 (5), 109055.https://doi.org/10.1016/j.celrep.2021.109055.

Muntaz, N., Jimmerson, L.C., Bushman, L.R., Kiser, J.J., Aron, G., Reusken, C., Darcis, J., 2020. Viral proton-sensing activity of sobivavirus against Zika virus. Antivir. Res. 164, 161-163. https://doi.org/10.1016/j.antiviral.2017.09.004.

Nalinskus, G.M., Paunescu, H., Moschos, S.A., Petraitis, D., Nalinskus, G., Ion, G.N.D., Spanfidelis, D.A., Nikolaoukakis, T.K., Drakakis, N., Tsatsakis, A., 2020. Comprehensive analysis of drugs to treat SARS-CoV-2 infection: mechanistic insights into current COVID-19 therapies. Int. J. Mol. Med. 46 (2), 467-488. https://doi.org/10.3892/ijmm.2020.4606.

Nunes-Santos, C.J., Kuenh, H.S., Rosenzweig, S.D., 2021. N-Glycan Modification in Covid-19 Pathobiology: in vitro structural changes with limited functional effects. J. Clin. Immunol. 41 (2), 335-344. https://doi.org/10.1007/s10875-020-00905-4.

Pitazono, A., Padley, B., Julien, T., Trouillet-Assant, S., Transversier, A., Errazuriz-Cerd, E., Pourion, J., Lesueur, F.X., Dubez, J., Pinelli, F., 2020. SARS-CoV-2 infection of human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in cell Rep. 32 (3), 107940. https://doi.org/10.1016/j.celrep.2020.107940.

Sahakijpijarn, S., Moon, C., Warnken, Z.N., Maier, E.Y., Devoire, J., Christensen, D.J., Koleng, J.J., Williams III, R.O., 2021. Development of remdesivir as a dry powder for inhalation by thin film freezing. Adv. Pharmacol. 111 (1), 10007. https://doi.org/10.1016/j.pharmaco.2021.111002.

Sheahan, T.P., Sims, A.C., Zhou, S., Graham, R.L., Pujirisius, A.J., Agotini, M.L., Leit, S.R., Granklinski, L.E., Dinnon, K.H., Yount, B.L., Agotini, M.L., Stevens, J.J., Chappell, J.D., Lu, X., Hughes, T.M., Guly, K., Martinez, D.R., Brown, A.J., Graham, R.L., Perry, J.K., Du Pont, V., Pitts, J., Ma, B., Feng, J., Ayers, B.J., Fisher, B., Babst, A., Devoire, J., June, 2015. Remdesivir: a review of its discovery and development leading to clinical evaluation for the treatment of Ebola virus disease. J. Med. Chem. 58 (13), 5248-5263. https://doi.org/10.1021/acs.jmedchem.5b00305.

Tao, S., Zhou, L., Zhang, H., Zhou, S., Amiralaei, S., Shelton, J., Ethesami, M., Jiing, Y., Amblard, F., Coats, S.J., Schinazi, R.F., 2019. Intracellular metabolism and potential pharmacokinetic impact of remdesivir analogues: a bottleneck to reach active drugs on HIV reverse transcriptase. Curr. Drug Metabol. 20 (12), 1259-1267. https://doi.org/10.2174/1389200220666180925134280.

Tao, S., Zhou, L., Zhang, H., Zhou, S., Amiralaei, S., Shelton, J., Ethesami, M., Jiing, Y., Amblard, F., Coats, S.J., Schinazi, R.F., 2019. Intracellular metabolism and potential pharmacokinetic impact of remdesivir analogues: a bottleneck to reach active drugs on HIV reverse transcriptase. Curr. Drug Metabol. 20 (12), 1259-1267. https://doi.org/10.2174/1389200220666180925134280.

Tao, S., Zhou, L., Zhang, H., Zhou, S., Amiralaei, S., Shelton, J., Ethesami, M., Jiing, Y., Amblard, F., Coats, S.J., Schinazi, R.F., 2019. Intracellular metabolism and potential pharmacokinetic impact of remdesivir analogues: a bottleneck to reach active drugs on HIV reverse transcriptase. Curr. Drug Metabol. 20 (12), 1259-1267. https://doi.org/10.2174/1389200220666180925134280.
Flint, M., McMullan, L.K., Chen, S.S., Fearna, R., Swaminathan, S., Mayers, D.L., Spiropoulou, C.F., Lee, W.A., Nichol, S.T., Cihlar, T., Bavari, S., 2016. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature 531 (7594), 381–385. https://doi.org/10.1038/nature17180.

Xie, X., Muruato, A., Lokugamage, K.G., Narayanan, K., Zhang, X., Zou, J., Liu, J., Schindewolf, C., Bopp, N.E., Aguilera, P.V., Plante, K.S., Weaver, S.C., Makino, S., LeDuc, J.W., Menachery, V.D., Shi, P., 2020. An infectious cDNA clone of SARS-CoV-2. Cell Host Microbe 27 (5), 841–848. https://doi.org/10.1016/j.chom.2020.04.004 e3.

Yan, V.C., Muller, F.L., 2020. Advantages of the parent nucleoside GS-441524 over remdesivir for Covid-19 treatment. ACS Med. Chem. Lett. 11 (7), 1361–1366. https://doi.org/10.1021/acsmedchemlett.0c00116.

Zandi, K., Amblard, F., Musall, K., Downs-Bowen, J., Kleinhard, R., Oo, A., Cao, D., Liang, B., Ollinger-Russell, O., McBryar, T., Bassit, L., Kim, B., Schinazi, R.F., 2020. Repurposing nucleoside analogs for human coronaviruses. Antimicrob. Agents Chemother. 65 (1). https://doi.org/10.1128/AAC.01652-20 e01652-20.

Zampino, R., Mele, F., Florio, L.L., Bertolino, L., Andini, R., Galdo, M., Rosa, R.D., Corcione, A., Durante-Mangoni, E., 2020. Liver injury in remdesivir-treated COVID-19 patients. Hepatol. Int. 14 (5), 881–883. https://doi.org/10.1007/s12072-020-10077-5.