Indian J Med Res 157, January 2023, pp 100-103
DOI: 10.4103/ijmr.ijmr_233_22

Quick Response Code:

Perspective

Can changing the prodrug moiety in remdesivir be a life-saving strategy in COVID-19 infection?

The emergence of the COVID-19 pandemic in November 2019 put the whole world in a state of turmoil as the death toll kept on surging across the globe with no cure available to tackle the emergency situation the infection posed[1]. At such time, drug repurposing seemed to be the most suitable alternative to face the crisis until new vaccines and drugs could be developed for treating symptoms of COVID-19 infection[2].

The antiviral drug remdesivir (RDV) (or GS-5734) got approval from the Food and Drug Administration (FDA) to treat infection caused by SARS-CoV-2 virus[3]. RDV exhibited potent antiviral activity against many members of *Coronaviridae* family, bringing it under consideration to be tested against the SARS-CoV-2 virus[1,4-7]. Initial clinical trials with RDV showed improved symptoms for COVID-19, subsequently leading to its Emergency Use Authorization by the The United States Food and Drug Administration (US-FDA) for the treatment of hospitalized patients[6]. However, data from most of the clinical trials, including the Solidarity Trial (NCT04321616) by the WHO, hinted that RDV might have little or no effect on all-cause mortality up to 28 days in hospitalized patients infected with SARS-CoV-2 virus[8].

Certain limitations regarding the clinical utility of RDV in COVID-19-infected patients question the relevance of its synthesis. These include poor oral bioavailability, rapid decomposition to parent nucleoside due to plasma instability and complex bioactivation together with complex synthesis process of RDV[9-11]. This short communication focuses on mechanisms/pathways limiting the use of RDV in the treatment of COVID-19 infection. It also highlights a new approach *i.e* the development of lipid analogues of RDV nucleoside (RVn, GS-441524), shown to overcome limitations posed for RDV to serve as an efficient therapeutic drug for treating COVID-19 infection in outpatient settings.

Remdesivir: Mechanism of bioactivation

RDV, administered intravenously, reaches different parts of the body through bloodstream, ultimately diffusing into cells. Ideally, the prodrug metabolizes intracellularly to its active metabolite, RVn-triphosphate by sequential hydrolytic steps[5]. First, RDV prodrug is acted on by esterases (namely, cathepsin A/CTSA and carboxylesterase 1/CES1) to form an alanine metabolite GS-704277 which is further hydrolyzed by phosphoramidases (such as histidine triad nucleotide proteins, HINTs 1-3) to monophosphorylated nucleotide (RVn-monophosphate). Owing to high polarity, RVn-monophosphate cannot diffuse back across the cell membrane, and is phosphorylated by kinases to ultimately form RVn-triphosphate, which interacts with SARS-CoV-2 RdRp (RNA-dependent RNA polymerase), thereby interrupting viral replication process[5,9].

Purpose of synthesis

RDV (or GS-5734) is a prodrug of a nucleoside monophosphate, structurally analogous to adenosine monophosphate[9,12,13]. It was synthesized from parent nucleoside, GS-441524 (or RDV nucleoside, RVn) to overcome two main limitations in the case of latter molecule. First, RDV has additional McGuigan prodrug moieties (phenol and L-alaninate ethylbutyl ester), conferring more enhanced cellular permeability compared to RVn[12,13]. Second, it could bypass the first phosphorylation step involving RVn, a rate-limiting step in RVn-triphosphate synthesis[5,14]. These factors were expected to enhance the antiviral efficacy of the prodrug by increasing RVn-monophosphate availability inside the cells, and speeding up intracellular RVn-triphosphate accumulation.

Challenges in administration of RDV

Despite its potent antiviral efficacy *in vitro*, RDV did not show the expected efficacy in rodent models[15]. High carboxylesterase activity in rodent serum can be a reason for this reduced efficacy, unlike humans/ primates with low levels of serum esterase activity[15]. However, efficacy evaluation studies in rhesus monkeys also revealed the rapid breakdown of RDV to parent nucleoside RVn[16]. Available data suggest that instead of the expected path for bioactivation as observed in *in vitro* studies, RDV is highly unstable in plasma, being rapidly hydrolyzed to parent nucleoside and alanine metabolite by ubiquitously present serum esterases, phosphatases and nucleosidases[9,11,16]. During the development of plasma level determination methods for RDV and its metabolites, observations revealed that RDV degradation always led to an increase in levels of GS-704277 and GS-441524; and GS-704277 degradation always led to an increase in the GS-441524 levels[11]. GS-704277 was also found to be nephrotoxic in rat models, contributing to renal adverse effects[10]. GS-441524 is the predominant metabolite in plasma after intravenous administration of RDV. Non-human primates like cynomolgus monkeys had higher levels of RVn-triphosphate (active metabolite) in lung tissue (1.39 nmol/g *vs.* 0.41 nmol/g) on the administration of RDV (10 mg/kg) compared to administration of GS-441524 alone (20 mg/kg) in a 30 min. i.v. dosing[17]. This efficacy can be attributed to the rapid distribution of RDV before elimination from plasma compared to more stable GS-441524 as was observed in peripheral blood mononuclear cells (32.2 µM *vs.* 8.59 µM, respectively)[17]. However, this also strengthens the possibility to enhance RDV efficacy furthermore by designing more plasma-stable prodrug moieties.

Tissue-specific expression and localization of enzymes involved in RDV bioactivation is also an aspect worth consideration. SARS-CoV-2 predominantly infects lungs and pharyngeal tissue, with type-II pneumocytes being affected the most. On the contrary, RDV bioactivation enzymes (*viz.* CTSA/CES1/ HINT1-3) are highly expressed in cells of the liver, GI (gastrointestinal) tract and kidneys but minimally expressed in pneumocytes[9]. Higher bioactivation in these organs may lead to adverse effects of prodrug in them, as observed in patients administered with RDV, making the liver, especially the site for dose-limiting toxicity[6,9]. This was confirmed by *in vitro* studies where RDV showed 50 per cent cytotoxicity at 15.2 µM concentration in Huh7.5 cells (human hepatocyte cell line) compared to concentration higher than 100 µM for Calu-3 cells (human lung epithelial cell line), limiting the utility of RDV in treatment of COVID-19 like respiratory tract infections[14].

Overcoming the barriers: Are the efforts good enough?

Since intravenous administration of RDV limits its utility to hospitalized patients, efforts have been made to develop its oral analogues that could reduce the risk of hospitalization by serving as intervention in the early stages of infection, consequently leading to the development of GS-621763, an orally bioavailable nucleoside analogue of RDV[18]. Having the same parent nucleoside, RDV is a monophosphoramidate prodrug, while GS-621763 is a triester prodrug[18]. GS-621763 showed a potent antiviral effect in both *in vitro* and *in vivo* studies with efficacy similar to molnupiravir on oral administration in mice model[18]. Phase I clinical trial of an oral form of RDV has been completed (CTRI/2021/03/031661) in India[19].

These efforts to increase the oral bioavailability of prodrug do not seem to solve problems related to plasma instability and complex bioactivation process. GS-621763 rapidly metabolizes to RVn in systemic circulation. Since hydrolysis of RDV to RVn seems to contribute to its reduced antiviral potency, synthesis of a molecule rapidly converting to RVn might be a nugatory approach[16]. Data from *in vivo* studies also confirm the same, with GS-621763 possessing less antiviral potency than RDV but similar to GS-441524[18]. Oral formulation of RDV might overcome the issue of poor oral bioavailability, but other limiting factors would still be a hurdle in achieving greater antiviral potency as well as overcoming adverse effects seen during RDV administration.

Oral lipid prodrugs of RDV

In recent years, a few oral lipid prodrugs have successfully entered the clinical trial phase, proving themselves to be promising candidates against different viruses. These include Brincidofovir (approved by FDA for treatment of smallpox virus), and Tenofovir exalidex or CMX157 (completed Phase 1 trial for the treatment of hepatitis B virus (NCT03279146))[20,21]. Recently, Schooley *et al*[14] synthesized three novel oral lipid prodrugs of RDV analogous to RVn-monophosphate, namely hexadecyloxypropyl-, octadecyloxyethyl- and 1-O-octadecyl-2-O-benzyl-sn-glyceryl-esters of RVn-5'-monophosphate (or HDP-P-RVn, ODE-P-RVn and ODBG-P-RVn,

respectively). These prodrugs had antiviral potency similar or greater than RDV and/or RVn against a panel of viruses in different cell lines[14,22]. Of the three, ODBG-P-RVn showed higher antiviral potency and more plasma stability with a half-life of 5 h during oral administration (doses of 13.2 mg/kg and 16.9 mg/kg) in Syrian hamster models[18]. In SARS-CoV-2-infected Vero-6 cells, ODBG-P-RVn achieved EC_{50} (half-maximal effect) and EC_{90} (90% effective concentration) concentrations of 0.14 and 0.16 μM, respectively, while RDV had EC_{50} and EC_{90} values of 1.13 and 7.05, respectively[14]. In addition, its absorption through intestinal lymph, bypassing portal vein and liver, delivers antiviral metabolites away from the liver, reducing the chances of hepatotoxicity[14,23].

Lipid prodrugs are able to follow physiological pathways of lipid metabolism, with high absorbance in the gastrointestinal tract even in their intact form[24]. These liponucleotides are stable in plasma and can enter cells rapidly[24]. The lipid portion of such prodrugs contributes to increased oral bioavailability as well as increased cellular uptake[25]. OBDG-P-RVn molecule had a half-life of more than 24 h in human plasma [K_2EDTA or sodium heparin (NaHep) as anticoagulant] when incubated for 24 h, compared to half-life of 69 min for RDV[14]. Unlike RDV, ODBG-P-RVn is readily cleaved to RVn-monophosphate by acid phospholipase C or acid sphingomyelinase, reducing steps required for prodrug bioactivation[14]. Still, *in vitro* efficacy data cannot be ignored where ODBG-P-RVn had cell-specific antiviral activity moderately lower or nearly equal to RDV but greater than RVn. Plasma stability of ODBG-P-RVn, however, might serve as an advantage as observed in the Syrian hamster model, being stable for more than 24 h after oral administration, and reaching therapeutic plasma levels *i.e.* above EC_{90} for SARS-CoV-2 at a dose of 16.9 mg/kg[14,22]. Despite all this, the lack of *in vivo* efficacy data necessitates assessment studies for the bioavailability, safety and efficacy of the ODBG-P-RVn molecule, to be considered a therapeutic option against COVID-19 infection in humans.

Conclusion

A few limiting factors of RDV affecting the antiviral potency restrain its therapeutic utility in treating COVID-19 patients. These include difficulty in synthesis, poor oral bioavailability, plasma instability and complex bioactivation process. Although different oral formulations of RDV are being considered, apart from improved oral bioavailability, none of these formulations seem to guarantee enhanced antiviral potency and/or improved clinical utility against SARS-CoV-2 virus. Oral lipid analogues of RDV, particularly OBDG-P-RVn offer to be a suitable alternative to address these issues as observed during *in vitro* studies. However, efforts to develop such analogues cannot be justified unless issues with regard to plasma stability and bioactivation are addressed. Success of oral lipid prodrugs in the past against different viruses, necessitates the need to carry out studies for the assessment of bioavailability, safety and efficacy of OBDG-P-RVn in humans[23,25]. The class of lipid prodrugs can be explored further to develop alternative therapeutic interventions in case of COVID-19 infection.

***Financial support & sponsorship*:** Nil.

***Conflicts of Interest*:** None

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Received January 27, 2022

References

1. Mahalmani VM, Mahendru D, Semwal A, Kaur S, Kaur H, Sarma P, *et al*. COVID-19 pandemic: A review based on current evidence. *Indian J Pharmacol* 2020; *52* : 117-29.
2. Office of the Commissioner. *Emergency use authorization*. *FDA; 12 August, 2022*. Available from: *https://www.fda.gov/emergency-preparedness-and- response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization,* accessed on August 13, 2022.
3. Ison MG, Wolfe C, Boucher HW. Emergency use authorization of remdesivir: The need for a transparent distribution process. *JAMA* 2020; *323* : 2365-6.
4. Brown AJ, Won JJ, Graham RL, Dinnon KH, Sims AC, Feng JY, *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.
5. Eastman RT, Roth JS, Brimacombe KR, Simeonov A, Shen M, Patnaik S, *et al*. Remdesivir: A review of its discovery and development leading to emergency use authorization for treatment of COVID-19. *ACS Cent Sci* 2020; *6* : 672-83.

6. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, *et al*. Remdesivir for the treatment of Covid-19 – Final report. *N Engl J Med* 2020; *383* : 1813-26.
7. Tao S, Zandi K, Bassit L, Ong YT, Verma K, Liu P, *et al*. Comparison of anti-SARS-CoV-2 activity and intracellular metabolism of remdesivir and its parent nucleoside. *Curr Res Pharmacol Drug Discov* 2021; *2* : 100045.
8. Ansems K, Grundeis F, Dahms K, Mikolajewska A, Thieme V, Piechotta V, *et al*. Remdesivir for the treatment of COVID-19. *Cochrane Database Syst Rev* 2021; *8* : CD014962.
9. Yan VC, Muller FL. Advantages of the parent nucleoside GS-441524 over remdesivir for covid-19 treatment. *ACS Med Chem Lett* 2020; *11* : 1361-6.
10. European Medicines Agency. *Summary on compassionate use: Remdesivir Gilead. Procedure No.: EMEA/H/K/005622/CU*. Foster City (CA): Gilead Sciences; 2020.
11. Xiao D, John Ling KH, Tarnowski T, Humeniuk R, German P, Mathias A, *et al*. Validation of LC-MS/MS methods for determination of remdesivir and its metabolites GS-441524 and GS-704277 in acidified human plasma and their application in COVID-19 related clinical studies. *Anal Biochem* 2021; *617* : 114118.
12. Mackman RL, Hui HC, Perron M, Murakami E, Palmiotti C, Lee G, *et al*. Prodrugs of a 1'-CN-4-Aza-7,9-dideazaadenosine C-Nucleoside leading to the discovery of remdesivir (GS-5734) as a potent inhibitor of respiratory syncytial virus with efficacy in the African Green monkey model of RSV. *J Med Chem* 2021; *64* : 5001-17.
13. Siegel D, Hui HC, Doerffler E, Clarke MO, Chun K, Zhang L, *et al*. Discovery and synthesis of a phosphoramidate prodrug of a Pyrrolo[2,1-f][triazin-4-amino] adenine C-Nucleoside (GS-5734) for the treatment of Ebola and emerging viruses. *J Med Chem* 2017; *60* : 1648-61.
14. Schooley RT, Carlin AF, Beadle JR, Valiaeva N, Zhang XQ, Clark AE, *et al*. Rethinking remdesivir: Synthesis, antiviral activity, and pharmacokinetics of oral lipid prodrugs. *Antimicrob Agents Chemother* 2021; *65* : e01155-21.
15. Pruijssers AJ, George AS, Schäfer A, Leist SR, Gralinksi LE, Dinnon KH, *et al*. Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. *Cell Rep* 2020; *32* : 107940.
16. Yan VC, Muller FL. Comprehensive Summary Supporting Clinical Investigation of GS-441524 for Covid-19 Treatment. OSF Preprints; 2020. Doi:10.31219/osf.io/mnhxu.
17. Mackman RL. Phosphoramidate prodrugs continue to deliver, the journey of remdesivir (GS-5734) from RSV to SARS-CoV-2. *ACS Med Chem Lett* 2022; *13* : 338-47.
18. Schäfer A, Martinez DR, Won JJ, Moreira FR, Brown AJ, Gully KL, *et al*. Therapeutic efficacy of an oral nucleoside analog of remdesivir against SARS-CoV-2 pathogenesis in mice. *bioRxiv* 2021. Doi: 10.1101/2021.09.13.460111.
19. Jubilant Pharma Limited. *Jubilant pharma announces the development of a novel oral formulation of remdesivir with successful completion of safety and pharmacokinetic/absorption studies in animals and healthy human volunteers [Press Release]; April, 2021*. Available from: *https://www.jubilantpharmova.com/Uploads/files/450pressfile_JubilantPharmova-PR19April2021.pdf*, accessed on May 5, 2021.
20. ClinicalTrials.gov. US National Library of Medicine. National Institute of Health. *A single dose study evaluating PK of TXL oral formulations in healthy subjects*. Available from: *https://clinicaltrials.gov/ct2/show/NCT03279146,* accessed on September 22, 2022.
21. Chan-Tack K, Harrington P, Bensman T, Choi SY, Donaldson E, O'Rear J, *et al*. Benefit-risk assessment for brincidofovir for the treatment of smallpox: U.S. Food and Drug Administration's Evaluation. *Antiviral Res* 2021; *195* : 105182.
22. Lo MK, Shrivastava-Ranjan P, Chatterjee P, Flint M, Beadle JR, Valiaeva N, *et al*. Broad-spectrum *in vitro* antiviral activity of ODBG-P-RVn: An orally-available, lipid-modified monophosphate prodrug of remdesivir parent nucleoside (GS-441524). *Microbiol Spectr* 2021; *9* : e0153721.
23. Hostetler KY, Beadle JR, Trahan J, Aldern KA, Owens G, Schriewer J, *et al*. Oral 1-O-octadecyl-2-O-benzyl-sn-glycero-3-cidofovir targets the lung and is effective against a lethal respiratory challenge with ectromelia virus in mice. *Antiviral Res* 2007; *73* : 212-8.
24. Markovic M, Ben-Shabat S, Keinan S, Aponick A, Zimmermann EM, Dahan A. Prospects and challenges of phospholipid-based prodrugs. *Pharmaceutics* 2018; *10* : 210.
25. Hartline CB, Gustin KM, Wan WB, Ciesla SL, Beadle JR, Hostetler KY, *et al*. Ether lipid-ester prodrugs of acyclic nucleoside phosphonates: Activity against adenovirus replication *in vitro*. *J Infect Dis* 2005; *191* : 396-9.