Research Article ISSN 2639-944X

Journal of Medical - Clinical Research & Reviews

Mismatch of *In Vitro* and *In Vivo* Antiviral Effect of Remdesivir against SARS-CoV-2

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Received: 24 Jun 2022; **Accepted:** 31 Jul 2022; **Published:** 05 Aug 2022

Citation: Ashok Chakraborty, Anil Diwan. Mismatch of *In Vitro* and *In Vivo* Antiviral Effect of Remdesivir against SARS-CoV-2. J Med - Clin Res & Rev. 2022; 6(8): 1-4.

ABSTRACT

A recent clinical trial (Clinical Trial: NCT04315948) by Florence Ader et al., published on the preprint server medRxiv (Posted April 12, 2022), reported that RDV was not associated with clinical improvement at day 15 or day 29, nor with a reduction in mortality, nor with a reduction in SARS-CoV-2 RNA. In cell culture studies with SARS-CoV-2 infection in a variety of cell types, the antiviral effect of RDV was satisfactory. However, the authors of this clinical trial (Published at Medxiv, clinical trial: NCT04315948 by Florence Ader et al., April, 22, 2022) state that the efficacy of the drug and its toxicity in humans is over predicted. However, we came up with our own evaluation from our most relevant experimental results.

Keywords

NV-CoV-2, GS-441524, GS-5734, *In vitro* models, *In vivo* models, Nonhuman Primates, Pharmacokinetics, Prodrug, Remdesivir.

Abbreviations

RDV: Remdesivir (GS-5734), SARS-CoV-2: Severe Acute Respiratory Syndrome Virus 2, RdRp: Ribonucleic acid (RNA)-dependent RNA-polymerases, WHO: World Health Organization, PHH: Primary Human Hepatocytes, HAE: Human Airway Epithelial, EBOV: Ebola Virus, HAE: Human Airway Epithelial, NHPs: Nonhuman Primates, PEG: Polyethylene Glycol, NV387-R: RDV-Encapsulated Biopolymer, NB387, Gilead RDV: RDV-in SBECD (sulfobutylether β-cyclodextrin).

Introduction

Remdesivir (RDV, GS-5734) is an FDA approved only antiviral drug against the severe acute respiratory syndrome virus 2 (SARS-CoV-2). It inhibits viral ribonucleic acid (RNA)-dependent RNA-polymerases (RdRp) [1]. Initially the antiviral potency of this drug is known for Ebola virus (EBOV), but later on its efficacy against other viruses, like Middle East Respiratory Syndrome (MERS)-CoV, SARS-CoV, and respiratory syncytial virus (RSV) have been explored [2].

In a randomized clinical trial in China with 237 SARS-CoV-2 infected, RDV shortens the time of clinical improvement (10 >) in them, however no such beneficiary on mortality rate. Similarly, other clinical trial, sponsored by the World Health Organization (WHO), found no benefit of RDV on in-hospital mortality of COVID-19 patients [3,4]. These contradictory results limit the use of RDV in COVID-19 patients.

PharmacoKinetic study of RDV in humans what was reported by Humeniuk et al. [5] are similar to our observation with rat model [6]. Plasma concentrations of RDV after day 1 of i.v. administration of the drug were undetectable in all the participants [5]. Furthermore, this trial was not placebo-controlled, and no evaluation of endpoints are there. Next, viral load assessment, plasma concentrations, intracellular concentrations, and excretion via urine of this prodrug RDV and its active metabolite GS-441524, should be considered for proper evaluation of the efficacy of the drug.

In brief, in this randomized controlled trial [7,8] the use of RDV did not show any clinical improvement nor a reduction in mortality.

Why Did RDV Fail?

Preclinical Assumptions Overestimate the Clinical Efficacy of RDV for COVID-19 and Ebola

Much of the initial results regarding the potency of RDV are from cell culture studies using primary human hepatocytes (PHH) and human airway epithelial (HAE) cells infected with SARS-CoV-2. The lowest EC50 for RDV found at the nanomolar range similar to against Ebola virus (EBOV)-infected cells in culture [9-12]. However, levels of active membrane-impermeable analogue NTP, nucleoside triphosphate (GS-443902), are about 4-fold higher in PHH (primary human hepatocytes) over the primary HAE (human airway epithelial) cells, when the culturing procedures and treatments of both the cell types were identical [9,13].

Conventional Cell Culture Protocols Fail to Account for the Complex Pharmacokinetics of Remdesivir *in vivo*

Differences between *in vivo* and *in vitro* conditions, such as time and magnitude of drug exposure, their distribution kinetics are the key factors for the discrepancies the drug results [14]. Indeed, Mackman et al. demonstrated a non-uniform distribution of the prodrug, RDV in African green monkeys and cynomolgus macaques when a high accumulation of GS-443902 and RDV metabolites were found in the liver and kidney, after the drug administration in them. This suggest the inability of conventional cell culture protocols to count for the effect found *in vivo* [15].

Prolonged Treatment with RDV in Nonhuman Primates (NHP) Fails to Extrapolate Clinically in Humans

The biochemical and immunological data indicate that the liver–related RDV-mediated toxicities are not counted during the experiments with nonhuman primates (NHPs) [3,11,16,17]. The dose of RDV a NHP can tolerate (10 mg/kg body weight) that equivalent dose to about 168 to 284 mg of RDV is not tolerable by humans [5,18]. The similar observation have been noted with other McGuigan prodrug, sofosbuvir (SOF) in human and monkey [3].

The effects of RDV is dose dependent, and a required antiviral effect of RDV could have been observed in humans if the safety issues can be controlled [18,19]. Further, RDV is unstable in presence of plasma. Plasma catabolize RDV to GS-441524 and decreases its effective concentration *in vivo*. The catabolism of RDV reduces the required exposure time to efficiently eliminate the virus.

Discussion

A detail investigation is warranted to understand the discrepancies of RDV's antiviral effect at preclinical and clinical level. Cell-based screening is not sufficient as the RDV is very sensitive to esterase and therefore uncontrolled tissue distribution and metabolism could occur [5,18,20].

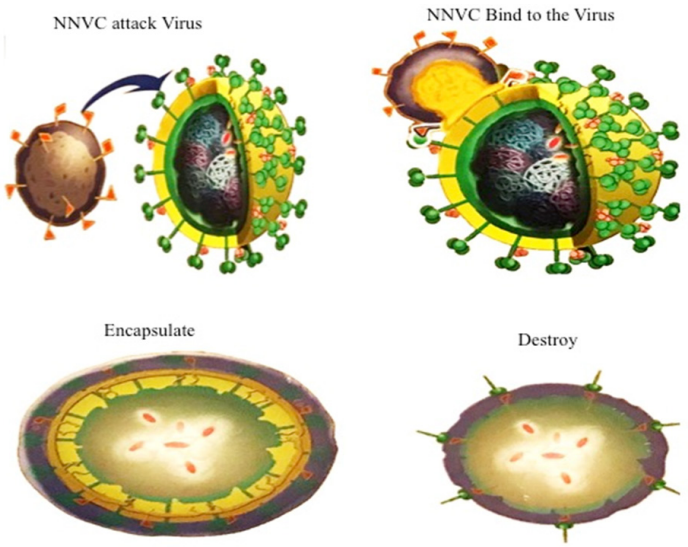
At the *in vivo* experiment, the results of McGuigan prodrug, RDV, in NHP models fails to explain the difference in results found with humans, presumably due to the species differences [3]. Our analysis opens up the insight when selecting the phosphate-prodrugs like RDV for clinical advancement.

Encapsulation of RDV by a PEG-Based Polymer

We have used a polyethylene glycol (PEG) based polymer and C16-alkyl pendants in the monomer unit. The PEG polymer accounts for the hydrophilic shell and the alkyl chains make a flexible core, similar to an immobilized oil droplet.

In the current study, the authors proposed a nanoviricide action mechanism wherein it latches on to the virus and by wrapping it around disables the virus to infect the host cells. Nanoviricide is a small particle, circulates in the body and simultaneously captures the virus that has already infected the cell (Figure 1).

Figure 1: NNVC binds and dismantle the virus. Nanoviricide can attack virus, can bind to the virus, can encapsulate the virus, and ultimately can destroy the virus.



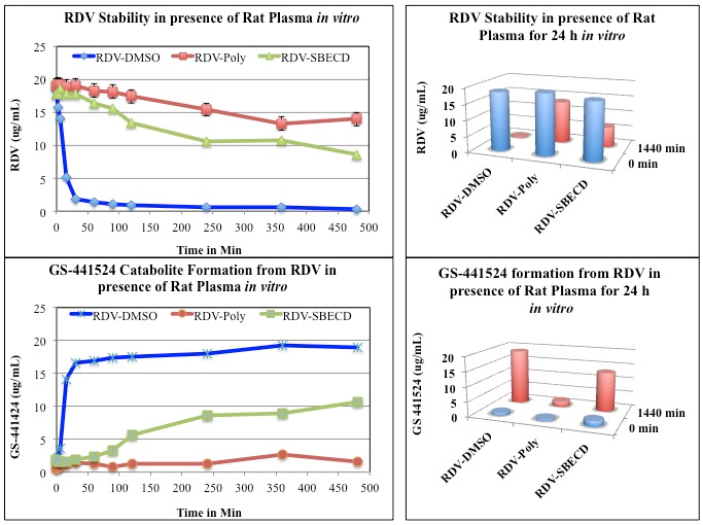
Encapsulated RDV is Stable in Presence of Plasma, *in vitro* and *in vivo*

We compared the RDV levels by liquid chromatography-mass spectrometry (LC-MS) in a time-dependent manner after incubating the free RDV and polymer encapsulated RDV (NV387-R) with rat plasma *in vitro*. Gilead Remdesivir were also used for comparison. RDV metabolite, GS-441524 was also measured to justify the RDV breakdown.

In fact, at up to 24 hours, the polymer-encapsulated RDV found stable, whereas free RDV catabolized fully in 30 min by rat plasma. The Gilead RDV (RDV-in SBECD) showed the stability better than free RDV (t1/2 = 8hrs) but did not last for long as RDV-NV387 (t1/2 =24 hrs) (Figure 2) [21].

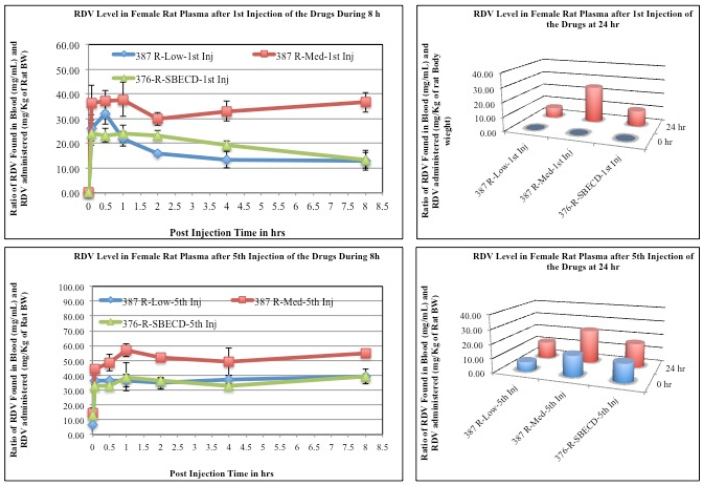
Figure 2: Stability of RDV in DMSO, in polymer NV-387 encapsulated, and in SBECD in presence of Rat plasma *in vitro*, and their comparison. The samples, RDV in DMSO, RDV-encapsulated in NV-387-Polymer, RDV in SBECD (Gilead) were tested for their stability in presence of Rat Plasma *in vitro*. At indicated time points the reactions were stopped by adding acetonitrile extraction mixture and assayed for RDV and/or

it's catabolite GS-441524 by LC-MS spectroscopy (Described in details in Methodologies section). The results show that the RDV alone has a very short life in presence of Plasma, but when encapsulated in NV-387-polymer it becomes very stable even after overnight incubation, compared to Gilead RDV test materials. GS-441524 metabolite formations are representative of RDV breakdown, and supportive to each other data. Here presented the data (Mean± SD) from 3 experiments done in duplicate. The experiments were repeated several times with similar conclusion.



In vivo experimental results were shown below where RDV level in female rat plasma were measured after 1st and 5th injection of the drugs (Figure 3) [6].

Figure 3: RDV values in Rat plasma after 1st and 5th injection of the drugs. The blood samples collected at different time points after drug adninistration i.v. to the animals, were collected and measured for RDV level as described in the method section. The values obtained as mg/mL were normalized by dividing with the RDV amount administered (mg/kg of rat body weight). Each data point is the mean (±SD) of 3 values and the experiment was repeated three times with similar results.



Conclusion

Our study showed that RDV alone has a very short life (t1/2 = 5-8 min) in presence of plasma, but encapsulation in our platform technology-based biopolymer NV-387 provides the stability of the RDV appreciably (t1/2= 24h). This observation counts the efficacy of our nanoviricide as a countable improvement over Gilead RDV for the treatment of SARS-CoV-2. In addition, the inherent chemistry involved in this biopolymer will not let the virus to escape even it mutates.

Acknowledgement

We acknowledge all our colleagues, Secretaries for their help during the preparation of the manuscript by providing all the relevant information. Thanks to Ms. Bethany Pond for her editorial assistant.

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