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A STUDY REPORT ON ANALYTICAL METHODS FOR REMDESIVIR ANTIVIRAL DRUG FOR COVID-19 PANDEMIC

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ABSTRACT

Corona virus wrapped with the layers of positive-sense RNAinfections controlled by the club-like projections from their surface responsible for a combination of disorders in the arrangement, all things considered. After the two verifiable scenes of Covids i.e., serious intense respiratory disorder Covid (SARS-CoV) in 2002 and Middle East respiratory disorder Covid (MERS-CoV) in 2012, this time world hit with its exceptionally pathogenic structure which is serious intense respiratory disorder Covid 2 (SARS-CoV-2). Covid infection(COVID-19) is a viral cascade event that starts from Wuhan in December 2019 and becomes a public health emergency of global interest as declared by the World Health Organization. Primarily, bats became an identifiable source of SARS-CoV-2 and their transmission to humans has occured through an unknown intermediate source. Escalation of this virus occurs very easily through human-to-human transmission via droplets or direct contact, with an estimated incubation period of 7-14 days. Fever is the ubiquitous symptom of SARS-CoV-2 followed by a dry cough. To screen the advancement of patient clinical reaction profile, just as address the difficulties related with remdesivir scientific techniques are essential. This review covers the analytical techniques produced for the identification and quantitative determination of remdesivir and its metabolites in biological samples which helps the analysts in growing new strategies for the investigation of remdesivir by thinking about the advantages and disadvantages of the recently detailed technique

Key words: Remdesivir, antiviral, COVID-19, SARS-CoV-2, analytical methods

1. INTRODUCTION

Coronaviruses are large, enveloped, positivestrand RNA viruses that can cause diseases ranging from the common cold to severe acute respiratory syndrome (SARS). The virus causing coronavirus disease 2019 (COVID-19) is a novel β-coronavirus which is now named as SARS-CoV-2 [1]. This virus has four essential structural proteins including the small envelope (E) glycoprotein, membrane (M) protein, nucleocapsid (N) protein, and spike (S) glycoprotein, and also three accessory (non-structural) proteins including papain-like protease (PLpro) and 3Chemotrypsin-like protease (3CLpro, also known as the main protease-Mpro), which are responsible for cleavage of viral polypeptide into functional units; and RNA-dependent RNA polymerase (RdRp), which is critical for viral replication and transcription [2]. SARS-CoV-2 penetrates the host cell via the binding of its S-protein with the angiotensin converting enzyme II (ACE-2) receptor, which is found in virtually all human organs in varying degrees [3]. In general, S protein, PLpro, 3CLpro, RdRp and ACE-2 are the most attractive targets for the development of new antiviral drugs against COVID-19 [4]. Although this disease is asymptomatic to mild in most people (approximately 80%), in the most severe cases, it can lead to pneumonia, acute respiratory distress syndrome, sepsis and septic shock, multi-organ failure, and even death [5]. Despite global containment measures to fight the current pandemic, the incidence of COVID-19 has continued to rise, with over 73.9 million confirmed-cases and over 1.6 million deaths worldwide as of 17 December 2020 [6]. COVID-19 poses a serious threat to global public health and a broadly effective therapeutic strategy could provide a key means of overcoming this crisis [7]. Unfortunately, there is currently no known effective treatment for COVID-19. Thus, drug repurposing (i.e., testing the efficacy of existing drugs used previously to treat other diseases) is a basic goal in order to develop a fast therapeutic approach for patients with COVID-19 [8]. One of these drugs is remdesivir, an RdRP inhibitor, which shows a broad spectrum of antiviral activity against many RNA viruses like Ebola virus, Marburg, MERS-CoV, SARS-CoV, respiratory syncytial virus and Nipah virus in vivo and in vitro studies, and thus it is being studied for post-infection treatment for COVID-19 [9-12]. Human studies of the pharmacokineticpharmacodynamics relationship of remdesivir and its metabolite appeared necessary in the context of COVID-19. Despite these study needs, to the best of our knowledge, only four studies have been reported for the analysis of remdesivir and its metabolites in biological samples. Therefore, there is an urgent need to improve the robustness of the available analytical methods and to establish new standardized methods, which must be fast, more sensitive, more accurate and more specific, to determine remdesivir and its metabolites in biological matrices (e.g., urine, serum, plasma, intracellular matrix, tissues). On the other hand, the maximum information (physical and chemical properties) about the drug is important and necessary to dispose of a starting point to develop the analytical method. For instance, the structures (Figure 1),

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acid/basic activity and hydrophobicity are useful to elucidate the composition of the mobile phase [13]. These parameters are listed in Table 1. To the best of our knowledge, up until now, no systematic report summarizing the analytical methods applied to remdesivir analysis has been achieved in the literature. This review article highlights the analytical methods used for the quantification and identification of remdesivir in biological matrices.

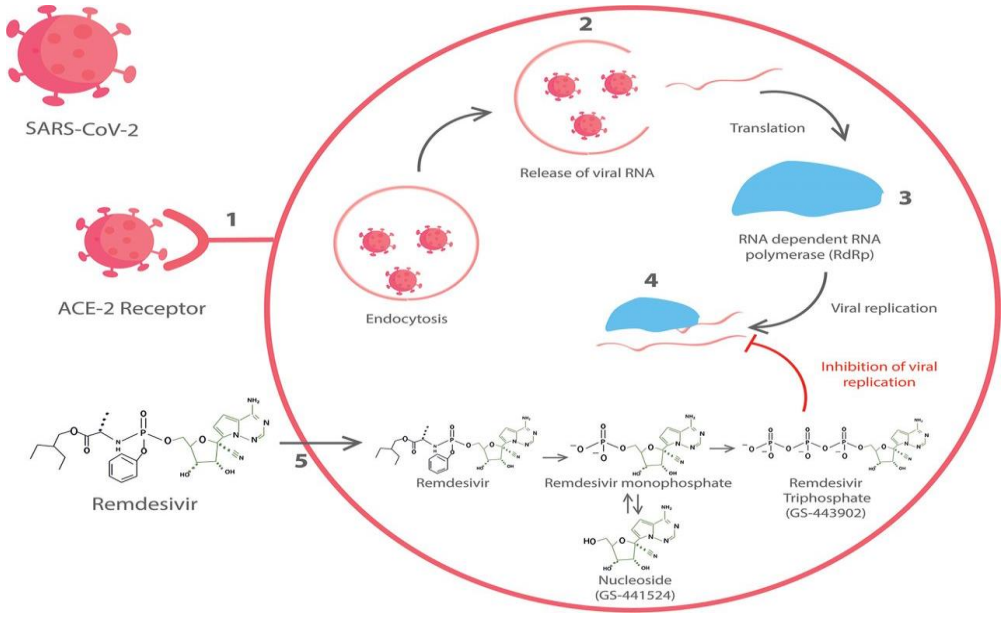

Figure 1. Remdesivir and its intracellular conversion.

2. REMDESIVIR

Remdesivir (Veklury® ; GS-5734) is a novel antiviral drug developed by Gilead Sciences, originally for the treatment of Ebola virus disease and Marburg virus infections [14]. Remdesivir, 2-ethylbutyl (2S)- 2-[[[(2R,3S,4R,5R)-5-(4-aminopyrrolo [2,1-f][1,2,4] triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl] methoxy-phenoxyphosphoryl]amino]propanoate (Figure 1A), is a single diastereomer monophosphoramidate prodrug of a nucleoside analog that perturbs viral replication. It is a white to off-white or yellow non hygroscopic solid, practically insoluble in water and soluble in ethanol [15]. The physicochemical properties of remdesivir are summarized in Table 1.

Table 1. Salient features of the studied drug

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Generic Name/Trade Name	Remdesivir (GS-5734)/Veklury
Drug Class	Antiviral (Small Molecule)
Molecular Formula	Coronavirus Infections
Molecular Weight	C27H35N6O8P
Exact Mass	602.59 g/moL
Water Solubility	602.22539 Da
pKa (strongestacidic/basicLog	0.339 mg/L
Po/wCharge at pH 7	10.23/0.65
Route Dose	2.0 1 0
Cmax (ng/mL) (i.v. 100 mg once daily)	Intravenous (i.v.) 200 mg i.v. loading dose over30min on day 1, then 100 mgi.v. daily over 30 min on days 5 to 10
EC50 AUCtau (ng/hour/mL) (i.v. 100 mg once daily)	2229 and 145 (GS-441524)
Ctrough (ng/mL)	ND 1585 and 2229 (GS-441524)
Bioavailability (%)	0 0.67-0.68
Tmax (hour)	ND (24-hr post dose) and 69.2 (GS-441524)
T1/2 (hour)	
Toxicity	0
Protein Binding (%)	1.51-2.00 (GS-441524)
Metabolic pathway(s)	~1 and ~27 (GS-441524)
Major route of elimination	
Extraction	ND 88-93.6% and 2% (GS-441524) CES1 (80%), Cathepsin A (10%), CYP3A (10%) Metabolism (for remdesivir) and Glomerular filtration and active tubular secretion (for GS-441524) Urine: 10% and 49% (GS-441524) Feces: ND and 0.5% (GS-441524)

2.1. Remdesivir mechanism of action

Remdesivir has a complex activation pathway (Figure1) Briefly, upon diffusion of remdesivir (Figure 1A) into the respiratory epithelial cell, it is first metabolized into an intermediate alanine metabolite (GS-704277; Figure 1B), which is further processed into nucleoside monophosphate derivative (GS-441524; Figure 1C), the major circulating metabolite of remdesivir, via a phosphoramidase-type enzyme. Ultimately, GS-441524 is rapidly converted by intracellular kinases to the pharmacologically active nucleoside triphosphate analog (GS-443902; Figure 1D), a final product of remdesivir activation, which has a prolonged intracellular half-life (T½ ~40 hours). Overall, remdesivir inhibits the RdRp activity of SARS-CoV-2 via non-obligate termination of RNA chains, after being activated to a triphosphate [16-17].

2.2. Efficacy of remdesivir against SARS-COV-2

Antiviral actions of remdesivir against SARS-CoV-2 have been evaluated in both cultured cells and animal models. Pruijssers et al. [18] reported that remdesivir potently inhibited SARS-CoV-2 replication in human lung cells and primary human airway epithelial cultures with a half maximal effective (EC50) concentration of 0.01 μM. Remdesivir was also found to have an EC50 of 0.77 μM against SARS-CoV-2 in Vero E6 cells [19]. Moreover, in vivo studies in a rhesus macaque model infected with SARS-CoV-2 was found to prevent disease progression with remdesivir [20]. These initial studies demonstrate that remdesivir is potently active against SARS-CoV-2 virus infection in vitro and in vivo, supporting its further clinical testing for treatment of COVID-19.

There are several randomized control trials currently being conducted to assess the efficacy and safety of this drug in patients with COVID-19 (https://clinicaltrials. gov). Some evidence suggests that compassionate use of remdesivir may cause some clinical improvement in patients with mild or moderate, or severe COVID-19 disease [21-24.] But, before making any conclusive statement about the efficacy of remdesivir for COVID-19 treatment, more randomized, placebo-controlled clinical trials and other scientific validation need to be performed.

2.3. Remdesivir pharmacokinetics

Table 1 summarizes the data on the pharmacokinetic properties of remdesivir for SARS-CoV-2. Remdesivir is administered via an intravenous (i.v.) infusion, in a total volume of up to 250 mL 0.9% saline over 30 to 120 min, with a loading dose of 200 mg once daily in patients ≥ 12 years old and weighing ≥ 40 kg, followed by a

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maintenance dose of 100 mg once daily for 5 to10 days [25]. This dose is also being evaluated in multicenter randomized trials. Remdesivir is not recommended for patients with an estimated glomerular filtrationrate of ≤ 30 mL/min, or for patients with an alanine aminotransferase level ≥ 5 times the upper limit of normal [26]. The most common adverse effects include gastrointestinal distress, elevated transaminase and bilirubin levels, and infusion site reactions [27]. Due to poor hepatic stability, remdesivir should not be given orally as bioavailability is expected to be low. Remdesivir is unstable in plasma and is widely distributed in many tissues, including the kidney, kidney medulla, and liver, but does not cross the bloodbrain barrier [28]. After i.v. administration, maximum plasma concentrations (Cmax) of remdesivir and its main metabolite (GS-441524) were 2,229 ng/mL and 145 ng/mL, respectively. Plasma T½ of remdesivir and GS441524 were 1 and 27 hours, respectively. Remdesivir is widely bound to human plasma proteins (88-93.6%). By contrast, metabolites GS-704277 and GS-441524 exhibit low plasma protein binding (< 2% bound). Remdesivir is a substrate for CYP2C8 (minor), CYP2D6 (minor), and CYP3A4 (minor), and is a substrate for organic anion transporting polypeptides 1B1 and P-glycoprotein transporters (minor). The majority of the remdesivir dose recovered in urine is GS-441524 (49%), while 10% is recovered as the unmetabolized parent compound [29].

3.ANALYTICAL METHODS

In 2020, Alvarez et al. [30] determined remdesivir and its metabolite GS-441524 in human plasma using an LC-MS/MS method with the help of a simple protein precipitation (PP) step using a mixture of methanol and Zinc sulphate (ZnSO4, 1 M). ZnSO4 reduces protein stability by altering the isoelectric points and by replacing protons on proteins, thereby lowering of the solution's pH. Therefore, the use of ZnSO4 makes the PP thoroughly and enhances the detection sensitivity of remdesivir at low concentration. When ZnSO4 is used as the sole reagent for extraction, a lot of inorganic salts move into the supernatant to contaminate the MS sources. The addition of methanol or acetone/methanol mixture to the precipitation step successfully prevents water and water-soluble salt into the supernatant, making the reconstituted samples definitely cleaner (13). In this work the authors used deuterated remdesivir13C6 as an IS. The system was operated in positive (+) ESI mode, and the following MRM transitions were used: 603.3→200.0 and 603.3→229.0 for remdesivir, 292.2→173.1 and 292.2→147.1 for GS-441524, and 609.3→206.0 for IS. The authors used a Kinetex® Polar C18 column (100 × 2.1 mm I.D., 2.6 µm) to separate analytes. The elution was performed with a gradient of 10 mM sodium formate buffer in 0.1% formic acid (A) and acetonitrile (B) starting from 0% of (B) to 100% in 2 min, at flow rate of 0.50 mL/min, and the total run time was 5 min per sample. The method linearity was over the range of 1-5,000 ng/mL for remdesivir and 5-2,500 ng/mL for GS-441524, with limit of detection (LOD), as the lower concentration with a signal/noise (S/N) ratio higher than three, of 0.3 and 2 ng/mL and lower limit of quantitation (LLOQ) of 1 and 5 ng/mL, for remdesivir and GS-441524, respectively. The major advantages of the method were the requirement for small plasma volume and simple sample preparation procedure, while the main limitation of study was slightly higher LLOQ value obtained for GS-441524 (5 ng/mL). After the optimization and validation according to European Medicines Agency guidelines, the method was successfully applied to a pharmacokinetic study in a COVID-19 patient after a single dose of remdesivir (200 mg i.v.).

Humeniuk et al. [31] also used an LC-ESI(+)- MS/MS method to determine plasma remdesivir concentrations. Quantification was performed using MRM of the transitions m/z 603.3→402.2 and m/ z 606.3→402.2 for remdesivir and an isotopicallylabeled IS (GS-829143), m/z 441.1→150.1 and m/ z 444.1→150.1 for metabolite GS-704277 and an isotopically-labeled IS (GS-829466), m/z 292.2→202.2 and m/z 295.2→205.2 for metabolite GS-441524 and an isotopically-labeled IS (GS-828840), respectively. The method was linear over the range of 4-4,000 ng/ mL for remdesivir, 2-2,000 ng/mL for GS-704277 and 2-2,000 ng/mL for GS-441524, respectively. Interassay precision, based on coefficient of variation for remdesivir, GS-704277 and GS-441524, ranged from 2.1% to 5.3%, and accuracy, based on interassay percent relative error for remdesivir, GS-704277 and GS-441524, ranged from – 9.8% to 9.5%. This study has some drawbacks, such as the lack of analysis of selectivity, sensitivity, robustness, and stability. However, these parameters are reported as fundamental performance characteristics for a method to be considered as validated.

Recently, Avataneo et al. [32] described a simple, rapid and sensitive UHPLC-ESI(+)-MS/MS, an fast technique (total run time < 2.5 min) which has the advantage of high sensitivity and high sample throughput over conventional LC-MS systems, method for the quantification of remdesivir and its main metabolite, GS-441524, in spiked human plasma. The MRM transitions were set at 603.15>200 (m/z), 292>163 (m/z) and 313.2>78.05 (m/z) for remdesivir, GS-441524 and 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline (as IS), respectively. After a PP procedure with a mixture of methanol:acetonitrile (50:50, v/v), the chromatographic separation of the analytes was achieved on an Acquity® HSS T3 column (50 × 2.1 mm I.D., 1.8 µm) with gradient elution of the mobile phase (A: water/formic acid 0.05% and B: acetonitrile/ formic acid 0.05%) at flow rate of 0.40 mL/min. Retention times were 0.98, 1.67 and 1.72 min for GS441524, remdesivir and IS, respectively. The LLOQ value for both the analytes was 0.98 ng/mL while the LOD values (S/N > 3) were 0.24 ng/mL for remdesivir and 0.98 ng/mL for GS-441524. The recoveries ranged from 87-118% (remdesivir) and 81-102% (GS441524). The established method was shown to be accurate, precise, sensitive, and linear. However, it is still necessary to

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develop a more sensitive method to measure the concentrations of remdesivir in human plasma for advanced pharmacokinetic profiles in low dose remdesivir. According to the authors, this method could be a useful tool for studying remdesivir and GS441524 clinical pharmacokinetics, particularly during the current COVID-19 outbreak.

The same UHPLC system was then employed by Tempestilli et al. [33] for the pharmacokinetic evaluation of remdesivir and GS-441524 in two critically ill patients who recovered from COVID-19. They used a PP technique (600 µL of methanol/ acetonitrile, 50:50, v/v) for pretreatment and cleanup of plasma samples. Using this method remdesivir and GS-441524 were simultaneously measured with a LOQ of 5.86 ng/mL for remdesivir and 1.96 ng/mL for GS-441524. Since most studies, including in vivo pharmacokinetics, have a large number of samples to analyze, run time per sample can be very important. The main advantage of the developed methods is the lack of a laborious sample preparation step, which results in a shortening of the analysis time. Although recoveries obtained by LC-MS/MS (30,31) and UPLC-MS/MS (32,33) were similar, UHPLC gave significantly better precision.

4. SUMMARY AND CONCLUSION

COVID-19, a highly infectious respiratory disease, is undoubtedly the most challenging pandemic in the current century. It has been shown that remdesivir is highly effective in stopping the replication mechanism of the coronavirus that causes COVID-19. This review provides for the first time a simplified and thorough evaluation of the analytical methods for the analysis of remdesivir from 2000 up to 2020. A survey of the literature reveals that only LCMS methods have been introduced for remdesivir determination in biological samples, which increases analysis costs to a considerable extent. To date, all the studies reported in literature have been performed in biological fluids, particularly in plasma samples. The sample clean-up procedure is a mandatory step in the whole analytical process, due to the low concentration of remdesivir and interference of complex matrix in biological samples. Surprisingly, all the authors have used conventional PP technique for the extraction of remdesivir and its metabolites. PP is a rapid, low cost and convenient extraction technique; however, it is nonselective and does not remove many of the matrix interferences. In addition, PP is not as rugged and reproducible sample perpetration procedure with LC-MS quantification due to the strong and inconsistent matrix effect. On the other hand, it is difficult to choose the ideal precipitating agents to remove interfering proteins from biological samples. Hence, to overcome such drawbacks, it is recommended that future trends should focus on the development of modern and more effective sample preparation techniques. Qualitative and quantitative determination plays an important role in ensuring the safety and efficacy of drugs in different matrices. To the best of our knowledge, no stability indicating method has been reported for the estimation of remdesivir impurities and degradation products present in pharmaceutical formulation. Thus, it is felt necessary to carry out forced degradation studies as per International Conference on Harmonization guidelines and design a selective and validated stabilityindicating HPLC method. According to the literature data, it can be concluded that both LC-MS/MS and UPLC-MS/MS methods provide acceptable analytical performance for remdesivir measurement but UPLC exhibited shorter analysis time, higher efficiency with better resolution and better precision. In all cases, the MS instrument was operated in the positive polarity. Furthermore, the protonated molecular ion [M+H]+ was chosen as a precursor ion for quantitation in all developed methods. Based on the cited literature, ESI is the most widely used ion source in the analysis of remdesivir and its metabolites in biological matrices by means of LC-MS(/MS) methods. These methods provide a powerful analytical tool for clinical therapeutic monitoring of remdesivir. However, MS apparatuses are usually quite expensive, and this cost may be prohibitive to clinical laboratories. As a result, despite many advantages of MS detection, the application of separation methods based on MS can be problematic in clinical practice. Unlike complicated analytical techniques, miniaturized analytical systems offer a low-cost, fast, easy-to-use, and on-site analysis method to explore the full potential of TDM. Also, on-site TDM has the potential to improve patient outcomes and extremely reduce health-care costs. Therefore, it is recommended that future trends should focus on the design and development of a highly sensitive, portable and miniaturized biosensor for monitoring of remdesivir. At last, it is hoped that this study provides new ideas and prospective for researchers involved in the development of new analytical methods, formulations, and quality and medical control of remdesivir.

5.CONFLICT OF INTEREST

The Authors declare no conflict of interest

6.ACKNOWLEDGMENT

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