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Development of an Assay for the COVID-19 Drug Remdesivir- Toward Proper Remdesivir Use in Patients with Pre-Existing Conditions-

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Pharmaceutical

■ Abstract

In May 2020, the antiviral drug remdesivir was granted special approval in Japan as a treatment for COVID-19. Due to limited experience using remdesivir throughout the world, little information had been collected on remdesivir pharmacokinetics, particularly in patients with renal dysfunction or other comorbidities. This article presents a mass spectrometer-based method for analyzing an active metabolite (GS-441524) of the COVID-19 therapeutic remdesivir. GS-441524 blood levels were measured in a variety of patients, population pharmacokinetics were analyzed, and a pharmacokinetic model was established that allows dose setting based on renal function. Monitoring drug blood levels can be of immense help when treating patients with certain pre-existing conditions, even for drugs that are not necessarily eligible for reimbursement of blood level monitoring fees.

1. Introduction

As of February 2022, 4 million people in Japan had developed COVID-19 and over 20,000 people had died from the disease. Despite this, the third round of vaccinations had yet to be rolled out and the sixth wave of COVID-19, the Omicron variant wave, was still raging. The antiviral drug remdesivir received special approval in Japan as a treatment for COVID-19 in May 2020. However, due to limited experience using remdesivir throughout the world, there was limited information on remdesivir pharmacokinetics, particularly in patients with renal dysfunction or other comorbidities.

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This article establishes a mass spectrometer-based analytical method for remdesivir and uses it to measure drug blood levels in various COVID-19 patients.[1)] Population pharmacokinetics are also analyzed, and a pharmacokinetic model is established that allows dose setting based on renal function.

2. Development of COVID-19 Therapeutics

During drug development, the clinical efficacy and safety of a candidate drug are evaluated through clinical trials from phase I to phase III. Before marketing the drug, a variety of information may also be gathered in clinical pharmacokinetic studies performed in patients with pre-existing conditions, drug interaction studies, and other studies when necessary. Only a limited number of patients can participate in clinical trials, and the information collected in trials and studies is used to inform how the drug should be used in clinical practice. In rare cases, drugs may be granted special approval without completing these standard steps. Japan's Pharmaceutical Affairs Act allows special approval to be granted for drugs that meet the following criteria.

"(i) pharmaceuticals for any urgent need to prevent the spread of disease or other health hazards that may seriously impact the lives and health of the general public, and for which no appropriate method is available other than using said pharmaceuticals; (ii) with respect to use, pharmaceuticals that are authorized to be sold, provided, or stored or displayed for the purpose of sale or provision thereof in a foreign country (limited to countries specified by Cabinet Order as those with a marketing approval system or other systems recognized as being of an equivalent level to that of Japan in terms of securing the quality, efficacy, and safety of pharmaceuticals)."

3. The COVID-19 Therapeutic Veklury

The COVID-19 therapeutic Veklury (generic name: remdesivir) is the third product to be granted special approval in Japan following two vaccines for H1N1 flu (2010). Veklury is a long-awaited COVID-19 antiviral drug. However, drugs granted special approval are adopted for clinical practice based on limited information about the drug without gathering complete findings from clinical trials. Remdesivir is excreted renally, but its pharmacokinetics have not been evaluated in patients with impaired renal function and it is not recommended for patients with estimated glomerular filtration rate (eGFR) levels below 30 mL/min. Nevertheless, university hospitals and other institutions may sometimes decide to use remdesivir following a consideration of the risks and benefits of treatment. Therefore, knowing blood levels of remdesivir in patients with impaired renal function will be extremely important for its use as a COVID-19 therapeutic, especially given that only a limited number of therapeutic agents are available for COVID-19. Pharmacokinetic data for remdesivir also needs to be collected from patients with impaired renal function.

4. An Assay for GS-441524, an Active Metabolite of Remdesivir

Remdesivir is an adenosine nucleoside analog prodrug that is distributed intracellularly where it undergoes metabolism by hydrolysis and is eventually phosphorylated to a nucleoside triphosphate form that is pharmacologically active. As an analog of adenosine triphosphate (ATP), this active metabolite of remdesivir competes with natural ATP substrate for incorporation into RNA strands that are newly synthesized by SARS-CoV-2 RNA-dependent RNA polymerase. Shortly after incorporation, the analog halts RNA strand elongation reactions that are essential to virus replication. However, this active nucleoside triphosphate form of remdesivir is produced intracellularly and almost undetectable in blood, hence the pharmacokinetic analysis presented here targets a nucleoside analog (GS-441524). The assay method for this nucleoside analog is described below.

Standards for remdesivir, GS-441524, and stable isotope-labeled remdesivir and GS-441524 ([$^{13}C_6$]-Remdesivir and [$^{13}C_5$]-GS-441524) were procured from Alsachim, a group company of Shimadzu. Methanol was used to prepare a mixture of [$^{13}C_6$]-Remdesivir (2.5 µg/mL) and [$^{13}C_5$]-GS-441524 (0.25 µg/mL) that was used as an internal standard (ISTD). Remdesivir and GS-441524 were added to commercially available EDTA 2K-treated human blood plasma to prepare calibration curves and quality control (QC) samples. The remdesivir calibration curve range was 100 to 5000 ng/mL and the GS-441524 calibration curve range was 5 to 500 ng/mL. Samples were prepared by combining 20 µL of 75 % IPA, 50 µL of blood plasma, 10 µL of ISTD, and 100 µL of acetonitrile, agitating thoroughly, centrifuging the mixture, and assaying the resulting supernatant.

Analysis was performed using Shimadzu's Nexera™ X2 (high-pressure gradient mixtures of 2 solvents) high-performance liquid chromatograph and Shimadzu's LCMS-8060 liquid chromatography-mass spectrometer. The LC analysis conditions are shown in Table 1 and the MS analysis conditions are shown in Table 2. The Shim-pack Scepter™ C18-120 (50 mm×2.1 mm, I.D. 1.9 µm) column was used for separation and analysis of the compounds being measured and the column oven was set to 40 °C. Water with 0.05 % formic acid was prepared and used as mobile phase A, acetonitrile with 0.05 % formic acid was prepared and used as mobile phase B, and gradient analysis was performed at a flowrate of 0.4 mL/min. The MRM transitions are shown in Table 3.

Analysis with the above conditions produced chromatograms of remdesivir, [U-Ring-$^{13}C_6$]-remdesivir, GS-441524, and [$^{13}C_5$]-GS-441524 as shown in Fig. 1. Calibration curves were prepared for remdesivir (range: 100 to 5000 ng/mL) and GS-441524 (range: 5 to 250 ng/mL) as shown in Figures 2 and 3. The calibration curves for remdesivir and GS-441524 both showed good linearity with coefficients of determination (R^2) > 0.999.

Table 1 LC Conditions

Liquid Chromatograph	
System:	Nexera X2
Column:	Shim-pack Scepter C18-120 (50 mm×2.1 mm I.D., 1.9 µm)
Temperature:	40 °C
Injection Volume:	2.0 µL (Co-injection 20 µL water)
Mobile Phase A:	0.05 % formic acid in water
Mobile Phase B:	0.05 % formic acid in acetonitrile
Gradient Program (B conc%):	5 % (0 – 0.30 min) → 30 % (0.35 min) → 70 % (1.50 min) → 90 % (1.80 – 2.80 min) → 5 % (2.90 – 4.50 min)
Flowrate:	0.4 ml/min

Table 2 MS Conditions

Mass Spectrometer	
System:	LCMS-8060
Ionization:	ESI(+)
Nebulizing Gas:	3 L/min
Drying Gas:	10 L/min
Heating Gas:	10 L/min
DL Temp.:	200 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C

Table 3 MRM Transitions for Remdesivir, GS-441524, and Stable Isotope-Labeled Remdesivir and GS-441524

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)
Remdesivir	603.05	272.10
	603.05	229.00
[$^{13}C_6$]-Remdesivir	609.05	278.20
	609.05	229.15
GS-441524	291.90	163.05
	291.90	173.05
[$^{13}C_5$]-GS-441524	296.90	164.10
	296.90	174.10

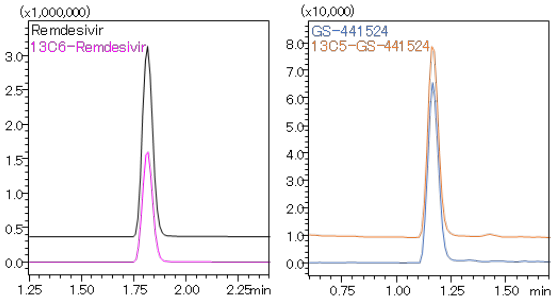

Fig. 1 Chromatograms of Remdesivir, [U-Ring-$^{13}C_6$]-Remdesivir (Left), GS-441524, and [$^{13}C_5$]-GS-441524 (Right)

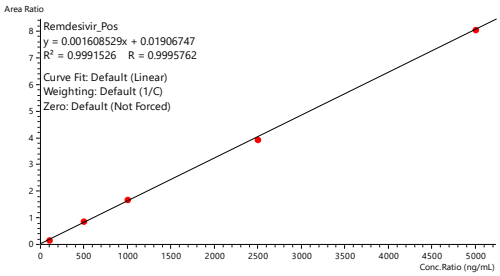

Fig. 2 Remdesivir Calibration Curve Range: 100 to 5000 ng/mL

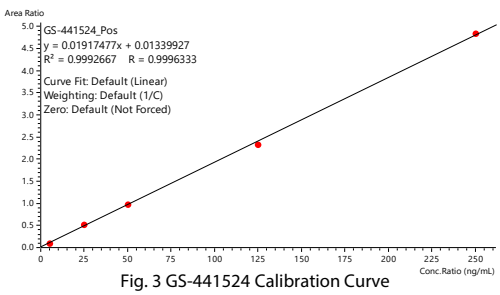

Fig. 3 GS-441524 Calibration Curve Range: 5 to 250 ng/mL

5. Clinical Specimen Analysis

In a joint research project between Kyoto University Hospital and Shimadzu Corporation, GS-441524 blood levels were measured in patients with renal and other disorders in an attempt to identify factors that influence inter-individual variation in pharmacokinetics. Blood was collected from 37 patients administered remdesivir at Kyoto University Hospital and GS-441524 blood levels were measured. The results showed higher GS-441524 blood levels in patients with renal dysfunction compared to patients with normal renal function. GS-441524 blood levels in patients with renal dysfunction are shown in Fig. 4.[1)] Population pharmacokinetics were then analyzed using the collected blood level data, eGFR was identified as a factor that affects GS-441524 clearance, and a model was established that predicts GS-441524 blood levels based on eGFR, as shown in Equation (1). Since high drug blood levels can pose a risk of side effects, simulations were performed using the pharmacokinetic model, and dosing was set based on renal function. This study was approved by the Ethics Committee of Kyoto University Hospital and the Graduate School of Medicine and Faculty of Medicine, Kyoto University (approval number: R2768).

The package insert for Veklury (remdesivir) states that remdesivir is not recommended for patients with severe renal dysfunction except in cases when the therapeutic benefits outweigh the risks. Dose setting based on the model established in this study suggests that remdesivir can likely be administered more safely in patients with severe renal dysfunction who have no choice but to use remdesivir. This is the first model in the world to be established that incorporates eGFR, an indicator of renal function, and is expected to lead to better individualized treatment with remdesivir for patients with pre-existing conditions such as renal dysfunction. Nevertheless, several factors must also be considered when interpreting the results of this study. First, this study does not measure blood levels of other remdesivir metabolites or remdesivir additives that pose a risk of renal damage. Second, the number of subjects included in this study, especially patients with severe renal dysfunction or receiving ECMO, is small, and further research is needed in a larger number of patients.

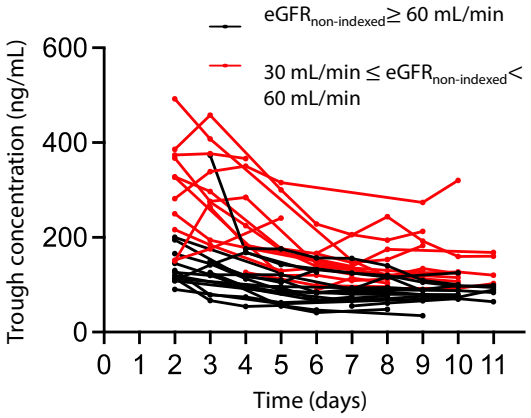

Fig. 4 Time-Course of Trough GS-441524 Concentrations Following Intravenous Administration of Remdesivir

Population Pharmacokinetics Analysis Model

$$CL\ (L/h) = 11.8 \times (eGFR_{non\text{-}indexed}/74.7)^{1.09}$$
$$Vd\ (L) = 382 \times (1\text{-}0.429 \times AGE_{\geq 75,\ V})$$ ・・・(1)

6. Conclusion

Japan has become a super-aged society and Japanese medical care is now changing dramatically. Japan is transitioning from an era of treating single diseases to an era of comprehensive treatment that accounts for comorbidities. Drug development normally involves conducting clinical trials in patients with a single disease, but drug pharmacokinetics may differ in actual clinical practice where comorbidities are common. Advanced treatment hospitals in particular can be forced to administer drugs to patients with pre-existing conditions based on limited information. For patients with pre-existing conditions, measuring drug blood levels can be of immense help in drug treatment, even for drugs that are not necessarily eligible for reimbursement of blood level monitoring fees. In the future, mass spectrometers are expected to play an increasingly active role in clinical practice.

Acknowledgments

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References

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