Effects of Rifampicin on the Pharmacokinetics and Safety of Carotegrast Methyl in Healthy Subjects: A Randomized 2 x 2 Crossover Study

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Abstract

Aims: To evaluate the effect of the combination of carotegrast methyl with rifampicin, a potent inhibitor of organic anion transporter polypeptide, on the pharmacokinetics (PK), safety and tolerability. Methods: In this 2 x 2 crossover study in 20 healthy Japanese adults, 10 subjects received carotegrast methyl 960 mg and rifampicin 600 mg on day 1, and received carotegrast methyl 960 mg on day 8. The subjects in the other sequence received the same treatments but in the opposite order. When the 90% confidence interval (CI) of the geometric mean ratio of the $AUC_{0\text{-}t}$ and C_{max} for carotegrast, the main active metabolite of carotegrast methyl, with/without rifampicin fell within the range of 0.80 – 1.25, it was deemed that no PK interaction occurred. Adverse events (AEs) were monitored. Results: The C_{max} and $AUC_{0\text{-}t}$ for carotegrast with/without rifampicin was 11724.5 ± 6097.6 vs 2620.1 ± 1843.0 ng mL^{-1}, and 55046.0 ± 23427.8 vs 9849.9 ± 4580.6 ng h mL^{-1}, respectively. The ratios (90% CI) of the C_{max} and $AUC_{0\text{-}t}$ with/without rifampicin were 4.78 (3.64 – 6.29) and 5.59 (4.60 – 6.79), respectively, indicating carotegrast has a PK interaction with rifampicin. The combination with rifampicin also increased the exposure of carotegrast and its metabolites. The incidence of any AEs with/without rifampicin was five (25.0%) and one (5.0%), respectively. Conclusion: Coadministration of carotegrast methyl with rifampicin significantly increased exposure of carotegrast compared with carotegrast methyl administration alone. However, no increase in the incidence of adverse drug reactions due to coadministration with rifampicin was observed.

INTRODUCTION

Carotegrast methyl (AJM300) is the first orally administrable small-molecule antagonist of α4-integrin to be approved worldwide for the induction therapy of ulcerative colitis.[1] In phase 2 and phase 3 clinical trials,[2,3] oral administration of carotegrast methyl 960 mg three times daily after meals for 8 – 32 weeks effectively induced a clinical response in moderately active ulcerative colitis patients who had an inadequate response or intolerance to at least 5-aminosalicylic acid. In these trials, carotegrast methyl was well tolerated and most adverse drug reactions were mild or moderate in severity. Although progressive multifocal leukoencephalopathy (PML) is known to be a fatal adverse drug reaction to natalizumab,[4-6] which is a humanized monoclonal antibody having a mechanism of action similar to that of carotegrast methyl, no such events related to carotegrast methyl have been reported so far. In order to reduce the risk of PML, the administration period should be no longer than 6 months. If the treatment is repeated, a drug holiday of at least 8 weeks between consecutive administrations is required.[7]

Carotegrast methyl is an ester prodrug of carotegrast, which is orally absorbed and metabolized mainly by carboxylesterase 1 in the liver rather than in the small intestine to its active metabolite, carotegrast.[8]

Carotegrast methyl is partly metabolized by cytochrome P450 (CYP) 3A4 to demethylated carotegrast methyl (M-I) and then M-II (Figure 1). Carotegrast methyl is mainly excreted in the feces as carotegrast and its glucuronidate conjugate and excretion in urine is very limited in healthy adults.[9]

Ulcerative colitis is a chronic inflammatory disease affecting the colon, and is a lifelong condition that develops early in life.[10-12] Patients treated with carotegrast methyl may require concomitant medications related to other underlying conditions. Therefore, it is important to evaluate the potential drug-drug interactions of carotegrast methyl. In the previous clinical study, we demonstrated that carotegrast methyl was a moderate inhibitor of CYP3A4 and that 14-day repeated oral administration increased exposure to CYP3A4 substrates such as midazolam and atorvastatin, suggesting that coadministration with carotegrast methyl may enhance the pharmacological activity of the drugs metabolized by CYP3A4. Carotegrast was shown to be a substrate for organic anion transporting polypeptide (OATP)1B1/1B3 in vitro, which is an uptake transporter expressed mainly in the liver.[13,14] This suggests that OATP1B1 and OATP1B3 may be involved in the uptake process of carotegrast in the human liver, and OATP1B1/1B3 inhibitors may increase the plasma carotegrast concentration by escaping hepatic metabolism and entering systemic circulation. Here, we report the results of a clinical study to evaluate the effects of rifampicin, a potent OATPs inhibitor, on the pharmacokinetics (PK) and safety of carotegrast methyl in healthy volunteers.

METHODS

Study population

Subjects were Japanese males aged between 20 and 46 years, with a body mass index between [?] 18.5 and $<$ 25.0 kg m^{-2} $^{-1}$. Eligible subjects had no clinically problematic abnormalities regarding their medical findings, vital signs, electrocardiogram, and laboratory tests, and the investigator determined that there were no problems that would have prevented participation in this study. The following subjects were excluded: those who had a previous or current history of functional disorders related to the liver, heart, kidney, lungs, blood, gastrointestinal tract, or any other disorders that would preclude their participation; a previous or current history of upper gastrointestinal disorders; a history of drug allergy; white blood cell count [?] 4000 μL^{-1}; neurological symptoms; a previous or current history of serious infectious diseases, including opportunistic infections within 1 year prior to administration of the study drug; ingestion of grapefruit, grapefruit juice or foods containing these ingredients within 8 days prior to the start of administration of the study drug; or ingestion of St. John's Wort or foods containing these ingredients within 15 days prior to the start of administration of the study drug.

Study design

This was a single-center, single-oral dose, randomized, open-label, three-step, 2 x 2 crossover phase 1 study conducted in 20 healthy adults between February and May 2017 in Japan (Figure 2). The study protocol and the informed consent form were approved by the institutional review board of Hakata Clinic. All participants gave written informed consent before initiation of any study-specific procedures. The study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki, and Good Clinical Practice guidelines.

Twenty subjects were randomized at the study site using medication numbers in permutated blocks and treatment-sequence assignments, with half the subjects assigned to each treatment sequence (sequence A and sequence B). Subjects were admitted to the study center on day -1. Subjects in sequence A received a single oral dose of carotegrast methyl 960 mg in combination with oral rifampicin 600 mg on the morning of day 1 after overnight fasting (period I). After being discharged on day 2, they were again admitted to the study center on day 7, and on the morning of day 8, received a single oral dose of carotegrast methyl 960 mg after overnight fasting (period II). Subjects were discharged on day 9. Follow-up observation was conducted on day 14. The subjects in the other group (sequence B) received the same treatments but in the opposite order.

Because of inexperience in terms of systemic exposure and safety when 960 mg of carotegrast methyl is

administered in combination with rifampicin, we decided to adopt sentinel dosing administration. This began with a small number of patients (two subjects) as Step 1, and sequentially moving to Step 2 (four subjects) and Step 3 (remaining 14 subjects) while confirming safety and evaluating PK at each step. Transition from Step 1 to Step 2, and Step 2 to Step 3 was determined based on the absence of the following safety criteria; (1) the same moderate or severe AEs in more than 50% of the subjects, (2) a serious AE (SAE), or (3) neurological symptoms suggestive of PML that could not be ruled out as having a causal relationship with the study drug during the period between the first dose of Period I (day 1) and two days after Period II (day 9) in each step. The medical advisor assessed the validity of the investigator's decision of moving to the next step, taking into consideration the PK results. The safety committee provided advice to the clinical trial sponsor from a third-party perspective, based on professional expertise, in order to ensure safety regarding the potential for PML to occur.

The Pharmaceuticals and Medical Devices Agency *Guideline on drug interaction for drug development and appropriate provision of information* recommends that rifampicin or cyclosporine should be considered for evaluating drug-drug interactions in humans if the study drug is a substrate of OATP1B1 and OATP1B3.[15]Rifampicin was selected as a potent inhibitor of OATP1B1 and OATP1B3 in this study since cyclosporine has been reported to inhibit P-glycoprotein in the gastrointestinal tract[16] and carotegrast methyl was shown to be a substrate for P-glycoprotein (data not shown). The guideline recommends[15] that the dose of inhibitors used in clinical drug interaction studies should be a dose that maximizes the likelihood of a drug interaction being exhibited; therefore, the dose of rifampicin was set at 600 mg. The dose of carotegrast methyl was set at 960 mg, which was the maximum dose used in healthy adults and patients with moderately active ulcerative colitis in previous studies, and this dose was safe and well tolerated.[3,8,9,17]

Two treatment periods were separated by a 7-day washout period based on more than five times the terminal elimination half-life ($t_{1/2}$) of carotegrast methyl and carotegrast, which were approximately 8.0 – 20.2 h and 10.0 – 15.6 h, respectively, when 960 mg of carotegrast methyl was orally administered in healthy adults. The $t_{1/2}$ of rifampicin 450 mg was 2.3 h.[18]Following this washout period, subjects returned to the study center for the next treatment.

Sample collection, analytical methods, and pharmacokinetic analysis

Blood samples were collected for PK analysis of carotegrast methyl, carotegrast, and other metabolites including M-I, M-II, and carotegrast glucuronide (carotegrast-gluc) at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, and 24 h post-dose on days 1 and 7. A blood sample was collected on day 14 when subjects visited for follow-up observation. Plasma concentrations of carotegrast methyl, carotegrast, M-I, M-II, and carotegrast-gluc were measured by validated methods using liquid chromatography-tandem mass spectrometry at Toray Research Center, Inc. (Tokyo, Japan). The linear analytical ranges of carotegrast methyl and its metabolites including carotegrast, M-I, and M-II were 0.5 - 500 ng mL^{-1}, and that of carotegrast-gluc was 2.0 - 200 ng mL^{-1}. Plasma concentrations of rifampicin were not measured in this study.

The primary PK parameters analyzed for carotegrast methyl and its metabolites included area under the time concentration curve (AUC) from time of dosing to time of last measurable concentration ($AUC_{0\text{-}t}$), AUC from time of dosing to infinity ($AUC_{0\text{-}inf}$), maximum drug concentration (C_{max}), time to C_{max}(T_{max}), $t_{1/2}$, and mean residence time from time of dosing to time of last measurable concentration ($MRT_{0\text{-}t}$).

Safety assessments

Safety and tolerability were assessed by monitoring the incidence, nature, and severity of AEs as well as by vital sign measurements, 12-lead electrocardiograms, clinical laboratory testing (hematology, chemistry, and urinalysis), and physical examinations.

Data analysis and statistical analysis

The sample size was calculated using the coefficient of variations (AUC: 43.7% and C_{max}: 46.8%) derived from the C_{max} and AUC of carotegrast after oral administration of carotegrast methyl.[17] The correlation coefficient was assumed to be 0.7. When the point estimate of the geometric mean ratio of the PK parameters

of carotegrast methyl obtained in the absence and presence of rifampicin was set to 1, indicating no drug interaction, the number of subjects required to have a 90% confidence interval (CI) of 0.8 – 1.25 was calculated to be 19 for AUC and 21 for C_{max} with 80% power. Considering the feasibility of conducting the trial at the study site, the number of subjects was set to 20.

The PK parameters were assessed in all subjects who received carotegrast methyl and whose PK data were adequate for the calculation of [?] 1 primary PK parameter (the PK analysis set). Safety was assessed in all subjects who received the study drug (the safety analysis set). Levels of analyte below the level of quantification were entered as 0 for calculations. Descriptive statistics were used to summarize demographics and safety parameters. The PK parameters of carotegrast methyl, carotegrast, M-I, M-II, and carotegrast-gluc were calculated based on the plasma drug concentration data from subjects who received carotegrast methyl alone or in combination with rifampicin using non-compartmental analysis. For plasma drug concentration and PK parameters, descriptive statistics and the two-sided 95% CIs were calculated. A natural logarithmic transformation of PK parameters except for T_{max} was applied for all statistical inference. The 90% CI for ratios of geometric means of logarithmic PK parameters was calculated by the following mixed effects model;

$\mathrm{Log_e}$ (PK Parameter) = μ + group + sequence + time point + subject + ε

μ: population mean, time point: duration of administration, subject: interindividual variation, ε: error

The geometric mean ratios and their 90% CI for the $AUC_{0\text{-}t}$ and C_{max} of carotegrast methyl and carotegrast when carotegrast methyl was administered in combination with rifampicin versus when carotegrast methyl was administered alone were calculated. When the 90% CI of the geometric mean ratio of both parameters fell within the range of 0.80 – 1.25, it was determined that there was no PK interaction. The same analysis was performed for the other metabolites including M-I, M-II and carotegrast-gluc and for other PK parameters including T_{max}, $t_{1/2}$, and $MRT_{0\text{-}t}$ as a reference. For T_{max}, a nonparametric test was performed. PK parameters were calculated using noncompartmental analysis with WinNonlin Professional Version 6.3 (Phoenix Corporation, Mountain View, California, USA). AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0. All data processing, summarization, and analyses were conducted using SAS software ver. 9.3 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Among a total of 66 subjects who gave informed consent, 20 subjects were randomly assigned to sequence A (n = 10) or sequence B (n = 10). All subjects received the study drugs in period I and period II (Figure 2). Baseline demographics were generally similar across all treatment groups (Table 1). There were no exclusions from the analysis, and all 20 subjects were included in the PK analysis set and safety analysis set. Twenty subjects received carotegrast methyl alone, and carotegrast methyl and rifampicin in combination. Plasma drug concentration profiles of carotegrast methyl, carotegrast, M-I, M-II and carotegrast-gluc after single oral administration of carotegrast methyl in the presence and absence of rifampicin under fasting conditions are shown in Figure 3. The PK parameters are shown in Table 2. Drug-drug interactions between carotegrast methyl and its metabolites, and rifampicin are shown in Table 3.

Carotegrast methyl

The mean ± standard deviation (SD) of C_{max} and $AUC_{0\text{-}t}$ for carotegrast with/without rifampicin was 11724.5 ± 6097.6 vs 2620.1 ± 1843.0 ng mL^{-1}, and 55046.0 ± 23427.8 vs 9849.9 ± 4580.6 ng h mL^{-1}, respectively. The geometric mean (95% CI) ± standard deviation (SD) of C_{max} of carotegrast methyl after a single oral administration of carotegrast methyl alone and in combination with rifampicin was 803.4 (572.7, 1126.9) ng mL^{-1} and 1670.4 (1252.7, 2227.3) and ng h mL^{-1}, respectively. The $AUC_{0\text{-}t}$ was 2199.1 (1674.1, 2888.7) and 4450.1 (3432.7, 5769.1) ng h mL^{-1}, respectively.

The C_{max} and AUC of carotegrast methyl were approximately doubled by coadministration with rifampicin with a statistically significant difference between the treatments ($p = 0.0005$ and 0.0004, respectively). The median (min – max) T_{max} of carotegrast methyl in the absence and presence of rifampicin was 1.0 (0.5 – 4.0) and 2.0 (0.5 – 4.0) h, respectively, and tended to be delayed by coadministration with rifampicin. The

geometric mean (95% CI) $t_{1/2}$ of carotegrast methyl after administration of carotegrast methyl alone and in combination with rifampicin was 2.8 (2.2, 3.5) h and 3.7 (3.1, 4.4) h, respectively. The geometric mean (95% CI) $MRT_{0\text{-}t}$ of carotegrast methyl after administration of carotegrast methyl alone and in combination with rifampicin was 2.2 (1.9, 2.5) h and 2.3 (2.1, 2.6) h, respectively, with no significant difference between the treatments.

Carotegrast

Carotegrast was the main active metabolite of carotegrast methyl, and its mean ± SD of C_{max} and AUC were more than double those of carotegrast methyl (1047.8 ± 846.8 vs 2620.1 ± 1843.0 ng mL^{-1}, 2583.0 ± 1522.9 vs 9849.9 ± 4580.6 ng h mL^{-1}, respectively). The geometric mean (95% CI) C_{max} of carotegrast increased approximately 4.8-fold from 2170.1 (1629.8, 2889.4) and 10380.6 (8133.2, 13249.0) ng mL^{-1} by coadministration of carotegrast methyl with rifampicin ($p < 0.0001$). Similarly, a 5.6-hold increase was observed in the geometric mean (95% CI) $AUC_{0\text{-}t}$ from 9051.3 (7477.7, 10956.1) and 50548.0 (41286.2, 61887.5) ng h mL^{-1} ($p < 0.0001$). The median (min – max) T_{max} of carotegrast after carotegrast methyl administration without/with rifampicin was 2.0 (1.0 – 4.0) h and 4.0 (2.0 – 4.0) h, respectively, which was statistically significant ($p = 0.0020$), indicating delayed absorption with coadministration of rifampicin. The geometric mean (95% CI) $t_{1/2}$ was 6.6 (4.9, 8.8) h and 4.9 (4.2, 5.8) h, respectively, indicating a tendency to decrease with coadministration of rifampicin. The geometric mean (95% CI) $MRT_{0\text{-}t}$ was 4.6 (4.1, 5.2) h and 5.2 (4.9, 5.6) h, respectively, indicating an increase with coadministration of rifampicin ($p = 0.0164$). For the other metabolites, M-I and M-II, the C_{max} and $AUC_{0\text{-}t}$ similarly increased as a result of the combination with rifampicin. Only the $AUC_{0\text{-}t}$ of carotegrast-gluc increased by coadministration with rifampicin and disappearance took longer than the others.

Drug interaction of carotegrast methyl with rifampicin

The geometric mean ratio (90% CI) of the C_{max} and $AUC_{0\text{-}t}$ of carotegrast methyl with coadministration of carotegrast methyl with rifampicin to that of carotegrast methyl alone was 2.08 (1.54 – 2.80) and 2.02 (1.53 – 2.67), respectively. The geometric mean ratios of the $t_{1/2}$ and $MRT_{0\text{-}t}$ considered as reference parameters were 1.35 (1.12 – 1.63) and 1.05 (0.98 – 1.14), respectively. For the T_{max}, Wilcoxon's signed-rank test did not show a significant difference ($p = 0.2847$) between carotegrast methyl with/without rifampicin.

The geometric mean ratio (90% CI) of the C_{max} and $AUC_{0\text{-}t}$ of carotegrast with coadministration of carotegrast methyl with rifampicin to that of carotegrast methyl alone was 4.78 (3.64 – 6.29) and 5.59 (4.60 – 6.79), respectively. The geometric mean ratios (90% CI) of the $t_{1/2}$ and $MRT_{0\text{-}t}$ considered as reference parameters were 0.75 (0.58 – 0.97) and 1.13 (1.04 – 1.22), respectively. The median $T_{max}$of carotegrast was extended from two to four hours by the coadministration with rifampicin (Wilcoxon signed test, $p = 0.0020$). Regarding M-I and M-II, the geometric mean ratios (90% CI) of the C_{max} after coadministration of carotegrast methyl with rifampicin to that of carotegrast methyl alone were 1.42 (1.15 – 1.75) and 3.57 (2.91 – 4.38), respectively, and those of the $AUC_{0\text{-}t}$ were 1.58 (1.30 – 1.92) and 6.05 (5.13 – 7.14), respectively. PK parameters of carotegrast-gluc were used as a reference because they deviated from the standards of incurred sample reanalysis. However, the geometric mean ratios (90% CI) of the $AUC_{0\text{-}t}$ of carotegrast-gluc after coadministration of carotegrast methyl with rifampicin to that of carotegrast methyl alone was 1.14 (0.99 – 1.30).

Safety

The incidence of any AEs observed in the carotegrast methyl alone group was one out of 20 (5.0%), and in the coadministration of carotegrast methyl with rifampicin group the incidence was five out of 20 (25.0%). The incidence of AEs for which a causal relationship with the study drug could not be ruled out was one out of 20 (5.0%) in the carotegrast methyl alone group and one out of 20 (5.0%) in the rifampicin combination group. AEs in the coadministration group with rifampicin for which a causal relationship with concomitant drug could not be ruled out occurred in two out of 20 subjects (10.0%). The only AE in the carotegrast methyl alone group was nausea (one of 20 subjects), while AEs in the coadministration group with rifampicin were blood bilirubin increased (two of 20 subjects), C-reactive protein increased (two of 20 subjects), and

viral upper respiratory tract infection (one of 20 subjects). All AEs were mild in severity. AEs for which a causal relationship with the study drug could not be ruled out were nausea in one out of 20 (5.0%) in the carotegrast methyl alone group and viral upper respiratory tract infection in one out of 20 (5.0%) in the rifampicin combination group. In the coadministration group with rifampicin, the AE for which a causal relationship with concomitant drugs could not be ruled out was blood bilirubin increased, which occurred in two out of 20 subjects (10.0%). Neither deaths nor the other SAEs were reported.

DISCUSSION

This 2 x 2 crossover phase 1 clinical study in healthy male adults demonstrated that coadministration of carotegrast methyl with rifampicin, a potent OATP1B1/1B3 inhibitor, increased exposure of carotegrast methyl and carotegrast, the main metabolite of carotegrast methyl. However, coadministration with rifampicin was shown to be safe and well tolerated at the dosage evaluated in this study.

Carotegrast methyl is an ester prodrug of carotegrast, which is the main component of the metabolites in the blood after oral administration. The geometric mean ratios (90% CI) of the $AUC_{0\text{-}t}$ and C_{max} of carotegrast in the presence of rifampicin to those in the absence of rifampicin were 5.59 (4.60 – 6.79) and 4.78 (3.64 – 6.29), respectively. Both 90% CIs were outside the range of PK equivalence of 0.80 – 1.25, suggesting that carotegrast has a PK interaction with rifampicin. Similarly, the $AUC_{0\text{-}t}$and C_{max} of unchanged carotegrast methyl in plasma, and M-I and M-II increased by coadministration of carotegrast methyl with rifampicin. The PK profiles of carotegrast methyl and its N-demethyl form, M-I, and carotegrast and its N-demethyl form, M-II were similar to each other, and their impact on drug-drug interactions was also comparable.

Carotegrast is a substrate for hepatic uptake transporters OATP1B1 and OATP1B3 in vitro;[1] therefore, inhibition of OATP1B1 and OATP1B3 by rifampicin resulted in escape from hepatic metabolism and increased systemic exposure. Carotegrast methyl may be involved as a substrate of OATP1B1 and OATP1B3 in the body, since carotegrast methyl itself showed inhibitory activity in vitro. Furthermore, the increase in carotegrast exposure may be partly due to the inhibitory activity of P-glycoprotein (P-gp) because carotegrast was a weak substrate of P-gp in in vitro study. It is reported that single-dose rifampicin increased the exposure of both lenvatinib[19] and venetoclax[20] by inhibiting P-gp.

The dose of 960 mg of carotegrast methyl used in this clinical study was the highest dose that has been used in clinical studies of carotegrast methyl to date,[3,8,9,17] and there is currently no clinical experience of administration conditions that resulted in even higher plasma concentrations than those achieved with 960 mg of carotegrast methyl. However, coadministration of carotegrast methyl with rifampicin in this study could elucidate previously unexplored areas in terms of systemic exposure and safety. Therefore, in this study, we adopted a sentinel dosing regimen starting from a small number of cases (two subjects) in Step 1, and gradually transitioned to Step 2 (four subjects) and Step 3 (14 subjects) while confirming safety and evaluating PK at each step. It should be noted that even coadministration of carotegrast methyl with rifampicin was safe and well tolerated at the dose of 960 mg even though systemic exposure increased. All AEs observed in relation to coadministration were mild, and neither SAEs nor AEs leading to treatment discontinuation were observed. In the clinical studies of carotegrast methyl in patients with ulcerative colitis, some patients received OATP1B1/1B3 inhibitors such as clarithromycin, atorvastatin, telmisartan, and tacrolimus as a concomitant medication, but there was no tendency for an increase in the incidence of AEs and adverse drug reactions due to the coadministration.[3] Furthermore, there was no increase in the incidence of AEs leading to discontinuation of treatment. However, it should be noted that there are very few examples of coadministration with an OATP1B1/1B3 inhibitor. Therefore, it is necessary to collect more information during post-marketing surveillance on the safety of carotegrast methyl when coadministered with drugs that inhibit OATP1B1 and OATP1B3.

CONCLUSION

Coadministration of carotegrast methyl with rifampicin, a potent OATP1B1 and OATP1B3 inhibitor, significantly increased the exposure of carotegrast compared with carotegrast methyl administration alone in

healthy adults. The active metabolite carotegrast was classified as a sensitive substrate of OATP1B1 and OATP1B3, and carotegrast methyl was a moderate substrate. However, no increase in the incidence of adverse drug reactions due to coadministration with rifampicin was observed.

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REFERENCES

1. Dhillon S. Carotegrast methyl: First approval. *Drugs* . 2022;82(9):1011-6. doi: 10.1007/s40265-022-01732-0.
2. Yoshimura N, Watanabe M, Motoya S, et al. Safety and efficacy of AJM300, an oral antagonist of α4 integrin, in induction therapy for patients with active ulcerative colitis. *Gastroenterology* . 2015;149(7):1775-83.e2. doi: 10.1053/j.gastro.2015.08.044.
3. Matsuoka K, Watanabe M, Ohmori T, et al. AJM300 (carotegrast methyl), an oral antagonist of α4-integrin, as induction therapy for patients with moderately active ulcerative colitis: a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Gastroenterol Hepatol* . 2022;7(7):648-57. doi: 10.1016/S2468-1253(22)00022-X.
4. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy.*N Engl J Med* . 2012;366(20):1870-80. doi: 10.1056/NEJMoa1107829.
5. McGuigan C, Craner M, Guadagno J, et al. Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. *J Neurol Neurosurg Psychiatry* . 2016;87(2):117-25. doi: 10.1136/jnnp-2015-311100.
6. Williamson EML, Berger JR. Diagnosis and treatment of progressive multifocal leukoencephalopathy associated with multiple sclerosis therapies. *Neurotherapeutics* . 2017;14(4):961-73. doi: 10.1007/s13311-017-0570-7.
7. Package insert of Carogra® Tablets. https://www.pmda.go.jp/PmdaSearch/iyakuDetail/ResultDataSetPDF/111890_2399018F1024_1_02Japanese. Accessed August 20, 2023.
8. Fukase H, Kajioka T, Oikawa I, Ikeda N, Furuie H. Food effect on a single high dose of carotegrast methyl, an oral antagonist of α4-integrin, in healthy male subjects: a randomised, placebo-controlled, double-blind study. *Clin Drug Investig* . 2020;40(3):237-47. doi: 10.1007/s40261-019-00879-1.
9. Fukase H, Kajioka T, Oikawa I, Ikeda N, Furuie H. AJM300, a novel oral antagonist of α4-integrin, sustains an increase in circulating lymphocytes: A randomised controlled trial in healthy male subjects.*Br J Clin Pharmacol.* 2020;86(3):591-600. doi: 10.1111/bcp.14151.
10. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* . 2012;380(9853):1606-19. doi: 10.1016/S0140-6736(12)60150-0.
11. Feuerstein JD, Cheifetz AS. Ulcerative colitis: epidemiology, diagnosis, and management. *Mayo Clin Proc* . 2014;89(11):1553-63. doi: 10.1016/j.mayocp.2014.07.002.
12. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet* . 2017;389(10080):1756-70. doi: 10.1016/S0140-6736(16)32126-2.
13. Nigam SK. What do drug transporters really do? *Nat Rev Drug Discov* . 2015;14(1):29-44. doi: 10.1038/nrd4461.
14. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* . 2009;158(3):693-705. doi: 10.1111/j.1476-5381.2009.00430.x.
15. Ministry of Health, Labour and Welfare, Guideline on drug interaction for drug development and appropriate provision of information. https://www.pmda.go.jp/files/000228122.pdf. Accessed April 8, 2023.

16. Lown KS, Mayo RR, Leichtman AB, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* . 1997;62(3):248-60. doi: 10.1016/S0009-9236(97)90027-8.
17. Kambayashi R, Goto A, Izumi-Nakaseko H, et al. Appraisal of ICH E14/S7B Q&As adopted in February 2022 using thorough QT/QTc study data for α4-integrin antagonist carotegrast methyl in Japanese healthy subjects. *J Pharmacol Sci* . 2022;150(3):191-9. doi: 10.1016/j.jphs.2022.08.007.
18. The package insert of Rifampicin Capsules. https://www.pmda.go.jp/PmdaSearch/iyakuDetail/ResultDataSetPDF/430 6164001M1216_1_15. Japanese. Accessed 18 June, 2023.
19. Shumaker RC, Aluri J, Fan J, Martinez G, Thompson GA, Ren M. Effect of rifampicin on the pharmacokinetics of lenvatinib in healthy adults. *Clin Drug Investig* . 2014;34(9):651-9. doi: 10.1007/s40261-014-0217-y.
20. Agarwal SK, Hu B, Chien D, Wong SL, Salem AH. Evaluation of rifampin's transporter inhibitory and CYP3A inductive effects on the pharmacokinetics of venetoclax, a BCL-2 inhibitor: results of a single- and multiple-dose study. *J Clin Pharmacol* . 2016;56(11):1335-43. doi: 10.1002/jcph.730.

FIGURE LEGENDS

Figure 1 Metabolic map of carotegrast methyl in humans

The metabolic map was generated based on a human mass balance study in healthy adults.

Figure 2 Study design

Figure 3 Study flow

Figure 4 Time course of mean + standard deviation of plasma concentration of (A) carotegrast methyl (B) carotegrast, (C) M-I, (D) M-II, and (E) carotegrast glucuronate (carotegrast gluc) after oral carotegrast methyl 960 mg administration in the presence () and absence (*) of oral rifampicin 600 mg.

TABLE LEGENDS

Table 1 Subject demographics

Table 2 Pharmacokinetic parameters of carotegrast methyl and its metabolites

Table 3 Drug-drug interactions between carotegrast methyl and its metabolites, and rifampicin

Table 1 Volunteer demographics

	Total no. of subjects N = 20	Sequence A N = 10	Sequence B N = 10
Age (year), median (min, max)	22.5 (20, 45)	22.0 (20, 45)	24.0 (21, 43)
Weight (kg), mean ± SD	64.2 ± 6.4	64.4 ± 8.2	64.1 ± 4.5
Height (cm), mean ± SD	173.0 ± 6.8	172.2 ± 7.7	173.8 ± 6.1
BMI (kg $m^{2\ -1}$), mean ± SD	21.6 ± 1.5	21.8 ± 1.8	21.4 ± 1.3

BMI, Body mass index; max, maximum; min, minimum; SD, standard deviation.

Table 2 Pharmacokinetic parameters of carotegrast methyl and its metabolites

				S_AJM300	CO_RIFAMPIC
Carotegrast methyl	C_{max} (ng mL^{-1})	C_{max} (ng mL^{-1})	Mean ± SD	1047.8 ± 846.8	1973.8 ± 1151.7
			Geometric Mean	803.4	1670.4
			95% CI	572.7, 1126.9	1252.7, 2227.3
	AUC_{0-t} (ng h mL^{-1})	AUC_{0-t} (ng h mL^{-1})	Mean ± SD	2583.0 ± 1522.9	5087.8 ± 2626.0
			Geometric Mean	2199.1	4450.1

				S_AJM300	CO_RIFAMPIC
			95% CI	1674.1, 2888.7	3432.7, 5769.1
	T_{max} (h)	T_{max} (h)	Median (min, max)	1.0 (0.5, 4.0)	2.0 (0.5, 4.0)
	$t_{1/2}$ (h)	$t_{1/2}$ (h)	Mean ± SD	3.1 ± 1.2	4.0 ± 1.5
			Geometric Mean	2.8	3.7
			95% CI	2.2, 3.5	3.1, 4.4
	$MRT_{0\text{-}t}$ (h)	$MRT_{0\text{-}t}$ (h)	Mean ± SD	2.3 ± 0.6	2.4 ± 0.6
			Geometric Mean	2.2	2.3
			95% CI	1.9, 2.5	2.1, 2.6
Carotegrast	C_{max} (ng mL^{-1})	C_{max} (ng mL^{-1})	Mean ± SD	2620.1 ± 1843.0	11724.5 ± 6097
			Geometric Mean	2170.1	10380.6
			95% CI	1629.8, 2889.4	8133.2, 13249.0
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Mean ± SD	9849.9 ± 4580.6	55046.0 ± 2342
			Geometric Mean	9051.3	50548.0
			95% CI	7477.7, 10956.1	41286.2, 61887.
	T_{max} (h)	T_{max} (h)	Median (min, max)	2.0 (1.0, 4.0)	4.0 (2.0, 4.0)
	$t_{1/2}$ (h)	$t_{1/2}$ (h)	Mean ± SD	8.8 ± 11.2	5.3 ± 2.8
			Geometric Mean	6.6	4.9
			95% CI	4.9, 8.8	4.2, 5.8
	$MRT_{0\text{-}t}$ (h)	$MRT_{0\text{-}t}$ (h)	Mean ± SD	4.8 ± 1.2	5.3 ± 0.8
			Geometric Mean	4.6	5.2
			95% CI	4.1, 5.2	4.9, 5.6
M-I	C_{max} (ng mL^{-1})	C_{max} (ng mL^{-1})	Mean ± SD	40.6 ± 30.3	50.7 ± 19.7
			Geometric Mean	33.0	46.9
			95% CI	24.5, 44.5	38.6, 57.0
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Mean ± SD	85.6 ± 48.7	124.2 ± 40.3
			Geometric Mean	73.7	116.5
			95% CI	56.4, 96.3	97.0, 139.9
	T_{max} (h)	T_{max} (h)	Median (min, max)	1.0 (0.5, 2.0)	1.0 (0.5, 4.0)
	$t_{1/2}$ (h)	$t_{1/2}$ (h)	Mean ± SD	1.1 ± 0.6	1.0 ± 0.7
			Geometric Mean	1.0	0.9
			95% CI	0.8, 1.3	0.7, 1.1
	$MRT_{0\text{-}t}$ (h)	$MRT_{0\text{-}t}$ (h)	Mean ± SD	1.8 ± 0.6	1.9 ± 0.7
			Geometric Mean	1.7	1.8
			95% CI	1.4, 2.0	1.6, 2.1
M-II	C_{max} (ng mL^{-1})	C_{max} (ng mL^{-1})	Mean ± SD	199.9 ± 146.4	641.5 ± 218.8
			Geometric Mean	168.3	600.1
			95% CI	130.3, 217.4	498.3, 722.7
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Mean ± SD	789.5 ± 367.7	4617.6 ± 1387.5
			Geometric Mean	723.7	4378.0
			95% CI	596.6, 877.8	3704.5, 5174.0
	T_{max} (h)	T_{max} (h)	Median (min, max)	2.0 (1.0, 4.0)	4.0 (2.0, 6.0)
	$t_{1/2}$ (h)	$t_{1/2}$ (h)	Mean ± SD	5.5 ± 1.8	3.7 ± 0.6
			Geometric Mean	5.3	3.7
			95% CI	4.6, 6.1	3.4, 4.0
	$MRT_{0\text{-}t}$ (h)	$MRT_{0\text{-}t}$ (h)	Mean ± SD	4.6 ± 0.9	6.8 ± 0.8
			Geometric Mean	4.5	6.7
			95% CI	4.1, 5.0	6.4, 7.1
Carotegrast-gluc	Carotegrast-gluc	C_{max} (ng mL^{-1})	Mean ± SD	803.7 ± 430.2	622.2 ± 243.2
			Geometric Mean	729.2	577.5
			95% CI	597.0, 890.5	477.9, 697.8

			S_AJM300	CO_RIFAMPIC
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Mean ± SD	2998.4 ± 1081.2	3414.8 ± 1085.3
		Geometric Mean	2855.6	3243.2
		95% CI	2473.8, 3296.3	2766.5, 3802.1
	T_{max} (h)	Median (min, max)	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)
	$t_{1/2}$ (h)	Mean ± SD	6.5 ± 2.9	6.0 ± 1.6
		Geometric Mean	6.0	5.8
		95% CI	4.8, 7.3	5.2, 6.6
	$MRT_{0\text{-}t}$ (h)	Mean ± SD	4.4 ± 1.0	5.8 ± 0.8
		Geometric Mean	4.3	5.7
		95% CI	3.8, 4.7	5.4, 6.1

$AUC_{0\text{-}t}$, area under the curve from time 0 to the last measurable concentration; carotegrast-gluc, carotegrast glucuronate; CI, confidence interval; C_{max}, maximum drug concentration; CO_RIFAMPICIN, coadministration with rifampicin (600 mg); max, maximum; min, minimum; S_AJM300, single oral administration of carotegrast methyl (960 mg); SD: standard deviation; $t_{1/2}$, half-life period; T_{max}, time to C_{max} maximum drug concentration

Table 3 Drug-drug interactions between carotegrast methyl and its metabolites, and rifampicin

Carotegrast methyl	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	2.08
		90% CI of the ratio	1.54, 2.80
		p-value**	0.0005
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Estimated ratio after inverse logarithmic conversion*	2.02
		90% CI of the ratio	1.53, 2.67
		p-value**	0.0004
	T_{max} (h)	Median (Min, Max) in carotegrast methyl alone	1.0 (0.5, 4.0)
		Median (Min, Max) in the combination with rifampicin	2.0 (0.5, 4.0)
		Test statistics	18.5
		p-value**	0.2847
	$t_{1/2}$ (h)	Estimated ratio after inverse logarithmic conversion*	1.35
		90% CI of the ratio	1.12, 1.63
		p-value**	0.0112
	$MRT_{0\text{-}t}$ (h)	Estimated ratio after inverse logarithmic conversion*	1.05
		90% CI of the ratio	0.98, 1.14
		p-value**	0.2594
Carotegrast	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	4.78
		90% CI of the ratio	3.64, 6.29
		p-value**	<.0001
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Estimated ratio after inverse logarithmic conversion*	5.59
		90% CI of the ratio	4.60, 6.79
		p-value**	<.0001
	T_{max} (h)	Median (Min, Max) in carotegrast methyl alone	2.0 (1.0, 4.0)
		Median (Min, Max) in the combination with rifampicin	4.0 (2.0, 4.0)
		Test statistics	27.5
		p-value**	0.0020
	$t_{1/2}$ (h)	Estimated ratio after inverse logarithmic conversion*	0.75
		90% CI of the ratio	0.58, 0.97
		p-value**	0.0677
	$MRT_{0\text{-}t}$ (h)	Estimated ratio after inverse logarithmic conversion*	1.13

Carotegrast methyl	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	2.08
		90% CI of the ratio	1.04, 1.22
		p-value**	0.0164
M-I	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	1.42
		90% CI of the ratio	1.15, 1.75
		p-value**	0.0094
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Estimated ratio after inverse logarithmic conversion*	1.58
		90% CI of the ratio	1.30, 1.92
		p-value**	0.0007
	T_{max} (h)	Median (Min, Max) in carotegrast methyl alone	1.0 (0.5, 2.0)
		Median (Min, Max) in the combination with rifampicin	1.0 (0.5, 4.0)
		Test statistics	37.0
		p-value**	0.0348
	$t_{1/2}$ (h)	Estimated ratio after inverse logarithmic conversion*	0.89
		90% CI of the ratio	0.70, 1.13
		p-value**	0.3877
	$MRT_{0\text{-}t}$ (h)	Estimated ratio after inverse logarithmic conversion*	1.09
		90% CI of the ratio	1.01, 1.19
		p-value**	0.0750
M-II	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	3.57
		90% CI of the ratio	2.91, 4.38
		p-value**	<.0001
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Estimated ratio after inverse logarithmic conversion*	6.05
		90% CI of the ratio	5.13, 7.14
		p-value**	<.0001
	T_{max} (h)	Median (Min, Max) in carotegrast methyl alone	2.0 (1.0, 4.0)
		Median (Min, Max) in the combination with rifampicin	4.0 (2.0, 6.0)
		Test statistics	51.5
		p-value**	0.0010
	$t_{1/2}$ (h)	Estimated ratio after inverse logarithmic conversion*	0.70
		90% CI of the ratio	0.63, 0.78
		p-value (Treatment Group)	<.0001
	$MRT_{0\text{-}t}$ (h)	Estimated ratio after inverse logarithmic conversion	1.49
		90% CI of the ratio	1.41, 1.57
		p-value**	<.0001
Carotegrast-glu	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	0.79
		90% CI of the ratio	0.67, 0.94
		p-value**	0.0272
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	Estimated ratio after inverse logarithmic conversion*	1.14
		90% CI of the ratio	0.99, 1.30
		p-value**	0.1158
	T_{max} (h)	Median (Min, Max) in carotegrast methyl alone	2.0 (1.0, 4.0)
		Median (Min, Max) in the combination with rifampicin	2.0 (1.0, 4.0)
		Test statistics	11.0
		p-value**	0.1719
	$t_{1/2}$ (h)	Estimated ratio after inverse logarithmic conversion*	0.98
		90% CI of the ratio	0.84, 1.14
		p-value (Treatment Group)	0.7938
	$MRT_{0\text{-}t}$ (h)	Estimated ratio after inverse logarithmic conversion	1.35
		90% CI of the ratio	1.26, 1.44

Carotegrast methyl	C_{max} (ng mL^{-1})	Estimated ratio after inverse logarithmic conversion*	2.08
		p-value**	<.0001

* The ratio was with/without rifampicin. ** Between the carotegrast methyl alone group and coadministration of carotegrast methyl with rifampicin

$AUC_{0\text{-}t}$, area under the curve from time 0 to the last measurable concentration; carotegrast-gluc, carotegrast glucuronate; CI, confidence interval; C_{max}, maximum drug concentration; max, maximum; min, minimum; $t_{1/2}$, half-life period; T_{max}, time to C_{max} maximum drug concentration

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