Evaluation of the potential drug-drug interactions of carotegrast methyl with midazolam, prednisolone, or atorvastatin in healthy adults

Shunji Matsuki[1], Ichiro Oikawa[2], Tetsuya Koyama[3], and Hiromitsu Imai[4]

[1]Affiliation not available
[2]Oita University Faculty Of Medicine Graduate School of Medicine
[3]EA Pharma Co Ltd
[4]Oita University Faculty of Medicine

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Abstract

Aims: To evaluate drug-drug interactions between carotegrast methyl, a CYP3A4 inhibitor, and other CYP3A4 substrates, midazolam, atorvastatin, and prednisolone. Methods: A total of 88 healthy volunteers orally received carotegrast methyl 960 mg three times daily for 14 days. A single oral (5 mg) or intravenous (0.017 mg kg^{-1}) midazolam, oral (5 mg) prednisolone, or oral (10 mg) atorvastatin was administered before, with, and after carotegrast methyl treatment. When the 90% confidence interval (CI) for the geometric mean ratios of the pharmacokinetic (PK) parameters with coadministration with carotegrast methyl (day 14) to those before carotegrast methyl administration was between 0.80 and 1.25, no PK interaction were deemed. Results: The C_{max} and $AUC_{0\text{-}t}$ of oral midazolam before administration of carotegrast methyl was 30.9 ± 9.8 ng mL^{-1} and 74.5 ± 21.9 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of midazolam on day 14 to those on day -1 was 1.86 (90% CI, 1.64 – 2.11) and 3.07 (90% CI, 2.81 – 3.35), which did not fall within the range of 0.80 – 1.25, suggesting that carotegrast methyl had a PK interaction with midazolam. Similar PK interactions were found for intravenous midazolam and atorvastatin, but not for prednisolone. The inhibitory effect of carotegrast methyl on CYP3A4-mediated metabolism of midazolam and atorvastatin had almost disappeared by 14 days after the end of administration. Conclusion: Carotegrast methyl was classified as a moderate CYP3A4 inhibitor in humans. Carotegrast methyl might enhance the action of drugs that are metabolized by CYP3A4.

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Evaluation of the potential drug-drug interactions ofcarotegrast methyl with midazolam, prednisolone, or atorvastatin in healthy adults

Short running title: drug-drug interaction of carotegrast methyl

Shunji Matsuki[1], Ichiro Oikawa[2], Tetsuya Koyama[3], Hiromitsu Imai[4]

1. Department of Clinical Research Center, Souseikai Fukuoka Mirai Hospital, Fukuoka, Japan. E-mail: shunji-matsuki@lta-med.com
2. Clinical Development Department, EA Pharma Co., Ltd., Tokyo, Japan. (Present address; Department of Clinical Pharmacology and Therapeutics, Oita University Faculty of medicine, Oita, Japan. E-mail: oikawaichiro@oita-u.ac.jp)

3. Clinical Development Department, EA Pharma Co., Ltd., Tokyo, Japan. E-mail: tetsuya_kouyama@eapharma.co.jp
4. Department of Medical Ethics, Oita University Faculty of Medicine, Oita, Japan. E-mail: imaih@oita-u.ac.jp

The authors confirmed that the Principal Investigator for this paper is Shunji Matsuki and that he had direct clinical responsibility for participants.

Contributor

HI, IO, and SM contributed to the study design, data interpretation, and were responsible for clinical trial management. IO performed the analysis of the data. All authors reviewed the draft and approved the final version of the manuscript for publication.

Data availability statement:

The study protocol and statistical analysis plan will be shared with those who request data sharing. Requests for data should be directed to the corresponding author. Requests will be reviewed, and scientifically sound proposals will be approved by the sponsor (EA Pharma Co., Ltd. and Kissei Pharmaceutical Co., Ltd.). In addition, an agreement for data sharing needs to be contracted between data requestors and the sponsor. Data will be shared two years after the article publication.

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IO and TK were employees of EA pharma. SM and HI had no conflicts of interest to disclose.

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The study protocol and the informed consent form were approved by the institutional review board of Hakata Clinic. The study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki, and Good Clinical Practice guidelines.

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Corresponding:

Tetsuya Koyama, BSc

EA Pharma Co., Ltd., Clinical Development Department,

2-1-1, Irifune, Chuo-ku, Tokyo, 104-0042. Japan

E-mail:*tetsuya_kouyama@eapharma.co.jp*

Telephone: +81(0)8037023694

What is already known about this subject

Carotegrast methyl (AJM300) is the first orally administrable small molecule of α4-integrin antagonist to be approved for the treatment of ulcerative colitis.

Carotegrast methyl inhibits CYP3A4 time-dependently in vitro.

What this study adds

In humans, carotegrast methyl was a moderate CYP3A4 inhibitor, and after 14 days treatment with carotegrast methyl, the combination with CYP3A4 substrates including midazolam and atorvastatin increased their exposure.

The inhibitory effect of carotegrast methyl on CYP3A4 had almost disappeared by 14 days after the end of administration.

ABSTRACT

Aims:

To evaluate drug-drug interactions between carotegrast methyl, a CYP3A4 inhibitor, and other CYP3A4 substrates, midazolam, atorvastatin, and prednisolone.

Methods:

A total of 88 healthy volunteers orally received carotegrast methyl 960 mg three times daily for 14 days. A single oral (5 mg) or intravenous (0.017 mg kg^{-1}) midazolam, oral (5 mg) prednisolone, or oral (10 mg) atorvastatin was administered before, with, and after carotegrast methyl treatment. When the 90% confidence interval (CI) for the geometric mean ratios of the pharmacokinetic (PK) parameters with coadministration with carotegrast methyl (day 14) to those before carotegrast methyl administration was between 0.80 and 1.25, no PK interaction were deemed.

Results:

The C_{max} and $AUC_{0\text{-}t}$ of oral midazolam before administration of carotegrast methyl was 30.9 ± 9.8 ng mL^{-1} and 74.5 ± 21.9 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of midazolam on day 14 to those on day -1 was 1.86 (90% CI, 1.64 – 2.11) and 3.07 (90% CI, 2.81 – 3.35), which did not fall within the range of 0.80 – 1.25, suggesting that carotegrast methyl had a PK interaction with midazolam. Similar PK interactions were found for intravenous midazolam and atorvastatin, but not for prednisolone. The inhibitory effect of carotegrast methyl on CYP3A4-mediated metabolism of midazolam and atorvastatin had almost disappeared by 14 days after the end of administration.

Conclusion:

Carotegrast methyl was classified as a moderate CYP3A4 inhibitor in humans. Carotegrast methyl might enhance the action of drugs that are metabolized by CYP3A4.

INTRODUCTION

Carotegrast methyl (AJM300) is a small-molecule of α4-integrin antagonist which received its first approval in Japan for the treatment of ulcerative colitis (UC).[1] Carotegrast methyl is an ester prodrug of carotegrast, which exerts an anti-inflammatory effect by blocking the interaction of α4β1 or α4β7 integrins and

their counter-receptors, VCAM-1 and MAd-CAM-1, followed by inhibiting leucocyte extravasation into inflammatory sites.[2] In phase 2 and phase 3 clinical trials,[3,4] oral administration of carotegrast methyl 960 mg three times daily after meals for 8 – 32 weeks effectively induced a clinical response in patients with moderately active UC 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 is a known fatal adverse drug reaction to natalizumab,[5-7] which is a humanized monoclonal antibody having a mechanism of action similar to that of carotegrast methyl, no events related to carotegrast methyl have been reported so far. Carotegrast methyl is currently being used as an induction therapy for patients with moderately active UC.

After a single oral dose of carotegrast methyl, the drug was absorbed and metabolized mainly by carboxylesterase 1 to carotegrast.[8] Carotegrast methyl is mainly excreted in the feces, and excretion in urine is very limited in healthy adults.[9] The elimination half-lives ($t_{1/2}$) of carotegrast methyl and carotegrast after a single dose (960 mg) of carotegrast methyl was 15.8 h and 15.6 h, respectively. The plasma drug concentration reached steady state on day 2 after administration of 960 mg three times daily for six days.[1]

UC 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. In the preclinical study, carotegrast methyl inhibited human liver microsome CYP3A4 time-dependently, suggesting a risk of drug-drug interactions with carotegrast methyl in humans. Therefore, we planned a clinical study to investigate drug-drug interactions focusing on CYP3A4, using representative substrates in healthy adult males in accordance with the Pharmaceuticals and Medical Devices Agency (PMDA) *Guideline on drug interaction for drug development and appropriate provision of information* .[13]

Here, we report the results of the clinical trial that evaluated CYP3A4-mediated drug-drug interactions following repeated doses of carotegrast methyl in healthy adults using midazolam, prednisolone, and atorvastatin as CYP3A4 substrates.

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}. Eligible subjects had no clinically problematic abnormalities regarding their medical findings, physiological examinations, 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 medical history of functional disorders related to the liver, heart, kidney, lungs, blood, gastrointestinal tract, or any other disorders that would preclude participation; a previous or current medical history of upper gastrointestinal disorders; a history of drug allergy; white blood cell count [?] 4000 μL^{-1}; neurological symptoms; a previous or current medical history of serious infectious diseases, including opportunistic infections within 1 year prior to administration of the 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 study was a repeated-dose, single-center, open-label, phase 1 study conducted between November 2015 and June 2016 in Japan. 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.

Subjects orally received 960 mg of carotegrast methyl three times daily for 14 days from day 1 to day 14. Carotegrast methyl was administered 30 min after each meal, but under fasting conditions in the morning on the day of blood collection. A single dose of midazolam (5 mg; po, 0.017 mg kg^{-1}; iv), prednisolone

(5 mg; po), or atorvastatin (10 mg; po) was administered under fasting conditions on day -1 (one day before the start of carotegrast methyl administration), day 7 (coadministration with carotegrast methyl), day 14 (coadministration with carotegrast methyl), day 28 (14 days after the end of carotegrast methyl administration) and day 48 (28 days after the end of carotegrast methyl administration). Because the median time for intravenous midazolam to reach the maximum drug concentration (T_{max}) was two hours, the drug was administered two hours after the administration of carotegrast methyl. Each coadministration cohort consisted of 20 subjects, 80 subjects in total, and the cohort to which carotegrast methyl alone was administered consisted of eight subjects. When the 90% confidence interval (CI) of the geometric mean ratio of the area under the concentration-time curve from time of dosing to time of last measurable concentration ($AUC_{0\text{-}t}$) and maximum concentration (C_{max}) of oral midazolam both with and without concomitant carotegrast methyl fell within the range of 0.80 - 1.25, it was determined that there was no pharmacokinetic interaction between the drugs, subsequent combination studies were not conducted.

Midazolam was selected for this study because it is a representative substrate of CYP3A4 listed in the guideline[13] and used in many clinical studies as a sensitive probe for drug-drug interactions mediated by human CYP3A4.[14-16] When given orally, midazolam is quickly metabolized via the first-pass effect, which is not only associated with CYP3A4 in the liver, but also in the small intestine.[17] On the other hand, intravenous midazolam, mainly metabolized in the liver by CYP3A4, is one of the most commonly used medications for inducing anxiolysis or sedation or both, prior to colonoscopy in patients with UC.[18] Therefore, whether carotegrast methyl interacted with intravenous midazolam was also investigated. Prednisolone is used for inducing remission of UC[19] and it is known to be a substrate of CYP3A4.[20-22] Atorvastatin is a substrate of CYP3A4[23-25] and a typical substrate of OATP1B1/1B3.[13] It has been reported[26,27] that the ratio of the contribution of CYP3A4 to the oral clearance of midazolam, atorvastatin, and prednisolone is 92%, 68%, and 18%, respectively.

In order to maximize the inhibitory effect of carotegrast methyl on CYP3A4, carotegrast methyl should be administered for more than ten days because the recovery half-life of CYP3A4 activity following 14-day treatment with St. John's Wort was reported to be 46.2 h,[28] and five times its half-life is 231 h (9.6 days). Therefore, we set a 14-day treatment period for carotegrast methyl. The follow-up period was four weeks after the end of administration of carotegrast methyl. Subjects were admitted to the study center on day -3 and discharged on day 15. The second admission was from day 27 to day 42.

Sample collection, analytical methods, and pharmacokinetic analysis

Blood samples were collected for pharmacokinetics (PK) analysis of oral midazolam, prednisolone, and atorvastatin at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 h post dose on days -1, 7, 14, 28, and 42. For PK analysis of intravenous midazolam, blood samples were collected at 0, 0.08, 0.17, 0.33, 0.5, 0.75, 1,1.5, 2, 4, 6, 8, 10, 12, and 24 h post dose on days -1, 7, 14, 28, and 42. Plasma concentrations of all analytes were measured by validated methods using liquid chromatography-tandem mass spectrometry. The linear analytical range of the assay was 20 - 20000 pg mL^{-1} for midazolam, 5 - 250 ng $mL^{-1}$for prednisolone, and 50 - 10000 pg mL^{-1} for atorvastatin.

The primary PK parameters analyzed for midazolam, prednisolone, and atorvastatin included the $AUC_{0\text{-}t}$, AUC from time of dosing to infinity ($AUC_{0\text{-}inf}$), and C_{max}. Additional PK parameters included T_{max}, and elimination half-life ($t_{1/2}$).

Safety assessments

Safety and tolerability were assessed by monitoring the incidence, nature, and severity of adverse events (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 of 20 was determined based on the reported PK parameters of atorvastatin. The coefficient of variances calculated based on a single-dose administration of atorvastatin 10 mg were 45.7% for AUC and

44.2% for C_{max}.[29] The correlation coefficient was assumed to be 0.7. When there is no drug interaction, the number of subjects whose 90% CI of the geometric mean ratio of PK parameters was 0.80 - 1.25 was calculated to be 20 for AUC and 19 for C_{max} with 80% power.

The PK parameters were assessed in all subjects who received more than one dose of the study drug and whose PK data were adequate for the calculation of more than one primary PK parameter (PK analysis set). Safety was assessed in all subjects who received more than one dose of the study drug. Levels of analyte below the level of quantification were entered as 0 for calculations. Descriptive statistics were used to summarize demographics and safety parameters. For plasma drug concentration and PK parameters, summary 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 + time point + subject + ε

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

For each interacting drug, the 90% CI of the geometric mean ratios of the $AUC_{0\text{-}t}$ and C_{max} of midazolam, prednisolone, and atorvastatin with coadministration of carotegrast methyl (days 7 and 14) or after the end of administration of carotegrast methyl (days 28 and 42) to those prior to the administration of carotegrast methyl (day -1) were calculated. When the 90% CI of the geometric mean ratio fell within the range of 0.80 - 1.25, it was determined that there was no PK interaction. 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 19.0. 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 190 subjects who gave informed consent, 88 subjects received the study drugs and 85 subjects completed the study (Figure 1). There were no exclusions from the analysis, and all 88 subjects were included in the PK analysis set and the safety analysis set. Baseline demographics were generally similar across all treatment groups (Table 1). When carotegrast methyl was repeatedly administered for 14 days from days 1 to 14, time course of the mean plasma concentration of oral midazolam, oral prednisolone, oral atorvastatin, and intravenous midazolam on days -1, 7, 14, 28, and 48 is shown in Figure 2. These PK parameters and drug-drug interaction between carotegrast methyl and CYP3A4 substrates are shown in Table 2 and Table 3, respectively.

Pharmacokinetics

Midazolam

The mean ± standard deviation (SD) of C_{max} and $AUC_{0\text{-}t}$ of oral midazolam before repeated administration of carotegrast methyl (day -1) was 30.9 ± 9.8 ng mL^{-1} and 74.5 ± 21.9 ng h mL^{-1}, respectively. The C_{max} and $AUC_{0\text{-}t}$ of midazolam after 14-day administration of carotegrast methyl (day 14) was 56.6 ± 14.4 ng mL^{-1} and 225.1 ± 50.0 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of midazolam on day 14 to those on day -1 was 1.86 (90% CI, 1.64 – 2.11) and 3.07 (90% CI, 2.81 – 3.35), which did not fall within the range of 0.80 – 1.25, suggesting that carotegrast methyl had a pharmacokinetic interaction with oral midazolam. The C_{max} and $AUC_{0\text{-}t}$ of midazolam 14 days after the end of carotegrast methyl administration (day 28) was 33.7 ± 11.5 ng mL^{-1} and 83.9 ± 26.1 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of midazolam on day 28 to those on day -1 was 1.08 (90% CI, 0.95 – 1.22) and 1.12 (90% CI, 1.02 – 1.22), respectively, which fell within the range of 0.80 - 1.25.

The $AUC_{0\text{-}t}$ of intravenous midazolam before repeated administration of carotegrast methyl (day -1), after the 14-day repeated administration (day 14), and 14days after the end of the repeated administration (day 28) was 48.8 +- 12.1, 73.7 +- 14.5, and 56.0 +- 12.3 ng h mL^{-1}, respectively. The geometric mean ratio of the

$AUC_{0\text{-}t}$ of intravenous midazolam on day 14 to those on day -1 was 1.53 (90% CI, 1.43 – 1.64), which did not fall within the range of 0.80 – 1.25, suggesting that carotegrast methyl had a pharmacokinetic interaction with intravenous midazolam. The geometric mean ratio of the $AUC_{0\text{-}t}$ of intravenous midazolam on day 28 to those on day -1 was 1.16 (90% CI, 1.08 – 1.24).

Prednisolone

The mean +- SD of C_{max} and $AUC_{0\text{-}t}$ of oral prednisolone before repeated administration of carotegrast methyl (day -1) was 156.1 +- 28.7 ng mL^{-1} and 571.6 +- 98.1 ng h mL^{-1}, respectively. The C_{max} and $AUC_{0\text{-}t}$ of prednisolone after 14-day administration of carotegrast methyl (day 14) was 138.0 +- 19.7 ng $mL^{-1}$and 631.0 +- 77.9 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of prednisolone on day 14 to those on day -1 was 0.89 (90% CI, 0.84 – 0.95) and 1.11 (90% CI, 1.08 – 1.15), respectively, which fell within the range of 0.80 – 1.25, suggesting that carotegrast methyl had no pharmacokinetic interaction with oral prednisolone. The C_{max} and $AUC_{0\text{-}t}$ of prednisolone 14 days after the end of carotegrast methyl administration (day 28) was 150.0 +- 28.6 ng mL^{-1} and 571.9 +- 96.5 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of prednisolone on day 28 to those on day -1 was 0.96 (90% CI, 0.91 – 1.02) and 1.00 (90% CI, 0.97 – 1.04), respectively.

Atorvastatin

The mean +- SD of C_{max} and $AUC_{0\text{-}t}$ of oral atorvastatin before repeated administration of carotegrast methyl (day -1) was 3.2 +- 1.5 ng mL^{-1} and 16.1 +- 6.2 ng h mL^{-1}, respectively. The C_{max} and $AUC_{0\text{-}t}$ of atorvastatin after 14 days administration of carotegrast methyl administration (day 14) was 4.0 +- 2.4 ng mL^{-1} and 33.8 +- 13.8 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of atorvastatin on day 14 to those on day -1 was 1.24 (90% CI, 1.01 – 1.52) and 2.10 (90% CI, 1.97 – 2.24), which did not fall within the range of 0.80 – 1.25, suggesting that carotegrast methyl had a pharmacokinetic interaction with oral atorvastatin. The C_{max} and $AUC_{0\text{-}t}$ of atorvastatin 14 days after the end of carotegrast methyl administration (day 28) was 3.3 +- 1.4 ng mL^{-1} and 17.9 +- 6.8 ng h mL^{-1}, respectively. The geometric mean ratio of the C_{max} and $AUC_{0\text{-}t}$ of atorvastatin on day 28 to those on day -1 was 1.06 (90% CI, 0.87 – 1.30) and 1.13 (90% CI, 1.06 – 1.20), respectively, which fell within the range of 0.80 - 1.25.

The T_{max} of atorvastatin and prednisolone with coadministration of carotegrast methyl was delayed compared with the T_{max} without carotegrast methyl coadministration. No change in T_{max} was observed when carotegrast methyl was combined with oral midazolam.

Safety

The incidence of any AEs when carotegrast methyl was combined with orally administered midazolam, prednisolone, atorvastatin, and intravenously administered midazolam, and for carotegrast methyl alone was 17/20 (85%), 5/20 (25%), 1/20 (5%), 16/20 (80%), 2/8 (25%), respectively. All AEs were mild in severity except for moderate upper limb fracture in combination with prednisolone and moderate influenza in combination with atorvastatin, all of which resolved except upper limb fracture. The most common AE was somnolence, which was reported in 17/20 (85%) of subjects receiving a concomitant oral midazolam and 15/20 (75%) of subjects receiving concomitant intravenous midazolam. AEs leading to discontinuation of the study were 1/20 (5%) in the combination with prednisolone and 1/20 (5%) in the combination with atorvastatin. Neither deaths nor the other serious AEs were reported.

DISCUSSION

Carotegrast methyl is a moderate time-dependent inhibitor of CYP3A4 in vitro. This phase 1 clinical study in healthy males demonstrated that repeated administration of carotegrast methyl increased exposure to CYP3A4 substrates such as midazolam and atorvastatin, but not to prednisolone, which is used for treating UC.

Carotegrast methyl affected the PK of midazolam, a typical substrate drug susceptible to PK interactions due to inhibition of CYP3A4[14-16], and increased the $AUC_{0\text{-}t}$of midazolam by 2.7-fold on day 7 and 3.1-fold on day

14, compared to midazolam administration alone (day -1). Based on these results, carotegrast methyl was classified as a moderate CYP3A4 inhibitor according to the guideline.[13] Increase in the $AUC_{0\text{-}t}$ of intravenous midazolam under coadministration with carotegrast methyl was also observed, but was lower than that with oral midazolam. The lower ratios seen compared to oral midazolam might be due to lack of metabolization by CYP3A4 in the gastrointestinal tract; this means the gastrointestinal CYP3A4 would be mainly involved in midazolam metabolism rather than liver CYP3A4. Based on the in vitro study, carotegrast methyl might increase exposure of a strong CYP3A4 probe substrate such as midazolam 8.3-fold in the blood and 1.5-fold in the gastrointestinal tract. On the other hand, in this study, the exposure of midazolam showed a 3.1-fold increase for oral administration and 1.5-fold increase for intravenous administration, suggesting that the contribution of CYP3A4 in the gastrointestinal tract was roughly double. The increase in exposure for intravenous administration of midazolam was 1.5-fold and no significant AEs were observed. Therefore, there are no major concerns regarding the use of carotegrast methyl in combination with midazolam as a sedative for endoscopy procedures.

The degree of increase in $AUC_{0\text{-}t}$ of the interacting drugs with coadministration with carotegrast methyl did not differ significantly between the different times of administration of midazolam (days 7 and 14). Carotegrast methyl inhibitory activity against CYP3A4 appeared to have reached almost steady state 7 days after repeated administration of carotegrast methyl, and evaluation on day 14, as set in this study, seemed reasonable. The carotegrast methyl inhibitory activity disappeared 14 days after the end of administration as was also reported for evacetrapib.[30]

Carotegrast methyl also affected the PK of atorvastatin, a moderate substrate drug susceptible to PK interactions due to inhibition of CYP3A4,[23-25] and increased atorvastatin exposure $AUC_{0\text{-}t}$ by 1.8-fold on day 7 and 2.1-fold on day 14, compared to atorvastatin administration alone (day -1). Carotegrast methyl did not affect the PK of prednisolone, which is a drug commonly used to treat UC colitis and known to be metabolized by CYP3A4.[20,21,31] The T_{max} of atorvastatin and prednisolone was delayed when coadministered with carotegrast methyl compared to the T_{max} in the absence of carotegrast methyl administration. No delay in $T_{max}$was reported when itraconazole was combined with prednisolone[20] or atorvastatin[32]. No delay in T_{max} was observed in combination with oral midazolam in liquid form. These results suggest that the delayed T_{max} may be caused not by metabolic inhibition, but by concurrent use with carotegrast methyl, which may affect the disintegration and dissolution of prednisolone and atorvastatin tablets, leading to delayed absorption.

In terms of the administration and dose of the interacting drugs used in this study, the tolerability in combination with carotegrast methyl was considered acceptable. Oral carotegrast methyl 960 mg three time daily for 14 days was also well tolerated.

CONCLUSION

Carotegrast methyl is a moderate inhibitor of CYP3A4 and repeated oral administration increased exposure to CYP3A4 substrates such as midazolam and atorvastatin in humans. However, no increase was observed with prednisolone. The inhibitory effect of carotegrast methyl on CYP3A4 had almost disappeared 14 days after the end of the repeated administration. Combination with carotegrast methyl may enhance the pharmacological activity of certain drugs metabolized by CYP3A4. The AEs observed at the dosages of the interacting drugs used in this study were mild in severity and well tolerated when used in combination with carotegrast methyl.

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FIGURE LEGENDS

Figure 1 Study flow

CO_ATV, combination of carotegrast methyl with atorvastatin; CO_MDZiv, combination of carotegrast methyl with intravenous midazolam; CO_MDZpo, combination of carotegrast methyl with oral midazolam; CO_PSL, combination of carotegrast methyl with prednisolone; iv. intravenous; po. per os; S_AJM, Carotegrast methyl alone

Figure 2 Time course of mean + standard deviation plasma concentration of (A) oral midazolam, (B) oral prednisolone, (C) oral atorvastatin, and (D) intravenous midazolam on days -1, 7, 14, 28, and 42, when carotegrast methyl was repeatedly administered for 14 days from days 1 to 14.

iv, intravenous; po, per os

TABLE LEGENDS

Table 1 Volunteer demographics

Table 2 Pharmacokinetic parameters of midazolam (po), prednisolone (po), atorvastatin (po), and midazolam (iv) which were administered on day -1, days 7, 14, 28, and 42, while carotegrast methyl was repeatedly administered for 14 days from days 1 to 14

Table 3 Drug-drug interaction between carotegrast methyl and CYP3A4 substrates

Table 1 Volunteer demographics

		CO_MDZpo (N = 20)	CO_PSL (N
Age (year), median (min, max)	Age (year), median (min, max)	25.5 (20, 42)	21.0 (20, 44
Sex, n (%)	Sex, n (%)		
	Male	20 (100)	20 (100)
Weight (kg), mean ± SD	Weight (kg), mean ± SD	63.5 ± 6.9	60.2 ± 7.2
Height (cm), mean ± SD	Height (cm), mean ± SD	168.9 ± 6.7	169.6 ± 5.8
Body mass index (kg $m^{2\ -1}$), mean ± SD	Body mass index (kg $m^{2\ -1}$), mean ± SD	22.3 ± 1.9	20.9 ± 1.8

CO_ATV, Combination of carotegrast methyl with atorvastatin; CO_MDZiv, Combination of carotegrast methyl with intravenous midazolam;

CO_MDZpo, Combination of carotegrast methyl with oral midazolam; CO_PSL, Combination of carotegrast methyl with prednisolone;

max, maximum; min, minimum; S_AJM, Carotegrast methyl alone; SD, standard deviation

Table 2 Pharmacokinetic parameters of midazolam (po), prednisolone (po), atorvastatin (po), and midazolam (iv) which were administered on day -1, days 7, 14, 28, and 42, while carotegrast methyl was repeatedly administered for 14 days from days 1 to 14

			Day -1	Day 7	Day 14	Day 28
Midazolam (po)	C_{max} (ng mL^{-1})	n	20	20	20	20
		Mean ± SD	30.9 ± 9.8	50.5 ± 14.1	56.6 ± 14.4	33.7 ± 11
		Geometric mean	29.6	48.6	54.9	31.9
		95% CI	25.8 – 33.9	42.6 – 55.6	48.7 – 61.8	27.1 – 37.
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	n	20	20	20	20
		Mean ± SD	74.5 ± 21.9	198.7 ± 52.2	225.1 ± 50.0	83.9 ± 26
		Geometric mean	71.6	192.9	219.9	80.0
		95% CI	62.5 – 82.0	171.9 – 216.4	198.3 – 243.9	68.8 – 93.
	T_{max} (h)	n	20	20	20	20
		Median (min, max)	0.5 (0.5, 1.0)	0.5 (0.5, 2.0)	0.5 (0.5, 2.0)	0.5 (0.5, 1
	$t_{1/2}$ (h)	n	20	20	20	20
		Mean ± SD	4.4 ± 0.9	4.2 ± 0.8	4.3 ± 0.9	4.1 ± 0.9
		Geometric mean	4.2	4.1	4.2	4.1
		95% CI	3.8 – 4.8	3.7 – 4.5	3.8 – 4.7	3.7 – 4.5
Prednisolone (po)	C_{max} (ng mL^{-1})	n	20	20	20	20
		Mean ± SD	156.1 ± 28.7	134.6 ± 21.3	138.0 ± 19.7	150.0 ± 2
		Geometric mean	153.6	133.1	136.8	147.6
		95% CI	141.0 – 167.4	124.0 – 142.9	128.2 – 145.9	135.5 – 16
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	n	20	20	20	20
		Mean ± SD	571.6 ± 98.1	591.9 ± 76.4	631.0 ± 77.9	571.9 ± 9

			Day -1	Day 7	Day 14	Day 28
		Geometric mean	562.7	586.7	625.9	563.8
		95% CI	515.5 – 614.2	549.7 – 626.3	587.9- 666.4	519.2- 612
	T_{max} (h)	n	20	20	20	20
		Median (min, max)	1.0 (0.5, 2.0)	1.5 (0.5, 3.0)	2.0 (0.5, 3.0)	1.0 (0.5, 3
	$t_{1/2}$ (h)	n	20	20	20	20
		Mean ± SD	2.2 ± 0.3	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.2
		Geometric mean	2.2	2.2	2.3	2.2
		95% CI	2.1 – 2.3	2.1 – 2.3	2.2 – 2.4	2.1 – 2.4
Atorvastatin (po)	C_{max} (ng mL^{-1})	n	20	19	19	19
		Mean ± SD	3.2 ± 1.5	3.1 ± 1.4	4.0 ± 2.4	3.3 ± 1.4
		Geometric mean	2.9	2.8	3.5	3.0
		95% CI	2.3 – 3.7	2.3 – 3.5	2.8 – 4.5	2.5 – 3.8
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	n	20	19	19	19
		Mean ± SD	16.1 ± 6.2	28.2 ± 12.1	33.8 ± 13.8	17.9 ± 6.8
		Geometric mean	15.2	26.4	31.7	17.0
		95% CI	13.1 – 17.7	22.3 – 31.3	26.8 – 37.6	14.5 – 19.
	T_{max} (h)	n	20	19	19	19
		Median (min, max)	0.5 (0.5, 3.0)	3.0 (1.0, 10.0)	2.0 (1.0, 10.0)	0.5 (0.5, 6
	$t_{1/2}$ (h)	n	20	19	19	19
		Mean ± SD	9.2 ± 1.6	5.8 ± 1.3	4.5 ± 0.8	8.5 ± 2.2
		Geometric mean	9.1	5.7	4.4	8.3
		95% CI	8.3 – 9.8	5.1 – 6.3	4.1 – 4.8	7.4 – 9.3
Midazolam (iv)	$AUC_{0\text{-}t}$ (ng h mL^{-1})	n	20	19	19	19
		Mean ± SD	48.8 ± 12.1	67.4 ± 13.6	73.7 ± 14.5	56.0 ± 12
		Geometric Mean	47.5	66.0	72.4	54.7
		95% CI	42.6 – 52.9	59.7 – 73.1	66.0 – 79.3	49.2 – 60.
	$t_{1/2}$ (h)	n	20	19	19	19
		Mean ± SD	4.6 ± 1.5	4.5 ± 1.2	4.6 ± 1.0	4.0 ± 1.3
		Geometric Mean	4.3	4.3	4.5	3.8
		95% CI	3.6 – 5.2	3.7 – 5.0	4.1 – 5.0	3.1 – 4.5

$AUC_{0\text{-}t}$, area under the curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum drug concentration; iv, intravenous; max, maximum; min, minimum; po, per os; SD, standard deviation; $t_{1/2}$, half-life period; T_{max}, time to C_{max}

Table 3Drug-drug interactions between carotegrast methyl and CYP3A4 substrates

			Day -1	Day 7	Da
Midazolam (po)	C_{max} (ng mL^{-1})	n	20	20	20
		Estimated ratio after inverse logarithmic conversion	—	1.65	1.8
		90% CI of the ratio	—	1.45 – 1.87	1.6
		p-value	—	<.0001	<.0
	$AUC_{0\text{-}t}$ (ng h mL^{-1})	n	20	20	20
		Estimated ratio after inverse logarithmic conversion	—	2.69	3.0
		90% CI of the ratio	—	2.47 – 2.94	2.8
		p-value	—	<.0001	<.0
Prednisolone (po)	C_{max} (ng mL^{-1})	n	20	20	20
		Estimated ratio after inverse logarithmic conversion	—	0.87	0.8
		90% CI of the ratio	—	0.82 – 0.92	0.8

			Day -1	Day 7	Da
		p-value	—	0.0001	0.0
	AUC_{0-t} (ng h mL^{-1})	n	20	20	20
		Estimated ratio after inverse logarithmic conversion	—	1.04	1.1
		90% CI of the ratio	—	1.01 – 1.08	1.0
		p-value	—	0.0471	<.(
Atorvastatin (po)	C_{max} (ng mL^{-1})	n	20	19	19
		Estimated ratio after inverse logarithmic conversion	—	1.00	1.2
		90% CI of the ratio	—	0.81 – 1.22	1.0
		p-value	—	0.9651	0.0
	AUC_{0-t} (ng h mL^{-1})	n	20	19	19
		Estimated ratio after inverse logarithmic conversion	—	1.75	2.1
		90% CI of the ratio	—	1.64 – 1.87	1.9
		p-value	—	<.0001	<.(
Midazolam (iv)	AUC_{0-t} (ng h mL^{-1})	n	20	19	19
		Estimated ratio after inverse logarithmic conversion	—	1.40	1.5
		90% CI of the ratio	—	1.30 – 1.50	1.4
		p-value	—	<.0001	<.(

AUC_{0-t}, area under the curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum drug concentration; iv, intravenous; po, per os

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