Amenamevir: Studies of Potential CYP2C8- and CYP2B6-Mediated Pharmacokinetic Interactions With Montelukast and Bupropion in Healthy Volunteers

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and clinical studies undertaken by Maruho (unpublished data held by the company). In healthy volunteers, amenamevir was rapidly absorbed following single oral doses with maximum plasma concentration \( (C_{\text{max}}) \) 1.33-2.5 hours independent of dose. During repeated dosing, \( C_{\text{max}} \) and the area under the concentration-time curve (AUC) decreased between the first dose (day 1), an intermediate dose (day 9), and the last dose (day 16), suggesting autoinduction of metabolism, probably via cytochrome P450 (CYP)3A4/5. AUC and \( C_{\text{max}} \) increased less than dose proportionally in dose-ranging studies. Plasma protein binding of \(^{14}\text{C}-\text{amenamevir} \) in humans was about 75%, and \( t_{1/2} \) about 7-8 hours. After a single oral dose of 200 mg \(^{14}\text{C}-\text{amenamevir} \), 74.6% of \(^{14}\text{C}-\text{radioactivity} \) was recovered in feces. These data are consistent with later pharmacokinetic analyses of studies in healthy volunteers and patients.

The metabolic profile of amenamevir was evaluated in vitro using pooled liver microsomes and cryopreserved hepatocytes of mouse, rat, rabbit, dog, and human origin. The major human metabolite was a monohydroxy derivative (AS1955888-00, Mo4, R5), which was also detected in all other species tested.

CYP isoforms involved in amenamevir metabolism were studied in vitro using human liver microsomes. Amenamevir’s metabolism correlated significantly with CYP2B6, CYP2C19, and CYP3A4/5 activity. The correlation was strongest with CYP3A4/5 (0.9236 \( r^2 \) coefficient of determination, \( P < .0001 \)), suggesting a major role for CYP3A4/5 in the metabolism of amenamevir, whereas correlation with CYP2B6 (0.3578 \( r^2 \), \( P = .0144 \)) and CYP2C19 was less marked (0.3489 \( r^2 \), \( P = .0160 \)). Correlation with CYP2C8 was weaker (0.1967 \( r^2 \), \( P = .0853 \)).

The potential for amenamevir to inhibit cytochrome metabolism was evaluated in vitro using human liver microsomes and CYP-selective substrates. Amenamevir had weak direct inhibitory activity against CYP2C8 (IC50 69 \( \mu \text{mol/L} \)) but no activity against CYP2B6 in the range of concentrations studied (0.1 to 100 \( \mu \text{mol/L} \) (IC50 > 100 \( \mu \text{mol/L} \)).

To investigate the potential of amenamevir to induce CYP2B6, CYP2B6 activity and gene expression were measured in human hepatocytes with or without pretreatment for 72 hours with amenamevir. After pretreatment with 2, 20, and 200 \( \mu \text{mol/L} \) amenamevir, CYP2B6 activity increased 1.4-, 1.9-, and 2.9-fold, respectively, and CYP2B6 gene expression increased by 1.9-, 4.5-, and 4.8-fold, respectively, compared with the negative control. Those results suggest that amenamevir has the potential to induce CYP2B6.

We report here 2 studies in healthy volunteers that were part of a series of investigations to elucidate potential interactions. Two probe substrates were selected: montelukast to investigate effects of amenamevir on CYP2C8 and bupropion to test effects on CYP2B6.

(1) Montelukast is an orally available leukotriene receptor antagonist used for the preventive treatment of asthma and seasonal allergic rhinitis. It is metabolized to its primary metabolite methyl-hydroxymontelukast (M6) via 36-hydroxylation by CYP2C8, which is also responsible for subsequent conversion to the secondary metabolite montelukast dicarboxylic acid (M4). CYP2C8 is thought to account for 70% to 80% of montelukast’s metabolism in vivo, with most of the remainder accounted for by CYP3A4-mediated conversion to M5a and M5b metabolites: less than 0.2% is eliminated in urine. This, together with its benign safety profile, makes it an appropriate choice as a CYP2C8 probe in healthy subjects.

(2) Bupropion is an orally available antidepressant and nonnicotine smoking cessation aid that is thought to exert its activity by reuptake inhibition of norepinephrine and dopamine and by noncompetitive antagonism of nicotinic acetylcholine receptors. It is metabolized in vivo to 3 primary active metabolites: hydroxybupropion, threohydrobupropion, and erythrohydrobupropion. Hydroxybupropion has around 50% of the activity of bupropion, but, as its \( C_{\text{max}} \) is 4-7 times greater and AUC about 10-fold greater, it is responsible for most of bupropion’s activity.

Threohydrobupropion concentrations are about 5-fold greater than that of bupropion, and erythrohydrobupropion concentrations are similar to those of bupropion, but they are only 20% as potent as the parent. Hydroxybupropion formation is closely correlated with CYP2B6 activity in human microsomes and with CYP2B6-specific N-demethylation of S-mephenytoin and can be 95% inhibited by a CYP2B6-specific monoclonal antibody. Bupropion’s other metabolites are formed independently of cytochrome activity. Thus, measurement of hydroxybupropion formation can be used to investigate CYP 2B6 metabolism of bupropion.

Subjects and Methods

Both studies were done concurrently at Hammersmith Medicines Research (HMR), London, after approval by both the Medicines and Healthcare products Regulatory Agency (MHRA) and the London-Brent Ethics Committee. The studies were conducted in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines and the ethical principles outlined in the Declaration of Helsinki.
The montelukast study (EudraCT no 2014-003955-73) lasted from December 2014 to April 2015, and the bupropion study (EudraCT no 2014-004656-59) from February to April 2015.

Subjects
Each study recruited the planned number of 24 healthy male volunteers aged 18-45 years, deemed healthy on the basis of medical history, medical examination, vital signs, electrocardiogram, laboratory safety tests on blood and urine, and urine tests for drugs of abuse (Table 1). During the study, smoking, alcohol, caffeine, all enzyme-inducing foodstuffs, and concomitant medications were prohibited. Subjects fasted overnight until a standardized light breakfast, which they finished 30 minutes before dosing. No food or drink was then allowed until 4 hours after dosing. Subjects took standard meals and drinks at 4, 10, and 24 hours after dosing and then at standard meal times on nondosing days. Safety tests on blood and urine, vital signs, and medical examination were done at appropriate intervals throughout each study.

Montelukast Study
This was a randomized, single-center, open-label, 2-way crossover drug-drug interaction study to investigate the effect of a single oral dose of amenamevir on the pharmacokinetics of a single oral dose of montelukast in 24 healthy men.

Each subject received montelukast 10 mg alone, followed 2 weeks later by montelukast 10 mg with amenamevir 400 mg, or vice versa; 400 mg amenamevir was selected because it was the projected therapeutic dose daily dose in Japan. Likewise, 10 mg montelukast was selected on the basis that it is the adult daily dose for treatment of asthma and seasonal allergies in adults and is well tolerated in healthy volunteers_18_.

Subjects were resident on the ward from the afternoon of the day before dosing (day –1) until day 4 and subsequently returned to give blood samples for pharmacokinetic analysis at 7 and 14 days after their dose of amenamevir. Plasma samples for analysis of montelukast and methyl-hydroxymontelukast were obtained predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, and 72 hours after dosing. Subjects returned for a final follow-up visit about 30 days after their last dose.

Bupropion Study
This was a single-center, open-label drug-drug interaction study to investigate the effect of amenamevir-mediated CYP2B6 induction on the probe substrate bupropion. Subjects received a single dose of 150 mg bupropion on days 1, 15, 22, and 29 and once-daily doses of 400 mg amenamevir on days 6-15 (Figure 1). Subjects were resident on the ward on 3 occasions: from the day before the first dose (day –1) until day 19; from day 21 until day 26; and from day 28 until day 33. Subjects returned for an outpatient visit on day 36 and a final follow-up visit on day 45. Blood samples for assay of bupropion and hydroxybupropion were taken before each dose of bupropion and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 36, 48, 60, 72, and 96 hours afterward (Supplementary Table S1).

Blood samples for assay of amenamevir were taken at predose on days 6-14, and before and frequently up to 24 hours after dosing with amenamevir on day 15.

Safety and Tolerability Assessments
Safety and tolerability assessments included adverse events, vital signs, 12-lead electrocardiogram, physical examination, and clinical laboratory tests.

Assays
Plasma concentrations of all compounds and metabolites were determined using validated liquid chromatography tandem mass spectrometry by Shin Nippon Biomedical Laboratories, Ltd (Tokyo, Japan) and Analytical Services International (London, UK). The lower limit of quantification (LLQ) was 5 ng/mL for amenamevir, 10 ng/mL for montelukast, 1 ng/mL for methyl-hydroxymontelukast, 2.5 ng/mL for bupropion, and 10 ng/mL for hydroxybupropion.

Blood samples for bupropion, hydroxybupropion, montelukast, and methyl-hydroxymontelukast were collected in lithium heparin tubes. Plasma was separated by centrifugation at ~1500g for 10 minutes at 4°C, then stored at –20°C or below until analysis by Analytical Services International as described below.

Blood samples for amenamevir and AS1955888-00 were collected in sodium heparin tubes and prepared as above before assay by Shin Nippon Biomedical Laboratories, as described by Adeloye et al._19_.

Bupropion
Preparation of Internal Standard and Calibrators. Bupropion, hydroxybupropion (calibrators), internal standard 1 (bupropion-d_9_), and internal standard 2 (hydroxybupropion-d_6_) were extracted from 100 μL of human plasma by protein precipitation. The internal standards were prepared by diluting 50 μL of bupropion-d_9_ stock + 200 μL hydroxybupropion-d_6_ stock with 20 mL acetonitrile + 20 mL deionized water to yield a final concentration of 125 ng/mL bupropion and 500 ng/mL hydroxybupropion. For calibration, a substock solution containing bupropion and hydroxybupropion was prepared by diluting 50 μL of bupropion stock (1 mg/mL) and 200 μL hydroxybupropion stock (1 mg/mL) in 20 mL of human plasma.
Table 1. Subject Demographics

|                          | Bupropion Subjects (N = 24) | Montelukast Subjects (N = 24) |
|--------------------------|-----------------------------|-------------------------------|
| Age (y) Mean (SD) range  | 32.6 (7.14) 20-45           | 30.8 (7.1) 20-43             |
| Race n (%)               |                             |                               |
| White                    | 18 (75.0)                   | 19 (79.2)                     |
| Black                    | 3 (12.5)                    | 4 (16.7)                      |
| Asian                    | 1 (4.2)                     | 0                             |
| Mixed race               | 2 (8.4)                     | 1 (4.2)                       |
| Ethnicity n (%)          |                             |                               |
| Not Hispanic or Latino   | 23 (95.8)                   | 24 (100.0)                    |
| Hispanic or Latino       | 1 (4.2)                     | 0                             |
| Mean (SD)                | 178.7 (5.32)                | 180.2 (8.1)                   |
| Height (cm) Range        | 169-188                     | 164-199                       |
| Mean (SD)                | 78.07 (9.46)                | 81.22 (12.24)                 |
| Weight (kg) Range        | 64.3-99.8                   | 61.9-104.9                    |
| Mean (SD)                | 24.46 (2.80)                | 25.00 (3.38)                  |
| BMI (kg/m²) Range        | 20.0-30.1                   | 19.4-30.0                     |
| History of smoking n (%) | 4 (16.7)                    | 4 (16.7)                      |
| Consumes any alcohol (%) | 15 (62.5)                   | 17 (70.8)                     |
| Units/week mean (SD)     | 5.8 (3.8)                   | 5.7 (4.2)                     |
| Alcohol consumption      | 1-12                        | 1-17                          |

BMI indicates body mass index.

*Includes only those subjects who drink alcohol.

Table 2. Bupropion and Hydroxybupropion Variability

|                | Concentration (ng/mL) | Mean (ng/mL) | Precision (%CV) | Accuracy (%) |
|----------------|-----------------------|--------------|-----------------|--------------|
| Bupropion      | QC1 5                 | 4.640        | 5.1             | 92.9         |
|                | QC2 75                | 69.86        | 4.2             | 93.2         |
|                | QC3 200               | 192.5        | 3.6             | 96.3         |
| Hydroxybupropion| QC1 20               | 19.24        | 7.5             | 96.2         |
|                | QC2 300               | 286.1        | 5.0             | 95.4         |
|                | QC3 800               | 781.1        | 4.9             | 97.3         |

QC indicates quality control.

Figure 1. Bupropion dosing intervals. A, dosing with amenamevir 400 mg once daily; B, dosing with bupropion 150 mg.

Table 2. Bupropion and Hydroxybupropion Variability

|                | Concentration (ng/mL) | Mean (ng/mL) | Precision (%CV) | Accuracy (%) |
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|                | QC2 75                | 69.86        | 4.2             | 93.2         |
|                | QC3 200               | 192.5        | 3.6             | 96.3         |
| Hydroxybupropion| QC1 20               | 19.24        | 7.5             | 96.2         |
|                | QC2 300               | 286.1        | 5.0             | 95.4         |
|                | QC3 800               | 781.1        | 4.9             | 97.3         |

QC indicates quality control.

Analytical Method. We then added to a 2-mL polypropylene tube 100 μL of calibrator or plasma sample, 50 μL of internal standard (125 ng/mL bupropion-d9 and 500 ng/mL hydroxybupropion-d6), 200 μL precipitating agent (acetonitrile), and 200 μL of deionized water.

The sample was vortexed for 2 minutes before centrifugation and 150 μL supernatant was transferred to an autosampler tube and submitted to analysis by liquid chromatography tandem mass spectrometry.

HPLC Conditions. The analytic column was an Onyx Monolithic C18 column (100 mm × 3 mm) (Phe-...omenex, Torrance, California). The mobile phase comprised methanol 1000 mL, deionized water 1000 mL, plus 10 g ammonium formate. Elution was iso-cratic at a flow rate of 300 μL/min.

MS Settings. An Applied Biosystems (Foster City, California) API4000 mass spectrometer was used with turbo ion spray and positive ionization. Mass ratios were 240.0/184.0 amu for bupropion, 249.0/185.0 amu for the bupropion standard, 256.0/167.0 amu for hydroxybupropion, and 282.0/130.0 amu for the hydroxybupropion standard.

Variability. Variabilities of bupropion and hydroxybupropion determinations are shown in Table 2.

Montelukast

Preparation of Internal Standard and Calibrators. Montelukast, montelukast 1,2-diol (calibrators), and montelukast-d6 (internal standard) were extracted from 100 μL of human plasma by protein precipitation. Working solutions at a concentration of 1000 ng/mL were prepared by diluting an aliquot (25 μL) of stock solution (1000 μg/mL) with analyze-free plasma up to a final volume of 25 mL.

Analytical Method. We added to a 2-mL polypropylene tube 100 μL of calibrator or plasma sample, 25 μL of internal standard (montelukast-d6), and 500 μL of...
Table 3. Montelukast and Montelukast 1,2-diol variability

|蒙特鲁卡斯特 |浓度 (ng/mL) |均值 (ng/mL) |精确度 (%CV) |准确性 (%) |
|---|---|---|---|---|
|QC1 |20.1 |20.14 |8.7 |100.2 |
|QC2 |150.4 |149.6 |7.0 |99.5 |
|QC3 |752.2 |727.7 |7.889 |96.747 |
|蒙特鲁卡斯特1,2-醇 |QC1 |2.0 |1.951 |13.3 |97.6 |
|QC2 |14.7 |13.70 |13.4 |93.2 |
|QC3 |73.5 |70.80 |15.0 |96.3 |

QC indicates quality control.

一个沉淀溶液（乙腈：甲醇，1:1）。该样本被涡旋2分钟后再离心。上清液被转移至一个聚丙烯自动采样管，并加入10 μL至高精度液相色谱串联质谱仪。由于药物、代谢物和内标均对光敏感，采取了步骤来最大限度地减少光暴露。

HPLC条件。所用色谱柱为MonoLith PR-18e柱（100 mm × 3.0 mm）。流动相由1000 mL乙腈、1000 mL超纯水及氨水5 g组成。梯度洗脱速度为300 μL/min。

MS设置。使用Applied Biosystems API4000质谱仪，采用快速离子喷雾和正电荷模式。质量比分别为586.4/422.1 amu（蒙特鲁卡斯特），592.3/427.1 amu（蒙特鲁卡斯特标准），602.4/438.0 amu（蒙特鲁卡斯特1,2-醇），以及592.3/427.1 amu（蒙特鲁卡斯特1,2-醇标准）。

变异。蒙特鲁卡斯特和蒙特鲁卡斯特1,2-醇的变异数据如表3所示。

样本大小

蒙特鲁卡斯特研究的样本大小为24人，基于统计功效计算选定。从已发表的研究中，Cmax和AUC0–∞之间的百分比变异系数（%CV）估计为20%至30%。7统计功效通过模拟（模拟次数为10,000）来估计。模型如下。该模型包括治疗（蒙特鲁卡斯特单药或蒙特鲁卡斯特与阿尼美维合用），序列，以及受试者作为随机效应。

\[ \ln(\text{PK parameter}_{ijkl}) = \text{Treatment}_i + \text{Session}_j + \text{Sequence}_k + \text{Subject}_l + e_{ijkl} \]

给定一个%CV为25，一个真实的均值比为1.0，以及接受区间为80%到125%的90%置信区间，24人样本量至少可以检测到86%的功效，以检测到其定义的相互作用。在实践中，受试者内的变异可能小于受试者间的变异。

一个样本大小为24人的样本被用于分析地巴وبر普酮研究基于统计功效计算。蒙特鲁卡斯特合用阿尼美维与蒙特鲁卡斯特单药的两组之前的研究中的单剂量150 mg地巴وبر普酮就其在受试者间的Cmax和AUC0–∞之间的%CV为15%和30%。20,21统计功效通过模拟（模拟次数为10,000）来估计。模型包括治疗（地巴وبر普酮合用阿尼美维与地巴وبر普酮单药），序列，以及受试者作为随机效应。

\[ \ln(\text{PK parameter})_{ij} = \text{Treatment}_i + \text{Subject}_j + e_{ij} \]

给定一个%CV为22.5（平均值为15%和30%），一个真实的均值比为1.0，以及接受区间为80%到125%的90%置信区间，24人样本量可以提供93%的功效。

药代动力学和统计分析

药代动力学参数使用WinNonlin（Certara, Princeton, New Jersey）版6.3进行计算。使用等价分析来检验相互作用。

为了评估阿尼美维对蒙特鲁卡斯特的影响，蒙特鲁卡斯特与阿尼美维合用组与蒙特鲁卡斯特单药组的AUC0–∞和Cmax被对数转换，并使用ANOVA分析治疗（蒙特鲁卡斯特单药或蒙特鲁卡斯特与阿尼美维合用），序列，以及受试者作为固定效应和受试者作为随机效应。通过将治疗差异的最小二乘均值及其90%置信区间转换为原始尺度来获得AUC0–∞和Cmax比值（地巴وبر普酮与阿尼美维合用相对地巴وبر普酮单药）。如果90%置信区间落在80%到125%的接受范围内，则认为阿尼美维对蒙特鲁卡斯特的药代动力学没有临床显著影响。对阿尼美维对蒙特鲁卡斯特1,2-醇的影响进行了类似的研究。

为了评估阿尼美维对CYP2B6活性的影响，地巴وبر普酮与阿尼美维（测试组）的AUC0–∞和Cmax被对数转换，并使用ANOVA分析治疗（地巴وبر普酮单药或地巴وبر普酮与阿尼美维合用），序列，以及受试者作为固定效应和受试者作为随机效应。通过将治疗差异的最小二乘均值及其90%置信区间转换为原始尺度来获得地巴وبر普酮合用与阿尼美维合用的地巴وبر普酮平均AUC0–∞和Cmax比值。若90%置信区间落在80%到125%的接受范围内，则认为阿尼美维对地巴وبر普酮的药代动力学没有临床显著影响。
was concluded if the 90% CI for both $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$ ratios fell within the prespecified interval of 80% to 125%. To assess the effect of amenamevir on hydroxybupropion, bupropion in combination with amenamevir (Test) was compared with bupropion alone (Reference) using the method described above.

To assess the recovery of CYP2B6 activity, the effect of amenamevir on bupropion was assessed on days 22 and 29 using the method described above. The 95% CIs for the difference in means (day 22 vs day 1 and day 29 vs day 1) were used to determine how long it took for CYP2B6 activity to return to normal.

Results

Montelukast Study
Mean age, weight, and body mass index were similar across treatment sequences. There were no notable differences in race, ethnicity, or the subjects’ usual cigarette smoking or alcohol intake habits.

From about 1 hour after dosing until 30 hours post-dose, mean plasma concentrations of montelukast were about 1.2-fold higher when montelukast was coadministered with amenamevir than when given alone (Figure 2, Supplementary Table S1). After both treatments, mean montelukast plasma concentrations approached LLQ at 24 hours after dosing, and were below the limit of quantification by 30–36 hours after dosing.

At all time points from 1 to 30 hours after dosing, mean plasma concentrations of methyl-hydroxymontelukast were 1.2- to 2.2-fold higher when montelukast was taken with amenamevir than when given alone (Figure 3, Supplementary Table S2).

Both after montelukast alone and combined with amenamevir, mean methyl-hydroxymontelukast plasma concentration approached LLQ at 20 hours after dosing, and was below the limit of quantification by 36–48 hours after dosing.

Both $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ of montelukast increased significantly, by about 22% (Tables 4 and 5), when combined with amenamevir (121.7% [114.8, 129.1]; 122.1% [116.2, 128.4] respectively), as did $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ of its primary metabolite hydroxymontelukast (121.4%, 90% CI [106.4, 138.5]; 125.6% [111.3, 141.7]). After coadministration of amenamevir, the time to peak montelukast concentration remained unchanged.
Table 4. Summary of Effects of Amenamevir on C\textsubscript{max} and AUC0-\textsubscript{\infty} of Montelukast and Methyl-Hydroxymontelukast Using Log-Transformed Values (N = 24)

| Analyte                  | Parameter       | Montelukast With Amenamevir | Montelukast | Ratio (%) | 90%CI       |
|--------------------------|-----------------|-----------------------------|-------------|-----------|-------------|
| Montelukast              | C\textsubscript{max} (ng/mL) | 505.9                       | 415.6       | 121.7     | 114.8, 129.1 |
|                          | AUC\textsubscript{0-\infty} (h·ng/mL) | 3418.5                     | 2799.2      | 122.1     | 116.2, 128.4 |
| Methyl-hydroxymontelukast| C\textsubscript{max} (ng/mL) | 23.3                        | 19.2        | 121.4     | 106.4, 138.5 |
|                          | AUC\textsubscript{0-\infty} (h·ng/mL) | 188.6                      | 150.2       | 125.6     | 111.3, 141.7 |

AUC\textsubscript{0-\infty} indicates area under concentration-time curve extrapolated to infinite time; C\textsubscript{max}, peak concentration.

Table 5. Summary of Montelukast Pharmacokinetic Parameters

| Parameter       | Montelukast Alone (N = 24) | Montelukast With Amenamevir (N = 24) |
|-----------------|-----------------------------|-------------------------------------|
| C\textsubscript{max} (ng/mL) | Mean 427.5                  | 521.5                               |
|                 | SD 103.2                    | 131.5                               |
| AUC\textsubscript{0-\infty} (h·ng/mL) | Mean 2820.6                  | 3460.2                              |
|                 | SD 869.6                    | 1114.0                              |
| AUC\textsubscript{0-\infty} (h·ng/mL) | Mean 2916.3                  | 3567.5                              |
|                 | SD 878.9                    | 1115.7                              |
| T\textsubscript{max} (h) | Median 3.00                 | 3.00                                |
|                 | Range 2.00-6.00             | 1.50-5.00                           |
| t\textsubscript{1/2} (h)     | Mean 5.05                   | 5.45                                |
|                 | SD 1.40                     | 0.92                                |
| CL/F (L/h)       | Mean 3.72                   | 3.04                                |
|                 | SD 1.08                     | 0.86                                |

AUC\textsubscript{0-\infty} indicates area under concentration-time curve extrapolated to infinite time; CL/F, apparent total body clearance from plasma; C\textsubscript{max}, peak concentration; t\textsubscript{1/2}, half-life; T\textsubscript{max}, time of peak concentration.

The elimination half-life of montelukast was slightly increased, and apparent total body clearance was slightly reduced after coadministration of amenamevir (neither was statistically significant).

Overall, 7 subjects (29.2%) reported 9 adverse events (AEs); each of those subjects reported an AE after 10 mg montelukast alone, and 5 subjects (33.3%) also reported an AE after 10 mg montelukast with 400 mg amenamevir. Most AEs occurred at least 72 hours after dosing, and only 1 subject required concomitant medication within 72 hours after dosing (paracetamol for pharyngitis).

All AEs were of mild or moderate intensity. Moderate AEs were more frequent after 10 mg montelukast alone (16.7% of subjects) than after 10 mg montelukast with 400 mg amenamevir (4.2% of subjects). Mild AEs were reported by more subjects (12.5%) after 10 mg montelukast alone than after 10 mg montelukast with 400 mg amenamevir (4.2% of subjects).

**Bupropion Study**

From about 2 to 6 hours after dosing, mean plasma concentrations of bupropion were lower when bupropion was taken with amenamevir than when it was taken alone, whether on day 1 (before amenamevir had been given), or on days 22 and 29 (a week or more after repeated amenamevir dosing had ended (Figure 4, Supplementary Table S3).

After all treatments, mean bupropion plasma concentrations were approaching the LLQ at 36 hours after dosing and were below LLQ by 96 hours after dosing.

Plasma concentrations of bupropion after a single 150-mg dose were about 16% lower after 10 days’ dosing with amenamevir than when it was given alone, as evidenced by the reduction in both C\textsubscript{max} and AUC\textsubscript{0-\infty} to about 84% (84.29%, 90%CI [78.00, 91.10]; 84.07%, 90%CI [78.85, 89.63], respectively) (Table 6). Plasma concentrations then recovered to pretreatment levels on days 22 and 29.

Bupropion concentrations on day 22 were similar to those on day 1 (ratio 104.07, 95%CI [94.74, 114.32]), indicating that the induction of CYP2B6 by repeated doses of amenamevir had remitted by 1 week after the last dose of amenamevir (Table 7).

Figure 5 shows the mean plasma concentrations of hydroxybupropion plotted against time. On day 1, the AUC of the primary metabolite hydroxybupropion was about 15-fold that of the parent molecule (Table 8). Coadministration of amenamevir with bupropion had no significant effect on plasma concentrations of hydroxybupropion.

Median time to peak concentration of bupropion was 3 hours on days 1, 15, 22, and 29 (Table 9). Mean t\textsubscript{1/2} was shortened by amenamevir on day 15 by around 2 hours compared with that on day 1; consistent with that finding and with the reduction in AUC of bupropion, apparent total body clearance of bupropion was slightly higher when given with amenamevir than when given alone.

Overall, 12 subjects (50.0%) reported AE. Headache was the most frequently reported AE. Cannula site
Table 6. Summary of the Effect of 10 Days’ Pretreatment With Amenamevir on C\textsubscript{max} and AUC\textsubscript{0-221e} on a Single Dose of Bupropion (Day 15, N = 24) with 90% CIs

| Parameter        | Bupropion With Amenamevir | Bupropion | Ratio (%) | 90%CI       |
|------------------|---------------------------|-----------|-----------|-------------|
| C\textsubscript{max} (ng/mL) | 76.94                     | 91.28     | 84.29     | 78.00, 91.10 |
| AUC\textsubscript{0-221e} (h·ng/mL) | 653.70                     | 777.61    | 84.07     | 78.85, 89.63 |

AUC\textsubscript{0-221e} indicates area under concentration-time curve extrapolated to infinite time; C\textsubscript{max}, peak concentration. Parameters have been log-transformed.

Table 7. Summary of Bupropion Concentrations Before, During, and After Induction of CYP2B6 by Amenamevir (N = 24) With 95% CIs

| Parameter        | Day 1 (Control) | Day 15 | Day 22 | Day 29 |
|------------------|----------------|--------|--------|--------|
| C\textsubscript{max} (ng/mL) | LS mean 91.28 | 76.94 | 92.21  | 95.00  |
| ratio vs control (%) (95%CI) | N/A          | 84.29 (76.74, 92.60) | 101.02 (91.96, 110.97) | 104.07 (94.74, 114.32) |
| AUC\textsubscript{0-221e} (h·ng/mL) | LS mean 777.61 | 653.70 | 785.52 | 841.36 |
| ratio vs control (%) (95%CI) | N/A          | 84.07 (78.12, 90.46) | 101.02 (93.88, 108.70) | 108.20 (100.55, 116.43) |

AUC\textsubscript{0-221e} indicates area under concentration-time curve extrapolated to infinite time; C\textsubscript{max}, peak concentration; LS mean, least-squares mean; N/A, not applicable. Parameters have been log-transformed.
Table 8. Summary of Hydroxybupropion Pharmacokinetic Parameters

| Bupropion Parameter | Day 1 Bupropion Alone (N = 24) | Day 15 Bupropion + Amenamevir (N = 24) | Day 22 Bupropion Alone (N = 24) | Day 29 Bupropion Alone (N = 24) |
|---------------------|---------------------------------|----------------------------------------|---------------------------------|---------------------------------|
| C_{max} (ng/mL)     | Mean 308.0                      | 315.1                                  | 310.8                           | 304.2                           |
|                     | SD 87.8                         | 96.8                                   | 79.5                            | 86.5                            |
| AUC_{0-tn} (h·ng/mL)| Mean 11,045.1                   | 10,807.2                               | 11,725.4                        | 11,623.6                        |
|                     | SD 3471.9                       | 3828.3                                 | 3814.3                          | 3858.9                          |
| AUC_{0-\infty} (h·ng/mL)| Mean 11,784.3               | 11,556.5                               | 12,754.5                        | 12,559.6                        |
|                     | SD 3882.3                       | 4263.4                                 | 4423.4                          | 4238.0                          |
| T_{max} (h)         | Median 8.00                     | 8.00                                   | 8.00                            | 8.00                            |
|                     | Range 4.00-10.08                | 5.00-24.00                             | 5.00-12.00                      | 3.00-12.02                      |
| t\_{1/2} (h)        | Mean 21.83                      | 21.42                                  | 23.70                           | 23.19                           |
|                     | SD 4.70                         | 4.94                                   | 5.76                            | 3.74                            |

AUC_{0-\infty} indicates area under concentration-time curve extrapolated to infinite time; AUC_{0-tn}, area under concentration-time curve up to last nonzero value; C_{max}, peak concentration; t\_{1/2}, half-life; t_{max}, time of peak concentration.

Table 9. Summary of Bupropion Pharmacokinetic Parameters

| Bupropion Parameter | Day 1 Bupropion Alone (N = 24) | Day 15 Bupropion + Amenamevir (N = 24) | Day 22 Bupropion Alone (N = 24) | Day 29 Bupropion Alone (N = 24) |
|---------------------|---------------------------------|----------------------------------------|---------------------------------|---------------------------------|
| C_{max} (ng/mL)     | Mean 94.5                       | 80.3                                   | 95.6                            | 97.8                            |
|                     | SD 25.8                         | 24.4                                   | 26.9                            | 24.5                            |
| AUC_{0-tn} (h·ng/mL)| Mean 764.5                      | 629.8                                  | 777.5                           | 825.7                           |
|                     | SD 246.9                        | 175.5                                  | 242.5                           | 235.7                           |
| AUC_{0-\infty} (h·ng/mL)| Mean 812.6                   | 675.7                                  | 820.9                           | 873.7                           |
|                     | SD 269.0                        | 188.7                                  | 253.1                           | 245.5                           |
| T_{max} (h)         | Median 3.00                     | 3.00                                   | 3.00                            | 3.00                            |
|                     | Range 2.00-6.00                 | 1.00-5.98                              | 2.00-5.00                       | 1.00-5.00                       |
| t\_{1/2} (h)        | Mean 10.92                      | 8.97                                   | 9.76                            | 11.87                           |
|                     | SD 6.09                         | 5.08                                   | 4.01                            | 5.24                            |
| CL/F (L/h)          | Mean 200.64                     | 236.46                                 | 199.59                          | 185.59                          |
|                     | SD 56.33                        | 57.20                                  | 62.04                           | 57.71                           |

AUC_{0-\infty} indicates area under the concentration-time curve extrapolated to infinite time; AUC_{0-tn}, area under concentration-time curve up to last nonzero value; CL/F, apparent total body clearance from plasma; C_{max}, peak concentration; t\_{1/2}, half-life; t_{max}, time of peak concentration.

Discussion

Montelukast

Coadministration of amenamevir with montelukast caused a 22% increase in both C_{max} and AUC of montelukast. The data failed to exclude a clinically significant drug-drug effect because the 90%CI of the log ratio montelukast versus amenamevir plus montelukast did not fall within the range 80% to 125% for both C_{max} and AUC. The increase in C_{max} is consistent with a reduction in first-pass intestinal and hepatic extraction. Apparent clearance and t\_{1/2} showed a trend toward reduction in rate of elimination of montelukast. Our results are consistent with the in vitro finding that amenamevir is a weak inhibitor of CYP2C8. An increase of 22% in plasma concentration of a drug that is a substrate of CYP2C8 would be of importance only for medicines with a very narrow therapeutic window, so reduction of the dose of concurrent medication is unlikely to be necessary in clinical practice.

Coadministration of amenamevir with montelukast was associated with a mean 22% increase in plasma C_{max} and AUC of montelukast’s major metabolite, methyl-hydroxymontelukast. That increase mirrored the 22% increase in C_{max} and AUC of parent montelukast and is likely due to the metabolite also being dependent on CYP2C8 for subsequent conversion. Thus, rather than concentration decreasing as might be expected, it increased in parallel due to inhibition in a manner consistent with the effect on the parent compound.

pain, rhinitis, back pain, and anxiety were reported by 2 subjects each. No other AE was reported by more than 1 subject.
Montelukast is metabolized not only by CYP2C8 but also by CYP3A4, of which amenamevir is both a substrate and inducer. However, CYP2C8 is believed to account for about 80% of montelukast’s metabolism: inhibition of CYP2C8 by gemfibrozil increases the AUC of montelukast 4-fold. The same study also showed that inhibition of CYP3A4 by itraconazole did not affect the metabolism of montelukast, in contrast with later findings: in 1 study, inhibition of CYP3A4 increased AUC of montelukast by 144%. Thus, current evidence is contradictory, but it is certainly conceivable that induction of CYP3A4 activity by amenamevir might reduce plasma concentrations of montelukast. However, amenamevir was given only as a single dose together with a single dose of montelukast in this study, and it is unlikely that CYP3A4 induction by amenamevir could have developed quickly enough to have influenced the results.

In respect to safety and tolerability, both single and combined dosing were equally well tolerated in the healthy men in this study.

**Bupropion**

Amenamevir 400 mg once daily for 10 days decreased both Cₘₐₓ and AUC₀₋∞ of bupropion by about 16%. The results did not exclude a significant effect of amenamevir on bupropion exposure, as the 90%CI of the least-squares geometric mean ratios (bupropion with amenamevir to bupropion) of Cₘₐₓ and AUC₀₋∞ did not fall within the prespecified interval of 80% to 125%. At 1 and 2 weeks (days 22 and 29) after the final dose of amenamevir, Cₘₐₓ, and AUC₀₋∞ of bupropion were similar to those on day 1, indicating that CYP2B6 activity had returned to pretreatment levels within 1 week after the last amenamevir dose. That is consistent with the observation that even extensive induction of CYP2B6 by rifampicin is fully reversed by 2 weeks after cessation of rifampicin treatment.

Prior treatment with amenamevir 400 mg once daily for 10 days did not affect Cₘₐₓ or AUC₀₋∞ of hydroxybupropion, which is bupropion’s main metabolite. Because amenamevir treatment did reduce AUC₀₋∞ of bupropion parent drug, there was a small change in the ratio of AUC₀₋∞ for hydroxybupropion:bupropion; before amenamevir the ratio was 15.2; after treatment with amenamevir, the corresponding ratio was 17.7. Those results are consistent with the findings of Laizure et al, who showed that the more powerful induction of CYP2B6 by rifampicin reduced AUC₀₋∞ of bupropion by 3-fold, but doubled AUC₀₋∞ of the hydroxybupropion metabolite.

The minor reduction (by 16%) in plasma concentrations of bupropion, coupled with no change in concentrations of the active metabolite hydroxybupropion, would be unlikely to warrant dose adjustment when amenamevir is coadministered with CYP2B6 substrates.

Our AE data showed that repeated doses of 400 mg amenamevir, given alone or with a single dose of 150 mg bupropion, were well tolerated in healthy men.

**Conclusions**

The minor increase (22%) of the concentration of montelukast and the similarly marginal reduction (16%) in plasma concentration of bupropion suggest that dose adjustment is unlikely to be necessary when amenamevir is coadministered with CYP2C8 or CYP2B6 substrates.

**Declaration of Conflict of Interest and Financial Disclosure**

The studies were sponsored by Maruho. The authors are employees of either Maruho or the clinical unit. The authors confirm that they have no conflict of interest.

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Supporting Information
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