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Baloxavir marboxil in Japanese patients with seasonal influenza: Dose response and virus type/subtype outcomes from a randomized phase 2 study

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ABSTRACT

Background: Baloxavir marboxil (baloxavir) is an antiviral drug that inhibits the viral "cap-snatching" step in virus RNA transcription initiation. In Phase 2 study, baloxavir significantly shortend the time to alleviation of symptoms (TTAS) and showed significantly greater reduction in influenza virus titer compared with placebo. This provides additional outcomes including efficacy against virus types/subtypes and pharmacokinetic/pharmacodynamic (PK/PD) analysis.

Methods: Subgroup analyses by virus types/subtype were conducted for the primary and key secondary endpoints. Blood samples were collected totally at 2 to 5 points including Day 2 after baloxavir dosing. PK/PD analyses were conducted for TTAS and change in virus titer using the liner model and the E_{max} model, respectively.

Results: The median TTAS in each baloxavir dose group was significantly shorter than in the placebo group for patients with A/H1N1pdm virus, and was numerically shorter than the placebo group for patients with A/H3N2 and type B virus. Baloxavir significantly reduced virus titer within 1 day after treatment: for A/H1N1pdm, A/H3N2, and B virus, all 3 doses of baloxavir marboxil reduced virus titer on Day 2 to a greater extent than placebo. No clear PK/PD relationships were found for the TTAS, but the larger reduction in virus titer was observed in increasing C_{24}.

Conclusion: These results support that baloxavir marboxil will be effective against a range of virus types/subtypes.

1. Introduction

Influenza type A and influenza type B viruses disseminate worldwide and cause an acute respiratory disease with typically mild symptoms (World Health Organization, 2018). In the United States, it is estimated that influenza virus infection leads to between 9.2 and 35.6 million illnesses a year (Centers for Disease Control and Prevention, 2018). However, the role of influenza B in this morbidity is often underestimated: although influenza B viruses circulate at lower levels than influenza A viruses, their frequency varies widely each season (Burnham et al., 2013). Influenza infection may be prevented via vaccination, but treatment with an antiviral drug, such as the neuraminidase inhibitor oseltamivir, within a few days of the onset of symptoms may be prescribed to any patient with influenza symptoms. Of note, observational studies have suggested that oseltamivir has lower clinical efficacy against influenza B compared with influenza A (Burnham et al., 2013). Therefore, there is an unmet medical need for antiviral drugs that are effective against a range of influenza virus types.

Baloxavir marboxil (formerly S-033188) is an antiviral drug that inhibits the viral "cap-snatching" step in virus RNA transcription initiation (Noshi et al., 2018). In vitro studies, baloxavir marboxil has demonstrated efficacy against a range of virus strains including seasonal and avian A/H5N1 or A/H7N9 influenza viruses (Noshi et al., 2018). In a Phase 1 study in healthy Japanese adults, after a single oral administration of 6 mg baloxavir marboxil, the mean plasma

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concentration of the active form of baloxavir marboxil at 24 h (6.92 ng/mL) exceeded the target mean estimated from nonclinical studies (6.85 ng/mL) (Koshimichi et al., 2018a), suggesting that baloxavir marboxil may be effective in clinical practice.

The randomized, double-blind, placebo-controlled Phase 2 study was conducted in Japan to evaluate the dose-response effects of a single oral dose of baloxavir marboxil in adult otherwise-healthy patients. Among 403 patients enrolled in the study, 400 patients were randomized to the 4 treatment groups (100 in each group), and 389 patients completed the study. The predominant influenza virus strain was the A/H1N1pdm subtype (67%); type B was 23%, and A/H3N2 was 9% of the total. Each of the baloxavir dose groups significantly shortend the time to alleviation of symptoms and showed significantly greater reduction in influenza virus titer compared with placebo. Patient demographic and baseline clinical characteristics, and dose-response clinical, virology and safety outcome of baloxavir marboxil was previously reported (Hayden et al., 2018); however there was no information presented regarding its effectiveness against specific influenza virus types/subtypes, such as type B and pharmacokinetics/pharmacodynamics (PK/PD).

Here, we provide additional important outcomes from the Phase 2 study including efficacy against virus types/subtypes, and PK/PD analysis of a single oral dose of baloxavir marboxil, -used for the selection of clinical dosage.

2. Materials and methods

2.1. Study design

This was a double-blind, multicenter, placebo-controlled study of patients in Japan conducted from December 2015 to April 2016. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and was approved by the institutional review board or ethics committee at each center. All patients provided written informed consent.

The study was registered at the Japan Pharmaceutical Information Center Clinical Trials Information (Japic CTI-153090). The study design has been reported by Hayden et al. (2018).

2.2. Study population

The study included male or female patients aged ≥ 20 years to < 65 years (at time of informed consent) with a diagnosis of influenza virus infection. Infection was confirmed by fever ≥ 38 °C, positive rapid influenza virus test with nasal or throat swabs, and at least 1 of the general (headache, feeling feverish or having chills, aches or pains of the muscles or joints, fatigue) and respiratory symptoms (cough, sore throat, nasal congestion) associated with influenza virus infection present with a severity of moderate or greater. The time between onset of symptoms (when body temperature first increased ≥ 1 °C from normal body temperature or presence of ≥ 1 general/respiratory symptom) and screening was ≤ 48 h.

Patients were excluded from the study if they had severe symptoms of influenza requiring inpatient treatment, or risk factors including pregnancy, chronic respiratory disease, major disorders, compromised immune system, body mass index ≥ 40 kg/m^2, and serious underlying diseases. Patients were also excluded if they had concurrent infections requiring antimicrobial treatment (excluding skin infections), had received another antiviral influenza drug within 7 days, or had previously received baloxavir marboxil.

2.3. Treatment protocol

All patients received a single oral dose of baloxavir marboxil on Day 1. Patients were randomized 1:1:1:1 to the following groups: 10 mg baloxavir marboxil, 20 mg baloxavir marboxil, 40 mg baloxavir marboxil, or placebo. Patients were given 3 tablets in total: a combination of 10 mg tablets, 20 mg tablets, and placebo tablets depending on the dose. Baloxavir marboxil tablets and matching placebo tablets were manufactured by Shionogi & Co., Ltd. (Osaka, Japan) and were stored at room temperature.

2.4. Efficacy assessment

The outcome measures assessed by the patient and recorded in an electronic patient diary were: body temperature (4 times daily until Day 3 and twice daily from Day 4 to Day 14), severity of 7 influenza symptoms (cough, sore throat, headache, nasal congestion, feeling feverish or having chills, aches or pains of the muscles or joints, fatigue; twice daily from Day 1 to Day 9, and then once daily from Day 10 to Day 14, on a 4-point rating scale [0 = none, 1 = mild, 2 = moderate, 3 = severe]), and the ability to perform daily activities (from Day 1 to Day 14, once daily, on a 10-point scale [0 = unable to perform daily activities at all, 10 = able to perform all daily activities as usual]).

The primary efficacy outcome measure was the TTAS. This was defined as the time between study drug treatment and the time when all 7 influenza symptoms were assessed as 0 (none) or 1 (mild) and sustained for at least 21.5 h (90% of 24 h).

The secondary efficacy endpoints reported in this publication are in composite symptom score from all 7 influenza symptoms, time to resolution of fever (defined as the time between study drug treatment and the time when body temperature was < 37 °C and sustained for at least 12 h), time to resumption of normal activities (defined as the time between study drug treatment and the time when the patient assessed activities as a score of 10), and incidence of influenza-related complications (defined as sinusitis, otitis media, bronchitis, or pneumonia reported as an adverse event after study drug treatment). Subgroup analyses by virus subtype were also conducted for the primary and secondary endpoints.

2.5. Virology assessment

Nasal or throat swabs were collected on Days 1, 2, 3 (if possible), 6, 9, and 15 (if requested by the investigator when influenza symptoms were ongoing). The virology testing facility (LSI Medience Corporation, Tokyo, Japan) performed virus typing and subtyping (2009 pandemic A/H1N1 [A/H1N1pdm], A/H3N2, B, or mixed infection), virus titer (50% tissue culture infectious dose [$TCID_{50}$]/mL), and viral RNA load (copy/mL) tests. The secondary virology efficacy endpoints reported in this publication are change from baseline in virus titer. In addtion, subgroup analyses by virus type/subtype were also conducted for these endpoints.

2.6. Pharmacokinetic and pharmacokinetic/pharmacodynamic assessments

Blood samples were collected totally at 2 to 5 points: Day 2 and between Days 5 and 7 after baloxavir marboxil administration, and additionally if possible, between 0.5 and 4 h post dose, Day 3 and between Days 12 and 18. Heparin sodium-containing tubes were used for blood collection, and centrifuged to collect the plasma (stored at −20 °C). Plasma concentrations of baloxavir marboxil and active form baloxavir acid were analyzed with a validated liquid chromatography–tandem mass spectrometry method. The plasma concentration of baloxavir acid at 24 h after dosing (C_{24}; the observed concentration at 20–28 h post-dose) was summarized by each dose.

PK/PD analyses were conducted for TTAS and change in virus titer using the linear model and the E_{max} model, respectively. The C_{24} was used as an exposure index in PK/PD analyses because it was used as the PK parameter bridging from nonclinical studies. The C_{24} for this study was simulated to justify the dose of baloxavir marboxil for the pivotal Phase 3 study (which was based on the efficacy and safety assessments and PK/PD analyses).

2.7. Statistical analysis

The target sample size was 400 (100 patients in each group), with women of childbearing age limited to a maximum of 150 study participants. Sample size was calculated to detect a significant difference between at least one treatment group and the placebo group with a pairwise comparison of TTAS after making adjustments for multiplicity using the Hommel method. Based on the results from previous influenza studies, the median TTAS in the placebo group was assumed to be 100 h and the hazard ratios versus placebo were assumed to be 0.65 for 40 mg, 0.7 for 20 mg, and 0.8 for 10 mg. With the assumption that the TTAS follows an exponential distribution, we determined that 99 patients per group would be required to provide 80% power in a Cox proportional hazards model with a two-sided significance level of 5% and a follow-up period of 14 days.

The analysis population was the intention to treat infected (ITTI) population. The ITTI population comprised all patients who received the study drug and who had a confirmed diagnosis (by rapid influenza virus test) of influenza virus infection.

For analysis of the primary endpoint, the hazard ratio, 95% confidence interval (CI) and *P* value of each treatment group versus placebo was calculated using the Cox proportional hazards model (which was the planned primary analysis method) with TTAS as the response variable, treatment group as the fixed effect, and the randomization factors (current smoking status and composite symptom score at baseline) as covariates. However, in a post hoc goodness-of-fit test, the assumption of proportional hazards for the Cox proportional hazards model was contradicted between each treatment group and the placebo group. From this result, it was concluded that the stratified generalized Wilcoxon test (which was the planned secondary analysis method) was more appropriate to evaluate group differences in the TTAS because the test does not require an assumption of proportional hazards and places greater weight on events that occur at earlier time points. The stratification factors in this test were current smoking status (smoking or non-smoking) and composite symptoms score (≤ 11 or ≥ 12) at baseline. Multiplicity adjustment was applied using the Hommel method for the primary analysis, but not for the secondary analysis of the primary endpoint. In addition, the median of TTAS and its 95% CI were calculated for each treatment group. Secondary efficacy endpoints and virology endpoints were compared between each treatment group and the placebo group using the stratified generalized Wilcoxon test, analysis of covariance, or the van Elteren test.

All analyses and tabulations were performed using SAS Version 9.2 and WinNonlin Version 6.2.1.

3. Results

3.1. Primary outcome measure

Baloxavir marboxil was effective over a range of doses. For patients with A/H1N1pdm, the TTAS in each baloxavir marboxil dose group was significantly shorter than in the placebo group ($p < 0.01$ for each dose group). In patients with A/H3N2 and type B virus, the median TTAS was numerically, but not statistically significantly, shorter than with placebo for all doses (Table 1).

For patients with a composite symptom score of ≤ 11 at baseline, the median TTAS was shorter than for patients with a score of ≥ 12, regardless of dose (Table 1). Neither smoking habits nor time to treatment from influenza onset appeared to affect the TTAS (Table 1). There was no consistent trend in vaccination status on the TTAS (Supplementary Table 1).

3.2. Virus titer

Baloxavir marboxil was effective regardless of virus type/subtype (Fig. 1). For A/H1N1pdm and the type B virus, the change from

Table 1
Time to alleviation of influenza symptoms (TTAS) in patients treated with baloxavir marboxil or placebo for all patients by virus type/subtype, composite symptom score at baseline, smoking habits, and time to treatment from influenza onset.

TTAS (hours)	Baloxavir marboxil			
	10 mg (n = 100)	20 mg (n = 100)	40 mg (n = 100)	Placebo (n = 100)
ITTI[a]				
N	100	100	100	100
Median	54.2	51.0	49.5	77.7
95% CI	47.7 to 66.8	44.5 to 62.4	44.5 to 64.4	67.6 to 88.7
p value[b]	0.009	0.018	0.005	–
Virus type/subtype				
A/H1N1pdm				
N	66	71	61	69
Median	52.9	47.1	48.2	70.6
95% CI	45.9 to 65.6	39.4 to 55.3	35.2 to 65.5	64.9 to 89.9
p value[b]	0.008	0.008	0.005	–
A/H3N2				
N	13	5	12	6
Median	66.0	65.8	45.4	100.0
95% CI	28.1 to 83.5	21.3 to 188.5	23.5 to 113.4	18.9 to 113.1
p value[b]	0.125	0.491	0.269	–
B				
N	21	23	24	23
Median	63.3	65.4	63.3	83.1
95% CI	44.5 to 82.3	46.4 to 73.2	43.3 to 69.8	58.1 to 92.8
p value[b]	0.215	0.661	0.160	–
Composite symptom score at baseline				
Score ≤11				
N	36	36	36	36
Median	47.6	39.5	44.2	65.7
95% CI	30.8 to 55.9	30.1 to 53.1	27.2 to 62.1	47.3 to 77.7
p value[b]	0.143	0.014	0.067	–
Score ≥12				
N	64	64	64	64
Median	64.0	62.4	54.6	84.2
95% CI	51.3 to 77.7	49.4 to 80.8	46.9 to 69.0	69.9 to 93.7
p value[b]	0.026	0.133	0.022	–
Smoking habits				
Smoking				
N	33	32	31	33
Median	52.7	58.8	50.4	79.0
95% CI	45.1 to 69.1	36.1 to 69.3	37.9 to 69.7	64.9 to 91.3
p value[b]	0.230	0.490	0.094	–
Nonsmoking				
n	67	68	69	67
Median	55.0	49.4	49.5	77.7
95% CI	47.7 to 69.8	43.0 to 61.7	39.0 to 65.5	64.2 to 90.7
p value[b]	0.018	0.024	0.015	–
Time to treatment from influenza onset				
0 to ≤12 h				
n	7	15	12	11
Median	51.6	41.4	77.9	81.6
95% CI	41.4 to 225.9	23.2 to 66.8	32.7 to 172.3	39.9, 103.0
p value[b]	0.918	0.185	0.289	–
> 12 to ≤24 h				
n	38	40	28	42
Median	52.4	63.9	53.5	86.4
95% CI	45.3 to 63.3.	46.2 to 80.8	37.9 to 88.8	67.6 to 98.5
p value[b]	0.006	0.117	0.044	–
> 24 to ≤36 h				
n	30	18	36	22
Median	65.8	49.2	44.8	69.0
95% CI	48.7 to 91.8	30.3 to 66.0	31.4 to 62.1	46.6 to 90.7
p value[b]	0.654	0.518	0.267	–
> 36 to ≤48 h				
n	25	27	24	25

(*continued on next page*)

Table 1 (*continued*)

TTAS (hours)	Baloxavir marboxil			
	10 mg (n = 100)	20 mg (n = 100)	40 mg (n = 100)	Placebo (n = 100)
Median	55.8	47.1	48.1	67.8
95% CI	27.2 to 73.2	27.2 to 62.3	28.6 to 69.0	63.4 to 83.1
p value[b]	0.412	0.170	0.145	–

Abbreviations: CI, confidence interval; TTAS, time to alleviation of influenza symptoms.

[a] Previously reported in Hayden et al. (2018).

[b] Stratified generalized Wilcoxon test versus placebo: stratification factors were smoking habit and composite symptom score at baseline.

baseline in virus titer was significantly larger in all 3 dose groups than in the placebo group on Day 2 ($p < 0.0001$ for each dose group for A/H1N1pdm virus, $p < 0.05$ for each dose group for the type B virus). A statistically significant difference in the change from baseline in virus titer for the A/H3N2 virus was also found on Day 2 in the 10 mg and 40 mg baloxavir marboxil dose groups ($p < 0.05$ for 10 mg and 40 mg doses). For A/H1N1pdm and A/H3N2, virus titer was close to the lower limit of quantification by Day 2.

3.3. Secondary efficacy outcomes

The mean composite symptom score from 36 h to 120 h after study drug treatment was significantly smaller for baloxavir marboxil compared with placebo (Fig. 2). The time to resolution of fever was

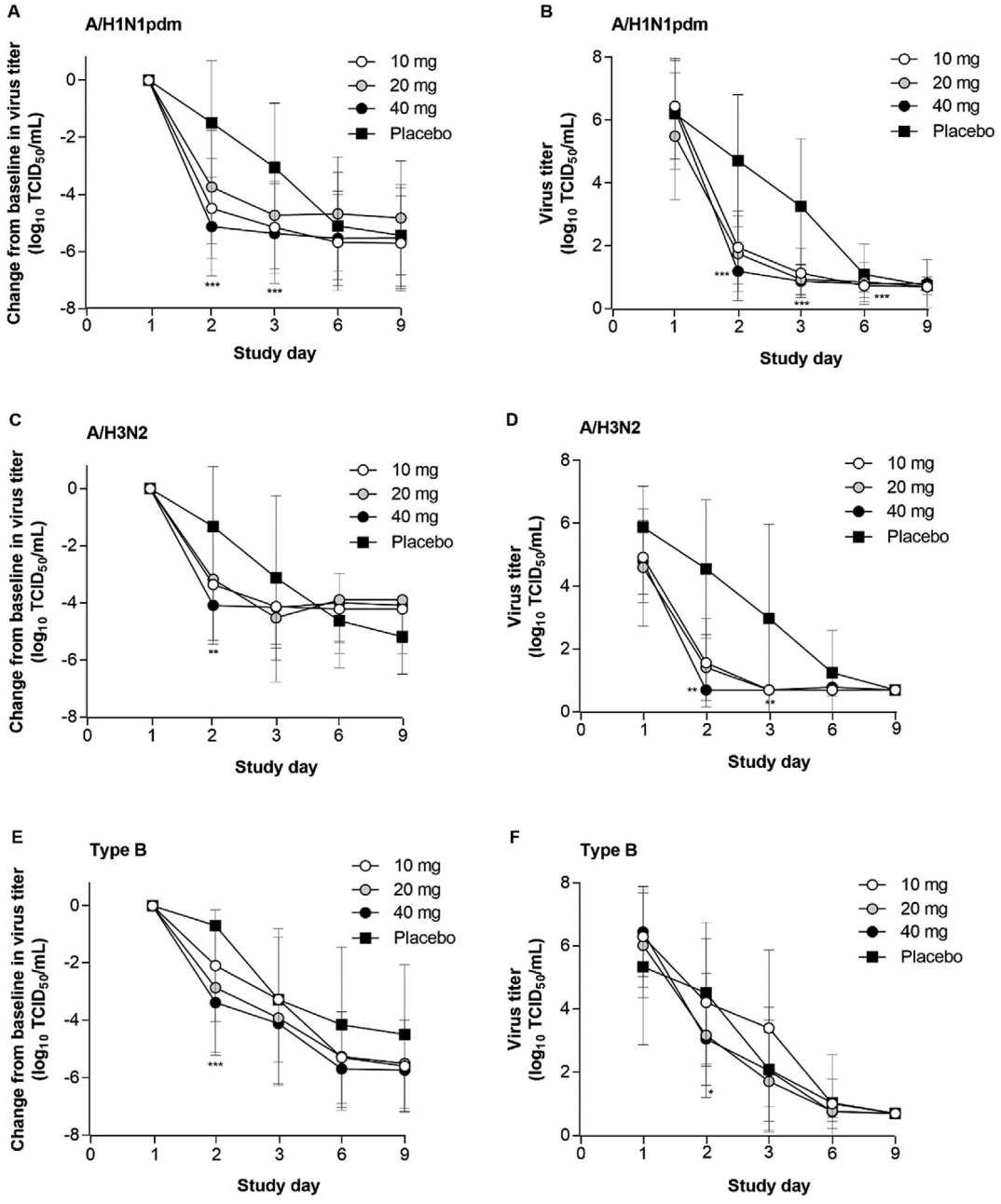

Fig. 1. Change from baseline in virus titer (**A**) (**C**) (**E**) and virus titer (**B**) (**D**) (**F**) (mean (± SD)) for influenza virus types/subtypes A/H1N1pdm, A/H3N2, and Type B. Limit of virus detection was 0.70 $\log_{10}$ $TCID_{50}$ per mL. Abbreviation: $TCID_{50}$, 50% tissue culture infectious dose; SD, standard deviation. ***$p < 0.05$ for all doses of baloxavir marboxil versus placebo; **$p < 0.05$ for 10 mg and 40 mg baloxavir marboxil versus placebo; *$p < 0.05$ for 20 mg baloxavir marboxil versus placebo. Note: Multiplicity adjustment was not applied to secondary endpoint analyses.

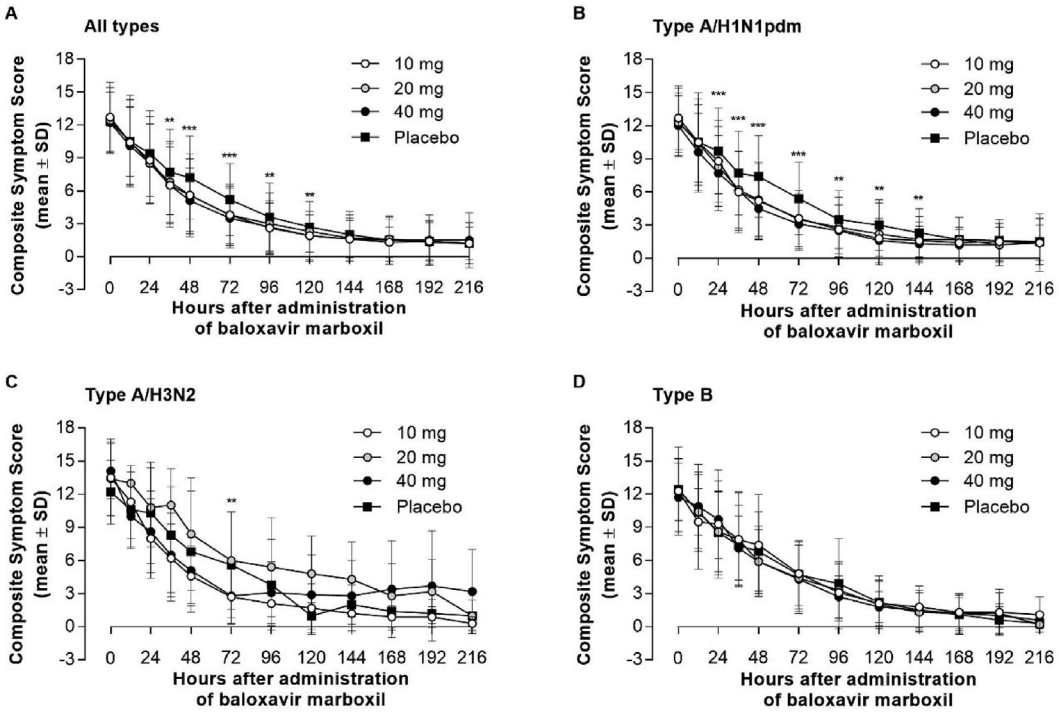

Fig. 2. Composite symptom score (mean (± SD)). Abbreviation: SD, standard deviation. ***p < 0.05 for all doses of baloxavir marboxil versus placebo; **p < 0.05 for 10 mg and 40 mg baloxavir marboxil versus placebo by analysis of covariance, with current smoking status and composite symptom score at baseline as covariates. Note: Multiplicity adjustment was not applied to secondary endpoint analyses.

Table 2
Secondary efficacy outcomes in patients treated with baloxavir marboxil or placebo for all patients by dose.

	Baloxavir marboxil			
Outcome Median hours (95% CI)	10 mg (n = 100)	20 mg (n = 100)	40 mg (n = 100)	Placebo (n = 100)
Time to resolution of fever	33.4[a] (26.9–38.1)	31.6[a] (26.9–35.8)	28.9[a] (24.5–34.7)	45.3 (35.6–54.0)
Time to resumption of normal activity	188.7 (152.2–225.9)	167.8[a] (123.9–207.7)	158.3 (131.7–200.7)	180.2 (155.9–242.9)
Time to alleviation of individual symptoms				
Nasal congestion	25.2[a] (19.0–47.2)	21.6 (13.4–30.5)	21.9[a] (16.0–28.7)	42.8 (22.9–68.3)
Aches or pains	31.2 (24.9–39.9)	29.9[a] (22.8–37.0)	25.4[a] (20.5–28.9)	41.9 (30.3–53.2)
Fatigue	32.0 (29.2–39.9)	31.3 (26.7–42.4)	31.1[a] (24.6–38.6)	42.7 (28.7–48.6)
Feeling feverish	24.7 (21.3–28.4)	29.4 (22.0–34.8)	23.0[a] (19.8–28.6)	28.8 (21.1–33.4)
Headache	42.2 (29.8–47.3)	37.0 (28.5–43.5)	37.9 (28.6–44.5)	43.7 (29.7, 53.6)
Cough	31.1 (21.3–41.5)	29.8 (21.9–32.9)	24.6 (16.1–29.4)	31.2 (20.9–51.4)
Sore throat	35.3 (21.2–49.8)	27.8 (19.9–32.1)	31.9 (17.3–43.0)	26.3 (16.5–45.2)

For time to resolution of fever, n = 100 for all groups.
For time to resumption of normal activity, n = 100 for 10 mg, 99 for 20 mg, and 98 for 40 mg and placebo.
For time to alleviation of individual symptoms, n was variable per group and symptom but was at least 38.
Abbreviation: CI, confidence interval.
[a] p < 0.05 for the stratified generalized Wilcoxon test versus placebo: stratification factors were smoking habit and composite symptom score at baseline.

significantly shorter for patients treated with all 3 doses of baloxavir marboxil compared with placebo (Table 2). The time to resumption of normal activity was significantly improved in 20 mg baloxavir marboxile dose group compared with placebo group (Table 2). The effect of baloxavir marboxil on the time to alleviation of individual symptoms was variable (Table 2). For example, nasal congestion, aches and pains, fatigue, and feverishness significantly improved with 40 mg baloxavir marboxil, whereas headache, cough, and sore throat were not significantly different to placebo at any dose.

For patients with A/H1N1pdm, the time to resolution of fever in the 20 mg and 40 mg baloxavir marboxil dose group was significantly shorter than in the placebo group (p < 0.0069 and < 0.001, respectively). In patients with A/H3N2 and type B virus, the median time to resolution of fever in the higher baloxavir marboxil dose groups was numerically, but not statistically significantly shorter than placebo (Supplementary Table 2). There were no statistical significant findings for the time to resumption of normal activity for any virus types (Supplementary Table 3).

There were 3 reports of influenza-related complications in 2 patients: 1 patient on 40 mg baloxavir marboxil reported bronchitis and otitis media, and 1 patient on placebo reported acute sinusitis. All complications were classified as mild/moderate, resolved, and were not considered related to the study drug.

3.4. Plasma concentration of the active form of baloxavir marboxil

The median C_{24} was 14.8 ng/mL for patients who received a 10 mg dose (n = 71), 29.6 ng/mL for patients who received a 20 mg dose

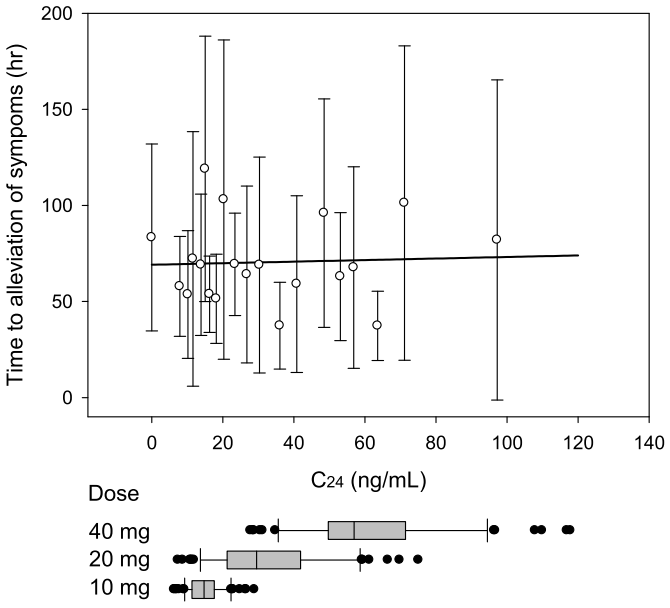

Fig. 3. Correlation between plasma concentration (C_{24}) of baloxavir acid and time to alleviation of influenza symptom (TTAS) with boxplots of C_{24} after 10 mg, 20 mg and 40 mg doses.

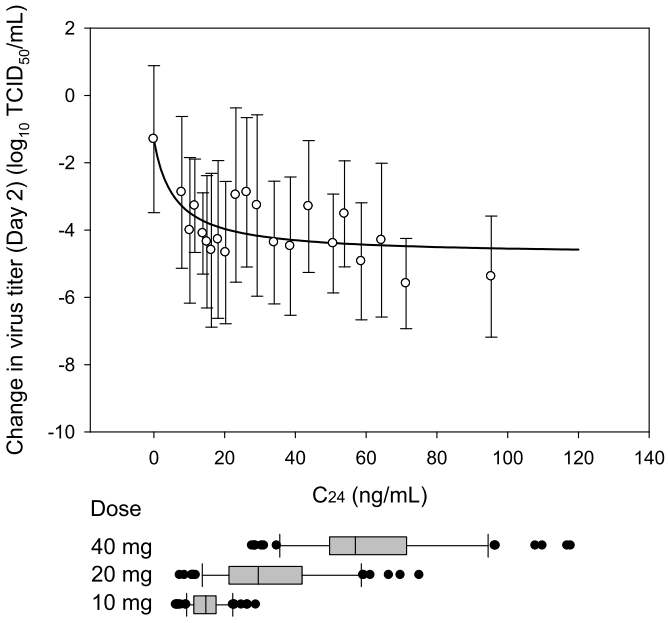

Fig. 4. Correlation between plasma concentration (C_{24}) of baloxavir acid and virus titer on Day 2, with boxplots of C_{24} after 10 mg, 20 mg and 40 mg doses.; Abbreviation: $TCID_{50}$, 50% tissue culture infectious dose.

(n = 67), and 57.1 ng/mL for patients who received a 40 mg dose (n = 64). The boxplots of C_{24} after each dose are shown at the bottom of Figs. 3 and 4.

The correlation between and TTAS and C_{24} of baloxavir acid were analyzed using linear model (Fig. 3). No clear PK/PD relationships were found for the TTAS. The correlation between changes in virus titer on Day 2 (24-h post dose) and the C_{24} of baloxavir acid were analyzed using the E_{max} model and is shown in Fig. 4. The reduction in virus titer increased with increasing C_{24}, and the C_{24} achieving 50% of the maximal response (EC_{50}) was 5.88 ng/mL. The mean C_{24} at the 10 mg dose exceeded the EC_{50}.

4. Discussion

This Phase 2 trial was the first to study the effectiveness of the new antiviral baloxavir marboxil in adult patients with a seasonal influenza virus infection. Importantly, our study found that a single oral dose of baloxavir marboxil was clinically and virologically effective over a range of doses and against a range of virus types/subtypes. In addition, all 3 doses of baloxavir marboxil were effective in rapidly reducing virus titer to almost the lower limit of detection 1 day after treatment. Our findings contribute to the body of evidence supporting baloxavir marboxil as a suitable alternative treatment to the neuraminidase inhibitors for influenza infection.

Our study found that even a low dose (10 mg) of baloxavir marboxil significantly shortened the TTAS and the time to resolution of fever, and produced a significantly larger change from baseline in virus titer within 1 day of treatment compared with placebo. For patients with an A/H1N1pdm infection, all doses of baloxavir marboxil had statistically significant shorter TTAS. In addition, for patients infected with influenza type B virus, all doses of baloxavir marboxil shortened the TTAS by about 20 h (note that it was likely that due to low patient numbers, this difference was not statistically significant), and 20 mg and 40 mg baloxavir marboxil produced a significantly larger change from baseline on Day 2 in virus titer compared with placebo. For patients with an A/H3N2 infection, there was no statistical difference due to limited number of patient enrollments but baloxavir marboxil also shortend the TTAS numerically compared with placebo and demonstrated significantly larger change from baseline in virus titer. These results were supported by the findings from a mouse model of influenza virus infection where baloxavir marboxil rapidly and profoundly reduced virus titer within 24 h of administration regardless of virus type/subtype (Unpublished results). We anticipate that baloxavir marboxil will be an effective oral antiviral therapy against a variety of virus types/subtypes, and that the strong antiviral effect might lead to the reduction of virus transmission. We propose that further investigations are required.

Based on PK analysis, the C_{24} of baloxavir at the 10 mg dose exceeded, by approximately 3 times, the EC_{50} in Emax models, which aligns with the clinical and virologic outcomes observed in this study. We found that the C_{24} was dependent on body weight, regardless of any differences in race/ethnicity (Koshimichi et al., 2018b). In addition, the C_{24} was 30%–40% lower in patients from Western countries compared with patients from Japan with the same body weight (Koshimichi et al., 2018b). When the difference in C_{24} due to body weight and the potential difference due to race/ethnicity difference (noted in the simulated population PK analysis) are taken into consideration, the exposure in the Western population may be reduced by up to half that of the exposure in the Japanese population. Since the body weight in Western population is heavier than that in Japanese population, the dose regimens based on weight is considered to reduce the difference of exposures between Japanese and Western population. We suggest that the estimated 10th percentile value of C_{24} for a weight-dependent dose, ie, 40 mg in patients weighing < 80 kg, or 80 mg in patients weighing ≥ 80 kg, in all populations is greater than the median C_{24} (14.8 ng/mL) for the baloxavir 10 mg dose observed in this study as shown in Fig. 5. To ensure maximal effectiveness, the single dose of 40 mg for patients weighing < 80 kg or 80 mg for patients with a body weight of ≥ 80 kg was chosen for the Phase 3 studies.

The predominant influenza virus strain during the 2015–2016 influenza season in Japan was the A/H1N1pdm subtype (49%); type B (44%; 56% of Yamagata lineage and 44% of Victoria lineage), and A/H3N2 (7%). (National Institute of Infectious Diseases, Japan, 2016). The strength of the study is that we treated a real-world seasonal influenza infection. We also confirmed infection before treatment and assessed a large range of efficacy outcomes, including the relationship of outcomes to plasma levels of the study drug. The study is limited because most patients were infected with influenza A/H1N1pdm or type B, and all were Japanese, which restricts generalizability to other

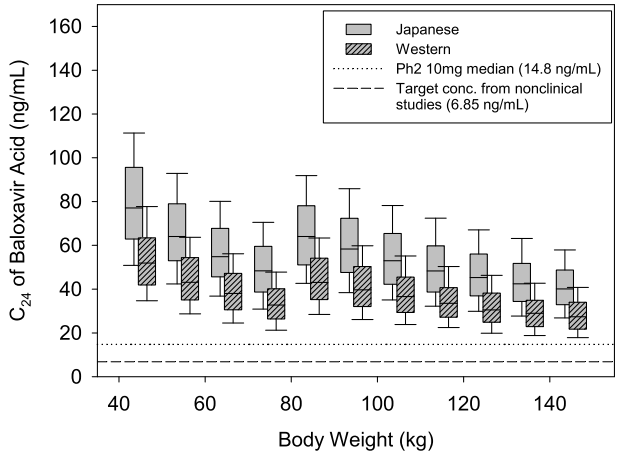

Fig. 5. Plasma concentration (C_{24}) of baloxavir acid in patients from Japan or Western Countries over a range of body weights. The 25th and 75th percentiles and the median are represented by the box plot; error bars are 10th and 90th percentiles. Abbreviation: SAD, single ascending dose study (Koshimichi et al., 2018a).

infections and populations. In addition, due to the small number of enrollments in each dose group, further clinical studies with more patient enrollment are proposed to obtain the robust clinical data in each subgroup.

5. Conclusion

We found that a single oral dose (as low as 10 mg) of baloxavir marboxil was clinically and virologically effective in adults with seasonal influenza virus infection against a range of virus types/subtypes. Taken together with the PK/PD results, a single oral dose of 40 mg (80 mg for patients with a body weight of ≥ 80 kg) baloxavir marboxil is expected to be effective in most patients over a wide range of body weights and a variety of races/ethnicities, and against a range of virus types/subtypes.

Declaration of interest

KK, TI, TS, CS, KT, and TU are employees of Shionogi & Co., Ltd. SP is an employee of Shionogi Inc. AW has received grant support and lecture fees from Shionogi, and consulting and lecture fees and grant support from other pharmaceutical companies. NH has received consulting and lecture fees from Shionogi. TI has no conflicts of interest to declare.

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Shionogi & Co., Ltd. was involved in the study design, data collection, data analysis, and preparation of the manuscript. Authors were involved in the study design and collection and interpretation of the data. KK contributed to the data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.01.012.

Glossary

ITTI	intention to treat infected
$TCID_{50}$	50% tissue culture infective dose
TEAE	treatment-emergent adverse event
TTAS	time to alleviation of influenza symptoms

References

Burnham, A.J., Baranovich, T., Govorkova, E.A., 2013. Neuraminidase inhibitors for influenza B virus infection: efficacy and resistance. Antivir. Res. 100, 520–534.

Centers for Disease Control and Prevention, 2018. Disease Burden of Influenza. https://www.cdc.gov/flu/about/disease/burden.htm, Accessed date: 1 February 2018.

Hayden, F., Sugaya, N., Hirotsu, N., et al., 2018. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. NEJM 379, 913–923.

Koshimichi, H., Ishibashi, T., Kawaguchi, N., et al., 2018a. Safety, tolerability, and pharmacokinetics of the novel anti-influenza agent baloxavir marboxil in healthy adults: Phase 1 study findings. Clin. Drug Invest. 38, 1189–1196. https://doi.org/10.1007/s40261-018-0710-9.

Koshimichi, H., Tsuda, Y., Ishibashi, T., et al., 2018b. Population pharmacokinetic and exposure-response analyses of baloxavir marboxil in adults and adolescents including influenza patients. J. Pharmaceut. Sci. https://doi.org/10.1016/j.xphs.2018.12.005.

National Institute of Infectious Diseases, Japan, Isolation/detection of influenza virus in Japan 2015/16 https://www.niid.go.jp/niid/ja/iasr-inf.html.

Noshi, T., Kitano, M., Taniguchi, K., 2018. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. Antivir. Res. 160, 109–117.

World Health Organization, 2018. Influenza (Seasonal). http://www.who.int/mediacentre/factsheets/fs211/en/, Accessed date: 1 February 2018.