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ORIGINAL ARTICLE

Pharmacokinetic and pharmacodynamic assessment of histamine H_3 receptor occupancy by enerisant: a human PET study with a novel H_3 binding ligand, [^{11}C]TASP457

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Abstract

Purpose Histamine H_3 receptor antagonists and inverse agonists have been extensively developed to treat sleep–wake, neurocognitive, and allied disorders. However, potential adverse effects, including insomnia, hampered the clinical use of these drugs, possibly due to their persistent interaction with the target molecules. The purpose of the present study was to estimate the pharmacokinetics and pharmacodynamics of enerisant, a novel antagonist and inverse agonist for histamine H_3 receptors.

Methods To measure the histamine H_3 receptor occupancy by enerisant, positron emission tomography studies using [^{11}C]TASP457, a specific radioligand for histamine H_3 receptors, were performed in 12 healthy men at baseline and at 2 h after oral administration of enerisant hydrochloride. For three of these subjects, two additional scans were performed at 6 and 26 h after the administration. Relationships between the receptor occupancy by enerisant and its dose and plasma concentrations were then analyzed.

Results Administration of enerisant hydrochloride decreased the radioligand binding in a dose-dependent manner. The estimated receptor occupancy values at 2 h varied as a function of its dose or plasma concentration. The time course of the occupancy showed persistently high levels (> 85%) in the two subjects with higher doses (25 and 12.5 mg). The occupancy was also initially high at 2 h and 6 h with the lower dose of 5 mg, but it decreased to 69.7% at 26 h.

Conclusion The target engagement of enerisant was demonstrated in the brains of living human subjects. The occupancy of histamine H_3 receptors by enerisant at 2 h can be predicted by applying the plasma concentration of enerisant to Hill's plot. The preliminary time-course investigation showed persistently high brain occupancy with high doses of enerisant despite the decreasing plasma concentration of the drug. Five milligrams or less dose would be appropriate for the treatment for narcolepsy with initially high occupancy allowing for effective treatment of narcolepsy, and then the occupancy level would be expected to decrease to a level to avoid this drug's unwanted side effect of insomnia at night, although further research is warranted to confirm the statement since the expected decrease is based on the finding in one subject.

Trial registration This study was retrospectively registered with ClinicalTrials.gov (NCT04631276) on November 17, 2020.

Keywords Histamine H_3 receptors · Receptor occupancy · Enerisant · Hysteresis · Positron emission tomography

Introduction

The histamine H_3 receptor in the central nervous system is a potential therapeutic target for neuropsychiatric disorders with impaired sleep–wake cycle and cognition [1]. Many compounds that work as antagonists and inverse agonists for histamine H_3 receptors have been extensively developed with an intent to treat these disorders. However, potential adverse effects such as cardiotoxicity, phospholipidosis, and insomnia hampered the clinical use of these

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drug candidates except for one compound, pitolisant, a histamine H_3 receptor antagonist [2]. Pitolisant has been used to treat narcolepsy and excessive daytime sleepiness, with its clinical use being approved in the European Union and the USA [3]. Insomnia at night is a potential adverse effect of pitolisant because it is a long-acting drug with a biological half-life of 11 h after an oral administration in humans, although pitolisant is known to have no other major side effects, apart from a possible relation to miscarriage [3].

The pharmacokinetics and pharmacodynamics of a drug can be studied in humans using positron emission tomography (PET), allowing for snapshot and time-course assessments of the target occupancy by the drug [4]. It was reported that at least 70% occupancy of histamine H_3 receptors by an antagonist during the day is needed to enhance wakefulness, whereas greater than 70–80% occupancy at night may result in insomnia [5]. Using PET, the receptor occupancy of pitolisant was 84% at 3 h after its oral administration at the standard dose of 40 mg [6]. The high receptor occupancy by pitolisant persisting into night times may be attributable to its slow clearance from plasma.

In the snapshot analysis at a single time point after administration of a drug, sigmoid curves can describe the relationship between the dose of the drug and its receptor occupancy. In the time-course analysis, these curves can also describe the relationship between the plasma clearance of drug concentrations and its receptor occupancy [7]. The sigmoid curve model assumes that the free concentrations of a drug in plasma and the brain are in equilibrium at all times. However, these sigmoid relationships may not necessarily hold; the drug's receptor occupancy that reflects its pharmacological effects may persist or be delayed despite a continuous fall of its plasma concentration.

Enerisant, [1-(4-{3-[(2*R*)-2-methylpyrrolidin-1-yl]propoxy}phenyl)-1H-pyrazol-4-yl](morpholin-4-yl)methanone, is a novel antagonist and inverse agonist for histamine H_3 receptors developed by Taisho Pharmaceutical Co., Ltd [8]. Enerisant has a high receptor binding affinity and potency with IC_{50} values of 0.3–3 nmol/L for human histamine H_3 receptors. Its binding selectivity to histamine H_3 receptors is more than 3,000-fold over the other histamine receptor subtypes. In rats, enerisant occupy of histamine H_3 receptors was dose-dependent, promoting cognition and wake states at low and high doses, respectively [8].

The purpose of the present study was to assess the pharmacokinetics and pharmacodynamics of enerisant. To accomplish this purpose, we performed PET experiments in two ways in healthy humans with a novel PET radioligand, [^{11}C]TASP457, that binds to H_3 receptors: first we evaluated the relationship between the oral enerisant dose and its histamine H_3 receptor occupancy at 2 h after the drug administration, when plasma drug concentrations reach a peak, and second we evaluated the plasma concentrations of enerisant and its receptor occupancy over time after administration of varying doses of enerisant. To be used in this PET study, we had developed [^{11}C]TASP457, which was found previously to bind selectively with a high affinity (in vitro $IC_{50} < 3$ nM for rats and monkeys) to histamine H_3 receptors [9]. [^{11}C]TASP457 shows a good brain penetration and fast kinetics, allowing accurate quantification of histamine H_3 receptors in humans [10]. We performed [^{11}C]TASP457 PET scans before and after oral administrations of enerisant hydrochloride to estimate its occupancy at histamine H_3 receptors. The time course of the occupancy was estimated by sequential PET measurements up to 26 h after a single oral administration of enerisant.

Method

Study design

This is an exploratory open-label uncontrolled study, in which [^{11}C]TASP457 was administered after a single oral administration of enerisant hydrochloride followed by PET experiments to evaluate enerisant's histamine H_3 receptor occupancy.

Study subjects

Twelve healthy men (age, 25.9 ± 3.9 years; body weight, 63.3 ± 6.3 kg; body mass index, 21.0 ± 1.5 kg/m^2) were recruited in the study. The inclusion criteria consisted of subjects with age (≥ 20 and < 40 years), body mass index (≥ 18.5 and < 25.0 kg/m^2), no abnormal findings in medical examination including vital signs, 12-lead electrocardiogram, and laboratory tests (hematology, blood chemistry, and urinalysis). Subjects with a history of neuropsychiatric disorders and alcohol or drug dependence were excluded. The full inclusion and exclusion criteria are reported in the supplementary material.

This study was approved by SOUSEIKAI Hakata Clinic Institutional Review Board and the Radiation Drug Safety Committee and the Institutional Review Board of the National Institute of Radiological Sciences, Japan, and was conducted in compliance with the ethical principles set forth in the Declaration of Helsinki, the standards stipulated in Article 14, Paragraph 3, and Article 80–2 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics, and Good Clinical Practice (MHW Ordinance No. 28 dated March 27,

1997). All subjects gave written informed consent prior to their inclusion in the study. This study was registered with Clinicaltrials.gov (NCT04631276).

Measurements of enerisant occupancy

We aimed at estimating the dose-occupancy relationship by measuring a wide range of receptor occupancies, guided by the information we had obtained earlier in our previous preclinical and clinical phase 1 studies (unpublished data). The dose range was set between 0.1 and 100 mg. The minimum dose of 0.1 mg was because the maximum plasma concentration after 0.1 mg dose was expected to be close to and slightly higher than the lowest detectible enerisant concentration. The maximum dose was set at 100 mg because no severe adverse event was observed with 100 mg daily doses for 7 days in the multiple-dose phase I study.

Twelve subjects received oral administration of prescribed doses of enerisant hydrochloride and were studied in two steps. First, we studied nine of 12 subjects to estimate the relationships between orally administered enerisant doses and receptor occupancy at 2 h, a time when plasma enerisant concentrations were previously shown to reach a peak after oral administration. Second, we studied three remaining subjects to evaluate the time course of the receptor occupancy at 2, 6, and 26 h after the administration of varying enerisant doses.

For the initial two subjects of the nine subjects, the dose was set at 25 mg, because, previously, 25 mg was the smallest dose that showed the plasma concentration to be over the pharmacologically effective concentration for 8 h after its administration. The doses for the remaining seven subjects were intended from 0.1 to 100 mg ensuring a wide range of occupancy.

The second step was guided by the findings from the first step for examining the relationships between plasma concentrations of enerisant and its histamine H_3 receptor occupancies over time up to 26 h. The doses for the three subjects were selected so as to yield occupancies of 90% at 2 h and 60–90% at 26 h.

Prescribed doses of enerisant were administered in tablet or liquid form (Taisho Pharmaceutical Co., Ltd., Tokyo, Japan) in the morning after overnight fasting. For doses 25 mg or more, 25 mg tablets in multiples were taken with 150 mL of water. For doses less than 25 mg, the drug substance powder (50 mg/bottle) was dissolved in purified water (50–500 mL) with the volume of administration adjusted to prescribed doses and was taken with an additional 150 mL of water.

Plasma enerisant concentration

Just before the PET experiments, blood samples (3 mL) were drawn from the antecubital vein and were subsequently sent to CMIC Pharma Science Co., Ltd for the measurement of plasma unaltered enerisant concentrations. Plasma concentrations of enerisant were determined by a validated liquid chromatography-tandem mass spectrometry method (API4000, AB SCIEX, Framingham, USA). The lower quantifiable limit was 0.0300 ng/mL.

Radiotracer synthesis

The precursor and standards for the radiosynthesis of [^{11}C]TASP457 used in this study were prepared as described elsewhere [9–11]. [^{11}C]TASP457 was radiosynthesized by O-alkylation of the 2-pyridone-containing precursor (desmethyl TASP457) with [^{11}C]methyl triflate [9]. At the time of administration, the radiochemical purity of [^{11}C]TASP457 was > 95%, and its molar activity was > 37 GBq/μmol.

PET procedures

PET studies were performed initially to establish baseline data without enerisant on board and then 2 h after administration of enerisant for all subjects. These two scans were performed on separate days with an average of 23 ± 9 days apart (range: 14–42 days). For the subjects in the second step, two additional PET studies were performed at 6 h and 26 h after the administration of enerisant. For each PET study, three-dimensional dynamic images were acquired with a PET camera (Eminence SET-3000GCT/X, Shimadzu, Kyoto, Japan) for 90 min in 33 frames of increasing scan duration (from 10 s to 5 min) after intravenous injection of [^{11}C]TASP457 (382 ± 10.3 MBq equivalent to 7.3 ± 3.3 nmol). All PET images were reconstructed using the filtered back projection method with a Gaussian filter with a kernel of 5 mm corrected for attenuation, randoms, scatter, and head motion. The reconstructed in-plane resolution was 7.5 mm in full width at half maximum, and voxel size was $2 \times 2 \times 2.6$ mm.

Plasma input functions were acquired by arterial blood sampling and radiometabolite analyses. Arterial blood samples were taken manually 33 times after injection of radioligand (at 10-s intervals up to 120 s; at 30-s intervals up to 3 min; at 1-min intervals up to 10 min; at 12, 15, 20, 25, and 30 min; and at 10-min intervals up to 90 min). Each blood sample was centrifuged to obtain plasma fractions, and the concentrations of radioactivity in whole blood and plasma were measured. The plasma-free fraction was measured by ultrafiltration (Centrifree, Merck Millipore, Billerica, MA).

The fractions of the parent radioligand and its radiometabolites in plasma were determined from seven samples

in each subject (at 3, 10, 20, 30, 50, 70, and 90 min). The supernatant of the centrifuged samples was subjected to high-performance liquid chromatography analyses (CAPCELL PAK C18: 5 µm, 10×150 mm, column; Shiseido Co. Ltd., Tokyo, Japan). Acetonitrile (90%; A) and phosphoric acid (0.01 M; B) were used for mobile phases (35/65 A/B) at a flow rate of 6.0 mL/min. Hill functions were used to interpolate the fraction of the parent to obtain arterial input functions.

PET data analysis

Time-activity curves were generated from data extracted from PET images. T1-weighted MR images acquired with a 3-T MRI scanner (Verio, Siemens, Germany; TE 1.9 ms, TR 2,300 ms, TI 900 ms, flip angle 9°, field of view 250 mm, acquisition matrix 256×256, slice thickness 1 mm) were coregistered to the corresponding PET images and used for their spatial normalization. All PET images were spatially normalized to a standard anatomic orientation on the basis of transformation parameters from the coregistered M.R. images. This transformation parameters were estimated using the 3 probability maps normalization function, which was developed in SPM8 [12] and implemented in PMOD (PMOD Technologies Ltd., Zurich, Switzerland). A template of preset volumes of interest was applied to the spatially normalized PET images to extract time-activity curves for the following 15 ROIs: the frontal, cingulate, parietal, occipital, and temporal cortices, hippocampus, amygdala, caudate, putamen, pallidum, thalamus, hypothalamus, substantia nigra, cerebellum, and pons. All image and kinetic analyses were performed in PMOD Audit Trail License version 3.6 (PMOD Technologies Ltd., Zurich, Switzerland). Regional total distribution volumes (V_T) [13] were estimated using Ichise's multilinear analysis (MA1) [14] with t^* of 30 min applied on the regional time-activity curves and arterial input function. Three exponential functions were used to interpolate the arterial input functions before applying them to the multilinear analysis.

Plasma concentration and receptor occupancy

The histamine H_3 receptor occupancy by enerisant was determined for each post-dose PET study using Lassen plot with the following equation [15]:

$$V_T^{\text{Baseline}} - V_T^{\text{enerisant}} = \text{Occ} \cdot (V_T^{\text{Baseline}} - V_{ND}) \quad (1)$$

where V_T^{Baseline} and $V_T^{\text{enerisant}}$ are the regional V_T. values at baseline and at 2 h, 6 h, or 26 h after administration of enerisant, respectively, V_{ND} is the non-displaceable distribution volume, and Occ is the occupancy. V_T values from all regions were included in the Lassen plots to estimate the histamine H_3 receptor occupancies.

The relationships between the plasma concentration or orally administered dose of enerisant and its receptor occupancy at 2 h were evaluated based on the following Hill equation [16]:

$$\text{Occ} = \text{Occ}_{\max} \cdot C^{\gamma} / (EC_{50}{}^{\gamma} + C^{\gamma}) \quad (2)$$

$$\text{Occ} = \text{Occ}_{\max} \cdot C^{\gamma} / (IC_{50}{}^{\gamma} + C^{\gamma}) \quad (3)$$

where $\text{Occ}_{\max}$ is the maximum occupancy, C is the dose of enerisant in Eq. 2 and plasma concentration of enerisant in Eq. 3, EC_{50} in Eq. 2 and IC_{50} in Eq. 3 are the C-values inducing 50% occupancy, and γ is the shape factor. We included the shape factor in the model, because we did not have a priori knowledge about enerisant if it follows the standard E-max model, the shape factor being 1 if it follows the model [17]. We fitted the data to this sigmoid model to simultaneously estimate EC_{50} or IC_{50} and γ with and without a constraint of $\text{Occ}_{\max}$ to 100%. Akaike information criterion [18] was used to determine which of the two (constraint or no constraint of $\text{Occ}_{\max}$) methods are more appropriate in estimating the occupancy and its 95% confidence interval.

Results

Enerisant decreased radioligand binding to H_3 receptors in a dose-dependent manner as assessed by PET scans initiated at 2 h after the drug administration (Fig. 1). The effect of oral administration of enerisant on [^{11}C]TASP457 time activity was to increase its rate of washout in all brain regions, fastest with 25 mg and slowest with 0.1 mg. Kinetic model-based MA1 analysis showed that enerisant decreased V_T values in all brain regions including the cerebellum and pons from those of the baseline, with their magnitudes of V_T decreases dependent on the dosage (Table 1). Estimation of V_T, receptor occupancy, and V_{ND} were all stable with positive linear relationships between the baseline V_T, and the magnitudes of enerisant-induced V_T decrease, as seen in the Lassen plots (Fig. 2). The higher the receptor occupancy at 2 h with enerisant administration, the higher both the plasma concentrations and doses of the drug (Table 2). The estimated V_{ND} was 3.3 ± 0.9 mL/cm^3. The model equation with or without fixed maximum occupancy well described the relationships between the dose of enerisant and its receptor occupancy at 2 h after administration (Fig. 3a) and between the plasma concentration of enerisant and its occupancy at 2 h (Fig. 3b). However, the model equation without fixed maximum occupancy yielded better fits to these plots with smaller AIC than did the model with the occupancy fixed at 100% (77.2 vs. 81.6 for the dose-occupancy relationship

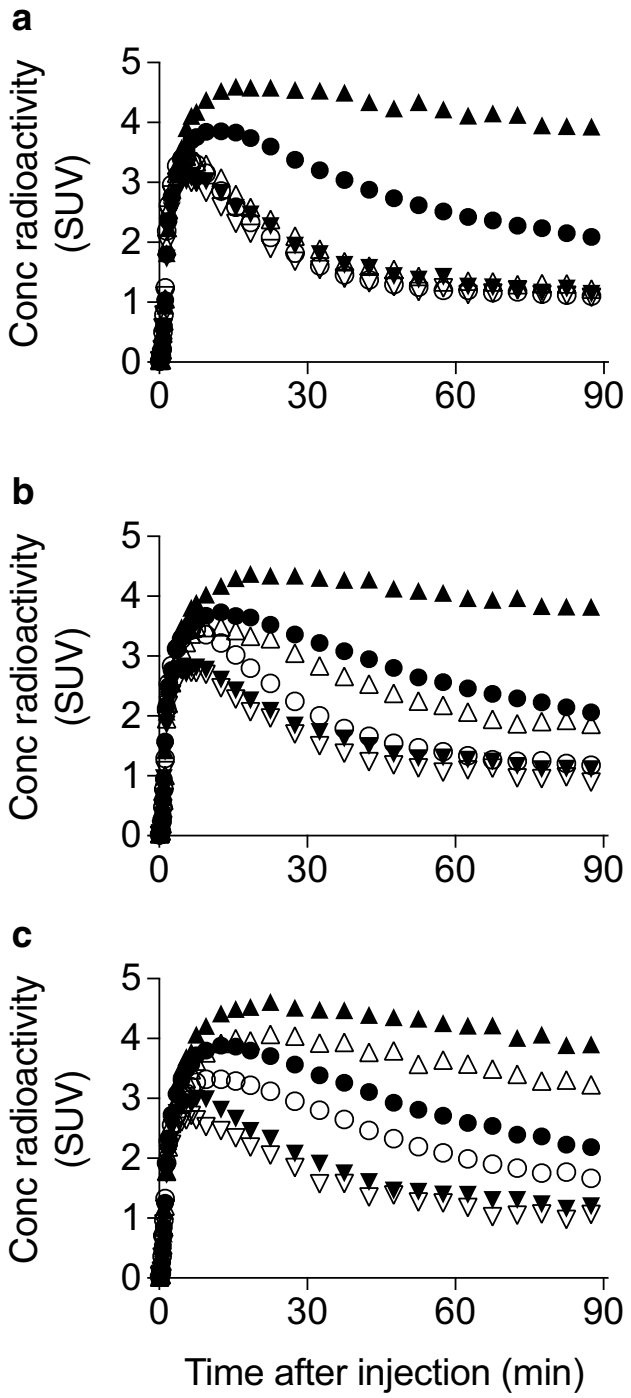

Fig. 1 Time-activity curves of [^{11}C]TASP457 PET in the brain regions at baseline (filled symbols) and 2 h after an enerisant administration (open symbols). The rates of washout were increased dose-dependently after oral administration 25 mg (**a**), 1 mg (**b**), and 0.1 mg (**c**) enerisant in the pallidum (up-triangle), cingulate cortex (circle), and pons (down-triangle)

Table 1 V_T values in brain regions at baseline and 2 h after an enerisant administration

Regions	V_T (mL/cm^3)/enerisant dose (mg)			
	0	0.1	1	25
Pallidum	16.56 ± 1.85	13.32 ± 1.24	6.52 ± 0.06	4.46 ± 0.51
Putamen	13.93 ± 1.22	11.05 ± 0.79	5.70 ± 0.02	4.25 ± 0.51
Hypothalamus	10.56 ± 1.22	7.94 ± 1.39	4.55 ± 0.14	3.93 ± 0.21
Substantia nigra	10.37 ± 1.13	8.02 ± 0.32	5.40 ± 0.29	4.17 ± 0.36
Caudate	10.33 ± 1.34	8.23 ± 1.52	3.97 ± 0.35	3.71 ± 0.27
Amygdala	9.87 ± 1.13	7.52 ± 0.04	4.97 ± 0.08	4.24 ± 0.41
Cingulate cortex	8.74 ± 0.86	6.93 ± 0.60	4.53 ± 0.02	3.96 ± 0.39
Thalamus	7.60 ± 0.57	6.18 ± 0.38	4.39 ± 0.07	4.17 ± 0.25
Hippocampus	7.50 ± 0.61	6.26 ± 0.10	4.71 ± 0.07	4.28 ± 0.35
Temporal cortex	6.58 ± 0.54	5.43 ± 0.16	3.96 ± 0.08	3.61 ± 0.34
Frontal cortex	6.45 ± 0.62	5.05 ± 0.38	3.66 ± 0.01	3.41 ± 0.26
Cerebellum	6.36 ± 0.47	5.15 ± 0.32	3.88 ± 0.12	3.59 ± 0.35
Occipital cortex	6.02 ± 0.44	4.96 ± 0.18	3.82 ± 0.01	3.59 ± 0.34
Parietal cortex	5.81 ± 0.45	4.61 ± 0.22	3.58 ± 0.04	3.37 ± 0.21
Pons	4.79 ± 0.47	4.26 ± 0.15	3.63 ± 0.20	3.82 ± 0.28

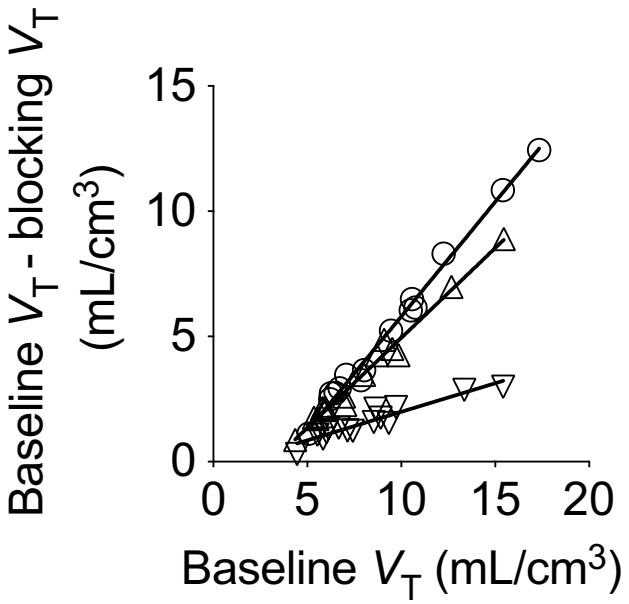

Fig. 2 Lassen plots showing positive linear relationships between the baseline V_T and the magnitudes of V_T decreases with enerisant administration. The largest slope was shown with 25 mg enerisant (open circle), followed in order by 1 mg (open up-triangle) and 0.1 mg (open down-triangle) enerisant

and 78.2 vs. 81.4 for the concentration-occupancy relationship, respectively). The estimated ED_{50} and plasma IC_{50} of enerisant were 0.33 mg and 0.93 ng/mL, respectively, with 93% of the maximum occupancy and 1.13 and 1.03 of the shape factors, respectively.

Table 2 Enerisant dose, occupancy, and enerisant plasma concentration

Step	Enerisant dose (mg)	Time after administration (h)	Occupancy (%)	Enerisant plasma conc (ng/mL)
1 ($n=9$)	25	2	91.4	113.0
	25	2	90.6	92.2
	1	2	71.8	3.5
	1	2	74.7	2.6
	0.1	2	22.8	0.25
	0.1	2	23.8	0.24
	0.2	2	21.9	0.54
	2.5	2	80.4	6.4
	0.4	2	56.0	1.2
2 ($n=3$)	12.5	2	90.1	31.5
		6	88.5	39.6
		26	85.6	11.0
	5	2	90.1	21.8
		6	87.9	13.9
		26	69.7	2.3
	25	2	94.0	53.0
		6	91.4	81.9
		26	89.5	19.9

Three subjects who took 25, 12.5, and 5 mg of enerisant were investigated up to 26 h after the single oral administration. Enerisant plasma concentration peaked at 6 h for the two subjects with higher doses (25 and 12.5 mg) and at 2 h for the subject with the lower dose (5 mg) (Fig. 4a, Table 2). The plasma concentrations at 26 h decreased from the peak but still higher than 10 ng/mL for the two subjects with higher doses and considerably lower at 2.3 ng/mL for the 5 mg subject (Fig. 4a, Table 2). On the other hand, the occupancy was persistently high (more than 85%) in the two subjects with higher doses (Fig. 4b, Table 2). The subject with 5 mg dose also showed high occupancy at 2 h and 6 h, but the occupancy decreased to 69.7% at 26 h.

Of the 12 subjects who received administration of enerisant hydrochloride, one subject had a mild adverse event. The subject was given 25 mg of enerisant and then developed hiccups for 3 h from 2 h after the administration. The subject recovered after drinking water and taking a rest. It was unclear whether this event was directly linked to enerisant although such a possibility could not be totally excluded. There were no other significant adverse events.

Discussion

In this study, we examined histamine H_3 receptor occupancy by enerisant at 2, 6, and 26 h after single oral administration of this investigational drug in healthy men. PET analyses with [^{11}C]TASP457 PET has shown that enerisant acts on the target, H_3 receptors in the human brain, and quantification of its receptor occupancy at different doses and times after administration is feasible. The occupancy at 2 h can be described as a function of the plasma concentration of unchanged enerisant. The time courses of the occupancy indicate that persistently high brain occupancy with high doses of enerisant despite the decreasing plasma concentration of the drug. However, 5 mg or less dose could be used to treat narcolepsy with the occupancy effectively high for several hours after administration and decreased thereafter, which may prevent insomnia at night. Further research is warranted to confirm the optimal dose since the expected decrease is based on the finding in one subject.

Enerisant dose-dependently decreased the radioligand V_T, reflecting that enerisant competes with [^{11}C]TSAP457 for specific binding to histamine H_3 receptors in the brains of living human subjects. This specific binding of enerisant to histamine H_3 receptors was in fact shown by in vitro binding assays with membranes of cells expressing human recombinant histamine H_3 receptors and membrane fractions of the rat cerebral cortex [8]. The affinities of enerisant for human and rat histamine H_3 receptors as assessed by IC_{50} values were 2.9 nmol/L and 14.5 nmol/L, respectively. Later, binding of enerisant to the central H_3 receptors was confirmed in living rats by an ex vivo analysis using a tritium-labeled radioligand for H_3 receptors, [^{3}H]N-α-methylhistamine, with ED_{50} determined as 0.78 mg/kg per oral administration of enerisant hydrochloride. Despite the lack of plasma enerisant concentration data in this rat study, a linear regression of the relationship between ED_{50} and IC_{50} analyzed in a separate experiment with and without a constraint to the origin led to the calculation of IC_{50} as 61 and 2.2 ng/mL, respectively. In the current study, the $ED50$ and plasma $IC50$ in humans were 0.33 mg per oral administration and 0.93 ng/mL, respectively. Accordingly, the dose and plasma concentration of enerisant required for an effective occupancy of histamine H_3 receptors in the human brain is lower than the dose predicted from the rat studies. This high clinical versus nonclinical drug potency may be due to species differences in the brain permeability and histamine H_3 receptor binding affinity of enerisant, requiring clarification by further pharmacokinetic investigations.

We investigated the time course of occupancy in three doses and one subject per dose up to 26 h after oral administration of enerisant. As only one time point of one subject showed decreased both plasma level and occupancy, our data is not sufficient to investigate the relationship between plasma concentrations of enerisant and its receptor occupancy over time after administration of varying doses of enerisant. Nevertheless, our data indicates

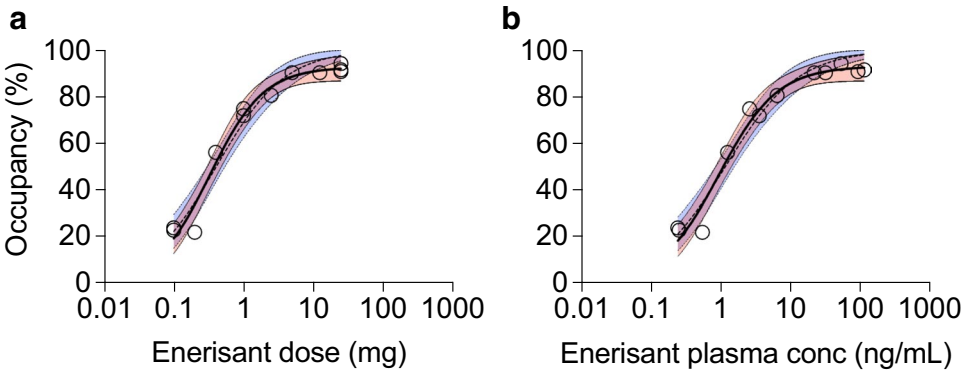

Fig. 3 Sigmoid relationships between enerisant dose and occupancy (**a**) and between the plasma concentration and occupancy (**b**) at 2 h after administration. Solid lines were from model equation without fixed maximum occupancy and dotted lines were from those with the occupancy fixed at 100%. 95% confidence interval areas were indicated in red and blue for those model estimations, respectively

persistently high brain occupancy with high doses of enerisant despite the decreasing plasma concentration of the drug. As 5 mg dose showed initially high occupancy at 2 h and 6 h, but the occupancy decreased to around 70%, 5 mg or less dose, such as 1–2.5 mg, would avoid unwanted side effect of insomnia at night. These doses showed initially a high occupancy to be potentially effective for narcolepsy (at 2 h, 72–75% for 1 mg and 80.4% for 2.5 mg, Table 2), while occupancy level should be less than 70% occupancy several hours later, although appropriate doses should be determined by taking duration of effective occupancy into consideration.

The present work has also shown that the histamine H_3 receptor occupancy of H_3 antagonist drugs can be evaluated by PET with [^{11}C]TSAP457, a novel PET ligand developed to be used in the current study. The inter-subject variability of the radioligand V_T was small, with a coefficient of variation around 10% or less in most of the regions with or without enerisant dosing. The receptor occupancy was also robustly estimated from the V_T values, with less than a 3% coefficient of variation among subjects. Although the test–retest variability has not yet been determined in this study, it is likely to be low based on the low between-subject variability (Table 1).

To quantify the histamine H_3 receptor density in the human brain, no reference region devoid of the receptor appears to be available. A previous occupancy study used the pons as a reference region for measuring the histamine H_3 receptor density by [^{11}C]MK-8278-PET [19]. In the current study, V_T values of [^{11}C]TASP457 in the pons decreased dose-dependently from 4.8 at baseline to 3.8 mL/cm^3 after 25 mg enerisant administration. The V_{ND} for [^{11}C]TASP457 estimated using Lassen plot was 3.3 mL/cm^3, which was much lower than V_T in the pons at baseline. Thus, the pons could not be employed as a reference region for the quantification of [^{11}C]TASP457 binding and receptor occupancy estimations, necessitating the implementation of a plasma input function for kinetic quantitative analyses.

One of the limitations of this study is that we have only investigated men due to limited volunteer availability. However, further study including both men and women will be needed to generalize our results. Second, only three subjects with three doses of enerisant have been investigated for the time course of the occupancy. A future study with a

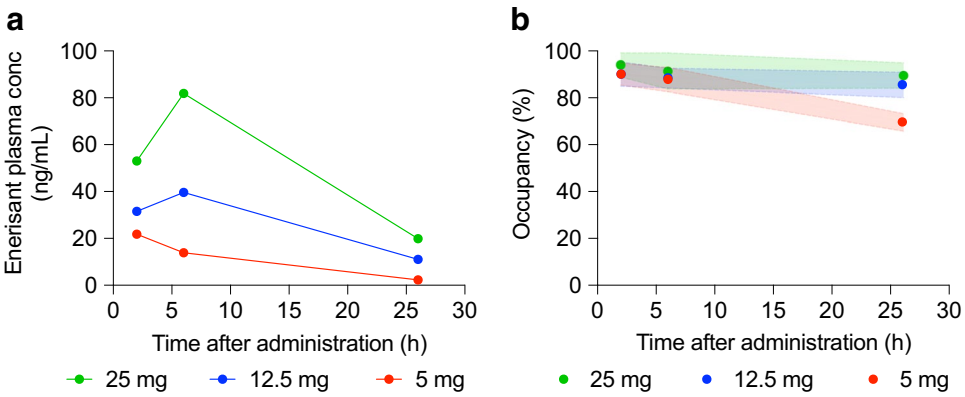

Fig. 4 Time course of enerisant concentration in plasma (**a**) and histamine H_3 receptor occupancy (**b**) with varying doses of enerisant (green, 25 mg; blue, 12.5 mg; red, 5 mg). 95% confidence intervals of occupancy estimation with the Lassen plots are indicated as the areas between the dotted lines in the corresponding color

greater number of subjects with lower doses of enerisant is warranted.

In conclusion, the pharmacokinetics and pharmacodynamics of an investigational new H_3 inverse agonist, enerisant, can be evaluated in humans by PET with our novel ligand [^{11}C]TASP457. The results of this study show that central H_3 receptor occupancy of enerisant at 2 h after its oral administration can be predicted from plasma concentrations of the drug. Time course of occupancy indicates that 5 mg or less doses would be appropriate for the treatment of narcolepsy with an initially high occupancy allowing for effective treatment of narcolepsy, which would decrease to a safe level to avoid this drug's unwanted side effect of insomnia at night. However, these findings are preliminary at present, and further studies appear warranted to confirm and expand on our current findings with larger samples of subjects, doses, and measurement time points including those over 26 h.

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Author contribution YK, TSh, MH, IN, and TSu contributed to the conception and design of this research. YK, KT, SK, CS, and YI participated in data acquisition and preparation. KK and MRZ were in charge of radioligand syntheses and metabolite analyses. YK, TSh, MI, MH, IN, and TSu undertook the analysis and interpretation of imaging data. YK and MI wrote the draft manuscript and managed the literature searches. All authors have made substantial intellectual contribution to the work and approved the final manuscript.

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Availability of data and material The datasets generated and analyzed on the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by SOUSEIKAI Hakata Clinic Institutional Review Board and the Radiation Drug Safety Committee and the Institutional Review Board of the National Institute of Radiological Sciences, Japan, and was conducted in compliance with the ethical principles set forth in the Declaration of Helsinki, the standards stipulated in Article 14, Paragraph 3, and Article 80–2 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics, and Good Clinical Practice (MHW Ordinance No. 28 dated March 27, 1997).

Consent to participate All subjects gave written informed consent prior to their inclusion in the study.

Consent for publication Written informed consent was obtained from all participants regarding publishing their data.

Conflict of interest Taisho Pharmaceutical Co., Ltd., MH, and TSu hold a patent for [^{11}C]TASP457 and related chemicals as histamine H_3 ligands (Japan patent JP2014047209A). TSh and IN are employees of Taisho Pharmaceutical Co., Ltd.

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