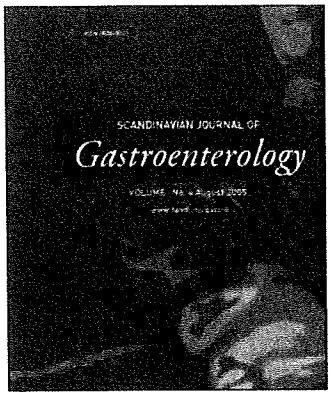

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ORIGINAL ARTICLE

A randomized, double-blind, placebo-controlled clinical trial of the effectiveness of the novel serotonin type 3 receptor antagonist ramosetron in both male and female Japanese patients with diarrhea-predominant irritable bowel syndrome

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Abstract

Objective. Irritable bowel syndrome is characterized by abdominal discomfort and/or pain associated with altered bowel habits. The neurotransmitter serotonin and serotonin type 3 receptors that are extensively distributed on enteric neurons in the human gastrointestinal tract play a role in increasing the sensation of pain and affecting bowel habits in patients with irritable bowel syndrome. The aim of this study was to evaluate the efficacy and safety of the serotonin type 3 receptor antagonist ramosetron hydrochloride in Japanese patients with diarrhea-predominant irritable bowel syndrome. ***Material and methods.*** In a double-blind, placebo-controlled, parallel group-comparative study with a 1-week run-in period, 539 patients with diarrhea-predominant irritable bowel syndrome meeting the Rome II diagnostic criteria received either 5 μg ramosetron hydrochloride ($n=270$) or placebo ($n=269$) once daily for 12 weeks. ***Results.*** Forty-seven percent of ramosetron hydrochloride-treated patients were monthly responders in the primary end-point, "Patient-reported global assessment of relief of irritable bowel syndrome symptoms", compared with 27% for placebos ($p<0.001$). The most frequently reported adverse event in the ramosetron hydrochloride-treated group compared with the placebo group was hard stool. ***Conclusions.*** Ramosetron hydrochloride 5 μg once daily is effective and well tolerated in the treatment of abdominal pain, discomfort and bowel habits in patients with diarrhea-predominant irritable bowel syndrome.

Key Words: *5-HT_3 receptor antagonist, irritable bowel syndrome, ramosetron, randomized controlled study*

Introduction

Irritable bowel syndrome (IBS) is a functional disease with persisting gastrointestinal symptoms, mainly abdominal discomfort and/or pain with abnormal bowel habits, not accompanied by organic diseases. IBS is an extremely common functional bowel disorder with an estimated prevalence of approximately 10–15% in the general population, and the annual incidence is probably 1–2% [1]. Kumano et al. reported that they found a 6.1% prevalence of IBS in total, with 7.8% in females and 4.5% in males, in a representative Japanese sample; these figures are similar to those reported in Western industrialized countries, when diagnosed using the Rome II criteria [2].

Although IBS is not a fatal disease, patients with IBS are forced to limit their behavior depending upon the extent of the symptoms, their social activity is restricted, and the health-related quality of life of IBS patients has been reported to be impaired to a level comparable with that of patients who have dialysis-dependent end-stage renal disease [3].

The mechanisms underlying the pathophysiology of IBS have not yet been fully elucidated [4]. Various psychogenic stresses have been considered to be associated with the occurrence of IBS and its symptoms. Such stress is considered to cause excitement of

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the descending nerve via a release of corticotropin-releasing hormone (CRH) and to cause abnormality of motility of the digestive tract and a lowering of the threshold of perception of the digestive tract via a transmitter such as serotonin (5-HT) [5,6]. In recent years, there has been increasing interest in the possible involvement of 5-HT in this syndrome. Serotonin type 3 (5-HT_3) receptors have been identified on the sensory neurons of the gut, and they mediate reflexes that control gastrointestinal motility and secretion, bowel function and the perception of pain [7]. In patients with IBS, 5-HT_3 receptor antagonists increase colonic compliance, slow colonic transit, improve stool consistency and are valuable therapeutic compounds for treatment [8,9].

Clinical studies with the selective 5-HT_3 receptor antagonist alosetron hydrochloride (alosetron) have shown that women with diarrhea-predominant IBS (D-IBS) taking 1 mg alosetron twice daily had significantly greater adequate relief of IBS pain and discomfort and significant improvement in bowel urgency, stool form and stool frequency compared to subjects taking a placebo [10–12]. Alosetron also provided moderate-to-substantial IBS global symptom improvement in a significantly greater proportion of women with D-IBS than the placebo [13]. A comparison study with the smooth muscle relaxant mebeverine hydrochloride showed that alosetron provided greater relief of pain and of some IBS bowel abnormalities [14]. However, alosetron is indicated only for women with severe, chronic D-IBS in whom conventional therapy has failed since serious gastrointestinal events, some fatal, including ischemic colitis and bowel motor dysfunction, have been reported with alosetron use.

Ramosetron hydrochloride (Ramosetron), a tetrahydrobenzimidazole derivative, is a potent and selective 5-HT_3 receptor antagonist, and since 1996 has already been on the market in Japan and some other Asian countries as an antiemetic for cancer patients in their chemotherapy [15,16]. In preclinical studies with rats, ramosetron dose-dependently suppressed defecation disturbance induced by the stress from restraint [17]. Ramosetron also dose-dependently suppressed accelerated defecation induced by CRH, which is believed to be associated with stress-related gastrointestinal dysfunction [18]. Moreover, ramosetron significantly increased the threshold of colonic pain induced by colonic distension [19]. Recently, the efficacy and safety of ramosetron in the treatment of patients with D-IBS meeting the Rome II diagnostic criteria has been evaluated in a multicenter, double-blind, dose-ranging study in Japan comparing three doses of ramosetron (1 μg, 5 μg and 10 μg once daily, orally) with a placebo [20]. It has been shown that both 5 μg and 10 μg ramosetron were effective doses for improving overall IBS symptoms, abdominal pain, discomfort and abnormal bowel habits. Neither ischemic colitis nor severe constipation, both of which were reported with alosetron use, was observed in treatment with ramosetron.

The present, double-blind, placebo-controlled, parallel group-comparative, phase III study was conducted to verify the superiority of ramosetron, 5 μg once daily, to a placebo and to evaluate its safety in Japanese patients with D-IBS.

Material and methods

Patients

The study was conducted from August 2004 to July 2005 at multiple centers in Japan. The study protocol was designed in accordance with the Declaration of Helsinki and approved by institutional review boards at all sites. Written, informed consent was obtained from all patients before they entered the study.

Patients were eligible if aged 20–64 years and diagnosed with D-IBS (at least 3 months of D-IBS symptoms as defined by the Rome II criteria). Organic diseases were ruled out by a sigmoidoscopic examination or barium enema in patients under 49 years of age and by colonoscopic examination or barium enema in patients over 50 years of age, performed after the onset of symptoms and within the last 5 years.

Patients were excluded if they had a history of surgical resection of the stomach, or intestine (excluding appendicitis or resection of benign polyps), if they had a history of or complication from inflammatory bowel disease, ischemic colitis or malignant tumors, if they had complications from infectious enteritis, hyperthyroidism or hypothyroidism, if they used drugs that could potentially affect the evaluation of efficacy of the study drug (patients who could have a washout period of ≥ 3 days before the start of the run-in period were acceptable), if they had a history of drug or alcohol abuse within the past year or were currently abusing them, if they were allergic to drugs, had severe depression or an anxiety disorder, if they had complications from a serious cardiovascular disease, respiratory disease, renal disease, hepatic disease, gastrointestinal disease (excluding IBS), hematological disease or neurological or psychiatric disease, or if investigational products had been administered within 6 months prior to the start of this study. Female patients who were pregnant, or who were lactating were also excluded.

Study design

During the 1-week run-in period, data on abnormal bowel habits and abdominal discomfort and/or pain were collected to ensure that patients had suitable symptom levels at the start of the study. Severity of abdominal discomfort and/or pain was assessed daily on a 5-point scale (0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Intolerable). Average daily scores of abdominal discomfort and/or pain during the run-in period were required to be ≥ 0.7 for patients to enter the treatment phase. Stool form (appearance) and stool frequency data were also monitored daily. Stool form data were scored on a 7-point ordinal scale according to the Bristol Stool Form Scale. Absence of stool was assigned a value of 0. Patients whose stool form was not type 1 or 2 and whose stool frequency was ≥ 3 times/week during the run-in period were enrolled, to exclude patients with predominant constipation.

Following the 1-week run-in period, eligible patients were randomly assigned 12 weeks of oral treatment with either placebo or ramosetron 5 μg tablets once daily taken before breakfast. Outpatient visits were scheduled at 2, 4, 8, and 12 weeks (or final treatment) to assess drug compliance and occurrence of adverse events.

Data collection

During the run-in period and treatment phase, patients recorded daily their IBS symptoms (severity of abdominal discomfort and/or pain, stool form, stool frequency, bowel urgency and feeling of incomplete bowel movement) on paper diary cards. Once every 7 days during the treatment phase, patients also provided weekly assessments of relief of overall IBS symptoms, abdominal discomfort and/or pain and abnormal bowel habits compared to the way they felt during the run-in period. At the same time, data input by telephone was employed to remind patients to enter data on a daily basis, or to ascertain whether the patient was experiencing a problem.

The primary end-point in the study was the monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms". A monthly responder was defined as a patient who had experienced "Completely relieved" or "Considerably relieved" for at least 2 weeks of the 4-week treatment (0: Completely relieved, 1: Considerably relieved, 2: Somewhat relieved, 3: Unchanged, 4: Worsened). The primary efficacy end-point was defined by the responder rate of the last 4 weeks of the treatment phase.

Secondary end-points included the "Patient-reported assessment of relief of abdominal discomfort and/or pain", the "Patient-reported assessment of improvement of abnormal bowel habits", and assessment of IBS symptoms (severity of abdominal discomfort and/or pain, stool form, stool frequency, bowel urgency and feeling of incomplete bowel movement).

A monthly responder for the "Patient-reported assessment of relief of abdominal discomfort and/or pain" was defined as above equally as "Patient-reported global assessment of relief of IBS symptoms". For "Patient-reported assessment of improvement of abnormal bowel habits", a monthly responder was defined as a patient who was "Nearly normalized" or "Considerably relieved" for at least 2 weeks of the 4-week treatment (0: Nearly normalized, 1: Considerably relieved, 2: Somewhat relieved, 3: Unchanged, 4: Worsened).

Statistical analysis

A sample size was calculated that would provide 90% power, with a two-sided level of significance of 0.05, to detect a difference of 15% in the primary end-point between the two groups of 27% for the placebo group and 42% for the ramosetron group. A total of 460 patients (230 patients per group) or more was planned to be randomized in the study, assuming a dropout rate of a few percent.

Efficacy analyses were conducted on the full analysis set (FAS). The FAS would consist of all randomized patients who received at least one dose of study medication and had at least one post-baseline efficacy measurement.

The primary end-point was the responder rate of "Patient-reported global assessment of relief of IBS symptoms", which was compared between treatment groups by means of the χ^2 test with a two-sided level of significance of 0.05. For secondary analysis, the primary end-point was analyzed, using the χ^2 test with gender as the stratification factor.

The other monthly responder rate parameters were analyzed similarly to the primary end-point. The weekly responder variables and the continuous variables (e.g. change in average daily scores from the baseline) were summarized at each week of treatment. The average daily scores were calculated for each subsequent week of study medication. If more than two daily scores were missing during any week of study medication, the average score for that week was also defined as missing.

Results

Study population and demographics

Five hundred thirty-nine of 676 subjects who provided written, informed consent to participate

in the study were enrolled in the treatment phase. A flowchart of patient progression through the study is presented in Figure 1. Of those who dropped out of the study before or during the run-in period, 48 did not meet the inclusion criteria, 3 withdrew consent, 1 had adverse events and 85 were excluded for other reasons.

A total of 229 (85%) of 270 and 223 (83%) of 269 of 539 randomized patients in the ramosetron and placebo groups, respectively, completed the study. Forty-one patients prematurely discontinued treatment in the ramosetron group and 46 in the placebo-treated group. Reasons for premature discontinuation from the study are indicated in Figure 1.

The demographic and baseline characteristics data for randomized patients were comparable for both treatments (Table I). Patients were predominantly male and in their 30–40s. Male patients seemed to have had IBS for a longer time than female patients.

Efficacy

Primary efficacy evaluation: patient-reported global assessment of relief of IBS symptoms. Forty-seven percent of ramosetron-treated patients were monthly responders at the final point (last 4 weeks) compared with 27% for placebo (treatment difference of 20 percentage points; $p<0.001$; Figure 2). The monthly responder rates at each month were also significantly higher in the ramosetron group than in the placebo group.

We further assessed weekly response rates to evaluate onset and sustainability of response (Figure 3). Patients assessed IBS symptoms every 7 days. According to their global assessment scores, patients who had complete or considerable relief were defined as weekly responders in that particular week. Ramosetron provided greater weekly response rates than placebo. Improvement was achieved by the first week of treatment and was sustained throughout the 12 weeks of treatment.

Figure 4 presents the monthly responder rates, stratified by gender, of "Patient-reported global assessment of relief of IBS symptoms". In male patients, monthly responder rates were significantly higher in the ramosetron group than in the placebo group at all time-points ($p<0.001$ for all points). Although the proportion of monthly responder rates in female patients was also higher in the ramosetron group compared with the placebo group at each time-point, a significant effect was only observed at month 2 ($p=0.031$). The magnitude of efficacy was comparable to or higher in female patients relative to male patients.

Secondary efficacy evaluation: bowel-related functions. A significantly greater proportion of patients treated

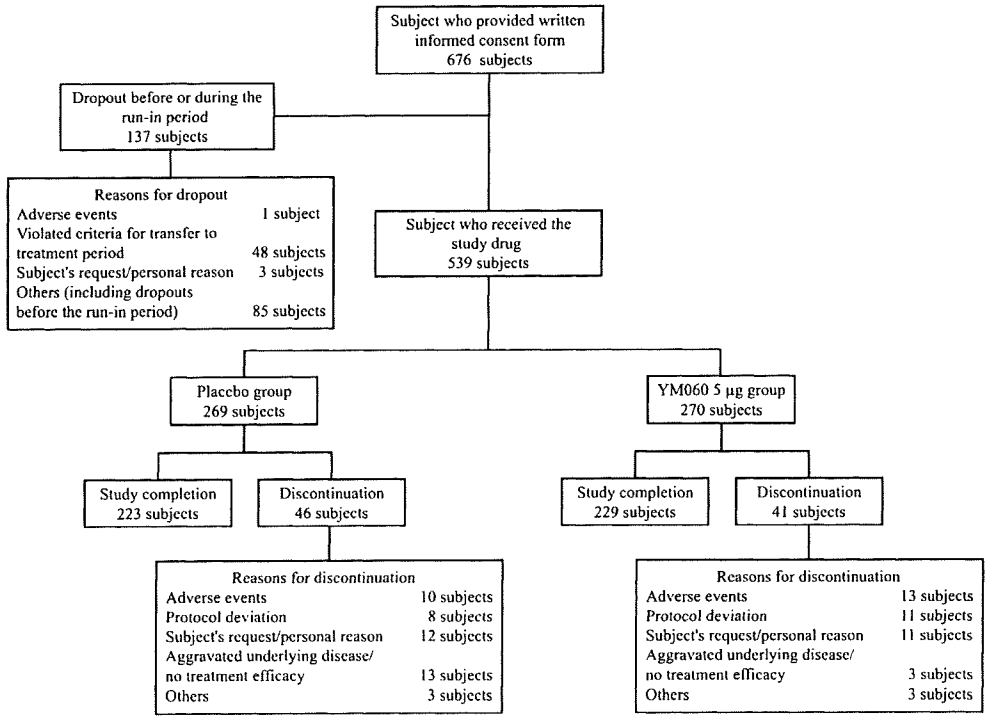

Figure 1. Flowchart of patient progression through the study. Reasons for dropouts and discontinuation from the study are indicated.

Table I. Demographics and baseline characteristics of participants.

Patient background			No. of subjects	Mean	SD	*t*-test
Age (years)	Total	Placebo	268	41.8	11.70	$t=1.089$, df $=535$
		5 μg	269	40.7	11.21	$p=0.276$
	Male	Placebo	226	41.9	11.38	$t=0.045$, df $=439$
		5 μg	215	41.8	10.84	$p=0.964$
	Female	Placebo	42	41.1	13.42	$t=1.958$, df $=94$
		5 μg	54	36.1	11.58	$p=0.053$
Duration of disease (months)	Total	Placebo	261	149.6	131.25	$t=0.337$, df $=522$
		5 μg	263	145.8	130.70	$p=0.736$
	Male	Placebo	219	155.4	128.76	$t=-0.207$, df $=427$
		5 μg	210	158.0	134.67	$p=0.836$
	Female	Placebo	42	119.7	141.41	$t=0.901$, df $=93$
		5 μg	53	97.3	100.88	$p=0.370$
Abdominal discomfort/pain	Total	Placebo	268	1.695	0.5991	$t=0.706$, df $=535$
		5 μg	269	1.658	0.6212	$p=0.480$
	Male	Placebo	226	1.677	0.6047	$t=0.893$, df $=439$
		5 μg	215	1.626	0.5991	$p=0.372$
	Female	Placebo	42	1.789	0.5660	$t=0.044$, df $=94$
		5 μg	54	1.783	0.6941	$p=0.965$
Stool form	Total	Placebo	267	5.323	0.6548	$t=0.094$, df $=529$
		5 μg	264	5.318	0.6430	$p=0.926$
	Male	Placebo	225	5.368	0.6413	$t=0.287$, df $=435$
		5 μg	212	5.351	0.6094	$p=0.774$
	Female	Placebo	42	5.084	0.6821	$t=-0.669$, df $=92$
		5 μg	52	5.184	0.7572	$p=0.505$
Stool frequency	Total	Placebo	268	2.752	1.3059	$t=0.511$, df $=535$
		5 μg	269	2.695	1.2726	$p=0.609$
	Male	Placebo	226	2.800	1.3152	$t=0.025$, df $=439$
		5 μg	215	2.797	1.3006	$p=0.980$
	Female	Placebo	42	2.493	1.2380	$t=0.862$, df $=94$
		5 μg	54	2.289	1.0721	$p=0.391$

Abdominal discomfort/pain: 0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Intolerable.
Bristol Stool Form Scale: 1: Separate hard lumps like nuts (difficult to pass); 2: Sausage shaped but lumpy; 3: Like a sausage but with cracks on its surface; 4: Like a sausage or snake, smooth and soft; 5: Soft blots with clear-cut edges (passed easily); 6: Fluffy pieces with ragged edges, a mushy stool; 7: Watery, no solid pieces, entirely liquid.

with ramosetron reported adequate relief of abdominal discomfort and/or pain at the final point (46% versus 33%, $p=0.005$; Figure 5a). Ramosetron also contributed to significantly greater improvement of abnormal bowel habits compared with the placebo (44% versus 24%, $p<0.001$; Figure 5b). For each assessment, the effects were observed by month 1 and were sustained throughout the treatment.

For each symptom, Figure 6 shows the effects of ramosetron on stool form, stool frequency and bowel urgency. Ramosetron hardened stool form (Figure 6a), decreased stool frequency (Figure 6b) and increased the rate of days without bowel urgency (Figure 6c) compared with the placebo. Improvement was observed within the first week and was sustained throughout the treatment. The rate of days without the sensation of incomplete bowel movement was also decreased in the ramosetron group compared with the placebo group (data not shown).

Safety

All 539 patients who received the drug (269 who received placebos and 270 who received ramosetron) were evaluated for safety.

One hundred and sixty-three patients (60.37%) in the ramosetron group and 141 patients (52.42%) in the placebo group reported adverse events. Table II shows the adverse events occurring with a frequency greater than 2% in the ramosetron group. Hard stool was the most frequently reported adverse event in the ramosetron-treated group compared with the placebo group and occurred in 20 (7.41%) of 270 patients in the ramosetron group compared with 2 (0.74%) of 269 patients in the placebo group. No adverse events were classified as severe, and most events were classified as mild in the ramosetron-treated group. Drug-related adverse events with a frequency greater than 1% in the ramosetron group compared with the placebo group were abdominal discomfort, abdominal distension, constipation, hard stool and an increase in blood bilirubin. Other

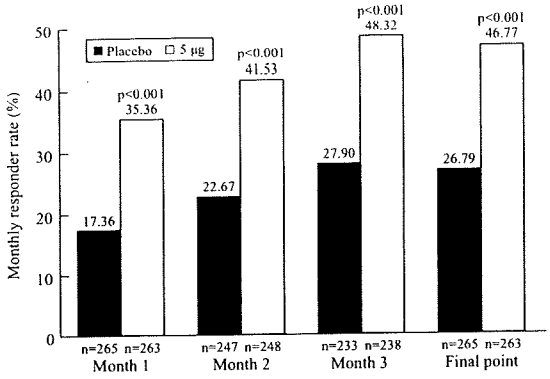

Figure 2. Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms". *P*-values were calculated using the χ^2 test.

adverse event profiles were similar in both groups. The incidence of drug-related adverse events was higher in females than in males. Drug-related adverse events for which the incidence in ramosetron-treated females was higher by 3% or more than that in males were abdominal distension, constipation, hard stool and a decrease in white blood cell count.

Thirteen patients (4.81%) in the ramosetron group and 10 patients (3.72%) in the placebo group discontinued the treatment because of adverse events. The adverse events associated with most of these discontinuations in ramosetron-treated patients were constipation (3 (1.11%)) and hard stool (3 (1.11%)).

Discussion

In this randomized, double-blind, placebo-controlled trial, the monthly responder rate based on "Patient-reported global assessment of relief of IBS symptoms" was higher in the ramosetron 5 μg group than in the placebo group, which verified the superiority of ramosetron 5 μg to placebos. It has been difficult to assess the effect of novel drugs for the treatment of IBS or other functional gastrointestinal disorders because of the multiplicity of symptoms and a high level of placebo effect. The Rome II working group guidelines recommended a patient-report outcome of the assessment as a primary end-point. Therefore, we used the "Patient-reported global assessment of relief of IBS symptoms" as a primary end-point. The primary end-point used in alosetron clinical trials was also the proportion of patients with adequate relief of IBS pain and discomfort for at least 2 weeks per month (defined as a monthly responder) for all 3 months [10–12]. Furthermore, in a recent report Camilleri et al. strongly insisted that global assessment should be a primary end-point in trials in IBS patients [21].

This study demonstrated the effects of ramosetron in both male and female Japanese patients with D-IBS meeting the Rome II diagnostic criteria. The statistically significant efficacy of ramosetron

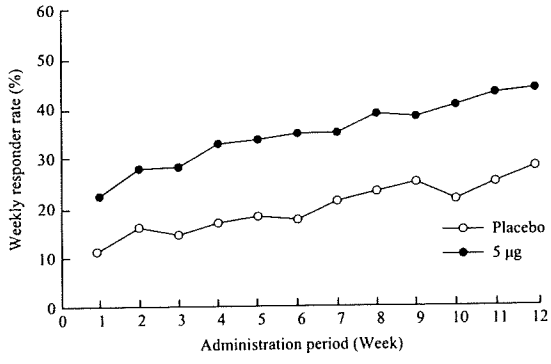

Figure 3. Weekly responder rate of "Patient-reported global assessment of relief of IBS symptoms". Weekly responders were patients whose scores were "Completely relieved" or "Considerably relieved".

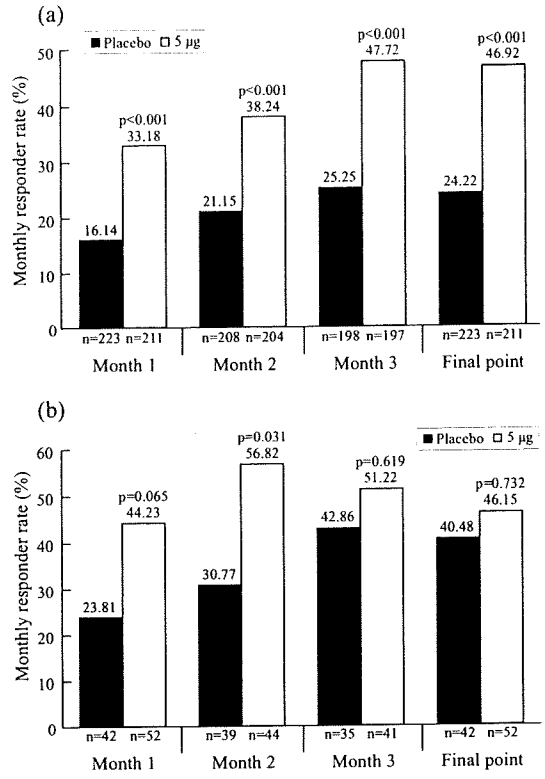

Figure 4. (a) Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms" in males. (b) Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms" in females. *P*-values were calculated using the χ^2 test.

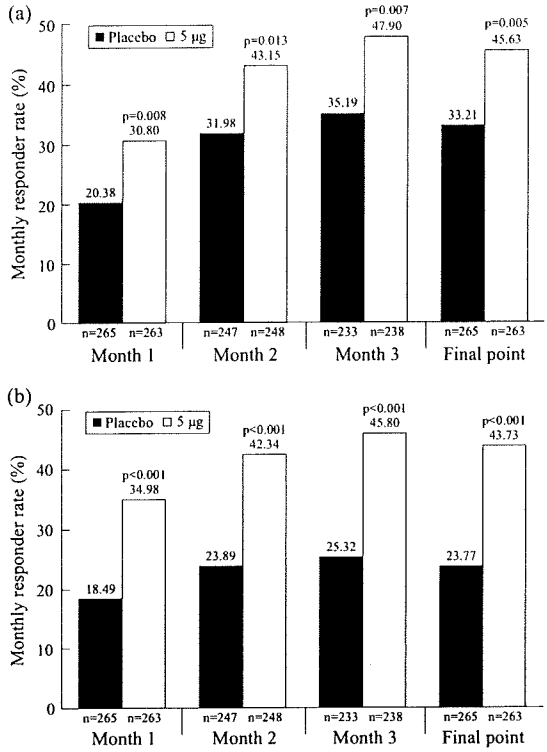

Figure 5. (a) Monthly responder rate of "Patient-reported assessment of relief of abdominal discomfort and/or pain". (b) Monthly responder rate of "Patient-reported assessment of improvement of abnormal bowel habits". *P*-values were calculated using the χ^2 test.

compared with placebos was observed at all time-points in male patients. In females, however, it was only observed at month 2. These findings may be related to the small sizes of female samples in this clinical study and a high level of placebo effect in females compared to males. The time-dependent increase in the placebo effect in females was greater than that in males. Chang & Heitkemper suggested that possible factors affecting gender differences in the response to treatment of patients with IBS included biobehavioral responses to stress, and sexual cycle, as well as differences in the roles and emotions between men and women. All of these factors may result in a variety of differences in clinical and physiological reactions [22]. Increased gastrointestinal symptoms and sensitivity of the colon during menses have been reported in clinical studies [23,24]. The mean age of female patients was around 40 years in this study, and the sexual cycle may have affected the efficacy assessment to some extent. A recent positron emission tomography study in patients with IBS reported gender differences in activation of brain networks concerned with cognitive, automatic and antinociceptive responses to

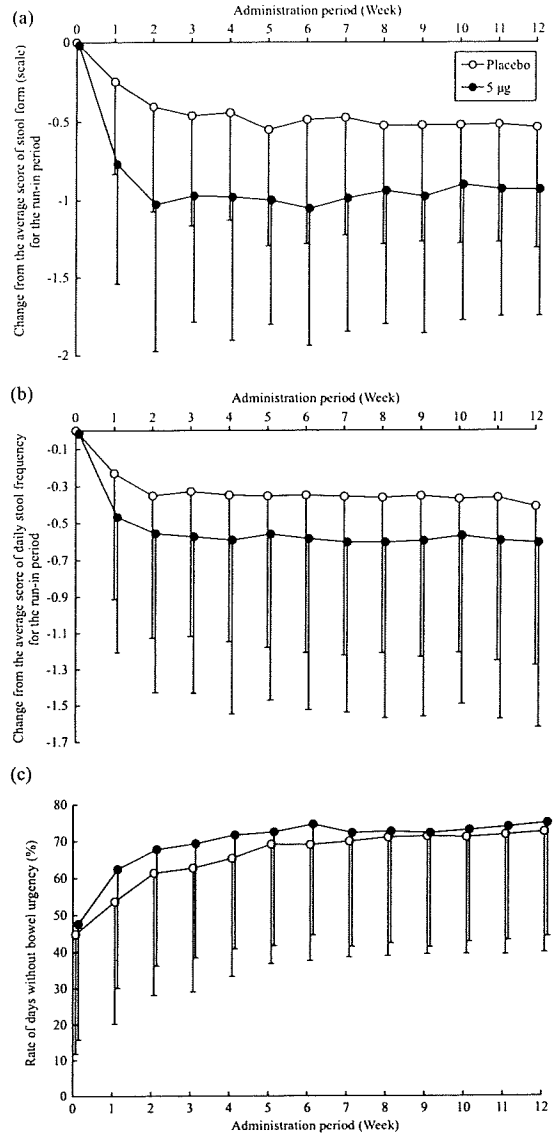

Figure 6. (a) Change in weekly average scores of stool form (change from the average score for the run-in period). (b) Change in weekly average of daily stool frequency (change from the average score for the run-in period). (c) Rate of days without bowel urgency (number of days without bowel urgency in each observation week/total number of entry days in the week).

aversive visceral stimuli or the psychological stress due to such visceral stimuli [25]. Likewise, there are physiological differences between male and female patients with IBS, but how these factors affect the response to placebos in each gender has not been elucidated. It is unclear how the differences in demographic characteristics between male and female patients affect the female patients' responses.

A clinical study of alosetron demonstrated efficacy in female patients but not in male patients [10]. The

Table II. Incidence of adverse events (incidence of 2% or higher in ramosetron group).

Symptom	Placebo	5 μg	*p*-value (χ^2 test, with continuity correction)
No. of safety analysis population	269	270	-
All adverse events	141 (52.42%)	163 (60.37%)	0.076
Gastrointestinal disorders	33 (12.27%)	70 (25.93%)	<0.001
Abdominal distension	4 (1.49%)	12 (4.44%)	0.077
Abdominal pain upper	5 (1.86%)	7 (2.59%)	0.775
Constipation	5 (1.86%)	14 (5.19%)	0.063
Hard stool	2 (0.74%)	20 (7.41%)	<0.001
Immune system disorders	4 (1.49%)	6 (2.22%)	0.754
Seasonal allergy	4 (1.49%)	6 (2.22%)	0.754
Infections and infestations	72 (26.77%)	53 (19.63%)	0.063
Nasopharyngitis	54 (20.07%)	40 (14.81%)	0.135
Investigations	37 (13.75%)	43 (15.93%)	0.557
Alanine aminotransferase increased	8 (2.97%)	7 (2.59%)	0.994
Blood bilirubin increased	3 (1.12%)	6 (2.22%)	0.505
Gamma-glutamyltransferase increased	10 (3.72%)	9 (3.33%)	0.993
White blood cell count increased	7 (2.60%)	13 (4.81%)	0.258
Respiratory, thoracic and mediastinal disorders	12 (4.46%)	16 (5.93%)	0.567
Upper respiratory tract inflammation	6 (2.23%)	10 (3.70%)	0.451

No. of subjects with events (incidence).

difference in the site of action between alosetron and ramosetron is a possible cause of the gender differences in the response to these drugs. Ramosetron acts only on peripheral tissues, whereas alosetron is reportedly transferred to the brain [26]. There are gender differences in the central pathophysiology of IBS [25,27]. In response to aversive visceral stimulation, women with IBS showed greater activation in the "limbic" area, which is frequently activated by emotional stimuli including the amygdala, as well as anterior and infragenual cingulate cortices. With regard to its central effects, alosetron was found to have an inhibitory effect on "limbic" (i.e. amygdala and ventral striatum) regions, and the inhibition of amygdala activity correlated with IBS symptom improvement. It is therefore conceivable that the greater efficacy of alosetron in women with IBS may be due to its inhibitory effects in the "limbic" region of the brain, which is more abnormally activated in women than men [28], whereas ramosetron does not act on the central nervous system. This difference in the site of action between alosetron and ramosetron may contribute to the result that ramosetron was effective in both male and female patients.

Alterations in mobility, secretion and visceral sensation are hallmarks of IBS. As all of these aspects of gastrointestinal function involve serotonin signaling, potential alterations in mucosal serotonin signaling have been explored as a possible mechanism of altered function and sensation in IBS [29,30]. A significant association was observed between D-IBS female patients and the serotonin reuptake transporter protein (SERT) polymorphisms, suggesting that the serotonin transporter is a potential candidate gene for D-IBS women [31,32]. However, it is not known whether differences in SERT polymorphisms between women and men with IBS contribute to the observed differences in clinical response to 5-HT_3 receptors antagonists.

Eighty percent of the patients enrolled in this trial were male. Kumano et al. reported that D-IBS is found more frequently among males than females, i.e. 67.5% of those with D-IBS are males; conversely, C-IBS is found much more frequently among females than males, i.e. 89.9% of those with C-IBS are females, in Japan [2]. It was reported that, in the United States, C-IBS is also found more among females, but the proportion of males with D-IBS is almost the same as that of females with D-IBS [33]. It is assumed that the difference in the ratio of males to females enrolled in the trial might be related to the differences in culture or lifestyle.

Adverse events that frequently occurred during the treatment with ramosetron were abdominal distension, constipation and hard stool, which are considered to be a classic effect of the 5-HT_3 receptor antagonist. The incidence of constipation due to the administration of alosetron was 29% ($n=8328$) [34], whereas such incidence due to the administration of ramosetron was only 5.19%. Thus, the incidence of constipation in the ramosetron group was lower than that in the alosetron group. Neither ischemic colitis nor severe constipation, which were reported with alosetron use, was observed in treatment with ramosetron. Although the pathogenesis of ischemic colitis in patients with IBS on treatment

with alosetron is uncertain, ischemic colitis might result from the constipation due to slow transit induced by alosetron [35]. The incidence of constipation in the ramosetron group is considered to be lower than that in the alosetron group, so ischemic colitis is unlikely to be caused by ramosetron.

In summary, it was shown that ramosetron is also significantly effective for male patients with D-IBS, unlike alosetron. It was also shown that ramosetron seems to be effective for female patients with D-IBS. Ramosetron was also proven to be a safe drug. Ramosetron is recommended as a novel drug for the treatment of Japanese patients with D-IBS due to its selective antagonistic action on 5-HT_3 receptors.

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Brain histamine H_1 receptor occupancy of orally administered antihistamines, bepotastine and diphenhydramine, measured by PET with ^{11}C-doxepin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- 'Bepotastine besilate' is a novel second-generation antihistamine developed in Japan and its antiallergic effects have already been demonstrated by various studies.
- However, only a few clinical studies regarding its sedative property are available.
- In addition, histamine H_1 receptor occupancy (H_1RO) of this new antihistamine has never been measured by positron emission tomography (PET).

WHAT THIS STUDY ADDS

- This paper provides the first measurement result of cerebral H_1RO of bepotastine besilate (approximately 15%) as determined by PET.
- This result is in accordance with the clinical classification of bepotastine as a second-generation antihistamine.
- In addition, the relationship between subjective sleepiness and cerebral H_1RO of this second-generation antihistamine is demonstrated for the first time using a placebo-controlled crossover study design.

AIMS

Antihistamines are frequently used for treating various allergic diseases, but often induce sedation. The degree of sedation can be evaluated by measuring histamine H_1 receptor occupancy (H_1RO) in the brain using positron emission tomography (PET). The aim was to measure H_1RO of bepotastine, a new second-generation antihistamine, and to compare it with that of diphenhydramine.

METHODS

Eight healthy male volunteers (mean age ± SD 24.4 ± 3.3 years) were studied after single oral administration of bepotastine (10 mg), diphenhydramine (30 mg) or placebo, by PET imaging with ^{11}C-doxepin in a crossover study design. Binding potential ratio and H_1ROs were calculated using placebo data and were compared between bepotastine and diphenhydramine in the anterior and posterior cingulate gyri (ACG and PCG, respectively), superior and inferior frontal cortices (SFC and IFC, respectively), orbitofrontal cortex (OFC), insular cortex (IC), lateral and medial temporal cortices (LTC and MTC, respectively), parietal cortex (PC), occipital cortex (OC) and sensorimotor cortex (SMC). Plasma concentration of each antihistamine was measured, and its correlation to H_1RO was examined.

RESULTS

H_1RO after bepotastine treatment was significantly lower than that after diphenhydramine treatment in all cortical regions ($P < 0.001$). Mean H_1ROs of bepotastine and diphenhydramine were 14.7% and 56.4%, respectively. H_1ROs of both bepotastine and diphenhydramine correlated to their respective drug plasma concentration ($P < 0.001$).

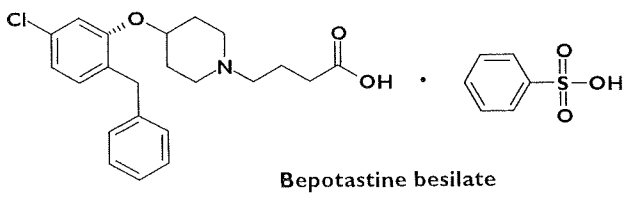
CONCLUSION

Oral bepotastine (10 mg), with its relatively low H_1RO and thus minimal sedation, has the potential for use as a mildly or slightly sedative antihistamine in the treatment of various allergic disorders.

Introduction

Histamine H_1 receptor (H_1R) antagonists, or antihistamines, are often used for treating allergic disorders such as seasonal rhinitis. Antihistamines mainly act on peripheral tissues, but can induce sedation as a central side-effect. This undesirable side-effect is caused by blockade of nerve transmission in the histaminergic neuron system which projects from the tuberomammillary nucleus in the posterior hypothalamus to almost all cortical areas [1–5]. First-generation antihistamines that can easily penetrate the blood–brain barrier (BBB), such as diphenhydramine and d-chlorpheniramine, tend to occupy a large proportion of postsynaptic H_1Rs as demonstrated by positron emission tomography (PET) [1, 6–8]. PET also reveals that second-generation antihistamines (mildly or slightly sedative antihistamines), such as cetirizine and olopatadine, can slightly penetrate the BBB and occupy some H_1Rs [1, 6, 9, 10]. Users who take these second-generation antihistamines at doubled or tripled doses to achieve desired effects may suffer from dose-related cognitive impairment. Third-generation antihistamines (truly nonsedative antihistamines), such as fexofenadine and ebastine, hardly penetrate the BBB and do not occupy H_1Rs even at high doses, as demonstrated by ^{11}C-doxepin PET [9]. Thus, the sedative property of antihistamines can be evaluated in terms of H_1R occupancy (H_1RO). Such variations in BBB permeability are caused by various factors, including differences in lipophilicity, molecular size and actions of drug transporters.

Bepotastine besilate ({d-(S)-4-[4-[(4-chlorophenyl)(2-pyridyl)methoxy]piperidino} butyric acid monobenzenesulphonate, betotastine besilate, CAS 125602-71-3, TAU-284 or Talion), a new second-generation antihistamine developed in Japan, is now used as an oral tablet for allergic rhinitis and chronic urticaria (Figure 1) [11–13]. Previous studies have demonstrated its excellent antiallergic effects compared with other antihistamines such as ketotifen, cetirizine, epinastine and terfenadine [14–18], whereas only a few studies have shown its central effects [18, 19]. Only one available animal behavioural study by Kato and colleagues has demonstrated that bepotastine is a highly specific drug to H_1R, having no significant binding affinity for histamine H_3, adrenergic α_1, α_2, β, dopaminergic D_2, serotonergic $5HT_2$, muscarinic or benzodiazepine receptors, and



Bepotastine besilate

Figure 1
Chemical structure of bepotastine besilate

that it poorly penetrates the BBB [19]. Takahashi and colleagues first conducted a double-blind, placebo-controlled, crossover study to measure subjective sedation and psychomotor activities following administration of bepotastine, cetirizine, fexofenadine and olopatadine [18], where bepotastine had the least sedative effect [18].

To date, we have measured H_1ROs of various second-generation and third-generation antihistamines, but not that of bepotastine. It is of great interest to examine H_1RO of bepotastine in humans. Thus, the primary aim of the present study was to measure subjective sedation and cerebral H_1RO of bepotastine and to compare the results with those of diphenhydramine, a typical sedative antihistamine [20], using a placebo-controlled crossover study design that would make the interpretation of results clearer and easier by minimizing potential errors due to intersubject variability [10]. Another aim was to determine whether bepotastine should be classified as a truly nonsedative or mildly sedative antihistamine.

Methods

The present study was approved by the Committee on Clinical Investigation at Tohoku University Graduate School of Medicine, Japan, and was performed in accordance with the principles of the Declaration of Helsinki. All experiments were performed at the Cyclotron and Radioisotope Centre, Tohoku University.

Subjects and study design

Eight male Japanese volunteers (mean age ± SD 24.4 ± 3.3 years), who provided written informed consent after receiving a detailed description of the study, were recruited. All subjects were in good health with no clinical history of major physical or mental illness, showed no abnormality in brain magnetic resonance imaging (MRI), and were not receiving any concomitant medication likely to interfere with the study results. Nicotine, caffeine, grapefruit and grapefruit juice were not allowed on the test day, and alcohol was not allowed on the test day or the day before PET measurement.

All subjects underwent PET measurement after single oral administration of bepotastine (10 mg), diphenhydramine (30 mg) or a lactobacteria preparation (6 mg) used as placebo in a three-way crossover study, with minimum wash-out intervals of 7 days between treatments. The lactobacteria preparation has been widely used as placebo in Japan, and its administration has produced no statistical difference between pre- and post-administration in previous cognitive studies [7, 9, 10, 21]. The present PET study was conducted in a single-blinded manner, and after drug administration each subject was asked to remain seated comfortably on a sofa. To determine bepotastine and diphenhydramine plasma concentrations, blood samples were collected from each subject

before drug administration and at 0, 60, 120 and 180 min post administration. Subjective sleepiness of each subject was also measured at 0, 60, 120 and 180 min post administration using the Line Analogue Rating Scale (LARS) [22] and the Stanford Sleepiness Scale (SSS) as used in previous studies [9, 23].

Measurement of plasma concentrations of bepotastine and diphenhydramine

Plasma concentrations of bepotastine and diphenhydramine were measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS) together with an electrospray ionization method [24]. LC was performed on an Agilent 1100 Series LC instrument (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an analytical column. The MS/MS system was the API 4000 (Applied Biosystems/MDS Sciex, ON, Canada). The Solid Phase Extraction (SPE) cartridge (OASIS HLB 3 ml/60 mg; Waters Corp., Milford, MA, USA) was pretreated with 2 ml of methanol, 2 ml of water and 2 ml of 0.2 M Na_2CO_3/HCl buffer (pH 11).

For measurement, an internal standard solution (10 μl) and methanol (10 μl) were added to each plasma sample (50 μl). To the resulting solution, 930 ml of 0.2 M Na_2CO_3/HCl buffer was added for bepotastine measurement, and 1000 μl of 0.1% formic acid containing acetonitrile/methanol (50 : 50, v/v) was added for diphenhydramine measurement. The mixture was applied onto the SPE cartridge after pretreatment as mentioned above. Separations were carried out on a high-performance liquid chromatography (HPLC) column [CAPCELL PAK C18 MG II (3 μm) 2.0 mmϕ × 100 mm; Shiseido Co., Ltd, Tokyo, Japan) at a flow rate of 0.2 ml min^{-1} and at a column temperature of 40 °C. The reconstituted extract (5 μl) was injected onto an HPLC system with mobile phases for bepotastine measurement including 10 mmol l^{-1} ammonium acetate and acetonitrile of varied concentrations, namely, 32% (0–9 min), 70% (9.5–12.5 min) and 32% (12.6–24 min), and with mobile phases for diphenhydramine measurement including 0.1% heptafluorobutyric acid and acetonitrile of varied concentrations, namely, 40% (0–7 min), 70% (7.5–10.5 min) and 40% (11–21 min).

Detection of bepotastine was based on fragmentation of the precursor ion (m/z = 389 to product ion m/z = 202 with collision energy of 29 eV for bepotastine, and m/z = 256 to product ion m/z = 167 with collision energy of 19 eV for diphenhydramine), and that of the internal standard was based on fragmentation of the precursor ion (m/z = 389 to product ion m/z = 201 with collision energy of 29 eV for bepotastine, and m/z = 270 to product ion m/z = 181 with collision energy of 17 eV for diphenhydramine) in positive multiple reaction monitoring (MRM) mode. Positive ions were detected using an API 4000 system at 550 °C nebulizer gas temperature, with 5000 V ion spray voltage, 68.9 kPa (nitrogen) curtain gas and Level 4 collision gas for bepotastine, and 206.8 kPa curtain gas and Level 4 collision gas for diphenhydramine. Chromatographic data for positive MRM were collected using Analyst software (ver. 1.2, Applied Biosystems/MDS Sciex) with cycle times of 1.010 s per cycle for bepotastine and 0.5200 s per cycle for diphenhydramine. The lowest detectable concentration was around 1 ng ml^{-1} for both antihistamines, and some values slightly under the threshold (only for diphenhydramine) were extrapolated. As for validation, the following items were checked for bepotastine and diphenhydramine, respectively: accuracy (100.7% and 102.2%), correlation coefficients to standard solutions ($r > 0.99$ for both), and coefficients of variation (CVs) of three different concentrations ($n = 5$) (1.7–2.3%, 1.8–3.8%).

For examination of the relationship between estimated binding potential ratio (BPR) of ^{11}C-doxepin and plasma concentration of each antihistamine, the areas under the curves (AUCs) of bepotastine and diphenhydramine were calculated for 0–180 min ($AUC_{0-3\,h}$) post administration (Table 1).

PET tracer and image acquisition

Doxepin is one of the tricyclic antidepressants that has binding affinity to other receptors such as muscarinic receptors to some extent. However, its affinity to histamine H_1Rs is much higher than to other receptors and is very high compared with other antidepressants [25]. Thus, doxepin's affinity to other receptor systems is negligible in this

Table 1

Plasma concentrations of bepotastine and diphenhydramine ($n = 8$)

Bepotastine time (min)	Mean (ng ml^{-1})	SEM	CV, %	Diphenhydramine time (min)	Mean (ng ml^{-1})	SEM	CV, %
0	0.0	0.0	**0.0**	0	0.0	0.0	**0.0**
60	78.0	17.3	**62.9**	60	6.2	2.3	**104.4**
120	71.0	4.6	**18.2**	120	16.3	2.1	**36.4**
180	82.6	8.4	**28.7**	180	19.7	1.2	**17.0**
$AUC_{0-3\,h}$	196.1	24.3	**35.0**	$AUC_{0-3\,h}$	32.3	4.0	**35.3**

CV, coefficient of variation; SEM, standard error of mean

imaging study, as also confirmed by experiments using histamine H_1R knock-out mice, where doxepin binding in the brain was nearly zero [26]. Thereafter, ^{11}C-doxepin has been used to evaluate the distribution of histamine H_1Rs. ^{11}C-doxepin kinetics in plasma and the brain are not affected by the sedative antihistamine d-chlorpheniramine using arterial sampling data combined with metabolite analysis [6].

In the present study, ^{11}C-doxepin was prepared by ^{11}C-methylation of desmethyl doxepin with ^{11}C-methyl triflate as described previously [10, 27]. ^{11}C-doxepin radiochemical purity was >99%, and its specific radioactivity at the time of injection was 120.9 ± 80.55 GBq μmol^{-1} (3268 ± 2177 mCi μmol^{-1}). ^{11}C-doxepin-containing saline solution was intravenously injected into each subject at 90 min post administration, a time close to T_{max} of both antihistamines (1.2 h for bepotastine and 2–3 h for diphenhydramine). The injected dose and cold mass of ^{11}C-doxepin were 135.4 ± 19.83 MBq (3.660 ± 0.536 mCi) and 1.587 ± 0.895 nmol, respectively, and the radiological dose was calculated based on a previous study on radiological exposure [28].

Approximately 60 min after ^{11}C-doxepin injection, the subjects were positioned on the coach of the PET scanner (SET2400W; Shimadzu Co., Kyoto, Japan) for transmission scan (6 min) and emission scan in the three-dimensional (3D) mode lasting for 15 min (70–85 min post injection of ^{11}C-doxepin) in a similar fashion to our previous work [10, 29]. PET brain images from a 15-min-long emission scan were corrected for scattering based on a previous study [30] and for tissue attenuation using post-injection transmission scan data according to previous work [31]. Brain images were reconstructed with a filtered back-projection algorithm, with the aid of a supercomputer SX-7 at the Information Synergy Centre, Tohoku University, Sendai, Japan. The brain images were then normalized by plasma radioactivity at 10 min post injection to yield images reflecting distribution volume (DV) based on our static scan protocol reported previously [10, 32]. Validation using venous sampling instead of arterial sampling was carried out by another group, giving no difference between venous and arterial sampling at 10 min post injection (M. Senda, personal communication, 23 August 2007).

Three brain images of each subject, following oral administration of bepotastine, diphenhydramine and placebo, were coregistered to an identical stereotaxic brain coordinate system using an MRI-T1 image of each subject, with the aid of Statistical Parametric Mapping (SPM2, Wellcome Department, UK) software package [33]. MRI images were obtained with a 1.5-T magnetic resonance (MR) scanner (HiSpeed, Ver. 9.1; General Electric Inc., WI, USA) at Sendai Seiryo Clinic (miyagi, Japan). T1-weighted images (Vascular TOF SPGR: TR/TE 50/2.4 ms, FA 45°, number of excitations 1, matrix size 256 × 256, spatial resolution: x, y, z = 0.86, 0.86, 20.0 mm, respectively) were collected from all subjects.

Regions of interest (ROIs) were first placed on the following brain regions on the T1 images that had precise anatomical information, i.e. anterior and posterior cingulate gyri (ACG and PCG, respectively), prefrontal cortices (PFC), orbitofrontal cortex (OFC), insular cortex (IC), temporal cortex (TC), parietal cortex (PC), occipital cortex (OC), primary sensorimotor cortex (SMC), thalamus, striatum, midbrain, pons, and cerebellum. ROI was defined for each cortical region by two to five circles with a diameter of 7.6 mm for each hemisphere in four to five consecutive brain transaxial slices, as indicated in Figure 2A. For the thalamus, striatum, pons and midbrain, the margin of each region was traced in MRI T1 images. An averaged value from all ROIs was used as a representative value of each region. Information on ROI location was automatically transferred to the coregistered three PET images reflecting DV, and the binding potential ratio (BPR) was calculated for each region using the following equation: BPR = [(DV of each region – DV of cerebellum)/DV of cerebellum] [8, 9]. Finally, H_1ROs of bepotastine and diphenhydramine were calculated for each cortical region using the following equation: H_1RO = [(BPR of placebo – BPR of antihistamine)/BPR of placebo] × 100. BPR brain images were also created by applying the same equation to each DV brain image [8–10, 34, 35]. BPR brain images were analysed statistically on a voxel-by-voxel basis using SPM2 [33], following spatial normalization and smoothing using the same method as in our previous work. Differences in parameter values between bepotastine, diphenhydramine and placebo (control) were statistically examined, and regional maxima of statistical significance ($P < 0.001$) were projected onto surface-rendered MRI-T1 standard brain images. Precise locations of statistically significant regions were identified with the Co-Planar Stereotaxic Atlas [36].

Statistical analysis

Differences in subjective sleepiness and BPR between bepotastine, diphenhydramine and placebo were examined using ANOVA followed by multiple comparison with Bonferroni correction. The difference in H_1RO between bepotastine and diphenhydramine was examined using paired Student's *t*-test. The relationship between plasma drug concentration (AUC) and H_1RO was examined using Pearson's correlation test. A probability of $P < 0.05$ was considered statistically significant. All statistical examinations were performed using SPSS for Windows 13.0.1 (Japanese version).

Results

Plasma concentrations of bepotastine and diphenhydramine

Mean plasma concentrations and AUCs of bepotastine and diphenhydramine are shown in Table 1. The peak mean

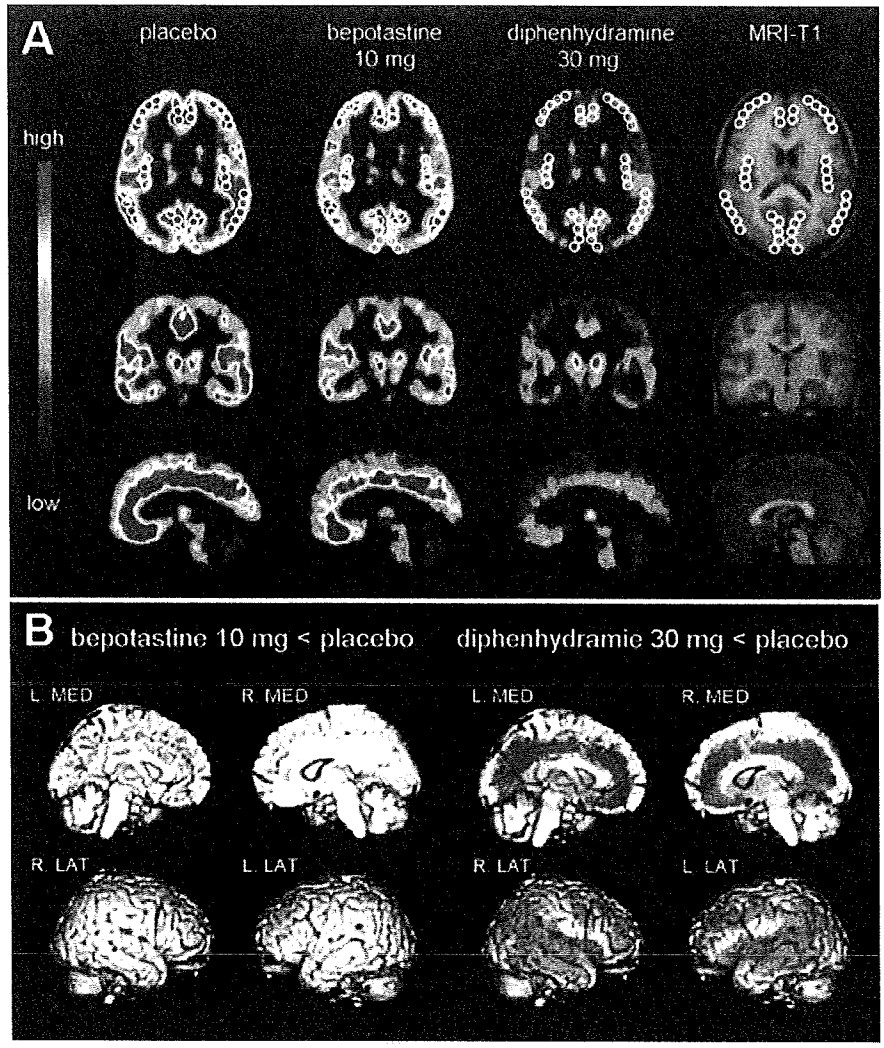


Figure 2

Binding potential ratio (BPR) images of ^{11}C-doxepin in the human brain (A) and results of voxel-by-voxel comparison (B). BPR of ^{11}C-doxepin was calculated in healthy male subjects ($n = 8$) by positron emission tomography following oral administrations of placebo (left), bepotastine (10 mg, middle) or diphenhydramine (30 mg, right), and their magnetic resonance imaging-T1 images (far right), demonstrated in the transaxial (top), coronal (middle) and sagittal (bottom) sections for each treatment condition, were compared (A). White circles in the transaxial images indicate the regions of interest (ROIs). The brain image of each subject was transformed to fit stereotaxic brain space (spatial normalization) and was averaged across each drug condition to generate the mean images displayed (A). The images demonstrate that diphenhydramine treatment results in BPR significantly lower than those of other drug conditions (B). Height threshold of voxel values was set at $P < 0.001$ and extent threshold was set at 10 voxel minimum. Results were not corrected for multiple comparisons. There are no areas with significantly lower BPR after bepotastine treatment compared with those after placebo treatment ('bepotastine 10 mg < placebo' in the left columns). In contrast, red colour shows areas with significantly lower BPR after diphenhydramine treatment compared with those after placebo treatment ('diphenhydramine 30 mg < placebo' in the right columns). In both columns, significant areas are demonstrated in four aspects, namely, left and right medial (L. MED and R. MED) and right and left lateral (R. LAT and L. LAT) aspects ($P < 0.001$, uncorrected, using SPM2) (B)

plasma concentration of bepotastine ranged from 60 to 180 min post administration because, in five of the eight subjects, it peaked at 60 min post administration, whereas in the other three subjects it peaked at 120 or 180 min post administration. Mean plasma concentration of diphenhydramine was maximal from 120 to 180 min post administration (Table 1).

Subjective sleepiness

Results of mean subjective sleepiness are shown in Figure 3. Mean subjective sleepiness of diphenhydramine peaked at 120–180 min post administration and that of bepotastine at 120 min post administration. Both LARS and SSS manifested similar patterns. Multiple comparison

following repeated ANOVA demonstrated that subjective sleepiness following diphenhydramine administration was significantly stronger ($P < 0.001$) than that of both bepotastine and placebo at 120 and 180 min post administration, and that of bepotastine was not significantly different from that of placebo (Figure 3).

Brain distribution of ^{11}C-doxepin

Radioactivity distribution patterns of ^{11}C-doxepin are shown in Figure 2A. The mean BPR image, averaged from eight subjects, following bepotastine treatment was similar to that following placebo treatment in an individual subject. Namely, high radioactivity was observed in ACG, PCG, PFC, OFC, IC, TC, PC, OC and SMC following both treatments, whereas the radioactivity distribution pattern following diphenhydramine treatment was much lower than that following the other two treatments (Figure 2A).

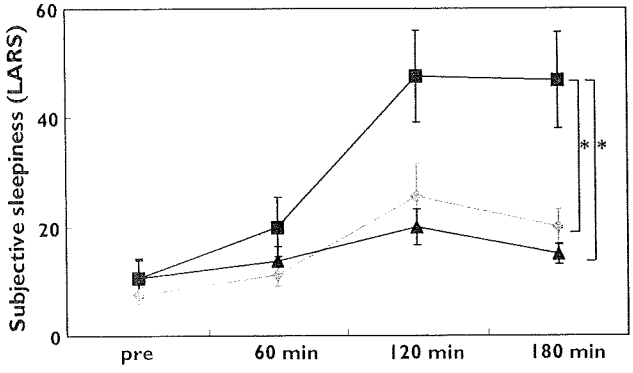


Figure 3

Subjective sleepiness evaluated using the Line Analogue Rating Scale (LARS). Eight healthy subjects were studied following oral administration of bepotastine (BEP, 10 mg), diphenhydramine (DIP, 30 mg) or placebo (PLA). *$P < 0.001$ by ANOVA followed by multiple comparison with Bonferroni correction. Error bars represent interindividual variability (SEM). BEP, (◈); DIP, (■); PLA, (▲)

Comparison of parametric BPR images (bepotastine vs. diphenhydramine)

Using SPM2 on a voxel-by-voxel basis, parametric brain BPR images following bepotastine or diphenhydramine treatment were statistically compared with those following placebo treatment. In Figure 2B, red areas show brain regions where BPRs were significantly lower ($P < 0.001$) following diphenhydramine treatment than those following placebo treatment (Figure 2B, right; Table 3). ACG, PFC and TC demonstrated significantly lower BPR after diphenhydramine treatment than after placebo treatment (Table 2). On the other hand, SPM analysis showed no brain areas where BPR was significantly lower after bepotastine treatment than after placebo treatment (Figure 2B, left).

ROI-based comparison of BPR and H_1RO

BPRs in H_1R-rich regions such as ACG, PFC, IC, TC, PC and OC were evaluated based on ROI analysis (Figure 4A). BPRs following bepotastine treatment were only slightly different from those following placebo treatment. However, BPRs following diphenhydramine treatment were significantly lower than those following placebo or bepotastine treatment ($P < 0.001$) for all cortical regions. In the thalamus, striatum, pons and midbrain, there was no significant difference in BPRs between diphenhydramine, bepotastine and placebo treatments.

H_1RO following diphenhydramine or bepotastine treatment was also calculated using H_1RO after placebo treatment as baseline (0%) (Figure 4B). Cortical mean H_1RO following bepotastine treatment was approximately 14.7% and that following diphenhydramine treatment was approximately 56.4%. H_1ROs following bepotastine treatment were significantly lower than those following diphenhydramine treatment ($P < 0.001$) in all cortical

Table 2

Binding potential ratios and histamine H_1 receptor occupancies in placebo, bepotastine and diphenhydramine conditions

Regions	BPR_{Pla} Mean	SD	BPR_{BEP} Mean	SD	BPR_{DIP} Mean	SD	95% CI Pla-DIP	BEP-DIP	H_1RO_{BEP} Mean	SD	H_1RO_{DIP} Mean	SD	95% CI DIP-BEP	C.E. BEP	C.E. DIP
Anterior cingulate gyrus (ACG)	0.78	0.22	0.66	0.11	0.33	0.09	0.28, 0.64	0.24–0.42	14.3	11.9	57.7	8.9	33.1, 53.8	0.54	−0.30
Posterior cingulate gyrus (PCG)	0.85	0.18	0.71	0.09	0.40	0.09	0.28, 0.61	0.18–0.43	14.8	12.3	52.0	9.7	24.1, 50.2	0.51	−0.57
Prefrontal cortex (PFC)	0.65	0.16	0.55	0.10	0.27	0.10	0.26, 0.51	0.20–0.37	14.2	12.2	59.3	12.2	31.7, 58.5	0.66	−0.28
Orbitofrontal cortex (OFC)	0.60	0.20	0.50	0.13	0.24	0.08	0.23, 0.51	0.18–0.35	15.4	11.8	60.5	6.9	34.1, 56.2	−0.01	−0.28
Insular cortex (IC)	0.80	0.18	0.66	0.09	0.36	0.09	0.29, 0.57	0.21–0.37	15.9	9.9	54.0	8.0	28.7, 47.4	0.52	−0.29
Temporal cortex (TC)	0.61	0.18	0.51	0.09	0.27	0.11	0.22, 0.48	0.21–0.28	14.4	12.6	57.2	11.0	30.6, 55.0	0.04	−0.20
Parietal cortex (PC)	0.54	0.17	0.44	0.13	0.21	0.12	0.22, 0.43	0.17–0.30	16.0	16.9	62.8	16.5	30.6, 62.9	0.34	0.16
Occipital cortex (OC)	0.49	0.10	0.43	0.06	0.27	0.08	0.15, 0.31	0.11–0.22	11.0	12.8	46.3	13.5	23.5, 47.0	0.31	0.06
Somatosensory cortex (SMC)	0.44	0.14	0.36	0.10	0.19	0.09	0.13, 0.38	0.06–0.29	16.5	15.9	57.6	21.6	18.9, 63.3	0.78	−0.18
Mean	0.64	0.14	0.54	0.12	0.28	0.07			14.7	1.6	56.4	5.0		0.48	−0.19

Results of statistical evaluation (*P*-values) are shown in Figure 3. BEP, bepotastine; BPR, binding potential ratio; C.E., correlation efficient of H_1 RO to plasma concentration of each antihistamine; 95% CI, 95% confidence interval; DIP, diphenhydramine; H_1RO, histamine H_1 receptor occupancy; Pla, placebo; SD, standard deviation.

Table 3

Regions with significantly lower specific binding following diphenhydramine treatment compared with those following placebo treatment

Regions	Brodmann's area	Hemisphere	x, y, z (mm)	Cluster size	*T*-value	*Z*-value	*P*-value
Superior temporal gyrus	22	R	54–56 16	29 543	11.55	6.41	<0.001
Medial temporal gyrus	21	L	−58-12-12	S.C.	11.41	6.37	<0.001
Precuneus	7	R	26–58 48	S.C.	11.08	6.29	<0.001
Medial temporal gyrus	21	L	−48 4–32	32	8.14	5.41	<0.001
Superior frontal gyrus	10	L	−24 60 12	104	7.53	5.18	<0.001
Superior frontal gyrus	8	L	−28 24 50	38	7.07	5	<0.001
Medial frontal gyrus	10	R	28 54 14	1342	8.53	5.55	<0.001
Medial frontal gyrus	9	R	42 6 40	S.C.	8.46	5.52	<0.001
Medial frontal gyrus	6	R	24 4 58	S.C.	8.98	5.7	<0.001
Inferior frontal gyrus	46	L	−40 44 12	38	8.96	5.69	<0.001

Cluster size is represented by the number of voxels (voxel size: 2.0 × 2.0 × 2.0 mm^3). S.C., the same cluster as above. Results are not corrected for multiple comparisons

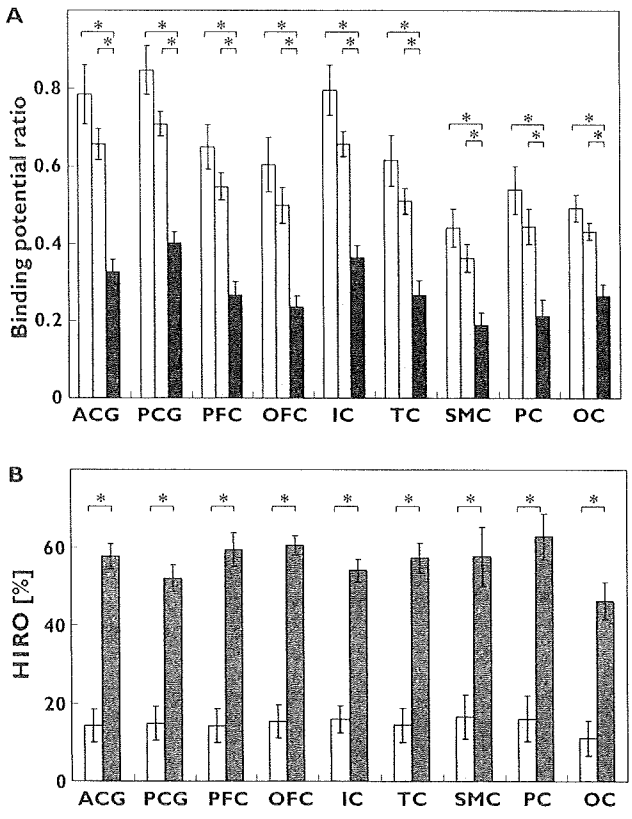


Figure 4

Region of interest (ROI)-based analyses of binding potential ratios (BPR) (A) and histamine H_1 receptor occupancy (H_1RO) (B) in the cortex. ROI measurements were performed in the anterior and posterior cingulate gyri (ACG and PCG, respectively), prefrontal cortex (PFC), orbitofrontal cortex (OFC), insular cortex (IC), temporal cortex (TC), sensorimotor cortex (SMC), parietal cortex (PC) and occipital cortex (OC) after treatments with placebo (PLA), bepotastine (BEP) and diphenhydramine (DIP). Comparison of BPRs shows differences in the sedative properties of the three drugs (A). H_1ROs due to BEP and DIP are shown, taking H_1RO due to placebo as 0% (B). *$P < 0.001$, ANOVA followed by multiple comparison with Bonferroni correction. Error bars represent interindividual variability (SEM). PLA, (□); BEP, (▨); DIP, (■)

regions. These data demonstrate that BPR following bepotastine treatment is substantially higher than that following diphenhydramine treatment in all cortical regions studied.

Relationships between subjective sleepiness, plasma drug concentration and H_1RO

Subjective sleepiness (AUC of LARS curve) did not significantly correlate to plasma concentration of bepotastine ($r = 0.04$), but did correlate well to plasma concentration of diphenhydramine ($r = 0.72$). H_1ROs following bepotastine administration significantly correlated to plasma concentration of bepotastine in ACG, PCG, PFC, IC and SMC, whereas those following diphenhydramine administration were all negatively correlated (Table 2). A similar trend was observed between cortical mean H_1RO and plasma concentration of both antihistamines (Figure 5A,B). However, when the baseline data are plotted together, mean H_1RO due to diphenhydramine tended to increase rapidly with diphenhydramine concentration, whereas that due to bepotastine gradually increased with bepotastine concentration (Figure 5A,B). H_1RO following bepotastine administration did not significantly correlate to subjective sleepiness ($r = 0.01$) (Figure 5D), whereas that following diphenhydramine administration negatively correlated to subjective sleepiness when the baseline data were plotted together (Figure 5C).

Discussion

Recently, molecular imaging techniques have been actively applied in clinical pharmacology research [1, 8–10, 37]. One of the primary aims of such work is to evaluate the relationship between doses of therapeutic drugs and their adverse reactions in terms of changes in 'endophenotypes' in the brain [38]. The primary aim of the present study was to clarify whether bepotastine belongs to second-generation antihistamines in terms of H_1RO. Another aim

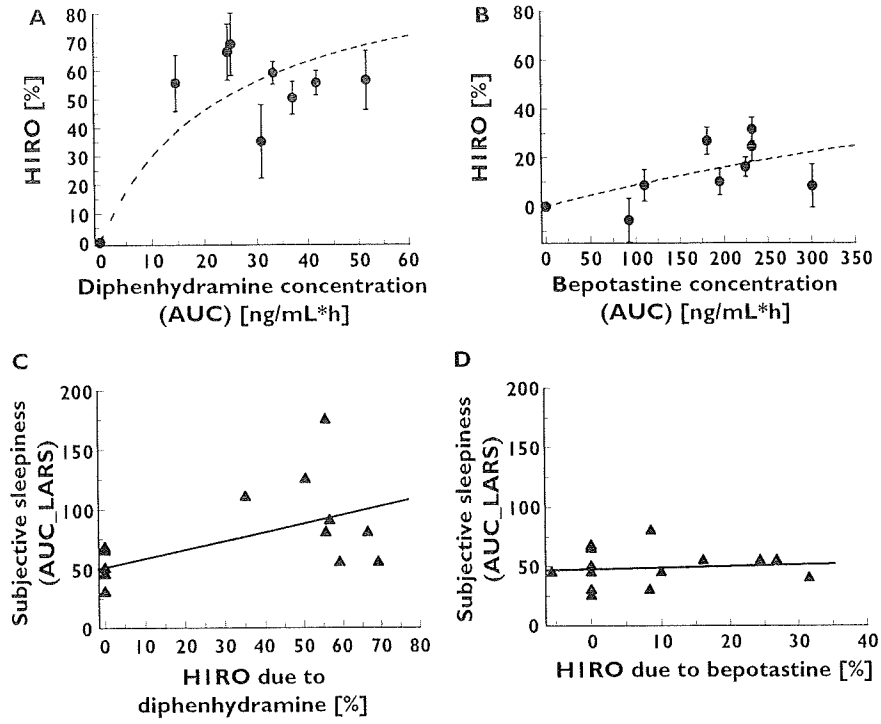


Figure 5

Relationship between mean H_1 receptor occupancy (H_1RO), plasma concentration and subjective sleepiness following administrations of bepotastine and diphenhydramine. Relationship between mean H_1RO (across brain regions) and plasma concentrations was examined for diphenhydramine (A) and bepotastine (B). Plasma concentrations of the two antihistamines are presented as area under the curve (AUC). H_1RO of diphenhydramine rapidly increases with plasma concentration, whereas H_1RO of bepotastine gradually increases with plasma concentration. Error bars represent intraindividual variability (SD). Dotted curves reflect the estimated curves of relationship between plasma concentration and the H_1RO analysed by the Michaelis–Menten equation (A and B). Subjective sleepiness (presented as AUC of line-analogue rating scale curve: AUC_LARS) demonstrates mild correlation to mean H_1RO due to diphenhydramine (C), whereas subjective sleepiness due to bepotastine demonstrates no correlation to mean H_1RO due to bepotastine (D)

was to determine whether bepotastine should be classified as a truly nonsedative or mildly sedative antihistamine, as previously discussed in the Consensus Group on New Generation Antihistamines (CONGA) [3].

In the present study, H_1ROs following a single oral administration of bepotastine (10 mg) or diphenhydramine (30 mg) were 14.7 and 56.4%, respectively. The relatively high H_1RO due to diphenhydramine agrees with those of other reported first-generation antihistamines [1, 6, 38]. Single oral administration of d-chlorpheniramine (2 mg) achieved 50–77% H_1RO, and this high H_1RO was associated with high prevalence of sleepiness and cognitive decline [6, 8, 39]. On the other hand, bepotastine's low H_1RO value suggests that this antihistamine can be classified into the category of second-generation antihistamines [6, 8, 9, 39–42, 38].

Thus, whether bepotastine can be classified as truly nonsedative or not is an additional point of clinical importance. In the present study, subjective sleepiness following bepotastine administration (10 mg) was negligible (Figure 3). This finding seems to be in accordance with that of Takahashi and colleagues [18], where bepotastine (10 mg) induced slight subjective sedation among healthy volunteers. In their study, the best performance was achieved following bepotastine treatment in a word-processing task compared with those following cetirizine (10 mg), fexofenadine (60 mg) or olopatadine (5 mg) treatment [18]. Another study [11] has demonstrated that results of serial arithmetic tests following oral administration of bepotastine (2.5, 5, 10 and 20 mg) showed no significant differences from those following placebo treatment. These clinical studies tried to demonstrate the nonsedative property of bepotastine, but they failed to include an active placebo (sedative antihistamine) to prove sensitivity of the selected tasks as recommended in the CONGA guideline [3]. An additional animal study [19] has demonstrated that bepotastine manifested a sedative profile similar to that of cetirizine and terfenadine. As a whole, these results suggest the low liability of bepotastine to produce sedative side-effects at a therapeutic dose of 10 mg. Considering the significant correlation between H_1RO and plasma drug concentration (AUC) found in the present study (Figure 5B), bepotastine may be classified as a mildly sedative antihistamine.

In general, H_1RO is used as an index of BBB permeability, but it could also be affected by gut absorption that can raise plasma drug concentration. At the molecular level, variation in BBB permeability has been determined by factors such as lipophilicity, molecular size and different actions of various drug transporters including P-glycoprotein (P-gp), an efflux pump expressed in capillary endothelial cells in the BBB [10]. Many lipophilic first-generation antihistamines are absorbed in full amount in the gut and can freely enter the brain tissue, whereas gut absorption and brain penetration of second-generation antihistamines tend to be reduced. For fexofenadine, a substrate of P-gp, both gut absorption and BBB permeability are highly reduced because of its low membrane permeability and high action of P-gp. For bepotastine, also a substrate of P-gp, the same extent of reduction as that of fexofenadine is observed [43]. The gradual increase in H_1RO with plasma bepotastine concentration, suggesting its relatively high membrane permeability (Figure 5B), may be associated with the action of P-gp in the BBB. On the other hand, gut absorption of bepotastine tends to be much higher than that of fexofenadine, presumably because of bepotastine's higher membrane permeability in the upper part of the small intestine where P-gp expression is low [43]. Such difference is one of the possible reasons for explaining the difference between bepotastine and fexofenadine. Another reason is that fexofenadine is transported not only by P-gp but also by the organic anion protein transporter family, further reducing its absorption in the gut [44, 45].

The limitations of the present method are as follows. The short scanning PET protocol would be useful especially in conducting a placebo-controlled crossover study because of the enormous mental and physical stress of volunteers observed in a previous study, where they were requested to complete four sets of 100-min-long PET examinations [34]. It is therefore important to develop a short scan protocol, although such protocols would include a larger amount of noise. Users of the PET system should consider these limitations. We note here that not exact values but, rather, approximations of DV and BP were measured in this study.

We were interested in the effects of an acute single dose of an antihistamine, and we planned to start PET scanning at the time point near T_{max} for each drug, but the tracer injection for diphenhydramine condition seemed to be slightly early since the plasma concentration of diphenhydramine was still increasing (Table 1). Therefore, H_1RO due to diphenhydramine might give slightly different values when measured in the equilibrium state. This might be the reason why H_1RO following diphenhydramine treatment did not correlate positively to the plasma concentration of diphenhydramine, contrary to our expectation based on a previous study using d-chlorpheniramine [8].

In summary, we have examined H_1RO of bepotastine (10 mg; 14.7%) and compared it with that of diphenhydramine (30 mg; 56.4%) given as a single oral administration, using PET in a placebo-controlled crossover study. It is suggested that oral administration of bepotastine (10 mg), with its low H_1RO and minimal sedation effects, is useful for treating various allergic disorders. As for the dose dependency of its sedative effects, another cognitive study involving an active placebo is needed in order to draw a definitive conclusion. The dose dependency of H_1RO should also be examined by PET measurements at higher doses.

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