Brain histamine H₁ receptor occupancy measured by PET after oral administration of levocetirizine, a non-sedating antihistamine

Kotaro Hiraoka, Manabu Tashiro, Thomas Grobosch, Marcus Maurer, Keiichi Oda, Jun Toyohara, Kenji Ishii, Kiiichi Ishiwata & Kazuhiro Yanai

Tohoku University Graduate School of Medicine, Department of Pharmacology, Sendai, Japan

Objective: Histamine H₁ receptor (H₁R) antagonists often have sedative side effects, which are caused by the blockade of the neural transmission of the histaminergic neurons. We examined the brain H₁R occupancy (H₁RO) and the subjective sleepiness of levocetirizine, a new second-generation antihistamine, comparing fexofenadine, another non-sedating antihistamine, as a negative active control.

Methods: Eight healthy volunteers underwent positron emission tomography (PET) imaging with [¹¹C]doxepin, a PET tracer that specifically binds to H₁Rs, after a single oral administration of levocetirizine (5 mg), fexofenadine (60 mg) or placebo in a double-blind crossover study. Binding potential ratios and H₁ROs in the cerebral cortices regions were calculated using placebo. Subjective sleepiness was assessed with the Line Analogue Rating Scale and the Stanford Sleepiness Scale.

Results: There was no significant difference between the mean brain H₁RO after levocetirizine administration (8.1%; 95% CI: -9.8 to 26.0%) and fexofenadine administration (-8.0%; 95% CI: -26.7 to 10.6%). Similarly, subjective sleepiness was not significantly different between the two antihistamines and placebo. Neither subjective sleepiness nor plasma concentrations was significantly correlated with the brain H₁RO of the two antihistamines.

Conclusion: At therapeutic dose, levocetirizine does not bind significantly to the brain H₁Rs and does not induce significant sedation.

Keywords: antihistamine, histamine H₁ receptor occupancy, levocetirizine, positron emission tomography, sedating side effect

1. Introduction

Histamine H₁ receptor (H₁R) antagonists, commonly known as antihistamines, are often used for treating allergic disorders such as seasonal rhinitis, urticaria and atopic dermatitis. The therapeutic efficacy of antihistamines on peripheral tissues is mediated via the peripheral H₁Rs, whereas their activity on the brain H₁Rs leads to central side effects such as sedation [1-3]. The latter is caused by the blockade of neuronal transmission in the brain histaminergic neuron system which projects from the tuberomammillary nucleus in the posterior hypothalamus to almost all areas of the CNS and commands general states of metabolism and consciousness [4].

First-generation antihistamines that can easily penetrate the blood–brain barrier (BBB), such as D-chlorpheniramime and diphenhydramine, are well known to cause sedation. Meanwhile, second-generation antihistamines such as cetirizine and
olopatadine can slightly penetrate the BBB and cause mild sedation in a dose-dependent manner. Among second-generation antihistamines, fexofenadine hardly penetrates the BBB and does not cause sedation. The variations in BBB permeability are due to various factors including differences in ionization, lipophilicity, the molecular size of the drug and transporter action [5-10]. In most second-generation antihistamines, hydrophilic functional groups (e.g., –COOH and –NH2) are introduced to reduce their BBB permeability in order to decrease their sedative effects. Most are substrates for P-glycoprotein, which functions as a drug efflux pump [5-10].

We previously investigated the brain H1R occupancy (H1RO) by many antihistamines using positron emission tomography (PET) and [11C]doxepin. This technique involves the intake of an oral antihistamine (the test drug) by healthy volunteers and assesses whether the test drug prevents binding of [11C]doxepin to the brain H1Rs. [11C]Doxepin is a radiopharmaceutical often used for imaging H1Rs in the brain [3]. The specificity of doxepin binding to H1R was previously confirmed using H1R gene-knockout mice [11]. Recently, doxepin was used to stabilize H1R and to determine its crystal structure because of its highest potency [12].

Our studies revealed that first-generation sedating antihistamines have H1ROs of > 50% [13,14]. Meanwhile, most second-generation antihistamines have H1ROs of < 20% at approved doses [15,16], although truly non-sedating antihistamines should not occupy H1Rs [17]. Shamsi and Hindmarch and McDonald et al. analyzed the literature regarding randomized placebo-controlled double-blind trials and calculated the incidence rates of the sedative effects of various antihistamines in terms of proportional impairment ratios [18,19]. Subsequently, we found that the proportional impairment ratios and H1ROs of examined antihistamines are significantly correlated [20,21]. Therefore, [11C]Doxepin-PET is a useful biomarker for evaluating the central side effects of antihistamines and CNS drugs with H1R antagonistic activity.

Levocetirizine, the R-enantiomer of cetirizine, is one of the most potent second-generation antihistamines [22-24]. Studies on histamine suppression revealed that 2.5 mg levocetirizine is comparable to 5 mg cetirizine, suggesting that the antihistamine potency of cetirizine may be attributable entirely to its R-enantiomer alone [25]. In addition to its potent antihistamine activity via the peripheral H1Rs, levocetirizine, along with fexofenadine, has also been reported as impairment-free for cognitive or psychomotor functions [3], as not having any effects on driving performance [26], as the only antihistamine with an impairment ratio of ‘0’ [18,19]. Indeed, in an open-label observational study of 7274 European patients treated with various antihistamines, the rate of somnolence in the levocetirizine group was similar to that of the fexofenadine group and less than half that of the cetirizine group [27]; a smaller study could not detect such a difference [28].

Although safety and tolerability of levocetirizine have been well documented, the brain H1RO of levocetirizine has not yet been investigated. The subjective documentation of drowsiness and objective CNS testing are limited in some situations by an individual’s motivation or familiarity with tests [29]. PET represents a major breakthrough, providing a sensitive reference method for quantifying CNS penetration. Furthermore, brain H1RO by antihistamines can be associated with psychometric and other tests of CNS function [3,21,29].

This study aimed to measure the cerebral H1RO and subjective sleepiness induced by levocetirizine. Fexofenadine, a non-sedating antihistamine, was used a negative active control. This study used a three-arm, placebo-controlled crossover design in order to minimize potential errors due to inter-subject variability.

2. Subjects and methods

This study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine and performed in accordance with the Declaration of Helsinki. PET imaging was performed at the Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

2.1 Subjects and study design

This study involved eight healthy male volunteers (mean ± standard deviation; 23.1 ± 2.9 years old), who provided written informed consent after receiving a detailed description of the study. All subjects had no clinical history of physical or psychiatric illness, showed no abnormal findings on the brain MRI and did not take any concomitant medications likely to affect the study results. Subjects were instructed to abstain from nicotine, caffeine, grapefruit and grapefruit juice on the test day or the day before PET imaging.

All subjects underwent PET imaging after a single oral administration of levocetirizine (5 mg), fexofenadine (60 mg) or a lactobacillus preparation (6 mg) often used as placebo in our hospital because its administration causes no statistical difference in cognitive assessments before and after administration [16,17,30,31]. Levocetirizine (5 mg) and fexofenadine (60 mg) are used in their legally approved and recommended single doses in Japan. Fexofenadine (180 mg) is a much more efficacious dose and is routinely utilized in the US. The test medications and placebo were given at 9:30 a.m. on the study day in a blind manner, that is, the eyes of the subjects were closed. The minimum washout interval between crossing-over medications was 7 days. After drug administration, each subject remained comfortably in bed. To measure the plasma concentrations of levocetirizine and fexofenadine, venous blood samples were collected from each subject before drug administration and 30, 60, 120 and 180 min post-administration. The subjective sleepiness of each subject was also measured before drug administration and 30, 60, 120 and 180 min post-administration using the Line Analogue Rating Scale (LARS) [32] and the Stanford Sleepiness Scale (SSS) [17,33].
2.2 Measurement of levocetirizine and fexofenadine plasma concentrations

Levocetirizine and fexofenadine plasma concentrations were determined by HPLC-mass spectrometry; the analytical column used was a Luna C8 (2) (30 × 2.1 mm; particle size, 5 µm; Phenomenex, Aschaffenburg, Germany). The HPLC system (Shimadzu, Duisburg, Germany) was coupled to a triple-quadrupole mass spectrometer (5500 QTrap; AB SCIEX, Darmstadt, Germany) with a turbo electrospray ion source operated in the positive ionization mode (ESI+). Samples were prepared for measurement by adding 400 µl IS solution (200 ng/ml solved in acetonitrile) to 100 µl plasma. Validation tests were performed as described by Peters et al. [34] and the guidelines of the Society of Toxicological and Forensic Chemistry [35]. The inter-day bias for both analytes and the three quality controls was < 3%. The bias and precision were < 3% for all control samples. The linear range of measurement was 0.2 – 750 ng/ml (R > 0.999). The lower limit of quantification of levocetirizine and fexofenadine was 0.2 ng/ml.

2.3 Radiosynthesis of [11C]doxepin and PET procedures

[11C]Doxepin was prepared by the 11C-methylation of desmethyl doxepin with [11C]methyl triflate as described previously [36] with some modifications [37]. The radiochemical purity of [11C]doxepin was > 97.8%, and its specific radioactivity at the time of injection was 53.4 ± 24.2 GBq/µmol (1440 ± 650 mCi/µmol). [11C]Doxepin-containing saline was intravenously injected into each subject 90 min after oral administration of the drugs, which approximately corresponds with the known T max of each antihistamine used. The injected dose [11C]doxepin was 331 ± 26 MBq (8.9 ± 0.7 mCi), and its cold mass was 7.4 ± 3.3 nmol.

[11C]Doxepin was injected 90 min after oral administration of the test drugs or placebo (11:00 a.m.). Sixty minutes after [11C]doxepin injection (12:00 a.m.), the subjects were positioned on the coach of the PET scanner (SET-2400W; Shimadzu, Kyoto, Japan) [38] for transmission scanning (6 min) and emission scanning in the three-dimensional mode for 15 min (70 – 85 min after [11C]doxepin injection) according to a simplified reference tissue model approach [37,39].

2.4 PET image analysis

PNEURO, a tool for brain PET/MR analyses in the dedicated software package PMOD (version 3.404; PMOD Technologies, Zurich, Switzerland) was used for the placement and evaluation of volumes of interest (VOI). The T1 MR images taken from each subject were initially segmented into grey matter, white matter and cerebrospinal fluid; grey matter probability maps were calculated. The PET images were matched rigidly to the MR images. The MR images were spatially normalized to the Montreal Neurological Institute (MNI) T1 template. The maximum probability atlas in MNI space which contains for every pixel a unique label number, corresponding to one of the VOIs [40], was transformed into the MR space. The cortical structures were intersected with the grey matter probability map at p > 0.3. The same VOIs templates were applied to all matched PET series to calculate the mean uptake value of each VOI. The VOIs were defined in the cortices, that is, temporal cortices (TPL), frontal cortices (FRL), occipital cortices (OCL), parietal cortices (PRl), anterior cingulate gyri (ACG) and posterior cingulate gyri (PCG). The cerebellum was defined as a reference region.

As a parameter for the specific H1R binding of [11C]doxepin in each cerebral region, we calculated the binding potential ratio (BPR) using a simplified reference tissue model method [39]. This approach allows for shorter PET scanning times as compared to other methods. BPR was estimated according to the following equation:

\[
BPR = \frac{\text{Mean uptake value during the 15 min scan of the reference region}}{\text{Mean uptake value during the 15 min scan of the cerebellum}} - 1
\]

Our previous studies demonstrate the rationale behind adopting BPR as a parameter for the specific H1R binding of [11C] doxepin [37,39,41]. H1RO as a percentage of the placebo control was calculated in each VOI using the following equation [14,16,41]:

\[
H_1RO = (1 - \frac{\text{BPR of antihistamine}}{\text{BPR of placebo}}) \times 100(\%)
\]

2.5 Statistical analysis

BPR among the levocetirizine, fexofenadine and placebo groups were examined by repeated measure ANOVA followed by a Bonferroni correction for multiple comparisons. The difference in H1RO between the levocetirizine and fexofenadine group was examined using a paired Student’s t-test. Differences in the changes of the LARS and SSS score from baseline among the levocetirizine, fexofenadine and placebo groups were examined by a repeated measure ANOVA with Bonferroni post hoc test. The relationship between the mean H1RO of the cortical regions and AUC of the changes in LARS and SSS score from baseline was examined using Pearson’s correlation test. The level of statistical significance was set at p < 0.05. All statistical analyses were performed on SPSS version 17.0 (SPSS, Chicago, IL, USA).

3. Results

3.1 BPR and H1RO

The averaged BPR images of the eight subjects following levocetirizine 5 mg, fexofenadine 60 mg, and placebo administration are shown in Figure 1. The BPR following levocetirizine 5 mg administration was similar to that following fexofenadine 60 mg and placebo administration. The BPRs in the cortical regions and the mean BPR of all regions are shown in Table 1. The order of BPR was fexofenadine 60 mg > placebo > levocetirizine 5 mg. The BPRs were not
In the present study, the mean cortical H1ROs following levocetirizine 5 mg and fexofenadine 60 mg administration were 8.1 and -8.0%, respectively. There were no statistically significant differences between the mean cortical H1ROs of levocetirizine versus fexofenadine. The clinical relevance of the significant differences in the occipital and parietal region H1ROs of levocetirizine and fexofenadine is not known. The negative mean cortical H1RO following fexofenadine 60 mg administration indicates that fexofenadine does not occupy H1Rs at all, which is consistent with the results of a previous study [17]. The brain H1RO of levocetirizine 5 mg is much lower than the previously reported H1ROs of sedating antihistamines; for example, the H1ROs following the administration of 2 mg d-chlorpheniramine and 30 mg diphenhydramine have been reported to be 76.8 and 56.4%, respectively [13,14]. The H1RO of 5 mg levocetirizine is equivalent to that of 10 mg ebastine (9.9%) [42], 10 mg loratadine (11.7%) [20] and 20 mg epinastine (13.2%) [43]. Our accumulated data on various antihistamines [3,21] suggest 20% H1RO as a threshold for the emergence of measurable and significant sedation; meanwhile, 50% H1RO could be a threshold for the emergence of apparent sedation. According to this cut-off standard, levocetirizine, which has an H1RO of 8.1%, can be classified as a non-sedating antihistamine at the recommended dose.

The H1RO findings were also confirmed by the subjective sleepiness results which were similar for all test groups, that is, neither levocetirizine 5 mg nor fexofenadine 60 mg showed any potential to cause significant sedation when compared to placebo, although there is a possibility that lack of a difference could certainly represent a type 2 error because of the small sample size. The result is further concordant with the relatively low H1RO of levocetirizine compared to those of sedating antihistamines [13,14].

Brain H1RO was not significantly correlated with plasma concentration in this study. In our previous studies, the H1ROs of bepotastine, diphenhydramine and d-chlorpheniramine were positively correlated with the plasma concentrations of their respective antihistamines [14,42], whereas those of ebastine and terfenadine were not [13,42]. In general, a large number of subjects in [11C]doxepin-PET protocols are needed to elucidate correlations with plasma drug concentrations for antihistamines with relatively low H1ROs, such as levocetirizine and fexofenadine.

The limitations of this study include the large variations in BPR and negative H1RO of fexofenadine. One possible explanation for these is the simplified protocol used in this study. We did not calculate distribution volumes of [11C]doxepin, which proved to be appropriate for estimating H1-R-[11C]doxepin binding from continuous PET scanning and arterial blood sampling [37]. Instead, we adopted BPR, which may indicate region-specific H1-R-[11C]doxepin binding; this was done to relieve subject burden by avoiding the long scanning time of PET and sequential arterial blood sampling. This simplified method could have included noise, consequently yielding some amount of measurement error.

Another limitation is the unverified test-retest reliability of [11C]doxepin-PET over days and weeks, although we evaluated the test-retest reliability of [11C]doxepin binding...
between the morning and afternoon [44]. The BP of $^{11}$C]doxepin in the cerebral cortex was slightly higher in the morning than the afternoon, suggesting that the endogenous release of histamine affects $H_1$R binding measured with $^{11}$C]doxepin in vivo. Because endogenous histamine release in the brain would be higher in the afternoon, the BP of $^{11}$C]doxepin in the cerebral cortex was slightly lower in the afternoon. In the study reported here, all measurements were performed at the same time of the day to avoid any variability in endogenous histamine release caused by the circadian rhythm. Interestingly, the other non-sedating antihistamine, bilastine 20 mg, was reported to have negative $H_1$RO (mean value $-3.92\%$ in the cortex), which was similar to that of fexofenadine. Therefore, it would be also possible to propose a new

### Table 1. Binding potential ratios and histamine $H_1$ receptor occupancies in levocetirizine, fexofenadine and placebo conditions.

<table>
<thead>
<tr>
<th>Regions</th>
<th>BPR&lt;sub&gt;levocetirizine&lt;/sub&gt;</th>
<th>BPR&lt;sub&gt;fexofenadine&lt;/sub&gt;</th>
<th>BPR&lt;sub&gt;placebo&lt;/sub&gt;</th>
<th>$H_1$RO&lt;sub&gt;levocetirizine&lt;/sub&gt;</th>
<th>$H_1$RO&lt;sub&gt;fexofenadine&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Temporal cortices (TPL)</td>
<td>0.21</td>
<td>0.05</td>
<td>0.24</td>
<td>0.04</td>
<td>0.24</td>
</tr>
<tr>
<td>Frontal cortices (FRL)</td>
<td>0.27</td>
<td>0.09</td>
<td>0.31</td>
<td>0.11</td>
<td>0.30</td>
</tr>
<tr>
<td>Occipital cortices (OCL)</td>
<td>0.25</td>
<td>0.06</td>
<td>0.30</td>
<td>0.03</td>
<td>0.28</td>
</tr>
<tr>
<td>Parietal cortices (PRL)</td>
<td>0.27</td>
<td>0.06</td>
<td>0.34</td>
<td>0.03</td>
<td>0.31</td>
</tr>
<tr>
<td>Anterior cingulate gyri (ACG)</td>
<td>0.41</td>
<td>0.10</td>
<td>0.47</td>
<td>0.09</td>
<td>0.45</td>
</tr>
<tr>
<td>Posterior cingulate gyri (PCG)</td>
<td>0.49</td>
<td>0.10</td>
<td>0.57</td>
<td>0.09</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean of all the regions</td>
<td>0.32</td>
<td>0.07</td>
<td>0.37</td>
<td>0.06</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*p < 0.05, paired t-test.

BPR: Binding potential ratio; SD: Standard deviation.

### Figure 2. Subjective sleepiness, change from baseline over time, evaluated using the Line Analogue Rating Scale (A) and the Stanford Sleepiness Scale (B). Error bars represent standard deviations.

FXD: Fexofenadine; LCZ: Levocetirizine; PCB: Placebo.
category of non-sedating antihistamines with negative H1RO values [45].

It has been reported that some second-generation antihistamines containing a carboxyl group are much more specific to H1R and show much lower affinity to the other aminergic receptors such as muscarinic cholinergic receptors. Recent studies on the structure of H1Rs have determined some residues of the anion-binding region including Lys191 and Lys179 are important for the H1R selectivity [12]. The unique carboxyl-group of fexofenadine, levocetirizine and cetirizine can interact with these anion-binding residues of H1R, which are not conserved in other aminergic receptor. As the tissue/ peripheral H1RO of levocetirizine is one of the highest among second-generation antihistamines, corroborated by its potent antihistamine activity in histamine-induced skin and nasal models [23-25], the precise evaluation of its brain H1RO using PET has been lacking. Our study demonstrates that the selectivity of levocetirizine to peripheral H1Rs is much higher than that to brain H1Rs which explains its potent clinical efficacy and low potential for sedative effects.

Previous studies reported that passive membrane permeability, Pgp-mediated efflux and high plasma protein binding influence the in vivo brain distribution of antihistamine drugs. Dextrocetirizine is estimated to be more permeable through the BBB than levocetirizine because the unbound fraction in the plasma for dextrocetirizine was ~ 2 times more than levocetirizine [46,47]. The stereoselective difference in the brain penetration of cetirizine would be due to the difference in the plasma protein binding, although further studies are needed to clarify in humans.

5. Conclusions

At therapeutic doses, levocetirizine does not bind significantly to the brain H1Rs and does not induce significant sedation. The H1RO for levocetirizine 5 mg of 8.1% presented in this paper is well within the established range of < 20% for non-sedating antihistamines. The result of this study brings additional confirmation that levocetirizine, when used in its recommended dose of 5 mg daily, does not penetrate the brain in large enough quantities to cause meaningful sedation.

Acknowledgments

This study was supported by research funding from GlaxoSmithKline. K Hiraoka did the human PET studies and wrote the manuscript. T Grobosh and M Maurer measured the plasma concentration of levocetirizine and fexofenadine and wrote the manuscript. M Tashiro, K Oda, J Toyohara, K Ishii, and K Ishiwata did the human PET studies. K Yanai designed the study and wrote the manuscript. We appreciate R Boev (Global Medical Affairs, UCB Farchim SA, Switzerland) for discussing the manuscript. The authors also thank Kunpei Hayashi for the radiosynthesis of [11C]doxepin and Hatsumi Endo for supporting PET imaging. This work was supported in part by Grants-in-Aid for Scientific Research from the Japan Society of Technology (‘Molecular Imaging’).

Declaration of interest

K Yanai and M Tashiro have received unrestricted and collaborative research grants support and lecture honoraria from manufactures of the second-generation antihistamines, including Sanofi, GlaxoSmithKline, Kyowa-Kirin and Mitsubishi Tanabe. M Maurer has received lecture honoraria from manufactures of the second-generation antihistamines, including Sanofi, GlaxoSmithKline and UCB. Other authors have no financial conflicts of interest.
Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


A very good meta-review of all published safety for H1 antihistamines.


A comprehensive review of positron emission tomography studies on sedative properties of antihistamines.


Verster JC, de Weert AM, Bijljes SL, et al. Driving ability after acute and sub-chronic administration of levocetirizine and diphenhydramine: a randomized, double-blind, placebo-controlled trial.
K. Hiraoka et al.

Psychopharmacology (Berl) 2003;169:84-90


Affiliation

Kotaro Hiraoka, Manabu Tashiro, Thomas Grobboch, Marcus Maurer, Keiichi Oda, Jun Toyohara, Kenji Ishii, Kiichi Ishiwata & Kazuhiko Yanai MD PhD

Author for correspondence

1Tohoku University, Cyclotron and Radioisotope Center, Division of Cyclotron Nuclear Medicine, 6-3, Aoba, Aramaki, Aoba-ku, Sendai, 980-8578, Japan
2Labor Berlin - Charité Vivantes GmbH, Department for Laboratory Medicine and Toxicology, Syber Str. 2, 13353 Berlin, Germany
3Allergie-Centrum-Charité at the Charité - Universitätsmedizin Berlin, Department of Dermatology and Allergy, Charitéplatz 1, 10117 Berlin, Germany
4Tokyo Metropolitan Institute of Gerontology, Research Team for Neuroimaging, Research Team for Neuroimaging, 35-2, Sakae-cho, Ibaraki-ku, Tokyo, 173-0015, Japan
5Tohoku University Graduate School of Medicine, Department of Pharmacology, 2-1, Seiryo-machi, Aoba-ku, Sendai, 980-8578, Japan

E-mail: yanai@cyric.tohoku.ac.jp