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貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：**研究報告書**

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1．助成金額： 60万 円

2．研究テーマ

抗ヒスタミン薬含有点眼剤使用後における脳内ヒスタミンH1受容体占拠率の評価：健常者における陽電子断層撮影法（PET）測定

3．成果の概要

第一世代と第二世代抗ヒスタミン類含有点眼薬をそれぞれに健康被験者に点眼し、その抗ヒスタミン成分の脳内への移行程度を陽電子断層撮影法（PET）で測定・評価した。第一世代抗ヒスタミン類含有点眼薬（商品名：ザジテン）が点眼後に高いH_1受容体占有率（40%超えた）を示していた一方、第二世代抗ヒスタミン類含有点眼薬（リザベン）がH_1受容体を占有せず、脳内にも透過しにくいとのことも分かった。従来、点眼薬は外用薬として認識されて、その中枢副作用・安全性への考慮が少なかったが、本研究より第一世代抗ヒスタミン含有点眼薬使用後に、中枢鎮静副作用の存在があるため、これらの医療品を使用する際に安全性への考慮もあるべきと結論した。

4．研究業績

（1）学会における発表　　無・(有)（学会名・演題）

Potential Central Sedative Effect of Antihistamine Eye-drops: Histamine H_1 Receptor Occupancy Measured by Positron Emission Tomography. 第5回日中薬理ジョントミーティーング（中国ウルムチ市）　2011年8月7~9日

（2）発表した論文　　㊙・有（雑誌名・題名）

5．指導責任者の意見（指導責任者がご記入・ご捺印ください）

張　冬穎先生は、今まで殆ど研究がされていない薬物の点眼からの脳移行性について

の研究を行った。世界で初めて PET を用いて鎮静性抗ヒスタミン薬含有点眼液からの

脳移行を証明した。現在、英語論文を執筆中で大変に努力したと評価できる。

指導責任者署名　谷内一彰　㊞

－日中医学協会助成事業－

抗ヒスタミン薬含有点眼剤使用後における脳内ヒスタミンH1受容体占拠率の評価：健常者における陽電子断層撮影法（PET）測定

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要旨：

Topical antihistamines are probably the best treatment option for various ocular allergies, thanks to their rapid action, safety and convenience of use. As the oral antihistamines are known to produce drowsiness, the present study was conducted to assess the possible influence of two antihistamine eye-drops, 0.05% ketotifen (Zaditen®) and 0.1% olopatadine (Patanol®), on the central nervous system (CNS) by measuring brain histamine H1 receptor occupancy (H_1RO) using positron emission tomography. Eight healthy adult subjects are recruited and a PET scan was performed 1.5 hr after 4 repeated local instillation of eye-drops (2 drops per eye, 30 min-interval) in a single-blind, placebo-controlled, crossover manner. H_1RO were calculated in several H_1R-rich cortical regions. We found that the H_1RO following ketotifen treatment is more than 20% and that following olopatadine was nearly zero. Our results provides the evidence for the first time that the first-generation antihistamine eye-drop, ketotifen, may potentially induce central sedation with higher doses, while olopatadine has no CNS influence, though the central side-effects have been rarely documented in the case of topical medications.

Key Words

olopatadine; Ketotifen; histamine H_1 receptor occupancy; positron emission tomography (PET); crossover study.

緒言：

Allergic conjunctivitis is a frequent condition as it is estimated to affect 20% of the population on an annual basis, with individuals in Italy, Japan, and other warm climates being more likely to have these conditions. Topical ophthalmic anti-allergy agent, antihistamine is now the first-line treatment option thanks to their rapid action, safety and convenience of use. However, recently years, with the improvement of the safety conscience regarding the drugs, sedation side-effect of Topical administration of antihistamine eye- or nasal- drops began to raise our attention. Topical administration of antihistamine induces subjective drowsiness in some users though objective evidence is still not available.

Oral or intravenous antihistamines were known to induce sedation, by blocking brain histaminergic system trough H1 receptors or other non-specific bindings. The sedative degree depends on the ability of

antihistamine in the circulation to penetrate blood-brain-barrier (BBB) and entered into the brain. In recent decade, researchers began to evaluate the sedative property of antihistamines using a more objective method, positron emission tomography (PET), by measuring brain H_1RO. The more drug enters brain, the more H_1 receptors should be occupied and thus induce sedation. H_1RO has been approved to as an index to reflect the sedation with the advantages of objectivity and quantify over the traditional method such as questionnaires or performance tasks. We therefore speculate that whether the sedation side effect of eye-drop also related with their occupying H_1RO in the brain. However, up to date, no study has been conducted regarding the central receptor occupying degree following topical administration of histamine eye-drops.

We designed a randomized, single-blind, placebo-controlled, crossover study and measured the brain histamine H_1RO following the local instillation of two commercially marked antihistamine eye-drops, ketotifen and olopatadine using PET and ^{11}C-doxepin. We aimed to compare and assess the possible sedative outcome of topical eye-drops in a point of view of molecular imaging, and to provide the doctors and patients information that might be useful in their guiding using or receiving such medications.

対象と方法：

Seven healthy Japanese volunteers (male, mean age ± SD: 23.1 ± 1.6 years) without history of allergy or any psychiatric diseases or of long-term taking H_1 antagonists, participated in this study. They showed no abnormality in brain magnetic resonance images (MRI). Drugs that might affect histamine response (such as sleep-aids, antidepressants or mast-cell stabilizers) were not allowed at least for 1 week prior to the study. Caffeine, tea, alcohol or grape juice was not allowed on the experiment day. This study was approved by the Ethics Committee on Clinical Investigation at Tohoku University School of Medicine and was performed in accordance with the policy of the Declaration of Helsinki. Informed consent was obtained from all the participants.

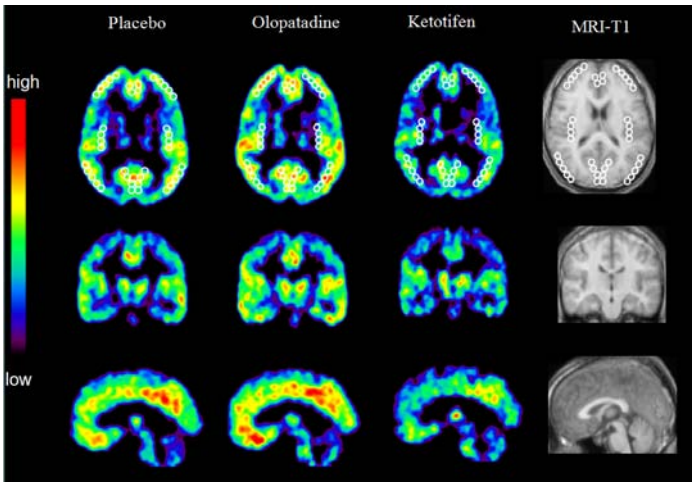
Each subject was randomly assigned to the treatment of 0.05% ketotifen (Zaditen® eye-drop, Novartis Pharma Corporation, Tokyo, Japan), 0.1% olopatadine (Patanol® eye-drop, Alcon, Inc, Tokyo, Japan) or a placebo (0.5% tranilast, Rizaben®, a mast-cell stabilizer, Kisei Pharma, Matsumoto, Japan) in a single-blind, crossover manner. The minimum washout period is 7 days. On the experiment day, the antihistamine-containing eye-drops or placebo, were instilled into both eyes of the subject (2 drops each eye with 5 min-intervals between each drop). Then the subject was asked to keep quiet in a supine position until the drug was adequately absorbed. Such instillation process would be repeated for 4 times with a 30 min break inserted (eg, around 0`, 30`, 60`. 90` after the first ocular instillation). During the break time, light music or walking in the room was permitted but reading or strenuous exercise was not. The label of the eye-drop was removed during the experiment to keep the subject blind to it. All the eye-drops were obtained commercially. After the fourth ocular instillation, subject was showed into the PET room and ^{11}C-doxepin-containing saline was injected intravenously. PET scan commenced about 70

min later with a SET2400W PET scanner (Shimadzu Co., Kyoto, Japan) according to our previous established static PET protocol. This protocol included a 15-min-long three-dimensional mode emission scan (70-85 min post ^{11}C-doxepin-injection) and a 6-min-long transmission scan thereafter.
In this study, one subject missed ketotifen-PET examination for irresistible personal reasons; two PET data (following ketotifen and placebo treatment, respectively) of another two subjects was eluted because of the low specific radioactivities of ^{11}C-doxepin below 20 GBq/μmol at the time of injection. Thus the sample sizes reduced to 5 for ketotifen and 6 for placebo, respectively, in our PET analysis.

Just prior to each time of ocular instillation (at 0`, 30`, 60`. 90` after instillation), subjective sedation was assessed by Line Analogue Rating Scale (LARS) and Stanford Sleepiness Scale (SSS). The LARS measurement assesses the sedation using a line scaled from 0, no sedation to 100, most marked sedation; SSS is composed of a 7 level self-report measure from feeling fully alert, level 1 to sleep onset soon, level 7. Subjects were asked to mark their present feelings on the line, and also select a statement to reflect their current level of alertness and sleepiness.

結果：

1. Brain distribution of ^{11}C-doxepin



After ^{11}C-doxepin injection, the radioligand was found apparently accumulated in H_1R-rich cortical regions, such as ACG and PCG, PFC, IC, LTC and MTC, PC, and OC. In the subjects treated with olopatadine or placebo, the ^{11}C-doxepin distribution patterns and intensity were similar. However, in the subject treated with ketotifen, radioactivity distribution appeared much lower than that in olopatadine or placebo (Figure). ^{11}C-doxepin, an H_1-antagonist, is known to compete with antihistamines for H_1R binding cites in the brain, which reflects inverse-proportionally the amount of antihistamines in the brain. Ketotifen-treated subjects appeared much lower specific binding of ^{11}C-doxepin compared with placebo- or olopatadine-treated subjects, suggesting that more ketotifen have entered into the brain instead.

2. Comparison of parametric of BPR images (Ketotifen vs. Olopatadine)

The parametric brain BPR images following treatment with ketotifen or olopatadine, were compared statistically on a voxel-by-voxel basis with those following treatment with the placebo using SPM5. In the ketotifen-treated subjects, ACG, PFC, PC, TC demonstrated significantly lower BPRs than those of the

placebo-treated subjects. Table 1 shows the detail coordinate information of these regions. In contrast, SPM5 analysis could not detect any area with significant lower BPR in the olopatadine-treated subjects than in the placebo-treated subjects.

3. **ROI-based comparison of BPR and H_1RO**

BPR in the different ROIs revealed significantly lower values in the case of ketotifen than in the case of olopatadine or the placebo in almost all the cortical regions studied except IFC and MTC ($P < 0.01$). No significant difference between olopatadine and the placebo was detected. H_1RO following ketotifen or olopatadine treatment was calculated considering the H_1RO after placebo treatment as baseline (0%). H_1ROs following ketotifen treatment were significantly higher than those following olopatadine treatment in all cortical regions studied. The mean H_1RO across all the cortical regions following ketotifen treatment was approximately 45.7% and that following olopatadine treatment was approximately -1.83%. The difference between mean cortical H_1RO following treatment with ketotifen and olopatadine was statistically significant.

3. Subjective sleepiness and their correlation with H_1RO

Individual subjective sleepiness is represented by the average scores of LARS and SSS data measured at approximately 0`, 30`, 60` and 90` min post-ocular illustration. Non-parametric analysis of Kruskal-Wallis test followed by Dunn`s multiple comparison failed to demonstrate statistical difference in sleepiness among the subjects treated with ketotifen, olopatadine or the placebo. Correlation analysis demonstrated that subjective sleepiness, as represented by area under the curve (AUC) of LARS measurement, showed moderate positive correlation ($r = 0.48$) with mean cortical H_1RO in the ketotifen treated subjects, but this correlation was not significant ($P = 0.16$). On the other hand, sleepiness did not correlate with H1RO in the case of olopatadine.

考察：

The primary aim of this study was to provide quantitative evidence via molecular imaging using ^{11}C-doxepin-PET on whether and to what extent the sedative effect happen in healthy subjects after anthistamine eyedrop instillation. We also compared this sedative effect of ketotifen with that of a second-generation antihistamine, olopatadine, which has been demonstrated to be a non-sedative. To the best of our knowledge, the present study is the first to verify the sedative effect using a direct measure of central occupancy with PET in human subjects. We found that the radioactivity distribution of the PET tracer, ^{11}C-doxepin, in subjects treated with ketotifen after instillation was much lower than that in olopatadine- or placebo-treated subjects. From this we know that ketotifen blocked a greater proportion of H_1Rs than olopatadine or placebo did at the time point examined. We confirmed these differences in terms of BPRs using both voxel-by-voxel and ROI-based comparison. As a result, most H_1R-rich brain regions demonstrated significantly lower BPRs in the ketotifen -treated subjects. On the other hand, there was no difference in BPRs between subjects treated with olopatadine and those treated with placebo. H_1RO of

ketotifen and olopatadine using H_1RO of each subject after placebo treatment as a baseline. Ketotifen and olopatadine H_1ROs at 12 h after dosing were 45% and 17%, respectively. In conclusion, topical instillation of ketotifen results in a predominant residual sedative effect, due to which high alertness demanding activities, such as driving, should be avoided, whereas the non-sedative olopatadine may have advantages over the first-generation antihistamines in the treatment of allergic conjunctivitis.

注：本研究は、2011年8月7～9日『第5回日中薬理ジョントミーティーング』にて口演発表。

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