Communication to the Editor

Allergen Stability and Immunological Reactivity during Co-dissolution and Incubation of House Dust Mite and Japanese Cedar Pollen SLIT-Tablets

Akiko Watanabe, a Takashi Yamamoto,a Hiroki Matsuara,b Hitoshi Matsuji,b Hiroshi Nakazawa,c Kaare Lund,d and Katsuyo Ohashi-Doi* a Torii Pharmaceutical Co., Ltd.; 3-4-1 Nihonbashi-Honcho, Chuo-Ku, Tokyo 103-8439, Japan; and b Papermill Medical; Ole Maaloes Vej 3, Copenhagen 2200, Denmark.

Received April 8, 2020; accepted July 12, 2020; advance publication released online August 4, 2020

Japanese allergic subjects are commonly sensitized to both house dust mite (HDM) and Japanese cedar pollen (JCP) and combined treatment with sublingual immunotherapy (SLIT) tablets is desirable. However, mixing extracts of two non-homologous allergens may compromise allergen stability and affect the clinical outcome. Therefore, we investigated the stability of major allergens and total allergenic reactivity of HDM and JCP SLIT-tablets following dissolution in human saliva or artificial gastric juice. Two fast-dissolving freeze-dried SLIT-tablets were completely dissolved and incubated at 37°C. Major allergen concentrations and total allergenic reactivity were measured. After mixing and co-incubation of HDM and JCP SLIT tablets in human saliva for 10 min at 37°C, there were no statistically significant changes in major allergen concentrations. In addition, no loss of allergenic reactivity of the mixed two SLIT-tablet solutions was seen. In contrast, complete loss of allergenic reactivity and detectable major allergen concentrations occurred when the two SLIT-tablets were dissolved and incubated in artificial gastric juice. These results demonstrate that HDM or JCP major allergens and the total allergenic reactivity of both SLIT-tablets measured here remain intact after dissolution and co-incubation in human saliva, supporting the possibility of a dual HDM and JCP SLIT-tablet administration regimen if clinically indicated. The complete loss of allergenic reactivity after incubation in artificial gastric juice can furthermore be taken to indicate that the immunological activity of the allergen extracts contained in the two SLIT-tablets is likely to be lost or severely compromised upon swallowing.

Key words house dust mite; Japanese cedar; sublingual immunotherapy-tablet; dual administration; allergen stability

INTRODUCTION

Allergic rhinitis (AR) caused by house dust mite (HDM) and Japanese cedar pollen (JCP) represents a significant and expanding health problem in Japan. An epidemiological study has shown that the prevalence of JCP-induced pollinosis has increased by 10% annually in recent years, and currently AR afflicts an estimated 40% of the Japanese population. Allergy immunotherapy (AIT) is a treatment that consists of repeated administrations of allergens to gradually establish and increase immunological tolerance to the sensitizing allergens. AIT is mainly performed as subcutaneous immunotherapy (SCIT) or as sublingual immunotherapy (SLIT) in the form of aqueous solutions of allergens or as sublingually administered tablets (SLIT-tablets). Unlike conventional pharmacotherapy, SLIT and SCIT address the basic immunological mechanisms of allergic disease and activates protective allergen-reactive pathways of the immune system. Therefore, in addition to providing relief from allergic symptoms, AIT is the only known treatment option with the potential to provide long-term post-treatment benefit and alter the natural course of the allergic diseases. Unlike SCIT, SLIT offers the convenience of home-administration with a reduced risk of severe systemic reactions which may make SLIT an appealing therapy, particularly for children.

Recently, SLIT in the form of fast-dissolving freeze-dried tablets has been developed for AR in Japan. Two SLIT tablets are available, CEDARCURE® (CDC) for JCP AR and MITICURE® (MTC) for HDM AR. These tablets were standardized in Japanese Allergy Units (JAU) by Japanese Society of Allergology based on major allergen concentration (CDC; Cry j 1, MTC; Der p 1 and Der f 1). As major allergens, the sensitization frequencies to these allergens are high: >90% of patients suffering from JCP pollinosis were reported to be sensitized to Cry j 1,1 and sensitization frequencies of >70% are typically reported for Der p 1 and Der f 1.2 In addition to being major allergens, Der p 1 and Der f 1 possess cysteine protease activity3) which may lead to loss of allergenic activity by proteolytic degradation if HDM extracts are mixed with extracts from other non-HDM allergen sources under conditions that allow cysteine protease activity. This is a potential challenge if extracts from multiple allergen sources are used for AIT, which is reflected by guidelines from the European Medicines Agency stating that allergens with proteolytic activities should not be used in mixtures.4)

The clinical efficacy of CDC and MTC has been demonstrated in large clinical trials.5-7) SLIT-tablets are held under the tongue for one minute and swallowed. The target organ for SLIT is the mucosal immune system, and fast release of allergens from the freeze-dried SLIT tablets following sublingual administration provides a high local concentration of allergens which facilitates the uptake of allergens by antigen-presenting cells resident in the sublingual mucosa.8) Gastrointestinal delivery of allergens is unlikely to be efficacious due to effective acidic and enzymatic breakdown in the stomach.

Japanese allergic subjects are commonly sensitized to both JCP and HDM and combined treatment with CDC and MTC is desirable. In this paper, the stability of major allergens and total allergenic reactivity of CDC and MTC when incubated separately or mixed together in human saliva under conditions that resemble SLIT was examined. The effect of swallowing on the integrity of MTC and CDC allergens was examined by incubation of the two tablets in artificial gastric juice.

MATERIALS AND METHODS

SLIT-tablets were dissolved separately or mixed together in a pool of human saliva collected from in-house volunteers, or
in assay buffer (100 mM phosphate, pH 6.8, 0.125% casein). In parallel, the SLIT-tablets were dissolved in artificial gastric juice (0.32% (w/v) pepsin, 0.03 mol/L NaCl, 0.084 mol/L HCl, pH = 1.2). The dissolved SLIT-tablets were incubated at 37°C. Samples were removed for analysis at t = 0 and t = 10 min. Major allergen content (Cry j 1, Der p 1, Der f 1, Der 2) was measured by enzyme-linked immuno sorbent assay (ELISA). Total allergenic reactivity was measured by competition ELISA using a pool of human sera essentially as described.9,10 All procedure employed in the study were approved by the Ethics Committees of TORII Pharmaceutical Co., Ltd. according to the Clinical Research Ethical Guidelines. The written informed consent was obtained from all subjects before the study. See Online Repository for additional experimental details.

RESULTS

The major allergen concentration following dissolution of CDC and MTC and incubation in human saliva is shown in Table 1. The major allergen concentration after dissolution in assay buffer served as the baseline (t = 0 min) control. Compared to the buffer control, no statistically significant changes in the major allergen concentrations were seen when CDC and MTC were dissolved separately in human saliva and incubated for 10 min at 37°C (Table 1). Likewise, when CDC and MTC were dissolved in human saliva, mixed together and co-incubated for 10 min at 37°C, no statistically significant difference in major allergen concentration compared to separate incubation of the SLIT tablets was detected (Table 1). These results show that human saliva itself had no negative impact on JCP

| Major allergen content | Buffer<sub>t=0min</sub> | Saliva<sub>t=10</sub> | MTC | CDC | MTC | CDC | MTC + CDC | MTC | CDC | MTC + CDC |
|------------------------|-------------------------|-----------------------|-----|-----|-----|-----|-----------|-----|-----|-----------|
| Der p 1                | 3.239 (0.37)            | 3.108 (0.20)          | 100.2                       | 0.960 (0.77)      | 99.1                       | 0.867 (0.73)      | 7.854 (0.63) | 93.9 (0.400) | 7.854 (0.63) |
| Der f 1                | 4.924 (0.56)            | 4.935 (0.77)          | 96.0                       | 0.517 (0.21)      | 96.0                       | 0.517 (0.21)      | 96.0 (0.20) | 96.0 (0.20) | 96.0 (0.20) |
| Der 2                  | 8.365 (0.43)            | 8.287 (0.73)          | 96.0                       | 0.517 (0.21)      | 96.0                       | 0.517 (0.21)      | 96.0 (0.20) | 96.0 (0.20) | 96.0 (0.20) |
| Cry j 1                | —                       | 6.607 (0.21)          | 96.0                       | 0.517 (0.21)      | 96.0                       | 0.517 (0.21)      | 96.0 (0.20) | 96.0 (0.20) | 96.0 (0.20) |

Major allergen concentrations (µg/mL) in after dissolution and incubation at 37°C of SLIT-tablets (MTC and CDC) in assay buffer, human saliva and artificial gastric juice. The data was expressed as the mean (standard deviation (S.D.)) (n = 4). N.D.; not detected.

Fig. 1. IgE-Reactivity in Saliva and Gastric Juice

IgE reactivity (A): HDM at t = 0, (B) HDM t = 10 min, (C) JCP at t = 0, (D) JCP t = 10 min. Red curve; Saliva, Blue curve; gastric juice. IgE-Reactivity was expressed relative to single dissolution (=100%, MTC or CDC) in saliva at the respective time-points. The data were expressed as the mean ± standard deviation (S.D.) (n = 4).
or HDM major allergen stability. The data furthermore demonstrate that even prolonged, compared to the recommended sublingual holding time, co-incubation in human saliva did not quantitatively reduce the concentrations of CDC or HDM major allergens released from CDC and MTC. None of the HDM or JCP major allergens examined could be detected after incubation of the SLIT-tablets in artificial gastric juice (Table 1). Total allergenic reactivity may be used as a measure for the potency of allergen extracts and can be determined by competitive ELISA. CDC and MTC were dissolved in human saliva (Fig. 1, red curves) or artificial gastric juice (Fig. 1, blue curves) and incubated separately or after mixing, respectively, at 37 °C. In Fig. 1, the inhibition of immunoglobulin E (IgE) binding to immobilized HDM (Figs. 1A, B) or JCP (Figs. 1C, D) by the mixed and co-incubated MTC and CDC solutions is shown relative to the IgE competition achieved with dissolved MTC or CDC alone. In human saliva, no loss of allergenic activity towards either HDM or JCP after co-incubation of the dissolved SLIT-tablets for 10 min was seen (compare red curves in Fig. 1). For HDM, IC_{50} = 0: 1511 and IC_{50} = 10 min: 1381 (Figs. 1A, B) and for JCP, IC_{50} = 0: 1049, IC_{50} = 10 min: 1089 (Figs. 1C, D). In contrast, immediate and complete loss of allergenic activity occurred when the two SLIT-tablets were co-incubated in artificial gastric juice (Fig. 1, blue curves). IC_{50} values could not be calculated.

DISCUSSION

In a recent clinical study, dual administration of CDC and MTC within 5 min of each other was demonstrated to be safe and induce similar immune responses as seen in clinical trials with CDC and MTC immunotherapy. Increased expression of allergen-specific antibodies (e.g. IgE and IgG4) is known to correlate with allergy immunotherapy, including SLIT, and is recognized as an immunological marker of treatment. Although direct evidence of stability of major allergens and allergenic reactivity has been lacking, the results in imply that the allergens of both CDC and MTC retain full allergenic activity when the SLIT-tablets are administered sublingually within a 5-min time interval. This is supported by the findings of this paper as no quantitative loss of major allergen concentration or reduction in total allergenic reactivity for either of the tablets was seen even after 10 min co-incubation of CDC and MTC in human saliva at body temperature. Additionally, the complete lack of measurable major allergen and total allergenic activity observed when CDC and MTC were dissolved and incubated in artificial gastric juice strongly indicates that once swallowed, the immunogenic effect of the allergens contained in the two SLIT-tablets is rapidly and completely lost and will in all likelihood not contribute to the immunological effect obtained through exposure of allergen to the sublingual mucosa.

Acknowledgments The authors thank Drs. Daichi Utsumi and Yuko Mitobe for expert technical assistance.

Conflict of Interest This work was funded by Torii Pharmaceutical Co., Ltd. AW, TY, HM, HN, KOD are employees of Torii Pharmaceuticals Co., Ltd. KL is a consultant for research project at Torii Pharmaceutical Co., Ltd.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES

1) Hashimoto M, Ngi H, Sakaguchi M, Inouye S, Imaoka K, Miyazawa H, Taniguchi Y, Kurimoto M, Yasueda H, Ogawa T. Sensitivity to two major allergens (Cry j I and Cry j II) in patients with Japanese cedar (Cryptomeria japonica) pollinosis. Clin. Exp. Allergy, 25, 848–852 (1995).
2) Becker S, Schlederer T, Kramer MF, Haack M, Vrtala S, Resch Y, Lupinek C, Valenta R, Gröger M. Real-life study for the diagnosis of house dust mite allergy—the value of recombinant allergen-based IgE serology. Int. Arch. Allergy Immunol., 170, 132–137 (2016).
3) Takai T, Kato T, Sakata Y, Yasueda H, Izhura K, Okumura K, Ogawa H. Recombinant Der p 1 and Der f 1 exhibit cysteine protease activity but no serine protease activity. Biochem. Biophys. Res. Commun., 338, 944–952 (2005).
4) European Medicines Agency. “Guideline on Allergen Products: Production and Quality Issues.” https://www.ema.europa.eu/documents/scientific-guideline/guideline-allergen-productsproduction-quality-issues_en.pdf, accessed 6 June, 2019.
5) Okubo K, Masuyama K, Imai T, Okamiya K, Stage BS, Seitzberg D, Konno A. Efficacy and safety of the SQ house dust mite sublingual immunotherapy tablet in Japanese adults and adolescents with house dust mite-induced allergic rhinitis. J. Allergy Clin. Immunol., 139, 1840–1848.e10 (2017).
6) Gotoh M, Yonekura S, Imai T, Kaneko S, Horikawa E, Konno A, Okamoto Y, Okubo K. Long-term efficacy and dose-finding trial of Japanese cedar pollen sublingual immunotherapy tablet. J. Allergy Clin. Immunol. Prac., 7, 1287–1297.e8 (2019).
7) Masuyama K, Okamoto Y, Okamiya K, Azuma R, Fujinami T, Riis B, Ohashi-Doi K, Natsui K, Imai T, Okubo K. Efficacy and safety of house dust mite sublingual immunotherapy-tablet in Japanese children. Allergy, 12, 2352–2363 (2018).
8) Mouingeon P, Mascarel L. Induction of tolerance via the sublingual route: mechanisms and applications. Clin. Dev. Immunol., 2012, 625474 (2012).
9) Du W, Fakatoo C, Yonemoto M, Matsuoka T, Masuyama K, Ohashi-Doi K. Comparison of the allergenic potency of house dust extract and house dust mite allergen extract for subcutaneous allergen immunotherapy. Biol. Pharm. Bull., 42, 601–606 (2019).
10) Kito H, Du W, Nakazawa H, Lund K, Ohashi-Doi K. The effective allergenic reactivity of house dust mite sublingual immunotherapy tablets is determined by tablet formulation. Biol. Pharm. Bull., 42, 1030–1033 (2019).
11) Ohashi-Doi K, Kito H, Du W, Nakazawa H, Ipsen H, Gudmann P, Lund K. Bioavailability of house dust mite allergens in sublingual allergy tablets is highly dependent on the formulation. Int. Arch. Allergy Immunol., 174, 26–34 (2017).
12) Gotoh M, Okubo K, Yuta A, et al. Safety profile and immunological response of dual sublingual immunotherapy with house dust mite tablet and Japanese cedar pollen tablet. Allergol. Int., 69, 104–110 (2020).
13) Ohashi-Doi K, Lund K, Ipsen H, Andersen PS, Mosbech H, Virchow JC, Kudo M, Stranzl T, Matsuoka T. Comparable responses of immunological markers in Japanese and European subjects after SQ HDM SLIT-tablet treatment. Allergol. Int., 69, 281–283 (2020).