Cannabis sativa as a clinically relevant nsLTP allergen in the Mediterranean region: a case series exemplifying different possible routes of sensitization

Alessandra Bennici[1], Daniel Grixti Soler[1], Giulia Marcassa[2,3], Gian Enrico Senna[2,3], Sebastiano Gangemi[1], Danilo Villalta[4], Paola Lucia Minciullo[1]

[1]Department of Clinical and Experimental Medicine, School and Operative Unit of Allergy and Clinical Immunology, University of Messina, Messina, Italy

[2]Asthma, Allergy and Clinical Immunology Section, University Hospital of Verona, Verona, Italy

[3]Department of Medicine, University of Verona, Verona, Italy

[4]Immunology and Allergy Unit, S. Maria degli Angeli Hospital, Pordenone, Italy

Summary

Cannabis is the most widely used drug worldwide sought for recreational and medicinal purposes. Cannabis allergy was first described 50 years ago but has become more frequently reported over the past decade due to a larger industrial and domestic cultivation, and an evolving legal status. However, it remains an infrequent cause of allergy in the Mediterranean European countries. We describe three clinical cases with primary sensitization to cannabis characterized by anaphylactic reactions. We hypothesize that in all three case reports, sensitization to Can s 3 nsLTP played a crucial role in the development of anaphylaxis, either directly following ingestion of hemp-containing food, or even indirectly through primary sensitization via involuntary exposure or occupation exposure to cannabis sativa.

Key words

Non-specific Lipid Transfer Proteins; cannabis sativa; cannabis allergy; can s 3

Impact statement

We report three clinical cases of primary sensitization to cannabis resulting in the development of non-specific lipid transfer protein allergy, confirmed with the Allergy Explorer 2 (ALEX2) Test.

Introduction

Cannabis allergy is generally described as an immediate hypersensitive reaction that occurs after exposure to any of multiple sensitization routes including active or passive smoking, pollen exposure, ingestion of cannabis-infused foods or beverages, and contact (1). Sensitization can occur in an occupational or non-occupational setting. The former tends to occur in cannabis industry workers, law enforcement personnel, and forensic technicians (2, 3). The most frequent allergic reactions involve the respiratory system, presenting with rhinoconjunctivitis and asthma. Other less common presentations are contact urticaria, generalized urticaria, and angioedema (4). Anaphylactic reactions to cannabis are rare but more likely when it is ingested. Can s 3 (cannabis nsLTP) has been identified as one of the ~~a~~ major allergens and sensitization to this molecule is frequently reported in patients presenting with cannabis allergy-related anaphylaxis (5) . Other allergens present in cannabis sativa are Can s 2 (cannabis profilin), Can s 4 (cannabis oxygen-evolving protein enhancer 2), and Can s 5 (cannabis PR-10), but their clinical relevance has not been yet defined (6). Cannabis' role in nsLTP allergy is not only as a primary sensitizer but also as a co-factor in other LTP food allergic reactions. Can s 3 is also responsible for cross-reactivity between cannabis and plant foods in a clinical entity known as the cannabis-fruit/vegetable

syndrome (1, 7). The cannabis-fruit/vegetable syndrome is not limited to oral manifestations but can present with anaphylactic reactions to food (8). In the Mediterranean region, nsLTP allergy is nearly always caused by primary sensitization to Pru p 3 (peach nsLTP) (9,10). We present three cases seen in two separate allergy and clinical immunology departments in Italy where Can s 3 seems to play the unlikely role of primary sensitizer in nsLTP allergy.

Clinical cases

Case 1

A 47-year-old man reached the Unit of Allergy and Clinical immunology of Messina in January 2020 with an allergic reaction following fennel ingestion. The patient reported two episodes characterized by OAS and respiratory involvement. In 2018, a few minutes after ingestion of raw fennel, the patient presented with tongue angioedema and shortness of breath, treated with intramuscular betamethasone with resolution of symptoms. In November 2019, after about 5-10 minutes from the ingestion of a mixed salad containing fennel and lettuce, he developed throat tightness, breathlessness, tongue and lip swelling. He was administered intramuscular betamethasone with scarce improvement, so he reached the nearest Emergency Department where he was treated with intravenous methylprednisolone, intramuscular chlorphenamine, and oxygen therapy, with gradual resolution of symptoms.

The patient's history was positive for persistent mild allergic rhinoconjunctivitis. In apparent good health, he denied taking any regular medications. Skin prick tests performed in January 2020 with commercially available standardized extracts, revealed a 10 mm wheal with flare for fennel and were negative for lettuce, peach, walnut, apple, peanut, pineapple, cabbage and lentil. An adrenaline self-injector was prescribed and the patient was educated on its correct usage. In

October 2021 in vitro multiplex allergy testing was carried out with ALEX2 Test which revealed sensitization for many nsLTP, as Can s 3 4,12 kU/L, Cor a 8 0,73 kU/L, Art v 3 0,71 kU/L, Api g 2 2,16 kU/L, Zea m 14 1,39 kU/L, and for Car c (cumin) 1,54 kU/L, and Pim a (anise) 2,05 kUL. Art v 1 was negative. Hazelnuts, celery, and corn were eaten and tolerated regularly. He did not eat cumin and anise as they were not part of his dietary habits.

In February 2023, specific IgE (sIgE) testing was carried out again using ImmunoCAP, with the following results: fennel 3,46 kU/L, peach 1,75 kU/L, Pru p 3, 3,21 kU/L, Art v 3 1,39 kU/L, hazelnut 0,80 kU/L, Artemisia 0,69 kU/L, corn 0,37 kU/L. sIgE to Can s 3 was not available. The sensitization to peach was considered new as was not detected in previous skin prick testing and ALEX2 test. Nevertheless, the patient insisted that he was still eating peach normally without any symptoms suggesting clinical allergy.

Given the positive sIgE reactivity to recombinant Can s 3, the patient was asked about previous cannabis use or exposure. He recalled two episodes of sneezing, conjunctival erythema, and nasal obstruction in the accidental exposure to marijuana smoke. The nsLTP cross-sensitivites in ALEX2 test, the relatively high values of Can s 3 with clinical relevance, and the absence of peach sensitization in vivo and in vitro tests at the time of clinical reactions, the absence of Art v 1 sensitization, suggest that Can s 3 was the primary sensitizer for nsLTP driven fennel allergy. Nonetheless, the remote possibility that fennel was the primary sensitizing agent in this LTP syndrome cannot be completely ruled out.

Case 2

A 48-year-old woman with a history of seasonal rhinoconjunctivitis and chronic spontaneous urticaria, was first seen in the Allergy and Immunology Unit of Pordenone in 2018 after suffering three previous episodes of anaphylaxis (with acute urticaria and bronchospasm) requiring treatment at the Emergency Department. All the episodes occurred after the association of a meal with different foods and physical exercise, but she lately tolerated all the foods involved in those reactions, even when in association with physical activity. In June 2018, skin prick tests and a multiplex specific IgE with ISAC assay were performed, highlighting positivity for many pollens and nsLTP (Ara h 9, Cor a 8, Jug r 3, Pru p 3, Art v 3, Pla a 3). Since there wasn't a clear correlation between assumption of nsLTP-containing foods and clinical reactions, even when she performed physical exercise, she was diagnosed with "idiopathic anaphylaxis". As a precautionary measure she was educated to avoid co-factors when eating foods appartaining to nsLTP to which she was already sensitized. An adrenaline autoinjector was prescribed and a regular inhalatory treatment was started, since a low-grade hyperreactivity appeared from the spirometry with methacholine provocation test. The patient came back to Allergy Unit in 2020, because she worked as a hemp seed farmer and was experiencing acute rhinoconjunctivitis, dyspnea and urticaria every time she had contact with *Cannabis Sativa* leaves; In the suspicion of Can s 3 allergy, ALEX2 test was performed and highlighted sensitization to many PR-10 (Bet v 1 25.41 kUA/L, Cor a 1 13.11 kUA/L, Ara h 8 2.71 kUA/L, Fag s 1-6.82 kUA/L, Alng 1 1.21 kUA/L, Gly m 4 6.8 kUA/L, Mal d 1 5.86 kUA/L), Cup a 1 (pectate lyase) 3.88 kUA/L, Fra a 1 + 3 (PR-10+LTP) 29.44 kUA/L and to many nsLTP: Cor a 8 (hazelnut) 6.35 kUA/L, Ara h 9 (peanut) 8.05 kUA/L, Art v 3 (mugwort) 5.05 kUA/L, Act d 10 (kiwi) 10.44 kUA/L, Zea m 14 (corn) 16.20 kUA/L, Mal d 3 (apple) 12.82 kUA/L, Pla a 3 (plane tree) 6.95 kUA/L, Pru p 3 (peach) 8.63 kUA/L, Vit v 1 (grape) 3.55 kUA/L, Api g 2 (celery) 22.74 kUA/L, and Can s 3 (hemp) 31 kUA/L.

She was subsequently instructed to wear a face mask and gloves while working with hemp and her daily treatment for asthma and chronic urticaria was confirmed. She has not experienced any respiratory symptoms or anaphylactic episodes since.

Case 3

A 45-year-old woman with no history of atopic disease was evaluated in the Allergy and Clinical Immunology Department of Pordenone in April 2023 following an anaphylactic episode (urticaria, dyspnoea, and dysphonia) five minutes after eating a homemade cake. There were no evident co-factors. She received oral prednisone, intravenous corticosteroids, antihistaminic therapy and eventually nebulized adrenaline; the latter was administered in the Emergency Department for the persistence of marked dysphonia, since highly concentrated inhaled epinephrine may offer an effective treatment option in imminent anaphylactic reactions. (31) As vital parameters were stable, no intramuscolar adrenaline was administered. Since hemp flour was one of the ingredients contained in the "culprit cake", an ALEX2 was performed. It highlighted sensitization to pollen PR-10 as Bet v 1 (2.32 kUA/L), Fag s 1 (3.15 kUA/L) and to Cup s (0.9 kUA/L); there was sensitization also for some nsLTPs: Ara h 9 (peanut,) 4.7 kUA/L), Pru p 3 (peach,) 1.21 kUA/L), Can s 3 (hemp,) 10.78 kUA/L). Given the positive sIgE reactivities listed above, a more detailed history was taken. Sensitization to PR-10 proteins, Pru p 3 and Ara h 9 had no clinical correlation, in fact the patient tolerated both peanut and peach after April 2023. Can s 3 had the highest sIgE titre in the results and was suspected to be the primary sensitizer; the patient claimed it was the first time she ate any food made with hemp, but she previously smoked weed for recreational purpose.

Discussion

LTP syndrome has three distinct clinical presentations. The first is characterized by sensitization to food without sensitization to pollen, the second involves primary sensitization to food with subsequent sensitization to pollen, and the third is characterized by primary sensitization to pollen and subsequent food allergy (10). In LTP allergy, peach generally represents the primary sensitizing agent in the Mediterranean area, while in the non-Mediterranean area such as in Central-Northern Europe, the responsible allergens are multiple, for example, plane tree, mugwort, and olive tree (11). In the three cases presented, the value for sIgE to Can s 3 was higher than that of any other nsLTP allergen including that of peach to which patient one only became sensitized at a later stage after the allergic reactions to fennel. The other two patients presented sensitization to Pru p 3 at presentation but without clinical manifestations.

A previous study showed that there are two IgE clusters to nsLTP polypeptides. The first one includes Pru p 3 and Mal d 3, two proteins that share an elevated structural sequence identity, while the second cluster includes 9 kDA nsLTP molecules and Can s 3 (12,13). We hypothesize that patient 1 belongs to the second cluster in which fennel nsLTP is a 9 kDA protein and Can s 3 represents the primary sensitizer to nsLTP. In a previous study on Italian patients with nsLTP allergy, the values for specific IgE to Pru p 3 were superior to the specific IgE to pollen nsLTP in most patients. Inhibition studies carried out on nsLTP reactors showed that mugwort and plane pollen commercial extracts were unable to significantly inhibit Pru p3 IgE reactivity. While considering the evidence for nsLTP allergy being a pollen-food syndrome as very thin, they would not rule out the possible sensitization to nsLTP via the airways (14). Patient 1 denied active smoking of marijuana, however, there have been previous descriptions of cases in which

sensitization occurred via passive inhalation or airborne pollen in Northern Italy and even in Northern Europe (8,10,11).

As confirmed by the case of patient 2, prolonged occupational exposure to cannabis typically results in occupational allergy, presenting with dyspnea, airflow obstruction, or contact urticaria (15,16). Cofactors such as alcohol and physical exercise might have had a role in the development of her early anaphylaxis episodes, unmasking a nsLTP allergy. The concomitant PR-10 sensitization could have had a protective role in the absence of co-factors (17) but the main role is probably due to continuous inhalation and cutaneous exposure to the primary sensitizer Can s 3. Early reports of allergic reactions to Cannabis sativa were of contact dermatitis after touching cannabis leaves or flowers (4). The first occupational allergy to hemp was described in 2008, in a forensic science technician who developed contact urticaria to cannabis, due to regular handling of cannabis (18). Later, other cases of occupational urticaria occurring after contact with Cannabis sativa were published (15). Despite this, unfortunately, it has not been established as a relevant allergen in the context of occupational exposures yet (3). Nowadays the evidence on allergic sensitization to cannabis consists mostly of case reports; consequently, it is not possible to establish the true burden of occupational allergy and different routes of exposure (i.e. inhaled, ingested, or skin contact) may result in different patterns of molecular allergic reactivity(19). The occupational pathophysiology of cannabis allergy is also complicated by the presence of other hazards as bacteria and irritants. Identification and validation of additional cannabis allergens useful in occupational exposure is certainly needed, as Can s 3 appears to be less relevant in occupational allergies (20).

Anaphylaxis-like reactions seem to be a common manifestation of Cannabis allergy and can occur in up to 20% of allergic patients; they can occur after ingestion of hemp seed or marijuana tea, or

less commonly after smoking (21). Patient 3 previously smoked marijuana in his life and he developed an immediate life-threatening reaction upon ingestion. We can hypothesize that Can s 3 is the primary sensitizer and previous smoke exposure played a crucial role in sensitization and development of subsequent food allergy. Can s 3 can lead to sensitization to other cross-reactive nsLTPs even via passive inhalation (22). Over time many case reports have reported cross-reactivities to different nsLTP foods in cannabis allergic patients, and the clinical entity cannabis-fruit/vegetable syndrome was born (23, 24). Fennel may be one of these cross-reacting foods, as suggested by patient 1 (25-28). Interestingly, the phenomenon of food allergic reactions occurring in individuals sensitized to Cannabis sativa has only been reported in Europe (4).

Conversely, sensitization to Can s 3 frequently occurs in the presence of other nsLTP sensitizations in patients who never had contact with hemp in their lifetime, confirming that Can s 3 sensitization can occur because of *in vitro* cross-reactivity to other nsLTPs (29). This does not appear to be the case in the three cases described who all tolerated peach. Moreover, it has already been demonstrated that in a group of 120 people with Cannabis allergy, only 66% were sensitized to Pru p 3; that means there is also a percentage of allergic patients primarily sensitized to Can s 3, with absence of sIgE for Pru p 3.(30)

In conclusion, these clinical cases show that in the Mediterranean region, also Can s 3 could represent the primary sensitizing allergen in a nsLTP syndrome in absence of laboratory and/or clinical manifestations of IgE-mediated reactions to peach, as already extensively reported in Northern-European Countries.

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Contributions

AB, DGS, PLM, DV, GM : conceptualization; AB, DGS, GM :writing - original draft; GES, SG, DV PLM: writing - review & editing; AB, DSG, GM: data curation; GES, SG, DV PLM: supervision.

Conflict of interests

The authors declare that they have no conflict of interests.

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