Anaphylaxis in a canine patient sensitized to human albumin while on honeybee immunotherapy

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Abstract: Venom immunotherapy (VIT) provides an opportunity to reduce life-threatening reactions to venomous insect stings. VIT requires stabilization with human albumin. This report describes a Labrador Retriever that developed anaphylaxis during VIT induction. Intradermal testing (IDT) identified a human albumin hypersensitivity. Four years later, re-testing revealed a persistent hypersensitization to human albumin. To identify if VIT is a risk factor for human albumin sensitivity, IDT was performed in 4 honeybee allergic dogs being treated with VIT, with 6 healthy and 6 atopic dogs as controls. One other dog reacted to human albumin at the testing concentrations. This dog was being treated with VIT but had no clinical manifestations. VIT containing human albumin may lead to sensitization of this protein and anaphylaxis.

Key words: Anaphylaxis, venom immunotherapy

Introduction

Venomous insect stings can cause severe reactions in companion animals ranging from localized pain/swelling at the site of the sting to systemic allergic reactions including anaphylaxis and death. These reactions are most commonly caused by Hymenoptera, particularly Apoidea (bees), Vespoidae (wasps, hornets, yellow jackets), and Formicidiae (fire ants).

The severity of the reaction will determine treatment recommendations. Treatment options include but are not limited to anti-histamines, glucocorticoids, epinephrine, and hospitalization with fluid and oxygen support. When anaphylaxis occurs from venomous insect stings, venom immunotherapy (VIT) provides an additional treatment option which may prevent or limit the severity of future reactions.
Venom Immunotherapy Leading to Human Albumin Sensitization

VIT is effective in human medicine in preventing systemic allergic reactions to hymenoptera2). Humans treated with VIT have a less than 3% risk of subsequent reactions to bee stings compared to 75% risk of subsequent reactions in untreated patients2). These statistics on response to VIT in the canine have not been established.

Currently, the availability for VIT in veterinary medicine is limited. ALK has been the only distributor of VIT to USA veterinarians for the past 4 years. Additionally, albumin is required to stabilize the VIT. As VIT is produced for the human market, human albumin is used in ALK’s product as the stabilizing protein1). Without the albumin stabilizer, the dosing and expiration of the product is not guaranteed by the manufacturer. The ALK venoms have been used by the veterinary field for VIT, in the USA, for the past 5 years. The product is provided in the form of a powder that must be reconstituted. The human albumin stabilizer is contained in the powder and cannot be separated from the VIT in the product provided by ALK. The concentration of human albumin in a reconstituted ALK venom vial is 0.06%1).

This case report describes a patient experiencing anaphylactic reactions during induction of Apis mellifera (honeybee) VIT believed to be caused by human albumin sensitivity. This paper also discusses additional research on human albumin sensitivity in healthy dogs, environmentally allergic dogs, and other dogs receiving honeybee VIT.

Case Report

The patient presented as a 5 month old female intact Labrador retriever 1 week after anaphylaxis due to a bee sting. The anaphylactic episode included collapse with uncontrollable vomiting and diarrhea. Venomous insect testing was postponed until 3 weeks post-anaphylactic reaction due to the post-sting refractory period that can lead to loss of sensitivity during testing3). At this time, an intravenous catheter was placed and the patient was sedated with dexmedetomidine (0.29 mg; 0.015 mg/kg; Dexdomitor; Zoetis, Kalamazoo, MI, USA). An intradermal allergy test (IDT) was performed utilizing venom from honeybee, wasp, white hornet, yellow jacket, and yellow hornet (ALK; Round Rock, TX, USA). A total of 0.05 mL was delivered for each intradermal injection via a 27-gauge needle. Saline (Greer® Laboratories; Lenoir, NC, USA) and histamine 0.005% (Histatrol; ALK, USA) acted as the known negative and positive controls. Briefly describing the honeybee venom testing: honeybee venom (0.01%; ALK, USA) underwent titration dilution with normal saline with phenol. The lowest concentration tested (0.00000001% (1 × 10⁻⁸)) was initially injected. If there was no positive reaction within 10 minutes, the venom was tested at a 10 fold increase in concentration until 0.001%, which the author’s consider an irritant. Wheal scores were based on objective scoring comparing the diameter of the known saline and histamine wheals. A positive reaction occurred when the diameter of the wheal was 75% greater than the mean of the diameter of the histamine and saline control wheals.

The patient had a positive wheal at 0.0001% for honeybee venom and VIT was recommended. The other venomous insects tested negative.

Since the VIT utilized human albumin as a stabilizer, which is a foreign protein, human albumin was obtained to test for a hypersensitivity. Canine albumin (ALK, USA) was also obtained to act as the negative control for human albumin. An IDT was performed as described above utilizing human albumin and canine albumin along with re-testing of honeybee venom containing human albumin. The same grading system was used to indicate a positive wheal reaction. The patient had
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a positive wheal to honeybee at 0.0001%, similar to the results obtained at initial testing. Human albumin (0.03%; ALK, USA) underwent titration dilution. The patient had a positive wheal at the initial testing concentration of 0.0003%. End-point titration was then performed utilizing a 10-fold decrease in concentration for each subsequent injection. The patient had a positive wheal down to and including 0.0000003% (3 × 10⁻⁷). All canine albumin wheals, which were tested up to 1.0%, were negative. This testing demonstrated a high sensitivity to human albumin. Due to the inability to remove human albumin from the VIT, it was elected not to pursue any further VIT treatment due to the belief that the VIT was sensitizing the patient to human albumin and leading to vomiting.

The patient was examined approximately 4 years after her initial testing for human albumin to see if a human albumin sensitivity was still demonstrable. She was approximately 5 years of age at re-examination. The patient had not had an episode of anaphylaxis since she was a puppy. However, the owner takes precautions to avoid venomous insects including limiting walks in the spring/summer. Also, the owner carries injectable diphenhydramine on the patient’s collar for emergencies at all times. Due to the constant fear of another anaphylactic reaction, the owner was hoping that VIT could be re-instituted.

Human albumin (0.03%; ALK, USA) underwent titration dilution and an IDT was performed as described above. The same objective scoring was performed to determine positive wheals. The first two intradermal injections were negative (0.00000003% (3 × 10⁻⁸); 0.0000003% (3 × 10⁻⁷)). The patient tested positive to human albumin at 0.0000003% (3 × 10⁻⁸) and 0.00003% (3 × 10⁻⁵), where the testing was stopped. This demonstrated a long-lasting human albumin sensitivity. The patient also underwent re-testing for honeybee venom as described above. The patient had a similar positive wheal at 0.0001%. Table 2 compares the initial testing to the testing 4 years later.

### Table 1. Venom immunotherapy schedule

| Injection no. | Dilution (%) | ml administered | Date     | Anti-histamine Pre-treatment | Adverse effects                                |
|---------------|--------------|-----------------|----------|-----------------------------|-----------------------------------------------|
| 1             | 0.0001%      | 0.05 ml         | 2/4/2014 | No                          | None                                          |
| 2             | 0.0001%      | 0.1 ml          | 2/11/2014| No                          | None                                          |
| 3             | 0.0001%      | 0.2 ml          | 2/18/2014| No                          | None                                          |
| 4             | 0.0001%      | 0.4 ml          | 2/25/2014| No                          | None                                          |
| 5             | 0.001%       | 0.05 ml         | 3/4/2014 | No                          | None                                          |
| 6             | 0.001%       | 0.1 ml          | 3/11/2014| No                          | None                                          |
| 7             | 0.001%       | 0.2 ml          | 3/18/2014| No                          | None                                          |
| 8             | 0.001%       | 0.4 ml          | 3/25/2014| No                          | None                                          |
| 9             | 0.01%        | 0.05 ml         | 4/1/2014 | No                          | None                                          |
| 10            | 0.01%        | 0.1 ml          | 4/8/2014 | No                          | None                                          |
| 11            | 0.01%        | 0.2 ml          | 4/15/2014| No                          | None                                          |
| 12            | 0.01%        | 0.4 ml          | 4/22/2014| No                          | None                                          |
| 13            | 0.01%        | 0.6 ml          | 4/29/2014| No                          | Vomited/Defecated 20 minutes post injection    |
| 14            | 0.01%        | 0.4 ml          | 5/13/2014| No                          | Vomited <30 minutes post injection            |
| 15            | 0.01%        | 0.2 ml          | 5/27/2014| Yes                         | None                                          |
| 16            | 0.01%        | 0.2 ml          | 6/10/2014| Yes                         | None                                          |
| 17            | 0.01%        | 0.2 ml          | 6/24/2014| Yes                         | Vomited 10 minutes post injection             |

The venom immunotherapy was diluted with normal saline with phenol.

Case Report: Supplementary Testing

To provide further evidence of the frequency of human albumin hypersensitivity being due to the exposure of VIT, a small study was performed by the authors. This supplementary testing did not include the patient above. The study protocol was approved by the Animal Dermatology Clinic Research Committee. The procedure below was described to the owners. The owners were provided with and signed consent forms...
prior to the procedure. Six healthy dogs, six dogs with environmental allergies diagnosed by a dermatologist, and four dogs being treated with maintenance honeybee VIT had an IDT performed utilizing human albumin (Table 3). Saline (Greer\textsuperscript{®}, USA) and histamine 0.005% (ALK, USA) again acted as the known negative and positive controls. Positive objective wheal scores were documented as previously stated. Each dog was initially injected with 0.05 ml of 0.0003% human albumin (ALK, USA). If the result was negative, 0.05 ml of human albumin was injected at 0.03%, which was the undiluted form. One dog that was being treated with maintenance VIT tested positive at 0.0003%. End-point titration was performed. This dog also tested positive down to and including 0.000000003% ($3 \times 10^{-8}$). However, there was no evidence that human albumin was a clinically relevant allergen in this patient as no reaction to VIT had been noted. The remaining dogs did not react at 0.0003% or 0.03%.

The four dogs that were being treated with honeybee VIT also underwent re-testing for honeybee (ALK, USA) and testing was performed as described above. Two of four dogs appeared to be less sensitive to honeybee compared to their initial testing based on concentrations that elicited a positive wheal, one of the dogs was considered the same sensitivity from their

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**Table 2.** Comparison of 2014 and 2018 testing to honey bee venom and human serum albumin

| % solution          | 2014     | 10\textsuperscript{2} | 10\textsuperscript{3} | 10\textsuperscript{4} | 10\textsuperscript{5} | 10\textsuperscript{6} | 10\textsuperscript{7} | 10\textsuperscript{8} |
|---------------------|----------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Honey bee (0.01%)   | Undiluted 0.01% (1 \times 10\textsuperscript{-3}) | Not tested             | +                      | –                      | –                      | –                      | –                      | –                      |
| Human serum albumin (0.03%) | Undiluted 0.03% (3 \times 10\textsuperscript{-2}) | Not tested             | +                      | +                      | +                      | +                      | –                      | –                      |

A positive reaction occurred when the diameter of the wheal was 75% greater than the mean of the diameter of the histamine and saline control wheals.

**Table 3.** Breed, age, sex, and weight of supplementary dogs

| Breed                        | Age | Sex | Weight (kg) |
|------------------------------|-----|-----|-------------|
| Healthy Dogs                 |     |     |             |
| 1. Mix Breed                 | 12 yr | MN | 5.0        |
| 2. Rottweiler                | 8 yr | MN | 45.5       |
| 3. Rottweiler                | 5 yr | FS | 37.7       |
| 4. Australian Cattle Dog     | 2 yr | FS | 5.5        |
| 5. Terrier Mix               | 5 yr | FS | 4.5        |
| 6. German Shepherd Mix       | 1 yr | FS | 20.5       |
| Atopic Dogs                  |     |     |             |
| 1. Labrador Retriever        | 2 yr | MN | 33.2       |
| 2. Terrier Mix               | 8 yr | MN | 20.0       |
| 3. Boxer                     | 3 yr | FI | 22.7       |
| 4. Miniature Pincher         | 8 yr | FS | 3.6        |
| 5. West Highland White Terrier | 8 yr | MN | 10.5       |
| 6. German Shepherd           | 9 yr | FS | 35.0       |

| Dogs currently on venom immunotherapy |     |     |             |
| 1. French Bulldog            | 10 yr | FS | 7.7        |
| 2. Poodle Mix                | 2 yr | FS | 23.2       |
| 3. Australian Cattle Dog     | 1 yr | FS | 19.1       |
| 4. Papillion Mix             | 3 yr | FS | 6.8        |

M: male; F: female; S: spayed; N: neutered; I: intact.
Table 4. Lowest concentration of honeybee venom that led to positive wheal formation prior to venom immunotherapy compared to the lowest concentration of honeybee venom that led to a positive wheal formation while patient was on maintenance venom immunotherapy.

| Venom Immunotherapy Patient | Lowest concentration at which honeybee venom led to a positive reaction prior to starting venom immunotherapy | Lowest concentration at which honeybee venom led to a positive reaction while on maintenance venom immunotherapy |
|-----------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| 1                           | 0.0000001% (February 2017)                                                                      | 0.00001% (July 2018)                                                                            |
| 2                           | 0.0001% (February 2018)                                                                          | 0.001% (July 2018)                                                                               |
| 3                           | 0.0001% (February 2018)                                                                          | 0.0001% (August 2018)                                                                           |
| 4                           | 0.0001% (October 2016)                                                                           | 0.000001% (August 2018)                                                                         |

initial testing, and one of the dogs was considered more sensitive compared to their initial testing (Table 4). The dog that was more sensitive was the same dog that reacted to the human albumin in the previous paragraph.

Discussion

This case report demonstrates and supports previous claims that utilizing VIT with human albumin can sensitize canine patients to this foreign protein. This is the first case report to the authors’ knowledge that demonstrates reactivity to human albumin in VIT in the canine. Furthermore, clinical systemic allergic reactions, such as vomiting, may occur. Although the authors believe that this patient was vomiting due to a hypersensitivity reaction to the human albumin, it was not possible to completely rule out a reaction the honeybee.

At the time of this study, there was not an available honeybee venom not containing human albumin to definitively make this claim. The case also demonstrates that human albumin sensitization can be long lasting. Despite VIT not being administered for approximately 4 years, this patient was still highly reactive to human albumin upon re-testing.

In the supplementary testing, one additional dog was sensitive to human albumin based on IDT. This dog was being treated with VIT, which utilized human albumin as a stabilizer. This patient was more sensitive to honeybee venom on re-test. The authors believe that the increased sensitivity in this additional patient was due to human albumin in the honeybee venom, though at this point there is no evidence it was a clinically relevant allergen as no adverse reactions had been noted at the clinic or reported by the owners. The owners of this patient elected to stop VIT due to concerns of reactions, based on IDT test results, to continued treatment with human albumin and the possibility of more severe reactions to an actual honeybee sting. Human albumin may lead to false assessment/failure of VIT.

In all testing, 2/5 patients being treated or previously treated with VIT were sensitized to human albumin. Only 1 of these patients had anaphylactic reactions. Veterinarians must be aware that administering VIT with human albumin may induce human albumin sensitivity, which can lead to anaphylaxis. Future VIT that does not utilize human albumin may allow for re-institution of VIT in these patients.

No authors have any conflict of interest.

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