Safety Evaluation of Allergen Specific Immunotherapy without an Induction Phase Using Calcium Phosphate Adjuvanted Allergenic Extracts in Atopic Dogs: A Retrospective Study of One Hundred Eighty Five Cases

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KEY WORDS: Monodose immunotherapy, ASIT, Allergen specific immunotherapy, Calcium Phosphate adjuvanted, Dogs, Atopic dermatitis.

ABSTRACT

Background

The treatment of atopic dermatitis is multimodal. When offending environmental allergens are identified, an allergen specific immunotherapy (ASIT) can be combined with other treatments, and it is the only treatment with a potential disease-modifying effect on allergic subjects. Traditionally, ASIT consists of subcutaneous administration of increasing doses of allergens up to a maintenance dose. The major drawback of such a protocol is the escalating phase, which presents a risk of incorrect dosage and lack of compliance from owners. There is a need for simplification of existing protocols to increase compliance and adherence.

Objective

To report the safety of a protocol of ASIT without an induction phase.

Method

Medical records of atopic dogs sensitized to common allergens and treated for at least 1 year with monthly subcutaneous injections of calcium phosphate conjugated allergenic extracts at maintenance dose without an escalating phase were reviewed. Signs of adverse reactions at the time of and 30 minutes

after each injection were reported.

Results

One hundred eighty five atopic dogs presenting a positive intradermal skin testing for domestic mites or pollens were included in the study . Each dog received a monthly subcutaneous injection of 0.8 ml of calcium phosphate adjuvanted allergenic extracts (Biogenix®, Ceva Biovac, France) at the following concentrations: mites 1/10000 w/v, pollens 1/1000 w/v. At the first injection, 104 dogs were not receiving any immunomodulating treatment, 41 were receiving oclacitinib, 22 cyclosporine, and 13 cetirizine. Except for one dog that displayed a reversible swelling at the injection site following the first injection, no adverse reactions were observed.

Conclusion

This protocol is safe. Thanks to the simplification (one vial for 1 year of treatment administered at the same dosage once a month), it could be a significant aid in prescribing ASIT. It may improve compliance, which would be helpful since 1 year is often necessary before ASIT efficacy can be measured on treated dogs.

BACKGROUND

Atopic dermatitis is a chronic inflammatory skin disease that can affect both humans and dogs, for which the treatment is multimodal. Most of the time, clinical features of atopic dermatitis are associated with IgE antibodies directed against environmental allergens.[1] When offending environmental allergens are identified, an allergen-specific immunotherapy (ASIT) can be added to other treatments.

ASIT consists of administering gradually increasing quantities of an allergen extract to an allergic subject to improve the symptoms associated with subsequent exposure to the causative allergen.[2] ASIT is the only treatment with a potential disease-modifying effect on allergic subjects. It can alter the response to an allergen and decrease the intensity of flares and clinical signs after contact with the allergen. Several methods are used with different doses, extracts administration, and schedules of allergen, but their standardization is not established yet. Currently, there is no consensus on the best protocol to use and no protocol has shown advantage over another one.[3] The precise mechanism of desensitization is not fully understood, but a combination of immune changes has been identified. Among them, during the induction phase, the early desensitization of mast cells and basophils is responsible for a decreased sensitivity to allergens and prevention of these cells from degranulation and systemic anaphylaxis.[4] The initial progressive escalating phase described in most protocols aims at this early desensitization and thus is considered to be a safe method.

Nevertheless, as with any therapy, one of the cornerstones is compliance. For this purpose, new protocols are described: among them, rush immunotherapy comprises an accelerated escalating phase undergone with hospitalization of the patient in order to reach the maintenance phase more quickly and safely.[5] Sublingual immunotherapy is increasingly used to facilitate administration by owners who can be reluctant to use injections on their dog, but requires daily administration.[6] A monodose protocol therapy was described a few years ago with a monthly injection of a maintenance dose of alum adjuvanted extract instead of an escalating phase.[7] In this study, a preventive concomitant treatment was performed using cetirizine as the lack of escalating phase with progressive increasing dosage was thought to potentially lead to adverse reactions. No studies are currently available on the safety of monodose ASIT (ie, without an escalating phase) with or without concomitant medication.

Using the same dose every month without an escalating phase would represent a good opportunity and a simple way to improve compliance and thus the success of ASIT. The aim of this retrospective study was to assess the safety of such a protocol in sensitized atopic dogs.

MATERIALS AND METHODS

The study was designed as a retrospective

study. Medical records from one practice were searched between October 2012 and October 2016 for dogs having received an ASIT without an induction phase.

Animals

Inclusion criteria comprised a clinical diagnosis of atopic dermatitis based on at least four major Prélaud's criteria.[8] Ectoparasitic diseases had been ruled out based on the negative results of multiple skin scrapings, coat brushings, and serological testing for sarcoptic mangement when necessary. All dogs had undergone, without improvement, an 8-week food trial using a novel home-cooked protein or commercial hydrolyzed diet. Exclusion criteria were dogs weighing less than 2 kg or presenting uncontrolled or controlled cardiac insufficiency or cardio-respiratory symptoms. All included dogs were recorded to be mono or poly-sensitized for the following allergens according to the results of the intradermal skin testing (IDT) they had undergone using a standard testing battery (Biogenix®, Ceva Biovac, France):

- *Dermatophagoides pteronyssinus*
- *Dermatophagoides farina*
- *Tyrophagus putrescentiae*
- *Lepidoglyphus destructor*
- *Acarus siro*
- Flea
- molds (*Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*)
- grasses (orchard, meadow fescue, ryegrass perennial, timothy)
- weeds (common mugwort, english plantain, lamb's quarter) and
- trees (oak, birch spring, black willow, olive, cypress).

For inclusion in the study, the records had to contain a precise report of safety assessment following the first injection as well as all following ones during the study period of at least 12 months.

ASIT Protocol

All included dogs received the following ASIT protocol: a calcium phosphate adjuvanted ASIT (Biogenix®, Ceva Biovac, France) containing a mixture of allergens specific for each dog and formulated based on the results of IDT. Mixtures contained as many allergens as needed according to the results of IDT, with the following composition:

- NaCl 9g/L
- Phenol 4g/L
- Glycerol
- Calcium phosphate and allergens (mites 1/10 000 w/v eq. 100 NU (Noon Unit) and pollens 1/1 000 w/v eq. 1000 NU)

Moulds and insect extracts were not used due to the risk of loss of antigenicity of the mixture.[9] A subcutaneous injection of 0.8 mL of the solution was administered at the first injection and then every 4 weeks during at least 1. The injection dose was the same for all dogs.

Concomitant Treatments

All concomitant treatments were allowed except for vaccination within 2 weeks. (at least 2 weeks from the ASIT injection).

Safety Assessments

Definition

Safety refers to ASIT-related reactions that occur far from the site of administration and include both life-threatening and non-life-threatening systemic adverse events.[10]

Clinical Adverse Events

The following clinical signs, compatible with adverse reactions, were recorded:

- Diarrhea
- abdominal pain
- facial urticaria
- erythema
- pruritus on the axillae, groin, paws and perianal area
- somnolence, and
- anaphylaxis.

RESULTS

Animals

The search identified 185 dogs fulfilling the inclusion criteria. They were aged from 6 months to 14 years old (median 4 years). Both sexes were equally represented: 92

females (64 neutered) and 93 males (15 neutered). The main breeds represented were:

- French bulldog (26)
- Labrador retriever (15)
- Jack Russel terrier (11)
- English bulldog (11)
- Staffordshire bull terrier (9)
- American Staffordshire terrier (8)
- Golden retriever (7)
- Mixed breeds (7)
- Cavalier king Charles (5)
- Bull terrier (5)
- Boxer (5), and
- German shepherd (5)

Body weights varied from 2.7 to 71.3 kg (median weight 17.7 kg).

Allergenic Extracts

The most frequent allergenic extract mixtures used were:

- *Dermatophagoides farina*e (DF) alone in 98 cases (53%)
- DF associated with *Dermatophagoides pteronyssinus* (DP) in 36 cases

Other combinations are detailed in Table 1 and were composed of mites allergenic extracts in 23 cases, pollen extracts in 5 cases, and of a mixture of pollens and mites in 6 cases.

Concomitant Treatment

At the first ASIT injection, 104 dogs had not been treated with systemic immunomodulating or anti-inflammatory drugs for at least 1 month, 41 dogs were received oclacitinib, 22 dogs cyclosporine, and 13 dogs cetirizine. Other medications or combinations of molecules are listed in Table 2.

Safety Assessment

No immediate or late systemic effects were reported at the first injection, and in most cases, no local side effects were observed. In one case, swelling was reported at the injection site following the first injection. It disappeared 3 days later without any treatment. However, as it was a small dog (2.7 kg body weight), dosage was then reduced to 0.5 mL and no other adverse reaction was observed

Table 1. *Allergen extracts used for ASIT*

Allergen extracts
Mite extracts
DF: n = 98
DF, DP: n= 36
AS, DF, TP: n= 5
AS, DF, DP, TP: n= 4
DF, TP: n= 3
AS, DF, LD, TP: n= 3
AS, TP: n= 2
DF, DP, LD: n= 2
TP : n= 1
DF, DP, TP : n= 1
AS, DP, LD, TP: n= 1
DF, DP, AS, TP, LD : n= 1
Pollen extracts
Weeds mixture: n= 1
Weeds mixture, birch: n= 1
Weeds mixture, plantain: n= 1
English plantain, birch: n= 1
Trees mixture, grass mixture, weeds mixture: n= 1
Mixed combinations
DF, Weed mixture: n= 17
DF, DP, Weed mixture: n= 4
DF, birch: n= 2

- *DF: Dermatophagoides farina*
- *DP: Dermatophagoides pteronyssinus*
- *AS: Acarus siro*
- *TP: Tyrophagus putrescentiae*
- *LD: Lepidoglyphus destructor*

Trees mixture:

- *Oak*
- *birch spring*
- *black willow*
- *olive*
- *cypress*

Grass mixtures:

- *orchard*
- *meadow fescue*
- *ryegrass perennial*
- *timothy*

Weeds mixture:

- *common mugwort*
- *English plantain*
- *lamb's quarter*

Table 2. *Treatment received at first injection:*

No treatment: n= 104
Monotherapy: n= 77
Oclacitinib: n= 41
Cyclosporine: n= 22
Cetirizine: n= 13
Methylprednisolone: n= 1
Combination of multiple treatments: n= 4
Cyclosporine & cetirizine: n= 1
Cyclosporine & oclacitinib: n= 1
Oclacitinib & cetirizine: n= 1
Prednisolone & azathioprine: n= 1

following this dose adjustment. During the 1 year follow up, no other adverse effect was reported in any of the dogs.

DISCUSSION

Traditionally, in veterinary medicine, ASIT consists of subcutaneous administration of progressively increasing doses of allergens up to a maintenance dose. The protocol usually proposed for adjuvanted allergenic extracts is a weekly injection of increasing doses of allergen given over a 1-month period until a maintenance dose is reached, followed by monthly injections of this maintenance dose.

For aqueous extracts, the initial phase can require several weekly injections. Such a protocol is directly adapted from human medicine, where the aim is to ensure a maximum efficacy while maintaining a good safety.

In human medicine, the escalating phase appears useful and increases the safety of protocols because in allergic subjects (especially in case of allergic asthma) the risk of severe systemic reaction (SR) exists. Systemic reactions vary from mild to severe or can even be anaphylaxic. The risk depends on the type of allergenic extract, injection schedule, and route of administration. For non-accelerated protocols, most surveys report a SR rate of 2-5% patients.[11] In a 4-year ASIT survey including 23.3 million injection visits, the SR rate was 0.1% only. A previous survey reported a rate of fatality of 1 in 2 to 2.5 million ASIT injections.[12]. Identified risk factors for SR were symptomatic and/or poorly controlled asthma and a high degree of skin test positivity.[12-13]

In veterinary medicine, systemic adverse reactions linked to ASIT are even more rarely reported. They have been reported to occur in about 1% cases, and include weakness, depression, anxiety, sleepiness, panting, hyperactivity, diarrhea, vomiting, urticaria, angioedema, collapse, and anaphylaxis.[14] Most cases of adverse reactions were reported with aqueous allergen extracts: in one study, one anaphylactic-like reaction was observed in a dog and 36 other dogs were lost to follow up, but the causes were not specified.[15] Another retrospective study of 100 cases using aqueous extracts reported an adverse reaction in 50% of dogs treated.[16] With alum adjuvanted extract, adverse reactions are less commonly reported (10-15% of cases).[17-19] These data support the good safety of ASIT in veterinary medicine, especially with adjuvanted extract, and suggest that it would be possible and still safe to use a monodose protocol (ie, without an escalating phase) with calcium phosphate adjuvanted extracts.

In 2007, a preliminary work studied the safety of an ASIT protocol without an escalation phase using subcutaneous administration of alum-precipitated allergen extracts combined with a preventive cetirizine treatment. No adverse reaction was reported in this pilot study.[7] The additional results of the present study confirm the good safety of a protocol without an escalating phase using calcium phosphate allergen extracts. No systemic effects were reported whether the dogs received a concomitant treatment or not, showing that concomitant treatments such as cetirizine or immune-modulating treatments such as cyclosporine, oclacitinib, or steroids did not influence the safety of this protocol. The composition of allergenic extracts did not influence the safety of the monodose ASIT either. There was no dif-

ference of tolerability between the 100 dogs treated with one allergen extract (98 for DF, 1 for TP, 1 for Weeds) and the 85 other dogs treated with a mixture of several allergens (maximum of 5 allergens).

An explanation for the good safety profile is the low dose of allergens in the extracts used for this protocol thanks to the calcium phosphate adjuvant. Indeed, in this study, allergenic extracts were composed of 1/10,000 w/v mite allergen extracts and a 1/1000 w/v pollen allergen extracts equivalent to 100 and 500 to 1,000 PNU (protein nitrogen unit) respectively, whereas in aqueous extracts, the concentrations of allergens are higher, ranging from 10,000 to 20,000 PNU in maintenance ASIT vials. Another explanation could be the nature of the adjuvant. In a previous study comparing low dose to standard immunotherapy efficacy using alum adjuvanted extracts, adverse reactions were reported in the same proportion within the two groups.[18] Another placebo-controlled trial using alum adjuvanted extracts reported adverse reactions in both the alum adjuvanted allergen extract group (3/27 dogs) and the alum adjuvanted placebo group (2/24 dogs).[19] These data suggest that the adverse reactions could be due to the alum adjuvant.

In our study, calcium phosphate adjuvant was used, and no adverse reactions were observed. Calcium phosphate adjuvant was first developed by Pasteur Institute for diphtheria, tetanus, pertussis and, poliomyelitis vaccines,[20] and was then substituted with alum salts. Depending on the studies, calcium phosphate adjuvant antigenicity is lower than alum salts, but several studies have confirmed their efficacy in human vaccination and desensitization. Calcium phosphate adjuvant provides a slow release of allergens at the injection site and is known to induce a low level of IgE (in contrast to alum adjuvant) while inducing progressively higher levels of IgG. Being a natural component and this low IgE reaction could therefore be an explanation for the safe profile of this adjuvant.[20] Finally, it is hypothetised that calcium phosphate adjuvant allows a better safety profile than alum adjuvant.

More so than adverse events, the major pitfall of ASIT is compliance, which is paramount for the success of hyposensitization. Indeed, according to ICADA guidelines, the onset of clinical benefit can appear after several months of treatment, and ASIT should be continued for at least 1 year to properly evaluate efficacy.[21] Moreover, most patients for which ASIT is effective appear to require several years of treatment,[22] which highlights the importance of a long-term compliance to the protocol. Only few data are available in veterinary medicine regarding the compliance of owners during ASIT treatment of their dog. Two retrospective studies conducted in the Norway and in the USA reported low compliance: 30-40% of dogs stopped the treatment after 3 to 6 months (corresponding to the initiation phase).[23,24]

It is our feeling that one of the major causes of non-compliance is the complexity of current protocols using an initiation phase. Weekly injections of increasing doses can lead to dosage errors and are not easy to schedule if injections are to be done in the clinics. Considering these conditions, owners can lose motivation after several weeks of treatment as no improvement occurs during the first months of treatment.

The protocol used in this study is much easier for both the clinicians and the owners: only one vial needed for 1 year of treatment with monthly injection. This protocol is a good tool to improve the compliance of owners during the first year of ASIT and, therefore, the the potential success of the treatment in allergic atopic dogs. The safety profile of this protocol allows its use with or without concomitant treatment and may also encourage clinicians to use ASIT in sensitized atopic dogs.

In this retrospective study, the efficacy could not be assessed. To best compare both the safety and efficacy of ASIT protocols

with and without an initiation phase, a blinded controlled prospective study assessing the occurrence of adverse events, as well as clinical criteria such as Canine Atopic Dermatitis Extent and Severity Index (CADESI), pruritus, and medication score would be necessary.

CONCLUSION

This retrospective study shows the safety of a monodose ASIT protocol. It is a very simple protocol which can increase compliance and hence the chance of success with this long duration treatment. These results cannot be extended to other protocols using aqueous extracts or other adjuvants. Further studies are needed to measure the real compliance of owners with this protocol over standard protocols. Finally, other controlled studies are needed to assess the efficacy of such a protocol and to compare it to the usual ASIT protocols.

Abbreviations

ASIT: Allergen specific immunotherapy, SR: Systemic reaction, IDT: Intradermal skin testing, NU: Noon unit, DF: *Dermatophagoides farina*e, DP: *Dermatophagoides pteronyssinus*, AS: *Acarus siro*, TP: *Tyrophagus putrescentiae*, LD: *Lepidoglyphus destructor*, H2R: Histamine type 2 receptor, PNU: Protein nitrogen unit.

DECLARATIONS

Ethics Approval and Consent to Participate

As this field study was using, without any modification, a protocol already described in the literature and routinely used by several clinicians in the country, no ethical approval was needed. See document for relevant french legislation in additional file.

Owner Consent: see document below.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

Prélaud P. works as a consultant for Ceva Company.

Drouet L. is working for Ceva Company.

Funding

Not applicable.

ACKNOWLEDGEMENTS

The authors are indebted to Ceva Biovac team for their help and support throughout this study.

Funding

Not applicable.

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