Research in Veterinary Science 118 (2018) 164–170

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Evaluation on the effects of 0.1% *Peumus boldus* leaf and *Spiraea ulmaria* plant extract combination on bacterial colonization in canine atopic dermatitis: A preliminary randomized, placebo controlled, double-blinded study

Domenico Santoro[a,*], Mary Bohannon[a], Kim Ahrens[a], Christelle Navarro[b], Hugues Gatto[c], Rosanna Marsella[a]

[a] Department of Small Animal Clinical Sciences, University of Florida, Gainesville, FL, USA
[b] Medical Department, Virbac, Carros, France
[c] Unlicensed Products Development Unit, Virbac, Carros, France

ARTICLE INFO

Keywords:
Canine
Antimicrobial peptides
Bacterial colonization
Plant extracts
Atopic dermatitis

ABSTRACT

Defective skin barrier characterize canine atopic dermatitis (AD). Pyoderma is the most common complication. Herbal compounds have been suggested as alternatives to control bacterial colonization for their effect on natural antimicrobial peptides (AMPs). This study evaluated the effects of 0.1% *Peumus boldus* leaf and *Spiraea ulmaria* plant extract combination on clinical signs, bacterial colonization and AMPs secretion in atopic dogs compared to placebo.

Twenty privately-owned atopic dogs were randomly divided in 2 groups (treatment: n = 10; placebo: n = 10) and their abdomen was sprayed every 24 h for 4 weeks. Total and inguinal clinical scores (CADESI-03), manual bacterial count, and skin washes for AMPs (cBD3-like and cCath) were performed on days 0, 14 and 28. AMPs were detected using in-house, previously-validated, canine-specific ELISAs. Data were statistically analyzed and a $p < 0.05$ was considered significant.

Clinical scores and AMPs secretion did not differ significantly between the two groups at any time point. A significant reduction of the clinical scores was seen in the placebo group at 14 and 28 days ($p < 0.04$). On days 14 and 28, a reduction in the bacterial count was seen in the treated group compared with placebo ($p < 0.009$ and $p = 0.04$, respectively). Compared to baseline, a reduction in *Staphylococcus* spp. was seen in the treated group after 14 days of treatment ($p < 0.03$).

These results show the efficacy of this plant extract combination against bacterial colonization, suggesting its potential usefulness in preventing bacterial infection in atopic dogs. The influence of this compound on AMPs secretion or other mechanisms should be further evaluated.

1. Introduction

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases in dogs. Canine AD is a chronic pruritic disease requiring life-long pharmacological management, with significant costs and potential side effects (Olivry et al., 2010, 2015). The pathogenesis of AD is not completely elucidated, although a skin barrier defect has been demonstrated in both dogs and people (Santoro et al., 2015a). Skin barrier impairment, genetic and immunological alterations, and altered interaction with the cutaneous microflora have been suggested as possible causes of recurrent infections in atopic dogs (Santoro et al., 2015a).

In the past decade, an alarming increase in bacterial resistance has been documented in both dogs and people with AD (DeBoer and Marsella, 2001; Kedzierska et al., 2008). These subjects require constant use of antimicrobials with the potential risk of selecting for resistant bacterial strains (Weese et al., 2015; Pitiriga et al., 2017). For this reason, researchers are looking into alternative treatment and preventative options for bacterial infections and colonization. Under this prospective, the use of metals and plant extracts have been hypothesized as good alternative options (Higaki et al., 1997; Higaki et al., 1999; Gloor et al., 2002; Lemire et al., 2013; Oktar et al., 2015). Plant extracts have also been considered with particular interest in the

* Corresponding author at: Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32610, USA.
E-mail address: dsantoro@ufl.edu (D. Santoro).

https://doi.org/10.1016/j.rvsc.2018.02.006
Received 14 November 2017; Received in revised form 5 February 2018; Accepted 18 February 2018

veterinary field. In fact, very recently, plant extracts have demonstrated a positive effect on natural immune defenses with the implied potential to reduce the insurgence of skin infection in atopic dogs (Marsella et al., 2010; Olivry et al., 2010; Olivry et al., 2015; Santoro et al., 2017).

The use of *Peumus boldus* (aka Boldo) and *Spiraea* (*Filipendula*) *ulmaria* (aka Meadowsweet) have been tested in vitro on healthy and atopic canine keratinocytes showing a positive effect on production and secretion of selected antimicrobial peptides (AMPs) without stimulating an inflammatory reaction (Santoro et al., 2017). This proof-of-concept study confirmed previous larger studies on the immunological properties of these plant extracts in people (Lanhers et al., 1991; Churin et al., 2008; Drummond et al., 2013).

Thus, due to the positive immunological properties of Boldo and Meadowsweet plant extracts and the lack of inflammatory stimulation, the aim of this randomized, double blinded, placebo controlled clinical trial was to evaluate the effects of 0.1% *Peumus boldus* leaf and *Spiraea ulmaria* plant extract combination on clinical signs, bacterial colonization, and AMPs secretion in privately-owned atopic dogs. The primary outcomes were the evaluation of bacterial colonization and the effect on AMP secretion using non-invasive techniques. The secondary outcomes included the effect of this compound on clinical signs of naturally occurring AD in dogs and the possible correlation among the variables analyzed.

2. Materials and methods

This study was a prospective, randomized, double-blinded, placebo-controlled study in privately owned atopic dogs. The study was approved by the Institutional Animal Care and Use Committee. All dogs entered the study with the owners' written informed consent.

2.1. Dogs

2.1.1. Inclusion criteria

Dogs with a previous diagnosis of non-seasonal AD were eligible to be enrolled. The diagnosis of AD was based on compatible history, and clinical signs and exclusion of other pruritic diseases according to Favrot's criteria (Favrot et al., 2010) and following recently published guidelines (Hensel et al., 2015). Additional topical therapy (e.g. shampoos or conditioners) was not permitted for the entire duration of the study.

2.1.2. Exclusion criteria

Dogs receiving glucocorticoids or other immunomodulatory drugs (e.g. cyclosporine or oclacitinib) or allergen specific immunotherapy were excluded from the study. The withdrawal period for topical, systemic and/or depot glucocorticoid treatment was two, four, and eight weeks, respectively. The withdrawal period for oclacitinib and systemic and topical calcineurin inhibitors was two weeks and eight weeks respectively. Dogs were also excluded from the study if a low number of total bacteria (< 500 colony forming units [CFUs]) or no *Staphylococci* spp. were cultured on the day of enrolment. If at any time point, a secondary infection developed and/or the severity of the disease warranted rescue therapy, the dog was withdrawn from the study and treated appropriately.

2.1.3. Intervention, randomization and masking

Each dog enrolled was randomized, using a computer software (www.randomizer.org), and allocated to a treatment or control group (ratio 1:1). The treatment consisted of a spray solution containing 0.1% *Peumus boldus* leaf and *Spiraea ulmaria* plant extract combination (Santoro et al., 2017). The control product consisted of the water base, but lacked the active ingredients. Both products were dispensed in identical bottles and were indistinguishable by appearance, smell and consistency. The clinician and all site personnel, with the exception of the treatment administrator, were blind to the treatment group assignments, as were the owner and the laboratory personnel. No other treatments, including antibiotics and antiseptics were allowed during the study.

2.1.4. Treatment

The treatment was administered at home by the owners daily. The owners were instructed to spray glabrous areas (axillae, inguinal area, ventral thorax, medial thighs) of each dog for a total of 10 pumps, corresponding to ~20 ml of product, every 24 h for 4 weeks. Licking was prevented by distracting the dog or placing an E-collar for the time needed for the product to dry. Each dog was clinically evaluated by the same investigator (RM) on days 0, 14, and 28.

2.2. Outcome measurements

The primary objectives of the study were to evaluate the effects of the spray formulation containing a mixture of 0.1% of Boldo and Meadowsweet extracts on bacterial load and on AMPs secretion in dogs with AD. The secondary objective was to evaluate the effect of this formulation on clinical lesions measured by the Canine Atopic Dermatitis Extent and Severity Index score (CADESI-03) (Olivry et al., 2007; Olivry et al., 2008).

2.2.1. Bacterial load evaluation

A sterile compact dry swab, consisting of a sterile swab and transporting media (HyServe GmbH & Co. KG, Uffing, Germany), was rubbed for 10 s on a well-defined standardized area (15 cm^2) of the ventral abdomen at each visit and plated on Columbia blood agar (BAP) and colistin and nalidixic acid (CNA) enriched plates (Remel Inc., San Diego, CA, USA). The first plate is an unselective medium for detection of both Gram + and Gram − bacteria (BAP); the second plate (CNA) is specific for isolating Gram + bacteria (CNA). A total of 100 µl of the transporting media and a minimum of three serial dilutions were plated for a total of 6 plates per evaluation. The dilutions were performed in order to ensure a representative number of CFUs, between 30 and 300, per plate (Markey et al., 2013). All plates were cultured at 37 °C for 24 h, and then the colonies were manually counted and identified based on characteristic morphological appearance for *Staphylococci* spp. (e.g. β-hemolysis, small white colonies, etc.). The final number of CFU/ml took into consideration the dilution factors.

Furthermore, the control and the plant extract solution were tested for direct antimicrobial activity. Briefly, isolated colonies of *Staphylococcus pseudintermedius* (ATCC 49444), *S. aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were suspended in sterile double distilled water to achieve an optical density equal to a McFarland 0.5 standard (Sensititre Inc., Westlake, OH, USA). The bacterial suspension was plated on BAP plates and treated (1 spray at ~5 cm from the plate) with either the control or the plant extract, or no treatment at all (positive control) and incubated at 37 °C for 18–20 h.

2.2.2. Skin washes

To evaluate secreted AMPs, the skin was washed using a sodium phosphate buffered solution, as previously reported, with few modifications (Harder et al., 2010). In brief, the skin was rinsed with 900 µl of 10 mM sodium phosphate buffer pH 7.4 containing 150 mM NaCl (skin rinsing buffer) in 2 standardized areas (2.5 cm^2) using a sterile upper half of a cut off 15 ml plastic centrifuge tube to demarcate the area. Another 900 µl was repeated in 2 other areas twice for a total of 6 areas (15 cm^2 total area) rinsed with 2700 µl in total. The skin rinsing buffer was then concentrated using Amicon® Ultra centrifugal filters Ultracell® - 3 K and stored at −80 °C until used for competitive inhibition ELISA for canine AMPs (cBD3-like and cCath).

2.2.3. ELISAs

Protein levels of AMPs (cBD3-like and cCath) in the skin washing