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First case report of ultrastructural cutaneous abnormalities in equine atopic dermatitis

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ABSTRACT

Atopic dermatitis (AD) is a common skin disease that affects humans and animals. Skin impairment has been described in human and canine AD. Equine AD is recognized in practice but little is known about its pathogenesis. As remarkable similarities exist across species in terms of cutaneous manifestations of AD, it was speculated that skin abnormalities may also exist in atopic horses. This case report describes the ultrastructure of the stratum corneum of two normal and two atopic horses. Biopsies were taken from sites predisposed to AD and examined using electron microscopy. Stratum corneum in normal samples was compacted with organized lipid lamellae while in atopic samples disorganized lipid lamellae, retained lamellar bodies and amorphous lipids were found. These changes are very similar to what reported in AD in other species. It is currently unknown whether these abnormalities in atopic horses are primary or secondary and their importance in allergen penetration.

1. Introduction

Atopic dermatitis in horses is a poorly defined and investigated disease. Although it is diagnosed in clinical practice (Hallamaa, 2009), the exact mechanisms of disease are inadequately understood. Affected horses start with dermatitis and pruritus at young age (1–3 years of age). Clinical signs are typically seasonal and tend to progress over the years. Affected horses are usually pruritic on their faces, ventral abdomen and flexural surfaces. Pruritus leads to self-trauma and that facilitates the development of secondary infections which further increase the severity of pruritus. Affected horses frequently require glucocorticoids therapy to obtain relief thus increasing their risk for developing laminitis. Self trauma and the need for treatment decrease the quality of life of affected horses and may interfere with their ability to perform.

A type I hypersensitivity to environmental allergens has been described in equine AD and has been thought to play a role in the disease (Frey et al., 2008). However, as allergen-specific IgE may also be seen in normal horses, the simple detection of them should not be used for diagnostic purposes and may represent a secondary finding (Jose-Cunilleras et al., 2001; Lorch et al., 2001a). Additionally, the various types of allergy testing (e.g., intradermal skin testing and serology testing) correlate poorly with each other, highlighting the need to better investigate the pathogenesis of this condition beyond the simple presence of allergen specific IgE (Lorch et al., 2001b).

Interestingly, many of the same body areas affected in human and canine AD (Terada et al., 2011) are also frequently involved in atopic horses (Hallamaa, 2009). These body areas are the periorbital, axillary, and inguinal areas and the flexural surfaces such as the antebrachial area. Interestingly these body areas are known to have a thinner stratum corneum (Barker, 1951). Increased permeability as measured by increased evaporation of water has been described in atopic humans (Loden et al., 1992; Seidnari and Giusti, 1995) and dogs (Hightower et al., 2010; Shimada et al., 2009). Excessively permeable skin is more prone to absorb allergens such as pollens therefore increasing the risk for allergic sensitization (Boralevi et al., 2008; Hudson, 2006). Chronic application of allergens on impaired skin leads to a TH2 driven response and IgE synthesis (Kondo et al., 1998; Pucheu-Haston et al., 2008; Wang et al., 2007; Yamamoto et al., 2007) thus skin impairment may be the key to the production of allergen specific IgE. Environmental allergens have been demonstrated to play an important role in aggravating AD in all species including horses (Hallamaa, 2009). Therefore, it is plausible to speculate that in horses with AD the skin itself is impaired, just as in humans and dogs, and that the IgE may be a secondary event.

In humans and dogs, impaired skin barrier function is linked to abnormalities in the ultrastructure of the upper layers of the epidermis (e.g., stratum corneum). More specifically, disorganization in stratum corneum, abnormal, discontinuous and decreased lipid lamellae have been described as well as retention of lamellar bodies in humans (Pilgram et al., 2001) and dogs (Marsella et al., 2010). The lamellar bodies are organelles that release their content at the

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junction between the stratum granulosum and corneum providing lipids and enzymes necessary for proper skin barrier formation. This process is crucial to provide adequate lipids to form the lipid lamellae in the intercellular spaces. In atopic skin a disturbed extrusion process has been described, possibly contributing to the lipid deficiencies and dryness of the atopic skin of humans (Fartasch et al., 1992; Melnik et al., 1988; Werner et al., 1987) and dogs (Marsella et al., 2010).

Therefore, the purpose of the present pilot study was to evaluate the ultrastructure of the upper layers of the epidermis in atopic horses and compare it to normal controls. The hypothesis tested was that the ultrastructure is altered in atopic horses when compared to normal controls and that abnormalities would be similar to what has been reported in other species.

2. Materials and methods

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida. Horses used for this study were privately owned and a consent form was signed by the owner prior to inclusion in the study.

2.1. Animals

Two normal horses with no history and evidence of skin disease and two atopic horses were used. No medications had been allowed either topically or systemically for 2 months prior to the inclusion in the study. For the atopic horses, a diagnosis of AD was based on history of seasonally relapsing dermatitis not responsive to aggressive insect control, supportive clinical signs and the documentation of high levels of allergen specific IgE on serology test (>150 AU, Heska, Fort Collins, CO, USA) and numerous strong positive reactions (subjectively scored at 3 and 4 where 0 is the score assigned to the negative control [saline] and 4 is the score assigned to positive control [histamine]) on intradermal skin test using a panel of 60 allergens 15–20 min after injection. Allergy testing was done in both horses after the peak of allergy season. One horse also had a history of seasonal (summer) chronic obstructive disease exacerbated by pollen exposure besides a seasonal history of AD. Skin cytology had been done on the atopic horses prior to inclusion in the study to monitor for the presence of a secondary infection and had been negative for bacteria or yeasts.

2.2. Skin biopsies

Two 4 mm biopsies were taken under local anesthesia obtained by subcutaneous injection of 1 ml of lidocaine per site from each horse. Skin sites biopsied included the flexural surface and inguinal area. Biopsied sites were sutured routinely.

2.3. Processing of skin biopsies

Skin samples were placed in phosphate buffered saline (PBS) and refrigerated. To enhance lipid visualization ultrastructurally within epidermal tissue, specimens were exposed to acrolein vapor (5% acrolein in PBS buffer pH 7.4) as the primary fixative for 1 h in ice, and post-fixed with 0.5% ruthenium tetroxide with 0.25% potassium ferrocyanide for 1 h in the dark at 4 °C. The specimens were then dehydrated by a series of alcohol and acetone rinses. Fresh epoxy resin mixture for low viscosity and high hardness was then prepared with 16.5 ml NMA, 25.7 ml Eponate (generic replacement for Epon 812), 9.3 ml DDSA, and 1.6 ml BDMA (accelerator). The samples were placed in a 2 part acetone and 1 part resin mixture for 45 min, then 1:1 and 1:2 mixtures for 1 h each, then embedded in a mold and left to polymerize in a 37 °C oven for 20 h. Samples were sectioned at 90 nm and stained with uranyl acetate and lead citrate. This methodology has been modified from Fartasch et al. (1992) and is widely accepted technique to increase visibility of lipids on electron microscopy.

2.4. Evaluation of TEM

Each grid (with section) was viewed, evaluated and photographed on a transmission electron microscope (Hitachi 7000) operated at 100 kV. Images of stratum corneum and granulosum were taken digitally at 5000×, 25,000×, 50,000×, and 100,000× using a Peltier-cooled Tietz 2k × 2k CCD camera mounted below the viewing chamber. Files were saved as Tagged Image Format Files. The upper and lower layers of the stratum corneum were evaluated. Emphasis was placed on the assessment of the intercellular spaces in the stratum corneum, the presence and location of lamellar bodies and the morphology of the lipid lamellae.

3. Results

3.1. Animals

At the time of the study the two normal horses were 9 years old (Andalusian mare) and 13 years old (Quarter horse gelding) and the two atopic horses were 8 years old (Friesian, stallion) and 12 years old (Andalusian mare). The Friesian stallion had evidence of AD at the time of the biopsies with evidence of disease on his face, the antebrachial area (Fig. 1) and inguinal area (Fig. 2) while the Andalusian mare was non lesional at the time of the study. The Andalusian mare also had seasonal chronic obstructive pulmonary disease. Both atopic horses later underwent immunotherapy for their atopic disease and showed marked reduction of disease severity and frequency of relapse of dermatitis after 1 year of immunotherapy further supporting a role of allergen induced atopic disease.

3.2. Description of EM findings

Noticeable differences in the ultrastructure of the upper layers of the epidermis were evident between the normal and atopic horses. Samples of the two healthy horses revealed a compacted stratum corneum and normal and well organized lipid lamellae (Fig. 3). The atopic samples had a stratum corneum with wider intercellular spaces and lipid lamellae were consistently disorganized even in non-lesional skin (Fig. 4). Disarrangements of lipid lamellae were more remarkable in lesional atopic skin. In the normal horses it was possible to see the organized assembly of lipid lamellae as the

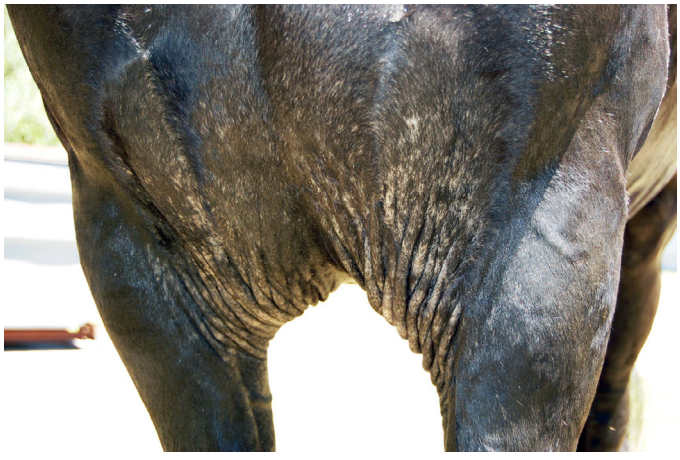

Fig. 1. Clinical lesions of atopic dermatitis in the antebrachial region of a Friesian stallion.