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SCIENCE SPEAKS

HOW DOES RABIES CAUSE AGGRESSION?

Researchers from the University of Alaska Fairbanks detailed molecular mechanism for how the rabies virus acts at a molecular level to induce specific behaviours and modifies host behaviour.

Their study shows that the molecules of this virus bind to nicotinic acetylcholine receptors — or proteins that respond to the neurotransmitter acetylcholine — thus impacting muscle control. That research showed how glycoprotein molecules of the virus bind to acetylcholine receptor molecules, which, in addition to influencing the signaling pathway that dictates muscle control, means that they can also replicate and infect the brain. More recent research also demonstrated that the glycoprotein molecule in rabies contains a sequence of amino acids that is very similar to an amino acid sequence found in snake venom. These amino acids act as inhibitors to the nicotinic acetylcholine receptors. The team stated that this is the first time that experimental evidence has been presented to show how rabies interacts with other cells in the nervous system to induce an altered behaviour that determines infected hosts to help spread the virus.

STUDY SHOWS HIGH PREVALENCE OF GAIT ABNORMALITY IN PUGS:

A study published in Veterinary Record assessed the gait abnormalities in pugs as 30.7 per cent. The most common sign of pain was a reluctance to walk (56.7 per cent). The researchers found a link between abnormal gait and age, with gait abnormalities being more prevalent in older pugs. An association between abnormal gait and dyspnoea was also found, with dyspnoea being significantly more common in pugs with gait abnormalities. The researchers said that whilst abnormal gait could be the result of orthopaedic conditions, it may also be a consequence of neurological issues.

RAW CHICKEN LINKED TO PARALYSING DISEASE IN DOGS:

Feeding dogs raw chicken meat could be linked to the rare paralysing condition, acute polyradiculoneuritis (APN), scientists from the University of Melbourne have said. APN is a debilitating disease that initially causes hind leg weakness, before progressing to the front legs, neck, head and face. Whilst dogs often recover in time without treatment, the disease can be fatal if paralysis spreads to the chest muscles. It is the canine equivalent of Guillain-Barré syndrome (GBS) in humans. The bacteria Campylobacter - found in undercooked chicken, unpasteurised milk products and contaminated water - is thought to be a trigger for GBS and is likely to be the reason for the dysregulation of the dog's immunity & the symptoms of paralysis. These bacteriological results were consistent with the hypothesis that the uncooked chicken meat was the source of the Campylobacter and as a result, triggered APN. Scientists also found a significant association between APN and smaller dog breeds. It is thought they may be more at risk as they are unable to eat larger bones, so owners tend to feed them more chicken necks.

EPIDERMAL LIPIDS & CUTANEOUS PERMEABILITY BARRIER HOMEOSTASIS: A MODERN APPROACH

THE EPIDERMAL BARRIER

Canine skin can be described as bricks and mortar, with epithelial cells making up the bricks and extracellular lipids and proteins making up the mortar. The anatomical/physical barrier of the skin consists of two parts. Some of this barrier function resides within the stratum corneum, but once this barrier has been breached the tight junctions found at the level of the stratum granulosum between the keratinocytes are the next level of defence. The first epidermal barrier is created by epidermal cornification, the end point of epidermal differentiation. An effective epidermal barrier requires intricate organization and formation of the keratin intermediate filaments and the intercellular lipids together with tight regulation of desquamation. The stratum corneum (SC) consists of a sheet of corneocytes embedded in an intercellular lipid matrix and is the primary barrier against pathogen entry. It is also largely responsible for the regulation of water loss from the body (transepidermal water loss- TEWL) and is also able to withstand physical forces. The stratum granulosum of the epithelium produces lamellar bodies, which contain necessary lipids and enzymes needed for differentiation and desquamation of epithelial cells. The lamellar bodies are extruded into the extracellular space and form organized stacks called lamellae, which help prevent water loss and allergen penetration. Assembly of the extracellular lamellar lipids is a crucial factor in maintaining permeability barrier function. Precursors of the extracellular lipid matrix, including phospholipids, glucosylceramides, sphingomyelin and cholesterol, are located in the lamellar granules of keratinocytes at the upper stratum spinosum and stratum granulosum, and originate from the Golgi apparatus. In human beings, the lipid matrix consists mainly of three lipid classes: ceramides (CER), fatty acids and cholesterol.

THE ROLE OF EPIDERMAL LIPIDS IN CUTANEOUS PERMEABILITY BARRIER HOMEOSTASIS:

Lipids are very important in barrier function, stratum corneum water holding, cohesion & desquamation of corneocytes and control of epidermal proliferation and differentiation.

SPHINGOLIPIDS:

Sphingolipids are a group of lipids containing the sphingoid base, which is formed by the condensation of an amino acid and a fatty acid. Sphingolipids were discovered in brain extracts in the 1870s and were named after the mythological Sphinx because of their enigmatic nature. The sphingoid bases are enzymatically modified to generate a wide range of biologically active sphingolipids, including ceramides, sphingomyelin, sphingosine-1-phosphate (S1P), ceramide-1-phosphate, and glycosphingolipids. The basic structure of the main epidermal sphingolipids, ceramides, is a sphingoid base with fatty acid connected by an amide bond.

CERAMIDES: A Real Solution for Healthy Skin

Ceramides are a major lipid constituent of the intercellular lamellar sheets, accounting for 40% to 50% of the SC lipids by weight. They play a key role in preventing transepidermal water loss (TEWL) and maintaining the water permeability barrier function of the skin. They also are partly responsible for the cohesion of corneocytes and therefore play a key role in the establishment of the SC barrier function for water evaporation. Canine SC has a CER profile closely resembling that of human beings. Ceramides structurally are heterogeneous. They are a complexed group of sphingolipids, containing derivatives of sphingosine bases in amide linkage with a variety of fatty acids.

These extremely complex skin CERs include numerous molecular subclasses consisting of a combination of sphingoid moieties, including sphingosine [S], dihydrosphingosine [DS], phytosphingosine [P] and 6-hydroxy-sphingosine [H], linked via an amide bond to a fatty acid moiety, which may be non-hydroxy [N], α-hydroxy [A], or ester-linked ω-hydroxy [EO]. At present, the following CER subclasses have been reported: CER[AH], CER[ADS], CER[AP], CER[AS], CER[EOH], CER[EOP], CER[EOS], CER[NH], CER[NDS], CER[NP] and CER[NS].

Differences in chain length, type and extent of hydroxylation, saturation etc. are responsible for the heterogeneity of the epidermal sphingolipids. It is well known that ceramides play an essential role in structuring and maintaining the water permeability barrier function of the skin. In conjunction with the other stratum corneum lipids, they form ordered structures. An essential factor is the physical state of the lipid chains in the nonpolar regions of the bilayers.

The stratum corneum intercellular lipid lamellae, the aliphatic chains in the ceramides and the fatty acids are mostly straight long-chain saturated compounds with a high melting point and a small polar head group. This means that at physiological temperatures, the lipid chains are mostly in a solid crystalline or gel state, which exhibits low lateral diffusional properties and is less permeable than the state of liquid crystalline membranes, which are present at higher temperatures.

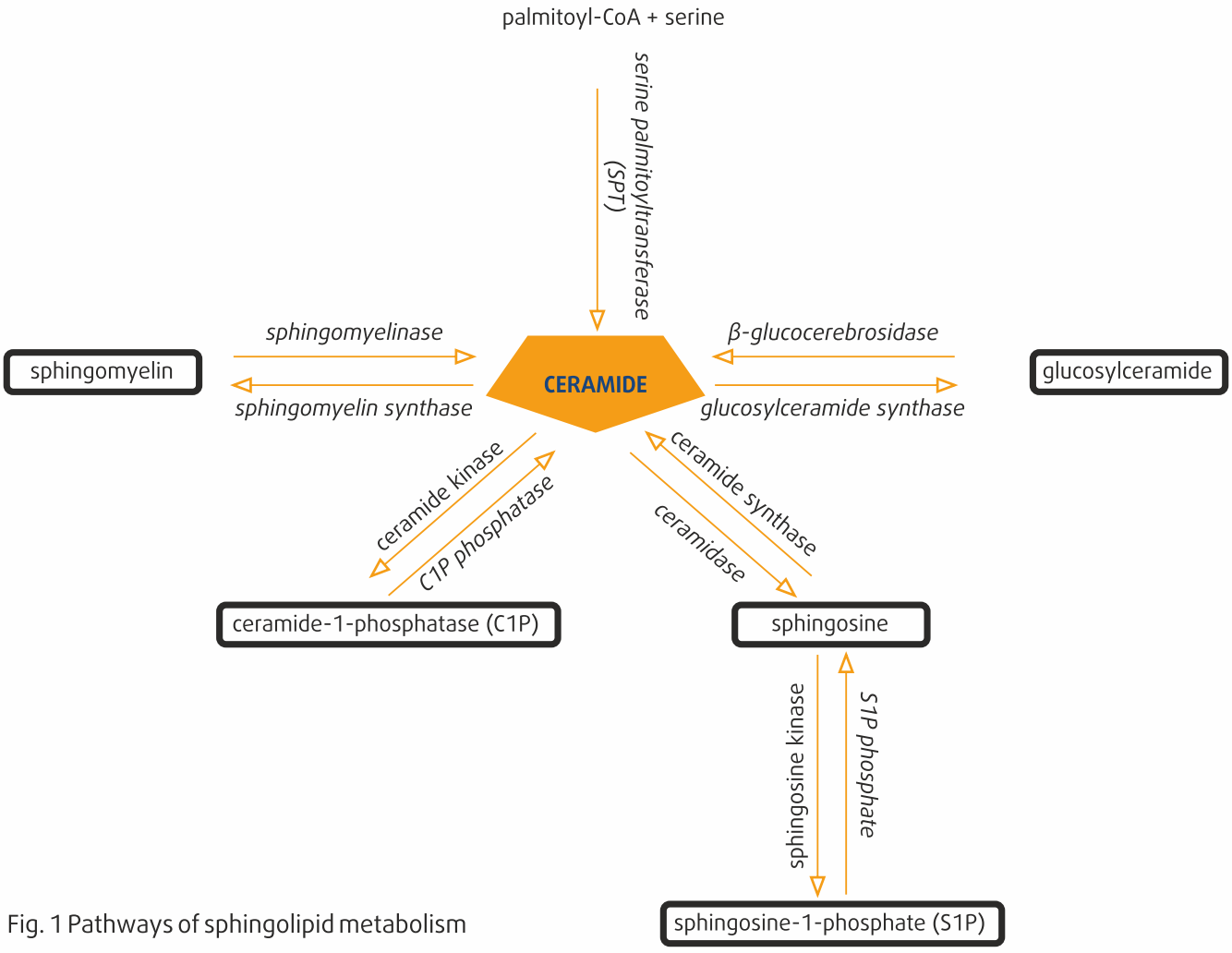


Fig. 1 Pathways of sphingolipid metabolism

The link between skin disorders and changes in barrier lipid composition, especially in ceramides, is difficult to prove because of the many variables involved. However, most skin disorders that have a diminished barrier function present a decrease in total ceramide content with some differences in the ceramide pattern.

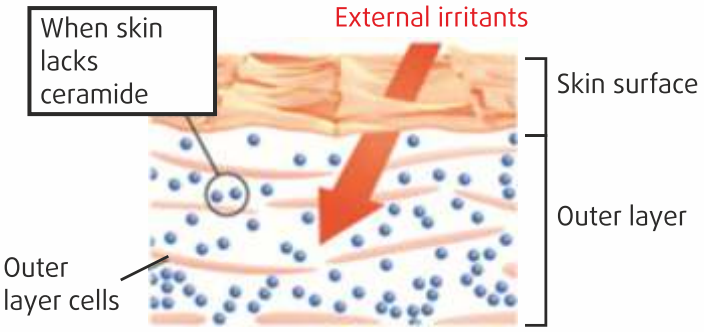


Diagram of cross section of skin

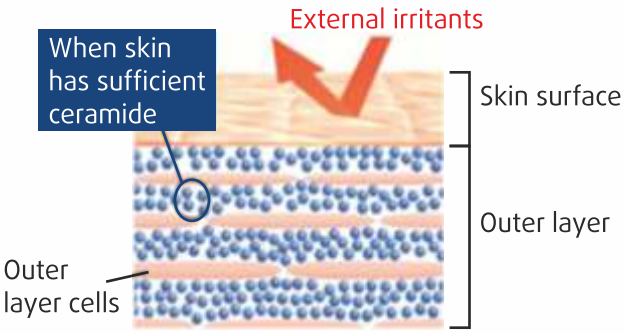


Diagram of cross section of skin

Ceramides species are differentiated by the length of the fatty acid or the sphingoid backbone. There are three pathways of ceramide generation in the skin: 1) *de novo* synthesis in the endoplasmic reticulum via serine palmitoyltransferase (SPT); 2) degradation of glucosylceramides by β- glucocerebrosidase; and 3) hydrolysis of sphingomyelin by sphingomyelinase. Ceramides synthesized in the endoplasmic reticulum are moved to the Golgi apparatus, where the transformation to glucosylceramides or sphingomyelin occurs. Next, these substances are transported from the Golgi apparatus into secretory vesicles – epidermal lamellar bodies, which fuse with the plasma membrane when the keratinocytes cross the line between the stratum granulosum and stratum corneum. In the intercellular space of the stratum corneum, glucosylceramides and sphingomyelin are converted back to ceramides via hydrolysis by β-glucocerebrosidase and sphingomyelinase, respectively. Ceramides may be modified to other biologically active sphingolipids, for example, sphingosine, which is phosphorylated to sphingosine-1-phosphate by sphingosine kinase and sphingomyelin, which is converted to sphingosylphosphorylcholine via sphingomyelin deacylase. Other than the structural function in the epidermis, sphingolipids also play an important role in epidermal signaling. Ceramides regulate such processes as proliferation, differentiation and apoptosis.

The o-acylceramides have distinctive structures not seen in other tissues. Their unique structures allow for their functionality in the epidermal permeability barrier. The ester-linked fatty acids in each of the three o-acylceramides (EOS, EOP, EOH) in normal healthy epidermis are predominantly linoleic acid.

In essential fatty acid deficiency, linoleate is replaced by oleate. This creates disorder in the intercelfular lamellae resulting in increased TEWL. Extracellular glycosylceramides and sphingomyelins are the precursors for SC ceramides. The enzymes beta-glucosylceramidase and sphingomyelinase are responsible for the hydrolysis and generation of ceramides from these precursors. Both precursors and enzymes are responsible for the epidermal permeability barrier homeostasis. Desquamation of skin cells is dependent on the transformation of cholesterol sulfate into cholesterol. It also is known that sphingomyelin derived ceramides are essential for normal barrier function homeostasis. Two epidermal sphigomyelins SM –I and SM-3 were found to be important precursors of two corresponding ceramides in the SC: ceramide NS (ceramide 2) and ceramide AS (ceramide 5), respectively. Other ceramide species, including the omega-hydroxyceramide species, which exhibit a different saturation level, are not derived from sphingomyelin.

OTHER ROLES FOR CERAMIDES:

In addition to their role in determining barrier properties, ceramides may play roles in other biochemical events in the skin. Ceramides were found to be present at significant levels both in the basal cells and in the upper epidermis. In basal epidermal cells and fibroblasts, ceramides were found at the cell nuclear envelope, the inner and outer mitochondrial membranes, at the golgi, the endoplasmic reticulum and at the plasma membrane. In the upper epidermis, ceramides were localized in the lamellar bodies, cornified envelope and, as already explained, within the intercellular space of the SC.

TRANSEPIDERMAL WATER LOSS:

Transepidermal water loss (TEWL) is the loss of water from the stratum corneum. It is one of the major factors responsible for dry, scaly skin and irritant dermatitis. The stratum corneum receives water from the dermis and some from the environment. All animals lose a small amount of water through the skin, called perspiratio insensibilis. But atopic dogs lose higher amounts of water than normal dogs do. It is thought that the higher water loss dries out and irritates the skin, but more important, it signifies that the skin barrier is not working properly. If water is leaking out, allergens may be penetrating the barrier.

FILAGGRIN:

Filaggrin is a structural protein that is fundamental in the development and maintenance of the skin barrier. Filaggrin is particularly important in the formation of the skin barrier, both for its fundamental role in terminal epidermal differentiation and for its implication in some of the most common dermatological diseases, such as atopic dermatitis (AD) and ichthyosis vulgaris. Filaggrin is an important structural protein that was first identified in 1977. Later, when it was found to produce aggregation and compaction of keratin intermediate filaments, it was named filaggrin, the acronym of filament-aggregating protein. This protein is synthesized as a giant precursor protein called profilaggrin, which is the main component of the keratohyalin granules in the stratum granulosum of the epidermis.

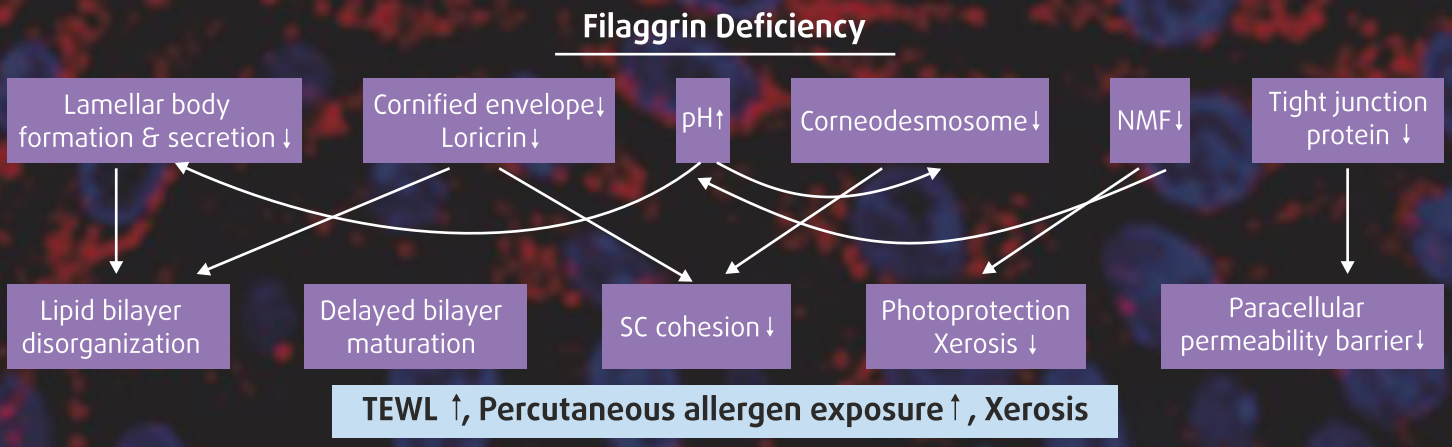
ROLE OF FILAGGRIN IN THE FORMATION OF THE EPIDERMAL BARRIER:

The main element of the skin barrier is the stratum corneum. This stratum is the end-product of the differentiation of keratinocytes, which, from the basal layer to the granulosum layer, are viable nucleated cells. These cells express various structural proteins as they mature. In the final steps of differentiation, the keratinocytes undergo marked changes in their structure, leading to their transformation into flat, anucleate squamous cells, the corneocytes. These corneocytes, which remain tightly bound together by corneodesmosomes, are covered by a cellular coating called the cornified envelope (CE), which has protein and lipid components that endow the cells with mechanical and chemical resistance. Between the cells there is a hydrophobic, lipidrich extracellular matrix arranged in a laminar bilayer. This organization of the stratum corneum has been called 'bricks and mortar', in which the keratinocytes are the bricks and the extracellular lipid matrix is the mortar. A calcium gradient exists in the epidermis, with low concentrations in the basal layer, even lower concentrations in the stratum spinosum, high levels in the stratum granulosum, and very low levels in the stratum corneum. This gradient is important in terminal keratinocyte differentiation. The higher concentration of calcium in the stratum granulosum causes the keratohyalin granules to release their contents, leaving the profilaggrin exposed to undergo processing and fragmentation into active filaggrin monomers. This free filaggrin binds to the intermediate keratin filaments, causing their aggregation and compaction, provoking the collapse and flattening of the cell. Simultaneously, the cell expresses a series of structural proteins that make up the protein portion of the CE. The bundles of keratin intermediate filaments aggregated by filaggrin bind to these structural proteins through the action of transglutaminases. The action of these enzymes on the lipids gives rise to the lipid portion of the CE and the extracellular matrix of the stratum corneum. Filaggrin continues to undergo processing by various proteases.

This proteolysis leads to the release of hygroscopic amino acids and their derivatives, which form natural moisturizing factor (NMF), responsible for water retention in the stratum corneum. The breakdown of some of these amino acids gives rise to 2 organic acids: trans-urocanic acid (UCA), a histidine derivative; and pyrrolidone-5-carboxylic acid (PCA), a glutamine derivative. These acids are 2 of the main factors responsible for maintaining the acid pH of the stratum corneum, which is essential for its role in the metabolism and organization of the lipids of the extracellular matrix, for its antimicrobial action, and for its regulatory role on enzyme activity and physiologic desquamation. In addition, UCA has a photoprotective effect against UV radiation18 and has been shown to be a key mediator in UV-B---induced immunosuppression.

THE EPIDERMAL BARRIER AND FILAGGRIN DEFICIT:

Filaggrin deficit has a major impact on the epidermal barrier, affecting the organization of the keratin filaments of the cytoskeleton and the structure of the CE. There is also a fall in the number of keratohyalin granules, a marked fall in NMF concentration (and thus in hydration of the stratum corneum), and alkalinization of the skin pH. In addition to these changes, recent ultrastructural studies have shown that a deficit of filaggrin is also associated with a generalized fall in the density of corneodesmosomes and of tight intercellular junctions, as well as with abnormalities in the architecture of the extracellular lipid matrix; these changes may produce a notable alteration of barrier function, provoked by abnormalities in the organization of the cytoskeleton (which affect lamellar body maturation and exocytosis, leading to a nonuniform distribution of secreted lipids and enzymes) and by the increase in the pH (which modulates the activity of those enzymes). Furthermore, the increase in the activity of certain proteases caused by the persistent elevation of the pH favors the release of proinflammatory mediators by keratinocytes; these mediators induce an inflammatory response mediated by type 2 helper T (Th2) cells even in the absence of allergens. For example, alkalinization of the skin pH increases the activity of the proteases responsible for the production of interleukin (IL) 1and IL-1 from their inactive proproteins generated by keratinocytes.



Together these epidermal components form an effective flexible epidermal barrier against the penetration of environmental irritants, allergens and infections and transepidermal water loss and dessication.

TOPICAL THERAPY FOR ATOPIC DERMATITIS (AD). A PRACTICAL OPTION? ARE EXPECTATIONS MET?

Valerie Fadok (USA) opened the workshop by describing the importance of topical therapy and shared her data from an open-label study on the effect of a sodium hypochlorite shampoo for the management of canine pyoderma. A product containing sodium hypochlorite with salicylic acid was assessed for the treatment of canine pyoderma in atopic dogs. The study focused on the efficacy of the shampoo in methicillin-resistant staphylococcal pyoderma. Other therapies needed to control signs of AD were allowed and owners were asked to bathe their dogs three times per week. Dogs were re-evaluated after 2 and 4 weeks. No antibiotics or other shampoos were allowed during the study. A client questionnaire with a scoring system of 0–3 for redness, crusting, odour, percentage of the body affected and severity of itch was used. After 2 weeks of bathing, a significant decrease in number of bacteria was found on cytology and by 4 weeks most dogs had no bacteria. In terms of clinical assessment, there was a statistically reduced clinical score at 2 and 4 weeks compared to baseline but no difference between weeks 2 and 4.

Ralf Mueller (Germany) reviewed several studies involving topical antibacterial therapy. The first study evaluated a chlorhexidine shampoo versus placebo in the treatment of canine pyoderma. Dogs were shampooed weekly; cytology and corneocyte bacterial counts were assessed before and after treatment with the placebo or chlorhexidine. There was a significant decrease in bacteria in both groups. The chlorhexidine shampoo decreased counts more but the difference at the end of the study between the placebo and chlorhexidine group was not statistically different. This suggests that mechanical removal of material with bathing is important regardless of the shampoo used. Topical antimicrobial therapy has also been applied to treatment of canine demodicosis.

Ralf Mueller reported on an unpublished study that used a titanium shower head with holes that produce foam-like water. It was theorized that this type of treatment might cause water to push oxygen into the tissue. This additional oxygen could potentially increase healing, decrease pruritus and have antibacterial activity. The recommended shampoo with the device contained basic cleansing ingredients.

The use of topical corticosteroids was reviewed next. Hydrocortisone Aceponate Topical Spray is considered the topical steroid of choice because it does not cause

systemic effects. Kerstin Bergvall (Sweden) has used this spray for the treatment of Malassezia-infected ears. This may sound counterintuitive as steroids are immunosuppressive, so one might expect that infection could worsen. However, if inflammation decreases then ceruminous gland secretion decreases and Malassezia numbers normalize.

Vanessa Schmidt reviewed a double-blind placebo-controlled study to evaluate the long-term maintenance use of hydrocortisone aceponate topical spray. The dogs recruited in this study were allocated to placebo or hydrocortisone aceponate groups. Dogs were allocated after they had been treated and were regarded as being stable or having a very low Canine Atopic Dermatitis Extent and Severity Index (CADESI) score. Dogs were randomized into two placebo and two hydrocortisone aceponate groups, twice a week. The outcome of the study was to look for recurrence of clinical symptoms measured as the median time until relapse. The time to relapse was significantly longer in the hydrocortisone aceponate group (median 115 days) compared to the placebo group (median 33 days). At day 50, more than 85% of the dogs in the placebo group had relapsed. This study supports the use of proactive anti-inflammatory treatment in atopic canine patients when the skin appears normal and the patient is stable to prevent the frequency of flares and reduce the severity of flares when they occur.

Genevieve Marignac commented that removal of biofilms with topical therapy is important but it is also important to maintain skin lipids, which may help the efficacy of some topical drugs. She reviewed a study involving lipids and the presence or absence of biofilms on the skin; changing the skin lipid composition can make it more difficult for a biofilm to develop. Atopy disrupts the stratum corneum, which may lead to a larger biofilm; small biofilms with a large diversity of microbes are optimal for normal skin. Shampoos and conditioners may affect skin biofilms differently: shampoo may help remove biofilms and conditioners may make heavy monomorphous biofilms more difficult to adhere.

Sergi Segarra (Spain) discussed the combination of sphingolipids and glycosaminoglycans (GAGs) for the topical treatment of AD. Two groups of compounds, GAGs and polysaccharides like chondroitin sulfate and A study was presented where treatments involved a combination of topical lipids and GAGs to see if this could be effective in the management of canine AD. Using cultures of human dermal fibroblasts, skin hydration and the potential for increased skin elasticity and for the proliferation and migration of fibroblasts was assessed using this combination. A significant increase in skin hydration, skin fibroblast proliferation and migration of fibroblasts was seen. Defects in the skin barrier of dogs with AD was the focus of another study. 8 The objective was to assess the changes induced by three different sphingolipid extracts that were produced by Bioiberica (Barcelona, Spain): sphingolipid extracts 1, 2 and 3. Using a well-developed in vitro model for canine skin, the morphology by histopathology and also quantification of lipids was analysed after exposure to the three different sphingolipid extracts. Data showed that sphingolipid extract 1 stimulates the production of ceramides. Sphingolipid extract 1 contributes to the formation of a well-organized stratum corneum and represents a potential therapy for improving the skin barrier function in AD. The main lipid in the sphingolipid extract 1 is a sphingomyelin, which also has antiinflammatory activity through inhibition of prostaglandin E2 production. The sphingolipid and GAGs were combined into a single product and were assessed in a randomized doubleblind placebo-controlled study using beagles that were experimentally sensitized to house dust mites. The control group had six dogs that received no treatment. The treatment group received the combination of sphingolipids and GAGs applied to different parts of the body twice weekly. CADESI scores, transepidermal water loss, pruritus, pruritus visual analogue scale (PVAS) scores and other clinical signs (time samples of biting, licking and scratching) were evaluated. Blood samples were collected and tape stripping was done to look at lipid profiles. A significantly lower CADESI was achieved at 1 week with the topical treatment compared to the control group. A significant increase in CADESI was observed in the control group from the beginning of the treatment to the end. A significantly lower global pruritus score was achieved after 8 weeks in the treatment group and the pruritus increased significantly only in the control group. There was a significant increase in skin polyunsaturated fatty acids with treatment after 8 weeks. There were no significant differences in any other parameters and the product appeared safe with no reported side effects. Topical formulations containing sphingolipids and GAG extracts may be useful for the management of canine AD either alone or in combination with other therapies. Future studies should include a larger number of patients with naturally occurring disease and formulations with sphingolipids and GAGs as single active ingredients.

(Source: Excerpts from panel discussion held as a part of workshop held in the WCVD (World Veterinary Dermatology Congress) in Bourdeaux in 2016; also published in Advances in Veterinary Dermatology. Authors: Marsella & Friberg, Topical therapy for atopic dermatitis. Compilation © 2017 ESVD and ACVD)

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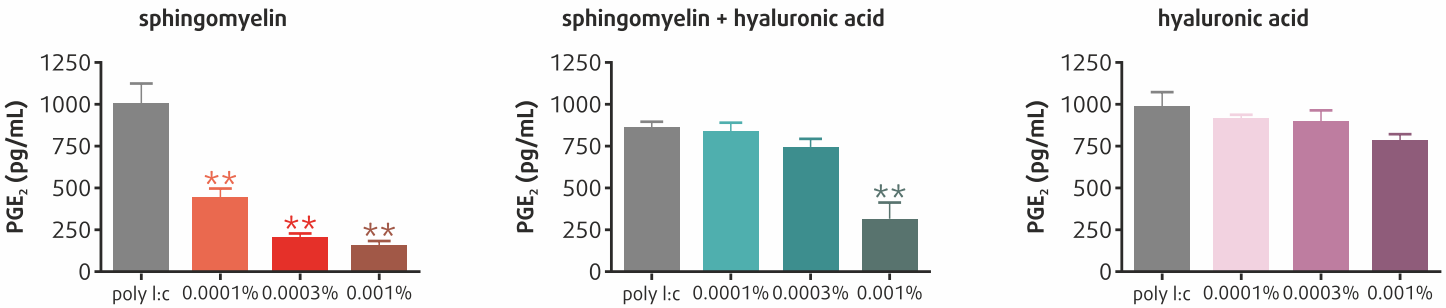
Effects of sphingomyelin and hyaluronic acid on prostaglandin E2 (PGE2) secretion in canine keratinocytes.

The present study was aimed to assess the inhibitory effects of sphingomyelin (SM) and hyaluronic acid (HA) on PGE2 secretion in canine keratinocytes cultures. The canine keratinocytes were isolated from skin biopsies and cultured either in the presence of sphingomyelin (SM) or hyaluronic acid (HA) or SM plus HA at increasing concentrations (0.001 mg/mL, 0.003 mg/mL and 0.01 mg/mL). Further, the cells were stimulated with poly-(I:C) at 10 µg/mL in order to induce PGE2 release. The PGE2 synthesis & release was studied by an enzymatic immunoassay. The results showed a significant inhibition of PGE2 secretion in the presence of SM at 0.001 mg/mL, 0.003 mg/mL and 0.01 mg/mL. It was concluded that the Sphingomyelin may have a therapeutic potential in the management of inflammatory skin diseases due to its ability to down-modulate PGE2 secretion in canine keratinocytes.



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