SCOPE

ECTOIN
THE NATURAL ALLERGY PROTECTION MOLECULE

SCIENTIFIC DOSSIER

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Overview

The ocular surface is facing various unspecific stress factors resulting in irritation and inflammation of the epithelia, causing discomfort to the patients. Ectoine is a bacteria-derived extremolyte with the ability to protect proteins and biological membranes from damage caused by extreme environmental conditions like heat, UV-light, high osmolarity, or dryness. Evidence from preclinical and clinical studies attest its effectiveness in treating several epithelium-associated inflammatory diseases, including the eye surface.

Ectoine forms a protective hydration shield around proteins and other biomolecules that is based on its strong binding capacities to water molecules. This mode of action is known as "preferential exclusion"; i.e., ectoine is preferentially excluded from the hydrate shield, leading to the alteration of the aqueous solvent structure. That effect protects proteins from damage and irreversible denaturation and stabilizes biological membranes.

Ectoine represents a widely applicable, well-tolerated and natural protective molecule against diverse harmful environmental influences such as heat, aridity or UV radiation. The cell protective and anti-inflammatory mode of action could be verified in various in vitro and in vivo studies.

In preclinical studies, ectoine was shown to protect lung and skin cells against the damage induced by toxic pollution particles and to prevent the subsequent activation of inflammatory cascades. Clinical trials have attributed topical applied ectoine effectiveness in upper airway inflammations such as acute pharyngitis/laryngitis, rhinosinusitis, rhinitis sicca, and acute bronchitis. In addition, several trials showed efficacy of ectoine in various diseases with barrier dysfunctions such as rhinitis sicca, chemotherapy induced mucositis, lung inflammation caused by environmental pollutants, prevention of upper respiratory infections, and atopic dermatitis. Moreover, studies on allergic rhinoconjunctivitis and dry eye syndrome have been published. A similar effect was observed in model systems for inflammatory bowel disease.

However, the studies presented in the clinical efficacy validation section of this dossier will focus on the efficacy of ectoin on the ocular surface i.e. on the tear film and corneal epithelium and provide a rationale on how the molecule works to deliver benefits as a protective agent against allergens.

Origin

Ectoine – C6 H10N2O2

Ectoine is a low molecular, cyclic amino acid derivative, which is produced by many different extremophilic microorganisms. Ectoine belongs to the class of compatible solutes also called extremolytes (osmolytes from extremophiles) and was first isolated by Galinski and colleagues from the bacterium Ectothiorhodospira halochloris found in Wadi Natrun, Egypt. [1]

In extremophilic microorganisms these low molecular weight compounds are accumulated in response to increased extracellular salt concentrations, but also as a response to other environmental changes, e. g. increased temperature. This is one of the ingenious strategies these organisms have developed to cope with harsh environmental conditions.

Extremolytes minimise the denaturation of biopolymers that usually occurs under conditions of water stress and are compatible with the intracellular machinery at high (>1 M) concentrations. Extremolytes have a wide range of applications due to their protection of biological macromolecules and cells from damage by external stresses.

One of the first extremolytes that was produced on a large scale is ectoine (trade name Ectoin®, a registered trademark of bitop AG, Germany). It is already used as a cell protectant in dermatological creams, skin care and as a stabiliser for proteins and cells in life sciences.

Ectoin® is used in Optase Allegro.

[1] Galinski, E.A., PFEIFFER, H.P. and Trüper, H.G., 1985. 1, 4, 5, 6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid: A novel cyclic amino acid from halophilic phototrophic bacteria of the genus Ectothiorhodospira. European Journal of Biochemistry, 149(1), pp.135-139.

Mode of action

This section was prepared based on technical and scientific documents supplied by bitop.

Ectoine stabilises biomolecules via a physical mechanism, called "preferential exclusion".[2] According to this theory, the protein stabilisation effects of osmolytes like ectoine are due to their effect on the solvent water leading to a preferential exclusion of the osmolyte from the protein surface and thereby to a preferential hydration of the protein. Because the surface area of globular proteins in the native state is smaller than in the denatured state, the equilibrium is shifted to the native state resulting in stabilisation of the native structure.

THE EFFECT IS BASED ON SEVERAL MECHANISMS:

1. Steric exclusion from the protein surface: this plays a role only in cases where the protective molecule is substantially larger than water.

2. Increase of the surface tension of water by the protective molecule: According to the Gibbs adsorption isotherm this must result in the exclusion of ectoine from the water-macromolecule interface.

3. Preferential hydration due to the solvophobic effect: Solvophobicity is a consequence of increased hydrophobic interactions caused by a solute molecule that enforces the water structure.

According to this concept of the osmophobic effect, the repulsion between the amide backbone of the protein and the osmolyte is due to the influence of the osmolyte on the water structure.

The osmolyte promotes the formation of water molecules in clusters. Thus the exclusion hypothesis attributes the stabilising effect of ectoine to changes in the surrounding water structure.

Ectoine is in contrast to e. g. sodium chloride a strongly kosmotropic (water structure forming) substance.

An investigation of the oxygen radial distribution function of pure water, sodium chloride and ectoine proved the stabilising effect of ectoine on the water structure **(Figure 1)**.

[2] Arakawa T, Timasheff SN. The stabilization of proteins by osmolytes. Biophys J 1985; 47(3):411-4.

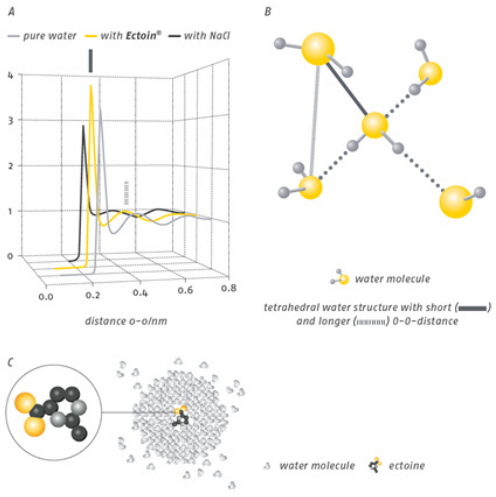

Figure 1: Effect of ectoine on the water structure

A) The radial distribution function of pure water (—) shows a maximum at approx. o.3nm (—), which demonstrates the direct interaction of two neighbouring water molecules. The smaller maxima at 0.45nm (|||||||||||)ond o.7nm demonstrote interactions of not neighbouring water molecules. The addition of NaCl (—) to water leads to a decrease in the maxima. In contrast, the solution containing ectoine (—) increases the maxima and thus enhances the tetrahedral water structure [3 J.

8) Tetrahedral structure of water, showing the interactions between water molecules at 0.3 nm (—) and 0.45 nm (|||||||||||) in the structure.

C) Complex formed by ectoine and water molecules.

Sodium chloride diminishes the interaction between water molecules. It destroys the water structure and is therefore chaotropic. In contrast to that, a solution containing ectoine increases the number of neighbouring water molecules. Thus, ectoine enhances the water-water-interactions. The tetrahedral structure of water is stabilised by ectoine. A molecular dynamic simulation gives further insight in the water structuring capabilities of ectoine. In this computer simulation, water diffusion out of water spheres was limited and decreased enormously by adding ectoine molecules to the sphere. Even a 5-fold longer simulation time showed a stable water structure form attributable to ectoine properties, which is superior compared with water itself and outstanding compared with water glycerol complex.

To explain this phenomenon, the total potential energy (Epot) was calculated. The Epot value of the water-ectoine mixture was smaller than of the water molecules per se, indicating the strong organising and complexing properties of ectoine. Furthermore, the Epot value of the water ectoine sphere remained constant even throughout a longer simulation time. These results also explain the stabilising effect of ectoine on proteins. The maintenance of the native state of a protein is a process driven by entropy, which results in the exclusion of hydrophobic moieties from contact with water. Stabilisation of the water structure leads to an increase of the hydrophobic interactions and therefore stabilises the whole protein structure. Due to the exclusion of ectoine from the hydration shell of biopolymers, a protective and stabilising shield is shaped around those biomolecules, which is termed the Ectoin® Hydro Complex **(Figure 2).**

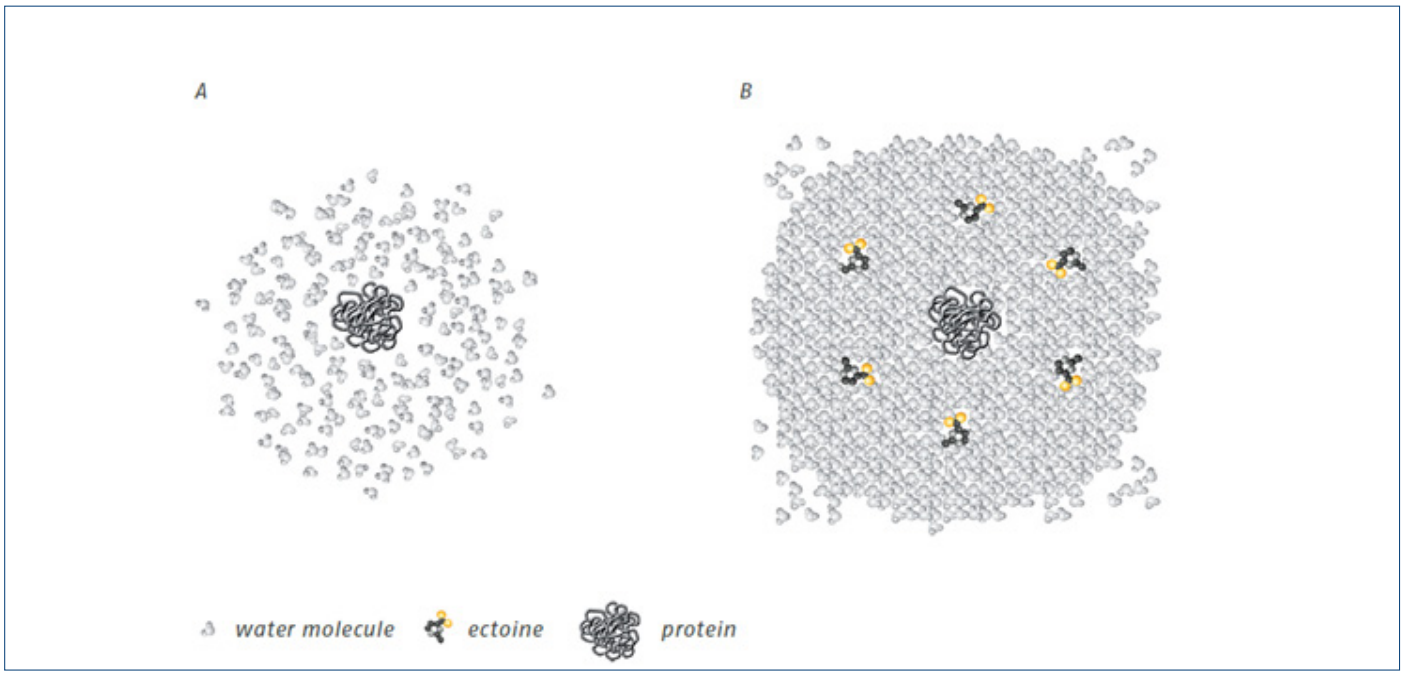

Figure 2: Stabilisation of biomolecules via ectoine according to the preferential exclusion model

A) Protein in water: the number of water molecules is small at the surface of the protein.

B) Protein in aqueous ectoine solution: the number of water molecules is increased by the formation of ectoine water complexes, hydrophobic interactions are increased and thus stronger stabilisation of the protein result.

The formation of ectoine water complexes and thus the kosmotropic effect of ectoine on the water structure shown above can stabilise lipid mono- and bilayers as well, which can be considered as a model for cell membranes. As shown in Figure 3, a lipid bilayer in water is stabilised by hydrophobic interactions of the apolar lipid tail and hydrophilic interactions of the polar lipid head groups to water. In an ectoine solution, the hydrophilic interactions are increased by the ectoine water complexes resulting in increased mobility of lipids and thus fluidity of lipid bilayers.

[3] Graf R, Anzali S, Buenger J, Pfluecker F, Driller H. The multifunctional role of ectoine as a natural cell protectant. Clin Dermatol 2008; 26(4):326-333.

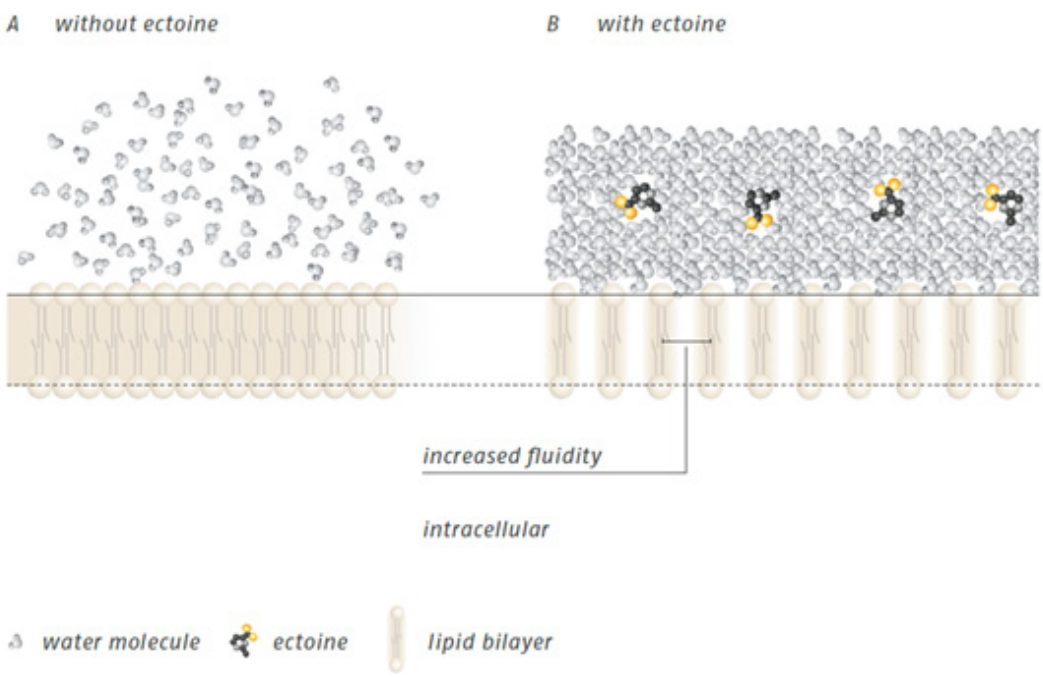

Figure 3: Membrane stabilisation and increase in membrane fluidity due to ectoine

A) Lipid bilayer in water: the bilayers are stabilised by hydrophilic interactions within head groups.

B) Lipid bilayer in aqueous ectoine solution: ectoine water complexes cause increased interactions of head groups with water and the membrane fluidity is increased.

The effect of ectoine on fluidity of lipid membranes was shown recently by film balance measurements of lipid monolayers (Figure 4). By increasing the surface pressure on a DPPC lipid monolayer in water the formation of rigid well-shaped domains can be observed at higher pressure. These rigid domains are much smaller in ectoine solutions. The higher the concentration of ectoine the smaller the rigid domains. The effect is already observed at the lowest concentration tested (1 mM).

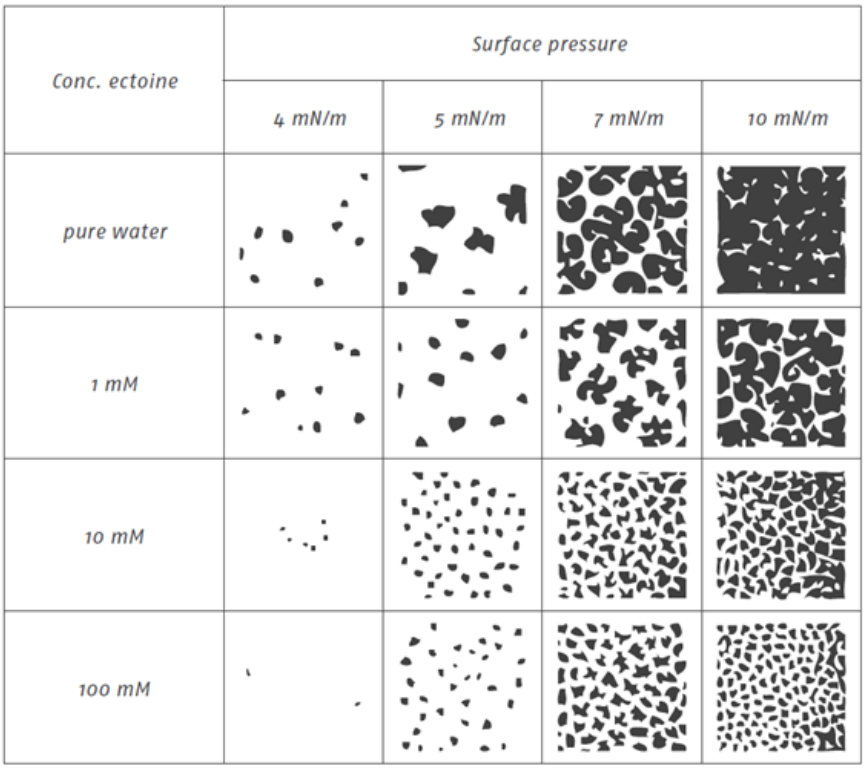

Figure 4: Fluorescence studies of film balance measurements

DPPC (Dipalmitoylglycerophosphatidylcholine) Monolayer at different surface pressure and ectoine concentrations. Rigid domains appear black, fluid domains bright. Without ectoine and a surface pressure of 10 mN/m, the lipid layer exists almost completely of rigid domains. With ectoine, more fluid domains are visible. This effect increases with increasing ectoine concentration. [4]

The physical state of the membrane influences the cell biology and its behaviour in response to external inputs. An increased fluidity of the membrane may for example induce the expression of stress-responsive, cell-protecting genes, such as heat-shock proteins, and reduce on-going inflammatory processes.[5,6] The modification of the distribution of membrane proteins in a more fluid membrane also alters their activity.

For example, integrins and selectins need a specific density and length to be efficient on the adhesion of reactive leukocytes, while lipoxygenases must be bound to the membrane to catalyse the release of the pro-inflammatory signal leukotrienes. The activation of Toll-like receptors in front of signals such as lipopolysaccharide (LPS) and tumor necrosis factor (TNF) a needs the formation of multimercomplexes. Several experiments have shown that physiologically relevant fluidisation of the membrane alters their functionality [6,7,8].

An increase in the membrane fluidity could reduce disease symptoms and accelerate healing. It is crucial for the efficient closure of wounds , and it has been suggested as mechanism for the very early effects of corticosteroids in asthma therapy and the beneficial effect of dietary moderate ethanol and polyunsaturated fatty acids intake in inflammatory diseases such as psoriasis, allergy, asthma and inflammatory bowel disease [12,13].

Extracellular membrane components (transmembrane proteins, lipids, extracellular matrix) are stabilised by ectoine in their native form. Without protective mechanisms, external and internal noxa can cause increased stress for cell membranes. Cells which are in direct contact with the environment like squamous epithelial cells, i. e. skin, upper airway, lung, and intestinal tract, are particularly endangered. The external stress leads to membrane damage, which causes water loss and inflammatory reactions in the tissue.

The **Ectoin®** Hydro Complex protects the cells against dehydration by accumulating water. Water molecules are bound more effectively near the membranes and form a stabilising and protecting complex. The impact of external pollutants on the cells is decreased by the stabilising effect of the **Ectoin®** Hydro Complex. It protects the cells from inflammation caused by environmental stress factors like dehydration, UV radiation, tensides or airborne particles.

The Ectoin® Hydro Complex protects the membrane and prevents the release of stress mediators (e. g. ceramides), which mediate inflammatory processes. Therefore, inflammation can be limited **(Figure 5).**

[5] Galinski EA, Pfeiffer HP, Truper HG. 1,4,5,6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid. A novel cyclic amino acid from halophilic phototrophic bacteria of the genus Ectothiorhodospira. Eur J Biochem 1985; 149(1):135-9.
[6] Vigh L, Maresca B, Harwood JL. Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 1998; 23(10):369-374.
[7] Gaborski TR, Clark A, Jr., Waugh RE, McGrath JL. Membrane mobility of beta2 integrins and rolling associated adhesion molecules in resting neutrophils. Biophys J 2008; 95(10):4934-4947.
[8] Kariko K, Weissman D, Welsh FA. Inhibition of toll-like receptor and cytokine signaling - a unifying theme in ischemic tolerance. J Cereb Blood Flow Metab 2004; 24(11):1288-1304.
[9] Pande AH, Qin S, Tatulian SA. Membrane fluidity is a key modulator of membrane binding, insertion, and activity of 5-lipoxygenase. Biophys J 2005; 88(6):4084-4094.
[10] Gojova A, Barakat AI. Vascular endothelial wound closure under shear stress: role of membrane fluidity and flow-sensitive ion channels. J Appl Physiol 2005; 98(6):2355-2362.
[11] Horvath G, Wanner A. Inhaled corticosteroids: effects on the airway vasculaturein bronchial asthma. Eur Respir J 2006; 27(1):172-187.
[12] Grimble RF. Dietary lipids and the inflammatory response. Proc Nutr Soc 1998;57(4):535-542.
[13] Goral J, Karavitis J, Kovacs EJ. Exposure-dependent effects of ethanol on the innate immune system. Alcohol 2008; 42(4):237-247

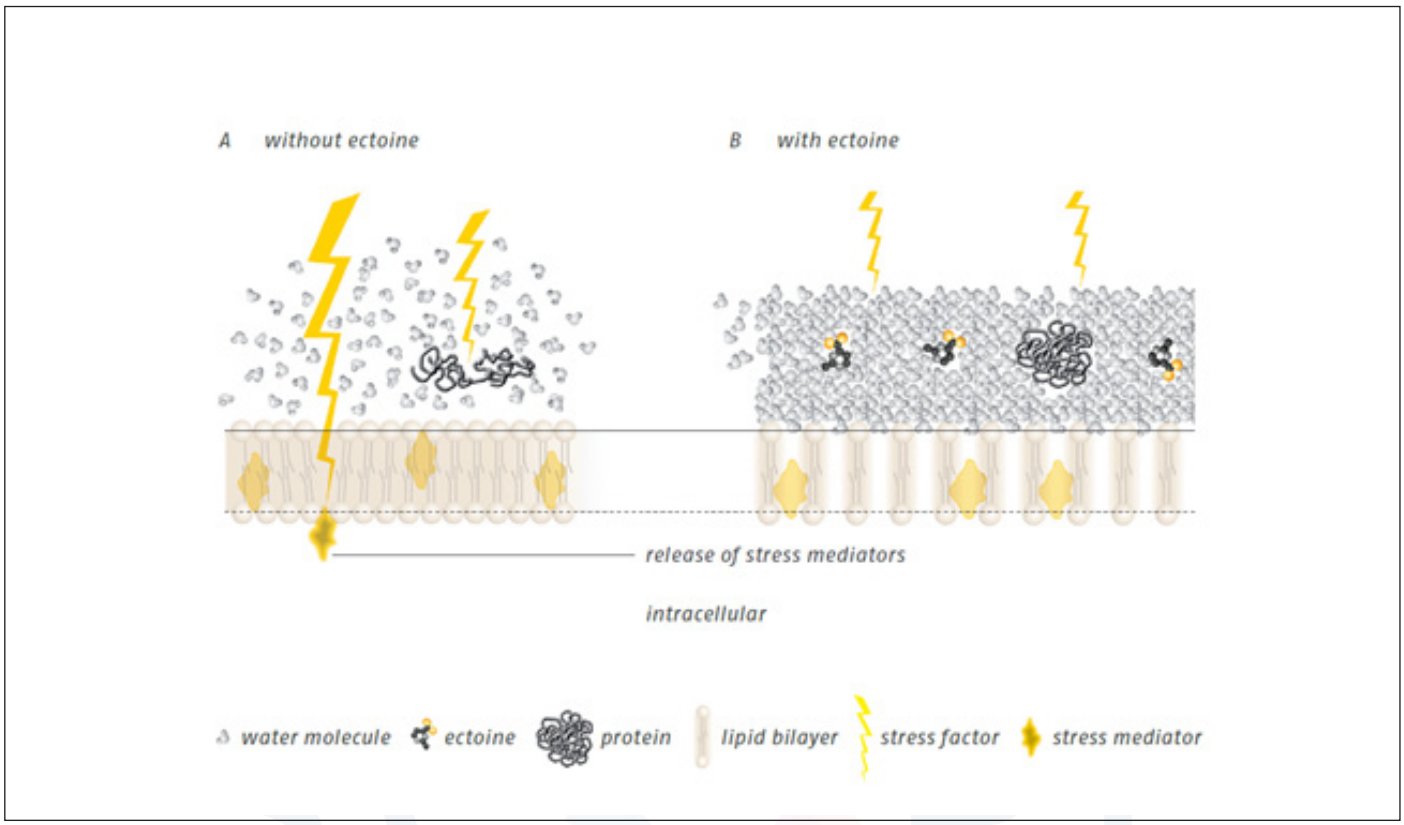

Figure 5: Prevention of release of stress mediators (e. g. ceramides) by ectoine

A) Without ectoine: external stress factors (like UV irradiation) cause membrane damage, water loss and release of stress mediators, which act as second messengers for inflammatory reactions.

B) With ectoine: the **Ectoin®** Hydro Complex protects the membrane against external stress factors; stress mediator release is prevented. The general protective and hydrating property of ectoine against various external stress factors is the basic mechanism of our medical device developments.

[14] Bünger J, Driller H. Ectoin: an effective natural substance to prevent UVA-inducedpremature photoaging. Skin Pharmacol Physiol 2004; 17(5):232-237. pretreated ectoine against an untreated control in per cent. The untreated control was determined as 0. The assay was performed 5 times .

Validation of mode of action

This section was prepared based on technical and scientific documents supplied by bitop.

Various experiments with model systems or cell cultures demonstrate the protective and stabilising effect of the Ectoin® Hydro Complex.

Protection of lipid membranes

Cell membranes are lipid double layers with integrated proteins and surface proteins. They possess specific ion channels, transport systems and receptors responsible for the signal transduction.The viability of the cells is highly associated with the efficiency of these systems. Many external factors, like temperature, radicals, pH, UV radiation, and tensides, can disturb the balance of the systems and damage the membrane. In a red blood cell assay, ectoine showed significant protective and stabilising effects on cell membranes.[14] This assay investigates the denaturising nature of different substances on erythrocytes. Membrane damage leads to a release of the haemoglobin and thus to a red colour in the surrounding media, which can be measured photometrically. Erythrocytes have been pretreated with 1% ectoine or lecithin as positive control for one hour and stressed with different tensides. Ectoine protects cell membranes against all kind of damaging tensides used in this test. It is even more efficient than the positive control lecithin (O-phosphatidylcholine), whose stabilising properties are well known (Figure 6).

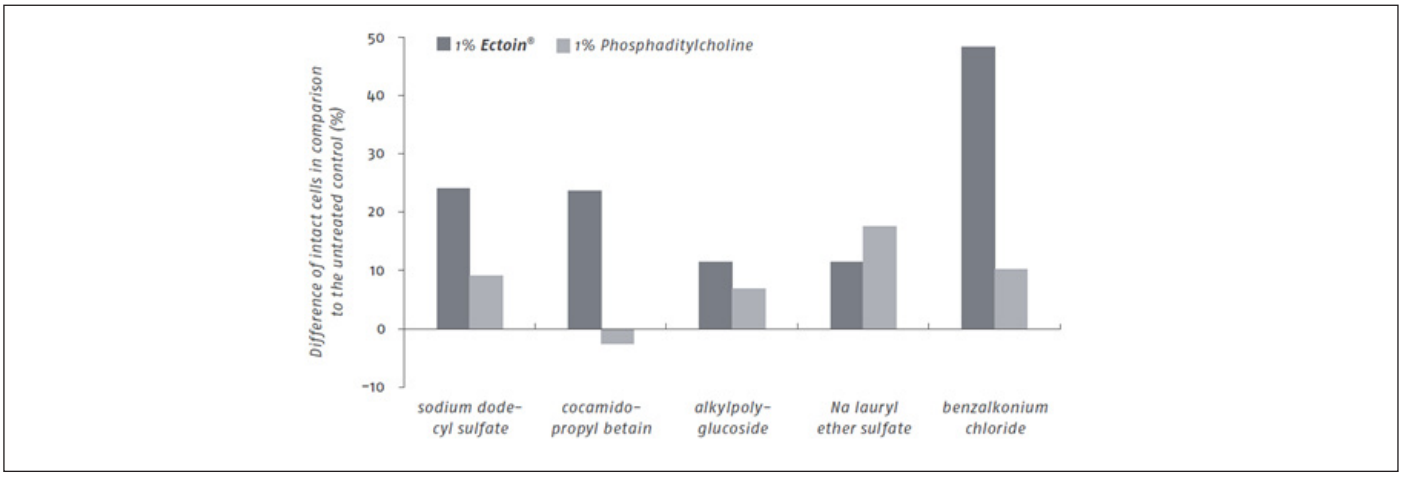

Figure 6: Membrane stabilising effect of ectoine Protective effect of ectoine on the membrane of human erythrocytes against sodium dodecyl sulfate (H50 = 29.63 ppm), cocamidopropyl betain (H50 = 41.45 ppm), alkylpolyglucoside (H50 = 131.76 ppm), sodium lauryl ether sulfate (H50 = 26.45 ppm) and benzalkonium chloride (H50 = 35.49 ppm). The figure shows the relative difference of cell lysis as a function of the concentration of pretreated ectoine against an untreated control in per cent. The untreated control was determined as 0. The assay was performed 5 times[15]

Graf and colleagues investigated the concentration and time dependency of this membrane stabilising effect of Ectoin®[16]. The higher the ectoine concentration the greater the protective effect against membrane damage. Prolonged incubation resulted in an increase in membrane stability of 30% after 6 hours and 60% after 24 hours. Thus, the longer the cells are pretreated with ectoine the greater the protective effect.

4.1.2 Decrease of inflammation process caused by external pollutants

The stabilising Ectoin® Hydro Complex also decreases inflammation processes caused by external pollutants like UV radiation[17],[18],[19]. The UVA-induced signal transduction is triggered by release of ceramides (second messenger) in the cell membrane and can be measured by activation of AP-2 and generation of ICAM-1 (Figure 7).

[15] Bünger J, Degwert J, Driller H. The protective function of compatible solute ectoin on the skin cells and its biomolecules with respect to UV-ratiation, immunosuppression and membrane damage. IFSCC Magazine 2001; 4(2):1-6.
[16] Graf R, Anzali S, Buenger J, Pfluecker F, Driller H. The multifunctional role of ectoine as a natural cell protectant. Clin Dermatol 2008; 26(4):326-333.
[17] Bünger J, Driller H. Ectoin: an effective natural substance to prevent UVA-induced premature photoaging. Skin Pharmacol Physiol 2004; 17(5):232-237.
[18] Grether-Beck S, Bonizzi G, Schmitt-Brenden H, Felsner I, Timmer A, Sies H, et al. Non-enzymatic triggering of the ceramide signalling cascade by solar UVA radiation. EMBO J 2000; 19(21):5793-800
[19] Grether-Beck S, Olaizola-Horn S, Schmitt H, Grewe M, Jahnke A, Johnson JP, et al. Activation of transcription factor AP-2 mediates UVA radiation- and singlet oxygen-induced expression of the human intercellular adhesion molecule 1 gene. Proc Natl Acad Sci U S A 1996; 93(25):14586-91

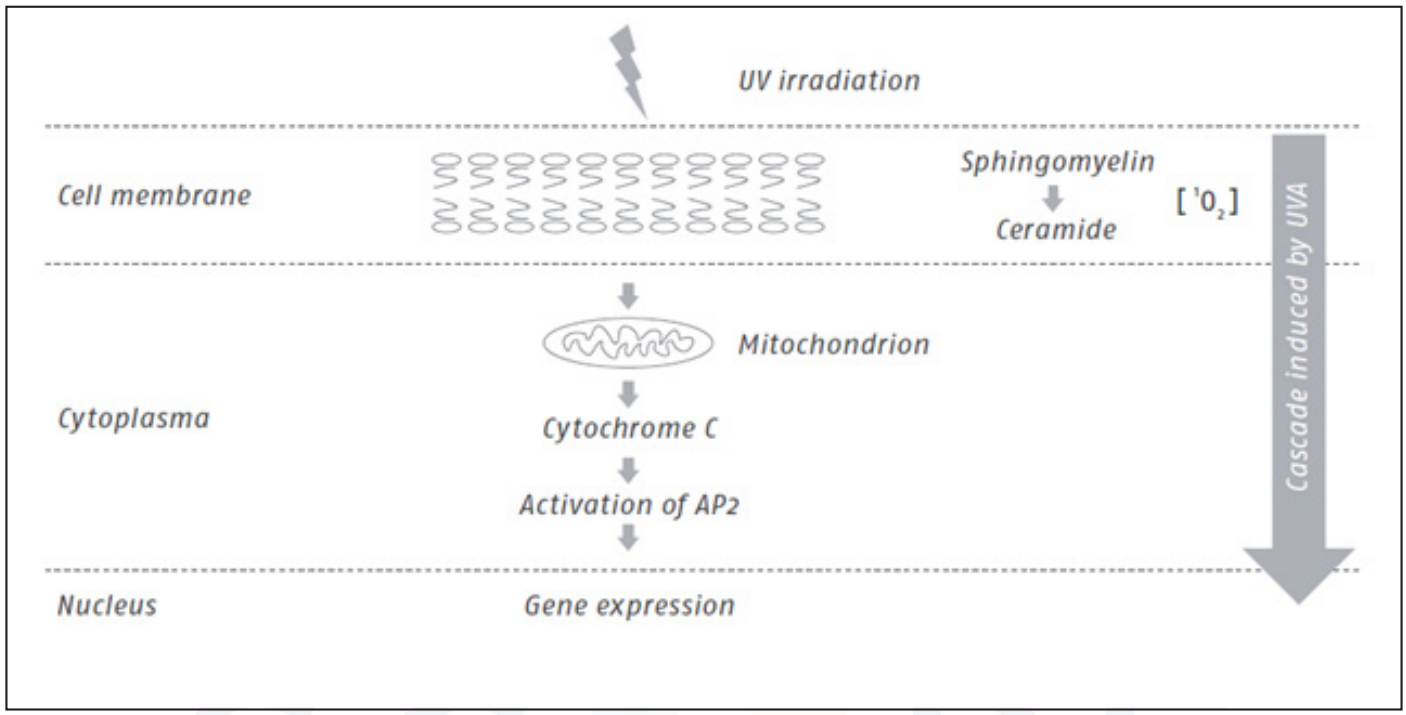

Figure 7: Model of UVA-induced reactions in human keratinocytes modified [17]

Human keratinocytes were pretreated with 1 mM ectoine for 24 hours and stressed via UVA radiation (30 J /cm2). The release of certain inflammation factors (AP-2, ICAM-1, ceramides) was measured. Pretreatment of human keratinocytes with ectoine leads to a significant decrease of second messenger release (Figure 8), AP-2 activation and ICAM-1 expression. Thus the Ectoin® Hydro Complex diminishes inflammatory processes caused by UVA radiation. Ceramide release is a general mechanism in inflammatory processes and is also postulated for e. g. lung cells.

[20] Graf R, Anzali S, Buenger J, Pfluecker F, Driller H. The multifunctional role of ectoine as a natural cell protectant. Clin Dermatol 2008; 26(4):326-333.

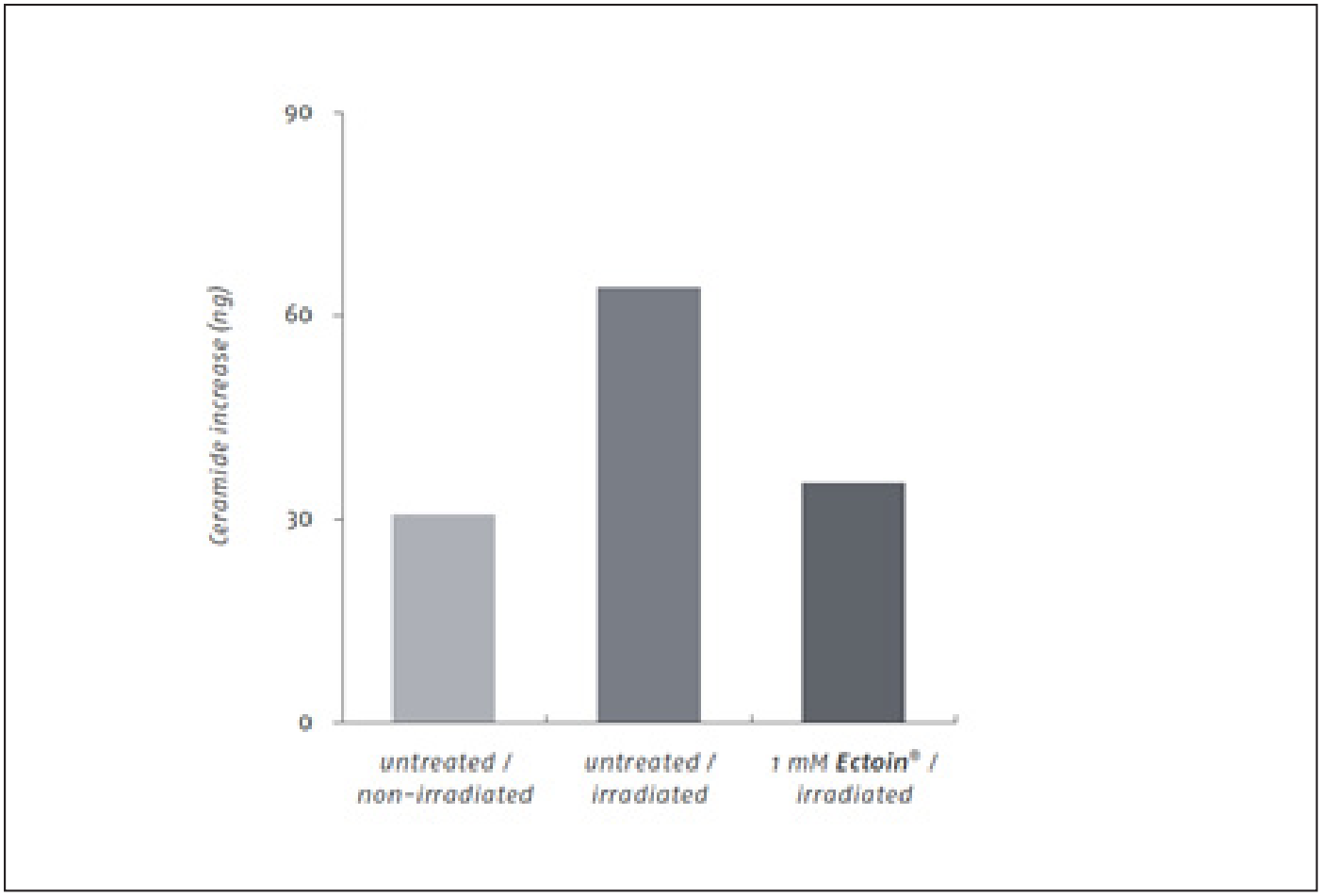

Figure 8: Effect of ectoine on second messenger release

UVA-induced ceramide release (ceramide split off membrane sphingomyelin) in ng. Cells were either pretreated with 1 mM ectoine or untreated. The experiment was repeated 3 times. Ectoine reduces the ceramide release after UVA irradiation

Clinical Efficacy Validation

Review Article: Ectoine in the Treatment of Irritations and Inflammations of the Eye Surface

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Abstract

The ocular surface is facing various unspecific stress factors resulting in irritation and inflammation of the epithelia, causing discomfort to the patients. Ectoine is a bacteria-derived extremolyte with the ability to protect proteins and biological membranes from damage caused by extreme environmental conditions like heat, UV-light, high osmolarity, or dryness. Evidence from preclinical and clinical studies attest its effectiveness in treating several epithelium-associated inflammatory diseases, including the eye surface. In this review, we analysed 16 recent clinical trials investigating ectoine eye drops in patients with allergic conjunctivitis or with other unspecific ocular inflammations caused by e.g. ophthalmic surgery. Findings from these studies were reviewed in context with other published work on ectoine. In summary, patients with irritations and unspecific inflammations of the ocular surface have been treated successfully with ectoine-containing eye drops. In these patients, significant improvement was observed in ocular symptoms of allergic rhinoconjunctivitis, postoperative secondary dry eye syndrome, or ocular reepithelisation after surgery. Using ectoine as an add-on therapy to antihistamines, in allergy patients accelerated symptom relief by days, and its use as an add-on to antibiotics resulted in faster wound closure. Ectoine is a natural substance with an excellent tolerability and safety profile thus representing a helpful alternative for patients with inflammatory irritation of the ocular surface, who wish to avoid local reactions and side effects associated with pharmacological therapies or wish to increase the efficacy of standard treatment regimen.

Full reference:

Bilstein, A., Heinrich, A., Rybachuk, A. and Mösges, R., 2021. Ectoine in the treatment of irritations and inflammations of the eye surface. BioMed Research International, 2021.

Downloadable version : Available at: https://www.hindawi.com/journals/bmri/2021/8885032/ Accessed on: April 2023

Clinical Study: Effects of ectoine containing nasal spray and eye drops on symptoms of seasonal allergic rhinoconjunctivitis

Anne M. Salapatek, Nina Werkhäuser, Basma Ismail, Ralph Mösges, Esther Raskopf, Andreas Bilstein

Abstract

Background: Patients are often dissatisfied with the symptom control obtained from available pharmacological treatments for seasonal allergic rhinoconjunctivitis (ARC). Therefore, patients seek for alternative, nonpharmacological options to treat their symptoms. Here, we assessed the efficacy of ectoine nasal spray and ectoine eye drops in comparison to placebo to prevent nasal and ocular symptoms following exposure to pollen in patients with ARC.

Methods: In this double-blind, randomized, placebo-controlled, cross-over study, 46 patients with ARC applied ectoine eye drops and nasal spray in immediate succession or placebo eye drops and nasal spray for 13 days before ARC symptoms were induced in an environmental exposure chamber. Primary endpoint was the baseline-adjusted area under the curve (AUC) posttreatment total nasal symptom score (TNSS) and the total ocular symptom score (TOSS) using analysis of covariance. Secondary endpoints were, amongst others, total nonnasal symptoms score (TNNSS) and nasal patency (measured using acoustic rhinometry).

Results: Treatment with both ectoine and placebo reduced TNSS, TOSS, and TNNSS upon allergen exposure. The analysis of parameters at baseline and after allergen exposure demonstrated that ectoine induced a clinically relevant improvement in ARC symptoms compared to placebo: the least square mean difference for baseline adjusted AUC was −1.87 for TNSS, −1.45 for TOSS and −2.20 for TNNSS. The mean change from baselineAUCof TNNSS for ectoine was also significantly greater than for placebo (−5.49 vs. −3.46; p = 0.011). Ectoine significantly improved the singular symptoms "sneezing," "watery eyes" and "itchy eyes" (p ≤ 0.021) as well as "itchy ear/ palate" (p = 0.036) in comparison to placebo. Mean cross sectional areas of the nasal cavity were reduced to a lesser extent after treatment with ectoine (−0.020 + 0.022) than with placebo (−0.047 + 0.029). The current study also demonstrated a very good safety profile of ectoine treatment. Few AEs with comparable numbers in both treatment groups were reported during the study, which were mild in severity and resolved without medical treatment.

Conclusion: The study suggests that ectoine is effective in reducing nasal and ocular symptoms associated with ARC. Being a natural, bacteria derived stress protection molecule functioning by a physical mode of action, it therefore represents an alternative nonpharmacological treatment option.

Full reference:

Salapatek, A.M., Werkhäuser, N., Ismail, B., Mösges, R., Raskopf, E. and Bilstein, A., 2021. Effects of ectoine containing nasal spray and eye drops on symptoms of seasonal allergic rhinoconjunctivitis. Clinical and Translational Allergy, 11(1), p.e12006.

Downloadable version: Available at: https://onlinelibrary.wiley.com/doi/full/10.1002/clt2.12006 Accessed on: April 2023

Clinical Study: Treatment of Allergic Rhinitis with Ectoine Containing Nasal Spray and Eye Drops in Comparison with Azelastine Containing Nasal Spray and Eye Drops or with Cromoglycic Acid Containing Nasal Spray

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1 Bitop AG, Stockumer Straße 28, 58453Witten, Germany 2 Private Health Centre, Institute for ENT Elmshorn, Hermann-Ehlers-Weg 4, 25337 Elmshorn, Germany

Objectives. Allergic rhinitis is a common disease with increasing prevalence and high impact on economic burden and comorbidities. As treatment with pharmacological drugs is not always satisfactory due to side effects and incomplete efficacy, alternative treatment strategies are needed. Ectoine is an osmolyte withmembrane stabilizing and inflammation reducing capacities. Nasal spray and eye drops containing ectoine are promising new treatment regimens for allergic rhinitis sufferers.

Design and Methods. The current two noninterventional trials evaluated the efficacy and safety of ectoine containing nasal spray and eye drops for treating allergic rhinitis in comparison with either azelastine or cromoglycic acid containing products. Nasal and ocular
symptom developments as well as judgment of tolerability and efficacy were assessed both by investigators and patients over a time period of one to two weeks.

Results. Both trials confirmed that ectoine containing products reduced nasal and ocular symptoms in allergic rhinitis patients. Results clearly demonstrated good safety profiles of the ectoine products comparable to those of azelastine and even better to those of cromoglycate products.

Conclusion. Ectoine containing nasal spray and eye drops are interesting new treatment strategies for sufferers of allergic rhinitis, combining both good efficacy and absence of side effects.

Full reference:

Werkhäuser, N., Bilstein, A. and Sonnemann, U., 2014. Treatment of allergic rhinitis with ectoine containing nasal spray and eye drops in comparison with azelastine containing nasal spray and eye drops or with cromoglycic acid containing nasal spray. Journal of allergy, 2014.

Downloadable version: Available at: https://downloads.hindawi.com/archive/2014/176597.pdf Accessed on: April 2023

Clinical Study: Retrospective study to evaluate the efficacy on vernal kerato-conjunctivitis (VKC) of 2% Ectoine versus 0.05% ketotifen eye-drops

Pia Allegri; Giuseppina Marrazzo; Chiara Ciurlo; Antonio Mastromarino; Silvia Autuori; Ugo Murialdo

Purpose: Ectoine is a strong water structure-forming solute. It exerts cell-protective, anti-inflammatory and anti-allergic activity. VKC is a rare, severe, challenging seasonal eye-allergic childhood disease managed in our referral hospital center. We underwent a retrospective case series review to evaluate and compare, when administered in pre-allergic and allergic period, the efficacy of this solution versus 0.05% Ketotifen eye drops (Ketoftil®) on signs and symptoms of VKC.

Methods: We evaluated records from 64 male pediatric subjects (mean age 8.5 y ± 2 months) and divided it into two groups of patients treated three times a-day with Ectoine 2% (Group A) and patients treated with 0.05% Ketotifen eye drops (Group B). The included patients were evaluated from February 2013 until November 2013 on three visits (at the beginning of treatment, 3 (±10 days) months later and 6 (±20 days) months).Main criteria of statistical evaluation were for VKC slit-lamp signs: Focal or diffuse conjunctival hyperemia; BUT; Modified Oxford scale; VKC grading (modified Bonini scale); and for VKC signs: VAS scale grading (ocular pain, itching, tearing, photophobia and foreign body sensation); Quick questionnaire on tolerance of the formulation. We compared the previous year treatment and evaluated the beginning of Cyclosporine eye drops treatment compared to the previous season and the different Cyclosporine dosages (0.25%-0.5%-1%-2%) used before

Results: When evaluating symptoms and signs of VKC patients, both groups showed a significant improvement ($p<0.0001$) from baseline and at three and six months after treatment. Although we found a significant difference ($p<0.0001$) between the two treatments on the tolerability at each time point. Furthermore both drugs delayed the beginning of treatment with Cyclosporine eye drops and reduced the concentration dosage.

Conclusions: Our case series review allowed us to establish that both topic treatments with 2% Ectoine as well as 0.05% Ketotifen are effective in improving signs and symptoms of VKC and to delay the adjuvant treatment with Cyclosporin. Therefore, we can conclude that Ectoine (a natural compound without any side effects) can be considered, in efficacy, equal to Ketotifen and is better tolerated by pediatric patients. Further studies with the inclusion of a bigger number of subjects are required.

Full reference:

Allegri, P., Marrazzo, G., Ciurlo, C., Mastromarino, A., Autuori, S. and Murialdo, U., 2014. Retrospective study to evaluate the efficacy on vernal kerato-conjunctivitis (VKC) of 2% Ectoine versus 0.05% ketotifen eye-drops. Investigative Ophthalmology & Visual Science, 55(13), pp.2492-2492.

Downloadable version: Available at: https://iovs.arvojournals.org/article aspx?articleid=2267842 Accessed on: April 2023

Mode of action study: Mode of action study Biophysical investigations of the structure and function of the tear fluid lipid layer and the effect of ectoine. Part A: Natural meibomian lipid films

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Abstract

The tear fluid lipid layer is the outermost part of the tear film on the ocular surface which protects the eye from inflammations and injuries. We investigated the influence of ectoine on the structural organization of natural meibomian lipid films using surface activity analysis and topographical studies. These films exhibit a continuous pressure–area isotherm without any phase transition. With the addition of ectoine, the isotherm is expanded towards higher area per molecule values suggesting an increased area occupied by the interfacial lipid molecules.

The AFM topology scans of natural meibomian lipid films reveal a presence of fiber-like structures. The addition of ectoine causes an appearance of droplet-like structures which are hypothesized to be tri-acyl-glycerols and other hydrophobic components excluded from the lipid film. Further the material properties of the droplet like structure with respect to the surrounding were determined by using the quantitative imaging mode of the AFM technique. The droplet-like structures were found to be comparatively softer than the surrounding. Based on the observations a preliminary hypothesis is proposed explaining the mechanism of action of ectoine leading to the fluidization of meibomian lipid films. This suggests the possibility of ectoine as a treatment for the dry eyesyndrome.

Full reference:

Dwivedi, M., Backers, H., Harishchandra, R.K. and Galla, H.J., 2014. Biophysical investigations of the structure and function of the tear fluid lipid layer and the effect of ectoine. Part A: Natural meibomian lipid films. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838(10), pp.2708-2715.

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Mode of action study: Biophysical investigations of the structure and function of the tear fluid lipid layers and the effect of ectoine. Part B: Artificial lipid films

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Abstract

The tear fluid lipid layer is present at the outermost part of the tear film which lines the ocular surface and functions to maintain the corneal surface moist by retarding evaporation. Instability in the structure of the tear fluid lipid layer can cause an increased rate of evaporation and thus dry eye syndrome. Ectoine has been previously shown to fluidize lipid monolayers and alter the phase behavior. In the current study we have investigated the effect of ectoine on the artificial tear fluid lipid layer composed of binary and ternary lipid mixtures of dipalmitoyl phosphatidylcholine (DPPC), cholesteryl esters and tri-acyl-glycerols. The focus of our study was mainly the structural and the biophysical aspects of the artificial tear fluid lipid layer using surface activity studies and topology analysis. The presence of ectoine consistently causes an expansion of the pressure–area isotherm indicating increased intermolecular spacing. The topology studies showed the formation of droplet-like structures due to the addition of ectoine only when tri-acyl-glycerol is present in the mixture of DPPC and chol-palmitate, similar to the natural meibomian lipids. Consequently, the hypothesis of an exclusion of tri/di-acyl-glycerol from the meibomian lipid film in the presence of ectoine in the subphase is confirmed. A model describing the effect of ectoine on meibomian lipid films is further presented which may have an application for the use of ectoines in eye drops as a treatment for the dry eye syndrome.

Full reference:

Dwivedi, M., Brinkkötter, M., Harishchandra, R.K. and Galla, H.J., 2014. Biophysical investigations of the structure and function of the tear fluid lipid layers and the effect of ectoine. Part B: artificial lipid films. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838(10), pp.2716-2727.

Downloadable version: Available at: https://www.sciencedirect.com/science/article/pii/S0005273614001795 Accessed on: April 2023

Mode of action study: The effect of compatible solute ectoines on the structural organization of lipid monolayer and bilayer membranes

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Abstract

Compatible solutes are small organic osmolytes responsible for osmotic balance and at the same time compatible with the cellular metabolism. Here, we have investigated the effect of the compatible solutes, ectoine and hydroxyectoine, on the fluid–rigid domain structure of lipid monolayer and bilayer membranes. Mainly saturated dipalmitoyl-phosphatidylcholine membranes exhibiting a clear le/lc phase transition were used. Fluorescence microscopy showed that ectoines added to the aqueous subphase expand and fluidize the lipid monolayers especially at surface pressures below 30 mN/m. The domain structure at the le/lc phase transition is sensitively modified leading to smaller but more numerous domains in the presence of ectoines. Hydroxyectoine was more efficient than ectoine. These results are explained by the replacement theory assuming that the ectoines are likely to be expelled from the membrane surface thus favoring the hydration of the lipid membrane. This effect reduces the line tension, which is the interfacial energy at the domain edges leading to reduced domain sizes and increased number of rigid domains. Isotherms of negatively charged phosphatidylglycerol membranes show a similar expansion, while unsaturated lipids are less affected. Mixed phosphatidylcholine/phosphatidylglycerol membranes exhibit the same effect on the line tension increasing the tendency for a phase separation. This could be shown also in bilayer vesicles, where the compatible solutes have only a minor effect on the lipid main phase transition in pure DPPC membranes but reduce the extent of the pretransition. In mixed DPPC/DPPG bilayer membranes ectoines cause a phase separation leading to the enrichment of expanded DPPC domains. In conclusion, our study gives for the first time evidence that ectoines have an effect on lipid membranes increasing the hydration of the surface and thus increasing the mobility of the lipid head groups and fluidizing the lipid layer accordingly. This increased fluidity may be of advantage for cell membranes to withstand extreme conditions like temperature or osmotic pressure and might also accelerate cellular repair mechanisms.

Full reference:

Harishchandra, R.K., Wulff, S., Lentzen, G., Neuhaus, T. and Galla, H.J., 2010. The effect of compatible solute ectoines on the structural organization of lipid monolayer and bilayer membranes. Biophysical chemistry, 150(1-3), pp.37-46.

Downloadable version: Available at: https://www.sciencedirect.com/science/article/abs/pii/S0301462210000359 Accessed on: April 2023

Overview: Ectoine in the Treatment of Irritations and Inflammations of the Eye Surface.

Table 1: Trials studying the treatment of allergic conjunctivitis with EED.

Authors, year, country, and type of publication	Indication	Study design	Patient distribution & treatment	Study population Age range Mean age	Description of therapy, duration, and dosage	Efficacy parameters	Main findings (ocular symptoms)	Side effects
Salapatek et al., 2011 [65], Canada, Conference presentation (manuscript accepted)	Allergic rhinoconjunctivitis	Randomized, double-blind, placebo-controlled, double crossover	46 patients	Adults Age range: 22-65 years Mean age: n.a.	14 days of treatment with either EED/Ectoine Nasal Spray (ENS) or control (3 times per day), wash-out (1 week without treatment), crossover of the groups, 14 days treatment (3 times per day)	Patient reported outcome: Sneezing, nasal congestion, itchy nose, runny nose, watery eye, itchy eye, red eye, itchy ear/palate Total Nasal Symptom Score (TNSS), Total Ocular Symptom Score (TOSS), Total Nonnasal Symptom Score (TNNSS)	Patients receiving ectoine treatment experienced a greater relief of overall ocular symptoms scores during posttreatment experimental exposure chamber (EEC) when compared to placebo. The TOSS significantly decreased to 12.64 ± 0.97 (-24.4%; $p = 0.0001$) in the ectoine group and to 14.09 ± 0.91 (-15.8%) in the placebo group. Individual ocular symptoms were more reduced after ectoine treatment than with placebo, with a greater relief for "watery eyes" ($p = 0.020$) and "itchy eyes" ($p = 0.021$)	6 AEs reported during EED/ENS treatment. During placebo treatment 5 AEs were reported. No SAE occurred.
Werkhäuser et al., 2014 [33], Germany, Peer-reviewed publication	Allergic rhinoconjunctivitis	Controlled, noninterventional, open-labelled, multicentre	48 patients Ectoine group: 22 Azelastine group: 26	Adults Age range: n.a. Mean age: 35 years	7 days of treatment with either EED: 1 eye drop per eye and 1 puff of the nasal spray per nostril 4 times per day, or azelastine (0.05 to 0.1 mg/L): 1 eye drop, 1 puff nasal spray, both twice per day	Investigator and patient assessment of nasal obstruction, rhinorrhoea, sneezing, nasal itching, conjunctivitis, eye itching, tearing, alate itching, TOSS, TNSS, efficacy judgement, tolerability judgement	The TOSS in the investigator assessment decreased significantly from V1 to V2 in both groups ($p < 0.001$ for EED, $p = 0.009$ for azelastine). TOSS values decreased in the ectoine group by 45.96% and by 44.98% in the azelastine group.	8 AEs in total, 2 in the ectoine group and 6 occurred in the azelastine group. No SAE occurred

Table 1 Continued

Authors, year, country, and type of publication	Indication	Study design	Patient distribution & treatment	Study population Age range Mean age	Description of therapy, duration, and dosage	Efficacy parameters	Main findings (ocular symptoms)	Side effects
Mrukwa-Kominek et al., 2018 [62], Poland, Conference presentation	Allergic conjunctivitis	Single-arm, open-labelled, noninterventional	30 patients Ectoine group (30)	Adults Age range: 21-75 years Mean age: 44.8 years	14-21 days of treatment One eye drop per eye up to 4 times per day	Assessment included McMonnies questionnaire, evaluation of therapeutic efficiency and adverse effects, best corrected visual acuity, intra ocular pressure, slit lamp examination with fluorescein eye stain test, ocular surface disease index, vision related quality of life	Decreases of TOSS values as assessed by patients were not significant Treatment with ectoine led to significant improvement for conjunctival redness, a reduction of follicular reaction and reduction of eyelid oedema, and a significant decrease of individual ocular symptoms. McMonnies questionnaire showed a 15% reduction of symptom score	Treatment tolerance in patients with allergic conjunctivitis was good with very few adverse effects
Allegri et al., 2014 [57], Italy, Conference presentation	Vernal keratoconjunctivitis (VKC)	Retrospective case series, controlled	64 patients Ectoine group (32) Ketotifen group (32)	Male children Age range: n.a. Mean age: 8.5 years	6 months of treatment Ectoine: 1 eye drop per eye, 3 times per day Ketotifen (0.05%) 1 eye drop per eye, 3 times per day	Assessment included VKC slit-lamp signs: Focal or diffuse conjunctival hyperaemia, tear break up time, modified Oxford scale, VKC grading (modified Bonferroni scale) and symptoms: VAS scale grading (ocular pain, itching, tearing, photophobia and foreign body sensation), quick questionnaire on tolerance of eye drops at instilment	The case series review showed that both treatments (2% ectoine and 0.05% ketotifen) are effective in improving signs and symptoms of VKC during allergic seasons. In tolerability rating, ectoine was significantly better rated ($p < 0.0001$)	None reported
Drozhzhyna and Troychenko,	Allergic conjunctivitis	Real-life, uncontrolled, noninterventional	30 patients Ectoine group (30)	Adults Age range: 18-65	7-14 days of treatment as prescribed (one eye	Assessment included Symptoms of conjunctival	After treatment, the scores for conjunctival	All patients experienced good

Table 1 Continued

Authors, year, country, and type of publication	Indication	Study design	Patient distribution & treatment	Study population Age range Mean age	Description of therapy, duration, and dosage	Efficacy parameters	Main findings (ocular symptoms)	Side effects
2015 [59], Ukraine, Publication				years Mean age: n.a.	drop per eye up to 4 times per day)	hyperaemia, lacrimation, and ocular itching. Conjunctival hyperaemia and oedema were evaluated by the ophthalmologists, whereas lacrimation and ocular itching were documented by the patients	hyperaemia, ocular itching, eyelid oedema, and lacrimation decreased significantly ($p < 0.05$). Eyelid oedema was significantly improved in all 30 patients ($p = 0.01$) and completely resolved in 22 patients at the end of the study	tolerance to ectoine eye drops, with no side effects being reported
Skrypnyk and Seidametova, 2017 [68], Ukraine, Publication	Allergic conjunctivitis	Randomized, controlled	34 patients Ectoine + standard of care (24) Standard of care (10)	Adolescents and adults Age range: 13-42 years Mean age: n.a.	Ectoine group: 2 weeks before onset of symptoms and during exacerbation as prescribed control group: traditional treatment from the moment of exacerbation	Symptoms were assessed on a 4-point scale: 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms	In the ectoine group, the symptoms of ocular itching, conjunctival hyperaemia, and oedema improved significantly faster compared the control group ($p < 0.05$)	No AE reported, a good tolerance of the eye drops was reported
Allegri et al., 2018 [56], Italy, Conference presentation	Vernal keratoconjunctivitis	Retrospective	Ectoine: 192	Children Age range: up to 10 years Mean age: 7.8 years	6 months of treatment time as prescribed (1 eye drop per eye, 3 times per day)	Assessment of the preventive administration of ectoine eye drops to shorten the duration of VKC relapses or to mitigate the attacks	8% of the included subjects had no relapse of VKC, 38% needed topical corticosteroid or cyclosporin treatment, but it was started 2 months later compared to previous years, 29% needed these topical drugs 3 months later, and 25% had a similar to previous year course	The treatment was well tolerated, and only 1 child had to stop it because of a local reaction to the eye drops

n.a.: not available.

IF YOU HAVE ANY QUERIES OR WOULD LIKE FURTHER INFORMATION PLEASE

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