

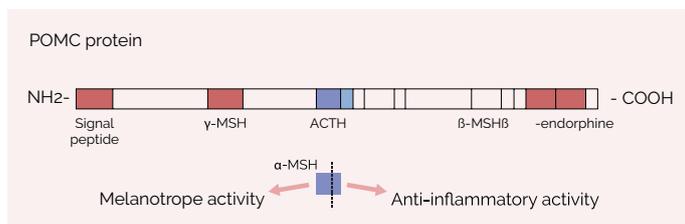
# NEURO CELLULAR SOOTHING CLINICAL STUDIES



## NEUTRAZEN – Soothing neurocosmetic

### NATURAL ANTI-INFLAMMATORY NEUROPEPTIDES: POMC DERIVATIVES

Some neuropeptides produced locally, within the skin, have natural anti-inflammatory activities. Proopiomelanocortin (POMC), a neuroendocrine protein found in the skin in response to local stressors, is an important precursor of such neuropeptides. To express its anti-inflammatory potential, POMC first needs to be processed in smaller pieces, called melanocortins (MSH) ( $\alpha$  and  $\beta$ ) and corticotropin (ACTH), which are recognized as key modulators of the cutaneous immune and inflammatory reactions. In vitro,  $\alpha$ -MSH downregulates the production of proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-. In contrast, the production of the cytokine synthesis inhibitor IL-10 is upregulated by  $\alpha$ -MSH. At the molecular level, part of the anti-inflammatory effects seems to be mediated by inhibition of nuclear factor kappa-b (NFkB) activity via the preservation and expression of IB protein.



The effects of the peptides derived from POMC are mediated through highly specific transmembrane receptors that can be detected on immunocompetent and inflammatory cells, as well as on skin cells. To date, 5 melanocortin receptors (MCR) have been identified.  $\alpha$ -MSH binds with a high affinity to MC1-R to exert anti-inflammatory activities. Evidence from experiments using MC1-R-selective synthetic agonists supports the idea that MC1-R activation contributes to the anti-inflammatory influences of melanocortin.

*Various skin cells are targets for the anti-inflammatory effects of  $\alpha$ -MSH, including monocytes, macrophages, dendritic cells, keratinocytes, melanocytes, fibroblasts, and endothelial cells.*

### NEUTRAZEN™ IS A LIPOPEPTIDE. LIPID COUPLING IS KNOWN TO FACILITATE PEPTIDE ABSORPTION THROUGH THE SKIN

Based on recent scientific evidence that POMC-derived neuropeptides exert anti-inflammatory activity in skin through binding and activation of the MC1-R, we have developed a series of biomimetic peptides derived from the sequence of  $\alpha$ -MSH and tested them for agonistic activity on that specific receptor. Among the molecules tested, Neutrazen™ distinguished itself for having excellent binding affinity for MC1-R, highly specific anti-inflammatory activities, and reduced potential for stimulating melanogenesis.

Neutrazen™ is a lipopeptide, composed of three aminoacids coupled to palmitic acid. Lipid coupling is known to facilitate peptide absorption through the skin.

In vitro, Neutrazen™ binds MC1-R with an affinity comparable to that of  $\alpha$ -MSH. However, and contrary to  $\alpha$ -MSH, Neutrazen™ had only weak melanogenic activity. In keratinocytes, Neutrazen™ down-regulates the production of UVB-induced inflammatory cytokines such as interleukin-8 (IL-8). In fibroblasts, Neutrazen™ inhibits the release of IL-1-induced IL-8 production, reinforcing its antiinflammatory potential.

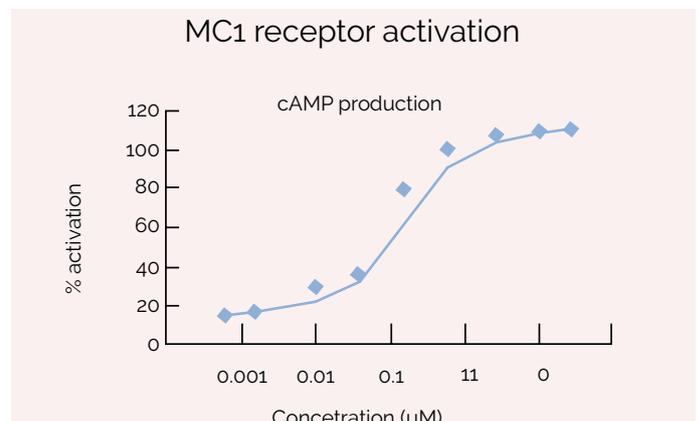
When tested in an ex vivo model of neurogenic inflammation, Neutrazen™ inhibited SP-induced TNF- $\alpha$  production and prevented vasodilation.

*The unique features of Neutrazen™ lie in its high selectivity for MC1-R, its demonstrated blockade of neurogenic inflammation in vitro and clinical, its excellent potential for skin penetration, and its good safety profile.*

*Neutrazen™ is a true neurocosmetic offering a soothing active for sensitive skin.*

### IN VITRO: MOLECULAR PHARMACOLOGY OF NEUTRAZEN: BINDING TO MC1-R

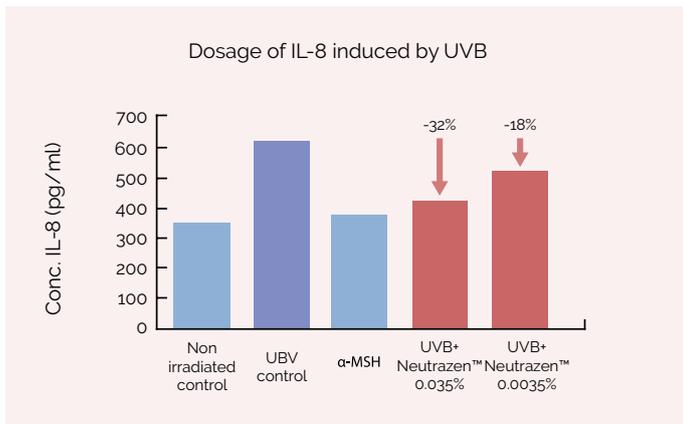
Binding of the POMC-derived neuropeptide  $\alpha$ -MSH to the melanocortin-1 receptor (MC1-R) leads to downregulation of proinflammatory cytokines such as IL-1, TNF-, and IL-6, upregulation of the cytokine synthesis inhibitor IL-10, inhibition of IL-8-induced biological responses in macrophages and neutrophils, as well as inhibition of adhesion molecule expression with subsequent reduction in leukocyte migration. Binding to MC1-R is crucial for the anti-inflammatory actions of  $\alpha$ -MSH. Thus the first step to validate any inflammatory modulating activity for Neutrazen™ was to measure its ability to bind to MC1-R. This was done on cells overexpressing MC1-R.



*Neutrazen™ binds the MC1-receptor with an affinity comparable to that of the natural ligand,  $\alpha$ -MSH, without stimulating melanin production.*

## IN VITRO: ANTI-INFLAMMATORY ACTIVITY: INHIBITION OF UVB-INDUCED IL-8 PRODUCTION

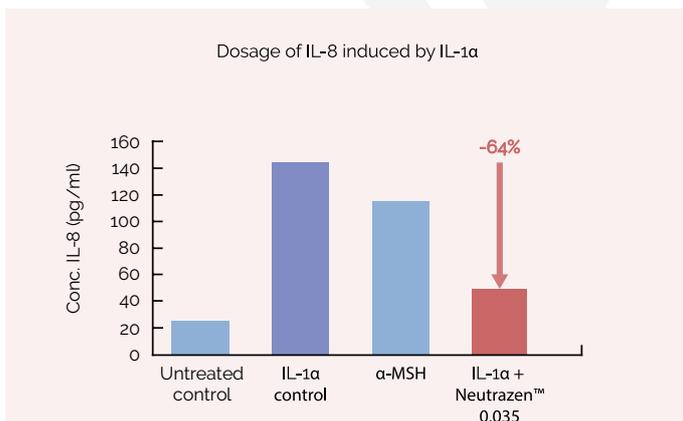
Keratinocytes respond to external aggressions such as UV, by producing interleukins acting as messengers to mobilize inflammatory cells. Interleukins act through specific receptors that are found at the surface of various cell types, including immune cells, making them very powerful mediators of inflammatory reactions. In the course of UV-induced inflammation, the first cytokines to be released are interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF). In an autocrine manner, IL-1 further promotes the recruitment of neutrophils into the inflamed tissue through the production of IL-8, acting as a kind of irresistible pheromone molecule to attract immune cells. In the skin, interleukin-driven inflammatory reactions are a major cause of erythema (redness) and edema (swelling). Alterations in cutaneous and systemic immunity also occur as a result of UV-induced inflammation and cytokine production.



*Neutrazen™ reduces UVB-induced inflammatory response and consequently may protect from the harmful effects of sun exposure*

## IN VITRO: ANTI-INFLAMMATORY EFFICACY: INHIBITION OF IL-1-INDUCED IL-8 PRODUCTION

IL-8 secretion is inducible in cultured human dermal fibroblasts following activation with the proinflammatory cytokine IL-1. As mentioned before, IL-8 mediates inflammatory reactions in the skin through the recruitment of neutrophils and other immune cells to invade injured or inflamed tissue. Interestingly, α-MSH may act as an inhibitory neuropeptide in this context highlighting its anti-inflammatory and immunomodulating potential in skin physiology.



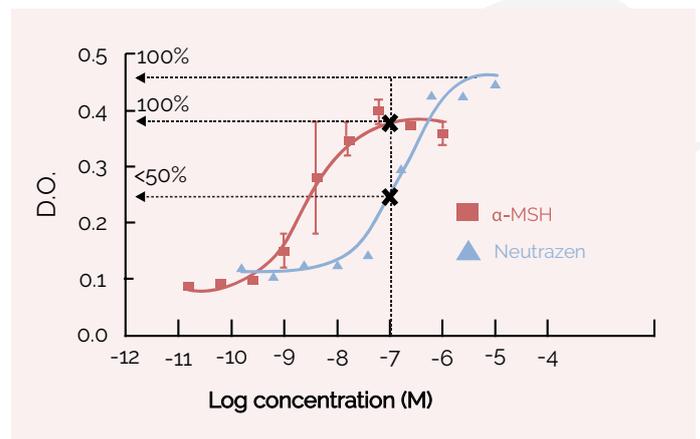
*Neutrazen™ dims the inflammatory cascade for optimal skin protection*

## CELLULAR PHARMACOLOGY OF NEUTRAZEN™: MELANOGENESIS IN VITRO

Neutrazen™ is derived from a family of neuropeptides called melanocortins, known to influence various biological activities, including:

- Stimulation of melanogenesis through the synthesis of melanin
- Reduction of inflammatory reactions

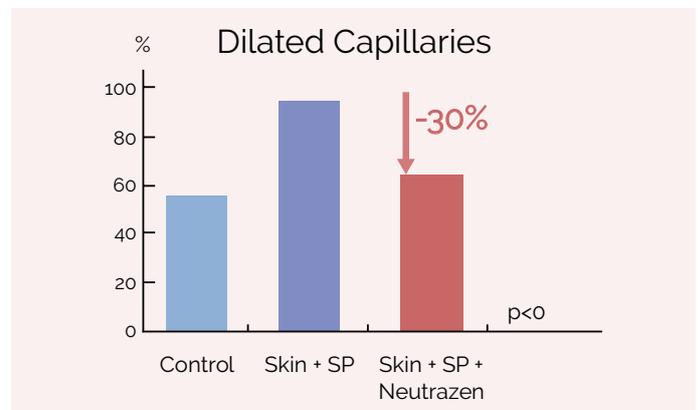
In a search for a molecule with high selectivity for modulation of inflammation we have tested Neutrazen™ against α-MSH in an assay for melanogenesis induction.



*This study demonstrated that Neutrazen™ only has weak melanogenic activity and does not significantly induce melanin synthesis when compared with the reference α-MSH. On the other end, Neutrazen™ can modulate inflammatory responses at concentrations that do not trigger melanin production. We thus conclude that Neutrazen™ is highly specific for MC1-R-mediated antiinflammatory activity.*

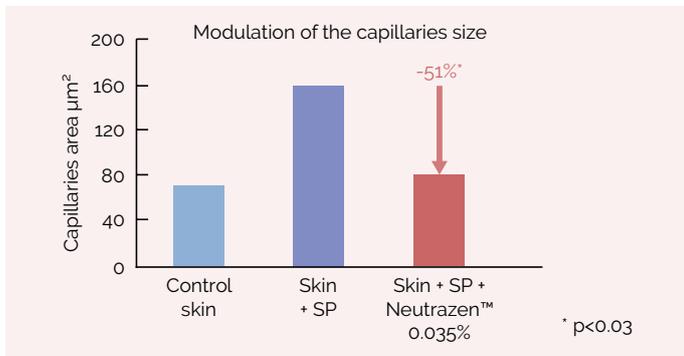
## EX VIVO: EVALUATION OF NEUROGENIC INFLAMMATION

Topical application of substance P induced vasodilation in the superficial dermis: there is an increased number and size of dilated capillaries.



Results: These effects of Substance P on skin explants were reduced in the presence of Neutrazen™:

- 30% for the number of dilated capillaries;
- 51% for the size of dilated vessels.

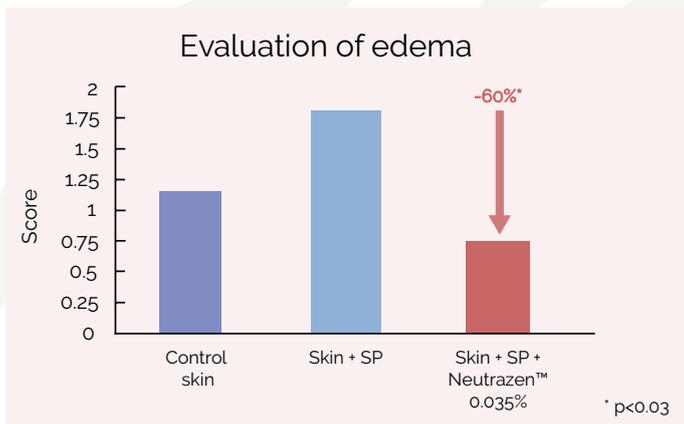
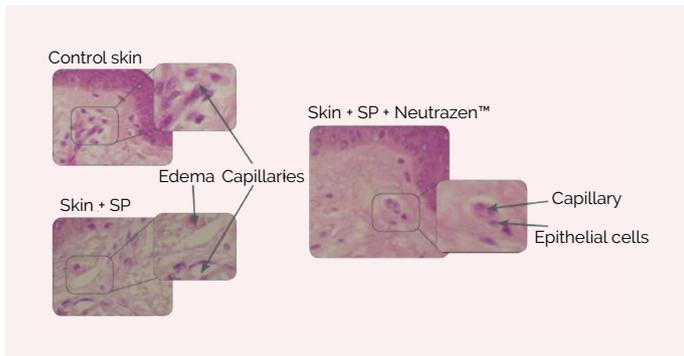


*Neutrazen™ can inhibit substance P-induced vascular effects in the skin.*

*Neutrazen™ has a direct impact on vasodilation and thus may reduce redness and swelling*

### EX VIVO: EFFECT OF NEUTRAZEN™ ON EDEMA

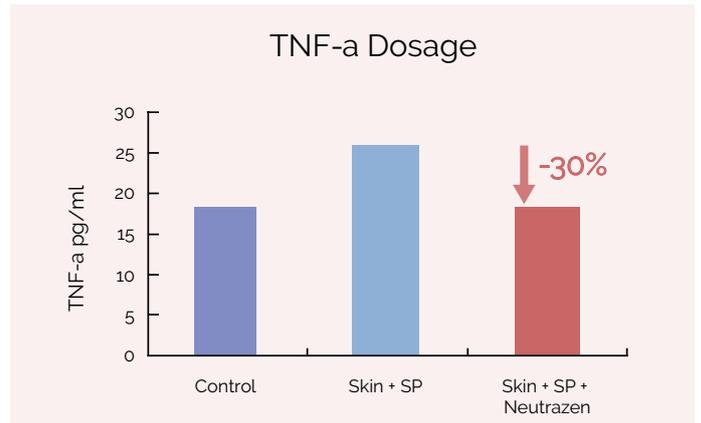
Application of substance P induces edema and vasodilation in the superficial dermis of skin explants. Edema is characterized by increased space between collagen bundles and by vessel dilatation in the vicinity of epithelial cells.



*Addition of Neutrazen™ to skin explants reduces edema formation and vessel dilation, in the presence of Substance P. Neutrazen™ reduces by 60% substance P-induced edema in skin explants.*

### EX VIVO: EFFECT OF NEUTRAZEN™ ON TNF-a

Tumor necrosis factor (TNF-a) is a potent pro-inflammatory cytokines that is produced in the course of substance P-induced neurogenic inflammation.

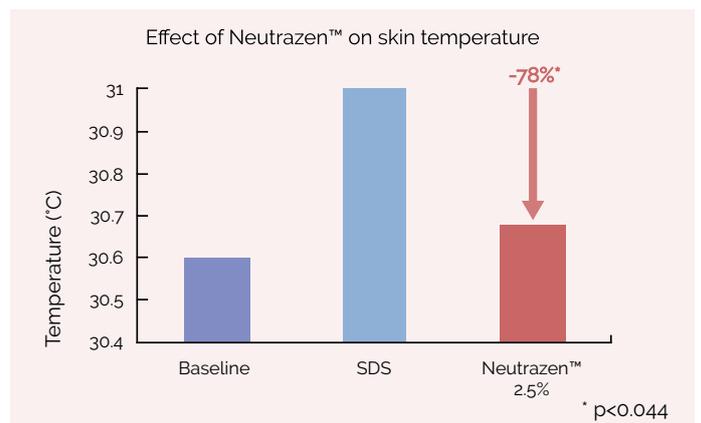


*In a cutaneous model of neurogenic inflammation, Neutrazen™ proved to have excellent antiinflammatory activities. In this model, Neutrazen™ inhibited vasodilation and edema formation, and prevented the release of TNF-. Knowing that neurogenic inflammation is linked to photoaging, Neutrazen™ offers a great potential in preventing the signs of premature aging of the skin.*

*Neutrazen™ inhibits a major inflammatory mediator for a powerful anti-inflammatory response*

### CLINICAL STUDIES: ANTI-INFLAMMATORY AND ANTI-IRRITANT EFFICACY STUDY

In this study, objective was to clinically evaluate and compare the anti-inflammatory and anti-irritant efficacy of a Neutrazen™ formulation when applied as a preventive care or a soothing care on chemically challenged skins. The chemical agent used for this challenge was sodium dodecyl sulphate 0.5% (SDS), an ionic surfactant known to be a skin irritant. SDS is used in numerous skin care products and is often responsible for skin reactions.



*A reduction of 78% is observed when Neutrazen™ is applied as a soothing care.*



*Neutrazen™ reduces redness in SDS-challenged skin.*

*Neutrazen™ successfully alleviated the signs of inflammation in chemically challenged, otherwise normal skins. Neutrazen™ may be useful in soothing care to help maintain a normal skin sensitivity threshold.*

### CONSUMER TEST ON MECHANICAL INFLAMMATION

BENEFITS	RESULTS AFTER 1 WEEK
The skin feels more comfortable	90%
The product reduces the irritating effect of shaving	83%
The product soothes razor burn	81%
The product calms skin tightness	80%
The product provides immediate soothing sensation	79%
The product improves skin redness	69%

*Neutrazen™ provides a fast action to reduce burning and irritated skin sensations*

### CONSUMER TEST ON MECHANICAL INFLAMMATION

BENEFITS	RESULTS AFTER 4 WEEK
The product improves skin redness	91%
The product soothes itching	79%
The skin is better protected from shaving irritation	79%
The product provides a refreshing sensation	77%
The product reduces tingling sensation	77%
The product reduces the severity of razor bumps	77%
The skin looks revitalized	71%
The product improves shaving comfort	71%
The product protects the skin from mechanical stress of shaving	64%

*Neutrazen™ soothes itching and reduces tingling sensation to provide better protection and skin comfort*

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