Philips BlueTouch
Scientific background
Philips – leading innovation in healthcare solutions

As a pioneering global company and number 1 in Home Healthcare, Philips delivers meaningful people-centric healthcare solutions.

We strive to improve people’s quality of life by identifying the barriers to their health and wellbeing and then creating solutions to overcome them.

To do this, we listen to the needs of consumers and healthcare professionals so that we can develop insightful innovations that truly improve people’s lives.

The result of years of intensive research, Philips BlueTouch brings innovation and ease of use to the management of back pain.

Delivered simply and easily through the use of a wireless wearable patch, Philips BlueTouch offers relief to patients who suffer from mild to moderate musculoskeletal back pain including neck, shoulders and/or lumbar spinal area. The wireless design provides maximum freedom of movement to enable patients to enjoy everyday activities.
Abstract

Blue LED light-induced endogenous nitric oxide (NO) production in human skin

Purpose
To study non-enzymatic NO release from NO derivates in an aqueous solution and in human skin irradiated with blue LED light (453 nm).

Method
• The release of NO in aqueous nitrite solutions (10 μM NaNO2) induced by blue LED irradiation was investigated at skin relevant pH values (pH 5.5).
• The NO developing in the solution was flushed out with inert gas according to the Kolbe electrolytic method and was determined with a chemiluminescence detector (CLD).

Results
• Using different LED light sources, significantly increased non-enzymatic NO production in nitrite solutions could be determined under conditions existing in human skin (pH 5.5).
• The blue LED light (453 nm)-induced release of NO could be increased by adding CuCl2 and could be inhibited with a specific Cu(I) scavenger.

Conclusion
Irradiation with blue LED light (453 nm) leads to a significant release of NO from nitrite solutions under skin-relevant conditions.

Background
Human skin contains relatively high concentrations of copper ions and nitrite and has a low pH value of 5.5 to 6, which allows the above-mentioned mechanism of blue LED light-induced NO generation from nitrite to take place. This theory should be verified by intact human skin tests.

Method
• The skin samples were incubated with Fe2+/DETC, irradiated with blue LED light (453 nm), and frozen in liquid nitrogen.
• The skin specimens were subjected to special electron paramagnetic resonance measurements, which demonstrated the presence of NO/Fe/DETC complexes and thereby the generation of NO at different layers.

Results
After irradiation with blue LED at a wavelength of 453 nm, increased NO release could be observed, also in deeper skin layers.

Conclusion
Blue LED light (453 nm)-induced NO release could also be proved in deeper layers of the human skin.

References:
Prof. Dr. C. Suschek, Dr. C. Opländer: Abschlussbericht zum BMBF-Verbundprojekt – „Desinfektion, Entkeimung und biologische Stimulation der menschlichen Haut durch gesundheitsfördernde Licht- und Plasmaquellen.“ Universitätsklinikum Aachen, 2010.
Abstract

Blue LED light-induced non-enzymatic nitric oxide (NO) production

Purpose

To study non-enzymatic NO release from nitrite and nitrosated proteins by irradiation with blue LED light (453 nm) compared with UVA light (360 nm).

Background

Bioactive NO can also be released non-enzymatically. This occurs through photolysis of nitrite (NO₂) and nitrosated proteins, e.g., S-nitrosoalbumin (SNO Alb), as was already proved by irradiation with UVA light.

Method

- A serum albumin solution (2% PBS – BSA solution) was examined with and without nitrite (50-100 μM) while irradiated with blue LED light (453 nm) and compared with irradiation with UVA light (360 nm).
- An increase in BSO nitrosation is an indication that the light source used decomposes nitrite photolytically.
- A decrease of protein nitrosation in the absence of nitrite during exposure shows that the wavelength used is able to release the NO locked in the nitroso BSA.
- The nitrosation of proteins was determined through chemiluminescence detection.

Results

- Without adding nitrite, the UVA-treated specimens showed a clear decrease in the amount of nitrosated proteins (positive control).
- By adding different amounts of nitrite, the nitrosation of BSA can be increased by UVA irradiation with the extent of the increase depending on the dose.

Conclusions

- Irradiation with blue LED (453 nm) did not show any increase in nitrosation of BSA in the presence of nitrite.
- However, a decrease in nitrosation depending on the irradiation intensity of the blue LED light could be proved.

References:

Abstract

Blue LED light with a wavelength of 453 nm is not toxic to endothelial cells and keratinocytes

Purpose

To evaluate the biological efficacy of different light sources on keratinocytes and endothelial cells in the human skin with a view to toxicity, proliferation and differentiation behavior.

Method

- Keratinocytes for the different experiments were isolated from skin resections and expanded. In addition, human microvascular endothelial cell line (HMEC -1) was used. The cells were cultivated and, after treatment with different parameters, they were analyzed with established molecular biological methods.
- To analyze cell proliferation, the AlamarBlue assay or the neutral red uptake assay were used.
- To establish a possible toxic effect of the treatments, the cells and cell nuclei were marked with fluorescent dyes and subjected to a microscopic morphological examination.
- In addition, the decomposition of apoptosis-specific PARP proteins (Poly (ADP Ribose) Polymerase) was demonstrated by means of the western blot technique.
- The differentiation degree of human keratinocytes was examined with specific markers ( involucrin, keratin1) in real-time PCR. The appropriate differentiation markers for endothelial cells are not known.

Results

- Irradiation of the cells with LED light at wavelengths of 412, 419 and 426 nm reduced proliferation on account of toxicity. The toxic effects increase at shorter wavelengths and higher doses.
- When a blue LED light source with a wavelength of 453 nm was used, a dose-dependent decrease in cell proliferation was also observed.
- The measurement of the energy status (ATP content) shows that the energy equivalents do not decrease but significantly increase at an irradiation dose of 100 J/cm².
- Contrary to the shorter wavelengths tested (412 – 426 nm), the proliferation reduction observed at a wavelength of 453 nm cannot be attributed to increased toxicity but, at least in the case of keratinocytes, can be attributed to increased differentiation.

Conclusion

Blue LED light with a wavelength of 453 nm is not toxic for skin cells up to an irradiation dose of 250 J/cm².

References:

Prof. Dr. Kolb-Bachofen V: Abschlussbericht zum BMBF-Verbundsprojekt „Desinfektion, Entkeimung und biologische Stimulation der menschlichen Haut durch gesundheitsfördernde Licht- und Plasmaquellen“. Universität Düsseldorf, 2010.
Abstract

The release of nitric oxide (NO) leads to vasodilatation

Background

NO is formed in the endothelial cells of blood vessels and triggers a series of biological processes through cyclic guanosine monophosphate signaling. NO is the only known endogenous radical that works as a signal transmitter. One of the most important effects triggered by NO is vasodilatation through smooth muscle relaxation.

Results

It could be demonstrated that NO activates soluble guanylate cyclase (sGC). NO binding to the heme domain of guanylate cyclase forms a nitrosyl-heme complex. By bonding with the enzyme guanosine triphosphate (GTP) and eliminating a phosphate domain, this configuration converts into the messenger substance cyclic guanosine monophosphate (cGMP). The intracellular increase in cGMP level triggers the activation of cGMP-dependent protein kinases PKG I and PKG II, with PKG I being mainly responsible for triggering vasodilatation. Currently three different signaling pathways are known through which the NO-triggered vasodilatation can take place. All three mechanisms lead to a decrease of the intracelluar calcium concentration, which causes relaxation of the flat musculature.

1. The activated PKG I phosphorylates various membrane proteins of the sarcoplasmic reticulum, such as the protein phospholamban. In their dephosphorylated state, phospholamban monomers inhibit the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) by binding to the cytoplasmic and membranous domains of the enzyme, thereby causing aggregation of the Ca\(^{2+}\) pumps. Phosphorylated phospholamban releases the previously bonded SERCA and activates it. This leads to rapid sequestration of intracellular calcium, which again reduces the influx of extracellular calcium into the sarcoplasmic reticulum.

2. The activated PKG I also phosphorylates the substrate of inositol 1,4,5-trisphosphate receptor-associated cGMP kinase (IRAG). The phosphorylation of IRAG leads to a strong inhibition of IP3-triggered Ca\(^{2+}\) release from the sarcoplasmic reticulum.

3. Moreover, NO can phosphorylate and activate through the PKG/Ca\(^{2+}\)- dependent potassium channels. The resulting hyperpolarization of the cell membrane reduces the effectiveness of the depolarization signal, thereby triggering vasodilatation.

References:

Abstract

The release of nitric oxide (NO) in the skin leads to a systemic increase in blood flow rates and a decrease in blood pressure.

Purpose

To investigate to what extent whole body UVA irradiation influences the blood pressure in healthy test subjects through continuous NO release.

Background

Human skin contains photolabile NO derivatives such as nitrite and S-nitrosated composites, which decompose as a result of UVA irradiation and release vasoactive NO.

Method

- An airtight chamber (16 cm²) with a UVA-transparent window was placed on the forearms of the test persons to collect the gaseous NO released from the skin. The NO and the nitrosated proteins formed after irradiation were quantified by chemiluminescence detection.
- To determine the biological activity of the blood plasma of non-irradiated and UVA-irradiated (20 J/cm², 30 min.) test subjects, the formation of cyclic guanosine monophosphates (cGMP) was determined in rat lung fibroblasts (RFL6 cells). In the presence of superoxide dismutase (500 U/ml) and isobutylmethylxanthin (0.6 mmol/l), the RFL6 cells (3x10⁵) were incubated with the blood plasma.
- In a further experiment, the RFL6 cells were additionally incubated with NO radical scavenger cPTIO.
- To determine the biological activity of the blood plasma of non-irradiated and UVA-irradiated (20 J/cm², 30 min.) test subjects, the formation of nitrosated proteins, test subjects were irradiated with UVA light (20 J/cm²) for 30 or 15 minutes. The control test subjects were not irradiated.
- In a further in-vivo experiment, healthy test subjects were treated with a cream containing isotope-marked nitrite (150 μmol in 20 ml) and then received a whole-body irradiation with UVA light (20 J/cm²). The amounts of ¹⁵N-marked S-nitrosated compounds were determined by cavity leak-out spectroscopy.

Results

- The gaseous NO from the skin increased four times through UVA irradiation compared to the non-irradiated control group.
- Applying nitrite-containing skin cream prior to irradiation increased the formation of photo-induced NO's significantly again.

Conclusion

Irradiation with UVA light leads to a systemic increase of S-nitroso compounds and increased release of NO. This correlates with the increased formation of cGMP, vasodilatation, systemic increase of the blood flow rate and a decrease of blood pressure.

References:

Abstract

Nitric oxide (NO) regulates the release of substance P in spinal cord synaptosomes

Purpose
To investigate to what extent NO directly affects the nerve endings and regulates synaptic transmission.

Background
The neuropeptide substance P plays an important role in the regulation of multiple physiological processes. Activation of the pain receptors is one of the factors triggering the release of substance P and this substance is also involved in the transmission of pain.

Method
• Rat spinal cord was used to isolate synaptosomes. The rat spinal cord was homogenized and centrifuged and resuspended. The protein content was determined by means of the method of Lowry et al. (1951).
• Next, the synaptosomes were superfused. 40 minutes after the superfusion started, the synaptosomes were depolarized with a high concentration of KCl (30 mM).
• Different reagents (100 μM in each case) were added to the standard perfusion medium (Krebs-Ringer buffer) 10 minutes before depolarization and their effects were investigated.
• The reagents were NO donor sodium nitroprusside (SNP), NO radical scavenger 1H-imidazol-1-yloxy-2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-3-oxide (cPTIO), cyanide ion donor sodium pentacyanoamminoferrate (II) (FeCN), NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) and two cyclic nucleotide derivates: N6,02'-dibutyryl adenosine 3',5'-cyclic monophosphate (Db-cAMP) and 8-bromoguanosine 3',5'-cyclic monophosphate (8-Br-cGMP).
• The concentration of substance P was determined with an EIA (enzyme immunoassay) kit. Here the antisemum identified the C-terminal sequencing of the peptides. The final absorption power was measured with a microplate reader.
• The concentration of cGMP was also determined with an EIA kit.
• The release of substance P was indicated by synaptosomes in femtomols per milligram protein. The basal release was defined as average quantity in two 5-minute fractions, which were taken immediately after depolarization (a 30 to 35 and a 35 to 40-minute fraction after start of perfusion).

Results
• Compared with the basal amounts, a 2.7 times higher release of substance P was observed by depolarizing the synaptosomes (control).
• This induced release of substance P was reduced to 60 percent by administering NO donor sodium nitroprusside (100 μM). This effect depended on the NP concentration.
• To confirm that the reduction of substance P release was attributable to the increased availability of NO, the experiment was repeated with NO donor (SNAP). Here similar results were obtained (55% reduction).
• In a further experiment, the NP-induced reduction of substance P release could be significantly decreased by adding NO radical scavenger (cPTIO).
• The addition of two cyclic nucleotide derivates showed that membrane permeable cGMP also leads to a significant reduction of the depolarization-induced release of substance P. No effect could be observed with cyclic AMP.
• It could also be observed that the NO amount released by sodium nitroprusside sufficed to activate the guanylate cyclase and was enough to increase the cGMP amount in the synaptosomes. NP brings about a significant increase of the cGMP amounts depending on the NP concentration. The concentration dependencies observed coincide with those that were observed for the NP-induced reduction of substance P release.
• The time after which an increased cGMP amount could be observed after NP administration coincides with the time after which the effect of NP on substance P release could be shown.

Conclusion
NO immediately affects the nerve endings and reduces the pain transmission by increasing the formation of cGMP and thereby reducing the release of substance P.

References:
Abstract

Nitric oxide (NO) reduces the development of inflammatory processes

Purpose

To investigate the effects of NO on the migration of neutrophil granulocytes in inflammatory processes.

Background

Neutrophil granulocytes circulate in blood and migrate into inflamed tissue. This is a process facilitated by adhesion molecules and chemokines. Four steps can be identified in the recruitment of neutrophil granulocytes to the center of an infection: selectin-facilitated rolling along the vessel endothelium, chemokine-facilitated increase in integrin affinity, stable integrin-facilitated adhesion to the endothelium and migration through the endothelial tissue.

Method

• For all experiments rats and mice were injected with a phosphate-buffered saline solution (PBS), a selective NO-synthase inhibitor aminoguanidine (Amino) or a non-selective NO-synthase inhibitor NG-nitro-L-arginine (Nitro). After 30 minutes the endotoxin carrageen (Cg) was administered intraperitoneally.
• For the in-vivo determination of the migration of neutrophil granulocytes, the rats and mice were killed and cells were taken from the abdominal cavity. The results were represented as the number of neutrophil granulocytes per abdominal cavity.
• Leukocyte rolling and adhesion were evaluated by microscopic examination of the mesenteric tissue. This involved an analysis of the interaction of the leukocytes with the luminal surface of the venulial epithelium.
• The apoptotic index of the neutrophil granulocytes harvested from the abdominal cavity was established.

Results

• The administration of both NO synthase inhibitors significantly increases the migration of neutrophil granulocytes to the induced inflammatory reaction.
• It may be assumed that the L-arginine/NOS pathway is involved in this process.

Conclusion

In inflammatory reactions a decrease of the migration of neutrophil granulocytes and induces their apoptosis.

References:

Abstract
The antioxidative effects of nitric oxide (NO) protect against cell damage and cell death, also in the central nervous system.

Purpose
To investigate the antioxidative effects of NO and the protective influence on the central nervous system.

Background
In vivo, NO develops in endothelial cells, astroglia and some neurons. By activating the guanylate cyclases and increasing the formation of cGMP, NO regulates cell-to-cell communication and the cerebral blood flow. Because of its chemical composition – a weak, nitrogen-centered free radical – NO could show antioxidative effects, which protect against, amongst other things, nerve damage. Several in-vitro and in-vivo studies support this hypothesis and show that NO is a strong antioxidative agent that protects dopaminergic neurons against reactive oxygen species and thereby against oxidative stress.

Antioxidative and neuroprotective properties of NO
• NO can protect against hydroxyl radical-induced DNA damage and against H₂O₂-induced cell death and has a large capacity to end radical-induced lipid peroxidation as vitamin E.
• Even in low concentrations, NO can modulate important components of the antioxidative protection, the glutathione metabolism for instance, by inducing an increase in the expression of enzymes that are key to the glutathione synthesis: g-glutamylcysteine synthetase (gGCS) and g-glutamyl transpeptidase.
• NO is a decisive factor in regulating the activity of heme oxygenase 1. The NO-induced increase in the activity of the enzyme leads to a high resistance of the endothelium against oxidative stress.
• When enzymes containing iron and thiol groups are nitrosylated by NO, this significantly changes their biological activity in the central nervous system. Redox reactions and the formation of reactive oxygen species are reduced, resulting in a decrease of oxidative stress. NO also deactivates cytochrome protease-induced apoptosis and neurotoxicity.

It was also shown that NO regulates the antioxidative thioredoxin system and the antipotic Bcl-2 protein through a cGMP-dependent mechanism, thereby providing protection against nerve degeneration.

Based on in-vivo and in-vitro cerebral preparations, it was also proved that NO acts as a free radical scavenger.

The antioxidative properties of NO protect against DNA damage, the oxidation of proteins and lipid peroxidation, also in neurons and brain cells.

Conclusions
The antioxidative effects of NO protect cells against DNA damage, the oxidation of proteins and lipid peroxidation, also in neurons and brain cells.

Results
Forming of reactive oxygen species in the central nervous system
• Free radicals are the inevitable byproducts of all electron-transmitted redox reactions, such as the various processes of the respiratory chain.
• It is suspected that free radicals such as •OH induce neurodegenerative pathways and develop as a result of ischemia and reperfusion-conditioned processes.

Neurobiology of NO
• Compared with the highly toxic hydroxyl radicals, the NO radicals are several orders of magnitude less reactive and thereby lead to opposite neurological processes.
• The effects of NO last long and NO can work over relatively long distances.

Reactions of unpaired electrons: • NO eliminates lipid, oxygen and thiol radicals
| I. | LIGO • | + + NO | LIGNO |
| II. | O₂ •– | + NO | [ONOO–] NO3– |
| III. | • OH | + NO | [HONO] |
| IV. | GS • | + NO | GSNO |
| V. | Peptide cis | + NO | Peptide cisNO |

Subsequently from Chiueh, 1999

References
Abstract

The anti-inflammatory effects of nitric oxide (NO) can protect against cell damage and cell death

Purpose

To investigate the effect of NO on ischemia and reperfusion-conditioned damage.

Background

Thanks to its antiapoptotic and anti-inflammatory properties, NO can have a protective effect. NO also influences the cell’s signaling pathways and modulates gene expression through inhibition of gene expression-regulating core proteins.

Results

Regulation by NO can have the following molecular effects:

- Ischemic damage results in a decrease of NO production, which can be compensated by the exogenous administration of NO or the stimulation of endogenous NO formation.
- NO protects against inflammation-conditioned cell damage and cell death.
- TNF-α stimulates the infiltration of neutrophil granulocytes during reperfusion.
- NO also induces the synthesis of inflammation-promoting chemokines in ischemic tissue.
- In addition, it activates transcription factor NF-κB, the gene regulating the inflammation response.
- The level of the pro-inflammatory cytokine TNF-α is reduced by NO.
- NO itself also inhibits the formation of NF-κB.
- NO is an important regulator of mitogen-activated protein kinases (MAPKs), which modulate gene and protein expression and thereby influence the signaling pathways of cell growth, differentiation and apoptosis.

References

Nitric oxide (NO) plays a decisive role in the repair of injured skeletal muscles

**Background**

The healing process of skeletal muscles consists of three phases:
1. Destruction: degeneration of muscle fibers and inflammation
2. Repair: phagocytosis of necrotic fibers. Regeneration of muscle fibers, forming of cicatricial tissue
3. Reconstruction: ripening of regenerated muscle fibers, contraction and readjustment of the cicatricial tissue, restoration of the functional performance of the muscle

NO appears to be an important regulatory factor in all phases of muscle repair.

**Results**

**Effects of NO in the degenerative phase:**
- During the inflammatory phase of a muscle injury, the local NO production increases.
- The NO released in the muscles reduces the cell damage due to inflammation by stimulating the apoptosis of inflammation-promoting cells and reducing the expression of cell adhesion molecules.
- NO also reduces the lysis of muscle cells induced by neutrophils and reduces the formation of reactive oxygen species.

**Effects of NO in the regeneration and reconstruction phases:**
- The formation of new blood vessels is an essential process in muscle regeneration. Angiogenesis is a precondition for the healing of injured muscles because it will restore the blood vessels, the blood flow and the oxygen supply to the tissue. NO also plays a decisive role in angiogenesis, as it acts as a vasodilator and activates various growth factors.
- NO regulates the expression of many genes that help repair localized damage, especially the many members of the matrix-metalloproteinase (MMP) family.
- The release of growth factor HGF as a result of muscle stretches depends on the presence of NO.
- NO activates MMPs that also trigger the release of growth factor HGF and thereby activates the satellite cells in the muscle.

**Conclusion**

NO is an important regulator in all three phases of healing injured skeletal muscles. NO modulates inflammatory processes, stimulates regeneration and counteracts fibrosis.

**References:**