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Authors
Meyskens, FL
Yang, S
Yang, Z
et al.

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Melatonin Can Attenuate HOCl-Mediated Hemolysis, Free Iron Release and Heme Degradation From Hemoglobin

Dhiman Maitra1, Michael P. Diamond2, Ghassan M. Saeed1, and Husam M. Abu-Soud1
1Wayne State University

In inflammatory diseases, where hypochlorous acid (HOCl) is elevated, iron homeostasis is disturbed, resulting in iron overload. Free iron is toxic since it can lead to generation of other free radicals through the Fenton reaction. Recently, we have shown that HOCl binds to the heme moiety of hemoglobin (Hb) and generates a transient ferric species in which ultimately leads to heme destruction, free iron release and protein aggregation. HOCl is generated by myeloperoxidase, utilizing chloride and hydrogen peroxide as substrates. Melatonin, a neurohormone, has been shown to act as an inhibitor for MPO and is also known to scavenge HOCl. Here we show that HOCl-mediated Hb destruction can be partially or completely prevented by pre-incubation of Hb with increasing concentrations of melatonin prior to the addition of HOCl, as judged by UV-Vis spectrophotometry and in-gel heme staining. Melatonin also prevented HOCl-mediated free iron release and protein aggregation. Similarly, pre-incubation of erythrocytes with increasing concentrations of melatonin prior to treatment with HOCl prevented HOCl-mediated loss of erythrocyte viability, indicating the biological relevance of this finding. Melatonin prevents HOCl-mediated Hb heme destruction by direct scavenging of HOCl or through the destabilization of the higher Hb oxidative states intermediates, ferryl porphyrin radical cation Hb-Fe(IV)=O"** (Compound I) and Hb-Fe(IV)=O (Compound II), that are formed through the reaction of HOCl with Hb. This work also indicates that Hb/melatonin system can function as a catalytic sink for HOCl, establishing a direct mechanistic link between melatonin and its protective effect in chronic inflammatory diseases.

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Neuronal Nitric Oxide Synthase (nNOS)/NO, An Accelerator of Melanoma Progression, is a Potential Target for Chemoprevention

Frank I. Meyskens2,7, Sun Yang2, Zheng Yang3, Haitao Ji4, Thomas I. Poulos1, and Richard B. Silverman4
1University of California, Irvine, 2Chao Family Comprehensive Cancer Center, 3Shandong Provincial Hospital, 4Northwestern University

As an important environmental carcinogen, UVR not only generates ROS, but also produces a large amount of nitric oxide (NO) in human skin. Utilizing a NO-donor (DETA) to mimic NO stress, we demonstrated that melanoma proliferation and invasion potential were significantly stimulated. Notably, long-term exposure of DETA/NO stress resulted in over-growth of primary normal human melanocytes with formation of foci in vitro. NO is predominantly generated in an enzyme-dependent manner via NO synthase (NOS). As melanocytes originate from the neural crest, we propose that neural NOS (nNOS) might play an important role in NO production in melanoma. Immunohistochemistry study using biopsies demonstrated marked elevation of nNOS expressions in melanoma and further trend analysis revealed that the staining scores of lesions are significantly associated with disease stage. More progressive stage samples exhibited higher nNOS levels. Significant
inductions of nNOS and elevated NO levels were evident after UVA or UVB radiation. Knockdown of nNOS with siRNA efficiently inhibited melanoma invasion potential, with marked reductions of c-Jun, Bcl-2 and MMP-1. Co-treatment of Ji-11, a nNOS inhibitor, efficiently diminished the overgrowth of melanoma stimulated by L-arginine in 3-dimensional skin reconstitutions. Additionally, a series of novel synthesized nNOS inhibitors were also tested, which represented a varied range of structures and distinct potentials and enzyme selectivity. The nNOS inhibitory potencies (represented by K_i/nNOS values) were correlated with their observed anti-melanoma activities, but not K_i/iNOS or K_i/eNOS, indicating that the inhibition of nNOS is crucial for reducing melanoma invasion. In toto, targeting nNOS with application of specific synthetic inhibitors to diminish NO stress represents an innovative and promising strategy for melanoma prevention and therapy.


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Redox Nanoparticle Enhances Effect of Anticancer Chemotherapy and Suppresses Cardio-toxicity

**Yukio Nagasaki** 1,2, **Yuki Ozaki** 1, and **Toru Yoshitomi** 1

1 University of Tsukuba; 2 MANA, NIMS

Utilizing the self-assembled core-shell-type polymeric micelle technique, high-performance nanoparticles possessing stable nitroxide radicals in the core and reactive groups on the periphery were prepared (Nitroxide Radical-containing Nanoparticles; RNP). The average diameter of the nanoparticles was ca. 40 nm, and the nanoparticles emitted intense electron paramagnetic resonance (EPR) signals. Owing to the confinement of the nitroxide groups in the core of the nanoparticles, it reduces its toxicity, prolongs its blood circulation time. Under the low pH environment in vivo, it was confirmed that RNP disintegrated to expose the nitroxide radicals outside of the particle. It is sharp contrast against low-molecular-weight TEMPOL, which is reduced and excreted rapidly from the blood stream. Recently, we have reported that RNP is effective to cerebral ischemia reperfusion injury. The RNP disintegrates in penumbra area due to the acidosis of ischemic region, followed by the scavenging reactive oxygen species (ROS) generated in this area. This strategy could be applied to other ischemic injuries such as renal and myocardial infarction. Quite recently, we have found that scavenging of ROS in tumor region by RNP improves efficiency of anticancer drug, doxorubicin (DOX), for example. RNP administration prior to the DOX-injection remarkably improved its efficiency. The RNP pre-administration also reduced its side effect. Actually, 25mg/kg intravenous administrations of RNP before and after the DOX-injection (20mg/kg) reduced significantly the lactate dehydrogenase (LDH) and the creatine kinase (CK) activity. Thus, the nanoparticle anti-oxidative treatment is promising as a new adjuvant for anti-cancer chemotherapy without severe side effect by anticancer drug.


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N-acetyl-L-Cysteine Restores Disrupted Skin Barrier Function in Flaky Tail Mice

**Kozo Nakai** 1, **Kozo Yoneda** 1, **Yoichiro Hosokawa** 1, **Tetsuya Moriuie** 2, and **Yasuo Kubota** 1

1 Kagawa University, Japan

Loss of filaggrin causes a poorly formed stratum corneum, and subsequently elicits disruption of skin barrier function that leads to enhanced percutaneous transfer of allergens with the incidence of atopic dermatitis. Flaky tail mice are filaggrin deficient mice in nature and have been used to investigate the role of filaggrin in skin pathophysiology. However, the mechanism remains unclear why loss of filaggrin causes skin barrier dysfunction. Epidermal growth factor receptor (EGFR), E-cadherin, occludin and SIRT1 are skin barrier-related proteins. We found that these skin barrier-related proteins are decreased in the skin of 8 week old flaky tail mice, of note, N-acetyl-L-cysteine (NAC) supplement increased these skin barrier-related protein expressions, implying the involvement of oxidative stress. Aging are known to accompany the increased levels of oxidative stress in various tissue including skin. The reduced expression of these protein expressions was not observed in 8 week old C57BL/6J control mice but observed in 32 week old C57BL/6J control mice. Our findings suggest that NAC may be considered as a future therapeutic agent in the treatments of atopic dermatitis that strengthens the skin barrier function.


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Oral Nanotheprapy by Redox Nanoparticle: Accumulation of Redox Nanoparticle in Colon Mucosa Improves Therapeutic Effect of Ulcerative Colitis

**Yukio Nagasaki** 1,2, **Long Binh Vong** 1, **Toru Yoshitomi** 1, and **Hirofumi Matsui** 1

1 University of Tsukuba; 2 MANA, NIMS

We have newly developed nitroxide-radical containing nanoparticle (RNP), which is constructed by amphiphilic block copolymer possessing stable 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) moity as a side chain of hydrophobic segment. Confinement of the nitroxide radicals in the core of the nanoparticle decreased its toxicity and rapid metabolism to improve blood circulation tendency. Because the nitroxide radical scavenges reactive oxygen species (ROS), we have investigated an antioxidant efficiency of RNP for cerebral, renal and cardiovascular ischemia-reperfusion injuries, intracerebral hemorrhage, Alzheimer's disease, cancer chemotherapy and gene delivery system so far and confirmed its high effect. In this study, we have applied this nanoparticle for oral nanotherapy. RNP was orally administered to ulcerative colitis model rats prepared by dextran sodium sulphate (DSS). Using fluorescence imaging and electron spin resonance (ESR) analyses, it is confirmed that accumulation of nitroxide radicals in mucosa membrane in colon increased more than two orders of magnitude as compared to low-molecular-weight TEMPO compounds. Especially, it is much increased in injured colon mucosa than that in normal colon mucosa. Although RNP is accumulated in the mucosa membrane, no uptake in blood stream was observed, which is ideal character because of avoidance of side effect in whole body. Oral administration of RNP (0.2 mM kg\(^{-1}\)) obviously suppressed disease activity index by reducing body weight loss, diarrhea, and feces bleeding. Colon length recovery, colonic epithelial architectural protection, and inhibition of inflammatory mediators such as, myeloperoxidase (MPO) activity, interleukin (IL)-1B and superoxide generation were also observed obviously in RNP treated mice. On the bases of these obtained results, oral nanotheprapy using RNP significant improves accumulation of drug in colon mucosa membrane to work effectively as anti-oxidative drug, suppressing side effect in whole body. Thus, oral RNP therapy is promising as new anti-oxidative therapy.