

Basic Science

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Heme oxygenase-1: An overlooked potential target in inflammatory skin disease?

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Studies of heme oxygenase in keratinocytes are few despite being an accessible target for pharmacological manipulation. We have used an immortalised human keratinocyte line to demonstrate the possibility of synergistically up-regulating keratinocyte heme oxygenase-1 (HO-1) as a novel means of treating inflammatory dermatoses such as acne, atopic dermatitis and psoriasis. HO-1 is the inducible rate-controlling enzyme that catalyses the conversion of heme to ferrous iron, bilirubin and carbon monoxide. HO-1 possesses anti-inflammatory, cytoprotective and immunosuppressive effects and hence limits tissue damage caused by reactive oxygen species.

The transcriptional activator, Nfr2, is intimately involved in the regulation of HO-1 gene expression. Cytosolic Nfr2 is phosphorylated and translocated into the nucleus in response to activation of mitogen-activated protein kinases (MAPKs). Most known inducers of HO-1 including antioxidants such as curcumin and carnosol target protein phosphorylation cascades.

Cobalt protoporphyrin IX (COPP) and metal salts on the other hand appear, at least in part, to target Bach-1 by promoting its nuclear export. Bach-1 is a transcriptional repressor, heterodimers of which normally suppresses transcription of the HO-1 gene by binding to the stress response elements within the promoter region. Using ELISA to detect HO-1 levels in lysates from HEK001 cells grown for 72 h without inducer followed by 24 h with different concentrations of inducer, we found that CoPP at 0.78 to 3.12 μM induced a >200 fold increase in HO-1 protein compared to baseline levels. Using the photosensitiser and heme precursor, aminolevulinic acid (ALA), the maximum increase in HO-1 production was 21 fold at 1 mM. When 100 μM ALA was combined with 100 μM cobalt chloride, the maximal increase in HO-1 production was 260 fold compared to baseline, 320 fold compared to 100 μM cobalt chloride alone and 32 fold compared to 100 μM ALA alone. When cobalt chloride was used alone the maximum induction of HO-1 protein production was 15 fold at 1mM.

We hypothesise that ALA and cobalt chloride affect different aspects of the HO-1 regulatory pathway and this results in a synergistic inducing effect as previously observed in hepatoma cells (Mitani et al. Biochem J 1993; 290: 819-25). Identifying other combinations of compounds that synergistically modulate aspects of HO-1 regulation represents a novel approach to the development of topical anti-inflammatories.

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Personalized approach to analysis of melanoma tumor heterogeneity with potential implications for therapy

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Background: Complex mixtures of molecularly distinct subpopulations occur in many malignancies, including melanoma. Differences in therapeutic effects on each clone may occur, in which case present cancer therapies may fail due to incomplete eradication of all malignant clones. Personalized cancer therapy will require the identification of therapeutic vulnerabilities among these tumor subsets in order to develop properly targeted therapies.

Methods: Here, multiparameter nuclear flow cytometry is used to identify melanoma clones which are then molecularly characterized with high-resolution genomic analysis using array-based comparative genomic hybridization (aCGH). From either fresh-frozen or paraffin-embedded specimens, melanoma nuclei were isolated from and subjected to flow cytometry using melanocyte-specific antibodies to separate melanoma cells from stroma and DNA content to separate individual tumor subpopulations. DNA extracted from isolated nuclear subpopulations was extracted and analyzed by aCGH.

Results: The feasibility of the outlined approach is demonstrated by the successful separation of melanoma from stromal nuclei as well as the separation of individual melanoma nuclear subpopulations by DNA content. aCGH analysis of DNA from isolated tumor subpopulations allowed successful identification of potentially targetable molecular aberrations in individual subsets of tumor cells. Importantly, these aberrations were often undetectable in unsorted, bulk tumors analyzed by the same high-resolution aCGH approach.

Conclusions: We demonstrate a feasible approach to in-depth molecular analysis of tumor subpopulations within clinical cancer specimens. This approach allows identification of potentially targetable molecular aberrations within individual tumor subsets, thus opening a possibility for a broad tumor targeting through design of individually-tailored therapeutic approaches.

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TBHQ represents a new class of antibacterial for topical application in dermatology

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Issues concerning the overuse of topical antibiotics and the emergence of resistance have led to a need for novel antimicrobials for dermatological applications. Repositioning known chemical entities with a history of use in man offers a more effective development route in terms of time, money and risk. 2-tert-butylhydroquinone (TBHQ) is a sterically hindered lipophilic hydroquinone used extensively as an antioxidant, especially to inhibit peroxidation of unsaturated fats.

The compound is a permitted direct food additive and a permitted cosmetic ingredient in both the US and EU. It therefore represents an excellent repositioning candidate. Experiments were carried out in order to evaluate its suitability as a topical anti-staphylococcal and anti-propionibacterial agent. In vitro tests showed that TBHQ was active against a panel of 10 antibiotic susceptible and resistant coagulase-negative staphylococci (MICs 0.98-7.8 mg/L) and 16 *Staphylococcus aureus* isolates including MRSA, VISA and GISA strains (MICs 1.95 – 7.8 mg/L). TBHQ was also active against strains of *Propionibacterium acnes* and *Propionibacterium granulosum* resistant to erythromycin, clindamycin and related antibiotics via target site mutation or methylation of 23S rRNA and/or to tetracyclines via target site mutation of 16S rRNA. MICs of TBHQ for 21 propionibacterial antibiotic susceptible and resistant isolates ranged from 1.95 to 15.6 mg/L; one isolate of *P. granulosum* showed reduced susceptibility (MIC 62.5 mg/L). Time to kill studies using suspensions of *P. acnes* NCTC737 in aqueous buffer showed that TBHQ at 31.25 mg/L is rapidly bactericidal. Current knowledge of its mode of action suggests that TBHQ affects multiple cellular processes including both direct effects due to TBHQ itself and indirect effects attributed to free radicals generated via oxidation to 2-tert-butylbenzoquinone.

TBHQ has physicochemical characteristics ideal for topical delivery and retains antimicrobial efficacy in the presence of salt and skin lipids. In view of its safety profile, physicochemical properties and antimicrobial potency, TBHQ represents a novel drug candidate for the local treatment of acne and/or the prevention of staphylococcal skin infections.

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