ulceration in the acute phase, as well as stiffness and fibrosis in the chronic phase. Deferoxamine (DFO) is an FDA approved iron chelator that has been shown to increase skin vascularization by stabilizing hypoxia-inducible factor 1-alpha levels and improve radiation-induced fibrosis. However, it is unclear whether there are other mechanisms underlying the therapeutic effects that DFO exhibits on the skin. Previous work has shown that DFO delivered transdermally improves healing in diabetic ulcer wounds by decreasing oxidative stress and cellular apoptosis. This study evaluates the effects of transdermal DFO on reactive oxygen species and cell death in the context of irradiation (IR) to skin.

Methods: CD-1 nude immunodeficient mice (n=8) were randomized to one of two conditions: 1. Control patch (IR only) (n=4) and 2. DFO patch (n=4). The study period consisted of a 14-day pre-IR period, followed by a 12-day course of fractionated IR which delivered a total of 30 Gray to all mice. The control group received patches without DFO and the DFO group received patches containing DFO throughout the experiment with patches changed daily. Mouse scalp skin was harvested one day after the completion of IR. Tissue samples were either fixed in 4% PFA, snap frozen in OCT, or directly stored at -80°C. Fixed samples were embedded in paraffin, sectioned, and stained for iron with Prussian blue. Snap frozen samples were cryosectioned at 10um, mounted, stained with dihydroethidium (DHE), and imaged with confocal microscopy. DHE detects reactive oxygen species. Stored samples were mechanically homogenized and enzyme-linked immunosorbent assays (ELISAs) were performed to measure levels of human Bax and Cleaved Caspase-3, markers of apoptosis.

Results: Treatment with DFO was associated with decreased levels of iron in the skin, confirming DFO’s role as an iron chelator. DHE fluorescence was more intense in the control patch group than in the DFO patch group (**p<0.0001), indicating that ROS generation decreased with DFO treatment. Similarly, apoptosis was highest in the control group and lowest in the DFO group as shown by higher human Bax protein (*p=0.028) and Cleaved Caspase-3 (**p=0.0038) levels.

Conclusions: DFO delivered transdermally leads to a dose-dependent decrease in reactive oxygen species and apoptotic markers. These results provide further insight into the mechanisms underlying its restorative effects on irradiated skin. Future work will investigate the relationship of reactive oxygen species and fibroblast subpopulations in the skin.

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Characterizing The Role Of Neutrophils In A Mouse Model Of Fibrodysplasia Ossificans Progressiva

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Purpose: Fibrodysplasia Ossificans Progressiva (FOP) is a debilitating congenital disease that causes heterotopic ossification (HO) of soft tissue during flares or after injury. Inflammation is crucial for the pathogenesis of disease and previous studies have demonstrated a characteristic acute influx of inflammatory cells including neutrophils. Additionally, there are no current biomarkers to identify flares. Neutrophils possess the unique ability to extrude their chromatin with degradative enzymes, termed neutrophil extracellular traps (NETs). Given the demonstrated ability for NETs and their byproducts to propagate further inflammation, specifically in diseases with characteristic spontaneous “flares”, like Rheumatoid Arthritis (RA), we hypothesize that neutrophils and NETs play a role in, and may serve as an early biomarker for FOP flares.

Methods: Neutrophils at post-injury day 1 and 3 from a tamoxifen-inducible cardiotoxin-mediated FOP mouse model, mice with the R206H genetic mutation in the Activin A receptor (ACVR1), were analyzed via single-cell RNA sequencing (scRNA). FOP neutrophil phagocytic and reactive oxygen species (ROS)-generating ability of peripheral and bone marrow granulocytes, respectively, (n=3/group) were measured. Immunofluorescent (IF) microscopy (n=2-4/group/time point) of NETosis markers citrullinated histone H3 (H3cit) and myeloperoxidase (MPO) at the site of HO formation was performed. Neutrophils were stimulated to NET in order to quantify (n=2-3/group) the sensitivity of neutrophils to NET in FOP and WT neutrophils. Finally, human plasma samples from FOP (n=10) and non-FOP controls (n=6) were analyzed for NETs by ELISA.
Results: Our scRNA data in FOP neutrophils demonstrated an association with Gene Ontology (GO) terms “immune system process” and “response to stimulus” while WT neutrophils associated with “biological regulation,” suggesting FOP neutrophils are more primed for an inflammatory response to stimuli. Further, KEGG pathway analysis demonstrated “TLR signaling” as one of the top 10 upregulated pathways. There was no significant difference in phagocytic ability (p=0.30) or ROS-generating ability (p=0.14) in FOP vs WT neutrophils. IF demonstrated that NETs are found along the sheaths of fascial planes. FOP neutrophils generate significantly more NETs (p=<0.0001) when stimulated with calcium ionophore (A23187). There was a trend of increased cfDNA in plasma from FOP patients compared to non-FOP controls that did not reach significance (p=0.12).

Conclusion: These studies demonstrate that FOP neutrophils may be hyper-responsive to external stimuli, and are more “primed” to undergo NETosis, even prior to NETosis induction. NETs were present in fascial planes, locally at sites where HO eventually forms in FOP. scRNA studies indicate that TLR signaling may play an important role in FOP neutrophils. Together, our data in FOP neutrophils suggest important differences from non-FOP neutrophils and that NETosis may play a pathogenic role in the formation of HO.

N-acetylcysteine (NAC) Addition To Anaesthetic Solution Decreases Wound Size And Promotes Wound Healing And Activation Of Hif1-alpha And Growth Factors - An Animal Model Study

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Purpose: N-acetylcysteine (NAC) is known for its direct and indirect anti-oxidative effects, while the chronic inflammation is a well-recognised factor impairing wound healing. The aim of the study was to evaluate if a pre-incisional NAC injection alters the process of wound formation.

Methods: The study was conducted according to ARRIVE guidelines. Each of 24 Sprague-Dawley rats had 2 rows of 3 incisions each planned on the dorsal side. In the first row (randomly chosen), injections with lidocaine and ephinephrine solution were made (control). In the second row injections contained lidocaine, ephinephrine and an addition of NAC in three different concentrations (0,015%, 0,03% and 0,045%). 11 timepoints photographic documentation of wounds was performed. Rats were sacrificed (6/timepoint) on the 3rd, 7th, 14th and 60th day after the wound creation. Then, scars/wounds were excised and preserved for histological and gene expression analyses. RT-qPCR included 94 targets related to wound healing process. Histological morphometric assessment included measurements of various parameters (e.g. width, depth, dermal proliferation area, contraction indexes). Photographic documentation underwent planimetric measurements (wound area, length, width) with ImageJ 1.48v. Mann-Whitney U and ANOVA Kruskal-Wallis tests were used for data statistical analysis.

Results: Photographic analysis showed wounds treated with 0,03% NAC concentration to have smaller wound lengths at all time points as compared with other groups, though statistically insignificantly. Also, scars pretreated with NAC solutions had significantly smaller area on the 3rd day and were narrower on the 4th day compared with all the other groups (p<0.05). We also showed that wounds pretreated with 0,03% NAC had a higher superficial concentration index (SCI) (p=0.03) and a larger dermal proliferation area (DPA) (p=0.04) in histological morphometric measurements on the day 7. Tissue samples treated with NAC showed higher expression of numerous genes vs control group. Upregulation was observed in tissue growth factors (FGF2, FGF10, IGF1, IGF2), selected cytokines and chemokines (TNF, IL1A, IL1B, TGF-B2, IL-10, ELANE), cell adhesion molecules (CDH1, ITA5) and remodeling factors (MMP2, CSK) (p<0.05). Constant upregulation of HIF1-alpha was observed at all timepoints (P<0.05).

Conclusion: NAC addition to pre-incisional anesthetic solution decreases wound size and width at an early stage of scar formation, acting as an activator of growth factors and cytokines involved in wound healing. Optimal results have been observed for the 0,03% NAC concentration. Conclusions are based on molecular, microscopic and macroscopic evidence.