

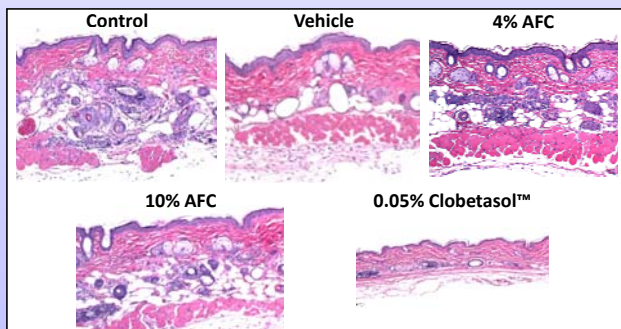
Long-Term Topical Treatment with Anti-Inflammatory N-acetyl-S-farnesylcysteine (AFC) Avoids the Skin Thinning and Inhibition of Wound Healing Associated with Glucocorticoids*

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INTRODUCTION

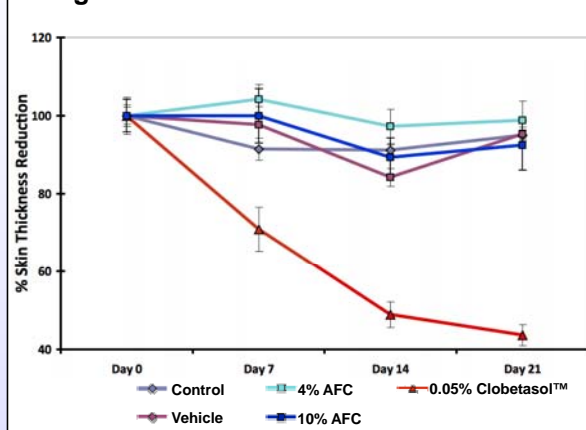
N-acetyl-S-farnesyl-L-cysteine (AFC) is a novel topical anti-inflammatory which acts by modulating the signaling of G-protein coupled receptors (GPCRs). As a structural mimic of a C-terminal post-translational modification making up the CAAX motif of prenylated G-proteins AFC competes with prenylated G-proteins for sites of interaction located within receptors, membrane-bound proteins and the α -subunit of heterotrimeric G-proteins (See Poster #126). AFC has been shown to be an effective inhibitor of inflammatory responses mediated by GPCRs in cultured macrophages, neutrophils and platelets. Its anti-inflammatory efficacy *in vivo* has been demonstrated using the mouse ear acute chemical irritation and delayed type hypersensitivity (DTH) models (Gordon et al. '08). AFC's activity was restricted to the treated ear, while treatment with the glucocorticoid (GC) dexamethasone was not, suggesting AFC would have a strong safety advantage over GCs by avoiding their systemic effects. However, long-term GC use also results in adverse skin effects such as skin thinning and the inhibition of wound healing. We now demonstrate long-term treatment of mouse skin with AFC at doses that produce maximal inhibition of inflammatory markers in the mouse ear model (Gordon et al. 08) does not cause either skin atrophy or inhibition of re-epithelialization. Thus, long-term use of AFC for inflammation potentially avoids both the systemic or skin adverse effects of GCs.

Fig. 1A Total Skin Thickness is Maintained After Long-Term Topical Treatment with AFC



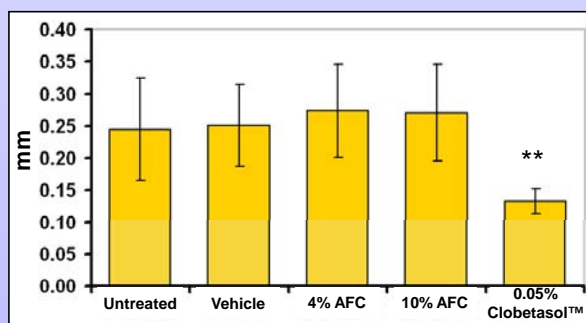
H&E stained histological sections of the skin from adult hairless mice (Skhr-1) were treated for each of 5 days, during a total of 3 weeks on the dorsal flank with either 10% AFC, 4% AFC or 0.05% clobetasolTM all dissolved in 70% EtOH/30% PEG, the vehicle or not treated. These are maximally inhibiting doses of AFC in the mouse ear model (Gordon et al. 08). No reduction is observed in the total thickness of the skin sections after treatment with both AFC doses compared to the skins untreated and vehicle treated mice. A substantial reduction in both the thickness of the dermis and epidermis of the clobetasolTM treated mouse skins is seen.

Fig 1B. Total Skin Thickness Over Time



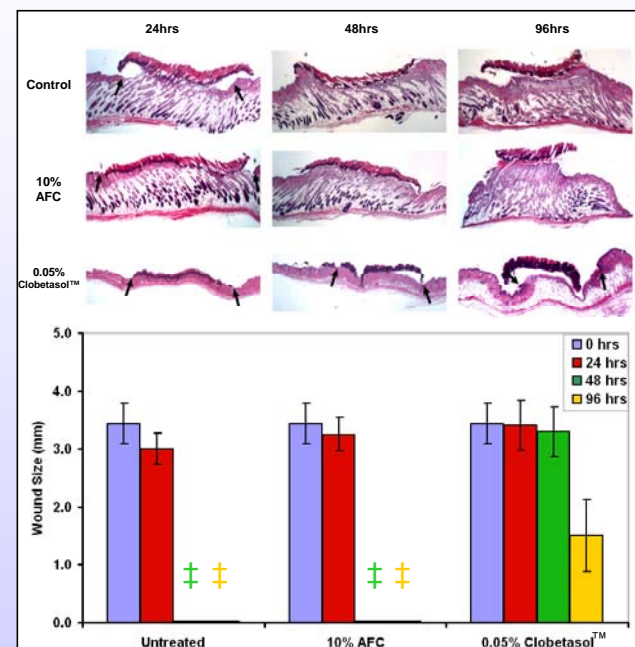
Quantification of total skin thickness with time using a MinotokaTM spring loaded caliper prior to the initiation of treatment and at the end of each week of treatment demonstrate no reduction in the control, vehicle or AFC treated skins. Conversely, clobetasolTM treated skins decrease over the 3 week period reaching maximum reduction of 40%.

Fig. 2 Epidermal Thickness is Maintained After Long-Term Topical Treatment with AFC



The epidermal thicknesses of sections of skin shown in Figure 1 were measured by image analysis from digitally captured photographs using ImageJ software. As with total skin thickness there is no reduction of epidermal thickness in the untreated, vehicle or AFC treated skins at the end of 3 weeks. There is a 50% reduction of the epidermal thickness in the clobetasolTM treated skins during the same period. (** Significance vs untreated)

Fig. 3 Long-Term Topical Exposure to AFC Does Not Inhibit Re-Epithelialization



Seven and half week old CD-1 treated mice were treated on depilated dorsal flanks daily for 2 weeks with 10% AFC (maximally active dose in mouse ear) or 0.05% clobetasolTM prior to selective mechanical removal of the epidermis with a slowly rotating felt wheel and once immediately after wounding with the respective agent. H&E stained histological of the skins harvested at various times after wounding show complete re-epithelialization by 48 hours in untreated and AFC treated skins (arrow marks the leading edges of the migrating epidermis). The clobetasolTM treated skins showed incomplete re-epithelialization in all the mice at 48 hours and 50% of the mice at 96 hours. ImageJTM software measurement of distance between the leading edges of the epidermis, shows no reduction in the size of the wound through 24 hours with untreated and AFC and 48 hours with clobetasolTM. The average size of the GC treated wound was 50% of the initial at 96 hours (± 100% healed).

SUMMARY/CONCLUSIONS

- Long-term topical treatment with the anti-inflammatory AFC does not produce skin atrophy, as does the potent glucocorticoid clobetasolTM.
- Long-term treatment with AFC at a dose that reduces wounding induced inflammation does not delay re-epithelialization as does the potent glucocorticoid clobetasolTM.
- Long-term use of AFC to treat chronic inflammatory conditions, such as rosacea, should avoid the adverse effects associated with glucocorticoids.