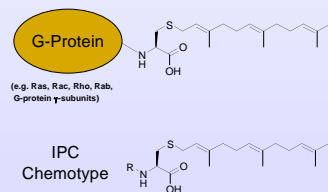


Introduction

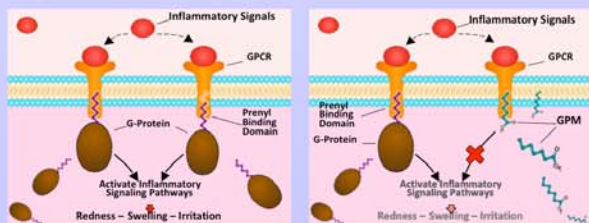
Using mouse models for acute dermal inflammation and allergic contact hypersensitivity we have previously demonstrated that the isoprenylcysteine (IPC) analog, *N*-acetyl-S-farnesyl-L-cysteine (AFC) is an effective topical anti-inflammatory *in vivo* comparable in activity to glucocorticoids and standard non-steroidal anti-inflammatory drugs (NSAIDs), but lower in potency (J.S. Gordon *et al.*, '08, JID, **128**: 643). To assess the anti-inflammatory potential of novel IPC analogs, a total of 126 chemically-diverse IPC analogs were synthesized with structural modifications made to four distinct regions: 1) the cysteine α -carboxyl, 2) the farnesyl side chain, 3) the sulfur and 4) the cysteine α -amino. Analogs were tested for three endpoints in mice: inhibition of TPA-induced ear thickness (edema), neutrophil infiltration (myeloperoxidase - MPO) and erythema (redness). Several analogs substantially more potent than AFC were identified for all three endpoints. Thus, IPC analogs could yield novel pharmaceutical leads for the treatment of specific inflammatory skin conditions. Our lead pharmaceutical candidate SIG989 is currently being developed as an anti-rosacea therapeutic agent.

Fig. 1 IPC Analogs Structural Mimics of G-Protein C-Terminus



G-proteins participate in eliciting inflammatory responses such as the release of pro-inflammatory mediators, and the migration and activation of inflammatory cells. Located near the end of each G-protein is a conserved cysteine residue modified with a prenyl tail (either 15 or 20 carbon side-chain). IPC analogs are structural mimics of the lipidated C-termini of the $G\gamma$ subunit of all heterotrimeric G-proteins, as well as that of small molecular weight GTPases such as Ras, Rho and Rac.

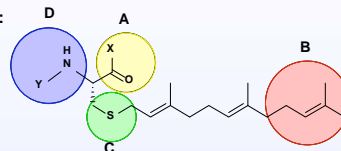
Fig. 2 IPC Analogs Mechanism of Action



IPC analogs through their novel mechanism of action successfully modulate inflammatory signaling by binding to GPCR prenyl-binding pockets, normally used by G-proteins to activate inflammation signaling pathways. Several other important signaling proteins, in addition to GPCRs, have recently been identified to possess prenyl-binding pockets or require a prenyl tail for binding representing other potential targets for IPC compounds.

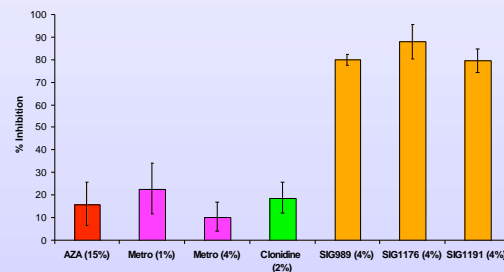
Fig. 3 Synthesizing 2nd Generation IPC Analogs for Pharmaceutical Development

2nd generation analogs:
 Carboxyl group
 Lipid tail
 Sulfur group
 Amino end



Using the naturally occurring farnesyl-cysteine structure, chemical modifications are made to four distinct regions of the molecule to generate novel IPC compounds. Compounds are screened in *in vitro* cell-based assays and then *in vivo* for anti-inflammatory activity using the mouse ear model of contact irritation.

Fig. 4 IPCs More Active than Current Topical Anti-Rosacea Agents vs Erythema

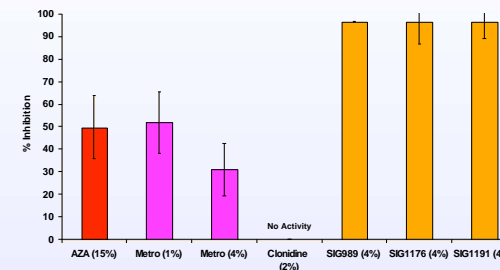


Using a mouse ear model of TPA-induced inflammation, compounds were tested for activity measuring for the reduction of erythema and compared to current topical anti-rosacea agents (azelaic acid (AZA), metronidazole (Metro) and clonidine). Results demonstrate IPC analogs are ~4-times more active in reducing chemically-induced erythema. Mice received 1.2 μ g/20 μ l TPA to each ear then the same volume of compound in EtOH after 5 minutes. Erythema measurements were taken at 24 hours after TPA treatment using a Konica Minolta CR400 and all compounds were tested in the concentration shown.

Summary/Conclusions

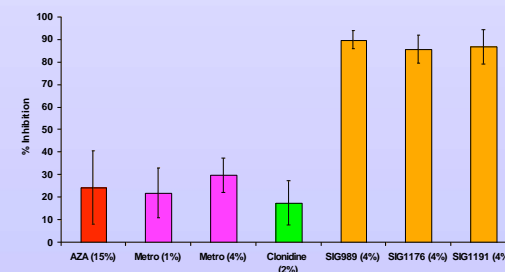
- Topical NSAID technology modulates G-protein signaling pathways by competing for prenyl-binding domains
- Novel, more potent IPC analogs are generated by introducing modifications to four distinct sites of the naturally occurring farnesyl-cysteine structure
- SIG989 as well as other IPC analogs exhibit superior activity to current topical rosacea and anti-erythema agents (metronidazole, azelaic acid clonidine)

Fig. 5 IPCs More Active than Current Topical Anti-Rosacea Agents vs Neutrophil Infiltration



Using a mouse ear model of TPA-induced inflammation, compounds were tested for activity measuring inhibition of myeloperoxidase (in tissue a marker for neutrophil infiltration in response to irritation) compared to current topical anti-rosacea agents (azelaic acid (AZA), metronidazole (Metro) and clonidine). Results demonstrate IPC analogs are 2 to 3 times more active in reducing chemically-induced neutrophil infiltration. Mice received 1.2 μ g/20 μ l TPA to each ear then the same volume of compound in EtOH after 5 minutes. 6 mm ear punches were taken and MPO measurements taken 24 hours after TPA treatment. All compounds were tested at the concentration shown.

Fig. 6 IPCs More Active than Current Topical Anti-Rosacea Agents vs Edema



Using a mouse ear model of TPA-induced inflammation, compounds were tested for activity measuring inhibition of edema compared to current topical anti-rosacea agents (azelaic acid (AZA), metronidazole (Metro) and clonidine). Results demonstrate IPC analogs are 3 to 4 times more active in reducing chemically-induced neutrophil infiltration. Mice received 1.2 μ g/20 μ l TPA to each ear then the same volume of compound in EtOH after 5 minutes. 6 mm ear punches were taken and weighed 24 hours after TPA treatment. All compounds were tested at the concentration shown.