COLONIC BIOACTIVATION AND POTENT TNFα INHIBITION OF PALI-2108 IN HUMAN CLINICAL STUDIES: A PROMISING PDE4 INHIBITOR PRODRUG FOR THE ORAL TREATMENT OF ULCERATIVE COLITIS

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PURPOSE / OBJECTIVES

Phosphodiesterase-4 (PDE4) is a key enzyme in cAMP hydrolysis and its inhibition elevates intracellular cAMP, downregulates inflammatory cytokines, and reduces the expression of cell adhesion molecules, thereby preventing local infiltration and activation of inflammatory cells. Approved PDE4 inhibitors include roflumilast for use in chronic obstructive pulmonary disease (COPD) and apremilast for psoriasis/psoriatic arthritis. Despite the development of subtype specific PDE4 inhibitors, oral administration and systemic distribution has resulted in central nervous system (CNS) toxicity, such as headaches, nausea or vomiting, leading to discontinuation of therapy and limiting potential efficacy. Targeted and better-tolerated oral PDE4 inhibitors hence remain an unmet need in IBD.

We investigated PALI-2108, an orally delivered, intestinally activated PDE4 inhibitor prodrug designed with a D-glucoronic sugar moiety (Figure 1). PALI-2108 minimizes systemic exposure until cleaved by the colonic bacterium enzyme β-glucuronidase, producing active drug PALI-0008, therefore reducing potential CNS toxicity. PALI-2108 proposed mechanism of action is outlined in figure 5.

MATERIAL & METHODS

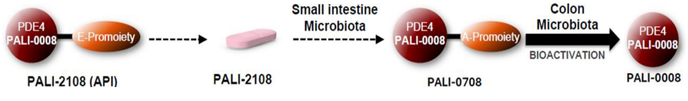
First, we evaluated prodrug conversion in human stool samples (6 healthy patients and 6 patients with UC) using LC-MS and the enzymatic activity was assessed by determining half-lives in samples from each individual donor (Figure 2). PALI-2108 was spiked into stool homogenate (100 μM) and homogenate was subsequently incubated for 24 hours. PALI-2108 and PALI-0008 concentrations were measured, and the prodrug-to-drug conversion percentage was reported.

Second, the effect of the active PDE4 inhibitor, PALI-0008, on lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNFα) production was evaluated using an ex-vivo peripheral whole blood assay. Whole blood from 12 healthy human donors were pre-treated with PALI-0008 followed by LPS challenge at 1ug/ml for 24 hours. TNFα production was measured and the IC_{50} of ampreliast and PALI-2108 was calculated (Figure 3).

Finally, a microbiome study was conducted with CosmosID, designed to evaluate the abundance of the beta-glucuronidase enzyme in microbiome samples from mice, dogs, healthy humans, and ulcerative colitis (UC) patients. Publicly available data for dog, mouse, and human whole genome metagenomic sequencing was obtained from the NCBI Sequence Read Archive. samples were uploaded to the CosmosID-HUB (app.cosmosid.com) for functional identification of genes, enzymes, and pathways utilizing the MetaCyc, Gene Ontology, and Enzyme Commission databases (Figure 4).

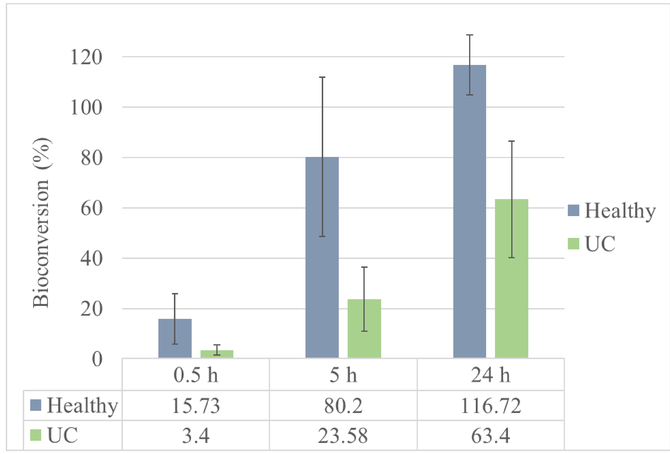
RESULTS

Figure 1. PALI-2108 is a novel small molecule prodrug of the parent PDE4 inhibitor PALI-0008. It is intestinally activated by the colonic bacterium enzyme β-glucuronidase, producing active metabolite PALI-0008, designed to minimize class-specific side-effects by reducing systemic exposure.



RESULTS

Figure 2. We demonstrated effective intestinal hydrolysis of PALI-2108 prodrug into active PDE4 inhibitor (PALI-0008) using routine stool samples from 6 healthy patients and 6 patients with UC. PALI-2108 was spiked into stool homogenate (100μM) and homogenate was subsequently incubated for 24 hours. PALI-2108 and PALI-0008 concentrations were measured and the prodrug-to-drug conversion percentage at 24 hours was a mean of 90.1% across samples. All subjects had conversion of greater than 30%. Conversion increased steadily over time beginning at 30 mins in normal healthy as well as potentially dysbiotic UC patients stool samples.



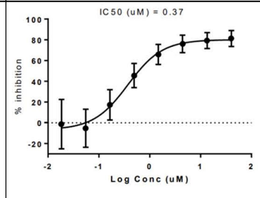
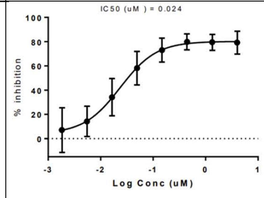
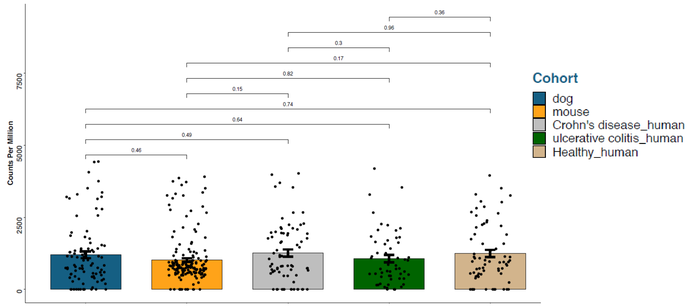
Donor	Sex	Apremilast (μM)	PALI-2108 (μM)
Mean		0.41	0.022
SE		0.065	0.003
Fit using 24-point avg.		0.37	0.024
95% conf. int.		0.29-0.47	0.018-0.030
			

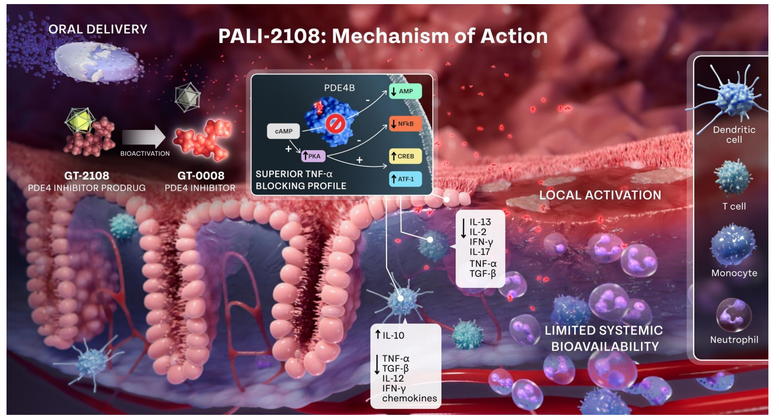
Figure 3. Pre-treatment of human whole blood from 12 healthy human donors with the PDE4 inhibitor PALI-0008 (seven-point titrations using three-fold serial dilutions starting at 4μM, in duplicate) followed by LPS challenge (1μg/ml) for 24 hours reduced the LPS induced TNFα production compared to non-pretreated samples. The IC50 values for PALI-0008 TNFα inhibition was 0.022μM. The PDE4 inhibitor apremilast showed a 20-fold **lower** potency (IC_{50} of 0.41μM) in the same assay.

Figure 4. Enzyme Commission (EC) Data from each database was subset to examine the difference in beta-glucuronidase-relevant genes, enzymes, and functional pathways between each group. The adjusted EC data shows no significant pairwise comparisons between any of the groups. Therefore, we demonstrated the relative abundance of beta-glucuronidase, the enzyme responsible for activating our prodrug, was found to be consistent across cohorts.



RESULTS

Figure 5. PDE4 is highly expressed in immune and proinflammatory cells. The inhibition of PDE4 prevents transcription factors, such as NF-κB, from inducing the expression of various proinflammatory mediators involved in IBD, such as TNFα and various interleukins. The PDE4 inhibitor PALI-2108 is orally delivered, and colon activated allowing for local activity with low systemic exposure.



SUMMARY / CONCLUSION

- PALI-2108, a novel orally delivered, intestinally activated PDE4 inhibitor prodrug, is effectively hydrolyzed into active PDE4 inhibitor (PALI-0008) ex-vivo in stool samples from healthy patients and patients with UC.
- PALI-2108 is successfully bio converted into its active form, PALI-0008, in NHV and UC patient stool, with a high conversion rate of 90.1% at 24 hours.
- The design of PALI-2108 ensures that activation occurs primarily in the colon, reducing systemic exposure and potential CNS side effects.
- By minimizing systemic exposure, PALI-2108 potentially addresses toxicity issues seen with other PDE4 inhibitors like roflumilast and apremilast.
- PALI-0008 shows significant inhibition of TNFα production in human whole blood, with an IC50 of 0.022 μM, indicating high potency.
- PALI-0008 demonstrates a 20-fold higher potency in reducing TNFα production compared to apremilast, highlighting its potential efficacy.
- The microbiome study shows consistent levels of β-glucuronidase across different species and conditions, supporting the activation mechanism of PALI-2108.
- The consistent enzyme abundance suggests that PALI-2108 could be effective across diverse patient populations, including those with UC.
- The targeted activation and reduced side effects of PALI-2108 may lead to better patient compliance compared to current PDE4 inhibitors.
- PALI-2108 addresses the unmet need for better-tolerated oral therapies in the treatment of inflammatory bowel disease (IBD).
- PALI-2108 is in development for moderate-to-severe UC and is advancing toward regulatory filing for first-in-human studies.

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