Investigating the Effect of Novel MRGPRX2 Antagonist in Inhibiting LL-37 Induced Rosacea

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Abstract

Rosacea is a chronic inflammatory skin disorder characterized by itching, redness, and impaired skin barrier function. It has been reported that mast cell activation plays a crucial role in its pathogenesis. This study investigates the therapeutic potential of GE1111, a novel MRGPRX2 antagonist, in rosacea using in vitro and in vivo models.

In the in vitro model, LL-37, a potent MRGPRX2 agonist, was used to stimulate LAD-2 mast cells, HaCaT keratinocytes, and RAW 264.7 macrophages. The effects on tight junction protein claudin 1, inflammatory cytokines, and macrophage phagocytosis were evaluated using immunohistochemistry, western blotting, RT-qPCR, and fluorescence imaging techniques.

A mouse model of rosacea induced by LL-37 was employed to assess the therapeutic effects of GE1111 in vivo. GE1111 treatment reduced mast cell degranulation associated with rosacea. It also decreased immune cell infiltration and MCP-1 levels, indicating reduced skin inflammation. GE1111 preserved the expression of the tight junction protein Involucrin and reduced the inflammatory mediator periostin, which was evaluated by immunohistochemical analysis. Gene and protein expression analysis supported these findings, showing suppression of inflammatory cytokines and MRGPRX2 signaling pathways in rosacea skin lesions.

In conclusion, GE1111 showed promising therapeutic potential in both in vitro and in vivo studies, suggesting its efficacy in targeting MRGPRX2-mediated interactions between mast cells, keratinocytes, and macrophages in rosacea.

Introduction

Rosacea's development involves a complex interplay of genetic, environmental, and immune factors. Among them, the antimicrobial peptide LL37 stands out as a key player, being upregulated in the skin of rosacea patients [1-3]. LL37 activates various immune cells, including mast cells, keratinocytes, and macrophages, triggering the release of pro-inflammatory substances that sustain the inflammatory response in rosacea [4-6]. Unfortunately, current treatment options primarily focus on managing the clinical symptoms rather than targeting the underlying mechanisms.

In recent developments, the Mas-related G protein-coupled receptor X2 (MRGPRX2) has emerged as a potential therapeutic target for inflammatory skin diseases [7-9]. MRGPRX2 is expressed on immune cells, particularly mast cells, and is involved in the regulation of itch and inflammation [9]. Preclinical studies targeting MRGPRX2 have shown promising results in other inflammatory skin conditions.

Building upon these findings, our research study aimed to investigate the therapeutic effect of GE1111, an MRGPRX2 antagonist, on LL37-induced rosacea. To achieve this, we employed both in vitro and in vivo models. By studying the effects of GE1111, we sought to gain a comprehensive understanding of its potential as a treatment option for LL37-induced rosacea.

Materials and Methods

In vitro model:
- mimics the immune cell environment found in vivo
- The Laboratory of Allergic Disease 2 (LAD-2) human mast cells
- The immortalized human keratinocyte line, HaCaT

1. Immunofluorescence Assay
2. Phagocytosis Assay
3. Western Blot
4. Real-Time PCR

LL-37-induced Mouse Model of Rosacea:
- wild-type C57 adult male mice, 6 to 8 weeks old
- vehicle control, disease control, lower treatment, and higher treatment groups

1. Enzyme-linked Immunosorbent Assay (ELISA)
2. Histology
3. Immunohistochemistry

Results

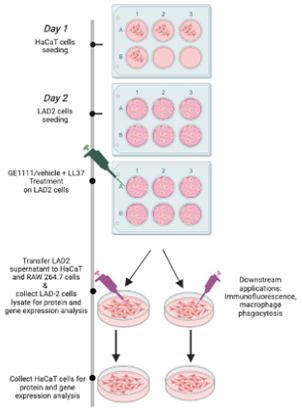

Figure 1: In-Vitro Model Experimental Design

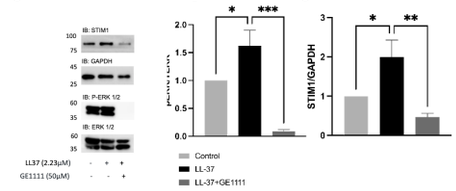

Figure 2: LAD-2 cells Western Blot

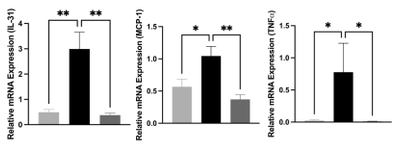

Figure 3: LAD-2 cells qRT-PCR

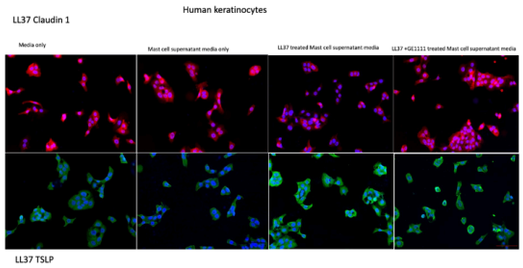

Figure 4: Immunofluorescence Staining of Claudin 1 and TSLP

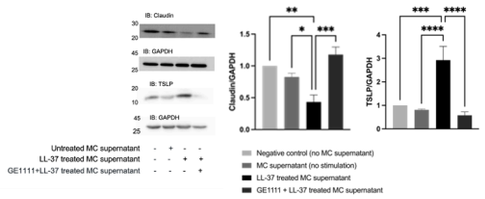

Figure 5: HaCaT cells Western Blot of Claudin 1 and TSLP

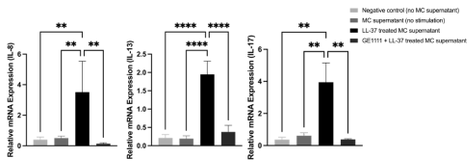

Figure 6: HaCaT cells qRTPCR of Claudin 1 and TSLP

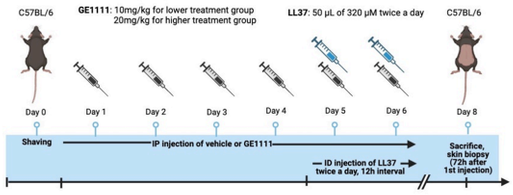

Figure 7: Animal Experiment Design

Discussion & Conclusion

MCs are an integral part of the immune system and play a crucial role in the pathogenesis and pathophysiology of Rosacea. GE1111 treated MC showed a significant reduction in the gene expression of IL-31 (**P < 0.01), MCP-1 (**P < 0.01) and TNF-α, (*P < 0.05) and protein expression of pERK and STIM1 as compared to LL-37 control MC. This trend was followed in HaCaT cells, in which immunofluorescence and western blotting of keratinocytes revealed that LL-37 mast cell supernatant-treated cells reversed the decreased claudin 1 expression and reduced TSLP expression compared to LL-37 treated disease control keratinocytes. Gene expression of IL-8, IL-13, IL-17 was also revealed to reduce with GE1111 treatment. In vivo, a model with mice also followed this trend in immunohistochemistry, gene and protein analysis, which was omitted in the poster due to the confidentiality of the project.

In conclusion, our study provides compelling evidence supporting the impact and involvement of MRGPRX2 and mast cell interactions with HaCaT keratinocytes and macrophages in the development and pathophysiology of Rosacea. This study also provides the potential therapeutic efficacy of the novel MRGPRX2 antagonist GE1111 in reducing the symptoms of LL-37-induced Rosacea.

References

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