TITLE:

In Silico Screening–Driven Intracellular signaling pathway modulation promotes inflammation resolution: A Translational in Silico–In Vivo Study

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INTRODUCTION:

Mucosal healing represents the primary therapeutic goal in Inflammatory Bowel Disease (IBD). The Il33/ST2 axis is involved in the pathogenesis of IBD, acting upstream of intracellular signaling pathways such as NF-κB and STAT3, which are critically involved in epithelial damage and repair processes.
In this context, we conducted a translational study in a DSS-induced murine model of colitis, to assess candidate molecules identified through an in-silico virtual screening approach, prioritizing compounds with potential activity on the IL-33/ST2 axis and its downstream signaling pathways.

AIMS AND METHODS

This study aims to explore the therapeutic potential of computationally identified molecules targeting the IL-33/ST2 axis can modulate downstream intracellular signaling pathways, particularly NF-κB and STAT3/phosphoSTAT3, and improve disease activity and survival in a Dextran Sodium Sulfate (DSS)-induced murine model of colitis. Mice were randomly assigned to five experimental groups, each receiving oral administration of DSS and a specific candidate molecule at defined concentrations (low concentration: 0,1 mg/Kg or high concentration: 0,5 mg/kg). Disease activity was monitored using the Disease Activity Index (DAI).
The experimental setting was structured on seven days of induction of Colitis in 25 mice (C57bl/6) by oral administration of DSS (2,5%). During recovery, mice received intraperitoneal

administration of selected candidate molecules at different concentrations on alternate days, for one week.
The computational workflow aimed to identify candidate molecules able to modulate the IL-33/ST2 interaction through a structure-based virtual screening approach. The IL-33/ST2 complex (PDB ID: 4KC3) was prepared and analyzed to define druggable binding sites, and virtual screening was performed using a hierarchical docking protocol followed by binding energy refinement and ADME/Tox prediction. Candidate selection was based on predicted binding affinity, quality of interactions at the IL-33/ST2 interface, and pharmacokinetic and toxicity profiles, enabling the selection of a small subset of compounds, which were subsequently validated in in vitro and in vivo experimental models.
At day 21, animals were sacrificed and biological samples, including colon, liver, blood, and gastrocnemius muscle, were collected. The Colon length was measured to assess differences among groups. The evaluation of protein expression of inflammatory mediators in colon homogenates was performed using Western blot, focusing on STAT3/phosphoSTAT3 and NF-κB as a downstream signaling pathway. Band intensities were quantified by densitometric analysis (ImageJ) and normalized to β-actin.

RESULTS

In the DSS-induced colitis model, all groups reached a peak DAI around day 8–9. During the recovery phase, mice treated with molecule 5 and molecule 6 (high dose) showed a more rapid and pronounced DAI reduction compared to DSS controls, reaching values below 1 by day 18–21, with statistically significant inter-group differences confirmed by two-way ANOVA ($p < 0.05$).
Treatment with molecule 5 and molecule 6 (high dose) was associated with a significant reduction in colon length compared to DSS control group ($p<0.01$), further supporting their differential impact on disease progression.
Survival analysis further highlighted treatment-dependent effects: while DSS control mice maintained 100% survival, animals treated with molecule 1 showed reduced survival rates (~20–50%), while molecule 5 and molecule 6 (high dose) preserved higher survival (~60–75%), indicating a more favorable safety and efficacy profile.
Western blot analysis of colon homogenates revealed a reduction in phosphorylated STAT3 (KDa: 91/86) and NF-κB (KDa:65) expression in treated groups compared to controls, with a more pronounced effect observed in mice receiving molecule 5 and molecule 6. Otherwise, the IL33 (KDa: 33) and ST2 (30–35 kDa) expression remain unalterated.

CONCLUSION

These findings demonstrate that targeting the IL-33/ST2 axis with identified compounds effectively modulates key downstream signaling pathways, particularly NF-κB and phosphoSTAT3/STAT3,

leading to improved disease activity in experimental colitis and supporting their translational potential in inflammatory bowel disease.
This study supports the potential of integrating in silico drug repurposing with in vivo validation to identify novel modulators of inflammatory signaling pathways involved in mucosal healing.

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