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Cellular and Molecular Impact of the Melanocortin Receptor Agonist PL8177 in Dextran Sulfate Sodium (DSS)–Induced Colitis in Rats

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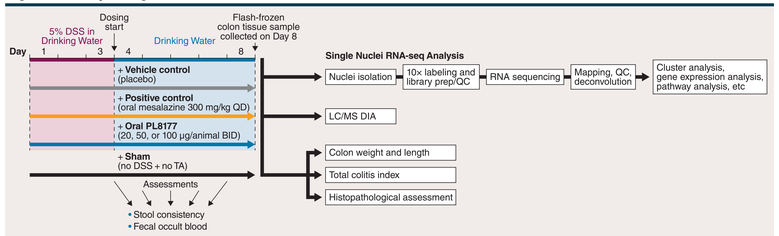
Introduction

- The melanocortin 1 receptor (MC1R)–specific agonist PL8177 and its main metabolite PL8435 have demonstrated MC1R binding affinity and functional activity that mirrors that of α-melanocyte stimulating hormone (α-MSH)[1,2]
- α-MSH has been demonstrated to be effective in reducing inflammation in numerous experimental models[1,3,4]
- Murine and human studies have found that MC1R is expressed on the colon luminal surface, and mouse models have demonstrated an important role for MC1R in a dextran sulfate sodium (DSS)–induced model of colitis[5]
- PL8177 has also shown significant protective effects against dinitrobenzene sulfonic acid–induced colitis in rats after intracolonic administration via cannula of 1.5- and 5-µg doses[1]
- High potency and a lack of systemic absorption make PL8177 a promising new candidate for clinical development of an oral formulation for the treatment of inflammatory bowel disease
- Delayed-release microparticles of PL8177 were developed for oral delivery, allowing the agent to withstand the acidic environment of the stomach and release the active drug directly into the colon
- Here we report the results of a study that investigated the effects of PL8177 on inflammation, cell population composition, and gene and protein expression in colons from a DSS–induced rat model of colitis
- The objective is to determine the effectiveness of PL8177 in this model and to characterize its underlying mechanism of action

Methods

- Male Wistar rats (each group n=6) received 5% DSS in drinking water for 3 days to induce colitis. Rats in the sham group had drinking water only (**Figure 1**)
- Doses administered were vehicle control (placebo)–filled capsules; PL8177-filled capsules at 20, 50, and 100 µg per animal (by oral gavage); or oral mesalazine 300 mg/kg (as positive control)

Figure 1. Study Design and Assessments



BID, twice daily; DSS, dextran sulfate sodium, LC/MS DIA, data-independent acquisition tandem mass spectrometer; QC, quality control; QD, once daily; RNA-seq, RNA sequencing; TA, test agent.

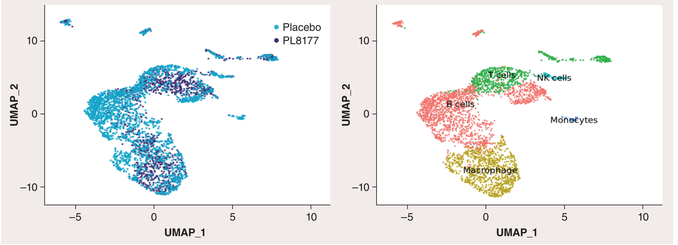
- At termination on day 8, 24 hours after the last dosing, colon tissues were harvested, dissected, and flash-frozen with liquid nitrogen
- Colon samples were analyzed for cytokine levels, single nuclei RNA sequencing (RNA-seq), and data-independent acquisition tandem mass spectrometry (LC/MS DIA)
- The generated RNA sequence was deconvoluted, mapped, and filtered. Resulting sequences were plotted with Uniform Manifold Approximation and Projection (UMAP) (https://arxiv.org/abs/1802.03426) and annotated using gene signatures for known cell types
- LC/MS DIA–based proteomic profiling was performed on 18 rat colon segments from 3 treatment groups. Data were aggregated at the protein level. Analysis was at the tissue level, not the cellular level
- Colitis was assessed by a disease activity index (diarrhea and rectal bleeding), colon length shortening, colon weight gain, and histopathological assessment
- Total colitis index was used to assess inflammatory damage
- Total colitis index scoring (score range 0–60) was based on independent observers examining and summing the scores from 3 sections from each colon per animal. Separate scored items consisted of abnormalities of mucosal architecture (0–4), extent of inflammation (0–4), erosion or ulceration (0–4), epithelial regeneration (0–4), percentage involvement (1 [1%–25%] to 4 [76%–100%]), and severity of colitis (graded semi-quantitatively from 0–20)

Results

Single Nuclei RNA-Seq

- Expression of canonical genes was used to annotate clusters into broad groups of immune cell types (**Figure 2**)
- UMAP analysis was performed to visualize clustering of single nuclei from different colon samples

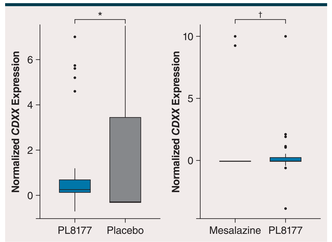
Figure 2. UMAP Plot Displaying Cell Type Clusters of Nuclei Derived From Placebo- and PL8177-Treated Colon Samples



NK, natural killer; UMAP, Uniform Manifold Approximation and Projection. Clustering enables rapid identification of batch effects and changes in cell type populations in treatment-naive diseased, and compound-treated diseased animals.

- Differential gene expression analysis showed significantly lower expression of *CDXX* genes in PL8177-treated macrophage cells compared to placebo (**Figure 3**)
- *Note that the gene name has been anonymized in recognition of currently insufficient certainty*

Figure 3. Differential Gene Expression Analysis Identified *CDXX* Gene as a Macrophage Cluster Marker



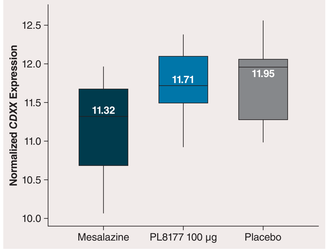
Figures show mean (SEM). *P<0.001 vs placebo; †P=0.148 vs mesalazine (by Wilcoxon signed rank test).

- Mesalazine and PL8177 have few nuclei with *CDXX* expression
- *CDXX* gene codes for a membrane protein that induces a signaling cascade to regulate immune development and inflammatory response
- Reduction of *CDXX* reduces the cell's ability to produce proinflammatory mediators
- Some nuclei appeared to be leaky and produced lower numbers of transcripts than expected
  - We are analyzing additional nuclei to generate sufficient numbers of high-quality nuclei to validate these findings

LC/MS DIA

- LC/MS DIA data analysis showed a trend towards low *CDXX* protein expression in mesalazine and PL8177 samples compared to placebo (**Figure 4**)
  - This analysis was performed at tissue level. Proteomic profiling of specific cell types will provide a more granular view of protein level changes

Figure 4. Box Plot of LC-MS DIA Data Showed a Trend Towards Low CDXX Protein Expression in Mesalazine- and PL8177-Treated Colon Samples



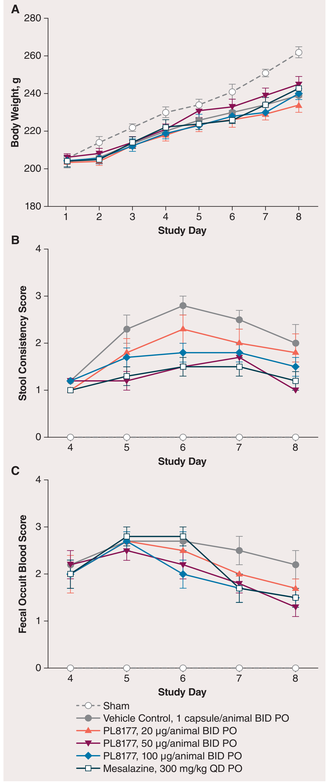
LC/MS DIA, data-independent acquisition tandem mass spectrometry. Median values shown.

- Additional analysis is ongoing and will be enhanced with additional snRNA-seq data
  - Improved snRNA-seq will enable more precise cell population determination

Body Weight, Stool Consistency, and Fecal Occult Blood

- Body weight gain was similar between the vehicle control (placebo) and treated groups (**Figure 5A**)
- Oral PL8177 at 50 µg/animal showed significant (P<0.05) improvement in stool consistency score from day 5 to day 8 and significant (P<0.05) improvement in fecal occult blood score on day 8 when compared to the vehicle group (**Figure 5B** and **C**)
- Oral PL8177 at 100 µg/animal had a significant (P<0.05) effect on stool consistency score on day 6
- Mesalazine also showed significant improvement vs vehicle control for days 5–8

Figure 5. Changes in (A) Body Weight, (B) Stool Consistency, and (C) Fecal Occult Blood in DSS Colitis–Induced Rats

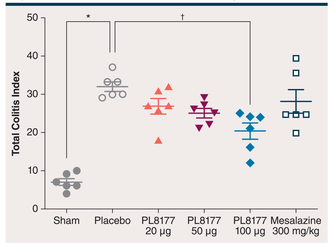


BID, twice daily; DSS, dextran sulfate sodium; PO, by mouth; QD, once daily.
Note: All animals, except those in sham group, received 5% DSS in the drinking water from day 1 to day 3, and then changed to normal drinking water for the following 5 days. Tissue harvest occurred on day 8.

Total Colitis Index and Histological Assessment

- There was a significant (P<0.05) improvement observed in the total colitis index for the PL8177 100-µg group compared to the vehicle control group, and all PL8177 cohorts showed greater improvement in the total colitis index compared to the mesalazine-treated cohort (**Figure 6**)

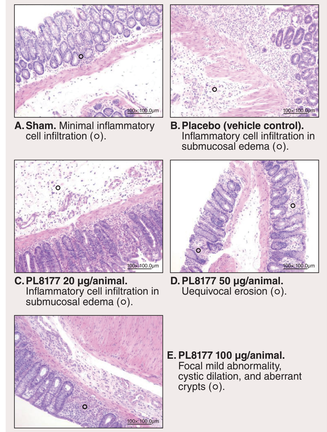
Figure 6. Total Colitis Index on Day 8



Values are mean (SEM). *P<0.05 vs sham; †P<0.05 vs vehicle control (placebo).

- The decline in the total colitis index for mesalazine-treated rats was less than that observed in any of the PL8177-treated rats
- Colon histopathological examination showed injury and prominent ulcerations to the mucosa of the distal colon that extended for 2.5–7 cm in treated inflamed rats. Submucosal focal edema and pronounced transmural thickening of the colonic wall were also observed (**Figure 7**)

Figure 7. Representative Colon Histological Sections of DSS Colitis–Induced Rats



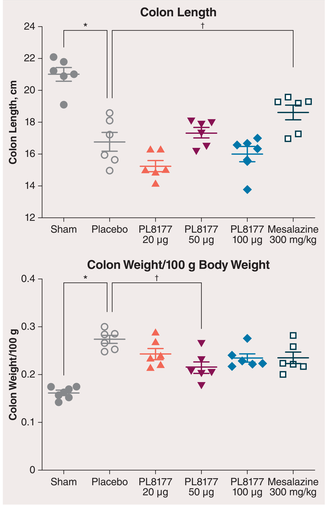
A. Sham. Minimal inflammatory cell infiltration (○).
B. Placebo (vehicle control). Inflammatory cell infiltration in submucosal edema (○).
C. PL8177 20 µg/animal. Inflammatory cell infiltration in submucosal edema (○).
D. PL8177 50 µg/animal. Unequivocal erosion (○).
E. PL8177 100 µg/animal. Focal mild abnormality, cystic dilation, and aberrant crypts (○).

DSS, dextran sulfate sodium.
Note: Sham (A) is no challenge and no treatment. Vehicle control (placebo) (B) is no treatment but DSS challenge. C, D, and E are DSS challenge and treatment with PL8177.

Colon Length and Weight

- Oral PL8177 (50 µg/animal) treatment showed a significant improvement in colon weight (53% reduction) (**Figure 8**)

Figure 8. Colon Length and Weight on Day 8



Values are mean (SEM). *P<0.05 vs sham; †P<0.05 vs vehicle control (placebo).

- Oral mesalazine 300 mg/kg (positive control) was associated with significant reduction in colon length, but only moderate improvement (35%) in colon weight gain

Conclusions

- Colon samples from the DSS rat model showed significantly lower expression of the *CDXX* gene in macrophages from PL8177-treated animals when compared to placebo. Protein levels of *CDXX* were likewise lower in PL8177-treated colon samples compared to the vehicle (placebo)
  - Lower *CDXX* expression reduces the production of proinflammatory mediators of disease
- Administration of oral PL8177 also produced significant improvement in markers of colitis compared to the vehicle group
  - Improvement in stool consistency score and blood score compared to vehicle
  - Improvement in total colitis index
  - Improvement in colon weight
- Reported results are consistent with the ultimate aim of using PL8177 to treat inflammatory bowel disease in humans

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References **1.** Spana C, Taylor AW, Yee DG, Makhlina M, Yang W, Dodd J. Probing the role of melanocortin type 1 receptor agonists in diverse immunological diseases. *Front Pharmacol*. 2018;9:1535. **2.** Dodd J, Jordan R, Makhlina M, et al. Pharmacokinetics of the melanocortin type 1 receptor agonist PL8177 after subcutaneous administration. *Drugs R D*. 2021;21(4):431-443. **3.** Ahmed TJ, Montero-Melendez T, Perretti M, Pitzalis C. Curbing inflammation through endogenous pathways: focus on melanocortin peptides. *Int J Inflam*. 2013;2013:985815. **4.** Wang W, Guo DY, Lin YJ, Tao YX. Melanocortin regulation of inflammation. *Front Endocrinol (Lausanne)*. 2019;10:683. **5.** Maaser C, Kannengiesser K, Specht C, et al. Crucial role of the melanocortin receptor MC1R in experimental colitis. *Gut*. 2006;55(10):1415-1422.