EQ504: A Potent Derivative of the AhR Ligand ITE Demonstrates Efficacy in Modulating Inflammation and Promoting Healing in DSS-Colitis

POSTER 1076

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Introduction

BACKGROUND

- The **aryl hydrocarbon receptor (AhR)** is a ligand-activated transcription factor that regulates various biological processes, including immune responses.
- Ligand-activated AhR results in pleiotropic effects on both immune cells (T cells, macrophages and dendritic cells, group 3 innate lymphoid cells) and gut epithelial cells.
- Due to its role in gut barrier function, inflammation, and immune cell differentiation, AhR activation is currently being investigated as a potential therapeutic strategy for **ulcerative colitis (UC)**[1].
- AhR binds a diverse array of ligands, however, few have demonstrated good drug-like properties leading to a lack of development candidates.
- **ITE** is a nontoxic, endogenous AhR agonist that has been shown to promote immune tolerance in colitis[1]. However, ITE has a very short half-life which limits its therapeutic potential.
- Indigo naturalis is a traditional Chinese medicine shown to be clinically effective at treating ulcerative colitis (UC)[2,3]. The active components of Indigo naturalis are indigo and indirubin. Indirubin has been shown to attenuate dextran sulfate sodium (DSS)-induced murine colitis[4].

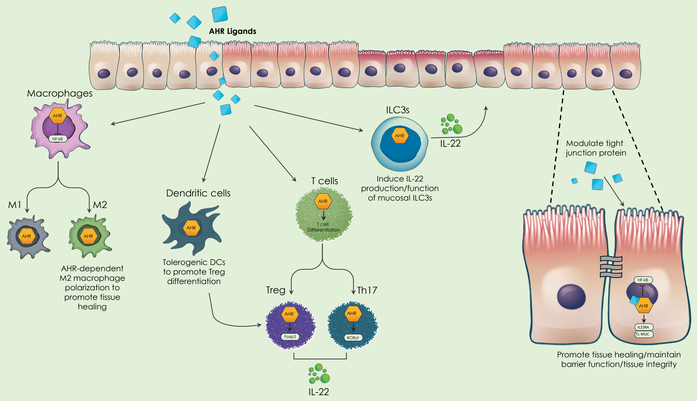


Figure 1. Schematic showing the pleiotropic effects of AhR activation on both immune and gut epithelial cells.

- **EQ504** is a novel ITE derivative with enhanced pharmacological properties, which ameliorates colitis through multiple AhR-dependent mechanisms, including regulation of inflammation and promotion of wound healing.

We highlight EQ504 as a potent AhR pathway activator with demonstrated therapeutic efficacy in alleviating colitis *in vivo*.

Methods

EROD (ethoxyresorufin-O-deethylase) Assay

HepG2 cells were plated in a 96-well black-walled, clear bottom assay plate and allowed to settle and adhere overnight. The next day, plated cells were treated with a titration of AhR ligands and incubated at 37°C for 24 hours. After 24 hours of treatment, cells were washed 2X with warm PBS and incubated with the EROD reagent mix prepared according to Schiwy et al., 2015 for 30 minutes. Cold methanol was added to stop the reaction, and the fluorescence signal was measured (540nM excitation, 590nm emission, 1000ms).

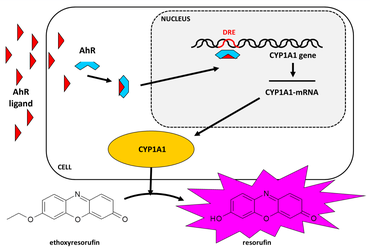


Figure 2. Overview schematic of the EROD assay.

Expression of AhR-regulated genes in immune cells

Total T cells were isolated from peripheral blood mononuclear cells (PBMCs) by magnetic negative isolation. Isolated total T cells were stimulated with STEMCELL Technologies' ImmunoCult™ Human CD3/CD28 T cell Activator overnight. Stimulated T cells were then treated with AhR agonists and CYP1A1, IL-10, and IL-22 gene expression was assessed by qPCR at specified timepoints.

DSS (Dextran Sodium Sulfate)-Induced Colitis Model

	Group	Route	Dose	Freq	N (61 total)
1	Naïve		-		5
2	Vehicle		-		7
3	EQ504	i.p.	1000 ug/kg	QD	7
4			100 µg/kg		7
5			10 µg/kg		7
6			1 µg/kg		7
7			0.1 µg/kg		7
8			0.01 µg/kg		7
9	CSA	p.o.	75 mg/kg	QD	7

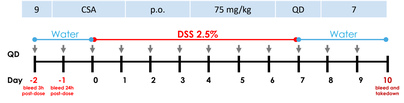
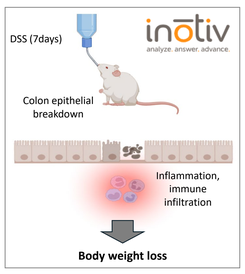


Figure 3. Dosing schedule for a 10-day mouse DSS-colitis study.



Efficacy of EQ504 (0.1 and 1mg/kg) was tested by systemic delivery (i.p.) in a mouse DSS-colitis model. Body weight was measured daily. Colon weight and length was assessed at necropsy. Tissue analysis included colon histology and gene expression (CYP1A1, IL-10, IL-22) from colon, liver, spleen, and blood. Cyclosporin A (CSA) was used as the positive control. The study and endpoint analyses were performed at Inotiv.

Results

EQ504 is a highly potent inducer of the AhR pathway

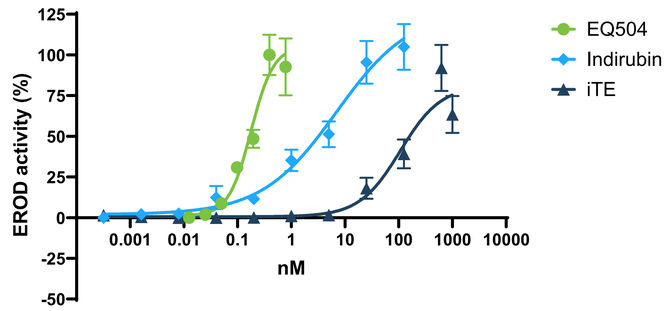


Figure 4. The relative potency of EQ504 was assessed in an EROD assay to measure the activity of CYP1A1. Treatment of HepG2 cells with EQ504 induces greater EROD activity as a result of increased CYP1A1 expression compared to its parent compound, ITE, or indirubin, one of the active components of indigo naturalis. EROD activity shown as relative percentage.

EQ504 induces greater expression of IL-10 and IL-22 in stimulated T cells compared to Indirubin

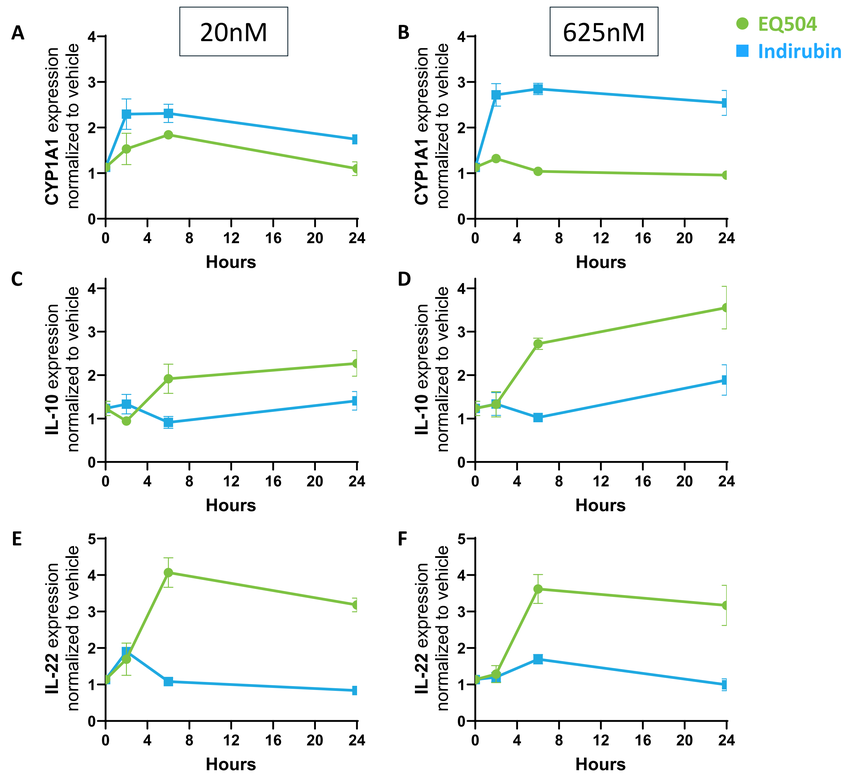


Figure 5. CYP1A1, IL-10, and IL-22 gene expression was assessed on stimulated total T cells following treatment with (A, C, E) 20nM or (B, D, F) 625nM of EQ504 or indirubin for 2, 6, or 24 hours. (A and B) Across both concentrations, indirubin induces greater expression of CYP1A1 compared to EQ504. Across both concentrations, EQ504 induces greater expression of (C and D) IL-10 and (E and F) IL-22. PK modeling (data not shown) from systemic delivery of 1mg/kg EQ504 after 24 hours in rat estimated colon and plasma exposure to be 400 – 800nM and 0 – 40nM, respectively. Concentrations for *in vitro* treatment of stimulated T cells were selected to approximate the average colonic exposure (625nM) and systemic plasma levels (20nM).

In vivo, EQ504 activates the AhR pathway in the colon of mice with DSS-induced colitis

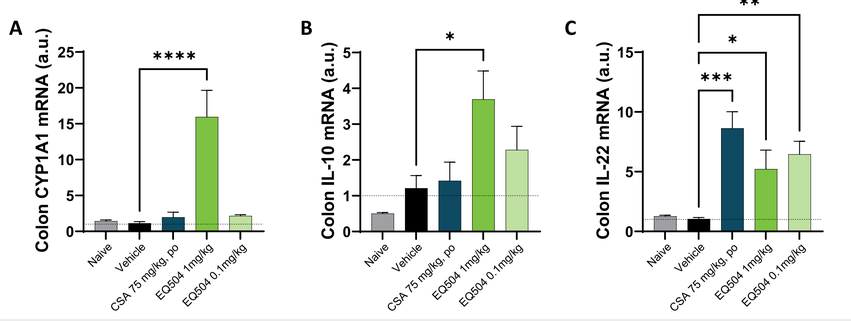


Figure 6. (A and B) EQ504 at 1mg/kg significantly upregulated gene expression of CYP1A1 and IL-10 in the colon compared to vehicle. (C) EQ504 at 1mg/kg and 0.1mg/kg significantly upregulated gene expression of IL-22. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle by One-Way ANOVA.

EQ504 protects change in body weight and preserves colon lengths in a DSS-induced mouse model of colitis

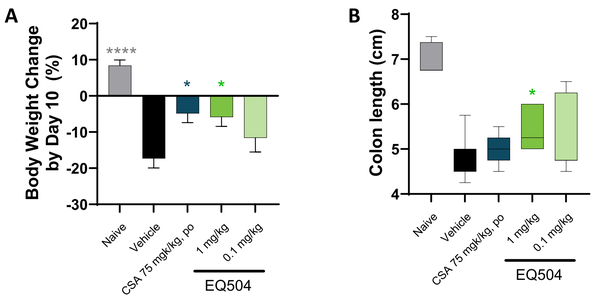
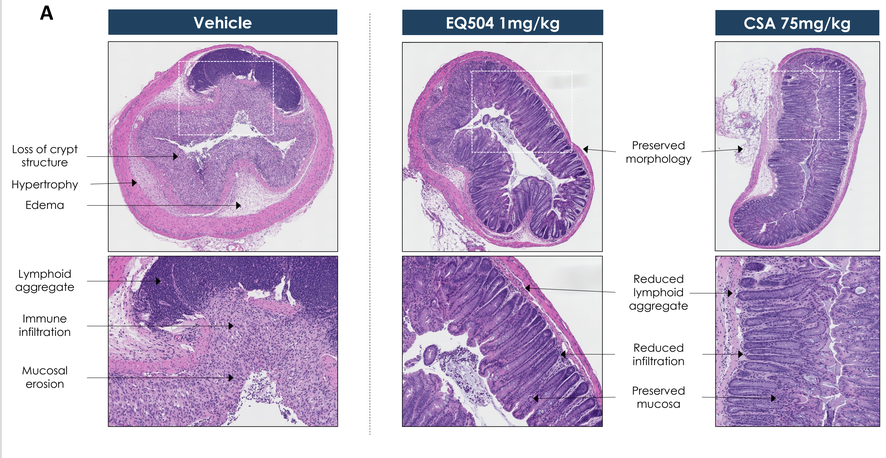


Figure 7. (A) Treatment with 1mg/kg EQ504 protects against a significant reduction in body weight due to DSS-induced colitis, comparable to 75mg/kg Cyclosporin A. (B) EQ504 at 1mg/kg significantly preserves colon length by Day 10 (peak disease). *p<0.05, ****p<0.0001 vs. vehicle by One-Way ANOVA

EQ504 ameliorates pathology during DSS-induced colitis



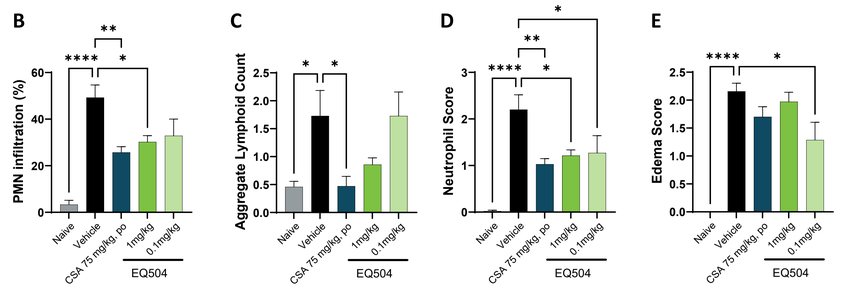


Figure 8. (A) Histology sections from the distal colon on Day 10 show that 1mg/kg EQ504 preserved morphology, reduced lymphoid aggregates, reduced immune cell infiltration, and preserved mucosa compared to the vehicle control. Quantification of (B) percentage of PMN infiltration, (C) aggregate lymphoid counts, (D) neutrophil score, and (E) edema score reveal that EQ504 significantly reduced measures of UC pathogenicity compared to the vehicle control. *p<0.05, **p<0.01, ****p<0.0001 vs. vehicle by One-Way ANOVA

Conclusions

- EQ504 is a more potent inducer of CYP1A1 activity compared to its parent compound, ITE.
- EQ504 is a stronger inducer of IL-10 and IL-22 gene expression compared to indirubin.
- **EQ504 effectively alleviates gut pathology in a mouse model of UC, suggesting its potential as a potent therapeutic for UC patients via modulation of immune and gut epithelial cell function.**

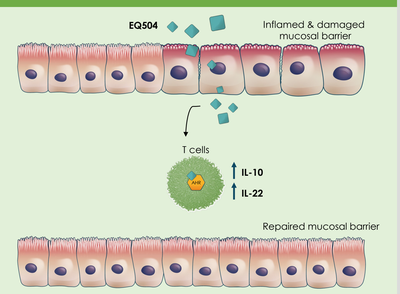


Figure 9. Schematic showing the impact of EQ504 on T cells & gut epithelial cells

Acknowledgments

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References

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Disclosures

This study was funded by Equillium, Inc.. The in vivo portion of the study was performed at Inotiv.

Richard Myers, A.J. Giovannone, Dalena Chu, Jeanette Ampudia, Valeria Marrocco, and Cherie Ng are currently employees and stockholders of Equillium. Mary Casis and Nisar Farhat are former Equillium employees who have contributed to the project. Stephen Connelly is currently an employee, stockholder, and officer of Equillium.

To request permission or to ask any questions about the poster, please contact dchu@equilliumbio.com.

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