EQ504, a Potent AhR Modulator, Promotes Epithelial Repair and Barrier Restoration Compared to Clinically Validated AhR Agonists.

POSTER 517

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Introduction

- The intestinal epithelium is a dynamic, single-cell barrier that maintains gut homeostasis by regulating permeability and coordinating mucosal defense. In ulcerative colitis (UC), epithelial barrier disruption leads to increased permeability, microbial translocation, and inflammation leading to tissue damage[1].
- The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor that integrates environmental and microbial signals to regulate intestinal immune homeostasis and epithelial barrier integrity[1].
- In intestinal epithelial cells, AhR activation induces CYP1A1 (indicates target engagement) and promotes barrier protection by upregulating IL22RA1, enhancing IL-22-mediated repair. It also regulates tight junction proteins (e.g., CLDN2) to control permeability, while suppressing NF-κB- and IFN-driven inflammatory mediators such as CXCL11 and IL-8[2].
- AhR agonists have demonstrated therapeutic potential in inflammatory disease. Tapinarof, a topical AhR modulators, is clinically effective in inflammatory skin diseases, supporting the therapeutic potential of AhR activation. Indirubin is a key bioactive AhR agonist component of *indigo naturalis*, which has demonstrated clinical efficacy in UC[3-4].
- In UC, AhR activation supports epithelial repair and barrier function, partly through IL-22[5]. However, how different AhR-targeting agents affect epithelial function is still not well understood.

➢ **EQ504 is a potent and selective AhR modulator designed to restore epithelial barrier function and promote mucosal homeostasis. Herein we demonstrate the activity of EQ504 in promoting healing of gut epithelial cells from inflammation and damage and compare its efficacy and potency to clinically validated AhR modulators.**

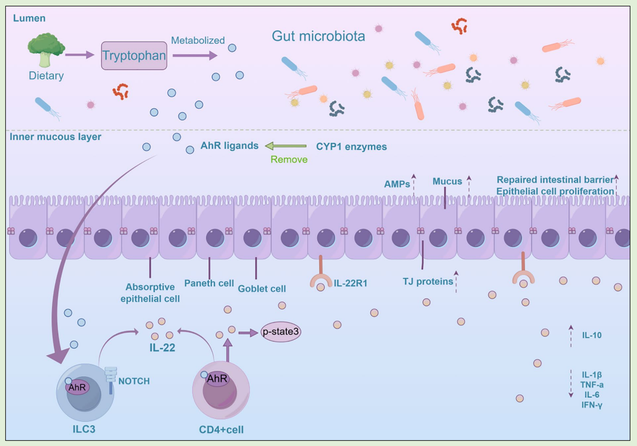


Figure 1. Mechanistic model of AhR signaling in intestinal homeostasis[6].

Methods

Gene Expression

- Caco2 cells were grown in 96- well plates for 7 days. Cells were then treated with a titration of the AhR modulators, EQ504 and Indirubin, or Vehicle. After 24h, cells were collected and processed for RNA isolation. Gene expression was analyzed by RT-qPCR.

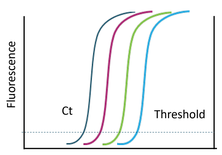


Figure 2. Reverse Transcription quantitative PCR (RT-qPCR)

HepG2-Lucia AhR Reporter Cells

- AhR activation was evaluated using HepG2 cells engineered with a Lucia luciferase reporter under control of AhR-responsive elements. Cells were plated and treated with compounds for 24 hours. Secreted luciferase activity was quantified from culture supernatants using a luminescent substrate and expressed relative to vehicle-treated controls

Epithelial Healing and Regeneration by In Vitro Scratch Assay

- T84 cells were plated on 24–well TC treated plates for 10 days until confluence. Once confluent, the cell monolayer was scratched using a 20µl tip, and the cells were treated with EQ504, Indirubin or Vehicle. The cells were imaged at day 3 and collected at day 6 for gene expression analysis by RT-qPCR.

Intestinal Epithelial Organoids

- Human intestinal epithelial organoids (Sigma) were grown as 3D dome cultures in organoid growth medium (OGM). For treatments, organoids were exposed to AhR agonists in differentiation medium (STEMCELL Technologies) for several days, and gene expression changes were assessed by qPCR.

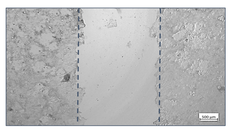
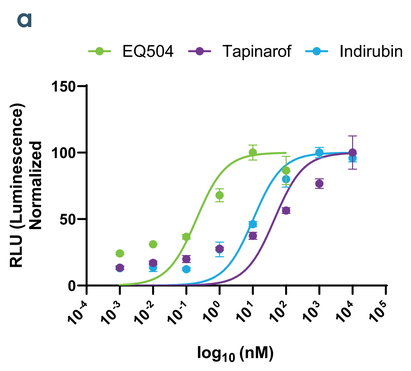


Figure 3. Scratch imaging and qPCR readout

Results

EQ504 is a Strong Inducer of CYP1A1 in Gut Epithelial Cells

- EQ504 potency was compared to Tapinarof and Indirubin using the HepG2-Lucia AhR reporter cell line and CYP1A1 gene expression analysis in the epithelial Caco2 cell line.
- Treatment of Caco2 cells with EQ504 or Indirubin led to upregulation of *CYP1A1*, confirming target engagement by these compounds.
- Comparison of EQ504 with Tapinarof and Indirubin showed that EQ504 is a potent molecule in engaging with the AhR target and inducing *CYP1A1* gene expression.



b

Compound	EC_{50} (nM)
EQ504	0.20
Tapinarof	45.21
Indirubin	10.39

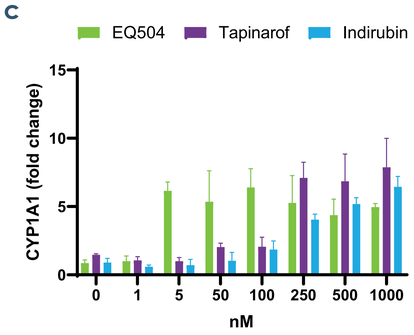


Figure 4. (a). Luminescence in HepG2-Lucia Reporter cells was measured following dose-dependent treatment of EQ504 and other AhR agonists. (b). Best-fit EC50 value of dose response curve. (c). Caco2 monolayer was treated with increasing concentrations of EQ504, Tapinarof, or Indirubin for 24h and CYP1A1 expression was quantified by Real-Time qPCR. DD Ct method was used to analyze the qPCR data with SDHA as reference gene.

EQ504 Promotes Scratch Repairs in T84 Gut Epithelial Cell Line

- Indirubin is a bioactive AhR modulator component of *indigo naturalis*, which is clinically validated in UC.
- A scratch assay was used to study the effect of AhR activation on gut repair, with EQ504 more potent than Indirubin in inducing recovery of the epithelial monolayer.
- RT-qPCR analysis during the repair period, showed increased expression of *IL22RA1* in cells treated with EQ504, and downregulation of *CLDN2*, a tight junction protein that creates cation channels and impacts the barrier function of the gut[2].

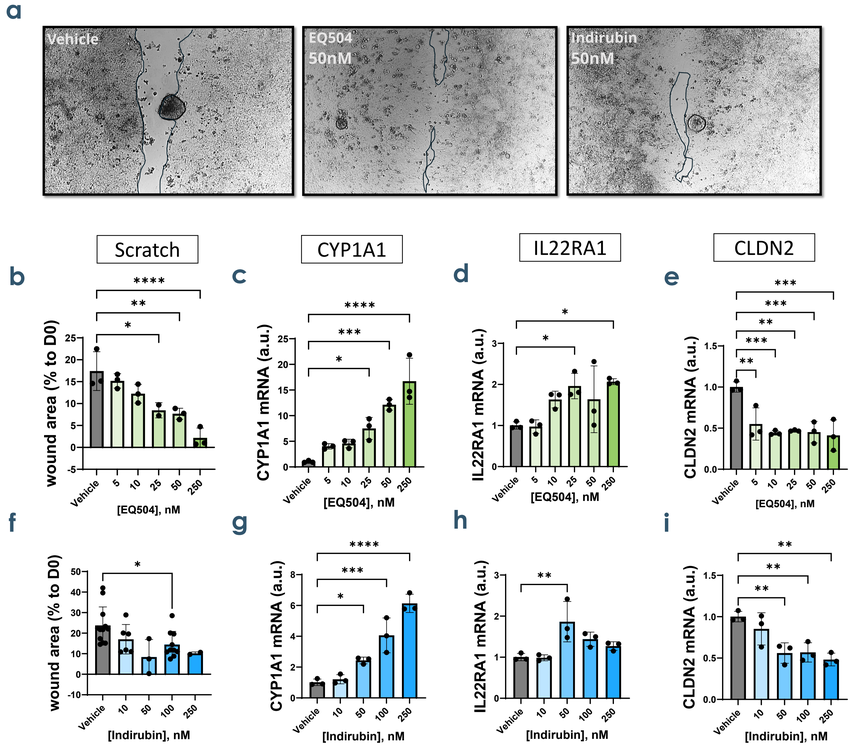


*Figure 5. (a) T84 monolayer scratched and imaged at day 0 and day 4. (b,f) Wound area quantification was analyzed using the Fiji "Wound Healing size tool master" macro at day 4 and normalized on the initial wound area (day0). ****p<0.0001, *p<0.05 vs D0 by Two-way ANOVA. c-e, g-i. CYP1A1 (c,g), IL22RA (d,h) and CLDN2 (e,i) gene expression in T84 cells scratched and treated with EQ504, Indirubin respectively or Vehicle for 6 days. DD Ct method was used to analyze the RT-qPCR data, and SDHA was used for normalization. ****p<0.0001 ,***p<0.001, **p<0.01, *p<0.05 vs Vehicle by One-way ANOVA.*

EQ504 Preserved Barrier Function Under Inflammatory Conditions

- EQ504 inhibits cytokine-induced expression of CXCL11, a downstream inflammatory marker in UC, in Caco-2 cells in vitro.
- CLDN2 and IL22RA expression were also modulated by EQ504 in Caco2 cells

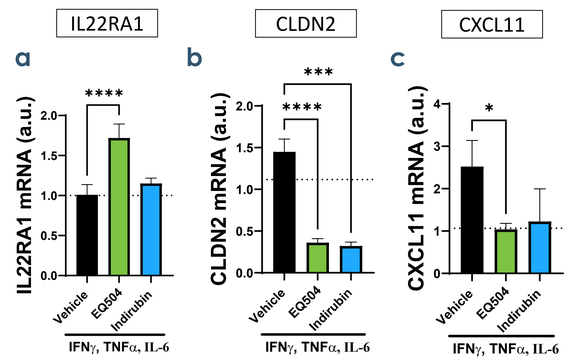


*Figure 6. CACO2 monolayer challenged with IFNγ 10ng/ml, TNFα 20 ng/ml and IL6 10ng/ml to assess recovery of leaky epithelial tight junctions CLDN2 (a), the anti-inflammatory cytokine receptor IL-22RA1 (b), and the pro-inflammatory chemokine CXCL11 (c) in the presence of 40nM EQ504 or Indirubin for 24h. Gene expression was analyzed by qPCR, DD Ct method was used to analyze the RT- qPCR data with SDHA as reference gene. and normalized on non-treated control (dashed line). ***p<0.001, **p<0.01, *p<0.05 vs Vehicle+ IFNγ, TNFa, IL6 by Two-way ANOVA.*

EQ504 Induces CYP1A1 Expression in Human Derived Tissues

- CYP1A1 expression was analyzed in Human Intestinal organoids derived from Normal or UC patients, treated in vitro for 3 or 10 days.

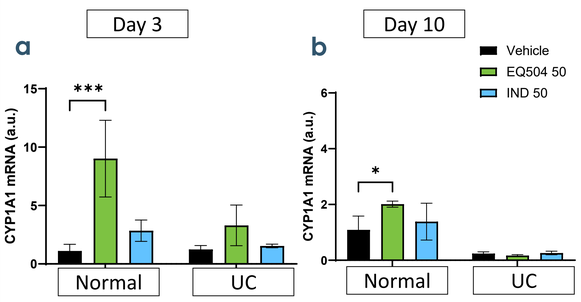


*Figure 7. Normal and Ulcerative Colitis (UC) Intestinal organoids were cultured using the 3D dome technique and treated with EQ504 or Indirubin for 3 (a) or 10 (b) days as indicated. DD Ct method was used to analyze the RT-qPCR data, and SDHA was used for normalization.***p<0.001, *p<0.05 vs Vehicle by One-way ANOVA.*

Dose-dependent Expression of CYP1A1 in Ex-vivo Treated Human Intestinal Explants, Confirming Target Engagement in Epithelial Cells by EQ504.

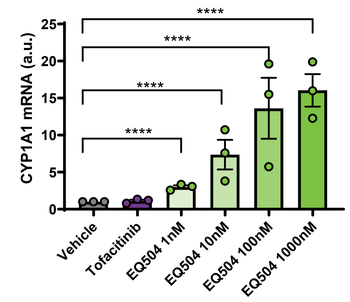
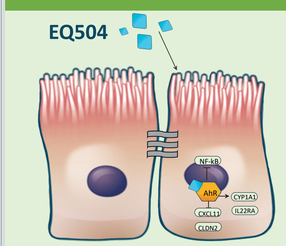


*Figure 8. Human-derived epithelial tissue explants were treated ex-vivo with EQ504 for 18h and CYP1A1 gene expression was analyzed by RT-qPCR. Tofacitinib treated at 1000nM as control. Scatter with bar plot graphs showing the effects of test formulations and controls on CYP1A1 gene expression within biopsies following culture. Data is presented as a fold change from vehicle control. Each dot represents a geometric mean donor result; each bar represents geometric mean ± geometric SD of all donors. 2way ANOVA with Dunnett's multiple comparison test was performed where ****p<0.0001.*

Conclusions



- **Consistent induction of CYP1A by EQ504 (compared to baseline) across multiple human epithelium models confirms strong AhR pathway engagement by EQ504 in Epithelial cells.**
- **EQ504 prevents upregulation of CLAUDIN2 (leaky gut claudin) and CXCL11 induced by stress like cytokines or injury.**
- **EQ504 promotes intestinal epithelial healing by upregulating IL22RA expression, increasing cellular responsiveness to the anti-inflammatory cytokine IL-22 produced by immune cells.**
- **EQ504 shows strong potency and clear AhR pathway engagement in epithelial cells, outperforming clinically validated AhR agonists and supporting its potential in inflammatory mucosal disease.**

Acknowledgments

We would like to thank the whole Equillium research team for continuous feedbacks and valuable input throughout the project. We especially acknowledge J. Ampudia and R.Q. Closser for generating the Lucia assay data.
Human organoids were licensed from Sigma-Aldrich, and the Human Explants studies were conducted in collaboration with Reprocell.

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Disclosures

This study was funded by Equillium, Inc. Mary Casis, Valeria Marrocco, and Stephen Connelly are currently employees of Equillium. Valeria Marrocco is a stockholder of Equillium. Stephen Connelly is currently an employee, stockholder, President and officer of Equillium.
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Presented at the American Association of Immunology, Boston, 2026