THE JAK INHIBITOR-TOFACITINIB INHIBITS PRO-INFLAMMATORY RESPONSES IN THE HUMAN INTESTINAL MUCOSA EX-VIVO

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Introduction: Tofacitinib, an orally administered pan-Janus kinase (JAK) inhibitor modulating the JAK-STAT (signal transducers and activators of transcription) signaling pathway, is used for the treatment of patients with ulcerative colitis (UC). Tofacitinib effects in the intestinal mucosa are still elusive. Aims: To identify tofacitinib effects in human intestinal mucosa and to evaluate patient’s response ex vivo.

Methods: Mucosal explants were obtained from patients with UC, Crohn’s disease (CD) and normal controls. Explants were treated with tofacitinib and phosphorylated STAT (p-STAT) levels were assessed. Lamina propria lymphocytes (LPL) were isolated and stimulated with anti-CD3/CD28 beads for 3 days. IFN-γ, TNF-α, IL-10 and IL-17A concentrations in supernatant of LPLs were analyzed by CBA. Statistical significance was calculated using paired t-test (p<0.05; n=14).

Figure 1. p-STAT1/3 are expressed in the lamina propria and expression decreases in response to tofacitinib. (A) Sections of normal colonic mucosal tissue were stained with anti-p-STAT1/3, anti-EpCAM and DAPI. Nuclear localization of p-STAT3, identified by co-staining with DAPI (arrows), is detected in the lamina propria (negative for the epithelial marker EpCAM). Mucosal explants from normal colonic tissue of a healthy control (B) and inflamed ileum of a patient with CD (C) were treated with 100µM tofacitinib. Sections were stained with anti-p-STAT1/3, anti-EpCAM and DAPI. Magnification x20.

Figure 2. Response to tofacitinib treatment ex-vivo is patient dependent. Mucosal explants from normal control, patients with CD or UC were treated with 100µM of tofacitinib. (A) p-STAT1/3 levels were assessed by Western blot of colonic mucosal lysates using anti-p-STAT1/3, anti-β-actin, anti-p-STAT3 and anti-p-STAT6 and compared to β-actin as a loading control. Densitometry analysis was performed using ImageJ. The results are presented as mean ± SD.

Figure 3. Tofacitinib inhibits cytokines production by LPL. LPLs isolated from normal colon of healthy control and inflamed colon of patient with UC and were activated in the presence or absence of tofacitinib with anti-CD3/CD28 beads for 3 days. IFN-γ, TNF-α, IL-10 and IL-17A concentrations in supernatant of LPLs were analyzed by CBA. Statistical significance was calculated using paired t-test (p<0.0001; n=14).

Figure 4. Tofacitinib prevented IL-13-induced decrease in TEER and increase Claudin2 levels. Polarized T84 cells were stimulated with IL-13 +/- tofacitinib. (A) Cells permeability was assessed by TEER measurements, expressed as a percentage of initial values. (B) Claudin2 mRNA levels was determined by quantitative real-time PCR. p-STAT1/3 and Claudin2 protein expression were assessed by Western Blot (C) and immunofluorescence (D).

Figure 5. Tofacitinib inhibits IL-13-induced increase in p-STAT6 and CLDN2 expression in human colonic organoids. Human colonic organoids generated from healthy controls were stimulated with IL-13 +/- tofacitinib. (A, B) p-STAT6 and CLDN2 protein expression were assessed by Western blot of colonic organoids lysates and compared to β-actin as a loading control (respectively). (C, D) Tofacitinib-treated colonic organoids were stained with anti-EpCAM, DAPI and anti-p-STAT6 or anti-CLDN2 (respectively). Visualisation by confocal microscopy. Original magnification x20.

Conclusions:
• Ex-vivo mucosal response to tofacitinib treatment is individual
• Tofacitinib ameliorates epithelial barrier disruption induced by IL-13
• Functional implications of JAK inhibition in the intestinal mucosa ex-vivo include inhibition of cytokines production by intestinal lymphocytes
• Mucosal explants from patients with CD respond to tofacitinib treatment. Thus, tofacitinib might be beneficial for CD patients.

Significance:
• Examination of drug effects in the ex-vivo intestinal mucosa may be evaluated as a tool for assessment of treatment effectiveness.

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