BACKGROUND

• The liver has both an arterial and a venous blood supply, with the greatest part of hepatic blood flow coming from the gut via the portal vein. The liver is therefore exposed to potentially harmful substances derived from the gut, including translocated bacteria, ethanol produced from microbiota, trimethylamine, and endotoxins.

• Altered gut barrier function allows the passage of bacteria-derived products into systemic circulation, causing a systemic inflammatory state leading to the development of comorbidities associated with obesity-like type 2 diabetes and non-alcoholic steatohepatitis (NASH; Figure 1).

Figure 1. Gut barrier dysfunction is core to the liver-gut axis, leading to metabolic disturbances

• Adherens junctions (E-cadherin) and tight junction proteins (zonula occludens, ZO-1) seal the junctions between intestinal epithelial cells and have a vital role in preventing translocation of harmful substances from the gut into the portal system to prevent inflammation.

Figure 2. Proprietary hydrogels and how they differentiate to functional fibers

- Superabsorbent hydrogels representing the GELESIS platform technology are orally administered and synthesized by cross-linking modified cellulose. Gelesis100, the first in this platform technology, demonstrated weight loss in patients with obesity and improvement in insulin sensitivity (Figure 2).
Only superabsorbent hydrogels made from food-grade building blocks (modified cellulose and citric acid)
- Biocompatible and biodegradable
- Able to absorb amount of water \(\sim 100\times\) its dry weight (superabsorbent)
- In fully hydrated state, \(\sim 1\text{–}2\) mm diameter with elasticity/firmness similar to leafy vegetables like lettuce
- Particles don’t cluster and maintain their 3D structure in upper GI tract; however, partially degrade in the large intestine

Figure 3. Gelesis hydrogel’s 3D structure generates orders of magnitude greater elastic response vs. functional fibers

\[ G' = \text{modulus of elasticity; } SGF = \text{simulated gastric fluid; } SIF = \text{simulated intestinal fluid; } SCF = \text{simulated colonic fluid.} \]

Previous pre-clinical work demonstrated that the 3D structure of Gel-B provided the mechanical (elastic modulus) and physical properties required to optimize human intestinal tissue proliferation and differentiation in ex vivo organ culture model

**OBJECTIVES**

We hypothesized that Gel-B with specific mechanical properties, designed to restore intestinal barrier function, would prevent translocation of unwanted substances into the blood after severe intestinal barrier injury.

**EXPERIMENTAL DESIGN**

**COLITIS INDUCTION:**

Dextran sulfate sodium (DSS) — mice were given 3% DSS (MP Biomedicals, Solon, OH) in their drinking water from Day 0–5 (Figure 4).

**IN VIVO STUDY:**

Male C57/Bl6 mice were purchased from Charles River Labs and were evaluated daily for survival, body weight, evidence of bloody stool, and diarrhea. Animals were given Vehicle or Gel-B (0.5%–4.0%) in their food from Days 5–19. An additional active control group was dosed via intraperitoneal injection (IP) every third day (Q3D) on Days 6–18 with anti-p40 mAb (Bioxcell, West Lebanon, NH).

**STUDY ENDPOINTS:**

On Day 19, all animals were fasted for 4 hours prior to FITC-dextran (40 kDa) dosing (25 mg/mL in PBS, Volume = 20 mL/kg; orally administrated). Exactly 3 hours post-FITC-dextran, animals were sacrificed and had blood collected for preparation of serum. Serum samples were assessed for relative fluorescent intensity using a fluorescent plate reader (Molecular Devices, San Jose, CA).

**TISSUE COLLECTION/HISTOLOGY:**

Mice were sacrificed with CO\(_2\) inhalation and colons were removed and trimmed to 5 cm in length. The most distal and proximal 2.0 cm sections of colon were each placed in Carnoy fixative for 2 hours; they were then switched to 100% ethanol for at least 72 hours prior to being paraffin embedded, sectioned at 5 \(\mu\)m, and stained for hematoxylin and eosin stain, or by routine immunohistochemical (IHC) or immunofluorescence (IF) protocols for E-cadherin and ZO-1, respectively.

**HISTOPATHOLOGY:**

All histology, IHC, and IF slides were evaluated in a blinded fashion by a board-certified veterinary pathologist. Incidence of epithelial barrier breaks observed with E-cadherin IHC were also recorded.

**STATISTICAL ANALYSIS:**

Data are presented as mean ± standard error of the mean (SEM). Semi-quantitative severity scores were analyzed by non-parametric ANOVA (Kruskal-Wallis H test with post hoc test) or t test (Mann-Whitney U test). Two-tailed tests were utilized, and significance was set at \( P \leq 0.05 \) for all tests.
RESULTS

SEVERITY OF INTESTINAL INJURY INDUCED BY DSS

- Weight loss, calculated as a percentage of the starting weight on Day 0, was observed in all groups to varying degrees beginning as early as Day 1 and peaking at around Day 11.
- Only the animals in the naive control group (Group 1) did not display dramatic reductions in weight.
- The Vehicle control group reached peak weight loss on Day 10 (-21.79%), then slowly recovered weight up until sacrifice on Day 19 (-5.03%).
- Treatment with anti-p40 at 10 mg/kg (Group 3) decreased peak weight loss compared with Vehicle control; AUC analysis revealed that the reduction in weight loss was statistically significant for animals dosed with anti-p40 (P <0.05).
- Treatment with Gel-B at 4.0, 2.0, 1.0, and 0.5% concentrations revealed a dose-dependent improvement in weight loss, with higher concentration of Gel-B resulting in attenuation of weight loss more so than lower doses, but no significant difference with Vehicle.

FITC-DEXTRAN FLUORESCENCE: MARKER OF GUT BARRIER FUNCTION

- Blood samples were evaluated for FITC-dextran fluorescence from animals in all groups and the mean blood fluorescence levels are shown in Figure 5.
- Vehicle control animals had very high fluorescent dextran mean blood levels (indicative of significant loss of intestinal barrier function) compared with naive control animals.
- The fluorescence levels of animals dosed with the active control group, anti-p40, were modestly lower than Vehicles.
- An obvious dose-dependent effect from treatment with increasing concentrations of Gel-B showed marked reduction in mean blood fluorescence compared with vehicles (Figure 5).

Fluorescent-labeled dextrans offer a simple tool for the evaluation of gut barrier function. It has been shown in E. coli that the majority of proteins are in the mass range of 25–120 kDa, which suggests that the most biologically significant proteins are in the range we selected for the dextran.

Figure 5. Gel-B prevents the spilling of small molecules into the circulation

The level of FITC-dextran in the serum of mice receiving DSS and Vehicle (mean ± SEM, 7.1 ± 4.1) was 3.4 times higher than in the naïve mice (2.1 ± 1.1), demonstrating a significant defect in the gut barrier function.

In turn, the mice assigned to Gel-B 1.0% to 4.0% had meaningful reduction in FITC-dextran serum levels compared with Vehicle-treated mice. The highest dose (4.0%) of Gel-B prevented spilling of dextran into the circulation, with serum levels numerically lower than the naïve group with an intact gut barrier (1.4 ± 0.4 compared with 2.1 ± 1.1, respectively).

Figure 6. Tight junctions regulating the seal between intestinal epithelial cells

E-CADHERIN IHC

The incidence of epithelial barrier breaks, assessed with E-cadherin IHC, found lower incidence of breaks in distal colon samples from anti-p40 (Group 3) and Gel-B 4.0% (Group 4)–treated mice compared with Vehicle-treated mice (Figure 6).

A reduction in epithelial barrier breaks in distal colon samples was observed in the highest dose of Gel-B (0.58 ± 0.15) compared with Vehicle-treated animals (0.86 ± 0.1)

ZONULIN OCCLUDENS-1 (ZO-1)

To verify the involvement of epithelial tight junctions, the expression of ZO-1 was assessed in C57BL/6J mice (5 per group) fed chow for 4 weeks, with or without Gel-B (Figure 7). Healthy mice treated with Gel-B (8%) showed increased expression of ZO-1, essential to tight junction assembly between epithelial cells.

Figure 7. Gel-B increased ZO-1 expression in intestinal epithelium cells
CONCLUSIONS

The study demonstrated in a model of severe intestinal injury that:

- Treatment with Gel-B reduced the translocation of 40 kDa dextran molecules into the circulation in a dose-dependent fashion
- Treatment with high-dose Gel-B restored the function of the gut barrier similar to the naïve group
- Potential mechanisms for the improved gut barrier function might include the regulation of tight junctions such as ZO-1 and E-Cadherin

More studies are ongoing in pre-clinical NASH models to better understand the mechanisms how Gel-B treatment restores gut barrier function and potentially prevents liver damage.

REFERENCES


DISCLOSURES

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