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Gelesis hydrogel reverses high fat diet-induced intestinal alterations and slows progression of hepatic steatosis in DIO mice

A. Silvestri, MSc[1], E. Chiquette, PharmD[2], A. Sannino, PhD[2], C. Demitri, PhD[2], M. Vitale, PhD[1], B. Jones, PhD[2], M. Rescigno, PhD[1]

[1]**Humanitas Clinical and Research Center – IRCCS**, via Manzoni 56, 20089 Rozzano (Mi) – Italy, [2]**GELESIS Inc.,** 501 Boylston, Boston MA - USA

BACKGROUND

- Therapies exploiting the gut liver axis may offer a unique treatment option for metabolic liver disorders.
- Gel-B, a novel orally administered hydrogel platform using a citric acid crosslinked, modified cellulose, was developed by Gelesis to restore gut barrier function.
- Prior animal experiments explored the protective effects of Gel-B against the development of non-alcoholic fatty liver disease (NAFLD) when co-administered with a high fat diet (HFD)(Silvestri *et al.* 2019).

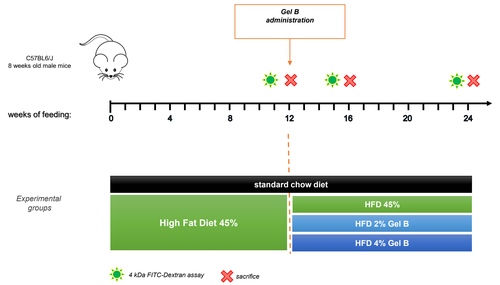
OBJECTIVE

- This study examines the therapeutic effects of Gel-B administration in diet induced obesity (DIO) mice with established NAFLD prior to treatment.

EXPERIMENTAL DESIGN

- Male C57 BL/6J *wild type* mice were fed HFD (45% lard) for 12 weeks (Figure 1).
- From week 12 to 24, mice were treated with either HFD alone (n=20), HFD+Gel-B 2% (n=18) or 4% (n=18).
- A control group (n=21) remained on chow alone.
- Body weight was monitored over time, epididymal adipose tissue (EAT) weight was recorded at 4 and 12 weeks treatment.
- Intestinal barrier integrity was evaluated using a FITC-dextran permeability assay and expression of zonula occludens-1 (ZO-1).
- Liver triglyceride (TG) accumulation was graded using a semi-quantitative scoring system on Oil red O-stained samples.

Figure 1. Study Design



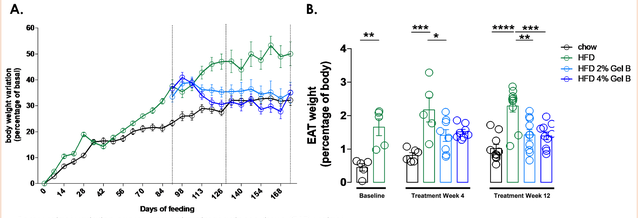
Experimental Design
C57 BL/6J *wild type* mice consumed a high fat diet (HFD) containing 45% lard for 12 weeks prior to treatment. A separate control group consumed standard chow diet for the entire study. Treatment groups included a HFD control, Gel-B 2% and Gel-B 4%. Animals were sacrificed at baseline, week 4 of treatment, and week 12 of treatment.

RESULTS

BODY WEIGHT AND ADIPOSE MORPHOLOGY

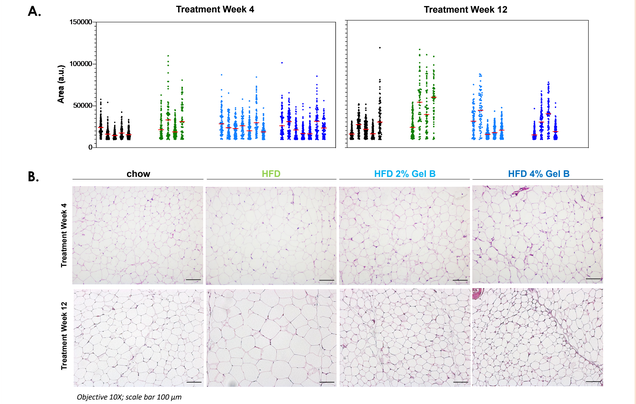
- High-fat diet feeding for 12 weeks induced a higher body weight gain (p=0.03) compared to chow diet fed animals.
- After 12 weeks of Gel-B 2 or 4% treatment, body weight was significantly reduced compared to mice on HFD alone (p=0.02 for 2% Gel-B and p<0.0001 for 4% Gel-B).
- HFD induced greater epididymal adipose tissue (EAT) accumulation than control diet (p<0.01), and treatment with 2 or 4% Gel-B significantly reduced EAT accumulation after 12 weeks (p=0.02 for 2% and p<0.0001 for 4% gel; Figure 2b).
- Adipocyte hypertrophy, induced by HFD, was significantly reduced by 2 and 4 % Gel B in 12 weeks treatment (Figure 3; p<0.0001 for both 2% and 4% Gel-B).

Figure 2. Body weight and EAT decreases with Gel-B treatment.



Body weight variation curves and epidydimal adipose tissue (EAT) weight.
a. Body weight curves expressed in percentage of basal. Both 2 and 4% Gel-B treatment resulted in significantly reduced body weight (p=0.02 for 2%; p<0.0001 for 4%; two-way ANOVA, Tukey's multiple comparisons test); **b.** EAT weight displayed in percentage of body weight (* p<0.5; ** p<0.01; ***p<0.001 one-way ANOVA Tukey's multiple comparisons test).

Figure 3. Reduction in adipocyte hypertrophy observed with Gel-B.

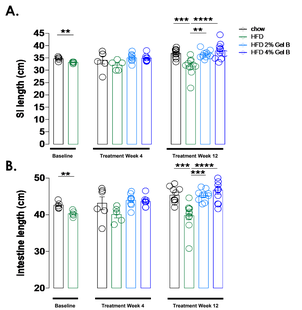


Adipocyte area distribution from epididymal adipose tissue.
a. Epidydimal adipocyte area distribution at 4 and 12 weeks treatment (p<0.0001 chow vs. HFD, HFD vs. 2%, and HFD vs. 4% at week 12; one-way ANOVA, Tukey's multiple comparisons test). **b.** Representative hematoxylin and Eosin stain images (scale bar = 100 µm).

INTESTINAL MORPHOLOGY AND BARRIER FUNCTION

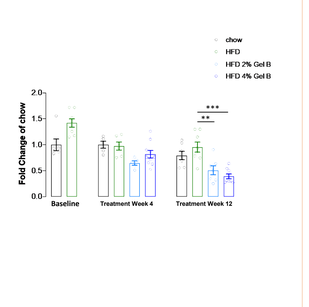
- High-fat feeding for 24 weeks induced intestinal atrophy (Figure 4; p=0.0037), increased intestinal permeability (Figure 5; p=0.008) and reduced ZO-1 expression (Figure 6; p=0.0084) compared to controls.
- Gel-B treatment prevented intestinal atrophy induced by high fat diet, mostly driven by preservation of the small intestine length (Gel-B 2% p=0.0017; 4% p<0.0001 by 12 weeks).
- Intestinal permeability, as measured by the amount of serum FITC-dextran (4 kDa) 4 hours after oral administration, was reduced in Gel-B groups compared to HFD at 12 weeks (Gel-B 2% p=0.0025; 4% p<0.0001).
- An upregulation of intestinal ZO-1 expression was measured in both Gel-B groups at 4 weeks (2% p=0.0052; 4% p=0.0003), though not significant at 12 weeks (2% p=0.1385; 4% p=0.0803).

Figure 4. Intestinal length.



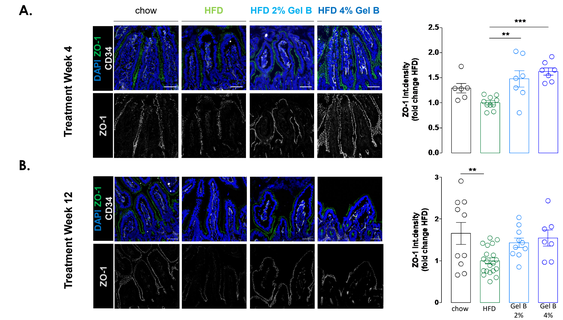
Intestinal length at 4 and 12 weeks treatment.
a. Small intestine length; **b.** total intestine length (** p<0.01, *** p<0.001, ****p<0.0001; t-test and one-way ANOVA Tukey's multiple comparisons test)

Figure 5. FITC-Dextran assay.



Intestinal permeability as measured by FITC-Dextran assay.
a. Permeability pre-treatment, after 4 and 8 weeks treatment expressed as fold change of chow and **b.** µg/ml (**p=0.0025; ***p<0.0001; t-test and one-way ANOVA Tukey's multiple comparisons test)

Figure 6. Zonula occludens-1 expression.

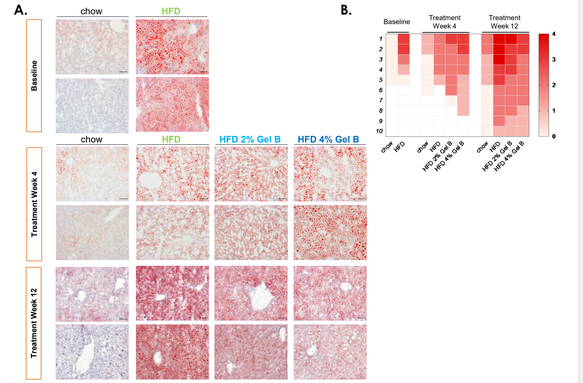


Gel-B restored intestinal epithelial tight junction ZO-1 expression.
a. Ileum tissue sections were stained with ZO-1 (green), CD34 (grey) and DAPI (blue) at 4 and 12 weeks treatment. **b.** ZO-1 intensity expressed in fold change of HFD after 4 and 12 weeks (**p<0.01, ***p<0.001; one way ANOVA with Tukey's multiple comparisons test).

HEPATIC STEATOSIS

- High-fat diet consumption for 12 weeks resulted in increased hepatic triglyceride (TG) accumulation compared to animals on chow alone (Figure 7).
- Treatment with Gel-B hampered TG accumulation at 12 weeks in a dose dependent manner:
  - 5/10 HFD mice had ≥ grade 3 accumulation.
  - 4/10 Gel-B 2% and 2/10 Gel-B 4% mice had ≥ grade 3 accumulation.

Figure 7. Hepatic triglyceride accumulation.



Gel-B effect on hepatic triglyceride accumulation.
a. Oil Red O representative stains before Gel-B administration, at 4 and 12 weeks. **b.** Stains were scored from 0 (no triglyceride – beige) to 4 (high accumulation of triglyceride – red). Each shaded square represents one animal.

CONCLUSIONS

- This study aimed to describe the effects of Gel-B treatment on mice with DIO and NAFLD induced via consumption of HFD (45% lard) for 12 weeks prior to treatment.
- After 12 weeks of Gel-B treatment:
  - Body weight and EAT decreased (Fig. 2), and associated adiposopathy was reduced, as evidenced by reversal of high fat induced adipocyte hypertrophy (Fig. 3)
  - Small intestine length was maintained when compared to the shorter length associated with HFD (Fig. 4), indicating protection from intestinal atrophy caused by chronic exposure to HFD.
  - Intestinal tight junctions exhibited restored integrity despite continued HFD. Specifically, HFD-induced intestinal permeability was reduced by Gel-B (Fig. 5), and a concomitant dose-dependent upregulation of the tight junction protein ZO-1 was observed (Fig. 6).
  - Hepatic triglyceride accumulation was attenuated in a dose-dependent manner.
- Together, these data support the hypothesis that Gel-B may protect against the deleterious metabolic effects of HFD. This protection most likely occurs via the small intestine, where HFD-associated pathologic permeability is reduced, ultimately resulting in the attenuation of hepatic triglyceride accumulation.
- Additional clinical trials in humans will be required to confirm these results.

REFERENCE/DISCLOSURES

- Silvestri, A. *et al.* 2019. LBP-33-Gelesis superabsorbent hydrogel prevents hepatic steatosis in a high fat diet-induced NAFLD pre-clinical model. Journal of Hepatology 70:e157-158.
- Disclosures: EC, AS, CD, BJ are employees of Gelesis. AS, MV, and MR have no disclosures to declare.

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