Butyric acid and derivatives: *In vitro* effects on barrier integrity of porcine intestinal epithelial cells quantified by transepithelial electrical resistance

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Background: IPEC-J2

- Intestinal porcine epithelial cells (IPEC)-J2
  - Immortal cell line
  - Derived from unsuckled neonate piglet jejunal intestine (Berschneider, 1989)
Barrier Integrity of the Intestine

- Intestinal epithelial cells form a monolayer
- Barrier function is semipermeable and vital to nutrient absorption, water retention, and preventing pathogen invasion

Gokulan et al., 2017
Intestinal epithelial cell monolayers *in vitro*

Differentiation:
Expression of tight junction proteins and polarized across apical and basal sides

Lea, 2015.
Butyric acid and derivatives

- Butyrate can be used as an energy source for enterocytes.
- *In vitro*
  - Butyrate and derivatives increase TEER in human and porcine intestinal cells (Peng et al., 2009; Yan and Ajuwon, 2017).
- *In vivo*
  - Dietary butyrate sodium butyrate improves pig growth performance (Manzanilla et al., 2006; Lu et al., 2008) and tributyrin improved intestinal morphology and barrier function (Hou et al., 2014; Wang et al., 2019).
Objective:

Determine the effects of butyric acid, sodium butyrate, monobutyrin, and tributyrin on transepithelial electrical resistance (TEER) of porcine epithelial cells *in vitro*.
a) Two electrodes—one on apical and one on basal side of monolayer—measure electrical resistance across monolayer of cells. b) Voltohmeter: Millicell ERS-2 model

https://ebrary.net/24380/health/measurement_transepithelial_electrical_resistance_teer
Materials and methods

• Culture conditions
  • IPEC-J2 seeded in 12-well transwell plates (Corning) at $5 \times 10^5$ cells/mL in DMEM + 5% fetal bovine serum, and 1% penicillin-streptomycin 24 h 37°C 5% CO$_2$
  • Cells cultured for 4-5 days for monolayer formation (TEER $\sim 1000$ Ω) then treated in duplicate
  • TEER measured at 24, 48, and 72 h post-treatment

• Completely Randomized Block Design
  • Dose as fixed effect, plate as random effect
  • Doses: Butyric acid, tributyrin—0, 0.5, 1, 2, 4 mM; Monobutyrin, sodium butyrate—0, 1, 2, 4, 8 mM (determined by MTT assay).
Calculations

Resistance of monolayer

\[ R_{\text{monolayer}} [\Omega] = R_{\text{sample}} - R_{\text{blank}} \]

Resistance is inversely proportional to the area of the membrane

\[ R_{\text{reported}} [\Omega \text{cm}^2] = R_{\text{monolayer}} [\Omega] \times \text{monolayer area} [\text{cm}^2] \]

(Srinivasan et al., 2015)
TEER of IPEC-J2 treated with butyric acid

TEER (Ω cm²)

Time post-treatment (hours)

0 mM 0.5 mM 1 mM 2 mM 4 mM
TEER of IPEC-J2 treated with sodium butyrate

TEER (Resistance) of IPEC-J2 cells treated with different concentrations of sodium butyrate (0 mM, 1 mM, 2 mM, 4 mM, 8 mM) over time post-treatment (0, 24, 48, 72 hours).

The graph shows the mean TEER values with error bars indicating standard deviations. Different letters above the bars indicate significant differences (a, b, c) according to statistical analysis.
TEER of IPEC-J2 treated with monobutyrin

TEER (Ωcm²)

Time post-treatment (hours)

0 mM 1 mM 2 mM 4 mM 8 mM

0 24 48 72
TEER of IPEC-J2 treated with tributyrin

- 0 mM
- 0.5 mM
- 1 mM
- 2 mM
- 4 mM

**Time post-treatment (hours):**
- 0
- 24
- 48
- 72

**TEER (Ω cm^2):**
- 0
- 1000
- 2000
- 3000
- 4000
- 5000
- 6000
- 7000
- 8000

Note: Significant differences indicated by different letters above the bars.
Conclusions

- Butyric acid and monobutyrin increased TEER at all timepoints post-treatment.
- Sodium butyrate increased TEER at 24 and 48 h post-treatment, but highest doses decreased TEER at 72 h post-treatment; 8 mM may be too high.
- Tributyrin tended to increase TEER at highest dose after 72 h with treatment.
  - not cleaved to monobutyrin/butyric acid without presence of lipase, may not affect cells *in vitro*
- **Butyric acid, monobutyrin, and sodium butyrate increase TEER in porcine epithelial cell monolayers in vitro**
References

https://ebrary.net/24380/health/measurement_transepithelial_electrical_resistance_teer


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Acknowledgments

- Miss Monika Hejna
- Dr. Yanhong Liu