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Enteric glial–mediated enhancement of intestinal barrier integrity is compromised by morphine

Brent D. Bauman, MD,[a] *Jingjing Meng, PhD,*[a] *Lei Zhang, PhD,*[a] *Amanda Louiselle, BS,*[b] *Eugene Zheng, BA,*[b] *Santanu Banerjee, PhD,*[a] *Sabita Roy, PhD,*[a] *and Bradley J. Segura, MD, PhD*[c,*]

[a] *Department of Surgery, University of Minnesota, Minneapolis, Minnesota*
[b] *University of Minnesota Medical School, Minneapolis, Minnesota*
[c] *Department of Surgery and Pediatrics, University of Minnesota, Minneapolis, Minnesota*

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ABSTRACT

Background: The opioid epidemic is a growing concern, and emerging evidence suggests that morphine use may be associated with sepsis. Enteric glial cells (EGCs) are the most numerous cell type in the enteric nervous system and regulate gastrointestinal function through the production of trophic factors, including glial-derived neurotrophic factor (GDNF). We sought to determine the effect of morphine on enteric glia and hypothesized that morphine contributes to EGC dysfunction and increased gut permeability.

Materials and methods: Rat intestinal epithelial cells (IECs) and EGC lines were purchased from ATCC. Immunocytochemistry was used to evaluate the impact of EGCs on IEC barrier proteins and detect the μ-opioid receptor. Co-culture assays were used to determine the effect of EGCs, GDNF, and morphine on barrier integrity. Quantitative polymerase chain reaction and western blotting were performed to determine the impact of morphine in GDNF production. Transepithelial resistance of IEC-6 cell monolayers was measured in the presence of EGC-conditioned media (EGC-CM) and morphine treated EGC-CM using electrical cell impedance sensing.

Results: EGC-CM enhanced tight junction organization in IECs. IEC barrier integrity was enhanced when co-cultured with unstimulated EGCs or with GDNF alone; this barrier protective effect was lost with morphine-treated EGCs. GDNF RNA and protein expression were decreased by morphine treatment. Transepithelial resistance was decreased in IEC confluent monolayers when exposed to morphine-treated EGC-CM compared with control.

Conclusions: Morphine compromises intestinal epithelial cell barrier function through a mechanism which appears to involve GDNF. Further studies are warranted to delineate the role of enteric glial cell function in opioid signaling and sepsis.

* *Corresponding author*. Department of Surgery and Pediatrics, University of Minnesota, 2450 Riverside Ave S, East Building MB505, Minneapolis, MN 55454. Tel.: +1 612 626 4214; fax: +1 612 624 6969.
E-mail address: bjsegura@umn.edu (B.J. Segura).
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Introduction

The opioid epidemic in the United States has been a growing source of concern for American physicians. According to recently published data from the Centers for Disease Control, 259 million opioid prescriptions were written in 2012 for chronic pain.[1] This number has increased from 2008, when 205 million opioid prescriptions were written which represents opioids given for all sources of pain.[2] Furthermore, in the emergency department, opioid prescriptions continue to increase and in 2011 numbered at 184.1 prescriptions per 100,000 compared with 82.5 per 100,000 in 2004.[3] Concomitantly to the increase in opioid prescriptions, there has been an alarming increase in opioid-related complications including sepsis and death. For example, in 2013, prescription opioids contributed to more deaths than all other illicit drugs combined (>16,200 *versus* 14,775).[3]

The common side effects of opioids have been well described and include nausea, vomiting, constipation, somnolence, and respiratory depression.[4] There is now a growing interest in elucidating the effects of opioid use on intestinal barrier function. Specifically, recent studies have demonstrated an association between opioid use and sepsis through animal model experiments demonstrating increased gut bacterial translocation in morphine-treated mice.[5,6]

Enteric glial cells (EGCs) are the most numerous cell population within the enteric nervous system and are identified by their staining positivity for GFAP.[7] Previous studies have identified that EGCs play a role in the integrity of the intestinal barrier function through the release of various trophic factors including glial-derived neurotrophic factor (GDNF).[7-10] It has been previously shown that in the murine models that have EGCs selectively abolished, there is decreased intestinal epithelial integrity leading to intestinal hemorrhage and necrosis.[8,11,12] Less is known, however, about the impact of opioids on enteric glial–mediated enhancement of intestinal barrier function. The purpose of our experiments was to determine the impact of morphine stimulation on EGC cell function. We hypothesized that EGC-mediated intestinal barrier enhancement would be lost when stimulated with morphine.

Methods

Cell culture

Disposable culture ware was purchased from Thermo Fisher Scientific (Waltham, MA). Intestinal epithelial cell (IEC)-6 and EGC/PK060399egrf cell lines were purchased from American Type Culture Collection (Manassas, VA) and the cells were cultured as the supplier recommends. IEC-6 cells are rat small intestinal epithelial cells. EGCs are rat enteric glial cells. Culture media were purchased from Gibco (Waltham, MA) and Sigma-Aldrich (St. Louis, MO). Both cell lines were cultured in Dulbecco modified eagle medium supplemented by penicillin/streptomycin (100μ/mL, Gibco) and 10% fetal bovine serum (FBS) (Gibco). IEC-6 cells were cultured with 0.1U/mL bovine insulin. Cells were cultured in a NAPCO Series 8000 DH incubator (Thermo Fisher Scientific) at 37.0°C at 5% CO_2.

Immunocytochemistry

μ-opioid receptor staining

EGCs were plated in eight-well chamber slides (Thermo Fisher Scientific). Cells were grown ×24 h and fixed with 4% paraformaldehyde. Cells were blocked with 5% FBS, washed with ice cold PBS and permeabilized with 0.1% Triton-x, and incubated overnight with primary antirabbit anti-μ-opioid receptor antibody (Abcam 10275, Cambridge, MA). Cells were washed three times with PBS and stained with goat antirabbit fluorescein isothiocyanate (FITC)–conjugated secondary antibody for 1 h (Jackson ImmunoResearch, West Grove, PA, #111-095-003). Cells were stained with DAPI-prolong gold antifade (Invitrogen) and analyzed for fluorescent antibody receptor expression.

IEC-6 tight junction protein staining

IEC-6 cells (ATCC, Manassas, VA) were cultured as recommended by the supplier and plated in the presence of normal medium or the supernatant of rat enteric glial cells for 72 h. Cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. After washing in PBS and blocking of nonspecific binding sites with 5% bovine serum albumin, tissues were incubated with polyclonal rabbit anti-occludin (used at 5 mg/mL, Invitrogen) in PBS with 5% bovine serum albumin for 120 min at room temperature. After washing, cells were incubated with rhodamine phalloidin (Invitrogen) and DyLight 488-conjugated AffiniPure Donkey antirabbit IgG (0.075 mg/mL, Jackson Lab, West Grove, PA) for 60 min. Cells were then washed and mounted under coverslips using ProLong Gold antifade reagent with DAPI (blue) (Invitrogen).

Transwell co-culture experiments

IECs (rat, ATCC IEC-6, Manassas, VA) were plated at a concentration of 2×10^5 cells/well in the apical chamber of the transwell plate (0.4 μm pore size; Corning, Kennebunk, ME). In the basal chamber of the transwell plate, EGCs (rat, ATCC EGC/PK060399egfr, Manassas, VA) were plated at a concentration of 8×10^4 cells/well. When EGCs were not plated into the well, EGC-conditioned media (EGC-CM, media from untreated or morphine-treated confluent EGCs plated in a separate six-well plate) was added to the well. If no EGCs or EGC-CM was present in the well, 2 mL of cell growth media was added (10% FBS). Final volumes were 500 μL to the insert and 2 mL to the well. In morphine-treated EGCs in the well, the cells were treated with 1-μM morphine 6 h after cell plating. GDNF was obtained from Abcam (187229, Cambridge, MA). When GDNF was added to the plate well (containing only 2-mL growth media), it was added at a concentration of 100 ng/mL immediately after adding media to the plate well.

The plate was incubated without disruption for 72 h to allow cell growth to confluence. At 72 h, all culture media were removed from the apical chamber and replaced with a 10 mg/mL 4 KDa FITC-Dextran (Sigma, St. Louis, MO) and 2.5 mg/mL 70 KDa rhodamine-dextran solution (Sigma, St. Louis, MO). After 4 and 24 h, the basal chamber media were