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Short-term effects of Poly(I:C) on gut permeability

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ABSTRACT

The intestinal barrier function depends on an adequate response to pathogens by the epithelium. Toll-like receptor 3 (TLR-3) recognizes double-stranded RNA, a virus-associated molecular pattern. Activation of TLR-3 with Poly(I:C), a synthetic agonist, modulates tissue repair and permeability in other epithelia; however, the effects of local luminal TLR-3 agonists on gut barrier function are unknown. The aim of this investigation was to evaluate short-term effects of Poly(I:C) on rat ileal and colonic *permeability ex vivo*. We also studied the acute effects of intrarectal administration of Poly(I:C) on colonic barrier function.

Ileum tissues displayed decreased transepithelial electrical resistance (TEER) 1 h after incubation with 200 μg/mL Poly(I:C); however, the mucosa-to-serosa transit of macromolecules (4.4 and 40 kDa dextrans – TD4.4 and FD40, respectively) remained unchanged. Conversely, colon tissue preparations stimulated with 200 μg/mL Poly(I:C) showed a decreased thinning of the mucosal layer after 2 h and a decreased transit of FD40 after 3 h, in comparison to controls. There was no change in colonic TEER after 3 h of treatment. In addition, colon tissue taken from rats 6 h after an intrarectal administration of 100 μg Poly(I:C) also showed decreased permeability to FD40 in the everted gut sac assay at 3 h post-extraction. Tissue morphology remained unchanged.

Our results suggest that an acute exposure to Poly(I:C) reduces colon permeability to macromolecules but increases ileum permeability to electrolytes/small molecules *ex vivo*. Although the mechanism associated to these effects needs further investigation, to our knowledge this is the first report of a direct effect of a TLR-3 ligand in intestinal barrier function and may be of significance to understand region-specific interactions between gut mucosa and microbiota.

1. Introduction

The intestinal tract wall is considered a major site of interaction between body and environment [1]. It regulates the entrance of water, electrolytes and nutrients from the lumen into the circulation, and also prevents penetration of harmful pathogens and noxious luminal substances [2]. Intestinal permeability to luminal contents is a dynamic and highly regulated process that depends on several elements, such as the surface mucous coat, epithelial factors and endothelial factors [2].

Toll-like receptors (TLRs) are a family of transmembrane receptors which recognize pathogen-associated and damage/danger-associated molecular patterns (PAMPs and DAMPs, respectively) [3,4]. Therefore, in addition to their role in innate immunity, TLRs also participate in injury-induced inflammation and tissue repair mechanisms. Generally, upon ligand recognition TLRs in the gut promote adequate signaling for the expression of antimicrobial peptides and proliferation of epithelial cells [5].

Also, healing of damaged intestinal epithelium appears to be mediated by a group of TLRs; for instance ligand-induced activation of TLR-2 enhances transepithelial electrical resistance (TEER) in isolated intestinal epithelial cells, which correlates with apical tightening and sealing of the tight junction protein zonula occludens 1 (ZO-1) [6]. However, under certain conditions, TLR signaling may have a negative impact on intestinal barrier function. For example burn injury in mice induces an early increase in intestinal permeability and barrier disruption. These events are markedly diminished in TLR-4 deficient mice, strongly suggesting that TLR-4 is required for thermal injury-related intestinal barrier dysfunction [7].

Similarly, intraperitoneal injection of TLR-3 ligand polyinosinic–polycytidylic acid (Poly(I:C)), a synthetic double-stranded RNA,

Abbreviations: FITC, fluorescein isothiocyanate; Poly(I:C), polyinosinic–polycytidylic acid; TEER, transepithelial electrical resistance; TLR-3, toll-like receptor 3; TRITC, tetramethylrhodamine isothiocyanate.

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induces severe small intestinal injury in mice, including intestinal wall attenuation and mucosal erosion. The effect is accompanied by increased levels of interleukin (IL) 15 [8]. On the other hand, subcutaneous, but not intragastric administration of Poly(I:C) protects against dextran sulfate sodium (DSS) – induced clinical symptoms (body weight loss, rectal bleeding, colon shortening) and inflammation in the mouse gut epithelium [9]. Interestingly, TLR-3 activation appears to have adverse effects on diverse epithelial barriers: exposure to West-Nile virus induces TLR-3-dependent secretion of the pro-inflammatory mediator tumor necrosis factor α (TNF-α) and transiently increases permeability of the blood–brain barrier in the mouse. Moreover, TLR-3 deficient mice have decreased blood–brain barrier permeability and a higher survival rate after virus infection [10]. Likewise, in human nasal epithelial cells, treatment with Poly(I:C) significantly reduced expression of the tight junction protein junctional adhesion molecule A (JAM-A) and induced secretion of pro-inflammatory cytokines IL-8 and TNF-α [11].

Epithelial barriers such as intestinal, nasal and the blood–brain barrier have several common features, *i.e.* they all regulate the movement of ions, molecules and cells between two body compartments, and are source of inflammatory mediators [12,13]. TLR-3 is expressed in human and murine intestine, not only in epithelial cells but also in dendritic cells [14] and in cells of the enteric nervous system [15–17]. Therefore, it is possible to suggest that TLR-3 activation may also mediate intestinal barrier dysfunction and consequently inhibition of TLR-3 activity could be used as a strategy to protect intestinal barrier integrity after trauma, infection or other pathological conditions. This investigation aimed to evaluate the *ex vivo* and *in vivo* effects of a short term Poly(I:C) exposure on the permeability of the rat intestine.

2. Methods

2.1. Animals

Male Sprague Dawley rats were maintained with food and water *ad libitum*, on a 12:12 h dark–light cycle with temperature at 20 ± 1 °C. A total of 35 rats were used at 60–70 days (300–350 g) and killing was performed by sodium thiopental overdose followed by decapitation. All the protocols for animal care and use included in this study were approved by the institutional ethics committee of the Pontificia Universidad Católica de Valparaíso and followed Conicyt directives.

2.2. Ussing chamber study

Rat colon or ileal samples were rinsed with saline (0.9% NaCl) and placed in oxygenated (95% O_2, 5% CO_2) Krebs buffer (1.2 mM NaH_2PO_4, 117 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11 mM glucose). The muscle layers were stripped away, leaving only the mucosa and submucosa. In the case of ileal preparations, these were found to produce a substantial amount of mucus; therefore, they were pre-incubated in oxygenated Krebs buffer at 37 °C for 20 min to clear some of this mucus. Preparations were then placed in Ussing chambers (Easy-Mount from Warner Instruments, Whitehall, PA, USA). Exposed area of 0.3 cm^2 as described previously [18] with oxygenated Krebs buffer maintained at 37 °C, and voltage-clamped at 0 mV (World Precision Instruments, Sarasota, FL, USA). In the case of colon preparations, these were immediately placed in the Ussing chambers (exposed area of 0.3 cm^2), as mentioned earlier. Short-circuit current responses were continuously monitored using LabScribe 2 software (World Precision Instruments, Sarasota, FL, USA). After stabilization, a 2 mV challenge was applied for 5 s in order to calculate baseline transepithelial resistance. Shortly after, Poly(I:C) (Tocris Bioscience, Bristol, UK) was applied on the mucosal side (0, 0.5, 5, 50 or 200 μg/mL), and transepithelial electrical resistance (TEER) was measured again every 30 min for up to 3 h. Treatments were assigned in a randomized manner. TEER data were expressed as % of baseline values.

2.3. Gut sac culture for histological assays

Rat colon samples were rinsed with saline and placed in oxygenated Krebs buffer as described before. Gut sacs measuring 2–3 cm were filled with 0.8 mL of Krebs buffer containing Poly(I:C) (0, 0.5, 5, 50 or 200 μg/mL), placed in a 15 mL centrifuge tube containing oxygenated Krebs buffer and incubated for 2 h at 37 °C. Sacs were then emptied, fixed overnight at 4 °C with 4% paraformaldehyde in phosphate buffered saline (PBS) and dehydrated in 30% sucrose in PBS. 10 μm cryosections were stained with hematoxilin and eosin and analyzed under a microscope (Nikon Eclipse, Melville, NY, USA). Microphotographs were taken to measure intestinal crypt length using Multi Gauge software (Fujifilm, Tokyo, Japan).

2.4. Everted gut sac culture for permeability assays

Rat colon or ileal tissue samples were rinsed with saline and placed in oxygenated Krebs buffer as described before. The tissues were everted by using a crochet hook (size 2.25 mm), thereby leaving the mucosa on the outer side. To prepare a sac, one end of a 2–3 cm gut segment was tied and the other end was affixed to a modified 2 mL cryotube, which allowed for repeated sampling. Each tube-intestine unit was then filled with a known volume of Krebs buffer and placed in a 15 mL centrifuge tube containing oxygenated Krebs buffer plus TRITC-dextran 4.4 kDa (TD4.4) 30 μg/mL, FITC-dextran 40 kDa (FD40) 30 μg/mL and Poly(I:C) (0, 0.5, 5, 50 or 200 μg/mL). Tissues were incubated at 37 °C and 60 μL samples were taken from the internal medium at different time points for up to 180 min. The internal media were diluted 1:10 before performing fluorimetric analyses (see below). At the end of the study, each sac was untied and the exposed mucosal area was measured.

2.5. Fluorimetric study

To establish the transit of TD4.4 and FD40 from the mucosal to the serosal side of the everted ileon and colon sacs, fluorescence of the internal medium (TD4.4: λ_{ex} 546, λem 575 and FD40: λex 490, λ_{em} 520) was measured in a FluoroMax-2 fluorometer (Jobin Yvon-Spex, Edison, NJ, USA). Standard curves were used to obtain the concentration of each fluorophore (TD4.4 limit of detection: 71 ng/mL – limit of quantification: 238 ng/mL; FD40 limit of detection: 2 ng/mL – limit of quantification: 7 ng/mL). The volume of medium and the exposed mucosal area in each sac were used to express fluorophore transit as pmoles/cm^2 at each sampling time.

2.6. Intrarectal administration of Poly(I:C)

Rats were handled daily for a week prior to the intrarectal procedure. The day before the experiment, rats were food deprived overnight but had free access to drinking water. Either saline or 100 μg of Poly(I:C) (total volume of 500 μL) were applied along 7 cm into the colorectal cavity using a lubricated round-edge, flexible plastic cannula. No sedation was required for this procedure. Rats were then returned to their home cage, given regular chow and monitored every hour until the time of killing. Six hours after intrarectal administration colon tissue was obtained and subjected to the everted gut sac permeability test as described before.