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ORIGINAL RESEARCH

Effect of xyloglucan associations with gelatin or gelose on *Escherichia coli*-derived lipopolysaccharide-induced enteritis in rats

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Abstract

Background: *Escherichia coli* is the predominant non-pathogenic facultative microbe of the human intestine, although some strains are diarrhoeagenic in humans. *E. coli*-derived lipopolysaccharide (LPS) induces diarrhoea, intestinal barrier impairment, bacterial translocation and intestinal inflammation. Associations with the mucoprotectant xyloglucan exhibit antidiarrhoeal effects. This study evaluated and compared the effects of xyloglucan in combination with gelatin or gelose (agar-agar) on jejunal permeability and inflammation using an *in vivo* rat model of *E. coli* LPS-induced enteritis.

Methods: Xyloglucan (12.5 mg/kg) plus gelatin (250 mg/kg) or gelose (250 or 500 mg/kg) were administered orally 2 hours before intraperitoneal injection with *E. coli* LPS. Following euthanasia, jejunal segments were removed for intestinal permeability measurement in Ussing chambers and inflammatory tone evaluation by myeloperoxidase activity assay.

Results: LPS administration increased jejunal permeability and increased mucosal inflammation in male Wistar rats. Xyloglucan plus gelatin 250 mg/kg and xyloglucan plus gelose 500 mg/kg significantly attenuated LPS-induced jejunal hyperpermeability and myeloperoxidase activity.

Conclusion: Xyloglucan, a known mucosal barrier protector, in combination with gelatin or gelose, has beneficial and comparable effects on intestinal permeability and inflammation following *E. coli* LPS insult in male rats.

Keywords: animal model, gelatin, gelose, lipopolysaccharide-induced enteritis, mucosal protector, xyloglucan.

Citation

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Introduction

Intestinal epithelial cells are a selectively permeable barrier with an important role in protecting against disease.[1,2] In acute intestinal infections, increased lipopolysaccharide (LPS)-induced intestinal permeability is one of the major mechanisms that promotes bacterial translocation.[3] LPSs are present in almost all Gram-negative bacteria and constitute the major component of bacterial outer surface membranes.[4] Although Gram-negative *Escherichia coli* is the predominant non-pathogenic facultative microbe of the human intestine, some strains have acquired virulence determinants conferring pathogenic and diarrhoeagenic properties.[5] Indeed, *E. coli* is the most common bacterial cause of paediatric diarrhoea and diarrhoea outbreaks worldwide.[6,7]

Experimental studies show that enteropathogenic *E. coli* infection in mice induces diarrhoea, intestinal epithelial barrier impairment and mucosal inflammation.[8] Intraperitoneal LPS from *E. coli* administration in rats also induces diarrhoea, bacterial translocation, mucosal inflammation and intestinal epithelial paracellular hyperpermeability,[9,10] underlying the pathogenicity of *E. coli* LPS.

Xyloglucan belongs to a class of products called mucoprotectants,[11] which act by forming a bioprotective film in the intestine.[12] In association with xyloglucan, gelatin prolonged the protective effect of xyloglucan in a rat model of cholera toxin-induced water secretion.[13] Xyloglucan–gelatin has been shown in clinical trials to be effective for the treatment of acute gastroenteritis in adults[14] and children.[15] Despite the widespread use of

gelatin in the food industry, in pharmaceutical and medical device formulations, and in personal care products, consumer acceptability has decreased due to its animal origin, underscoring the need to identify a suitable replacement.[16] Gelose, a polysaccharide obtained from agar-agar, is widely used in the biomedical field and exhibits gel-forming properties similar to those of gelatin.[17] Herein, we aimed to evaluate and compare the effects of xyloglucan–gelatin and xyloglucan–gelose associations on *E. coli* LPS-induced enteritis using a rat model of intestinal hyperpermeability and mucosal inflammation.

Materials and methods

Animals

Wistar rats (200–225 g) purchased from Janvier Labs (Le Genest St Isle, France) were used in all experiments. Male rats were used to avoid natural changes in intestinal permeability that occur in female rats according to the oestrous cycle.[18] Animals were kept at a constant temperature (22±1°C) and maintained on a 12-h light–dark cycle. Food (Harlan Global Rodent Diet; Harlan Bioproducts, Gannat, France) and tap water were available *ad libitum*.

Ethics

This study followed the ARRIVE guidelines for animal research. All experimental protocols described in this study were approved by the local Animal Care Use Committee (Comité d'Ethique de Pharmacologie-Toxicologie de Toulouse – Midi-Pyrénées, France).

Experimental design

Experiments were performed on groups of 8-10 rats and replicated once. After an overnight fast, animals were injected intraperitoneally with 250 µL of sterile 0.9% saline (vehicle) with or without 1 mg/kg of *E. coli* 0111:B4-derived LPS (Sigma). Test compounds were administered orally 2 hours before LPS administration and comprised xyloglucan (12.5 mg/kg) plus gelatin (250 mg/kg), xyloglucan (12.5 mg/kg) plus gelose (250 mg/kg), or xyloglucan (12.5 mg/kg) plus gelose (500 mg/kg). Six hours after LPS administration and following euthanasia, jejunal segments were removed for intestinal permeability measurement in Ussing chambers and for inflammatory tone evaluation by myeloperoxidase (MPO) activity assay.

Intestinal epithelial paracellular permeability measurement

Intestinal segments from the proximal part of the jejunum were mounted in Ussing chambers (Physiologic Instruments) and incubated with Kreb's solution (Sigma) constantly oxygenated with carbogen (95% O_2, 5% CO_2) in the presence of fluorescein isothiocyanate (FITC)–dextran (4000 MW, 0.022 g/ml, Sigma) in the mucosal compartment. Tissue integrity was checked continuously through electro-physiological measurements, and electrical resistance was monitored. After 2 hours of incubation, the serosal compartment was sampled to follow the epithelial paracellular passage of FITC–dextran by fluorescence intensity measurement using a microplate reader (Spark).

MPO activity assay

MPO activity, a marker of polymorphonuclear neutrophil primary granules, was determined in proximal jejunal tissues based on the method of Bradley et al.[19] After euthanasia, jejunal samples were removed and snap frozen until determination of MPO activity. Briefly, jejunal segments were homogenized using a Polytron homogenizer before undergoing three cycles of freezing–thawing and centrifugation. The supernatant was discarded, and the pellet was resuspended in an MPO-releasing detergent. Finally, suspensions were sonicated on ice and centrifuged. Supernatants were assayed spectrophotometrically for MPO activity and protein concentration using a Lowry assay (Bio-Rad Detergent Compatible Protein Assay, Bio-Rad, Ivry, France), whereas the pellets were discarded.

Statistical analysis

GraphPad Prism 8.3 (GraphPad, San Diego, CA, USA) software was used for statistical analyses. The mean and standard error of the mean (SEM) of intestinal permeability and MPO activity values were calculated. Comparisons between different test compounds were performed by one-way analysis of variance (ANOVA) followed by the Mann–Whitney test. Statistical significance was set at $p<0.05$.

Results

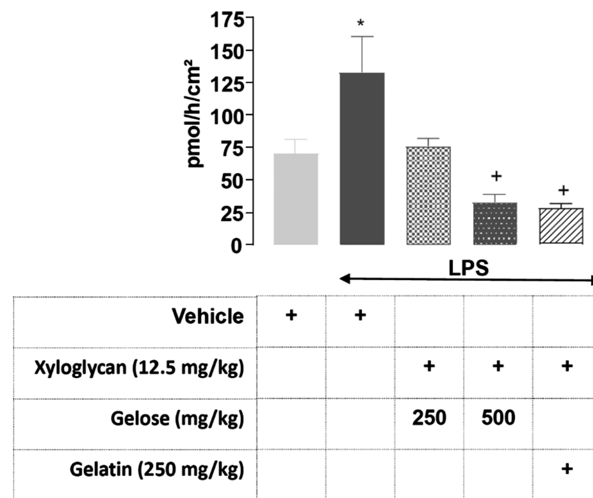
Effect of test compounds on intestinal paracellular permeability

LPS administration caused a significant ($p<0.05$) increase in jejunal paracellular permeability compared with control (vehicle) (132.5±27.48 *versus* 70.01±10.56 pmol/h/cm^2) (Figure 1). Pretreatment with xyloglucan (12.5 mg/kg) in association with gelatin (250 mg/kg) or gelose (500 mg/kg) prevented LPS-induced intestinal paracellular hyperpermeability compared with LPS alone ($p<0.05$), whereas xyloglucan in association with gelose (250 mg/kg) failed to prevent this effect (Figure 1).

Effect of test compounds on mucosal inflammation

MPO activity in the jejunum, reflecting intestinal mucosal inflammation, was significantly increased follow-

Figure 1. Comparative effect of oral pretreatment with xyloglucan plus gelatin *versus* xyloglucan plus gelose associations on *Escherichia coli* lipopolysaccharide (LPS)-induced intestinal epithelial paracellular hyperpermeability in rat jejunum (n=8–10/group). *$p<0.05$ *vs* vehicle; $^{+}p<0.05$ *vs* LPS.



Vehicle	+	+			
Xyloglycan (12.5 mg/kg)			+	+	+
Gelose (mg/kg)			250	500	
Gelatin (250 mg/kg)					+

ing LPS administration ($p<0.05$ *versus* control) (Figure 2). This LPS-induced inflammatory effect was significantly reduced ($p<0.05$) by pretreatment with xyloglucan (12.5 mg/kg) in association with gelatin (250 mg/kg) or gelose (500 mg/kg) (Figure 2). In contrast, the effect on MPO activity of pretreatment with xyloglucan (12.5 mg/kg) plus gelose (250 mg/kg) did not achieve statistical significance (Figure 2).

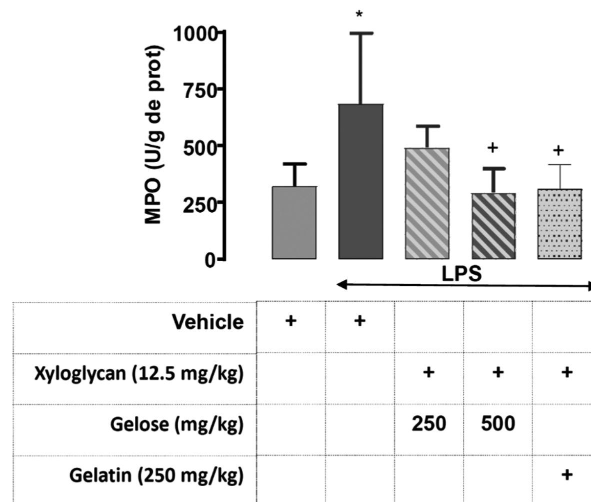
Discussion

In this study, we showed that oral treatment with xyloglucan in association with gelatin or gelose at the highest test dose (500 mg/kg) prevented *E. coli* LPS-induced intestinal epithelial barrier impairment and mucosal inflammation in male rats. Previously, in an *in vitro* model, *E. coli* LPS increased the intestinal paracellular permeability of Caco-2 cells as reflected by a reduction in tight junction proteins, such as ZO-1, occludin and claudin 1 mRNA expression, and enhanced the expression of pro-inflammatory cytokines (TNF, IL-1β, IL-6).[20] *In vivo*, intraperitoneal administration of LPS at the dose used herein (1 mg/kg) also altered gut paracellular permeability, induced bacterial translocation and released pro-inflammatory cytokines such as TNFα, IL-6 and IFNγ in the gut mucosa of rats.[9] Previous studies have shown that oral administration of xyloglucan 12.5 mg/kg prevents intestinal hyperpermeability induced by intraperitoneal administration of *E. coli* LPS in adult rats,[21] underlying the ability of this mucoprotectant to prevent impairment of gut barrier function.

Animal and human studies have demonstrated positive effects of xyloglucan in association with other components. Gelatin, for instance, acts with xyloglucan to favour increased intestinal bioavailability and prolong its protective activity. Xyloglucan in association with gelatin was shown to decrease the bacterial adsorption of *E. coli* in a co-culture of Caco–Goblet cells.[22] *In vivo*, the association of xyloglucan and gelatin 250 mg/kg given orally prolonged the protective effect of xyloglucan against cholera toxin-induced water secretion in rat gut lumen.[13] The efficacy of xyloglucan plus gelatin associations has also been tested in human acute infectious diarrhoea conditions involving tight junction disruption and subsequent increase in gut paracellular permeability.[23] In a randomized, open-label multicentre study in adults with acute diarrhoea, treatment with xyloglucan plus gelatin rapidly improved the main symptoms (number of Bristol Stool Scale (BSS) type 6 and 7 stools, nausea, abdominal pain) with an effect equal to or better than that of diosmectite or *Saccharomyces bouliardii*.[14] A randomized, open-label multicentre study in children with acute infectious gastroenteritis reported a faster onset of action and significantly greater reduction in the number of BSS type 6 and 7 stools with xyloglucan–gelatin plus oral rehydration solution (ORS) compared with ORS alone.[15]

In the current study, which used xyloglucan and gelatin at doses known to exhibit efficacy against *E. coli* LPS-induced hyperpermeability (xyloglucan 12.5 mg/kg)[21] and cholera toxin-induced water hypersecretion (xyloglucan

Figure 2. Comparative effect of oral pretreatment with xyloglucan plus gelatin *versus* xyloglucan plus gelose associations on *Escherichia coli* lipopolysaccharide (LPS)-induced jejunal inflammation in rats (*n*=8–10/group). $^{*}p<0.05$ *vs* vehicle; $^{+}p<0.05$ *vs* LPS.



Vehicle	+	+			
Xyloglycan (12.5 mg/kg)			+	+	+
Gelose (mg/kg)			250	500	
Gelatin (250 mg/kg)					+

12.5 mg/kg plus gelatin 250 mg/kg) in rats,[13] we showed that the xyloglucan–gelatin association prevents *E. coli* LPS-induced hyperpermeability and mucosal inflammation as reflected by MPO decrease. The ability of the combination to protect against *E. coli* LPS-induced intestinal hyperpermeability may be attributed to the chemical structures of these components. Xyloglucan, as an aqueous solution, is highly viscous, has broad pH tolerance and adhesiveness, and is not absorbed in the intestine.[24] Its chemical configuration gives it a 'mucin-like' molecular structure, thus conferring optimal muco-adhesive properties.[24] The positive effect of xyloglucan and gelatin in combination may arise from interactions occurring in the stomach between the secondary hydroxyl groups present in the structure of xyloglucan, which confer an anionic charge,[25] and gelatin, which has a positive charge at pH <6.[26] This enhances the formation of a protective polymeric film that prevents gut hyperpermeability. Even if we cannot exclude an inflammatory systemic effect of *E. coli* LPS intraperitoneal administration, the prevention of mucosal inflammation observed with xyloglucan–gelatin in our study may be the consequence of preventing gut hyperpermeability. Indeed, previously, it was shown that blockage of *E. coli* LPS-induced intestinal hyperpermeability prevents bacterial translocation and mucosal inflammation,[9] highlighting the crucial role of gut barrier integrity for preventing a subsequent mucosal inflammatory response.

A further objective of this study was to evaluate the effects of xyloglucan in association with gelose on *E. coli* LPS-induced intestinal adverse effects and compare them with the effects of xyloglucan plus gelatin in this model. The rationale for this objective was based on increasing societal demand to replace animal-based compounds with plant-based compounds due to religious prohibitions or vegetarian choices by consumers or zoonotic threats.[16] Gelose, a polysaccharide obtained from agar-agar, presents good biocompatibility, safety and gel-forming properties similar to those of gelatin. As an alternative to gelatin, gelose is widely used in the biomedical field.[17] A recent randomized, double-blind multicentre study in children with acute gastroenteritis recorded a significant reduction in the total number of BSS type 6 and 7 stools and improvement in associated clinical symptoms (apathy, vomiting, flatulence, blood in stool) with xyloglucan–gelose plus ORS compared with placebo plus ORS.[27]

In our study, *E. coli* LPS-induced gut hyperpermeability and mucosal inflammation was attenuated with xyloglucan 12.5 mg/kg in association with the higher (500 mg/kg) dose of gelose. We hypothesize that the polymeric bioprotective film resulting from association with gelose 250 mg/kg was not sufficiently strong to protect the intestinal epithelium in our model, where test compounds were administered acutely (2 h before LPS administration). Previously, xyloglucan plus gelose at doses of 10 mg/kg (similar to those used herein) and 5 mg/kg, respectively, was tested against *Salmonella enterica* and *Enterococcus hirae* oral infection in rats.[28] Both infectious agents induced intestinal histopathological features, including neutrophil infiltration as reflected by MPO increase and intestinal paracellular hyperpermeability.

Treatment with xyloglucan–gelose prevented both effects. Discrepancies in the efficacy of the gelose dose between the study by Esposito et al.[28] and our study may be explained by protocol differences. Whereas Esposito et al. administered xyloglucan–gelose chronically before and after inoculation with infectious agents,[28] in our study, treatment with the xyloglucan–gelose combination was acute.

Conclusion

Taken together, these data indicate that gelatin or gelose in association with xyloglucan provide effective and comparable protection of the intestinal barrier, thus reinforcing the rationale for use of both combinations in human treatment.

Contributions: All authors contributed extensively to the work presented in this paper. HE and VT contributed to conception, design, interpretation of the data, drafting, reviewing the manuscript and supervising the experiments. CB performed the experiments and contributed to data acquisition and analysis. All authors have approved the manuscript submission.

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