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Glucagon-like peptide 2 prevents down-regulation of intestinal multidrug resistance-associated protein 2 and *P*-glycoprotein in endotoxemic rats

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ABSTRACT

Multidrug resistance-associated protein 2 (Mrp2, ABCC2) and P-glycoprotein (P-gp, ABCB1) constitute essential components of the intestinal biochemical barrier that prevent incorporation of food contaminants, drugs or toxic metabolites into the blood stream. Endotoxemia induced in rats by administration of bacterial lipopolysaccharide (LPS) results in elevated intestinal permeability and toxicity of xenobiotics in part associated with down-regulation of expression and activity of Mrp2 and P-gp. We evaluated the protective effect of glucagon-like peptide 2 (GLP-2), a peptide hormone with enterotrophic properties, on Mrp2 and P-gp alterations induced by single i.p. injection of LPS (5 mg/kg b.wt.) to rats. Two different protocols of GLP-2 administration, namely prevention and reversion, were examined. The prevention protocol consisted of 7s.c. injections of GLP-2 (125 μg/kg b.wt.) administered every 12 h, starting 60 h before LPS administration. The reversion protocol consisted of 2 doses of GLP-2, starting 3 h after LPS injection. Intestinal samples were collected 24 h after LPS administration and expression (protein and mRNA) and activity of Mrp2 were evaluated in proximal jejunum whereas those of P-gp were studied in ileum. GLP-2 completely neutralized down-regulation of expression of Mrp2 and P-gp and loss of their respective activities induced by LPS under prevention protocol. GLP-2 was also able to prevent internalization of both transporters from the apical membrane of the enterocyte to intracellular compartments, as detected by confocal microscopy. LPS induced an increase in IL-1β and oxidized glutathione tissue levels, which were also counterbalanced by GLP-2 administration. In contrast, the reversion protocol failed to attenuate Mrp2 and P-gp down-regulation induced by LPS. We conclude that GLP-2 can prevent down-regulation of intestinal expression and activity of Mrp2 and P-gp in endotoxemic rats and that IL-1β and oxidative stress constitute potential targets of GLP-2 protective effects.

1. Introduction

The small intestine functions not only as an organ that absorbs water and nutrients but also functions as a biochemical barrier that prevents incorporation of food contaminants, drugs or toxic metabolites into the blood stream. Once in the intestinal lumen, xenobiotics can reach the circulation by paracellular or transcellular pathways. The paracellular pathway is associated with the passive transport through the intercellular space and is dependent on the tight junction integrity. The transcellular pathway is associated with the passive or active transport across cell membranes and is highly dependent on the existence of membrane transporters. Together, these two pathways constitute the intestinal biochemical barrier, an important intestinal function that impacts directly on drug availability and toxicity. ATP binding cassette (ABC) transporters localized to the apical membrane of the enterocyte constitute an important component of the transcellular

Abbreviations: ABC, ATP binding cassette; BBM, brush border membranes; cAMP, adenosine 3′,5′-cyclicmonophosphate; CDNB, 1-chloro-2,4-dinitrobenzene; CID, chemotherapy-induced diarrhea; DAPI, 4,6-diamino-2-phenylindole; DNP-SG, dinitrophenyl-S-glutathione; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; GLP-2R, GLP-2 receptor; GSH, reduced glutathione; GSSG, oxidized glutathione; IL, interleukin; iNOS, inducible nitric oxide syntase; LPO, lipid peroxidation; LPS, lipopolysaccharide; Mrp2, Multidrug resistance-associated protein 2; NF-κB, nuclear factor kappa B; PGCR, protein G couple receptor; P-gp, P-glycoprotein; PXR, pregnane X receptor; R123, rhodamine 123; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrotic factor alfa; ZO-1, zonula occludens-1

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barrier of the intestine since they pump dietary contaminants and other potentially toxic xenobiotics back into the lumen. Multidrug resistance-associated protein 2 (Mrp2, ABCC2) and P-glycoprotein (P-gp, ABCB1) are among the most active. The former is mainly expressed in the duodenum and proximal jejunum decreasing to the distal part and ileum, and its expression is maximal at the tip of the villi decreasing towards the crypt (Mottino et al., 2000). Mrp2 presents high affinity for organic anions preferentially conjugated with glutathione, glucuronic acid or sulfate. P-gp presents a different distribution since it is mainly expressed in the distal small intestine and colon. In contrast to Mrp2, it preferentially transports hydrophobic or cationic molecules. Together, they constitute a well extended protection against the absorption of xenobiotics of varied nature.

Our group reported an increase in Mrp2 expression and activity in jejunum from mother rats during lactation when compared to virgin females (Mottino et al., 2001). Maximal effects were observed at 15–21 days post-partum. Later on we demonstrated that the trophic factor glucagon-like peptide 2 (GLP-2) was able to induce intestinal Mrp2 expression and activity in female rats (Villanueva et al., 2010). Because secretion of GLP-2 is increased during lactation (Jacobs et al., 1981) we postulated that this hormone was involved in Mrp2 up-regulation during lactation. GLP-2, a 33 aminoacid-long peptide, is synthesized by the enteroendocrine L cells of the distal intestine. GLP-2 is co-secreted with GLP-1 after food ingestion, but while GLP-1 exerts its main action on endocrine cells of the pancreas, GLP-2 acts almost exclusively on the intestine, mainly on the proximal small intestine (Drucker and Yusta, 2014). GLP-2 binds to a receptor belonging to the PGCR superfamily (protein G couple receptor), named GLP-2R, which further activates adenylyl cyclase. GLP-2 action on the enterocytes seems to be indirect since GLP-2R is not present in these cells but in myofibroblasts, enteroendocrine cells and enteric neurons (Connor et al., 2016). This action is exerted under physiological conditions such as development, lactation, epithelial regeneration, etc, and involves hypertrophy and hyperplasia of the ephitelium, resulting in increased intestinal surface, villus height and nutrient absorption (Drucker and Yusta, 2014). Secretion of GLP-2 was also found to be increased under certain pathological conditions of the intestine, such as short bowel syndrome (Tappenden, 2014), gastric bypass (Cazzo et al., 2016) and inflammatory bowel diseases (Kissow, 2015). GLP-2 action is mainly based on epithelial regeneration, increased nutrient absorption, reduction of intestinal inflammation and enhanced intestinal epithelial barrier function (Drucker and Yusta, 2014; Kissow, 2015).

Increased intestinal permeability caused by intestinal tight junction dysfunction has been associated with intestinal inflammatory diseases (Turner, 2009). It seems that enhanced permeability represents a risk factor for the development of inflammatory disease, but it may also be involved in its progression (Turner, 2009). Alterations in ABC transporters localized apically may be also associated to intestinal diseases. For example, mice with *mdr1a* deficiency develop colitis spontaneously (Panwala et al., 1998). P-gp is involved in regulating the interaction of the intestine with bacterial products (Collett et al., 2008). Also, restoration of P-gp expression, after down-regulation, in Caco-2 cells was causally linked to attenuation of bacterial lipopolysaccharide (LPS) cell internalization, and consequently to cell protection (Yan et al., 2017). The potential beneficial effect of GLP-2 in counteracting down-regulation of ABC transporters in intestinal disease has never been explored. We hypothesize that GLP-2 may prevent/revert the alterations of intestinal barrier associated with loss of ABC transporter activities, particularly those of Mrp2 and P-gp. To test this hypothesis we selected the model of LPS-induced endotoxemia in rats. It represents a simple, well studied model of sepsis in which down-regulation of the expression and activity of Mrp2 and P-gp has been already reported (Kalitsky-Szirtes et al., 2004; Moriguchi et al., 2007). GLP-2 was administered using a prevention protocol (previous and simultaneous administration) and also a reversion protocol with the trophic factor being administered after LPS.

2. Materials and methods

2.1. Chemicals

Glucagon-like peptide 2 (GLP-2) was purchased from Abcam (Cambridge, UK); 1-chloro-2,4-dinitrobenzene (CDNB), phenylmethylsulfonyl fluoride, pepstatin A, leupeptin and rhodamine 123 (R123) were purchased from Sigma-Aldrich (St. Louis, MO, USA); Lipopolysaccharide (LPS), MK571 and PSC833 were purchased from Santa Cruz Biotecnology (Dallas, Texas, USA). All other chemicals were of analytical grade purity.

2.2. Animals and experimental design

Adult female Wistar rats (200–220 g b.wt.) were purchased from Centro de Medicina Comparada-Instituto de Cs. Veterinarias del Litoral (UNR-CONICET), Esperanza, Santa Fe, Argentina. Animals had free access to food and water and received human care as outlined in the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*. LPS treatment consisted of a single i.p. injection of LPS (5 mg/kg b.wt.). GLP-2 treatment consisted of s.c. injections of recombinant rat GLP-2 (125 μg/kg b.wt.), under two different experimental designs. In the first one, GLP-2 was administered every 12 h during 72 h, starting 60 h before LPS administration (prevention protocol, Fig. 1A). The second one consisted of only two doses of GLP-2, starting 3 h after LPS injection (reversion protocol, Fig. 1A). Control group was treated with sterile saline (vehicle of LPS) and PBS (vehicle of GLP-2) in accordance with the two different protocols. Animals were fasted 12 h before treatments ended, which occurred 24 h after LPS or saline injection (Fig. 1A). Samples were collected under intraperitoneal anesthesia [ketamine: 100 mg (0.42 mmol)/kg b.wt.; xylazine: 15 mg (0.07 mmol)/kg b.wt.].

2.3. Sample collection

The first 8 cm of the small intestine, starting from the pyloric valve, were discarded. The remaining small intestine was collected, carefully rinsed with ice-cold saline. The first portion (fraction 1, aprox 20 cm length), considered the proximal jejunum, was used for Mrp2 studies. The following portion (fraction 2, aprox. 20 cm length), considered the distal jejunum, was used for analysis of cytokines, antioxidant enzymes and oxidative stress markers. The remaining fraction (fraction 3, aprox. 30 cm length), considered the ileum, was used for P-gp studies. The segments were placed in saline at 4 °C until isolation of mucosa tissue or intestinal sacs. Aliquots of the intestinal segments were opened lengthwise, the mucus layer was carefully removed, and the mucosa was obtained by scraping and weighed. The tissue thus obtained was used for preparation of total homogenate and brush border membranes (BBM).

For intestinal total RNA extraction, aliquots from fractions 1 and 3 were rinsed with ice-cold saline and the whole tissue was immediately placed in liquid nitrogen.

For light and confocal microscopy studies, small rings were cut from these same regions of the intestine, gently frozen in liquid nitrogen-cooled 2-methylbutane and kept at −70 °C until use in slice preparation.

2.4. Western blot studies

BBM were prepared as described before (Mottino et al., 2000). Protein concentration was measured by Lowrýs method (Lowry et al., 1951). BBM samples were used the same day for assessment of Mrp2 and P-gp expression. Western blot studies were performed as described (Mottino et al., 2000) using antibodies specific for Mrp2 (M2 III-6, Alexis Laboratories, San Diego, CA, USA), P-gp (H-241), villin (H-60) and β-actin A-2228 (Sigma–Aldrich). Equal protein loading and transfer

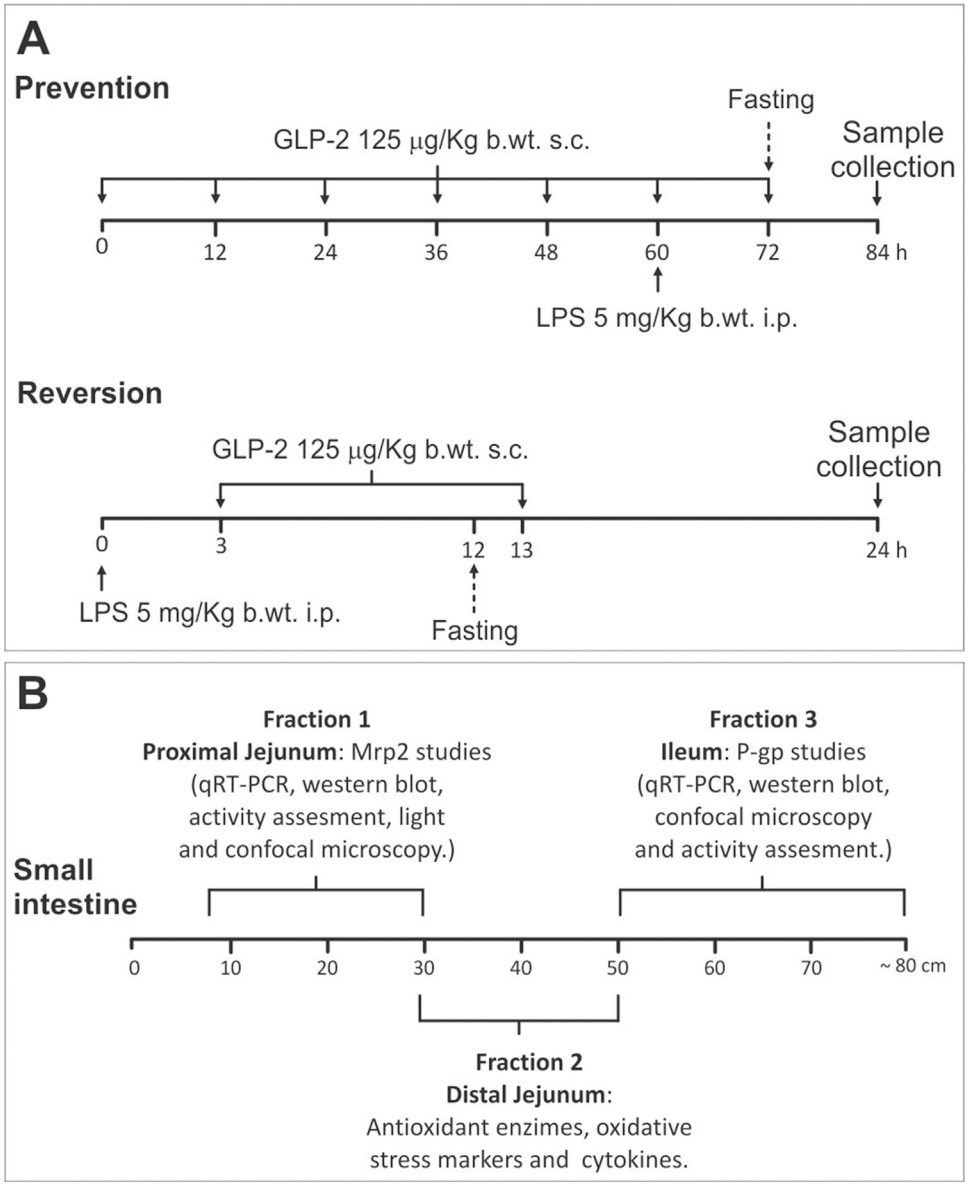

Fig. 1. Protocols for LPS and GLP-2 treatments and sample collection.
A. Prevention and reversion protocols. B. Studies performed in the different fractions of the small intestine.
LPS treatment consisted of a single i.p. injection of LPS (5 mg/kg b.wt.), whereas GLP-2 treatment consisted of 7 (prevention protocol) or 2 (reversion protocol) s.c. injections of recombinant rat GLP-2 (125 μg/kg b.wt.). Animals were fasted 12 h before sample collection.

was checked by Ponceau S staining of the membranes. Immunoreactive bands were quantified with Gel-Pro Analyzer software (Media Cybernetics, Inc., Bethesda, MD).

2.5. Mrp2 and P-gp activity studies

Proximal jejunum and ileum (fractions 1 and 3, respectively) were used for everted sac preparation. Segments from proximal jejunum (3 cm length) were everted and incubated for 20 min with 100 μM CDNB, with and without addition of the Mrp inhibitor MK571 (10 μM) as described (Mottino et al., 2001). The model Mrp2 substrate, dinitrophenyl-S-glutathione (DNP-SG), is generated endogenously by passive diffusion of CDNB into the enterocyte followed by conjugation with glutathione. Samples of the mucosal compartment were obtained every 5 min and analyzed for DNP-SG content by HPLC as described (Mottino et al., 2001).

Segments from ileum (4 cm length) were everted, filled with 12.5 μM R123 and incubated for 30 min with and without the addition of the P-gp inhibitor PSC833 (10 μM). Samples of the mucosal compartment were obtained every 5 min and subjected to fluorescence analysis (λex = 485 λem = 535).

2.6. RNA isolation and quantitative real-time PCR studies

Total mRNA was isolated using TRIzol® reagent (Invitrogen Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA synthesis and real-time PCR studies were performed as described (Villanueva et al., 2010). Primer sequences and reaction conditions are summarized in Table 1.

2.7. Light and confocal microscopy studies

Frozen rings from proximal jejunum and ileum were sectioned (thickness, 5 μm) and fixed as described (Arias et al., 2009). Mrp2 and zonula occludens-1 (ZO-1) were detected in jejunum sections by using anti-Mrp2 and anti-ZO-1 (61-7300, Zymed Laboratories, South San Francisco, CA) antibodies. P-gp and occludin were detected in ileum sections by using anti-P-gp and anti-occludin (33-1500, Zymed Laboratories, South San Francisco, CA) antibodies. Detection was completed by incubation with appropriate Cy2- or Cy3-conjugated donkey anti-IgGs (Jackson Immuno Research Laboratory, Inc., West Grove, PA). Cell nuclei were detected by using 4,6-diamino-2-phenylindole (DAPI) (Arias et al., 2009). All confocal studies were performed with a Nikon

Table 1
qRT-PCR nucleotide primers sequences for rat genes, temperature and time for amplification program.

Gene	Primers		Amplification program			
	F	R	Denaturation	Annealing	Elongation	Cycles
mrp2	accttccacgtagtgatcct	acctgctaagatggacggtc	95 °C (15s)	57 °C (30 s)	72 °C (30s)	40
mdr1a	gaggcttgcaaccagcatt	ctgttctgccgctggattt	95 °C (15s)	50 °C (30 s)	72 °C (30 s)	40
18S	gtaacccgttgaaccccatt	ccatccaatcggtagtagcg	95 °C (15s)	55 °C (30 s)	72 °C (30 s)	40

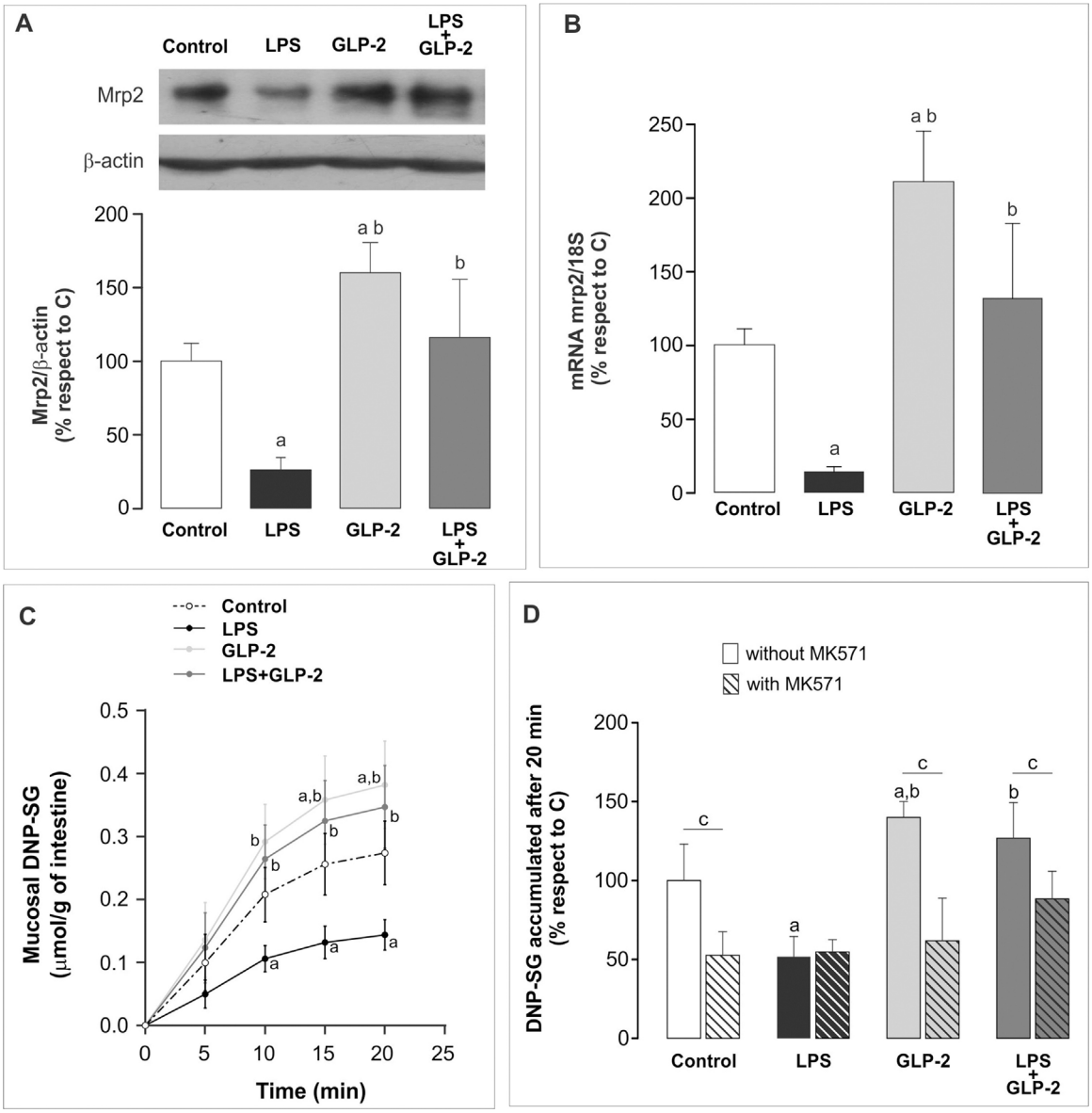

Fig. 2. Intestinal Mrp2 expression and activity.
A. Western blot analysis of Mrp2 expression in BBM obtained from rat proximal jejunum. Image shows a representative band for each experimental group. Bars graph represents the densitometric analysis expressed as percentage of control group (N = 4). B. qRT-PCR analysis of *mrp2* mRNA expression in proximal jejunum. Results are referred to 18s rRNA and expressed as percentage of control group (N = 4). C. Time-course of DNP-SG accumulation in the mucosal compartment of intestinal sacs (N = 6). D. Mucosal accumulation of DNP-SG after 20 min with or without the addition of the Mrp inhibitor MK571 (N = 6). Results are expressed as percentage of the control group without inhibitor.
Data are presented as means ± S.D. a: significantly different from Control group ($p < 0.05$); b: significantly different from LPS group ($p < 0.05$); c: significant difference from incubations without Mrp inhibitor ($p < 0.05$).

(Tokyo, Japan) C1 Plus microscope. To ensure comparable staining and image capture performance for the different groups belonging to the same experimental protocol, intestinal slices were prepared on the same day, mounted on the same glass slide, and subjected to the staining procedure and microscopy analysis simultaneously. Additionally, sectioned intestinal rings were stained with hematoxylin and eosin for light microscopy examination (Axiovert 25, Carl Zeizz, Oberkochen, Germany).

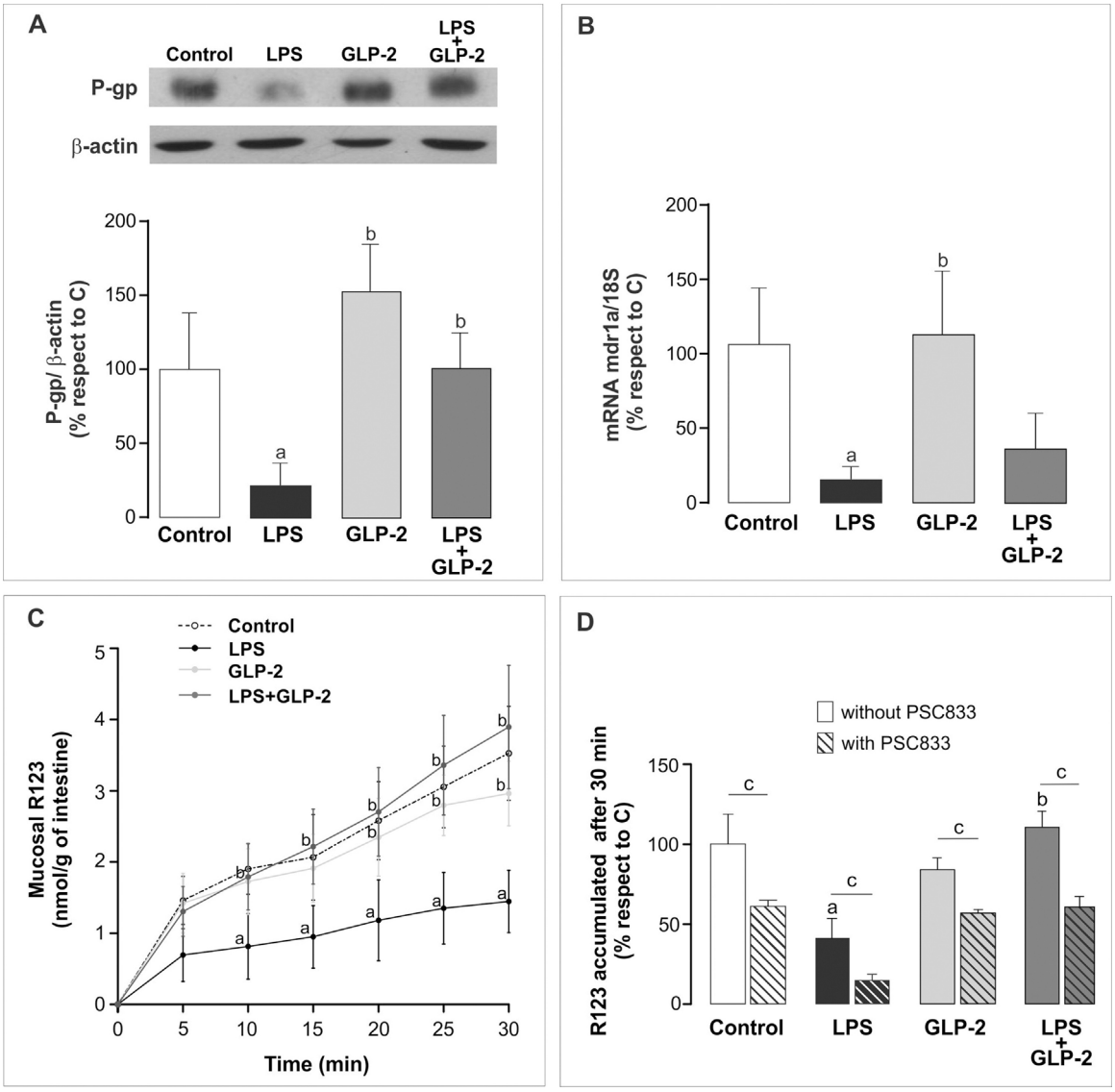

Fig. 3. Intestinal P-gp expression and activity.

A. Western blot analysis of P-gp expression in BBM from rat ileum. Image shows a representative band for each experimental group. Bars graph represents the densitometric analysis expressed as percentage of control group (N = 4). B. qRT-PCR analysis of *mdr1a* mRNA expression in ileum. Results are referred to 18s rRNA and expressed as percentage of control group (N = 4). C. Time-course of R123 accumulation in the mucosal compartment of intestinal sacs (N = 6). D. Mucosal accumulation of R123 after 30 min with or without the addition of the P-gp inhibitor PSC833 (N = 6). Results are expressed as percentage of the control group without inhibitor.

Data are presented as means ± S.D. a: significantly different from Control group ($p < 0.05$); b: significantly different from LPS group ($p < 0.05$); c: significant difference from incubations without P-gp inhibitor ($p < 0.05$).

2.8. Assessment of anti-oxidant enzyme activities and oxidative stress markers

Mucosal aliquots were homogenized in saline (1:3 P/V) and centrifuged at 2000*g* for 5 min. Supernatants, hereafter referred as mucosal supernatants were subjected to determination of superoxide dismutase and catalase activities as described previously (Londero et al., 2017). Assessment of oxidative stress markers was performed by determination of the glutathione redox balance and lipid peroxidation (LPO). For evaluation of the glutathione redox balance two volumes of mucosal supernatants were mixed with one volume of 10% sulfosalicylic acid and centrifuged at 5000*g* for 5 min, and the supernatants were immediately used in the assessment of total and oxidized (GSSG) glutathione (Griffith, 1980). Reduced glutathione (GSH) was estimated as the difference between total glutathione and GSSG. The oxidative stress index was estimated as the GSSG/GSH ratio. LPO was evaluated by measuring thiobarbituric acid reactive substances (TBARS) using the procedure of Ohkawa et al. (1979). Mucosal supernatants were mixed with 10% trichloroacetic acid and then centrifuged at 3000*g* for 10 min. The clear supernatant was mixed with 0.7% thiobarbituric acid and heated at 95 °C for 1 h and used in LPO assessment as described by Lambertucci et al. (2017). Protein concentration in mucosal supernatants was determined by Lowrýs method (Lowry et al., 1951).

2.9. Cytokine quantification

Mucosal aliquots were homogenized in PBS (1:3 P/V), sonicated 6 times for 20 s with an ultrasonifier Sonics Vibra-cell VCX 130 (Newtown, CT, USA), centrifuged at 15000*g* for 10 min (4 °C), and the supernatants used for cytokine quantitation with the commercial ELISA kits Rat IL-1 beta Tissue Culture ELISA Ready-SET-Go! (88-6010, Affymetrix) and Rat Interleukin-6 ELISA Kit (KRC0062, BioSource International). Measurements were carried out according to manufacturer's protocol and referred to protein content of the homogenates.

2.10. Statistical analysis

Data are presented as means ± S.D. Statistical analysis was performed using Studenťs *t*-test or one-way analysis of variance, followed by Bonferroni's test (when more than two groups were compared). Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Effect of LPS and GLP-2 on Mrp2 and P-gp expression and activity

3.1.1. Prevention protocol

Mrp2 protein expression was down-regulated by LPS treatment as expected from previous studies reporting alteration of mRNA and transport activity (Kalitsky-Szirtes et al., 2004). In our experimental conditions, such decrease was of 74% respect to control rats (Fig. 2A). In contrast, and as was also expected from our previous studies (Villanueva et al., 2010), GLP-2 treatment increased Mrp2 levels in the brush border membrane (60% over control group). Importantly, the prevention protocol was able to counteract the effect of LPS as Mrp2 expression did not differ from that of the control group. Fig. 2B shows similar patterns of mRNA variations in response LPS and/or GLP-2, suggesting transcriptional regulation by both agents. Consistent with the changes observed in Mrp2 expression, transport function evaluated *in vitro* was impaired by LPS (about 48% decrease at 20 min) and exacerbated by GLP-2 (about 40% increase at 20 min), while co-treatment showed similar results to Control group (Fig. 2C and D). Addition of an Mrp inhibitor to the assays confirmed participation of an Mrp protein in the mucosal accumulation of DNP-SG, except for the LPS group (Fig. 2D).

Regarding P-gp expression and as expected from previous studies (Moriguchi et al., 2007), LPS treatment decreased its protein expression by 78% when compared to control rats (Fig. 3A). In contrast to Mrp2 results, GLP-2 treatment had no effect on P-gp protein expression when administered alone. However, it was able to prevent the down-regulation induced by LPS. Fig. 3B shows that *mdr1a* mRNA levels are regulated in similar fashion as P-gp protein except for the co-treated group which did not differ from control or LPS group, suggesting that the protection mechanism could be at least in part post-transcriptional. Activity studies correlated well with protein expression studies. As shown in Fig. 3C and D, LPS induced a decrease in R123 serosal to mucosal transport (about 41% decrease at 30 min), while no difference was observed in GLP-2 or LPS + GLP-2 group when compared to Control group. Fig. 3D shows that all groups exhibited a decrease in transport activity after addition of a P-gp inhibitor, thus confirming participation of this transporter.

3.1.2. Reversion protocol

Administration of GLP-2 after LPS-induced endotoxemia has been established (see protocol in Fig. 1) failed to demonstrate any reversion of the alterations in Mrp2 or P-gp expression as detected by Western blot studies (Supplementary data Fig. A and B). Similarly, mRNA analysis for both transporters also shows no difference between LPS and LPS + GLP-2 groups (Supplementary data Fig. C and D), consistent with protein analysis. Additionally, under this same protocol GLP-2 administered alone also failed to induce any change in transporters expression with respect to the control group. In view of these negative results no further experiments were performed to characterize the effect of the reversion protocol on transporters localization or activity.

3.2. Effect of LPS and GLP-2 on intestinal structure and morphology

Fig. 4A shows hematoxylin-eosin representative images of the different experimental groups. Both GLP-2 groups present conserved intestinal morphology as compared with control animals. Although we did not perform any quantitative analysis, GLP-2 groups likely present longer villi, consistent with the known trophic properties of GLP-2 (Villanueva et al., 2010). In contrast, the intestinal sections in rats treated with LPS alone show signs of edema produced by inflammation. In spite of this alteration, the structure of the villi looked complete and the apical membrane preserved. To confirm preservation of this particular feature of the intestinal mucosa, we performed Western blot detection of villin, a structural protein associated with microvilli formation. Fig. 4A shows that the content of villin in BBM was similar in all four experimental groups.

3.3. Effect of LPS and GLP-2 on Mrp2 and P-gp localization

Confocal microscopy representative images of Mrp2 (red) together with the tight junction protein ZO-1 (green) and of P-gp (green) together with the tight junction occludin (red) are shown in Fig. 4B. The nuclei of the enterocytes are shown in blue. In control group detection of both Mrp2 and P-gp is restricted to the luminal surface of the enterocytes. Similar results are observed for GLP-2 group indicating that GLP-2 treatment has no effect on the localization of these transporters. However, LPS group shows an overall decrease in the detection of Mrp2 and P-gp together with irregular distribution of the corresponding signals. This seems to be more noticeable for Mrp2. Yellow arrows point out luminal surface regions were both transporters are almost absent, while the corresponding signal is still present in other regions (red arrows). White arrows point out regions where Mrp2 and P-gp are detected in the proximity of the cell nucleus, suggesting transporter internalization. Images from LPS + GLP-2 group show patterns of distribution of both transporters similar to those in control group consistent with a preventive effect exerted by GLP-2 on expression and localization of Mrp2 and P-gp.

3.4. Effect of LPS and GLP-2 on pro-inflammatory cytokines

Tissue levels of IL-1β and IL-6 where evaluated in the middle region of the small intestine. Fig. 5A shows that IL-1β levels were significantly increased by 94% in response to LPS treatment over control value. Although GLP-2 had no effect on basal level of this cytokine, it was able to suppress the increase produced by LPS so that no difference was observed with respect to control or GLP-2 group. IL-6 levels were not affected by LPS or GLP-2, either administered alone or in combination (data not shown).

3.5. Effect of LPS and GLP-2 on antioxidant enzymes and oxidative stress markers

The cellular redox status was also studied in the middle region of the small intestine. Fig. 5B shows that catalase activity was reduced in LPS and LPS + GLP-2 groups by 51% and 62% respectively when compared to control rats, which indicates that co-administration of GLP-2 was unable to restore this measure. GLP-2 administered alone had no effect on catalase activity. Superoxide dismutase activity was not affected by LPS or GLP-2 used either alone or in combination (data not shown). Determination of TBARS neither showed changes in the different groups (data not shown). Fig. 5C shows that the GSSG/GSH ratio increased in LPS-treated rats by 141% respect to control animals, with no change for the groups administered with GLP-2 either alone or together with LPS, indicating that GLP-2 was able to prevent LPS-induced alteration of the glutathione balance. The alteration in this balance observed for the LPS group was likely associated with increased accumulation of the oxidized specie as it was increased by 450% over controls when expressed per mg of protein. Consistent with prevention exerted by GLP-2, cellular GSSG in LPS + GLP-2 group was not different from the control group.

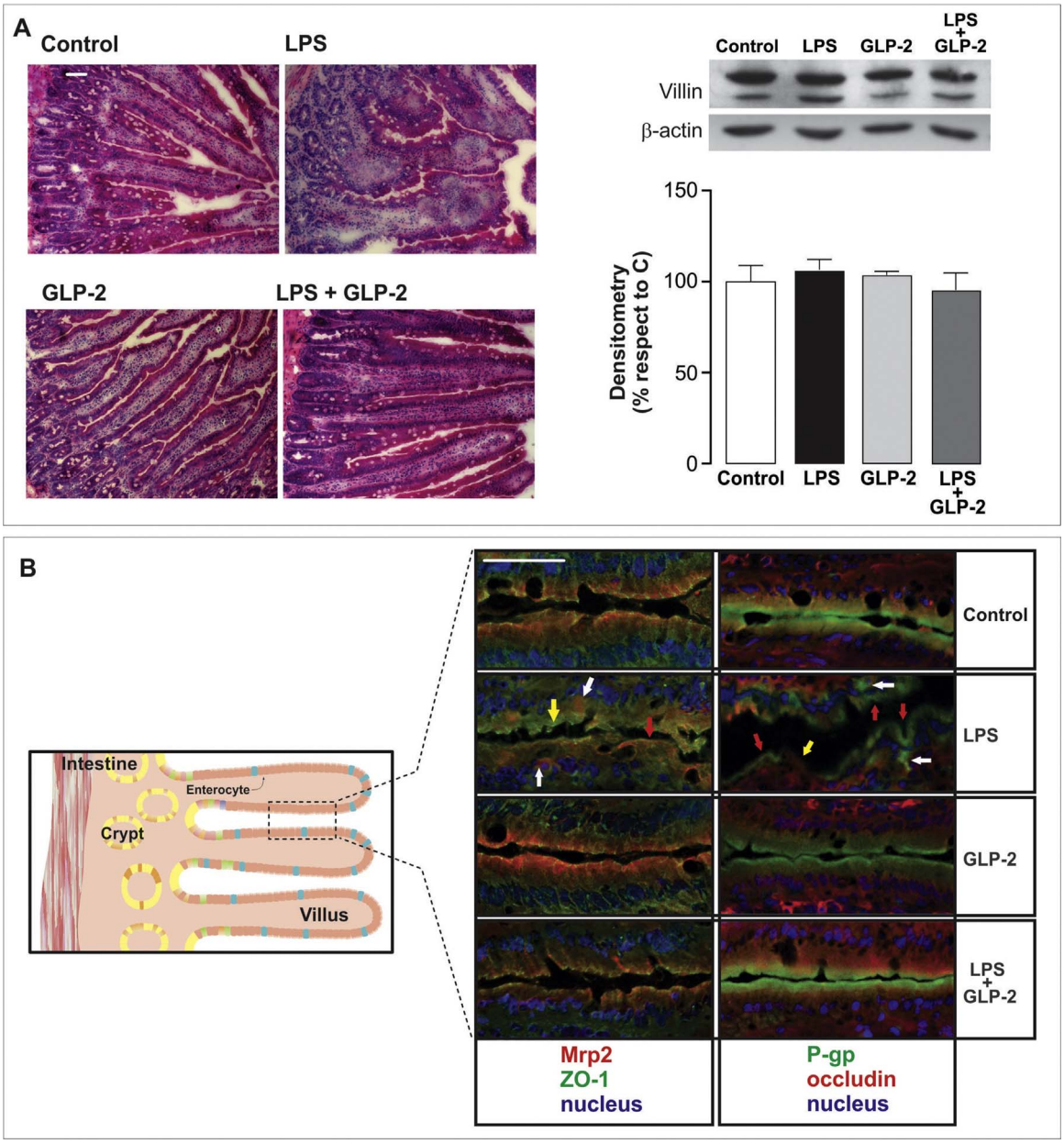

Fig. 4. Intestinal tissue integrity and transporter localization.
A. Hematoxylin-eosin microscopy images of proximal jejunum and Western blot analysis of villin expression in BBM from the same region. B. Confocal microscopy images of Mrp2 (red) and the tight junction protein ZO-1 (green) and of P-gp (green) and the tight junction occludin (red). The nuclei were stained with DAPI and are shown in blue. Samples were obtained from jejunum for Mrp2 and ileum for P-gp. White arrows indicate regions where the transporter was internalized. Yellow arrows indicate apical membrane regions where the transporter is absent. Red arrows indicate regions were transporter fluorescence is detected in the apical membrane.
All images are representative of at least 4 different preparations per group. Scale bars are indicated in white and correspond to 50 μm.

4. Discussion

The actions of the enterotrophic hormone GLP-2 on the intestine and other tissues, including the interaction to its receptor and subsequent signaling pathways, have been extensively studied (Connor et al., 2016; Drucker and Yusta, 2014). These actions are associated with physiological relevant processes such as development, lactation, tissue regeneration, etc. Recently, GLP-2 was assigned a clinical role as potential therapeutic agent based on findings demonstrating its ability to protect the gut in several models of intestinal injury (Drucker and Yusta, 2014). The beneficial effects were associated with alleviation of inflammation and oxidative stress and with induction of crypt and mucosal proliferation. Several studies demonstrated the beneficial effect of GLP-2 on preservation/reconstitution of intestinal permeability, which additionally involves preservation of tight junction integrity (Cameron et al., 2003; Cameron and Perdue, 2005; Dong et al., 2014). However, none of them considered the role GLP-2 in the regulation of the transcellular barrier associated to drug transporters such as the ABC proteins. This constitutes a subject of toxicological and pharmacological relevance since ABC transporters localized apically restrict the oral absorption of food contaminants and therapeutic drugs (Dietrich, 2003).

Transport function of ABC transporters is affected in several intestinal diseases in humans, in particular those including local or systemic inflammation such as ulcerative colitis and Crohńs disease (Arana et al., 2016; Blokzijl et al., 2007). Similar alterations were found in experimental models in rats presenting inflammation and oxidative stress such as those generated by LPS or Indomethacin administration

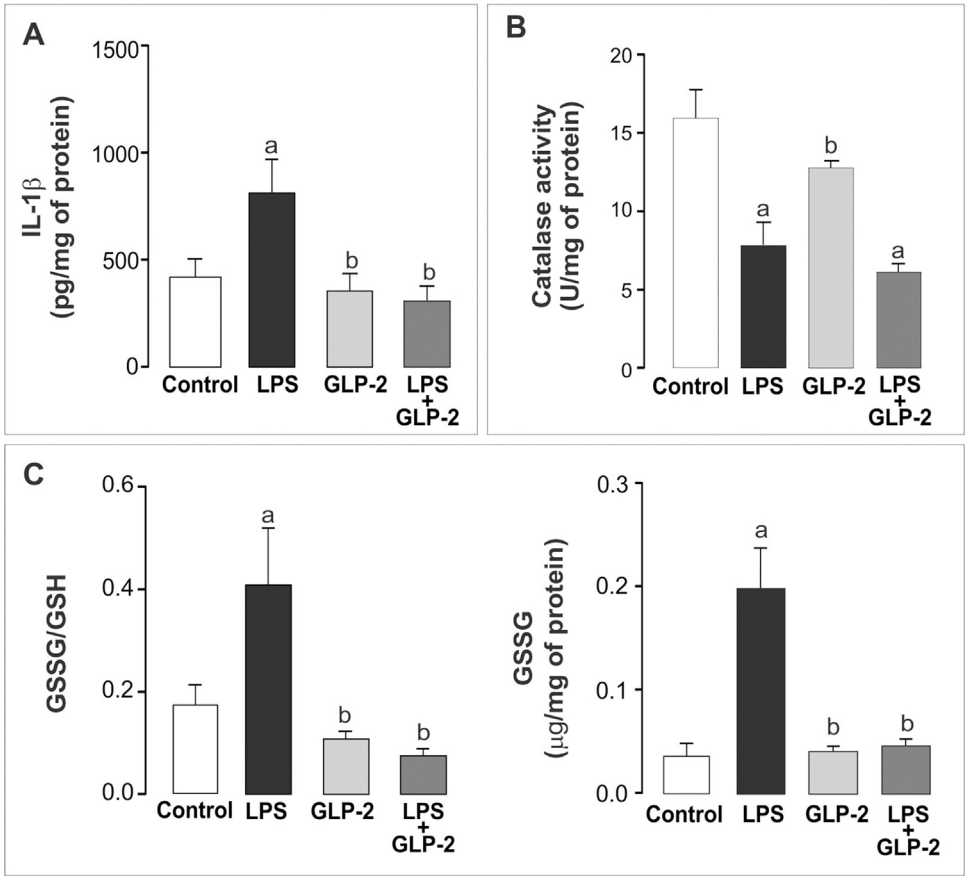

Fig. 5. Inflammation and oxidative stress markers. A. Intestinal content of the cytokine IL-1β detected in rat distal jejunum. B. Catalase activity detected in distal ileum. C. Cellular GSSG/GSH ratio (left panel) and GSSG content (righ panel) in distal jejunum. Data are presented as means ± S.D. (N = 4). a: significantly different from Control group ($p < 0.05$); b: significantly different from LPS group ($p < 0.05$).

and two-stage five-sixth nephrectomy (Iida et al., 2015; Kalitsky-Szirtes et al., 2004; Naud et al., 2007). The potential beneficial effect of GLP-2 in counteracting ABC transporter alterations has never been explored. To explore such possibility we chose an inflammation model induced by i.p. administration of a single dose of LPS in rats. It has been demonstrated that this model presents decreased expression and activity of intestinal Mrp2 and P-gp (Kalitsky-Szirtes et al., 2004; Moriguchi et al., 2007). We found that GLP-2 was able to prevent LPS-induced down-regulation of expression and activity of both Mrp2 and P-gp, though the mechanism of protection may not be the same.

Transcriptional regulation of ABC transporter proteins is usually associated to the activation of nuclear receptors, like pregnane X receptor (PXR), constitutive androstane receptor and farnesoid X receptor (as reviewed by Kullak-ublick and Becker, 2003; Tocchetti et al., 2016). PXR is the most studied and is considered a master regulator of metabolic enzymes, such as CYP3A and GST, and drug transporters like MRPs and P-gp, resulting in a synchronized induction of both systems to detoxify a wide spectrum of substrates. In contrast, the action of GLP-2 is less universal. While it was able to increase intestinal Mrp2 expression both at mRNA and protein levels, it was unable to induce P-gp expression. We have previously demonstrated that the inducing properties of GLP-2 in rats were more evident in jejunum, more specifically at the upper regions of the villus, and dependent on increased levels of intracellular cAMP (Villanueva et al., 2010). More recently, we found in Caco-2 cells that increased intracellular cAMP levels mediate transcriptional induction of MRP2 through PKA-dependent activation of the transcription factors c-JUN and ATF-2, ultimately interacting to specific regions in *mrp2* promoter (Arana et al., 2015). Lack of effect of GLP-2 on P-gp expression may be related to the absence of interactions of these same transcription factors with the *mdr1a* promoter or alternatively, to the fact that GLP-2 exerts its actions mainly at the proximal regions of the small intestine whereas P-gp studies were performed in distal ileum. Whether GLP-2 can induce P-gp expression in proximal jejunum was not explored in this study.

Although we found an overall correlation between protein content as detected by Western blotting and mRNA levels as detected by qRT-PCR, participation of post-transcriptional mechanisms in the actions of GLP-2 and LPS may be also implicated. In addition, either transcriptional or post-transcriptional mechanisms may involve participation of intermediates. For example, an effect of GLP-2 counteracting the inflammation and oxidative stress generated by LPS could explain, at least in part, its beneficial effects. The fact that inflammation and oxidative stress impair ABC transporter function in different tissues (Petrovic et al., 2007) and that GLP-2 protects the intestine from alterations associated with inflammation and oxidative stress (Drucker and Yusta, 2014) support such possibility.

In an attempt to characterize the mechanisms involved in the beneficial actions of GLP-2 on transporter function, we first considered the eventual occurrence of post-translational regulation. Several studies demonstrated that ABC transporters are subject to post-translational regulation associated with misslocalization (Roma et al., 2008). Impairment of transport activity due to endocytic internalization of ABC transporters is one of the possible mechanisms (Mottino et al., 2007; Sekine et al., 2008). We analyzed the localization of Mrp2 and P-gp by confocal microscopy. The tight junction proteins ZO-1 and occludin and the nuclei were also labeled and used as reference of eventual alterations of Mrp2 and P-gp. We noted a significant alteration in the pattern of localization of both transporters in the LPS group, not present in the other groups. Alterations consisted of irregular detection of the transporters in the surface of the villus, with partial loss of fluorescence signal. Additionally, fluorescence detection of both Mrp2 and P-gp in proximity of the nuclei suggests endocytic internalization of the transporters. These findings could explain loss of transporter activity in addition to the down-regulation detected by Western blotting. Mrp2 is

likely the most affected since its fluorescence was almost undetectable on the surface of the villus. This may explain why the presence of an Mrp inhibitor in the activity assays did not further decrease DNP-SG transport in the LPS group. Notably, in spite of the severity of these alterations, localization of Mrp2 was completely normalized by GLP-2 under the preventive protocol. More importantly, transport function of both transporters was completely normalized by this same protocol.

Mrp2 and P-gp are expressed at the surface of the enterocyte. Inflammatory processes may result in loss of the intestinal epithelium to variable degree, the upper regions of the villi being the most susceptible. We evaluated if LPS treatment affected the epithelium integrity and whether GLP-2 was able to protect from such alterations. This is of particular interest considering that GLP-2 was found to regulate epithelial proliferation and regeneration under several physiological and pathophysiological conditions (Cazzo et al., 2016; Drucker and Yusta, 2014; Kissow, 2015). Hematoxylin-eosin images show that the villi from LPS group presented irregular shape and look wider than those from control rats, most likely as a consequence of edema and consistent with an inflammatory process. However, these same images together with Western blot detection of villin suggest preservation of the integrity of the villus surface. These results indicate that loss of Mrp2 and P-gp function in LPS group may not result from erosive alterations of the epithelium but rather to more specific regulations acting on both transporters. Absence of apparent alterations in the structure of the villi in LPS + GLP-2 group is consistent with protective effect of GLP-2, as previously reported (Sigalet et al., 2007; Zhang et al., 2008).

Inflammation and oxidative stress resulting from different pathophysiological situations are known to regulate intestinal ABC transporters, including Mrp2 and P-gp (Arana et al., 2016; Ikemura et al., 2009). Although the signaling pathways of these regulations are only partially understood, they usually result in transcriptional modulation of the ABC transporters. LPS causes inflammation and oxidative stress, with participation of mediators such as the pro-inflammatory cytokines IL-1β, IL-6 and TNFα, NF-κB and iNOS (Bhattacharyya et al., 2004). Thus, it is possible that one or more of these mediators were involved in down-regulation of Mrp2 and P-gp as currently observed for the LPS group. In an attempt to evaluate this hypothesis, we measured inflammation and oxidative stress makers 24 h after administration of LPS or its vehicle. Notably, GLP-2 was able to prevent the LPS-induced increases of IL-1β and GSSG/GSH ratio detected in mucosal homogenate. Thus, these findings may be causally related with prevention of alteration of Mrp2 and P-gp. However, more direct evidence is necessary to confirm this postulate.

In contrast to the prevention protocol, the reversion protocol consisting of administration of two doses of GLP-2, after LPS has been injected, failed to demonstrate any compensatory effect on alteration of Mrp2 and P-gp expression. Inflammatory and oxidative stress processes may be already triggered by the time the first dose of GLP-2 was administered. For example, the levels of pro-inflammatory cytokines were found to be increased as soon as 3 h after LPS administration (Li et al., 2016; Moriez et al., 2005). It is possible that high levels of GLP-2 need to precede those alterations in order to exert a protective action, a condition compatible with the prevention protocol. In the particular case of Mrp2, GLP-2 administered alone failed to produce any change in protein or mRNA expression, in contrast to the induction observed for the prevention protocol. Clearly, more than two doses of GLP-2 are needed to modulate Mrp2 expression. This may additionally explain why the reversion protocol failed to restore Mrp2 alterations.

A long-acting analog of GLP-2, teduglutide, was approved for the treatment of short bowel syndrome in USA and Europe (Gattex and Revestive, respectively; NPS Pharmaceuticals, Shire North American Group), and different clinical studies were performed since (Carter et al., 2017; Jeppesen et al., 2013; Madsen et al., 2013). This medication was proven efficient to improve growth and nutrient absorption deficiencies and at the same time to reestablish the epithelial barrier function. Our work suggests that modulation of two relevant apical efflux transporters may represent an additional beneficial effect of GLP-2 under inflammatory or oxidative stress conditions, contributing to restore the transcellular barrier, which in turn restricts absorption of potentially toxic compounds.

GLP-2 has also been suggested as a potential therapeutic agent to prevent chemotherapy-induced diarrhea (CID) (Mayo et al., 2017). CID is a high-incidence side effect of chemotherapy that significantly reduces the quality of life of patients and usually causes an interruption or termination of the treatment. CID is thought to be a consequence of mucositis, which in turn has been associated to activation of Toll-like receptors. The observation that GLP-2 can prevent inflammation and ameliorate intestinal morphology alterations induced by LPS suggests that GLP-2 could also prevent mucositis, and supports the idea that it may be suitable for CID alleviation.

In conclusion, we demonstrated for the first time that GLP-2 can prevent down-regulation of intestinal expression of Mrp2 and P-gp and loss of their respective activities in endotoxemic rats. GLP-2 was also able to prevent internalization of both transporters from the apical membrane of the enterocyte to intracellular compartments. Increased production of IL-1β and oxidative stress in LPS group were also counterbalanced by GLP-2 thus constituting potential targets of GLP-2 beneficial effects.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tox.2017.08.007.

References

Arana, M.R., Tocchetti, G.N., Domizi, P., Arias, A., Rigalli, J.P., Ruiz, M.L., Luquita, M.G., Banchio, C., Mottino, A.D., Villanueva, S.S.M., 2015. Coordinated induction of GST and MRP2 by cAMP in Caco-2 cells: role of protein kinase A signaling pathway and toxicological relevance. Toxicol. Appl. Pharmacol. 287, 178–190. http://dx.doi.org/10.1016/j.taap.2015.06.003.

Arana, M.R., Tocchetti, G.N., Rigalli, J.P., Mottino, A.D., Villanueva, S.S.M., 2016. Physiological and pathophysiological factors affecting the expression and activity of the drug transporter MRP2 in intestine. Impact on its function as membrane barrier. Pharmacol. Res. 109, 32–44. http://dx.doi.org/10.1016/j.phrs.2016.04.014.

Arias, A., Villanueva, S.S.M., Ruiz, M.L., Luquita, M.G., Veggi, L.M., Pellegrino, M., Vore, M., Catania, V.A., Mottino, A.D., Pellegrino, J.M., Vore, M., Catania, V.A., Mottino, A.D., 2009. Regulation of expression and activity of rat intestinal multidrug resistance-associated protein 2 by cholestatic estrogens. Drug Metab. Dispos. 37, 1277–1285. http://dx.doi.org/10.1124/dmd.108.025643.thus.

Bhattacharyya, J., Biswas, S., Datta, A.G., 2004. Mode of action of endotoxin: role of free radicals and antioxidants. Curr. Med. Chem. 11, 359–368. http://dx.doi.org/10.2174/0929867043456098.

Blokzijl, H., Vander Borght, S., Bok, L.I.H., Libbrecht, L., Geuken, M., Van Den Heuvel, F.A.J., Dijkstra, G., Roskams, T.A.D., Moshage, H., Jansen, P.L.M., Faber, K.N., 2007. Decreased P-glycoprotein (P-gp/MDR1) expression in inflamed human intestinal epithelium is independent of PXR protein levels. Inflamm. Bowel Dis. 13, 710–720. http://dx.doi.org/10.1002/ibd.20088.

Cameron, H.L., Perdue, M.H., 2005. Stress impairs murine intestinal barrier function: improvement by glucagon-like peptide-2. J. Pharmacol. Exp. Ther. 314, 214–220. http://dx.doi.org/10.1124/jpet.105.085373.

Cameron, H.L., Yang, P.C., Perdue, M.H., 2003. Glucagon-like peptide-2-enhanced barrier function reduces pathophysiology in a model of food allergy. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G905–G912. http://dx.doi.org/10.1152/ajpgi.00231.2002.

Carter, B.A., Cohran, V.C., Cole, C.R., Corkins, M.R., Dimmitt, R.A., Duggan, C., Hill, S., Horslen, S., Lim, J.D., Mercer, D.F., Merritt, R.J., Nichol, P.F., Sigurdsson, L., Teitelbaum, D.H., Thompson, J., Vanderpool, C., Vaughan, J.F., Li, B., Youssef, N.N.,

Venick, R.S., Kocoshis, S.A., 2017. Outcomes from a 12-Week, open-label, multicenter clinical trial of teduglutide in pediatric short bowel syndrome. J. Pediatr. 181, 102–111. http://dx.doi.org/10.1016/j.jpeds.2016.10.027.

Cazzo, E., Gestic, M.A., Utrini, M.P., Chaim, F.D.M., Geloneze, B., Pareja, J.C., Chaim, E.A., Magro, D.O., 2016. Glp-2: a poorly understood mediator enrolled in various bariatric/metabolic surgery-related pathophysiologic mechanisms. Arq. Bras. Cir. Dig. (São Paulo) 29, 272–275. http://dx.doi.org/10.1590/0102-6720201600040014.

Collett, A., Higgs, N.B., Gironella, M., Zeef, L.A.H., Hayes, A., Salmo, E., Haboubi, N., Iovanna, J.L., Carlson, G.L., Warhurst, G., 2008. Early molecular and functional changes in colonic epithelium that precede increased gut permeability during colitis development in mdr1a(−/−) mice. Inflamm. Bowel Dis. 14, 620–631. http://dx.doi.org/10.1002/ibd.20375.

Connor, E.E., Evock-Clover, C.M., Wall, E.H., Baldwin, R.L., Santin-Duran, M., Elsasser, T.H., Bravo, D.M., 2016. Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals. Domest. Anim. Endocrinol. 56, S56–S65. http://dx.doi.org/10.1016/j.domaniend.2015.11.008.

Dietrich, C.G., 2003. ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut 52, 1788–1795. http://dx.doi.org/10.1136/gut.52.12.1788.

Dong, C.X., Zhao, W., Solomon, C., Rowland, K.J., Ackerley, C., Robine, S., Holzenberger, M., Gonska, T., Brubaker, P.L., 2014. The intestinal epithelial insulin-like growth factor-1 receptor links glucagon-like peptide-2 action to gut barrier function. Endocrinology 155, 370–379. http://dx.doi.org/10.1210/en.2013-1871.

Drucker, D.J., Yusta, B., 2014. Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. Annu. Rev. Physiol. 76, 10. http://dx.doi.org/10.1146/annurev-physiol-021113-170317. (1-10.23).

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106, 207–212.

Iida, A., Ouchi, S., Oda, T., Aketagawa, J., Ito, Y., Takizawa, Y., Tomita, M., Hayashi, M., 2015. Changes of absorptive and secretory transporting system of (1–3) beta-D-glucan based on efflux transporter in indomethacin-induced rat. Eur. J. Drug Metab. Pharmacokinet. 40, 29–38. http://dx.doi.org/10.1007/s13318-014-0174-0.

Ikemura, K., Iwamoto, T., Okuda, M., 2009. Altered functions and expressions of drug transporters in liver, kidney and intestine in disorders of local and remote organs: possible role of oxidative stress in the pathogenesis. Expert Opin. Drug Metab. Toxicol. 5, 907–920. http://dx.doi.org/10.1517/17425250903008525.

Jacobs, L.R., Bloom, S.R., Dowling, R.H., 1981. Response of plasma and tissue levels of enteroglucagon immunoreactivity to intestinal resection, lactation and hyperphagia. Life Sci. 29, 2003–2007.

Jeppesen, P.B., Pertkiewicz, M., Forbes, A., Pironi, L., Gabe, S.M., Joly, F., Messing, B., Loth, S., Youssef, N.N., Heinze, H., Berghöfer, P., 2013. Quality of life in patients with short bowel syndrome treated with the new glucagon-like peptide-2 analogue teduglutide – analyses from a randomised, placebo-controlled study. Clin. Nutr. 32, 713–721. http://dx.doi.org/10.1016/j.clnu.2013.03.016.

Kalitsky-Szirtes, J., Shayeganpour, A., Brocks, D.R., Piquette-Miller, M., 2004. Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats. Drug Metab. Dispos. 32, 20–27. http://dx.doi.org/10.1124/dmd.32.1.20.

Kissow, H., 2015. Glucagon-like peptides 1 and 2: intestinal hormones implicated in the pathophysiology of mucositis. Curr. Opin. Support Palliat. Care 9, 196–202. http://dx.doi.org/10.1007/400_2009_35.

Kullak-Ublick, G.A., Becker, M., 2003. Regulation of drug and bile salt transporters in liver and intestine. Drug Metab. Rev. 35, 305–317. http://dx.doi.org/10.1081/DMR-120026398.

Lambertucci, F., Motiño, O., Villar, S., Rigalli, J.P., de Luján Alvarez, M., Catania, V., Martín-Sanz, P., Carnovale, C.E., Quiroga, A.D., Francés, D.E., Ronco, M.T., 2017. Benznidazole, the trypanocidal drug used for Chagas disease, induces hepatic NRF2 activation and attenuates the inflammatory response in a murine model of sepsis. Toxicol. Appl. Pharmacol. 315, 12–22. http://dx.doi.org/10.1016/j.taap.2016.11.015.

Li, Z., Zhang, X., Zhou, H., Liu, W., Li, J., 2016. Exogenous S-nitrosoglutathione attenuates inflammatory response and intestinal epithelial barrier injury in endotoxemic rats. J. Trauma Acute Care Surg. 80, 977–984. http://dx.doi.org/10.1097/TA.0000000000001008.

Londero, A.S., Arana, M.R., Perdomo, V.G., Tocchetti, G.N., Zecchinati, F., Ghanem, C.I., Ruiz, M.L., Rigalli, J.P., Mottino, A.D., García, F., Villanueva, S.S.M., 2017. Intestinal multidrug resistance-associated protein 2 is down-regulated in fructose-fed rats. J. Nutr. Biochem. 40, 178–186. http://dx.doi.org/10.1016/j.jnutbio.2016.11.002.

Lowry, H.O., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275. http://dx.doi.org/10.1016/0304-3894(92)87011-4.

Madsen, K.B., Askov-Hansen, C., Naimi, R.M., Brandt, C.F., Hartmann, B., Holst, J.J., Mortensen, P.B., Jeppesen, P.B., Pertkiewicz, M., Forbes, A., Pironi, L., Gabe, S.M., Joly, F., Messing, B., Loth, S., Youssef, N.N., Heinze, H., Berghöfer, P., 2013. Quality of life in patients with short bowel syndrome treated with the new glucagon-like peptide-2 analogue teduglutide – analyses from a randomised, placebo-controlled study. Regul. Pept. 32, 713–721. http://dx.doi.org/10.1016/j.clnu.2013.03.016.

Mayo, B.J., Stringer, A.M., Bowen, J.M., Bateman, E.H., Keefe, D.M., 2017. Irinotecan – induced mucositis: the interactions and potential role of GLP – 2 analogues. Cancer Chemother. Pharmacol. 79, 233–249. http://dx.doi.org/10.1007/s00280-016-3165-9.

Moriez, R., Salvador-Cartier, C., Theodorou, V., Fioramonti, J., Eutamene, H., Bueno, L., 2005. Myosin light chain kinase is involved in lipopolysaccharide-induced disruption of colonic epithelial barrier and bacterial translocation in rats. Am. J. Pathol. 167, 1071–1079. http://dx.doi.org/10.1016/S0002-9440(10)61196-0.

Moriguchi, J., Kato, R., Nakagawa, M., Hirotani, Y., Ijiri, Y., Tanaka, K., 2007. Effects of lipopolysaccharide on intestinal P-glycoprotein expression and activity. Eur. J. Pharmacol. 565, 220–224. http://dx.doi.org/10.1016/j.ejphar.2007.02.058.

Mottino, A.D., Hoffman, T., Jennes, L., Vore, M., 2000. Expression and localization of multidrug resistant protein mrp2 in rat small intestine. J. Pharmacol. Exp. Ther. 293, 717–723.

Mottino, A.D., Hoffman, T., Jennes, L., Cao, J., Vore, M., 2001. Expression of multidrug resistance-associated protein 2 in small intestine from pregnant and postpartum rats. Am. J. Physiol. Gastrointest. Liver Physiol. 280, G1261–G1273.

Mottino, A.D., Hoffman, T., Crocenzi, F.A., Sánchez Pozzi, E.J., Roma, M.G., Vore, M., 2007. Disruption of function and localization of tight junctional structures and Mrp2 in sustained estradiol-17beta-D-glucuronide-induced cholestasis. Am. J. Physiol. Gastrointest. Liver Physiol. 293, G391–402.

Naud, J., Michaud, J., Boisvert, C., Desbiens, K., Leblond, F.A., Mitchell, A., Jones, C., Bonnardeaux, A., Pichette, V., 2007. Down-regulation of intestinal drug transporters in chronic renal failure in rats. J. Pharmacol. Exp. Ther. 320, 978–985. http://dx.doi.org/10.1124/jpet.106.112631.ance.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Panwala, C.M., Jones, J.C., Viney, J.L., 1998. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J. Immunol. 161, 5733–5744.

Petrovic, V., Teng, S., Piquette-miller, M., 2007. Vanja Petrovic, Shirley Teng, and Micheline Piquette-Miller. Rev. Lit. Arts Am. 7, 99–111.

Roma, M., Crocenzi, F., Mottino, A., 2008. Dynamic localization of hepatocellular transporters in health and disease. World J. Gastroenterol. 14, 6786–6801.

Sekine, S., Ito, K., Horie, T., 2008. Canalicular Mrp2 localization is reversibly regulated by the intracellular redox status. Am. J. Physiol. 295, G1035–G1041. http://dx.doi.org/10.1152/ajpgi.90404.2008.

Sigalet, D.L., Wallace, L.E., Holst, J.J., Martin, G.R., Kaji, T., Tanaka, H., Sharkey, K.A., 2007. Enteric neural pathways mediate the anti-inflammatory actions of glucagon-like peptide 2. Am. J. Physiol. Gastrointest. Liver Physiol. 293, G211–G221. http://dx.doi.org/10.1152/ajpgi.00530.2006.

Tappenden, K.A., 2014. Pathophysiology of short bowel syndrome. J. Parenter. Enter. Nutr. 38, 14S–22S. http://dx.doi.org/10.1177/0148607113520005.

Tocchetti, G.N., Rigalli, J.P., Arana, M.R., Villanueva, S.S., Mottino, A.D., 2016. Modulation of expression and activity of intestinal multidrug resistance-associated protein 2 by xenobiotics. Toxicol. Appl. Pharmacol. 303, 45–57. http://dx.doi.org/10.1016/j.taap.2016.05.002.

Turner, J.R., 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799–809. http://dx.doi.org/10.1038/nri2653.

Villanueva, S.S.M., Arias, A., Ruiz, M.L., Rigalli, J.P., Pellegrino, J.M., Vore, M., Catania, V.A., Mottino, A.D., 2010. Induction of intestinal multidrug resistance-associated protein 2 by glucagon-like Peptide 2 in the rat. J. Pharmacol. Exp. Ther. 335, 332–341. http://dx.doi.org/10.1124/jpet.110.171041.

Yan, J.K., Gong, Z.Z., Zhang, T., Cai, W., 2017. Sodium butyrate attenuates soybean oil-based lipid emulsion-induced increase in intestinal permeability of lipopolysaccharide by modulation of P-glycoprotein in Caco-2 cells. Biochem. Biophys. Res. Commun. 482, 791–795. http://dx.doi.org/10.1016/j.bbrc.2016.11.112.

Zhang, W., Zhu, W., Zhang, J., Li, N., Li, J., 2008. Protective effects of glucagon-like peptide 2 on intestinal ischemia-reperfusion rats. Microsurgery 28, 285–290. http://dx.doi.org/10.1002/micr.20491.