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ORIGINAL ARTICLE

Basic Study

Glutamine prevents oxidative stress in a model of portal hypertension

Gilmara Pandolfo Zabot, Gustavo Franco Carvalhal, Norma Possa Marroni, Francielli Licks, Renata Minuzzo Hartmann, Vinícius Duval da Silva, Henrique Sarubbi Fillmann

Gilmara Pandolfo Zabot, Gustavo Franco Carvalhal, Henrique Sarubbi Fillmann, Department of Surgery, School of Medicine, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS 90610-000, Brazil

Norma Possa Marroni, Francielli Licks, Renata Minuzzo Hartmann, Department of Gastroenterology, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, RS 90035-903, Brazil

Vinícius Duval da Silva, Department of Pathology, School of Medicine, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS 90610-000, Brazil

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Correspondence to: Gilmara Pandolfo Zabot, Ms, Sci, Department of Surgery, School of Medicine, Pontifical Catholic University of Rio Grande do Sul; Duque de Caxias Street, 1012/19, Porto Alegre, RS 90610-000, Brazil. gilmarapandolfo@terra.com.br
Telephone: +55-51-999357780
Fax: +55-51-34787000

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Abstract

AIM

To evaluate the protective effects of glutamine in a model of portal hypertension (PH) induced by partial portal vein ligation (PPVL).

METHODS

Male Wistar rats were housed in a controlled environment and were allowed access to food and water *ad libitum*. Twenty-four male Wistar rats were divided into four experimental groups: (1) control group (SO) - rats underwent exploratory laparotomy; (2) control + glutamine group (SO + G) - rats were subjected to laparotomy and were treated intraperitoneally with glutamine; (3) portal hypertension group (PPVL) - rats were subjected to PPVL; and (4) PPVL + glutamine group (PPVL + G) - rats were treated intraperitoneally

with glutamine for seven days. Local injuries were determined by evaluating intestinal segments for oxidative stress using lipid peroxidation and the activities of glutathione peroxidase (GPx), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) after PPVL.

RESULTS

Lipid peroxidation of the membrane was increased in the animals subjected to PH ($P < 0.01$). However, the group that received glutamine for seven days after the PPVL procedure showed levels of lipid peroxidation similar to those of the control groups ($P > 0.05$). The activity of the antioxidant enzyme GTx was decreased in the gut of animals subjected to PH compared with that in the control group of animals not subjected to PH ($P < 0.01$). However, the group that received glutamine for seven days after the PPVL showed similar GTx activity to both the control groups not subjected to PH ($P > 0.05$). At least 10 random, non-overlapping images of each histological slide with 200 × magnification (44 pixel = 1 μm) were captured. The sum means of all areas, of each group were calculated. The mean areas of eNOS staining for both of the control groups were similar. The PPVL group showed the largest area of staining for eNOS. The PPVL + G group had the second highest amount of staining, but the mean value was much lower than that of the PPVL group ($P < 0.01$). For iNOS, the control (SO) and control + G (SO + G) groups showed similar areas of staining. The PPVL group contained the largest area of iNOS staining, followed by the PPVL + G group; however, this area was significantly smaller than that of the group that underwent PH without glutamine ($P < 0.01$).

CONCLUSION

Treatment with glutamine prevents gut mucosal injury after PH in rats.

Key words: Portal hypertension; Endothelial nitric oxide synthase; Glutamine; Glutathione peroxidase; Inducible nitric oxide synthase; Lipid peroxidation

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Core tip: Portal hypertension (PH) is characterized by an increased portal pressure gradient. The progressive increase in portal pressure leads to the formation of portosystemic shunts and intestinal hypoxia. Many enzymes have been implicated in this process, among them endothelial nitric oxide synthase and inducible nitric oxide synthase. Some substances, such as glutamine, have been studied as protective agents against oxidative stress. In an experimental model of PH induced by partial portal vein ligation, we have found that glutamine reduced lipid peroxidation and preserved intestinal glutathione peroxidase activity, suggesting a protective role of this amino acid in the setting of PH.

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INTRODUCTION

Portal hypertension (PH) is a clinical entity defined by a haemodynamic increased portal pressure gradient, directly related to the portal blood flow and vascular resistance[1,2]. Anatomically, the causes of PH can be classified as pre-hepatic (portal vein thrombosis or splenic), intra-hepatic (cirrhosis) and post-hepatic (Budd-Chiari syndrome)[3].

PH triggers disorders of the gastrointestinal tract, compromising the function of the small intestine. Gastrointestinal bleeding is present in 80%-90% of patients with PH due to the progressive dilatation of the vessels, which rupture and lead to haemorrhage[4]. Such a high bleeding rate points to the need to study the pathophysiology of PH to minimize its consequences. The partial ligation model of the portal vein is the model most widely used to study pre-hepatic PH; it was developed by Sikuler in 1985, and several experimental studies have shown that, in animals, partial portal vein ligation (PPVL) produces abnormalities that are equivalent to those of PH in humans[5].

The development of hyper dynamic circulation can cause structural changes in the intestinal wall as well as spontaneous bacterial infections and sepsis[6]. The increase in splanchnic vascular resistance and venous flow leads to congestion in the portal system, causing an intermittent intestinal hypo perfusion[7]. Oxidative stress damages the integrity of the intestinal mucosa and leads to lipid peroxidation[8,9]. Likewise, there is an increased production of nitric oxide (NO) in this hyper dynamic state, producing nitrosative stress[10]. NO is the main mediator of cytotoxic immune cells and plays a messenger/modulatory role in many important biological processes[11]. However, it is highly toxic, especially in cases of oxidative stress and in situations of antioxidant system deficiency[12]. The physiological and pathophysiological effects of the nitric oxide isoforms [endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS)] are related to their concentration levels[13]. When operating at low concentrations, these isoforms behave as messengers and cell protection factors (antioxidants), interacting with transition metals and other free radicals. Under high concentrations and upon forming dinitrogen trioxide (N_2O_3) or peroxynitrite (ONOO), NO behaves as an active species of nitrogen, responsible for numerous cytotoxic actions in a framework known as nitrosative stress[14]. A high NO concentration was

observed in PH, this increase being intimately related to the development of hyper dynamic circulation and oxidative stress[15].

To protect against the damage posed by oxidative stress, we need a system to prevent the formation of free radicals and neutralize oxidative damage, a function performed by antioxidants. The enzymatic system is one of the most important defence systems because it catalytically removes free radicals and other reactive species. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are some of the most prominent antioxidant enzymes. The peroxidase GPx enzyme is characteristically located in the cytosol and mitochondrial matrix[16-18].

Similar to the enzymes involved in removing free radicals, there are other substances with antioxidant properties capable of preventing oxidative chain reactions, such as those involved in lipid peroxidation (LPO). These substances are known as non-enzymatic antioxidant defences[19,20].

Glutamine is the most commonly found free amino acid in plasma, acting on oxygen-free radicals and playing an important role in attenuating inflammatory bowel disease[21]. It is used for hepatic synthesis of urea, for renal ammoniagenesis and for gluconeogenesis and also serves as the main respiratory fuel for many cell types[8]. Thus, it is vital to the regulation of the intracellular oxidative balance[22].

The progression of different diseases is triggered by oxidative stress. In PH, it promotes the development of collateral circulation. Thus, antioxidant therapy appears to be a promising strategy to minimize the complications of PH[15].

The objectives of this study were to evaluate the effects of glutamine in rats with pre-hepatic PH submitted to an experimental model of PPVL, to assess LPO and determine the activity of the enzyme GTx in the intestine. Additionally, we aimed to quantify the expression of the enzymes eNOS and iNOS in the intestine through immunohistochemistry.

MATERIALS AND METHODS

Ethics

Animal care was performed in compliance with the normative resolution 04/97 of the Research and Ethics Committee of the Health Research Group and Graduate Teaching Hospital of Porto Alegre (Hospital de Clínicas de Porto Alegre-HCPA)[23].

Animals

Twenty-four male Wistar rats weighing between 250 and 350 grams, were used from the State Foundation for Research and Production in Health (FEPPS-RS). The animals were divided into 4 groups of 6. During the experiment, the animals were kept in plastic boxes of 47 cm × 34 cm × 18 cm lined with wood, in a cycle of 12 h light/dark and temperature between 20 and 25 ℃. Water and feed are given *ad libitum*.

Surgical procedures

After trichotomy, rats were anaesthetized with ketamine and xylazine solution [45 mg/kg intraperitoneally (ip)]. After midline laparotomy, the bowel loops were removed gently and were covered with gauze moistened with saline, and then, the portal vein was isolated. A 20G needle was placed on the portal vein and tied with 3.0 silk sutures. Subsequently, the needle was gently removed, and the occurrence of portal vein thrombosis was noted[5]. Sham-operated (SO) control animals underwent the same procedures but without partial ligature of the portal vein. After surgery, the animals were treated with glutamine or saline solution, depending on the group**.** Glutamine was administered beginning on day 8 after surgery, intraperitoneally, at a dosage of 0.75 g/kg for 7 d[12]. Control animals received vehicle (saline, 0.9% NaCl) in a volume of 0.6 mL intraperitoneally for the same period. At the end of the experiments, intestinal segments (10 cm) were removed for histological examination and biochemical studies.

Experimental groups

Rats were randomly allocated into the following groups: (1) control group (SO): rats were subjected to the simulation of surgery and vehicle administration (NaCl) (n = 6); (2) control + glutamine group (SO + G): rats were subjected to the simulation of surgery and glutamine administration (n = 6); (3) PH group (PPVL): rats were subjected to PPVL and vehicle administration (NaCl) (n = 6); (4) PPVL + glutamine group (PPVL + G): rats were subjected to PPVL and glutamine administration (n = 6).

Assessment of lipid peroxidation

Thiobarbituric acid reactive substances: Tissue samples were placed in test tubes, and solutions were added in the following order: 0.75 mL of 10% trichloroacetic acid (TCA), 0.25 mL of homogenate, 0.5 mL of 0.67% thiobarbituric acid (TBA), and 0.25 mL of distilled water. Thiobarbituric acid reactive substances (TBARS) were measured by heating the homogenate with thiobarbituric acid and then measuring the consequent formation of a coloured product in a spectrophotometer at 535 nm. The coloration is due to the presence of malondialdehyde and other substances from biological lipid peroxidation[24].

GTx activity

The determination of selenium glutathione peroxidase was based on the method of Guntzler Flohé and consisted of measuring the nicotinamide adenine dinucleotide phosphate dehydrogenase (NADPH) consumption rate in a system containing total glutathione (GSH), wherein the oxidation is recorded spectrophotometrically at a wavelength of 340 nm. The

GPx activity can be studied by measuring the NADPH consumption rate in a system containing GSH[25].

This technique consists of determining the activity of the enzyme spectrophotometrically by measuring the rate of oxidation of NADPH in a reaction.

To this end, 2.7 mL of phosphate regulating solution of Na^+ and K^+ (100 mmol/L, pH 7.0) was placed in a quartz cuvette with 50 μL of NADPH (10 mmol/L), 150 μL of butylhydroperoxide (BOOH) (10 mmol/L) and 50 μL of glutathione reductase (12 U/mL). The mixture was read for 1 min and was identified as the baseline, followed by the addition of 50 μL of GSH (100 mmol/L) and 50 mL of homogenate. The samples were incubated at 25 ℃ for 5 min and then absorbance was read at 340 nm. The activity was expressed in nmol/min/mg protein[25].

Evaluation of eNOS and iNOS

For the preparation of the slides and subsequent immunohistochemical analysis, 3-μm-thick sections were prepared using a microtome (Leica SM 2000R, Germany). The sections were placed on slides pre-treated with HistoGrip (Zymed, United States) and were left in the oven at 60 ℃ for 24 h.

The sections were then deparaffinized by incubation with xylene for 10 min three times, followed by rehydration with a sequence of decreasing concentrations of ethanol (absolute, 90%, 80% and 70%) for 3 min per dilution. Next, the sections were washed three times in distilled water.

Antigen retrieval was performed by heating in a pTLINK platform (DAKO) and then treating the slides for 40 min at 98 ℃ with the Envision Flex high pH antigen retrieval solution (DAKO). Immediately thereafter, the slides were washed with PBS buffer at a pH of 7.2. Endogenous peroxidase was blocked by incubation in a solution of 3% H_2O_2 in methanol for two 15-min intervals, followed by washing three times with PBS buffer at a pH of 7.2.

The sections were then incubated using a Sequenza (Thermo Shandon, United States) immunostaining station, were left for 2 h at room temperature and then were diluted with the dilution solution (Antibody Diluent with background reducing components, Dako, United States) and the following antibodies: Anti-eNOS (Thermo Scientific, PA3-031A, United States) 1:800, anti-iNOS (PA5-16855 Thermo Scientific, United States) 1:800. After incubation with the primary antibody, the sections were washed three times with PBS buffer at a pH of 7.2. For the amplification of the antigen-antibody reaction for anti-eNOS, we used the HRP Envision Flex System (Dako, United States) in accordance with the manufacturer's recommendations. Next, the slides were washed with PBS buffer at a pH of 7.2 and were incubated with diaminobenzidine solution (Dako Liquid DAB Substrate Chromogen System, United States) for 5 min. After washing in distilled water, the slides were counterstained with Harris haematoxylin for 1 min and were washed with water until complete removal of the dye, followed by incubation in a 37-mmol/L ammonia solution for 15 s. Finally, the slides were dehydrated in absolute ethanol (four 2-min incubations) and were treated twice with xylene for 5 min. The slides were then mounted with Entellan synthetic medium (Merck, Germany)[20,26,27].

Analysis of digital images

We used a digital analysis system composed of a Zeiss Axioskop 40 microscope (Oberkochen, Germany) with Neofluar lenses connected by a Roper Scientific video camera (Media Cybernetics, Rockville, United States) to a computer with an Image Capture Pro kit (Media Cybernetics, Rockville, MD, United States) capture card. Image-Pro Plus version 7.0 (Media Cybernetics, Rockville, United States) was used to analyse the digital images. Images were captured in the TIFF (True Image File Format) format without compression by the same examiner with the same light intensity pattern for all photos. Images were captured of at least fifteen random, non-overlapping fields for each histological slide at 200 × magnification (44 pixel = 1 μm). The hot spot method was used to select fields on slides with focal positivity for the markers. Colour selection was performed interactively by three trained observers and was then applied to all samples by the automated digital image analysis system. The initial area considered was 0.01 cm.

Statistical analysis

Quantitative data were initially described as the means and SE. For comparison of the groups, one-way analysis of variance (ANOVA) was used, followed by the SNK post hoc test (Student-Newman-Keuls). The level of significance for the experiment was $P = 0.05$. Data were analysed using SPSS software version 22.0. A biomedical statistician performed the statistical review.

RESULTS

Evaluation of lipid peroxidation

Oxidative stress, quantified by gut membrane lipid peroxidation was increased in the group of animals subjected to PPVL ($P < 0.01$). On the other hand, animals that received glutamine for seven days after the procedure exhibited levels of lipid peroxidation similar to those of the control groups (animals not subjected to PPVL and animals receiving glutamine without PPVL). These levels were also significantly different from those of the group of animals that was submitted solely to PPVL ($P < 0.01$; Figure 1).

GTx activity

The activity of GTx was decreased in the gut of animals subjected to PPVL compared with that of animals not subjected to PPVL ($P < 0.01$; Figure 2). Importantly,

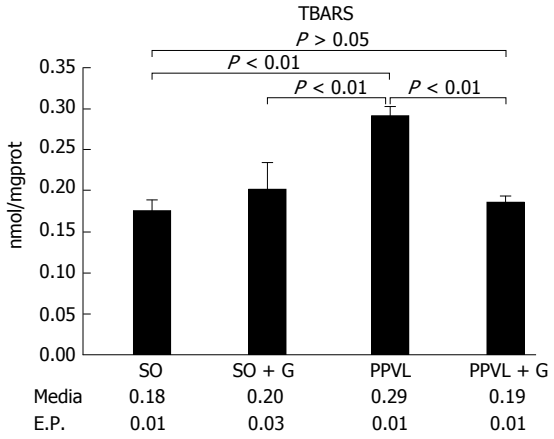

Figure 1 Lipid peroxidation (thiobarbituric acid reactive substances). TBARS: Thiobarbituric acid reactive substances; SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine.

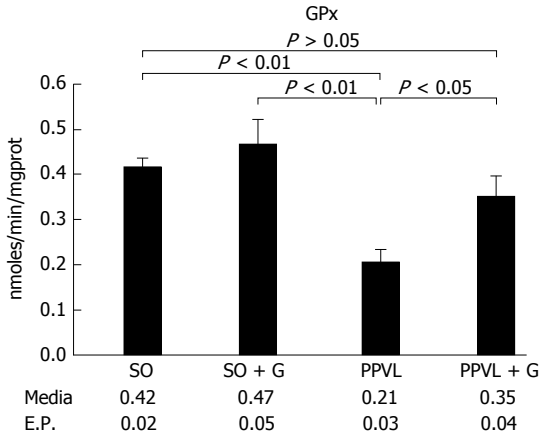

Figure 2 Glutathione peroxidase. SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine; GPx: Glutathione peroxidase.

the group that received glutamine for seven days after PPVL showed similar GTx activity to that of groups without PPVL. The difference in GTx activity in animals subjected to PPVL and in those that received glutamine after PPVL ($P < 0.05$), suggests that glutamine is a protective factor for PH.

Evaluation of eNOS

For digital analysis of the images, the program Image-Pro Plus version 7.0 (Media Cybernetics, Rockville, United States) was used. They were captured in the True Image File Format (TIFF) format, with at least ten fields, without random overlap, for each histological slide at 200 × (44 pixel = 1 μm). Slides with focal positivity for the markers were selected by the hot spot method. The initial area considered was 0.01 cm. The sum of the stained areas was calculated for each group. The control (SO) and control + G (SO + G) groups presented with similar areas of staining. The PPVL group presented the largest area. The second group with larger staining areas was the PPVL + G, but with values much lower than those of the PPVL group, resembling the area found in the control groups with and without glutamine. These results were statistically significant ($P < 0.01$). These results are shown in Figures 3 and 4.

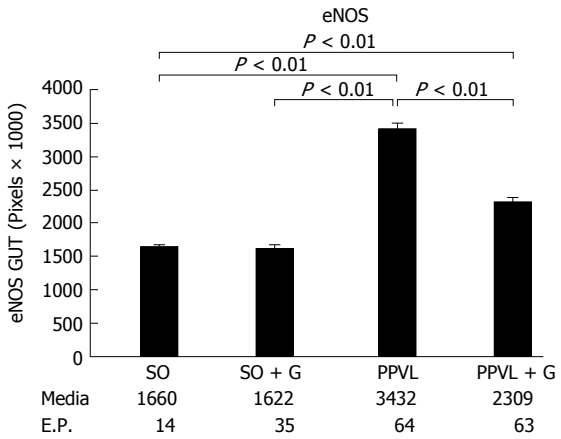

Figure 3 Immunohistochemical expression of endothelial nitric oxide synthase. SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine; eNOS: Endothelial nitric oxide synthase.

Evaluation of iNOS

Figures 5 and 6 show the immunohistochemical expression of iNOS. The control (SO) and control + G (SO + G) groups presented with similar areas of staining ($P > 0.05$). Regarding eNOS, the PPVL group also presented the largest stained area. The second group with a larger staining area was the PPVL + G, again with values much lower than those of the PPVL group. These results were also statistically significant ($P < 0.01$).

DISCUSSION

Glutamine promotes intracellular oxidative balance[28]. It is a precursor of glutathione, one of the most important non-enzymatic cellular antioxidants, and is the most abundant free amino acid in plasma, acting on oxygen-free radicals[29].

Other researchers have used the PPVL model to study pre-hepatic PH[5]. Our work demonstrates that glutamine treatment exerts important protective effects in pre-hepatic PH in an animal model. We have shown that after PPVL glutamine: (1) reduced oxidative stress determined by the lipid peroxidation of the intestinal mucosa; (2) maintained levels of GTx activity; and (3) reduced the expression of eNOS and iNOS.

We have observed greater levels of lipid peroxidation in the group of animals that underwent PPVL. Glutamine significantly decreased lipid peroxidation in the PPVL model. Schimpl *et al*[8] evaluated pre-hepatic PH with a model of common bile duct ligation (CBDL). They evaluated the effects of glutamine and allopurinol on bacterial translocation in PH and obstructive jaundice. These authors concluded that, in PH and common bile duct ligation (CBDL) in rats, there was significant

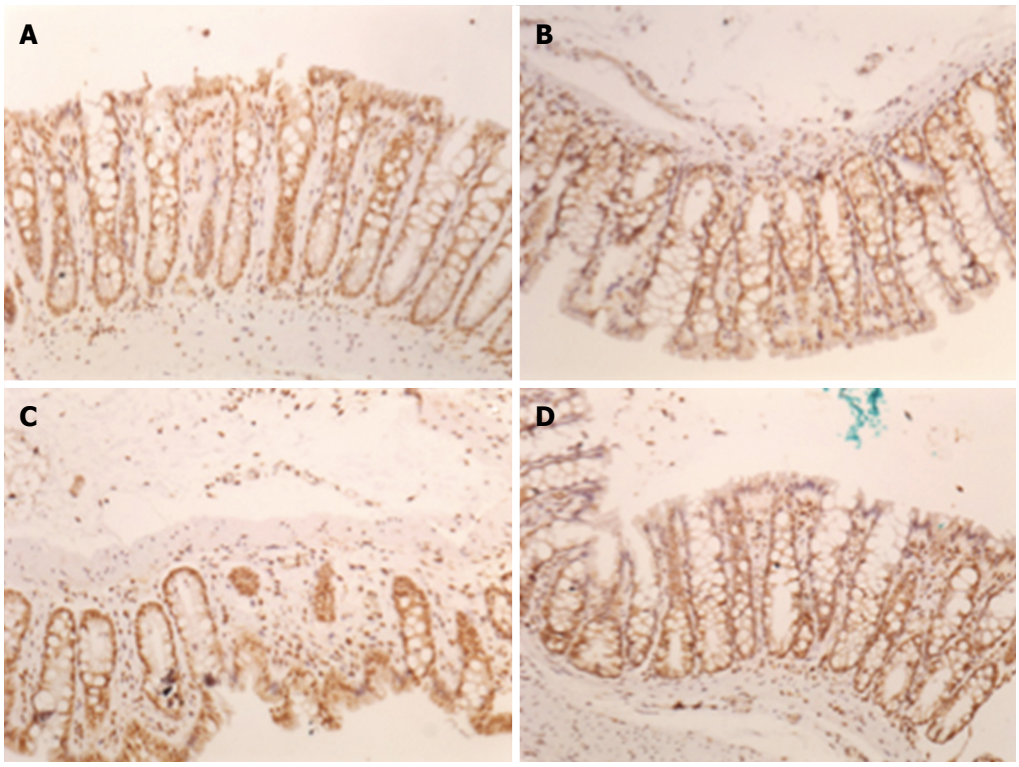

Figure 4 Digital images of the immunohistochemical expression of endothelial nitric oxide synthase (photomicrography, × 200). A: SO; B: SO + G; C: PPVL; D: PPVL + G. SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine.

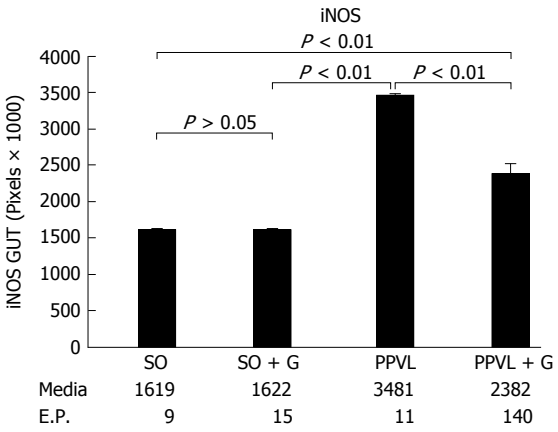

Figure 5 Immunohistochemical expression of inducible nitric oxide synthase. SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine; iNOS: Inducible nitric oxide synthase.

bacterial translocation, ileal mucosal lipid peroxidation, and neutrophil derived myeloperoxidase (MPO) activity. Bacterial translocation, ileal mucosal malondialdehyde (MDA) concentrations and MPO activities were significantly reduced by the combined use of allopurinol and glutamine.

Huang *et al*[30] studied the oxidative stress related to intrahepatic PH after ligature of the bile duct in rats. They identified an increase in the MDA levels in plasma as well as reduced levels of plasma GTx. We have found reduced levels of GTx activity in animals submitted to PPVL compared with controls (SO and SO + G). When glutamine was administered intraperitoneally to animals submitted to PPVL, there was a less pronounced reduction if GTx activity levels. Rodríguez-Vilarrupla *et al*[17] stressed the importance of antioxidants as enzymatic systems in PH syndrome. The effects of glutamine on eNOS activity were already investigated Marques *et al*[22]; however, these authors have used a model of PH gastropathy. They have also evaluated the activity of the nitric oxide (eNOS) by immunohistochemistry. In their study, lipid peroxidation and NO were significantly increased in PPVL, but the addition of glutamine has attenuated eNOS expression[22]. Oxidative stress was also evidenced by Gonzales *et al*[31] in a pre-hepatic PPVL rat model, through an increased concentration of TBARS. Additionally, these authors have shown a decrease in the antioxidant enzymes SOD, CAT and glutathione peroxidase (GTx). Moreover, they studied the antioxidant role of haem oxygenase-1 (HO-1) and suggested a beneficial role of HO-1 overexpression.

eNOS favours the decrease of blood pressure and assists in the inhibition of platelet aggregation. iNOS forms NO induced by certain cytotoxins, being closely related to defensive body processes[14]. In our study, the PH group (PPVL) showed the largest area of staining for eNOS. The PPVL + G group had the second highest amount of stained areas, but with a mean value much lower than that of the PH group ($P < 0.05$). For iNOS, the control (SO) and control + G (SO + G) groups showed similar areas of staining. The

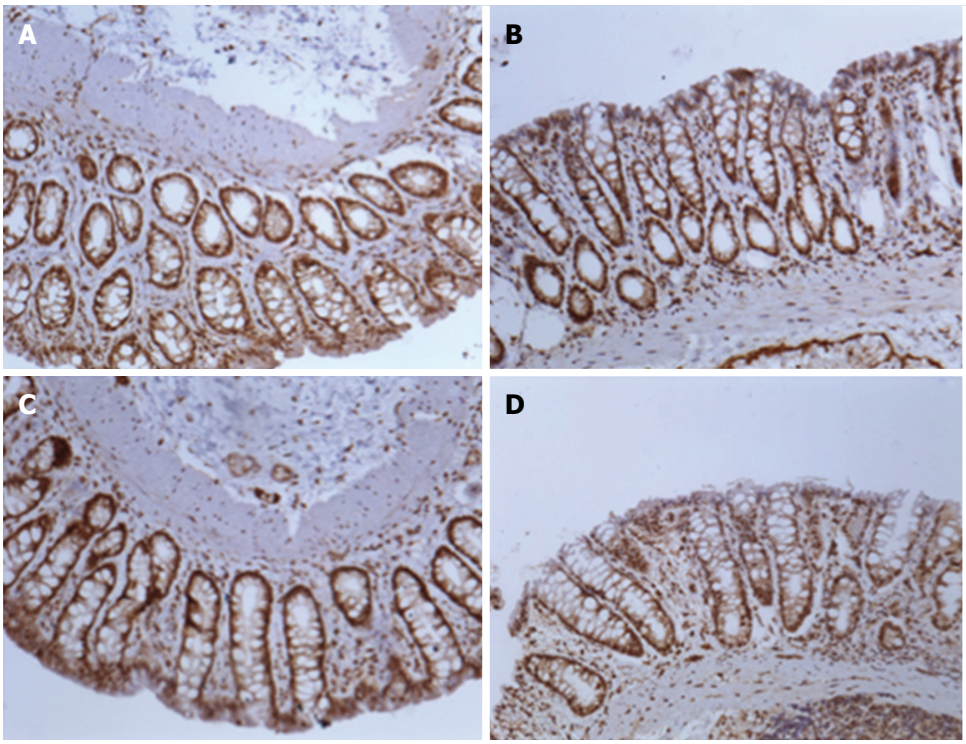

Figure 6 Digital images of the immunohistochemical expression of inducible nitric oxide synthase (photomicrography, × 200). A: SO; B: SO + G; C: PPVL; D: PPVL + G. SO: Control group; PPVL: Partial portal vein ligation; G: Glutamine; iNOS: Inducible nitric oxide synthase.

PH group (PPVL) contained the largest area of iNOS staining, followed by the PPVL + G group; however, this area was significantly lower than that of the group that underwent PH without glutamine (PPVL).

Jalan *et al*[32] tested the hypothesis that ammonia modulates human hepatic stellate cell activation *in vitro* and *in vivo*, in a BDL model, using l-ornithine phenylacetate. This substance significantly reduced the plasmatic levels of ammonia and portal pressure, which was associated with increased eNOS activity[32].

Iwakiri *et al*[14] stated that eNOS and iNOS have different roles; most commonly, eNOS prevents the occurrence of disease whereas iNOS favours its progress.

Kajita *et al*[33] observed that iNOS expression in vascular resident macrophages contributed to the circulatory dysfunction of splanchnic vascular smooth muscle contractions in PH rats[33]. Xu *et al*[34], in a study with male Sprague-Dawley rats submitted to intra-hepatic PH induced by the injection of CCl_4, noted that iNOS contributes to the haemodynamic alterations of PH secondary to increased levels of NO[34].

Oxidative stress is also responsible for hepatic encephalopathy. Pilar Carbonero-Aguilar *et al*[35], suggest that it may be resultant from increased ammonia concentration in the brain.

Arias *et al*[36], using an experimental model through the triple portal vein ligation method, demonstrated, for the first time, a relationship between inflammation, astrocyte damage and neurons and cerebral metabolic compromise.

The use of antioxidants in the prevention or treatment of various diseases related to oxidative stress seems to be a viable alternative in the attempt to minimize or reverse damage. Glutamine was initially used prophylactically in patients undergoing radiotherapy, resulting in a decreased incidence and decreased severity of actinic enteritis[37]. This substance was most extensively investigated in relation to the oxidative stress caused by exercise. Cruzat *et al*[38] showed that, in exhaustive exercise, there is a significant reduction in serum glutamine. This decrease is accompanied by a significant increase in the inflammatory activity and oxidative stress levels measured by the lipid peroxidation rate. Glutamine has also been beneficial as a nutritional supplement in severely debilitated patients, such as those with multiple trauma or in those undergoing major surgery[39,40]. Antioxidant therapy improves the prognosis and reduces the overall complication rates in debilitated patients[41]. Tang *et al*[42] utilized TPN containing glutamine and recombinant human growth hormone in the postoperative care of patients after PH surgery. They found that this supplementation enhanced immune function, modulated the inflammatory response, and prevented the intestinal membrane atrophy.

This is the first publication describing the role of glutamine in the intestinal oxidative stress in an animal model of PPVL.

In conclusion, the present study demonstrates that intraperitoneally administration of glutamine

at a dose of 0.75 g/kg during 7 d, starting on the 8th postoperative day after PPVL, prevented lipid peroxidation and maintained GTx levels. The expression of eNOS and iNOS were reduced upon intraperitoneal glutamine during the 7 d after PPVL in rats. Our work confirms the findings of previously published experimental research on glutamine as a protective factor in murine models of PH. Additional research is necessary to ascertain the benefits of glutamine as protective element against intestinal disorders secondary to PH in humans.

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COMMENTS

Background

Portal hypertension (PH) is often secondary to the obstruction of the intra- or extra-hepatic portal flow. The progressive increase in pressure in the portal system leads to dilation of the blood vessels, with consequent formation of porto-systemic shunts. The development of hyperdynamic circulation can cause structural changes in the intestinal wall and lipid peroxidation as well as oxidative stress. The physiological and pathophysiological effects of nitric oxide isoforms are related to their concentration levels. Glutamine has been investigated as a potential preventive agent against damage caused by inflammatory processes caused by oxidative agents.

Research frontiers

Being the most abundant amino acid in the plasma, glutamine has many roles including the regulation of macrophage activity, the modulation of reactive oxygen species, the hepatic synthesis of urea, renal ammoniagenesis, and gluconeogenesis, serving also as fuel for many cell types.

Innovations and breakthroughs

The first studies with glutamine were in patients undergoing radiotherapy, when there was a decreased incidence and severity of actinic enteritis associated with its use. It was also observed that glutamine plays a major role in the immune defence barrier of the intestinal mucosa through its participation in the formation of immunoglobulins, especially IgA. Furthermore, it was demonstrated that glutamine decreases the inflammatory enterocolitis induced by methotrexate and reduces bacterial translocation in animals with abdominal sepsis. These data demonstrate that glutamine prevents lipid peroxidation after partial portal vein ligation (PPVL) in a murine model.

Applications

This study suggests that glutamine reduces oxidative stress by decreasing lipid peroxidation and by preserving glutathione peroxidase (GTx) levels. The immunohistochemical expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) were reduced upon intraperitoneal administration of glutamine for seven days starting on the eighth postoperative day after PPVL in rats.

Terminology

PH: a clinical entity defined by a haemodynamically increased portal pressure gradient that is directly related to the portal blood flow and vascular resistance. Lipid peroxidation: oxidative stress that causes an alteration in cell membrane permeability that favours bacterial translocation and the activation of the inflammatory response. GTx: an antioxidant enzyme that catalytically removes free radicals and other active oxidative species. eNOS and iNOS: isoforms of nitric oxide, which is a mediator of cytotoxic immune cells that have messenger/modulator roles in many important biological processes.

Peer-review

It is a well-writen paper. The current work evaluates the protective effects of glutamine in a model of PH induced by PPVL.

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