Prevention of bacterial translocation using Glutamine and Melatonin in small bowel ischemia and reperfusion in rats

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BACKGROUND: *Ischemia/reperfusion (IR) injury of the intestine is a major problem in abdominal pathological condition and is associated with a high morbidity and mortality. The purpose of the study is to determine whether Glutamine and Melatonin can prevent BT of small intestinal IR injury in rats.*
METHODS: *Forty Wistar-Albino rats with a weight of 200 to 250 g were used in the study. They were randomly divided into four groups (n = 10 for each group): sham operated group (Group I), IR group (Group II), IR+ Glutamine treatment group (Group III) and IR+ Melatonin treatment group(Group IV). All animals were given 10^{10} E. Coli by orogastric intubation 12 hours before sampling. Seventy-two hours after the first operation, mesenteric lymph node and blood samples were obtained and cultured. Two cc blood samples were obtained for a Polymerase chain reaction study. A piece of terminal ileum was also sampled for histopathologic examination.*
RESULTS: *Mesenteric lymph node and blood cultures of all control animals were positive for microbiological growth, and polymerase chain reaction results were positive in seven of the eight rats. Histopathologically, edema, vasodilatation and inflammatory cell infiltration were found to be less in the other groups in comparison to the control group. The incidence of bacterial translocation was decreased in all treatment groups as compared to the control group.*
CONCLUSIONS: *Glutamine and Melatonin reduced the incidence of BT in intestinal I/R. rats. These results suggest that Glutamine and Melatonin would be clinically useful in the treatment of intestinal I/R injury.*

KEY WORDS: Bacterial translocation, Glutamine, Ischemia/reperfusion injury, Intestine, Melatonin

Introduction

The primary functions of the intestine are to absorb nutrients and exclude food debris, bacteria and their products. The maintenance of these functions relies on the integrity of mucosal and barrier of intestine. Gut barrier failure, leading to the passage of viable enteric bacteria and endotoxins across the intestinal mucosal barrier to the mesenteric lymph nodes (MLN) and distant organs, has been termed bacterial translocation (BT) [1,2].

Ischemia-reperfusion (I/R) of the gut is a common event in a variety of clinical conditions, such as trauma, burn, septic shock, heart or aortic surgery, and liver or small bowel transplantation [3,4]. Intestinal I/R induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxins from within the bowel into the blood, an event that may initiate a systemic inflammatory response and the secretion and activation of inflammatory mediators, including cytokines, and development of remote organ damage and systemic shock [5-8].

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We aimed to investigate the effects of Glutamine and Melatonin on the BT incidence in rats submitted to bowel ischemia reperfusion injury.

Materials and methods

Forty Wistar-Albino rats with a weight of 200 to 250 g were used in the study. All of the experimental protocols were performed according to the guidelines for the ethical treatment of experimental animals.

Animals and experimental protocol

The rats were housed individually in cages and allowed free access to standard rat chow and water before and after the experiments. The animal rooms were windowless and under controlled temperature (22 ± 2°C) and lighting conditions. The animals were made to fast overnight before the experiments, but were given free access to water. They were randomly divided into four groups (n = 10 for each group): sham operated group (Group I), IR group (Group II), IR+ Glutamine treatment group (Group III) and IR+ Melatonin treatment group(Group IV). They were anesthetized by ketamine HCl 50 mg/ml and xylazine HCl 20 mg/ml applied intramuscularly to the back part of the right leg at 0.25 ml/100 g live weight. The operating field on the abdomen was shaved just before the operation, cleaned with 10% providone-iodine, and covered in a sterile way but leaving the incision area exposed. Using sterile instruments, a laparotomy was performed through an abdominal midline incision. In the Sham group(group 1), the rats received a 3-centimeter medium-length-wise laparotomy, and the small intestine was exposed. Subsequently, the superior mesenteric artery (SMA) was identified and dissected, and then the peritoneal cavity was closed. In the IR group, the rats underwent intestinal ischemia for 60 minutes through occlusion of the SMA with a micro vascular clamp. The occluding clamp was removed after ischemia for a reperfusion period for 2 hours. The sham and group 2 did not undergo any treatment. The related agent was given to the other groups for three days before sampling. Glutamine (Gln) 0.75 g/kg/day was given by intraperitoneal route to the Glutamine (Gln) group, and melatonin 20 mg/kg/day was given IM to the melatonin group. Twelve hours before sampling all animals were given 1 ml of the solution containing *Escherichia coli* 10^{10} colony-forming units per milliliter by orogastric intubation. The abdomen was opened again using a sterile technique 72 hours later. For PCR analysis, 2 ml blood samples were drawn from the IVC, MLN and blood samples obtained for culture and tissue samples from the terminal ileum were collected.

Microbiological study

Blood samples for culture were put into Pedi-Bact culture bottles and incubated at 37 °C. MLN samples were put into brain heart infusion agar for culture after crushing and homogenizing with forceps. The PCR of blood samples from test and control animals was performed as described earlier [17]. Briefly, DNA extraction from blood samples was carried out with a commercial DNA extraction kit (Wizard Genomic DNA Purification Kit; Promega, Madison, WI, USA). The presence of *E. coli* genomic DNA in the extracted samples was sought in PCR assays, and PCR products were electrophoresed on 1.5% agarose gel.

Histopathological study

The terminal ileum samples taken from rats were fixed for 24 hours in 10% formalin. The intestinal segments were divided into pieces 0.5 x 0.5 x 0.5 cm in size and were processed for routine histopathologic examination. The intestinal tissues of each animal were obtained in separate blocks. Sections of 6 to 7 μ, prepared from all tissue samples, were examined by light microscopy. For histopathological evaluation edema, vasodilatation and inflammatory cell infiltration were scored from 0 (slight) to 3 (severe).

Statistical analysis

The chi-square test and Fisher's exact test were used for statistical comparison of the results pertaining to the experimental groups. In the evaluation, $p > 0.05$ was accepted as insignificant and $p < 0.05$ as significant.

Results

Microbiological evaluation showed that all blood and MLN cultures were positive in group II. Nine PCR result

Table I - *MLN culture, blood culture and PCR results*

	MLN culture				Blood culture				PCR			
	Negative		Positive		Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%	N	%	N	%
Group I (n=10)	8	80	2	20	8	80	2	20	9	90	1	10
Group II (n=10)	0	0	10	100	0	0	10	100	1	10	9	90
Group III (n=10)	8	80	2	20	10	100	0	0	9	90	1	10
Group IV (n=10)	8	80	2	20	8	80	2	20	7	70	3	30

TABLE II - *Statistical analysis of MLN culture, blood culture and PCR results*

	MLN culture	Blood culture	PCR
G_1-G_2	**0.006**	**0.006**	**0.01**
G_1-G_3	1.00	0.56	1.00
G_1-G_4	1.00	1.00	0.62
G_2-G_3	**0.006**	**0.006**	**0.01**
G_2-G_4	**0.006**	**0.006**	**0.27**
G_3-G_4	1.00	0.56	0.11

Fisher's exact test, p<0,05 is significant (significant values are in bold).

TABLE III - *Statistical analysis of histopathological results*

	Edema	Inflammatory cell infiltration	Vasodilatation
G_1-G_2	**0.001**	**0.005**	**0.02**
G_1-G_3	0.56	0.27	0.27
G_1-G_4	0.46	0.46	0,46
G_2-G_3	**0.001**	**0.02**	**0.02**
G_2-G_4	**0.005**	**0.01**	**0.01**
G_3-G_4	1.00	1.00	1.00

Kolmogorov-Smirnov test, p<0.05 is significant (significant values are in bold).

were positive in group II. Positive PCR result in groups I, III and IV were 1, 1, 3 respectively (Table I). The difference between MLN and blood cultures of group II and other groups was significant (p<0.05). The difference between the PCR results of groups I and II was significant (p<0.05) and the difference between groups II and III was also significant (p<0.05) (Table II). As shown in Fig. 2, edema, vasodilatation and inflammatory cell infiltration were higher in group II than in others. The statistical analysis of the histopathological results is shown in table III.

Discussion

Bacterial translocation (BT) is defined as the passage of intestinal bacteria and bacterial products from the intestinal lumen to the extraintestinal sites, such as the mesenteric lymph node (MLN), the blood, and distant tissues[9-18]. BT is reported to occur after thermal injury, hemorrhagic shock, intestinal obstruction, pancreatitis, intestinal ischemia-reperfusion inury, obstructive jaundice, portal hypertension, chirrosis and Crohn's disease [9-11,13,16,18,41].

Bacterial translocation is precipitated by bacterial overgrowth disturbing the normal ecologic balance [19,20], host immunedysfunction inciting pro and anti-inflammatory cytokines balance [21], and mucosal barrier dysfunction, favoring oxidants release [22].

Many agents used to prevent BT. Glutamin, Enisoprost, Vit C and E, zinc, melatonin, Levamisol, tungsten supplemented diet, and probiotic L.Plantarum 299V are shown to decrease BT [23-28]. Gurleyik et al. [29] reported PGE1 supplementation in jaundiced rats significantly prevents BT by maintaining the structural integrity of the mucosa. Ozen et al. [20] investigated the effects of melatonin on the healing of colonic anastomosis in a rat model of peritonitis, and reported that melatonin protects tissues against sepsis induced oxidative damage.

Melatonin is an agent that promotes sleep and is produced at night by the pineal gland. While produced primarly in the pineal gland, melatonin can also be found in cells of the bone marrow and gastrointestinal tract and plays a fundamental role in the neuroimmunoendocrine system [30-34,41].

The gastrointestinal tract is continuously exposed to toxins, microbes, microbial products, and other antigens. The intestinal epithelium has several important functions in protecting an organism from the potential deleterious effects of these agents acting as a physical barrier that limits the entry of luminal substances and microorganisms into the lamina propria [35]. Berg et al. [36] have described bacterial translocation at the passage of viable bacteria through the epithelial mucosa into the lamina propria, and then to the MLNs and possibly other tissue. Melatonin can protect gastrointestinal mucosa gainst damage by stimulating the immune system and fostering microcirculation and epithelial regeneration. Melatonin is a direct antioxidant and also decreases free radical levels by stimulating the activities of enzymes involved in antioxidative defense. Melatonin prevents circulatory failure in rats with endotoxemia by inhibiting the release of TNF-α [37]. TNF-α is a multifunctional cytokine produced primarly by activated monocytes and macrophages and plays a crucial role in the initiation and continuation of mucosal inflammation and immunity [38,39].

During the last 20 years, the polymerase chain reaction (PCR) is used to detect the genetic material of many infectious agents in various milieus at high sensitivity [40]. Measures of BT are blood cultures,MLN cultures, bacterial sintigraphy with 99mTc-Labelled E. Coli and PCR [40]. PCR is a superior way of determining BT [17].

Glutamine (GLN) is the primary metabolic fuel of small intestinal enterocytes, and has been shown to be an essential metabolic component of the proliferative response of enterocytes. Glutamine (Gln) is a conditionally essential nutrient during serious injury or illness, and plays a vital role in tissue metabolism [42,43]. Recently, Gln has been demonstrated to protect against I/R injury of gut, heart and skeletal muscle.

Studies showed that glutamine supplementation of TPN improved survival after gut I/R injury [44,45], reduced atrophy of intestinal mucosa in rats on total parenteral nutrition [46], prevented intestinal mucosal injury accompanying short bowel, small bowel transplantation, chemotherapy, and radiation [47,48].

Gln is a precursor for synthesis of nucleic acids and glutathione. Gln has been demonstrated to protect against I/R injury of gut, heart and skeletal muscle and its possible mechanism of action is partially related to the preservation of the content of glutathione (GSH).[49,50] GSH is an important endogenous antioxidant that protects against oxygen free radical injuries [51,54]. Gn is the main fuel for rapid proliferating and dividing cells such as enterocytes and lymphocytes. It can maintain the metabolism of intestinal mucosal cells directly or indirectly, promote hyperplasia of epithelial cells of ileum and colon, maintain the structure and function of small intestinal mucosal and reduce the increment of intestina permeability [52,53].

In our I/R model, the SMA of rats was clamped for 60 min, and the rats were killed 72 h after intestinal I/R. Mesenteric lymph nodes, and blood were then cultured quantitatively. Almost all MLN had positive cultures and grew significantly great numbers of enteric bacteria, spread to the blood, liver and spleen in I/R-PN group. The most common bacterium discovered from solid viscera was E. coli, other species included enterococcus, pseudomonas, proteus, and staphylococcus.

As shown in the present study, bowel ischemia and reperfusion promoted bacteria translocation. In addition, when compared to the sham, this phenomenon was significantly higher for MLN and serum in all other groups.

Conclusions

Glutamine and Melatonin reduced the incidence of BT in intestinal I/R. rats. These results suggest that Glutamine and Melatonin would be clinically useful in the treatment of intestinal I/R injury.

Riassunto

La lesione ischemica dell'intestino seguita da riperfusione costituisce un problema maggiore in numerose condizioni patologiche dell'addome e si associa con elevata morbilità e mortalità. Lo scopo di questo studio è quello di determinare se la Glutamina e la melatonina sono in grado di prevenire la trasmigrazione batterica nell'intestino tenue nei ratti.

Per lo studio sono stati impiegati 40 ratti Wistar Albini del peso medio di 200-250 g, distrubuiti a random in quattro gruppi (10 per ogni gruppo): quelli del I gruppo sottoposti ad un falso intervento; il secondo gruppo sottoposto soltanto alla lesione ischemica; il III gruppo trattato con Glutamina dopo la lesione eschemia; il IV gruppo trattato analogamente con Melatonina. A tutti gli animali è stata somministrata una carica di 10^{10}B.coli mediante intubazione gastrica 12 ore prima di raccogliere i campioni da esaminare.

72 ore dopo il primo intervento sono stati prelevati linfonodi mesenterici e campioni di sangue e posti in coltura. Due cc di sangue sono stati utilizzati per lo studio della reazione PCR. Un brammento di ileo terminale è stati parimenti prelevato per lo studio istopatologico.

I linfonodi mesenterici e le culture di sangue di tutti gli animali controllati sono risultati positivi per crescita microbica, ed i risultati della PCR sono stati positivi in 7 degli otto ratti. Istologicamnete è stato osservato edema, vasodilatazione ed infiltrazione di cellule della flogosi in misura inferiore negli altri gruppi in paragone al gruppo di controllo. L'evidenza della transolcazione batterica è risultata diminuita in tutti i gruppi trattati rispetto ai gruppi di controllo.

In conclusione la Glutamina e la Melatonina riducono l'incidenza della traslocazione batterica nell'intestino di ratto sottoposta ad ischemia/riperfusione, e questi risultati suggeriscono che sia la Glutamina che la Melatonina potrebbero essere utili clinicamente nel trattamento del danno intestinale da ischemia/riperfusione.

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