Original article

The prophylactic effect of L-Arginine in an experimental model of acute ischemic colitis. A histopathological and biochemical (malondialdehyde levels) evaluation

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SUMMARY

The aminoacid L-Arginine being the sole donor of nitric oxide synthesis in cases of intestinal ischemia, seems to play a protective role during experimental acute colon ischemia and reperfusion. We studied the effect of pretreatment with the aminoacid L-arginine in 144 Wistar rats who were subjected to experimental acute ischemic colitis (A.I.C.) and reperfusion of varying time periods (30, 60, 90 minutes). L-arginine was studied in comparison with Molcydomine and Caseine, two substances used for intestinal protection. Serum malondialdehyde (MDA) levels, a reliable marker which indicate the degree of tissue damage after ischemia-reperfusion, were lower in the L-arginine group, while the same group presented the lower level of histopathological damages. We conclude that pretreatment with L-arginine in rats subjected to A.I.C. attenuates the histopathophysiological damages.

Key words: L-Arginine, Reperfusion injury, Intestinal ischemia, Malondialdehyde, Experimental model.

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INTRODUCTION

Acute ischemic colitis (A.I.C.) is a medical emergency caused by an abrupt decrease of oxygen and nutrients delivery to the bowel, due to arterial embolism, thrombosis or low cardiac output. The therapy of the disease consists of conservative measures or surgery, both aiming to relieve the cause of ischemia, thus reperfusing the involved intestinal segment.[1,2] Paradoxically, this post-therapeutic bowel reperfusion seems to aggravate tissue injury. New drugs protecting the bowel during both the two phases of A.I.C. (Ischemia and Reperfusion) have to be introduced, in the clinical practice. L-Arginine is an essential aminoacid as sole donor for the endogenous synthesis of NO[3-6] with a possible, but unclear, beneficial impact on the intestinal function and integrity.[7,8] The aim of this study was to investigate the enteroprotecting role of L-arginine during A.I.C., by comparing it with the efficacy of exogenous vasodilators (molcidomine) or inert nitrogen-containing molecules (caseine).

MATERIAL - METHODS

Our study was carried out on male Wistar rats (n=144) weighing 200-300 gr. Rats were kept under standardized conditions for food, water, light and temperature. All animals were fed standard rat chow and water ad libitum until before surgery.

The rats were anesthetized with an intramuscular in-

jection of 30mg/kg ketamine hydrochoride and 1.5 mg/kg midazolame. After skin shaving and preparation of the abdominal wall with alcohol solution 70%, a cross-like laparotomy was performed. The small bowel was exteriorized and the ligament of Treitz cut to expose the superior mesenteric artery (SMA).[9] The SMA was dissected free. An atraumatic microvascular clamp was then placed across the SMA just after its origin from the aorta for occlusion. Care was taken in order not to clamp the superior mesenteric vein. Mesenteric ischemia was confirmed when the mesenteric pulsations were lost and the intestines became pale. The bowel was returned to the abdominal cavity and the incision was closed. After 30, 60 ,90 minutes of ischemia -depending on the subgroup- a relaparotomy was performed and the microvascular clamp on the SMA was removed for 90 mins of reperfusion. Mesenteric reperfusion was confirmed with the restoration of pulsation and color. The bowel was then returned inside the abdominal cavity. At the end of 90 mins, the animals were sacrificed.

Experimental design

Totally 144 Wistar rats were used in this study divided into 4 groups:

Group A, the ischemic control group (n=36), underwent laparotomy with clamping of SMA (9) according to the following pattern: Subgroup A1 (n=6) and A4 (n=6) for 30 min, A2 (n=6) and A5 (n=6) for 60 min, A3 (n=6) and A6 (n=6) for 90 min. For subgroups A1, A2 and A3 reperfusion period was 90 min. Subgroups A4, A5 and A6 were not subjected to reperfusion. After animal sacrifice, samples of blood were taken in order to determinate values of hematocrite, blood gases and electrolytes and serum malondialdehyde (MDA). Moreover the colon was removed and sent for pathologic evaluation.

Group B, (n=36) was given preoperatively L-Arginine in a dose of 800 mg/kg, divided in 3 equal doses, 22, 8 and 1 hour before operation. The procedure was the same as for group A, i.e. subgroups B1, B2, B3, B4, B5, B6.

Group C, (n=36) was given preoperatively Molcydomine in a dose of 4 mg/kg. The procedure was the same with group B, i.e. subgroups C1, C2, C3, C4, C5, C6.

Group D, (n=36) was given preoperatively hydrolysed Caseine in a dose of 1600 mg/kg. The procedure was the same as for group B, i.e. subgroups D1, D2, D3, D4, D5, D6.

All substances were given per os.

Statistical analysis

The statistical analysis and results were based on Student's t-test.

RESULTS

Group A results (control group) confirm the experimental pattern function.

Hematocrite, electrolytes and blood gases values in group A (venous blood) are presented in Table I.

Average MDA values per subgroup are presented in Table II.

Histopathological evaluation

All colon specimens were kept in tubes with a solution of 10% phormaldheyde and transferred to the pathology department where they were sectioned, fixed and stained with hematoxyline-eosine. Then, they were blindly examined by one pathologist.

In the L- Arginine group (group B) the results of the histopathological examinations showed an almost intact epithelium, a mild increase of inflammatory components inside the chorium and completely intact basal crypts (Fig. 1).

In group C, where molcydomine was given, the epithelium was almost intact. However, we observed a partial loss of intestinal wall nodes and a mild inflammation of the chorium while the basal crypts remained intact (Fig. 2).

In the Caseine group (group D) we observed damages which varied from ulceration of the intestinal mucosa with edema, mild inflammation of the chorium and cap-

Table 1.

Normal values	60 min ischemia	90 min ischemia
Ph=7.00-7.06	7.07	7.18
PCO_2=74-104 mmHg	65	75
PO_2=27-29 mmHg	<20	<20
HCO_3=19-29 mM	21.4	29.7
O_2 sat=29-31%	<9.3	15.5
Na=138 mM	137	157
K=5.1 mM	8.7	4.8
Ca=1.12 mM	1.19	0.7
Hct=45-47%	59	71

Table 2.

Ischemia								
	Control		**L-argine**		**Molcydomine**		**Caseine**	
30’	2.42	**A4**	2.53	**B4**	2.65	**C4**	4.15	**D4**
60’	4.70	**A5**	3.10	**B5**	3.10	**C5**	3.54	**D5**
90’	3.00	**A6**	2.92	**B6**	3.04	**C6**	4.67	**D6**
Reperfusion								
	Control		**L-argine**		**Molcydomine**		**Caseine**	
30’+90’	3.70	**A1**	3.00	**B1**	2.90	**C1**	3.85	**D1**
60’+90’	4.70	**A2**	4.50	**B2**	5.51	**C2**	3.43	**D2**
90’+90’	5.32	**A3**	3.07	**B3**	5.63	**C3**	3.54	**D3**

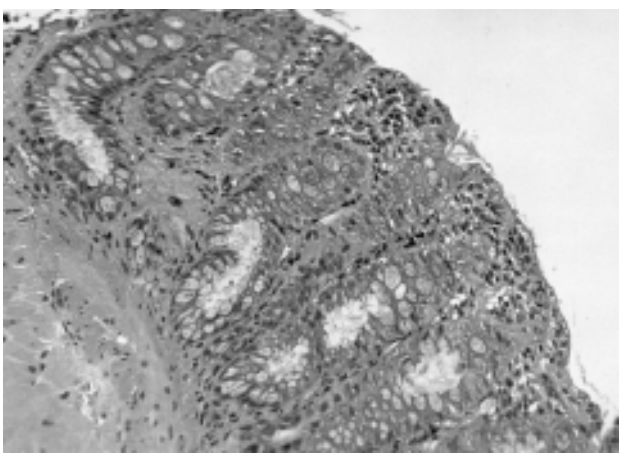

Figure 1. Minor histopathological damages in the L-arginine group.

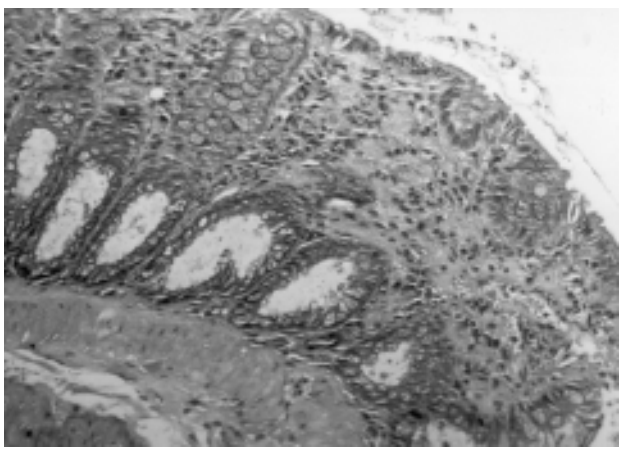

Figure 2. Mild damage was observed in the Molcydomine group.

illary dilation (Fig. 3) to grave ulceration with serious loss of basal crypts (Fig. 4,5).

Statistical evaluation

The statistical evaluation for the groups without reperfusion showed that L-arginine produce better results from the caseine group (p~0.001) and lower MDA levels in comparison to molcydomine. No statistical differences were observed between the control and the arginine group and the only exception was at 60 min of ischemia (p~0.01).

In the second part of our experiments (reperfusion for 90 min) the arginine group showed better results after 90 min of ischemia in comparison to the control group (p~0.001) and to molcydomine (p~0.001). Also the arginine group showed lower MDA levels in comparison to the control group and better profile from the other agents.

DISCUSSION

Acute ischemic colitis (A.I.C.) is a relatively common disease, but its pathogenesis is not fully understood. A dysregulation of various vasoactive substances (such as nitric oxide-NO), which participate in the control of intestinal perfusion, has recently been implicated as the major underlying mechanism of A.I.C.

The mortality rate is of 70-90%, if not promptly treated.[6-10] The currently available thrapeutic modalities (vasodilators, therapeutic angiography and surgical embolectomy) all have as common target, the reperfusion of the ischemic tissue with oxygenated blood. However, accordingly to many authors this reperfusion has been observed to aggravate the intestinal injury. Also the time play an important role and for example a tissue damage produced by 3 hrs of ischemia and 1 hr of reperfusion is more severe, than that of 4 hrs of pure ischemia.

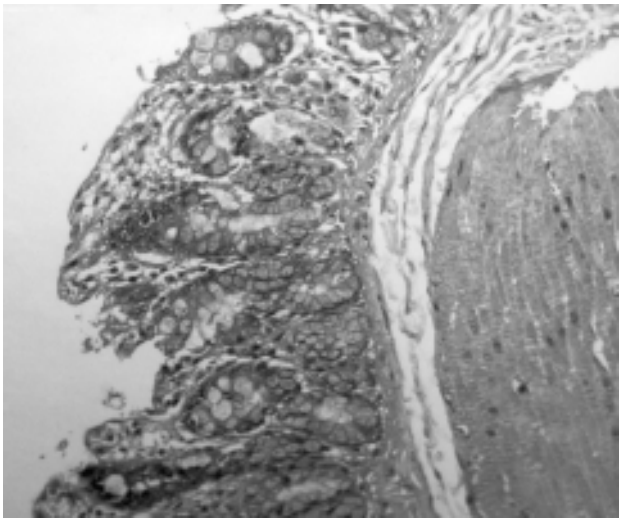

Figure 3. Mild inflammation and capillary dilation in the Caseine group.

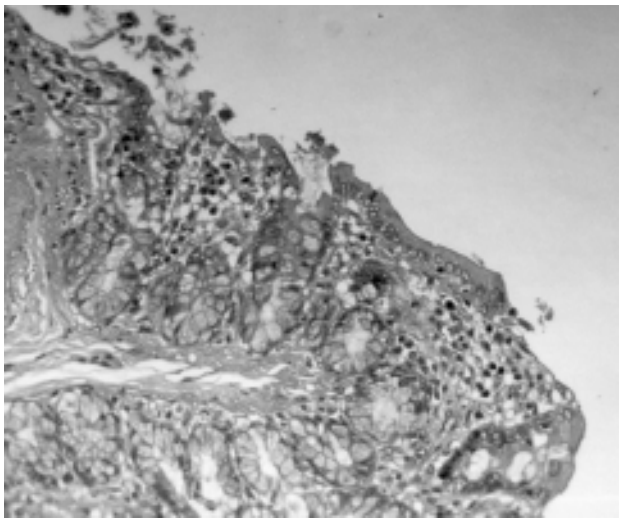

Figure 4. Grave ulceration and loss of basal crypts in the Caseine group.

During the last decade, a growing body of evidence underlined the negative role of decreased synthesis of nitric oxide (N0) in the pathophysiology of A.I.C.[10] Nitric oxide seems to act protectively during the ischemic period, with its vasodilative and antithrombotic properties. It produces myochalasis of the intestinal muscularis propria, while inhibiting leucocytes aggregation and activation.[11-18] This concept led the investigators, searching for novel enteroprotecting agents for A.I.C., to elucidate the possible beneficial effect of the L-arginine, as it is the sole precursor in the endogenous nitric oxide synthesis. Furthermore, it has been reported that L-arginine (as well as ornithine) catabolised into polyamines, seems to induce the repair of the intestinal mucosa, after ischemia.

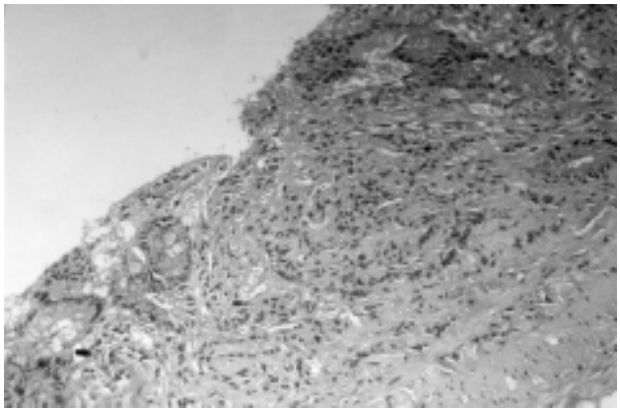

Figure 5. More grave damages in the Caseine group.

In our study, we developed a reproducible and reliable experimental model of A.I.C. in rats and we created 3 major animal groups, in each of which we administered 3 different potentially enteroprotective substances: L-arginine (as an endogenous vasodilator), molsidomine (as an exogenous vasodilator) and caseine (as an inert nitrogen-containing molecule). In all groups, apart of the histological study, we determined hematocrite, blood gases and serum malondialdehyde levels (MDA), which is a sensitive index for the nocious lipid peroxidation. Also in the literature they are few articles on MDA levels after ischemia - reperfusion in a rat model.[19] Most articles focus results on a pathological scale of intestinal tissue damages or measure tissue MDA levels.[20] On the other hand we focused more at MDA levels in serum, than in tissue samples, because we were mainly concerned about the clinical setting of A.I.C. prognosis and treatment, in which tissue sampling from the involved bowel segment cannot be easily applied. We finally demonstrated that the group given L-arginine suffered a less severe injury after experimental A.I.C. of various duration and we cocluded that, although the use of L-arginine does not prevent ischemic bowel damage, it promotes the quicker repair of the intestinal mucosa. This is not attributed only in the NO-production (since molcydomine had a lesser efficacy), but it resulted from a probable "synergistic" effect of both the NO and polyamines impact on bowel integrity and function, due to the central role of L-arginine in two different metabolic pathways.

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