

Protective effects of ulinastatin on intestinal injury during the perioperative period of acute superior mesenteric artery ischemia

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Abstract. – OBJECTIVE: This work aims to explore the protective effects of ulinastatin on intestinal injury during the perioperative period of acute superior mesenteric artery ischemia (ASMAI).

PATIENTS AND METHODS: 28 patients undergoing revascularization were divided into 2 groups, with 14 cases each. The cases in the observation group (OG) were treated with ulinastatin 300,000 U intravenously 30 min before the operation, and continuously treated with 300,000 U every 4 hr thereafter until 24 hr of the operation, while those in the control group (CG) were not given the intervention of ulinastatin. Patients' circular intestinal fatty acid binding protein (I-FABP) levels were measured at the following time points to reflect the intestinal injury: 30 min before the operation, before revascularization, then 1, 12 and 24 hr after the operation. The white blood cell counting (WBC), serum alanine aminotransferase (ALT), serum creatinine (Cr), D-dimer, and serum endotoxin (ET) were also measured simultaneously for the analysis of the significance of their values with the intestinal injury.

RESULTS: There were no significant differences ($p > 0.05$) in ischemia duration, length of the affected intestinal segments, WBC, ALT and Cr levels at the above time points between the 2 groups, and all the indicators of the 2 groups, including the mean circular I-FABP levels before the operation and the revascularization, showed no significant difference ($p > 0.05$). After the blood supply was restored, the I-FABP levels in OG dropped significantly as compared with those in CG. The pattern of circular ET levels appeared the similar manner as the circular I-FABP levels did.

CONCLUSIONS: Our study showed a protective effects of ulinastatin on intestinal injury during the perioperative period of ASMAI, as revealed by the circular I-FABP levels which mainly happened after the blood supply was restored.

Key Words:

Acute superior mesenteric artery ischemia, Revascularization, Ulinastatin, Perioperative period, Intestinal injury.

Introduction

Acute superior mesenteric artery ischemia (ASMAI) is a life-threatening vascular emergency in abdominal surgeries, which has high rates of misdiagnosis, missed diagnosis and death¹. Advances in accurate diagnostic modalities and aggressive therapeutic interventions have spared a great proportion of these patients from extensive intestinal resection. However, its perioperative morbidity and mortality remain high, ranging from 30% to 60%²⁻⁶, and perioperative organ dysfunction and sepsis are very common^{4,6}.

Better understanding of the pathophysiology of ASMAI achieved after decades of extensive researches on this issue has provided us a deeper insight into the local and generalized organ dysfunction in this setting. As we now know, due to an initiation of a proinflammatory cascade, intestinal injury won't stop rightly after the re-establishment of the mesenteric blood flow, leading to the migration of neutrophils and other inflammation cells into intestinal tissue and the consequent inflammatory reaction, which was postulated as main responsibility for the formation of severe mucosal lesions⁷. Activated inflammation cells in turn produce inflammatory cytokines such as tumor necrosis factors (TNFs), interleukins (ILs), platelet-activating factor (PAF) and leukotrienes in great quantities in a cascade manner⁸, and meanwhile the complement system is also activated^{9,10}. These cytokines and complements subsequently released into the circulation from the disrupted intestinal mucosal barrier, combined with other mechanisms such as bacteria translocation, are responsible for the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndromes (MODS)¹¹.

Duration of the ischemia is unquestionably the key determinant of the tissue damage, and early accurate diagnosis is the most essential factor for improving the clinical outcome of this disease. Unfortunately, as the principle complains and clinical signs are usually nonspecific in the majority of ASMAI cases at its onset, delayed diagnosis and treatments are not uncommon. Biomarkers for accurate early diagnosis are still lacking. Classically, leukocytosis, increased serum amylase, metabolic acidosis and more recently, elevated circular lactate have been proposed as markers indicating ASMAI. However, accumulating evidence shows that the discrepancy between the common usage of these markers and the certainty of diagnosing ASMAI is extremely wide due to inadequate sensitivity and specificity¹¹⁻¹⁴. Intestinal fatty acid binding protein (I-FABP) is exclusively located at the luminal pole of intestinal epithelial cells with relatively large quantities, whereas absent in other tissues. Being highly sensitive and sufficiently specific, it has recently emerged as a promising biomarker for the early diagnosis of intestinal injury in a variety of clinical settings, including ASMAI¹⁵⁻¹⁷.

Ulinastatin (UTI), a glycoprotein acting as a urinary trypsin inhibitor, was first identified and purified from human urine by a Japanese research group in 1980¹⁸. Because many factors are present in the inflammatory cascade, with its protease-inhibiting property, UTI has been proven an effective anti-inflammatory substance that protects tissues and organs against neutrophil-mediated injury, and is considered to be a reasonable treatment in a variety of clinical setting involving multiple organs due to systemic inflammatory reaction¹⁹⁻²⁵. Although the molecular mechanism(s) by which UTI exerts its organ-protective activity is not fully understood, accumulating data from animal studies and clinical investigations have shown that UTI is capable of protecting multiple organs against inflammation-induced dysfunction^{19,21,25}. In ASMAI, where neutrophil-mediated injury is also intensively involved in the process of intestinal injury, especially during the reperfusion stage after revascularization, UTI is hypothesized as a reasonable treatment. However, to date, perioperative use of UTI in ASMAI and its possible protective effects against intestinal injury in this setting have not been fully studied.

In the present study, we recruited ASMAI patients undergoing revascularization, with serum

I-FABP alongside with other laboratory hematological and metabolic indicators to determine the clinical stage. Those patients were randomly allocated to 2 groups, receiving intravenous administration of UTI or not, respectively. The aim of the study was to investigate whether UTI was capable of improving the clinical outcome for ASMAI patients subjected to revascularization.

Patients and Methods

General Information

28 cases of ASMAI patients, receiving therapeutic treatments and revascularization measures in affiliated Tongji Hospital of Tongji University from July 2007 to June 2011, were chosen as the subjects, including 15 males and 13 females, aging from 52 to 86 years old with the average age of 70.0 ± 9.4 years. The most common symptom of the patients was the sudden abdominal pain, 21 cases (75%); other common symptoms were nausea (61%), vomiting (49%), bloating (47%) and diarrhea (27%), mostly the manifestations of gastrointestinal disorders.

All patients were informed consent, and divided into 2 groups in accordance with the principle of randomization. Observer Group (OG) had 14 cases, 8 males and 6 females, with average age as 72.3 ± 9.1 years. The average time from the onset of symptoms to surgical treatment was seen as the average time from onset to treatment time, and for OG, the time was 6.2 ± 1.7 hr. Before the surgery, 12 cases in OG showed atrial fibrillation, 8 cases had a history of coronary artery disease, 8 cases had a history of hypertension and 1 case had a history of diabetes mellitus. Control Group (CG) had 14 cases, 7 males and 7 females, with average age 71.7 ± 9.8 years, the time was 6.6 ± 1.3 hr. Before the surgery, 11 cases had atrial fibrillation, 7 cases had coronary artery disease, 6 cases had hypertension, 2 cases had diabetes mellitus. There were no significant differences in the fields of gender, age, time from onset to treatment ($p > 0.05$). All the patients were considered to be ASMAI patients after the Risk Factors Analysis, combined with clinical manifestations, and finally confirmed by Computed Tomography Angiography (CTA).

Treatment

All the patients underwent superior mesenteric arteriography to make clear the ranges of the blocked blood vessels, and laparotomy was also

carried out for the determination of whether the ischemic bowel resection should be done according to the intraoperative findings in the laparotomy. The ranges of the blocked blood vessels were used to reflect extent of disease. Before the determination of revascularization, all the patients were administered with 5000 U heparin intravenously, and the revascularization methods included 20 cases of thrombectomy, 3 cases of endarterectomy, 5 cases of balloon dilatation and stent angioplasty, 2 cases, undergoing endarterectomy while with large vessel wall defects, also underwent patch plasty. When the color of the ischemic bowel restored to normal, the circulation of pathological bowel could be seen as restored. To confirm the patency of the previously occluded vessel and the reestablishment of the blood supply to the affected bowel, another round of intraoperative mesenteric arteriography was performed. The OG patients received intravenous infusion of 300,000 U UTI: Ulinastatin, Urinary Trypsin Inhibitor (Techpool Bio Pharmaceutical Co., Ltd., Guangzhou, China, 100,000 U/ampule) 30 min before the surgery, then given once every 4 hr during the consequent 24-hr continuous observation. The CG was not given with UTI. Low molecular weight heparin was given to both groups postoperatively for 7 days and intravenous thrombolytic for 24 hr.

Detection Indicators

All the patients were blood collected for indicators monitoring at 1 hour before the surgery, before revascularization, 1, 12 and 24 hr after the surgery. The collected blood samples were centrifuged immediately 3000 rpm for 10 min with Hitach Himar CF15R Low-Temperature High-Speed Centrifuge and the extracted serums were preserved under -70°C until tested. I-FABP is mainly distributed in the cytoplasm of epithelial cells at the tip of the small intestinal mucosa microvilli, when the necrosis of the epithelial cells happens, I-FABP firstly would enter the portal vein and the lacteal, then be released into the circulation⁴, and considered

capable of reflecting the intestinal injury in the early stage of intestinal ischemia. In this study, detection of I-FABP in the circulation was used to reflect intestinal injury. The rest of the monitoring indicators were as the following: white blood cell count (WBC), liver function (alanine aminotransferase, ALT), renal function (creatinine, Cr), D-dimer, plasma endotoxin (ET). ALT was measured by continuous monitoring procedure; Cr was detected by picric acid method, with the picric acid kit provided by Beckman Coulter Company (Miami, FL, USA), and used Beckman DXC800 Automatic Biochemical Analyzer; D-dimer was detected using immune turbidimetry method, SYSMEX (Kobe, Japan) CA1500 Automatic Coagulation Analysis; I-FABP was determined by enzyme-linked immunosorbent assay (ELISA), and the kit was purchased from R&D Company (San Diego, CA, USA); endotoxin was measured by matrix azo chromogenic Limulus experiment, purchased from Chinese Horseshoe Crab Reagent Manufactory, CO., Ltd., Xiamen.

Statistics Analysis

Using SPSS 11.0 software for the statistical analysis (SPSS Inc., Chicago, IL, USA), data were presented as mean $\pm$ standard deviation ($\pm$ s). Non-paired *t*-test analysis was used for the analysis of difference between CG and OG; 2 Factor ANOVA analysis was used for the analysis of group difference; $p < 0.05$ was used for the indication of statistically significant difference.

Results

The results of onset to treatment time, the involved bowel length, operation time of the 2 groups were shown in Table I. No statistically significant difference was found ($p > 0.05$) between the above indicators.

Changes in results of WBC, ALT, Cr, D-dimer, ET and I-FABP levels in 2 groups were shown in Table II. While there were no statistical signifi-

Table I. Onset to treatment time, the involved bowel length and operation time of the 2 groups.

Group	Cases	Onset to treatment time (h)	Involved bowel length (m)	Operation time (h)
OG	14	6.2 ± 1.7	1.25 ± 0.33	5.4 ± 1.2
CG	14	6.6 ± 1.3	1.20 ± 0.37	5.6 ± 1.0

Table II. C Change results of WBC, ALT, Cr, D-dimer, ET and I-FABP level of the 2 groups in perioperative period.

Group	Cases (n)	WBC ($\times 10^9/L$)	ALT (U/L)	Cr ($\mu\text{mol/L}$)	D-dimer (mg/L)	ET (Eu/mL)	I-FABP (ng/ mL)
CG	14						
1h before		1.0 $\pm$ 0.3	28.5 $\pm$ 15.6	83.8 $\pm$ 26.3	5.08 $\pm$ 1.23	1.979 $\pm$ 1.0670	1850.5 $\pm$ 107.6
Before revascularization		1.8 $\pm$ 0.6	33.4 $\pm$ 18.5	78.1 $\pm$ 24.6	7.05 $\pm$ 1.18	4.437 $\pm$ 1.021	2016.3 $\pm$ 124.5
1 h after		1.9 $\pm$ 1.0	36.9 $\pm$ 19.3	80.1 $\pm$ 25.1	6.58 $\pm$ 3.20	5.179 $\pm$ 1.967*	1797.2 $\pm$ 124.5*
12 h after		2.0 $\pm$ 0.9	34.5 $\pm$ 11.7	89.1 $\pm$ 21.9	3.06 $\pm$ 1.21	4.979 $\pm$ 1.471*	1481.6 $\pm$ 43.8*
24 h after		1.8 $\pm$ 0.7	40.2 $\pm$ 19.9	88.1 $\pm$ 16.8	1.08 $\pm$ 0.83	3.410 $\pm$ 1.257*	996.2 $\pm$ 54.1*
OG	14						
enrollment		0.4 $\pm$ 0.2	33.6 $\pm$ 14.1	80.1 $\pm$ 18.6	4.09 $\pm$ 0.93	0.605 $\pm$ 0.317	645.4 $\pm$ 77.1
1h before		0.8 $\pm$ 0.3	30.8 $\pm$ 14.5	78.3 $\pm$ 20.9	5.24 $\pm$ 1.46	1.824 $\pm$ 0.857	1901.5 $\pm$ 105.1
Before revascularization		1.7 $\pm$ 0.5	34.6 $\pm$ 16.4	84.8 $\pm$ 25.1	7.48 $\pm$ 2.21	4.026 $\pm$ 1.455	1960.9 $\pm$ 116.4
1 h after		2.1 $\pm$ 0.6	40.2 $\pm$ 19.1	79.7 $\pm$ 19.4	5.14 $\pm$ 2.96	2.194 $\pm$ 0.658	1306.8 $\pm$ 74.5
12 h after		1.6 $\pm$ 0.6	38.7 $\pm$ 14.8	86.3 $\pm$ 22.8	2.15 $\pm$ 1.41	1.028 $\pm$ 0.379	522.3 $\pm$ 34.6
24 h after		1.5 $\pm$ 0.7	35.5 $\pm$ 20.1	81.3 $\pm$ 18.7	0.93 $\pm$ 0.29	0.627 $\pm$ 0.391	106.2 $\pm$ 24.5

*Compared with the OG, $p < 0.05$.

cance in WBC, ALT, Cr and D-dimer level in the 2 groups ($p > 0.05$), there existed statistically significant difference in I-FABP and ET level at the points of postoperative 1, 12 and 24 hr.

Except for the deaths of 5 cases (mortality rate 17.9%), all the rest recovered and left hospital. The reasons for the death were: 2 cases of MODS, 2 cases of respiratory failure by repeated lung infections, 1 case of sudden acute myocardial infarction.

Discussion

Due to the lack of specific symptoms, ASMAI is easy to be misdiagnosed and omitted-diagnosed, and for its rapid progress and the lack of effective treatment measures, it has a high mortality¹⁻⁶. A population-based survey found that only 1/3 ASMAI cases got accurate diagnosis before the preoperative period or death²⁶.

Intestinal mucosa is the organ with rich blood supply and vigorous metabolic activities, which is also easily vulnerable to metabolic disorders caused by hypoxia, and reperfusion injury often occurs when reperfusion is recovered due to abnormal oxygen metabolism, oxygen free radicals and calcium overload, endothelial swelling, and other reasons²⁷. Especially in recent years, studies have shown that the over-expression of adhesion molecules, activation of cytokine and complement, would, directly, or through the way of affecting neutrophils to make infiltrate into tissues, cause inflammatory damages. It's recog-

nized as the mechanism of tissue continuous injury after reflow²⁸. And a large number of these factors released into the circulation are the root cause of SIRS, even MODS.

The base of revascularization is undoubtedly also the basis of the treatment of ASMAI. Based on this basis, how to prevent sustained intestinal injury as soon as possible becomes the focus for further treatment. Such damages are mediated with by a number of factors and a number of links, so it is difficult to control the disease progress by resisting a certain factor. UTI is an efficient and broad-spectrum protease inhibitor from human body, and could inhibit the activities of trypsin, α -chymotrypsin, hyaluronidase, neutrophil elastase, cathepsin G and protein hydrolase *in vitro*, etc²⁹. A large number of studies have confirmed that UTI could inhibit the generation of inflammatory factors, block the activities of inflammatory cytokines on leukocytes, inhibit the activation of neutrophil which would then release elastase and lead to the damages of tissues. It also could play a protective effect on multiple organs through indirect inhibition of the generation of the complement system and interleukin³⁰. In the situation of the multiple trauma, shock and sepsis, UTI could prevent the development of the disease into MODS, and reduce mortality^{20,25}. The results of extracorporeal circulation researches suggested that UTI could realize the role of lung protection after reperfusion by reducing the expression of adhesion molecule and selectin gene³¹.

Researches have shown that I-FABP could reflect early-stage intestinal mucosal damages,

while be not implicated in systemic stress, not only much more sensitive and specific than conventional indicators (including WBC, ALT, Cr), but also than lactic acid, which could reflect the metabolic state¹³, even better than the D-dimer which reflects thrombosis/fibrinolysis system^{14,32}. In this study, I-FABP was used to reflect the extent of the affected intestinal mucosa, which was mainly due to the supply disorder of oxygen and nutrients caused by ischemia, and the application of UTI exhibited no significant protection of the intestinal mucosa at this stage, for there were no significant differences of I-FABP levels at the relative time points in the 2 groups, revealing that the stage of bowel injury was not reduced or reversed because of the application of UTI. While after revascularization, the difference between the 2 groups appeared immediately, and tended to increase with time-going. Because the intestinal injury at this stage was mainly caused by reperfusion, whose characteristic mainly expressed as the expression of adhesion molecules, production of inflammatory cytokine, activation of complement, infiltration of neutrophil and releasing of protease, and UTI could directly inhibit the production of these molecular and cellular factors, or directly affect neutrophils, inhibiting its infiltration into the tissues and then causing tissue inflammatory damages. Therefore, the protective effect of UTI on intestinal mucosal injury was mainly reflected in the stage of perfusion recovery after circulation reconstruction.

Impaired intestinal mucosal barrier could also lead to the result that intestinal bacteria releases endotoxin into the circulation, then cause endotoxemia. This study also found that even at a relatively early stage of ASMAI (within 6 hr), before the rising of conventional indicators (including WBC, ALT, Cr), endotoxin levels would increase with the aggravation of the intestinal mucosa and time extension, exhibiting a positive correlation with the I-FABP levels. The endotoxin is one of the components of Gram-negative bacterial cell wall. Animal studies have shown that endotoxin could either directly, or under certain conditions, cause or exacerbate the damages of liver, kidney, lung and other organs²¹⁻²⁴, and is an important material basis for the final development into SIRS and MODS of varieties of clinical conditions³³. In this study, all the patients had no other source of infection, so the endotoxin in the circulation mainly came from the intestinal tract.

Endotoxin's entering into the circulation would increase the risk of MODS. Although

revascularization could be realized through a variety of ways, the prognosis of ASMAI is still not optimistic, with as high as 60%-80% mortality in perioperative period. The major cause of death is MODS, a condition that can be postulated as a result of the endotoxemia caused by intestinal reperfusion injury after circulation recovery and intestinal mucosal barrier damages.

When intestinal acute ischemia happens, the mucosal layers with abundant metabolic activities would be first to be affected. Studies have shown that the intestinal mucosal epithelial cells can occur irreversible changes in only about 40 min³⁴. Obviously revascularization can hardly be achieved in such a short period due to the complicate and evasive nature of ASMAI, but as long as the timely intervention is carried out and intestinal blood flow is restored before the intestinal muscularis and serosa are involved inside, necrosis of the mucosa can still be repaired by regeneration, and the intestine could be preserved. However, the clinical manifestations are often atypical and non-specific in this time window, as in our study, the patients mainly showed spastic abdominal pain, associated with bowel dysfunction such as nausea, vomiting, diarrhea and without peritonitis. At its early stage, when damage caused by ischemia is still confined in intestinal mucosa, routine laboratory tests (including blood, liver and kidney functions, etc.) are often negative, as shown in this study, and appropriate treatment is thus frequently overlooked during this time window.

In this study, the speculative diagnoses of all the suspected cases were clarified by imaging studies, and combined with I-FABP, a sensitive and specific indicator, early diagnosis was established and the appropriate treatment of ASMAI was rightly carried out. UTI administered in the perioperative period, exerted its significant protective effect by inhibiting the sustained intestinal injury in the postoperative stage after the blood supply was restored.

Conclusions

These results suggested that after the success of revascularization, UTI had a protective intestinal function, could reverse intestinal continuous damage, and demonstrate some therapeutic potential in the prevention of postoperative MODS.

In this study, limited by the limited samples, the specific protection mechanism involved need further research to clarify.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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