



RAPID COMMUNICATION

Diagnostic value of plasminogen activity level in acute mesenteric ischemia

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Abstract

AIM: To investigate the changes in plasminogen activity level during mesenteric ischemia.

METHODS: We performed laparotomy in 90 female Wistar-Albino rats (average weight 230 g). In sham groups (SL) (Groups I and II) the superior mesenteric artery (SMA) and vein (SMV) were explored, but not tied. In SMA groups (Groups III and IV) the SMA was ligated, and in SMV groups (Groups V and VI) the SMV was ligated. On re-laparotomy 2 mL of blood was drawn at 1 h in groups I, III and V, and at 3 h in groups II, IV and VI. Plasminogen levels were assessed and comparisons were made between groups and within each group.

RESULTS: The mean plasminogen activity in the SL group was significantly higher than SMA (25.1 ± 10.8 vs 11.8 ± 4.6 , $P < 0.001$) or SMV (25.1 ± 10.8 vs 13.7 ± 4.4 , $P < 0.001$) groups both at 1 h and at 3 h (29.8 ± 8.9 vs 15.1 ± 5.7 , $P < 0.0001$; 29.8 ± 8.9 vs 14.2 ± 2.9 , $P < 0.0001$). There were no significant differences between the values of SMA and SMV groups at 1 h ($P = 0.28$) and at 3 h ($P = 0.71$). In each group, plasminogen activity levels did not change significantly between the two measurements performed at 1 h and 3 h.

CONCLUSION: We conclude that blood plasminogen activities decrease during early phases of both arterial and venous mesenteric ischemia which may be a useful marker for early diagnosis.

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Key words: Mesenteric ischemia; Necrosis; Activity level

INTRODUCTION

Successful treatment of acute mesenteric ischemia depends on restoration of circulation before the development of intestinal necrosis, and hence on early diagnosis^[1-4]. However, laboratory signs are non-specific even after the development of necrosis and the rate of mortality is still over 50% even in experienced centers^[5-7].

Plasminogen is a protease synthesized in the liver. It is a member of the fibrinolytic system and its plasma level are stable. Plasma plasminogen level is known to decrease in several conditions such as disseminated intravascular coagulation, severe hepatic failure, thrombotic disorders, thrombolytic treatment, and primary and secondary fibrinolysis^[8,9]. Plasminogen is the most important element of the fibrinolytic pathway and our literature search has not revealed any studies examining the change in plasminogen level during acute mesenteric ischemia. Thus, in the present study, the potential use of plasminogen level for the early diagnosis of acute mesenteric ischemia has been investigated.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of Haydarpasa Training and Research Hospital. A total of 90 female Wistar-Albino rats, with an average weight of 230 g (range: 190-270 g), were fed with standard food and water at room temperature, and were allocated into 6 study groups, each having 15 rats.

Surgical procedure

Rats were placed in a bowl containing a cotton pad soaked with diethyl ether for about 3 min and then anesthetized with ketamine 50 mg/kg, IM. After shaving and sterilization of the abdominal wall with povidone

iodine solution, laparotomy was performed through a midline incision. In all groups the superior mesenteric artery (SMA) and vein (SMV) were explored and 3/0 silk suture materials were placed around the vessels. In sham laparotomy groups (Groups I and II) sutures were not tied. In the SMA occlusion groups (Groups III and IV) the SMA was ligated; while in the SMV occlusion groups (Groups V and VI) the SMV was ligated. Abdominal walls were closed with continuous 3/0 silk sutures.

Blood sampling

Exploration and ligation of the artery and the vein were taken as zero time point. Re-laparotomy was performed at 1 h in groups I, III and V, and at 3 h in groups II, IV and VI, and 2 mL of blood was drawn from the heart with a 21G syringe.

Tests and assessments

Two-milliliter blood samples, collected at 1 h and at 3 h, were centrifuged in citrated tubes for 15 min at 4500 r/min. They were then stored at -20°C until being assayed. Plasminogen was assessed as chromogenic enzyme activity by the Streptokinase method by using a Dade Behring Coagulometry. SMA occlusion, SMV occlusion and Sham laparotomy (SL) groups were compared with respect to plasminogen activity levels at 2 time points. Also plasminogen activities at 1 h and at 3 h were compared within each group.

Statistical analysis

The statistical analyses were performed with SPSS (Statistical Package for Social Sciences) for Windows 10.0. Wilcoxon test was used to compare the data obtained at different time points. Kruskal-Wallis H analysis was used to compare three groups and Mann Whitney U Test was used for pair wise comparisons between groups. The results were evaluated at a confidence interval of 95%, and *P* values less than 0.05 were considered statistically significant.

RESULTS

During the first hour of ischemia, the small intestine and right colon were pale and edematous at laparotomy in SMA- and SMV-ligated rats. These structures turned dark red and/or purple by the end of the third hour. At 1 h, the mean plasminogen activity in the SL group was significantly higher compared to the SMA or SMV occlusion groups (25.1 ± 10.8 vs 11.8 ± 4.6 or 13.7 ± 4.4 , $P < 0.001$). Although plasminogen activity levels in the SMA occlusion group were lower than the SMV occlusion group, the difference between the two was not statistically significant. Also, at 3 h, plasminogen activity in the SL group was significantly higher compared to the SMA and SMV occlusion groups (29.8 ± 8.9 vs 15.1 ± 5.7 or 14.2 ± 2.9 , $P < 0.0001$). Plasminogen activities in the SMA occlusion group were higher than those in the SMV occlusion group with no significant difference.

In each group, plasminogen activity levels did not change significantly between the two measurements performed at 1 and 3 h. Plasminogen activity levels within and between groups are presented in the Table 1.

Table 1 Plasminogen activity levels (%) of groups at two time points

Time points	Sham group	SMA occlusion group	SMV occlusion group	<i>P</i> value
1st h	25.1 ± 10.8	11.8 ± 4.6	13.7 ± 4.4	$P < 0.001$
3rd h	29.8 ± 8.9	15.1 ± 5.7	14.2 ± 2.9	$P < 0.0001$
<i>P</i> values	$P = 0.21$	$P = 0.088$	$P = 0.71$	

SMA: Superior mesenteric artery; SMV: Superior mesenteric vein.

DISCUSSION

Both metabolic and morphologic changes are observed during mesenteric ischemia. As reported by Brown *et al*, structural changes start to occur within the first 10 min of ischemia^[10]. It has also been reported that within 30 min of ischemia a remarkable amount of fluid accumulates, both in the basement membrane and between cells resulting in paleness of villi, and also inflammatory cells accumulate^[11,12]. Thus, laboratory markers with high sensitivity and specificity that can be utilized 1 to 2 h prior to the occurrence of irreversible changes would be beneficial in the early diagnosis of acute mesenteric ischemia.

Most of the previous studies examining intracellular enzymes released from ischemic bowel failed to identify any marker sensitive or specific to disease for early diagnosis^[13-15]. The most likely explanation for the failure to identify such an early marker is that these enzymes usually appear in the blood only after irreversible ischemia has occurred in the bowel and that they are taken to the liver through the portal system to be metabolized. Murray *et al*^[16] demonstrated an increase in the blood levels of D(-)-lactate, a product of bacterial metabolism, 2 h following superior mesenteric artery occlusion. However, D(-)-lactate is also known to be produced in increased amounts in several conditions associated with bacterial proliferation, such as non-ischemic obstruction, intestinal perforation and peritonitis. So, D(-)-lactate does not seem to be specific for this disease.

Inorganic phosphate, which is the most important anion at the intracellular space, has also been investigated in models of mesenteric ischemia^[17-20]. A significant rise in serum phosphate level occurred after 4 h of ischemia, which was not helpful for an early diagnosis^[19]. Regional intestinal ischemia is reported to be detected and monitored by means of a microdialysis catheter placed in the peritoneal cavity or the bowel lumen by measuring lactate, pyruvate, glycerol and glucose levels^[21].

Plasma markers of coagulation and fibrinolysis such as fibrinogen, soluble fibrin, D-Dimer, tissue plasminogen activator/plasminogen activator inhibitor complex levels, have previously been studied in intestinal ischemia^[22-26]. However, the change in plasminogen levels, a fibrinolytic marker, has not been studied during acute mesenteric ischemia. In the present study, the role of plasminogen as a novel and specific marker for the early diagnosis of mesenteric ischemia was investigated.

Plasminogen is a single-chain glycoprotein that is produced in the liver and found in plasma under normal conditions; it has a half-life of 2.2 d. Average plasma level

is 21 mg/dL and its enzymatic activity ranges between 75% and 150% in humans. The level of plasminogen activity in rats changes with age and sex of the animal, but it is much lower than those in humans. The parameters we measured in this study are comparable with those in the literature^[26-29]. Plasminogen binds to fibrin as soon as a fibrin clot forms. That is, the fibrinolytic system is activated as coagulation continues. Plasminogen is converted to active plasmin by tissue plasminogen activator (t-PA), which is present in many tissues as well as in plasma and endothelial cells^[30-31]. During intestinal ischemia coagulation and fibrinolysis are activated. Fibrin production is coupled with the conversion of plasminogen to plasmin by t-PA released from hypoxic intestinal endothelium^[20].

In this rat model of acute mesenteric ischemia produced by arterial and venous occlusion, we measured blood plasminogen activity levels after 1 h and 3 h of ischemia and compared the plasminogen activities within and between groups. After 1 h of ischemia, a significantly lower activity of plasminogen was observed in both arterial and venous occlusion groups compared to the sham group, and the difference was maintained up to 3 h of ischemia. No significant differences between plasminogen activity levels after 1 h and 3 h of ischemia were present within the groups. The decrease in plasminogen levels may be attributed to the increased consumption of plasminogen by conversion to plasmin, due to the increased t-PA activity and fibrinolysis.

These data suggest that blood plasminogen activities are decreased during the early phase of both arterial and venous mesenteric ischemia, and plasminogen activity levels may be a useful marker for the early diagnosis of this disease. However, more research is required to exclude other possible causes of the fall in plasminogen activities.

COMMENTS

Background

Acute mesenteric ischemia has no specific marker for early diagnosis and thus, it is mortal in half of the cases. In this study, we investigated significance of changes in plasma plasminogen activity level during mesenteric ischemia.

Research frontiers

In this study we found a significant reduction in plasminogen activity level during early phases of both venous and arterial mesenteric ischemia. However, we need more research to understand its value in human mesenteric ischemia and also we should find equipment in laboratories for urgent plasminogen level determination so that the test could be helpful before bowel necrosis developed.

Innovations and breakthroughs

Several parameters have been studied for early diagnosis of mesenteric ischemia but none of them are found to be valuable. As plasminogen is a member of the fibrinolytic pathway we thought its plasma level could change in the condition and this change could be diagnostic.

Applications

Plasminogen level determination may be helpful in early diagnosis of mesenteric ischemia before bowel necrosis develops and we can manage the patient early preventing mortality.

Terminology

Mesenteric ischemia is an acute condition resulting from occlusion of either mesenteric artery or vein. It is recommended early management is necessary

because after the development of bowel necrosis there is not much to do for the recovery of the condition. Plasminogen is a protein synthesized in liver which is known as a member of the fibrinolytic pathway.

Peer review

The research is very interesting since the author's demonstrated that blood plasminogen activities decrease during early phases of both arterial and venous mesenteric ischemia, which may be a useful marker for early diagnosis. It appears that many potential cases could be lost or misrepresented by the range on values in normal and abnormal groups.

REFERENCES

- Whang EE, Ashley SW, Zinner MJ. Small Intestine. In: Brunnicardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Pollock RE (eds). *Schwartz's principles of surgery*, 8th ed. New York: The McGraw-Hill Companies, 2005: 1017-1054
- Lock G. Acute mesenteric ischemia: classification, evaluation and therapy. *Acta Gastroenterol Belg* 2002; **65**: 220-225
- McKinsey JF, Gewertz BL. Acute mesenteric ischemia. *Surg Clin North Am* 1997; **77**: 307-318
- Ouriel K, Green RM. Arterial Disease. In: Schwartz SI, Shieres GT, Spencer FC, Daly JM, Fischer JE, Galloway AC (eds). *Principles of Surgery*, 7th ed. New York: The McGraw-Hill Companies, 1999: 931-1004
- Nonthasoot B, Tullavardhana T, Sirichindakul B, Suphapol J, Nivatvongs S. Acute mesenteric ischemia: still high mortality rate in the era of 24-hour availability of angiography. *J Med Assoc Thai* 2005; **88** Suppl 4: S46-S50
- Acosta-Merida MA, Marchena-Gomez J, Hemmersbach-Miller M, Roque-Castellano C, Hernandez-Romero JM. Identification of risk factors for perioperative mortality in acute mesenteric ischemia. *World J Surg* 2006; **30**: 1579-1585
- Jonas J, Bottger T. Diagnosis and prognosis of mesenteric infarct. *Med Klin (Munich)* 1994; **89**: 68-72
- Rutherford EJ, Skeete D, Schooler WG, Fakhry SM. Hematologic principles in surgery. In: Townsend CM, Beauchamp RD, Evers BM, Mattox KI (eds). *Sabiston Textbook of Surgery* 17th ed. Philadelphia: Elsevier Saunders, 2004: 113-136
- Berg LH. Chemistry of Coagulation. In: Anderson SC, Cockayne S (eds). *Clinical Chemistry Concepts and Applications*, 1st ed. Philadelphia: WB Saunders, 1993: 613-632
- Brown RA, Chiu CJ, Scott HJ, Gurd FN. Ultrastructural changes in the canine ileal mucosal cell after mesenteric arterial occlusion. *Arch Surg* 1970; **101**: 290-297
- Mitsudo S, Brandt LJ. Pathology of intestinal ischemia. *Surg Clin North Am* 1992; **72**: 43-63
- Robbins SL. Ischemic bowel disease. In: Manke D (ed). *Basic Pathology*, 4th ed. Philadelphia: WB Saunders, 1987: 526-528
- Kurland B, Brandt LJ, Delany HM. Diagnostic tests for intestinal ischemia. *Surg Clin North Am* 1992; **72**: 85-105
- Krausz MM, Manny J. Acute superior mesenteric arterial occlusion: a plea for early diagnosis. *Surgery* 1978; **83**: 482-485
- Boley SJ, Feinstein FR, Sammartano R, Brandt LJ, Sprayregen S. New concepts in the management of emboli of the superior mesenteric artery. *Surg Gynecol Obstet* 1981; **153**: 561-569
- Murray MJ, Barbose JJ, Cobb CF. Serum D(-)-lactate levels as a predictor of acute intestinal ischemia in a rat model. *J Surg Res* 1993; **54**: 507-509
- Feretis CB, Koborozos BA, Vyssoulis GP, Manouras AJ, Apostolidis NS, Golematas BC. Serum phosphate levels in acute bowel ischemia. An aid to early diagnosis. *Am Surg* 1985; **51**: 242-244
- Koksal N, Ozturk A, Titiz MI. Changes in serum phosphate levels in experimental acute mesenteric ischemia. *Haydarpa Numune Tip Dergisi* 1998; **37**: 9-13
- Jamieson WG, Lozon A, Durand D, Wall W. Changes in serum phosphate levels associated with intestinal infarction and necrosis. *Surg Gynecol Obstet* 1975; **140**: 19-21
- Lores ME, Canizares O, Rossello PJ. The significance of elevation of serum phosphate levels in experimental intestinal ischemia. *Surg Gynecol Obstet* 1981; **152**: 593-596

- 21 **Sommer T**, Larsen JF. Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischaemia. *Br J Surg* 2004; **91**: 855-861
- 22 **Acosta S**, Nilsson TK, Bergqvist D, Bjorck M. Activation of fibrinolysis and coagulation in non-occlusive intestinal ischaemia in a pig model. *Blood Coagul Fibrinolysis* 2004; **15**: 69-76
- 23 **Kulacoglu H**, Kocaerkek Z, Moran M, Kulah B, Atay C, Kulacoglu S, Ozmen M, Coskun F. Diagnostic value of blood D-dimer level in acute mesenteric ischaemia in the rat: an experimental study. *Asian J Surg* 2005; **28**: 131-135
- 24 **Acosta S**, Nilsson TK, Bjorck M. D-dimer testing in patients with suspected acute thromboembolic occlusion of the superior mesenteric artery. *Br J Surg* 2004; **91**: 991-994
- 25 **Kurt Y**, Akin ML, Demirbas S, Uluutku AH, Gulderen M, Avsar K, Celenk T. D-dimer in the early diagnosis of acute mesenteric ischemia secondary to arterial occlusion in rats. *Eur Surg Res* 2005; **37**: 216-219
- 26 **Schoots IG**, Levi M, Roossink EH, Bijlsma PB, van Gulik TM. Local intravascular coagulation and fibrin deposition on intestinal ischemia-reperfusion in rats. *Surgery* 2003; **133**: 411-419
- 27 **Karges HE**, Funk KA, Ronneberger H. Activity of coagulation and fibrinolysis parameters in animals. *Arzneimittelforschung* 1994; **44**: 793-797
- 28 **Laudes IJ**, Chu JC, Sikranth S, Huber-Lang M, Guo RF, Riedemann N, Sarma JV, Schmaier AH, Ward PA. Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. *Am J Pathol* 2002; **160**: 1867-1875
- 29 **Nobukata H**, Ishikawa T, Obata M, Shibutani Y. Age-related changes in coagulation, fibrinolysis, and platelet aggregation in male WBN/Kob rats. *Thromb Res* 2000; **98**: 507-516
- 30 **Rijken DC**. Relationships between structure and function of tissue-type plasminogen activator. *Klin Wochenschr* 1988; **66** Suppl 12: 33-39
- 31 **Teesalu T**, Kulla A, Asser T, Koskiniemi M, Vaheri A. Tissue plasminogen activator as a key effector in neurobiology and neuropathology. *Biochem Soc Trans* 2002; **30**: 183-189

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