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Influence of splanchnic vascular infusion on the content of endotoxins in plasma and the translocation of intestinal bacteria in rats with acute hemorrhage necrosis pancreatitis

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INTRODUCTION

The main reason for the death of the patient with acute hemorrhage necrosis panc reatitis (AHNP) is pancreatic infection and multi-organ failure caused by endot oxemia and intestinal bacterial translocation [1-7]. However, the patho genesis of endotoxemia and intestinal bacterial translocation remains a question [8-10]; moreover, no effective method of prevention and cure for it has been found till now[11-15]. In the present study, we infused low dose dopamine and low molecular weight dextran through the catheters to abdominal aort a and portal vein, and observed its influence on the endotoxin concentration in plasma and the rate of translocation of intestinal bacteria in AHNP rats.

MATERIALS AND METHODS

Animals

A total of 48 Sprague-Dawley rats weighing 295-320 g were divi ded into 4 groups (with 12 rats in each group): Group A (healthy rats), Group B (AHNP rats), Group C (femoral artery and femoral vein infused rats), and Group D (abdominal aorta and portal vein infused rats).

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Experimental methods

Bacterial labeling Following the way previously described by Wells, we di rectly labeled $O_{55}B_5$ Escherichia coli (*E. coli*) with flourescei n isothiocyanate to prepare the solution of 5×10^6 cfu/L tracer.

Induction of AHNP model and blood vessels catheter insertion and infusion Firstly, all rats were deprived of food 24 h before laparotomy, and were given a gavage of fluorescein-labeled *E.Coli* (0.7 mL/100 g) 12 h later, then they w ere kept for 12 h before being anesthetized ip with 2% pentobarbital sodium (0.15 mL/100 g). Secondly, after the abdominal hair was removed and t he abdominal cavity was opened through a midline laparotomy, the pancreas was exposed and 5% taurodexycholic acid sodium solution (0.15 mL/100 g; Sigma) was sl owly injected into the pancreatic duct with retrograde pressure. Five min la ter, hemorrhage, necrosis, swelling and exudation appeared in the pancreas. Thir dly, 2 h after the AHNP models were completed, the animals of group B were infused continuously with saline through the catheters which were connected to the fe moral artery and femoral vein and portal vein (the catheter was inserted from th e ileocolic vein to the main trunk of portal vein), the animals of group C were infused continuously and alternately with low dose dopamine ($5\ \mu g \cdot kg^{-1} \cdot min^{-1}$) and low molecular weight dextran (1.5 mL through catheters inserted into femoral arte ry and femoral vein, while being infused continuously with saline through the portal vein. The animals of Group D were infused continuously and alternately wi th low dose dopamine and low molecular weight dextran through the portal vein and abdominal aorta (catheters were inserted from the femoral artery and above the junction between the abdominal aorta and abdominal cavity artery), while being infused continuously with saline through the femoral vein. The total amount of infusion (6 mL/100 g) was the same in each group. Lastly, after 4 h of sustained infusion, the abdominal cavity was found to be hemorrhagic on beingre-open ed, and it was more evident in both Group B and Group C than in Group D. The pan creas showed pathological changes such as hemorrhage, necrosis, swelling and exu dation, while Group A (control group) had no pathological changes.

Indicators and methods

Plasma endotoxin One mL vein blood was put into the heparin-containing tes t tube under aseptic and non-pyrogenic conditions, and after being centrifuged 500 rpm at for 10 min, the plasma was absorbed and preserved in a refr igerator at -20℃. The content of plasma endotoxin was investigated by quantitative azo stromatic coloration limulus test microassay (kit from Institute of Medi cine in Shanghai).

Investigation of mesentric lymph mode (MLN) labeled-bacteria MLN of the ileocecum were excised, weighed, triturated and diluted into 10% tissue plasma a nd was observed under fluorescein microscope for the existence of labeled-bacteria. The bacterial translocation rate was also calculated.

The microcirculation of pancreas and mesentery The diameter of pancreatic and mesenteric small vein at the ileocecum end was directly measured under the microscope and recorded on the video-camera.

Pathological changes in the intestinal mucosa: Observed under light and transmission electron microscopy.

RESULTS

Changes in content of plasma endotoxin and rate of translocation of labeled-bac teria in MLN (Table 1)

Table 1 Changes in plasma endotoxin concentration ($\bar{x} \pm S_{\bar{x}}$, EU/mL) and the rate of translocation of labeled-bacteria in MLN

Group	Plasma endotoxin (2 h)	Plasma endotoxin (6 h)	Rate of translocation of labeled-bacteria (%)
A	0.023 ± 0.004	0.033 ± 0.006	0
B	0.028 ± 0.002	0.340 ± 0.038	91
C	0.025 ± 0.007	0.270 ± 0.048	83
D	0.027 ± 0.001	0.103 ± 0.018	33

There was a significant statistical difference between group D and either group B or C ($P < 0.05$), regarding the content of plasma endotoxin and translocation rate of MLN labeled-bacteria. However, there is no statistical difference betwe en group B and group C ($P > 0.05$), while a significant difference exists when group a is compared with either group B, C, or D ($P < 0.05$).

Microcirculatory changes in pancreas and mesentery (Table 2)

Table 2 Change in the mesenteric small vein diameter after 2 h and 4 h AHNP

Group	2 h MVD	6 h MVD
A	0.66 ± 0.04	0.63 ± 0.04
B	0.69 ± 0.05	1.03 ± 0.05
C	0.72 ± 0.03	1.09 ± 0.05
D	0.77 ± 0.07	0.68 ± 0.05

Between 2 h and 4 h after AHNP, in Group B and Group C, the diameter of panc reatic and mesenteric small vein increased significantly ($P < 0.05$), and the velocit y of blood was observed to be retarded or even blocked, while there was no siguificant increase in the diameters of pancreatic and mesenteric small veins in group D ($P > 0.05$) and no st atistical difference regarding the velocit y of blood stream between group D and group A ($P > 0.05$).

Pathological changes in intestinal mucosa

Optical microscopic observation It was seen that a large-number of mucos al chorionic epithelium were exfoliated, the upper parts of villus intestina were in significant edema, the central chylectasia was expanded, the blood vessels congested, the proprietary membrane was in moderate edema and the inflammatory cells infiltrated in group B and group C. While the damage of mucosa in group D w as alleviated as compared to group B or group C, it was seen that only the villus became shorter, the proprietary membrane was in edema and the inflammatory cel ls infiltrated.

Electronmicroscopic observation Rarefaction and exfoliation of the epithe lium microvilli of intestinal mucosa, exudation of matrix vacuolar degeneration of mitochondria, swelling of endoplasmic reticulum, and break-down of epitheliu m bridges were observed in groups B and C; and in group D only slight dera n gement of intestinal mucosa epithelium and slight swelling of mitochondria and endoplasmic reticulum were seen.

DISCUSSION

An extensive amount of experimental and clinical work reveals that the disorder of pancreatic microcirculation, the production of many inflammatory mediators an d cytokines and the translocation of intestinal bacteria are all thought to play a critical role in the pathogenesis of acute hemorrhage necrosis pancreatitis [15-26]; furthermore, the disorder of splanchnic organic microcirculation, especially the disorder of pancreas microcirculation in AHNP is closely connected with the production of many inflammatory mediators and cytokines [26-37]. Dopamine has been seen to possess complicate pharmacological function s[38-40], in above 10 μg • kg^{-1} • min^{-1}, alpha adr energic receptors are additional ly activated, causing splanchnic vascular contraction. At a dose range of 1-4 μg • kg^{-1} • min^{-1}, the effect is predominantly on dopaminergic receptors, leading to splan chnic dilatation. At the 4-10 μg • kg^{-1} • min^{-1}, beta a drenergic receptors are increasi ngly stimulated, which stops the increase in microvascular permeability caused by histamine and bradykinin. It also activates dopaminergic receptors leading to an increase of blood flow in splanchnic organs. Low molecular weight dextran can lower blood viscosity and hemaglutination leading to a halt in microvascular th rombogenesis in portal vein system[41-42]. Therefore, low dose dopamine and low molecular weight dextran can be used to improve splanchnic

microcirculat ion. With the aid of catheters inserted into the aorta and portal vein, we i nfused low dose dopamine and low molecular weight dextran continuously and alter natively, which enhances the drug concentration in pancreas, liver and intestin al tract. The results have revealed that by this method the microcirculation was improved, and the content of endotoxin and the rate of intestinal bacterial t ranslocation were decreased with increasing blood supply to the pancr eas and intestinal tract of AHNP rats. This effect can be related to the followi ng factors: Firstly, low dose dopamine and low molecular weight dextran can dire ctly improve the ischemic status of intestinal tract and inhibit the damage of t he barrier function of intestinal mucosa. It can also inhibit the pancreatic hem orrhage, necrosis and decrease the production of inflammatory mediators by impro ving microcirculation of pancreas, liver, and intestinal tract. On the other hand they can lighten the damage of inflammatory mediators and endotoxin on intesti nal tract by enhancing the ability of the liver in clearing from inflammatory me dia tors and endotoxin. Hence, the method can alleviate the injury of intestinal mucosa and protect its barrier function, and inhibit endotoxemia and translocation of the intestinal bacteria, and so indicate that the disorder of microcircul a tion of pancreas and intestinal tract and liver are critically important to endo toxemia and the bacterial translocation from the intestine. The study also shows that the infusion through catheter to femoral vein and artery has no obvious in fluence on the content of endotoxin in plasma and the bacterial translocation in AHNP rats, the reason of which may be related to the low concentration of drugs in pancreas, liver and intestinal tract.

To achieve clearance of inflammatory mediators in patients with AHNP, in addition to drainage and removing the necrotic tissues by op e ration, we can also infuse low dose dopamine and low molecular weight dextran in to the abdominal cavity after operation through catheter inserted either from t he right gastroduodenal vein to the portal vein or from the femoral artery, which can improve the microcirculation disorder of pancreas, liver and intestinal t ract. Moreover, we can infuse enzyme inhibitors and other anti-inflammatory med iators through the catheter to the portal vein, so as to eliminate inflammatory mediators before they reach the liver.

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