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Research Article

Effect of splanchnic nerve transection on liver injury in dogs with acute necrotizing pancreatitis

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Abstract

Objective: This study investigated the effect of bilateral greater splanchnic nerve (GSN) transection on hepatic injury during acute necrotizing pancreatitis (ANP) in dogs.

Methods: Twenty-four healthy adult beagle dogs were randomly divided into a sham operation group (SO group, n=8), ANP model group (ANP group, n=8), and ANP with bilateral GSN transection group (GSNT group, n=8). ANP was induced by sodium taurocholate and trypsin infusion into the pancreatic duct; dogs in the GSNT group underwent bilateral GSN transection immediately following ANP induction. Serum tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), pancreatic amylase (AMY), alanine aminotransferase (ALT), and aspartate transaminase (AST) were monitored dynamically. The dogs were sacrificed on day 7 after the operation, pancreatic and hepatic tissues were harvested for pathology, and the expression of p-NF-κB p65 in liver tissues was measured by western blotting.

Results: Serum AMY, TNF-α, IL-1β, IL-6, AST, and ALT levels did not differ significantly between the three groups at 2h before the operation ($P>0.05$). Serum TNF-α, IL-1β, IL-6, AMY, ALT, and AST levels, pancreatic and hepatic pathological scores, and p-NF-κB p65 expression in liver tissues were significantly higher in the ANP group than in the SO group after the operation (all $P<0.05$). Serum TNF-α, IL-1β, IL-6, ALT and AST, pancreatic and liver pathological scores, and p-NF-κB p65 protein expression in liver tissues were lower in the GSNT group than in the ANP group after the operation (all $P<0.05$), whereas serum AMY levels did not differ significantly between these two groups ($P>0.05$).

Conclusion: Bilateral greater splanchnic nerve transection alleviates liver injury during ANP in dogs.

Keywords Pancreatitis, Acute necrotizing; Hepatic injury; Ssplanchnic nerve

Introduction

Acute necrotizing pancreatitis(ANP) is a serious systemic disease with a high mortality rate of approximately 20% [1,2]. Liver injury is a serious and sometimes fatal complication of ANP. Studies [3] show that the incidence of liver injury associated with acute pancreatitis (AP) can be as high as 60%, increasing to 80% in the presence of ANP. Because liver injury persists throughout the course of ANP, the treatment of liver injury caused by ANP is crucial to the prognosis of patients [4].

The liver injury caused by ANP is mainly related to the massive activation of pancreatin, microcirculation disorders of the pancreas and liver, apoptosis, activation of inflammatory cytokines, and pancreatitis related ascites [4]. In addition, nuclear factor-kappa B (NF-κB) plays an important role in the release of inflammatory cytokines and in mediating hepatocyte apoptosis [5]. Liver injury complicated by ANP presents with liver enzyme changes in clinical practice, and is characterized by early liver function abnormalities (within 24 hours after ANP onset) and hepatocyte necrosis (the main pathological change of the liver detected by light microscopy) [4,6].

The sympathetic and parasympathetic pathways function both in synergy and opposition in the same organ, and the balance between the two plays an important role in regulating homeostasis. In addition, the immune system and the visceral nervous system show complex interactions, and the latter acts as a bridge linking the immune system and the central nervous system (CNS) [7]. The preganglionic fibers of the sympathetic nerves that innervate the liver originate from the greater splanchnic nerve, whereas the parasympathetic nervesare derived from the vagus nerve. Many studies have focused on the relationship between the nervous and immune systems since Borovikova first suggested the concept of the "cholinergic anti-inflammatory pathway" in 2000 [8], and the medical community has reached a consensus on the concept [9]. Michael et al. [10] demonstrated that, in sepsis, tension of the sympathetic nerves exceed that of the vagus nerves, and over excitation of the sympathetic nerves launches a systemic, proinflammatory, out-of-control response. These authors also proposed the concept of "sympathetic excitotoxicity

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in sepsis," which is characterized by neurogenic priming of the systemic proinflammatory response. Out-of-control inflammation is the chief pathological factor underlying a variety of deadly ANP complications, and the involvement of the cholinergic anti-inflammatory pathway and sympathetic excitotoxicity in sepsis suggests that the ANP inflammatory response can be controlled by relevant neurological intervention. Relevant studies on the neuroregulation of hepatic injury complicated by ANP have not been published to date. In this study, we hypothesized that transecting the bilateral GSN of dogs with ANP would improve the sympathetic-vagus imbalance and hepatic injury, thereby preventing an excessive inflammatory response. This would provide experimental evidence of the efficacy of sympathetic nerve transection for the clinical treatment of early ANP.

Methods

Ethics statement

This study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Henan University of Science and Technology (Permit No. 2018-0023). All surgical procedures were performed under intravenous propofol anesthesia to minimize the suffering of experimental animals.

Experimental animals and grouping

Twenty-four healthy beagle dogs weighing 10–15 kg were provided by Henan University of Science and Technology Experimental Animal Center. They were raised for 5 weeks before the experiment under observation to ensure the experimental dogs were in good health. The dogs were divided randomly into three groups: sham operation group (SO group, n = 8); ANP model group (ANP group, n = 8); and ANP model + bilateral greater splanchnic nerve (GSN) transection group (GSNT group, n = 8). The animals fasted for 12 h prior to the surgical procedure.

Preparation before ANP induction

The dogs were anesthetized via intramuscular injection of 15 mg/kg Zoletil (Virbac Ltd., Carros, France) and maintained with continuous intravenous administration of 10 mg/kg/h propofol (Guorui Pharmaceutical LLC., Sichuan, China). Under sterile conditions, a 7–9 cm median abdominal incision was created. In the GSNT group, abdominal organs were pushed to the right side with wet saline gauze, exposing the left adrenal gland in the abdominal cavity. The loose fat tissue behind the left adrenal gland was carefully separated to expose a bright white-like bundle of fibers. The left GSN was identified and dissociated, and then marked by threading. The right GSN was dissociated in the same way and marked by threading. The following celiac operations were similarly performed in all groups. In the plane of the main pancreatic duct trunk and approximately 12–14 cm from the pyloric sphincter, a 1.5–2.0 cm incision was made in the contralateral duodenum of the pancreatic duct; the accessory pancreatic duct was located, and a 5 cm epidural catheter was inserted parallel to the main pancreatic duct.

ANP induction

Dogs in the SO group were infused with saline at 0.5mL/kg using a micropump (TCL-II New Technology, Ltd., Guangxi, China) into the pancreas via the pancreatic duct at a pressure of 7.6kPa (infusing speed: 19mL/h). The ANP model dogs were induced by an infusion of 0.5 /kg 5% sodium taurocholate (Sigma-Aldrich, USA) with 3,000U/kg trypsin (Amresco, USA) into the pancreatic duct. The common biliary duct was clamped during the infusion. Pancreatic glands exhibited slight edema in the SO group; however, in the ANP and GSNT groups, they displayed quick congestion, edema, and were covered with flake-like bleeding and local dimming. In previous studies, we demonstrated that it is possible to create a necrotic pancreatitis model under 7.6 kPa with a constant pressure injection of 50 g/L sodium taurocholate (0.5/kg) and trypsin (3,000 U/kg) [11].

GSNT model execution

The GSNT group underwent bilateral GSN transection immediately following ANP induction. In all experimental dogs, the duodenal incision was sutured and the abdomen was closed layer by layer. Vital signs, urine volume, and defecation frequency and volume were observed and recorded. Clinical symptoms were managed in a timely manner and postoperative anomalies were recorded.

Maintenance support measures

All dogs were deprived of food and drink after the experimental model was completed and placed in a standard animal laboratory with clean-air circulation at room temperature (23°C) and 61% humidity. At the initiation of fasting, the total parenteral nutrition solutions comprised 7% vamin (SSPC, 9.4g/1,000), 20% intralipid (SSPC), and 50% glucose. The nonprotein calorie consumption was 50 kcal (209.2kJ/kg) and nitrogen intake was 0.3g/kg/d. The total volume of intravenous injection solution was 70/kg/d. The energy index supported with glucose and fat emulsion was 1:1. Multivitamins and electrolytes were also included in total parenteral nutrition solutions. Natural saline was injected by 250 ml/kg during operation time and 8 h postoperatively, and then injected with 120–150 ml/kg.

Specimen collection and testing methods

Samples of 3 ml venous blood were collected from all dogs, and serum was separated by centrifugation (3,000 r/min for 15 min) at 2 h preoperatively and at 12 and 24 h and 3, 5, and 7 days postoperatively. Samples were stored at -80°C for further testing after quick-freezing with liquid nitrogen. Tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) concentrations were detected using the double antibody sandwich enzyme-linked assay method in accordance with the kit operating instructions of TNF-α, IL-1β, IL-6 (RD co., USA). The levels of pancreatic amylase (AMY), alanine aminotransferase (ALT), and aspartate transaminase (AST) were detected with a veterinary automatic biochemical analyzer using 0.5 serum. Dogs in each group were sacrificed on day 7 after surgery; three specimens of pancreatic and hepatic tissues were obtained per dog. All specimens were fixed with 4% formaldehyde, paraffin-

embedded, sliced, and stained with hematoxylin-eosin (H&E). The tissues were inspected by light microscopy. Histological scoring was performed in a blinded manner using the Schmidt scoring method [12] to assess the severity of pathological lesions of the pancreas and the one point count method [13] for evaluating the severity of pathologic liver lesions. In case of inconsistent results, a third physician was consulted for decision making.

The expression of p-NF-κB p65 in the liver was detected by western blotting. Protein was extracted from liver tissues using RIPA buffer containing a protein phosphatase inhibitor (Beijing Solarbio Technology Co., Ltd). Coomassie Brilliant Blue staining was used for protein quantification. Western blotting was performed by probing with antibodies according to the manufacturer's instructions. The primary antibody was anti-phospho-NF-κB p65 (Ser536) rabbit mAb (sc-33020, 1:200, Santa Cruz Biotechnology, USA), and the secondary antibody was peroxidase-conjugated goat anti-rabbit IgG (1:5,000, Proteintech Co., Ltd). The target protein level was normalized to that of GADPH (ab181603, 1:1,0000, Abcam, USA). Bands were visualized by enhanced chemiluminescence, and the Image Lab gel imaging system was used for calculating the relative band intensity.

The presence of ascites or adhesions, blood conditions, and pancreatic pathology (for animals that died prematurely, the time and cause of death were recorded) were also recorded.

Statistical analysis

SPSS 19.0 software was used for data analyses. The data were expressed as the mean ± SD. The levels of serum TNF-α, IL-1β, IL-6, AMY, ALT, and AST in each group before the operation, the pathological scores of the pancreas and liver, and the expression levels of p-NF-κBp65 in the liver after the operation were analyzed by one-way ANOVA. Differences in serum TNF-α, IL-1β, IL-6, AMY, ALT, and AST levels in each group after the operation were analyzed by repeated measurement ANOVA. Pairwise comparisons between groups were performed using the LSD-t test. A *P* value of <0.05 indicated a statistically significant result.

Results

Dogs in the SO and GSNT groups lived for 7 days, whereas in the ANP model group, one dog died of respiratory failure caused by lung injury on day 5 and another died of multiple organ failure caused by fulminant AP on day 6 after the operation. All the data were collected including those of dead dogs for statistical analysis.

Preoperative 2 h serum TNF-α, IL-1β, IL-6, ALT, and AST concentration and AMY activity in the SO, ANP, and GSNT groups

The serum concentrations of TNF-α, IL-1β, IL-6, ALT, and AST, as well as AMY activity did not differ significantly between the three groups at 2 h before the operation. (P>0.05, Figure 1AB, Figure 2AB, Figure 3AB).

Postoperative serum AMY activity in the three groups

Postoperative serum AMY activity did not change significantly in the SO group. However, postoperative serum AMY activity increased significantly in the ANP and GSNT groups and differed significantly from that in the SO group at each time point (P<0.05). There was no significant difference between the ANP group and the GSNT group (P>0.05; Figure 1A).

Postoperative serum TNF-α, IL-1β, IL-6, AST, and ALT concentrations in the three groups

In the ANP and GSNT groups, peak serum levels of TNF-α, IL-1β, IL-6, AST, and ALT were recorded during the initial postoperative 24 h (Figure 1A-C), and then slowly declined.

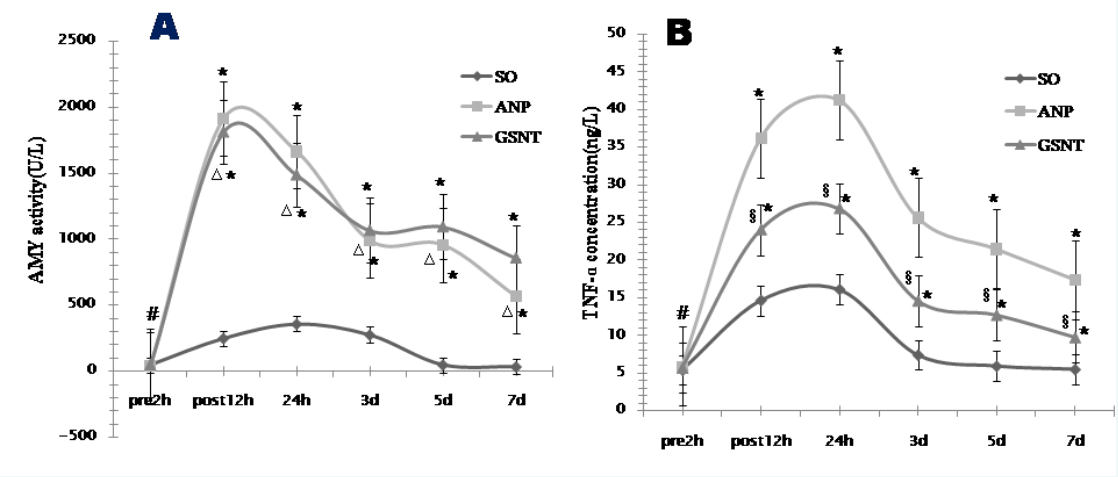

Figure 1 At 2 h preoperatively and at each postoperative time point, the serum activities of pancreatic amylase (AMY), and concentrations of tumor necrosis factor alpha (TNF-α) in all dogs in the SO, ANP, and GSNT groups showed variable trends. # no significant differences between the three groups with regards to serum levels of AMY, and TNF-α 2 h preoperatively (P>0.05, A, and B). * compared with the SO group (P<0.05, A, and B). compared with the ANP group (P>0.05, A). § compared with the ANP group (P<0.05, B). SO: sham operation group; ANP: acute necrotizing pancreatitis model group; GSNT: acute necrotizing pancreatitis model with bilateral greater splanchnic nerve transection group.

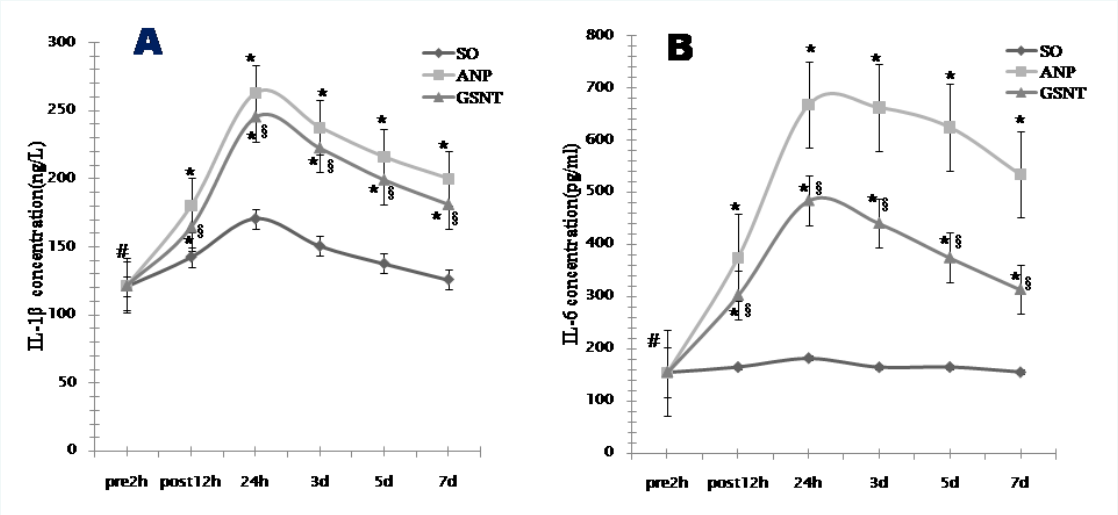

Figure 2 At 2 h preoperatively and at each postoperative time point, the serum concentrations of interleukin-1β (IL-1β), and interleukin-6 (IL-6) in all dogs in the SO, ANP, and GSNT groups showed variable trends. # no significant differences between the three groups with regards to serum levels of IL-1β, and IL-6 2 h preoperatively (P>0.05, A, and B). * compared with the SO group (P<0.05, A, and B). § compared with the ANP group (P<0.05, A, and B). SO: sham operation group; ANP: acute necrotizing pancreatitis model group; GSNT: acute necrotizing pancreatitis model with bilateral greater splanchnic nerve transection group.

At different postoperative intervals (12 h, 24 h, 3 d, 5 d, and 7 d postoperatively), the levels of serum TNF-α, IL-1β, IL-6, AST, and ALT significantly increased in the ANP and GSNT groups compared with the SO group (P<0.05, Figure 1B, Figure 2AB, Figure 3AB).

Based on the ANP model, we further transected the GSNT and observed postoperative change in the cytokines, and liver enzyme indicators in the GSNT group. We found that postoperative serum TNF-α, IL-1β, IL-6, AST, and ALT concentrations in the GSNT group at 12 h, 24 h, 2 d, 3 d, 5 d, and 7 d postoperatively were significantly lower compared with those in the ANP group (P<0.05, Figure 1B, Figure 2AB, Figure 3AB).

Pathological changes

Dogs were sacrificed on day 7 after the operation, and pancreatic and hepatic tissues were harvested for observation and pathology scoring.

General pathology changes were as follows: in the SO group, the pancreas was pink, soft, and showed no abnormalities; the liver was brownish red, soft, and elastic; there were no ascites; mild adhesions were detected, and intestinal blood flow was normal. In the ANP model group, the pancreas showed edema, hemorrhage, and necrosis; the liver showed a slightly darker color, obvious edema, visible patchy bleeding, and infectious foci, as well as intestinal flatulence; severe adhesions and peristalsis movement were observed, and some dogs showed obvious hemorrhagic ascites. In the GSNT group, the pancreas and liver both showed a moderate amount of bleeding and noticeable edema, and necrosis was observed in the pancreas; the intestinal adhesions were smaller and intestinal flatulence was inconspicuous.

Pathological pancreatic lesions were scored under a light microscope, and the results showed that the scores were significantly higher in the ANP group than in the SO group and the GSNT group (both P<0.05; Figure 4A). Histopathological changes in the pancreas were as follows: in the SO group, the pancreas was normal, whereas the ANP model group showed a large number of red blood cells in the pancreatic interstitium, inflammatory cell infiltration, abscess formation, and peripancreatic fat tissue necrosis, as well as severe pancreatic and vascular injury. The GSNT group showed a moderate amount of red blood cells in the pancreatic interstitium, inflammatory cell infiltration, small structure destruction, no obvious bleeding, and tissue necrosis or atrophic degeneration were partially visible within the pancreas and peripancreatic tissues; pathological changes were milder than those observed in the ANP model group (Figure 4 B, C1, C2, D1, and D2).

Scoring of pathological liver lesions under a light microscope showed that the lesion scores were significantly higher in the ANP group than in the SO group and the GSNT group (both P<0.05; Figure 5 A). Histopathological changes in the liver were as follows: in the SO group, there were no significant histopathology changes in liver tissues. In the ANP group, hepatic cord disruption, hepatocyte degeneration and necrosis, extensive inflammatory cell infiltration, and punctate hemorrhage were visible. In the GSNT group, the hepatic cord structure was normal, and inflammatory cell infiltration was only observed in the portal area; sporadic punctate hemorrhage and necrosis were detected infrequently (Figure 5 B, C1, C2, D1, and D2).

Expression of p-NF-κBp65 in liver tissues

On postoperative day 7, the expression of p-NF-κBp65 in liver tissues was higher in the ANP and GSNT groups than in the SO group, and the difference was statistically significant (both

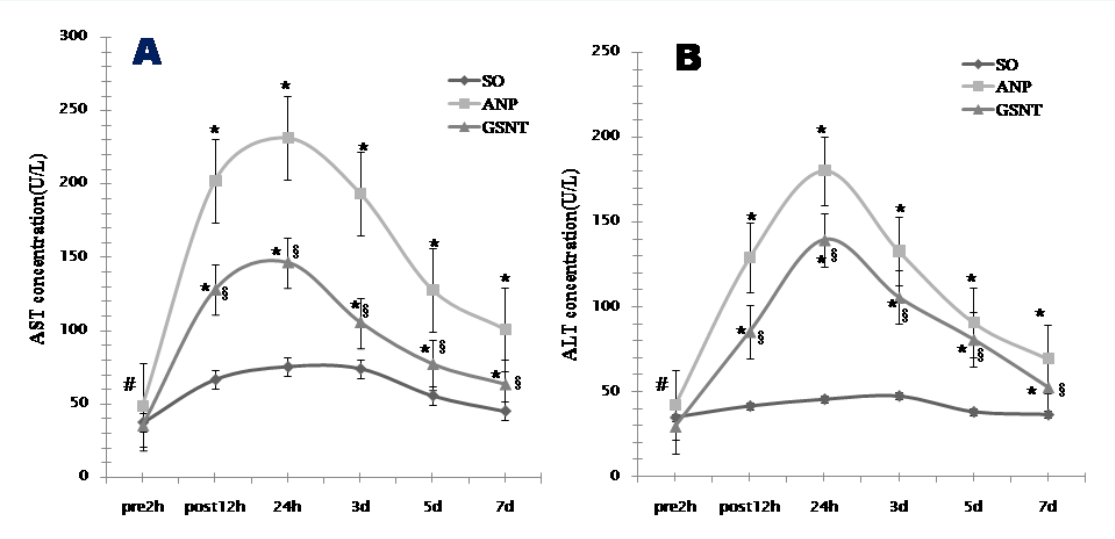

Figure 3 At 2 h preoperatively and at each postoperative time point, the serum concentrations of aspartate transaminase (AST), and alanine aminotransferase (ALT) in all dogs in the SO, ANP, and GSNT groups showed variable trends. # no significant differences between the three groups with regards to serum levels of AST, and ALT 2 h preoperatively (P>0.05, A, and B). * compared with the SO group (P<0.05, A, and B). § compared with the ANP group (P<0.05, A, and B). SO: sham operation group; ANP: acute necrotizing pancreatitis model group; GSNT: acute necrotizing pancreatitis model with bilateral greater splanchnic nerve transection group.

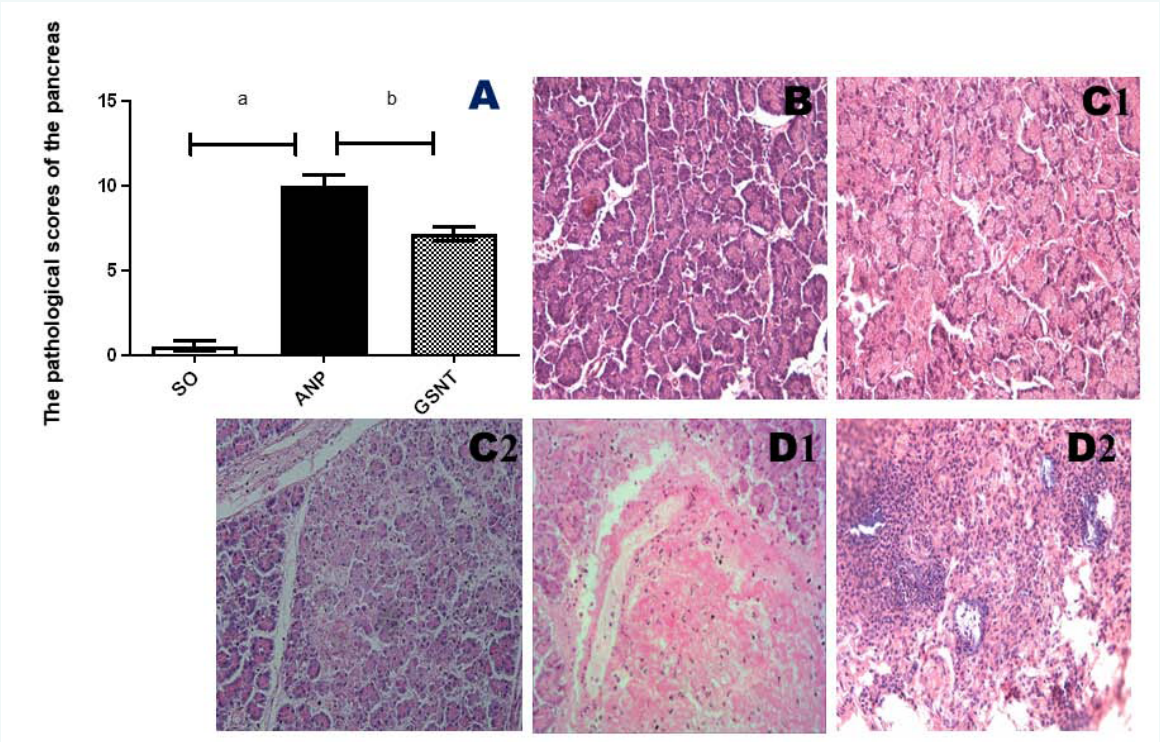

Figure 4 Dogs in each group were sacrificed on day 7 after the operation and the pathology of the pancreas was observed histologically. A, Pathological scoring of all groups of surviving dogs after 7 days. a Compared with SO (P<0.05); b compared with GSNT (P<0.05). B, SO (normal histology of pancreas); C1, GSNT (pancreatic edema); C2, GSNT (some small lobular acinar structure destruction or atrophic degeneration were partially visible, inflammatory cell infiltration, no obvious bleeding); D1, ANP (pancreatic gland cell degeneration necrosis, circumjacent bleeding, and inflammatory exudate formation); D2, ANP (abscess formation, inflammatory cell infiltration, hemorrhage, and pancreatic gland bubble structure not obvious; hematoxylin-eosin, ×200). SO indicates the SO group (n = 8); ANP, ANP the model group (n = 6); GSNT, ANP model with bilateral greater splanchnic nerve transection group (n = 8).

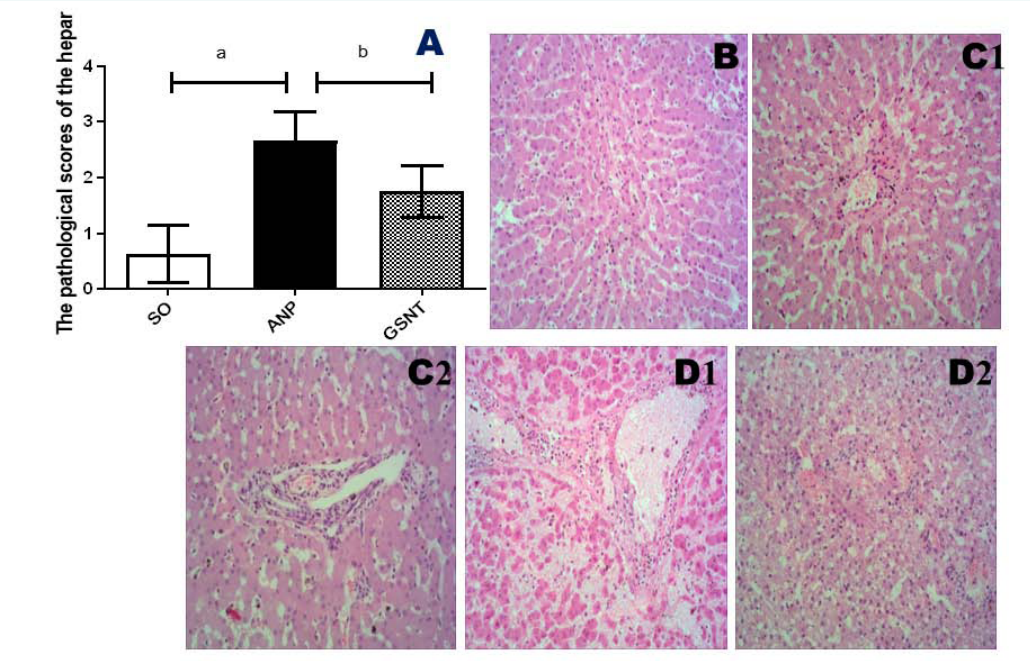

Figure 5 Dogs in each group were sacrificed on day 7 after the operation and the pathology of the liver was observed histologically. A, Pathological scoring of all groups of surviving dogs after 7 days. a Compared with SO (P<0.05); b compared with GSNT (P<0.05); B, SO (normal histology of the liver); C1 and C2, GSNT (inflammatory cell infiltration in the portal area, hepatic edema, punctate hemorrhage); D1, ANP (the destruction of the hepatic cord structure and patchy dissolute necrosis, a large inflammatory infiltration); D2, ANP (degeneration and necrosis of hepatocytes, rupture of hepatic cord structure and patchy hemorrhage; hematoxylin-eosin, ×200). SO indicates SO group (n = 8); ANP, ANP model group (n = 6); GSNT, ANP model with bilateral greater splanchnic nerve transection group (n = 8).

P<0.05). The expression of p-NF-κBp65 was higher in the ANP group than in the GSNT group (P<0.05; Figure 6).

Discussion

AP is a common acute abdominal disease, and its incidence has been increasing in recent years. In approximately 20% of patients, AP progresses to ANP [2]. In patients with ANP, the liver is one of the most frequently involved extra-pancreatic organs, and the rate of incidence of liver injury associated with ANP can reach up to 88.9% [3,4]. In addition, the pathogenetic factors of ANP, such as biliary tract disease , excessive drinking, and hyperlipemia [14], can directly cause liver injury. Hepatocyte damage can further accelerate the progression of ANP and is positively correlated with the severity of ANP [15].

Despite an abundant blood supply and strong compensatory function, the liver is the most frequent site of injury in ANP compared with other organs outside the pancreas. One reason for this is that the liver is adjacent to the pancreas, and the inflammatory exudate from ANP can easily spread to the liver through hepatic duodenal ligaments and the Glisson sheath [16]. In addition, pancreatic enzymes, inflammatory cytokines and endotoxins that are produced during the development of AP enter the liver through the portal vein, which makes the liver the first line of defense for ANP; the rich blood supply of the liver results in a higher probability of injury [4]. Almost all inflammatory mediators produced during pancreatitis need to pass through the liver before entering the systemic circulation. Severe liver

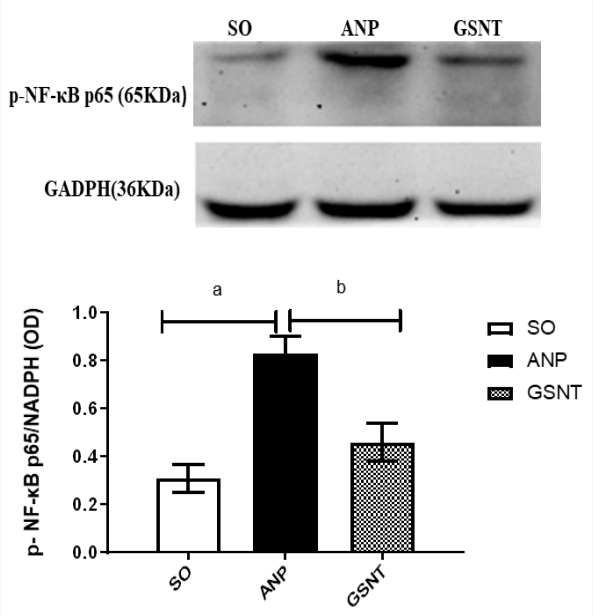

Figure 6 Dogs in each group were sacrificed on day 7 after the operation and the expression of p-NF-κB p65 in liver tissues was detected by western blotting. [a] ompared with SO (P<0.05); [b] compared with ANP (P<0.05); SO indicates SO group (n = 8); ANP, ANP model group (n = 6); GSNT, ANP model with bilateral greater splanchnic nerve transection group (n = 8).

function impairment (liver failure) is one of the factors causing high ANP mortality. Liver parenchymal cell injury impairs the detoxification function of the liver, thereby negatively affecting the clearance of inflammatory mediators and cytokines produced by ANP, which further aggravates the disease itself [15].

The pancreas is located in the retroperitoneal space and is rich in nerve tissue. The retroperitoneal space contains the kidneys, pancreas, and large abdominal blood vessels, as well as the GSN trunk, and visceral ganglia/plexus are located in the adjacent peripancreatic zone. In ANP, the main and most direct form of inflammation consists of spread from the retroperitoneal space; the peripancreatic ganglia/plexus and impulse signals are stimulated and transferred to the CNS via the visceral afferent nerve, thereby affecting the immune system response to ANP. The liver, which is rich in visceral nerves, is also affected by this neuropathic reflex. To date, studies of liver injury associated with ANP have mainly focused on humoral regulation, such as cytokines and proteins related to inflammatory pathways [17]; however, there are few studies on the role of neural regulation. Compared with humoral factors, neuromodulation has the unique advantage of being faster and easier to control. Along with the developing "sympathetic excitotoxicity" and "cholinergic anti-inflammatory pathway" [8,9,10], scholars have explored the role of the sympathetic and vagus nerves using various inflammatory models. However, the effect of sympathetic denervation on liver injury associated with ANP has not been reported so far. Examining the effect of GSN transection on liver injury with ANP is important to improve the prognosis of ANP patients.

In this study, biochemical indicators (AMY, TNF-α, IL-1β, IL-6, AST, and ALT) and pathological scores in pancreatic and hepatic tissues were significantly higher in the ANP and the GSNT groups than in the SO group after the operation, indicating that the canine ANP liver injury model was successfully established. The establishment of a stable animal model is the foundation and premise for the success of experimental research. Previous studies mostly used rats to generate an ANP liver injury model; however, this model has several shortcomings. First, the anatomy of rats differs from that of humans. In this study, we used large beagle dogs, in which the pancreatic tissues are well developed and the tissue structure and function are closer to those of the human body, which could simulate the human condition. In addition, the survivability of the dog is relatively tenacious and the survival time is relatively stable, which can simulate the evolutionary process of the inflammatory response *in vivo*. The survival time of the rat is short, and studies on the rat ANP model generally last less than 3 days. Our research group developed an ideal ANP model after preliminary experiments [11]. The ANP liver injury model established in this experimental study was easy to operate, the detection indexes were suitable, and the mortality of the experimental animals was low. These factors facilitated a long-term study consisting of relevant experiments and provided a relatively ideal animal model for further studies of the pathogenesis of ANP liver injury or drug intervention.

The expression of p-NF-κB p65 was significantly higher in the ANP and GSNT groups than in the SO group and significantly lower in the GSNT group than in the ANP group, which was consistent with the changes in serum TNF-α, IL-1β, IL-6, AST, and ALT levels and pathological liver injury severity score. The same trend was observed in studies of liver injury associated with pancreatitis [13,18]. The production of inflammatory mediators is regulated by the transcriptional factor NF-κB. The classic inflammatory factors *in vivo*, such as TNF-α, IL-1β, and IL-6, are regulated by NF-κB [19]. NF-κB plays a central role in regulating cytokine gene expression during AP, and may be an important switch in the cytokine cascade reaction [20]. These findings suggested that the activation of NF-κB may be an important mechanism underlying ANP liver injury. In this study, bilateral GSN transection inhibited the activation of NF-κB, thereby attenuating liver injury. NF-κB is a transcriptional regulator that is composed of two subunits, p50 and p65, and it regulates the transcription of factors related to immunity, inflammation, and hyperplasia. The promoter regions of various proinflammatory cytokines such as TNF, IL-6, and IL-1 contain binding sites for NF-κB. Non-activated NF-κB binds to the inhibitor of nuclear factor kappa-B (IκB) protein and exists in the cytoplasm of most cells. External stimuli lead to the phosphorylation and degradation of IκB by IκB kinase. The activated p65/p50 complex (i.e., p-NF-κB p65) is translocated into the nucleus, where it binds to DNA and induces the transcription of downstream inflammatory genes [21], promoting the release of cytokines. Cytokines further activate NF-κB, forming a positive feedback loop and releasing a large number of inflammatory cytokines, resulting in a cytokine network cascade reaction that leads to systemic inflammatory response syndrome and MODS, and even death [22].

The visceral nervous system is composed of two principal pathways, the parasympathetic pathway and the sympathetic pathway, which act either in synergy or in opposition to mediate basic physiological responses. In the physiological state, the sympathetic and parasympathetic pathways can act on the same organ, and the balance between the two plays an important role in the regulation of homeostasis. There are also complex interactions between the immune system and the visceral nervous system; the visceral nervous system acts as a bridge linking the immune system and the CNS. In this study, the levels of serum TNF-α, IL-1β, IL-6, AST, ALT and liver pathology scores significantly decreased in response to bilateral greater splanchnic nerve transection in the GSNT group compared with the ANP group, suggesting that sympathetic denervation improves liver injury during ANP. There are several potential underlying mechanisms as follows: ① "sympathetic excitotoxicity" is removed. The sympathetic excitement can stimulate the liver α adrenergic receptor and upregulate the expression of inflammatory factors such as TNF-α and IL-1β, and IL-6, thereby aggravating liver damage [10, 23]. Transection of the bilateral greater splanchnic nerves had an effect similar to blocking the afferent nerve fibers of the liver-controlling sympathetic nerve. ② the "cholinergic anti-inflammatory channel" is activated. Under normal circumstances, the liver is jointly controlled by the sympathetic and parasympathetic nerves; bilateral greater splanchnic nerve transection can activate the parasympathetic nerve because the antagonistic effect of the sympathetic nerve is removed, and the

"cholinergic anti-inflammatory pathway" may play a role. The main target cells of the cholinergic anti-inflammatory pathway are macrophages, and the liver contains the largest mononuclear macrophage group, namely, Kupffer cells, which account for approximately 80% of the total macrophages in the body [24]. Activation of macrophages is an essential part of the inflammatory response to liver injury in ANP. Studies [25] show that vagus nerve excitation can inhibit macrophage activation and NF-κB activation, which play an important role in ANP liver injury. In this study, the expression of activated NF-κB (i.e., p-NF-κB p65) in liver tissues was significantly decreased after bilateral greater splanchnic nerve transection. ③Improve gastrointestinal tract motor function. Bacterial overgrowth in the ileus plays a major role in the pathogenesis of pancreatic infection [26]; therefore, amelioration of intestinal dysmotility and stasis during the early period of ANP is important in reducing the risks associated with serious complications. The celiac plexus is a major interchange for autonomic fibers, receiving many of the thoracic splanchnic nerve fibers as they course toward the abdominal organs. In fact, the beneficial effect of epidural anesthesia has been attributed to blockade of a sympathetic nerve, which contributes to the recovery of gastrointestinal tract motor function [27]. Our previous studies show that perirenal space blocking of splanchnic nerve in the pancreatic region using 1% lidocaine on ANP treatment can ameliorate intestinal dysmotility and stasis during the early period of ANP [28]. Transection of GSN can more accurately block sympathetic nerve fibers, which supply the pancreas, liver and intestinal tract, thereby reducing the migration of enterogenous endotoxins to the liver and liver damage. Inhibit neurogenic inflammation caused by pain. ANP is often accompanied by persistent and severe abdominal pain, which can stimulate sensory nerve endings, release substance P, calcitonin gene-related peptides, and other polypeptides, which promote vascular dilatation, increase capillary permeability, and produce inflammatory effects. Splanchnicectomy is performed in the clinic to treat intractable pain caused by iatrogenic and retroperitoneal malignancies [29].

In conclusion, bilateral GSN transection may delay pathophysiological deterioration and relieve damage to the pancreas and liver in ANP dogs. This was associated with inhibition of sympathetic function and stimulation of parasympathetic function, resulting in inhibition of NF-κB activation, which prevented the amplification of the inflammatory cascade. Advances in imaging technology and in-depth studies of anti-inflammatory agents may support blocking of the GSN as a potential clinical treatment for ANP.

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