Beneficial effects of N-acetylcysteine on sodium taurocholate-induced pancreatitis in rats

Gokhan Yagci¹, Husamettin Gul², Abdurrahman Simsek³, Varol Buyukdogan¹, Onder Onguru³, Nazif Zeybek³, Ahmet Aydin⁴, Muidat Balkan³, Oguzhan Yildiz², and Dervis Sen¹

¹Department of General Surgery, Gulhane Military Medical Academy, 06018 Etlik, Ankara, Turkey
²Department of Pharmacology, Gulhane Military Medical Academy, Etlik, Ankara, Turkey
³Department of Surgical Pathology, Gulhane Military Medical Academy, Etlik, Ankara, Turkey
⁴Department of Pharmaceutical Toxicology, Gulhane Military Medical Academy, Etlik, Ankara, Turkey

Background. Acute pancreatitis (AP) is a complex disease associated with significant complications and a high rate of mortality. Although several mechanisms are put forward, oxidative stress seems the most important early event in the pathophysiology of AP. Therefore, we evaluated the beneficial effects of N-acetylcysteine (NAC), a strong antioxidant, in experimental AP.

Methods. Forty-nine Sprague-Dawley rats were used. Acute pancreatitis (AP) was induced by the intraductal infusion of sodium taurocholate. Rats were divided into seven groups (each containing seven rats): control, sham-operated (saline-treated, 3.5 and 12h), non-treated AP (3.5 and 12h) and NAC-treated AP (3.5 and 12h). Treated rats received intraperitoneal (i.p.) NAC 1000mg/kg 24h before and just before the induction of pancreatitis.

Results. Rats with AP had extensive parenchymal and fat necrosis and NAC treatment at 12h reduced tissue necrosis significantly (P < 0.05). NAC treatment at 12h reduced leukocytic infiltration significantly (P < 0.05). Edema and hemorrhage were significantly increased in the AP groups when compared to controls (P < 0.001). NAC treatment reduced edema and hemorrhage at both 3.5 and 12h slightly but not significantly. The total pathological mean score was significantly increased in the AP groups (P < 0.05) and it was reduced by NAC treatment (P < 0.05). NAC treatment decreased plasma amylase and lipase levels significantly (P < 0.05). While glutathione peroxidase (GPx) activity of pancreatic tissue was similar in the NAC-treated and AP groups, hepatic tissue GPx activity was lower in the AP groups, and NAC treatment restored it (P < 0.05). NAC had no effect on pancreatic superoxide dismutase level. In the NAC-treated rats, the serum NO₂/NO₃ (nitrite/nitrate) level was significantly increased in the 3.5-h group when compared to the respective AP group (P < 0.05). NAC treatment also significantly reduced the serum concentration of the lipid peroxidation product, malondialdehyde, at 12h (P < 0.05).

Conclusions. NAC treatment had beneficial effects in sodium taurocholate-induced AP in rats. It reduced pancreatic tissue necrosis and lipid peroxidation. In our study, the mechanism underlying the beneficial effects of NAC seemed to be its antioxidant activity, either by increasing hepatic GPx activity, or by a direct scavenging effect on free radicals, thus enhancing the production of and/or inhibiting the degradation of nitric oxide.

Key words: N-acetylcysteine, sodium taurocholate, pancreatitis, rats, nitrite/nitrate, glutathione peroxidase activity.

Introduction

Acute pancreatitis (AP) is still a life-threatening disease despite the development of new diagnostic and therapeutic tools. In most patients, AP is associated with remote organ failure, sepsis, and a high death rate. Current therapeutic procedures have been focused on the management of complications rather than the cause of the disease. Therefore, new and/or improved therapeutic concepts have been used to investigate the targeting of possible pathophysiologic mechanisms such as oxidative stress¹⁻³ and polymorphonuclear leukocyte infiltration.⁴ There is increasing experimental and clinical evidence that these mechanisms play an important role in the disease process.

Acute pancreatitis (AP) is characterized by acinar cell injury, with extensive tissue necrosis, edema, inflammatory infiltrate, and hemorrhage within the
pancreatic gland. Previous reports have shown a relationship between the clinical severity of AP and the incidence of acinar cell death. Necrosis is the characteristic finding in the more severe forms, such as the model of sodium taurocholate-induced pancreatitis. N-acetylcysteine (NAC), a thiol (sulfhydryl-containing) compound, and a precursor of reduced glutathione (GSH), has been widely investigated as an antioxidant compound in a variety of experimental studies, such as in conditions characterized by decreased GSH or oxidative stress, cancer, and heart disease. Because of its hepato-protective activity, NAC has also been used extensively in the management of acetaminophen poisoning.

We evaluated the beneficial effects of NAC in a rat model of sodium taurocholate-induced necrotizing pancreatitis by assessing pancreatic enzymes, antioxidant status, lipid peroxidation, and nitric oxide (NO) production. We showed improvement in pathological features in this model of pancreatitis in rats.

Methods

Animals

Male Sprague-Dawley rats (Breeding Colony of Research Center, Gulhane Military Medical School, Ankara, Turkey), weighing 200 to 250g, were used. The animals were housed under a 12-h light/dark cycle (lights on from 08:00 h) at a constant ambient temperature (24 ± 1 °C), with normal rat chow and water ad libitum. All animal experiments were conducted according to the guidelines of the local animal use and care committees, and the animals were killed according to the National Institutes of Health guidelines for the care and handling of animals.

Experimental design

Forty-nine rats were divided into seven groups, each contained seven rats. Group I (control), group II (sham-operated 0.9% saline-injection group, exsanguinated after 3.5h [sham 3.5-h group]), group III (sham-operated 0.9% saline-injection group, exsanguinated after 12h [sham 12-h group]), group IV (sodium taurocholate-induced pancreatitis, exsanguinated after 3.5h [AP 3.5-h group]), group V (sodium taurocholate-induced pancreatitis, exsanguinated after 12h [AP 12-h group]), group VI (sodium taurocholate-induced pancreatitis and NAC-treated rats, exsanguinated after 3.5h [NAC 3.5-h group]), and group VII (sodium taurocholate-induced pancreatitis and NAC-treated rats, exsanguinated after 12h [NAC 12-h group]). NAC was administered at a dose of 1000mg/kg (i.p.), 24h before and just before the induction of pancreatitis. An equal volume of isotonic saline was administered (i.p.) to the others. Before the animals were killed, they were anesthetized and an abdominal incision was made. The animals were killed by exsanguination, and abdominal aortic blood was collected in heparinized syringes. Samples were immediately centrifuged at 3000rpm for 10min, and the plasma was stored at −70°C until assayed.

Induction of pancreatitis

All animals were deprived of food for at least 10h prior to induction of pancreatitis. Anesthesia was induced with vaporized ether and maintained by an i.p. injection of ketamine 40mg/kg (Ketalar; Parke-Davis and Eczacibasi, Istanbul, Turkey). The abdomen was shaved and prepared with povidine-iodine, and laparotomy was performed into the pancreatic duct. A microaneurysm clip was placed on the bile duct close to the liver. Another clip was placed around the common biliopancreatic duct at its entry into the duodenum to avoid reflux of enteric contents into the duct. A 28-gauge microfine intravenous needle attached to a 1-cc U-40 insulin syringe (B. Braun Medical, Barcelona, Spain) was introduced into the common biliopancreatic duct, and freshly prepared 3% sodium taurocholate (Sigma-Aldrich Chemie, Steinheim, Germany) was injected into the common biliopancreatic duct (1.5ml/kg body weight) under a steady pressure at a speed of 0.15ml/min. After the injection, the microclips were removed, and the abdomen was closed in two layers. All procedures were performed under sterile techniques and conditions. Animals were killed under light ether anesthesia by exsanguination 3.5 or 12h after the induction of AP. Severity of AP was evaluated by serum amylase and lipase levels and pathology.

Assays

Serum amylase and lipase activity were measured using a Hitachi 917 autoanalyzer (Boehringer Mannheim, Mannheim, Germany).

Pathological scoring

For the pathological examination, pancreatic tissue was fixed in 10% neutral formaldehyde buffer for at least 24h and embedded in paraffin, using routine pathological methods. Sections (5-µm) were used for conventional pathological staining with hematoxylin-eosin and were evaluated under light microscopy by a pathologist who was familiar with pancreatic pathology, but unaware of the treatment group. He scored the tissues using a scale for edema, inflammatory infiltration,