Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Hepatic effect of NAC on sevear acute pancteatise of rats

Fang Chen[1*], Ye-Jiang Zhou[2]

[1]Surgical Emergency Center, People's Hospital, Sichuan Province 610072, China
[2]General Surgery, Luzhou Medical School Affiliated Hospital, Luzhou 646000, China

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 15 October 2013
Accepted 15 December 2013
Available online 20 February 2014

Keywords:
NAC
SAP
Liver injury
Protection

ABSTRACT

Objective: To analyze the hepatic protection of n-acetyl cysteine (NAC) on severe acute pancreatitis (SAP). **Methods:** SD rats were randomly divided into control group, SAP group and NAC group. SAP AHO method was adopted to establish the model, 2 h after modeling, rats in NAC group had intraperitoneal injection of NAC (200 mg/kg). Ten rats from each group were sacrificed in every 6 and 12 h at different time points respectively. Liver damage, liver function and serum amylase, AST, ALT and malondialdehyde (MDA) were determined. **Results:** Serum amylase, AST, ALT and MDA content in SAP, NAC group at each time point were significantly higher in the control group ($P<0.05$), serum amylase, AST, ALT and MDA content in NAC group rats were lower in the SAP group significantly ($P<0.05$); Microscopic examination showed that the liver injury in rats and the NAC group significantly reduced in the SAP group. **Conclusions:** NAC provides effective protection against liver damage to SAP, protective from SAP liver injury.

1. Introduction

Severe acute pancreatitis (SAP) pathogenesis has not been understood completely. It progresses rapidly, and it is easy to cause many viscera damages, with a high fatality rate[1-3]. The main death reasons is multiple organ dysfunction syndrome. The liver is one of the main SAP involvement outside of the pancreatic organ. Liver damage degree and the degree of the acute pancreatitis with SAP were positively correlated, and influences the course of the disease and prognosis[4-6]. n-acetyl cysteine (NAC) can reduce glutathione precursor cells to clear oxygen free radicals, reduce the synthesis and release of cytokines, and has extensive organ protection[7-9]. To observe protective effect of NAC on SAP liver damage, we established of SAP rats model, to observe the NAC liver damage before and after treatment.

2. Materials and methods

2.1. Experimental animals

A total of 60 SD rats were selected from the animal experiment center, weighting (220±10) g, aged 8 to 10 weeks, both male and female unlimited. They were breed at (23±3)℃ room temperature freely, following the regulations of Administration of Experimental Animals. A total of 60 rats were randomly divided into control group, SAP group and the NAC group with 20 in each.

2.2. Establishment of SAP

Based on the method of AHO[10], SAP rats model was established. After 12 h fasting, all rats had intraperitoneal injection of 20% urethane anesthesia (0.5 mL/100 g), ventral midline incision in the abdomen, small artery clip temporarily blocked bile duct of liver door side, duodenum perforation, with catheter via duodenum retrograde injection of 1% sodium taurocholic acid (0.1 mL/100 g, 0.1 mL/min). 10 min later artery clamp was removed, tube was drawn, the abdominal wall was sutured to establish the animal model of acute pancreatitis. Duodenum and the pancreas of rats in control group was only gently turned into the abdomen,

*Corresponding author: Fang Chen, Chengdu, M.M., Chief Physician, Surgical Emergency Center, People's Hospital, Sichuan Province 610072, China.
Tel: 18981838127
E-mail: chengfangss@163.com
Foundation project: It is supported by Scientific Research Fund Project Of Health Department, Sichuan Province (No: 090498).

without drug. 2 h after SAP model modeling, NAC group was intraperitoneal injected with NAC (200 mg/kg).

2.3. Observation

6 and 12 h after modeling 10 rats were sacrificed in each group; 3 mL blood was extracted from the right ventricular, using the HF-220 fully automatic biochemical analyzer. Serum amylase, AST, ALT, plasma MDA content was determined according to glucosinolates barbiturate method. Left liver tissue in three groups was collected at each time point, followed by conventional fixation, embedding, section and HE staining for immunohistochemical staining. They were observed under light microscopy, and brown the nucleus and(or) cytoplasmic was positive. Five horizons were selected randomly to calculate positive means.

2.4. Statistical analysis

Using SPSS12.0 statistics software, data was expressed as mean±SD, and analyzed by t test. $P < 0.05$ was regarded as statistically significant difference.

3. Results

3.1. Serum amylase, AST, ALT and MDA change at different time points

Six and 12 h after modelling, serum amylase, AST, ALT and MDA content were significantly higher than that of control group (P<0.05); serum amylase, AST, ALT and MDA content in NAC group at each time point were significantly lower than SAP group (P<0.05) (Table 1, 2).

Table 1
UAG changes at different time points (IU/L).

Group	n	6 h	12 h
Sham group	30	1211.32±129.61	1154.15±l22.45
SAP group	30	5863.54±364.11$^{*\triangle}$	6823.25±402.87$^{*\triangle}$
NACgroup	30	2801.21±239.54*	2933.24±421.12*

Compared with sham group, *P<0.05; with NAC group $^{\triangle}P$<0.05.

3.2. Pathological changes of liver tissue at different time points under light microscopy

The rats of sham group presented normal cell morphology at different time points. The SAP liver cells swelled under light microscopy 6 h after molding. Some liver cell swelled, several cells showed nuclei shriveled, fratured scattered; 12 h after molding, some swelling Kupffer cells nuclei shrivel, fracture around neutrophils were observed. 6 h after molding, liver cells in NAC group were swelling, occasional hepatocellular eosinophilic change was observed; 12 h later visible swelling of kupffer cells was rare, blood deficiency in liver mainly was observed (Figure 1).

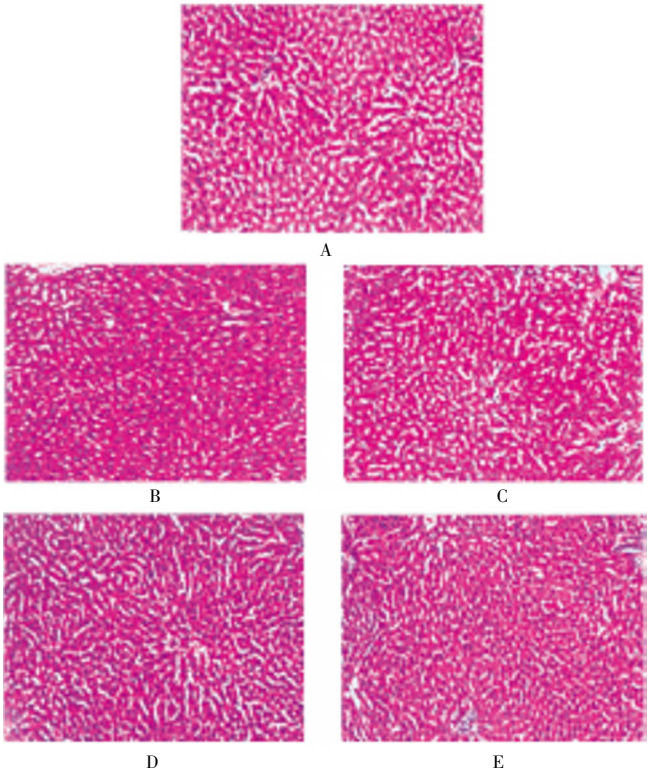

Figure 1. Pathological changes of liver tissue at different time points under light microscopy (×200).
A: Sham group of liver; B: SAP Group at 6 h; C: SAP Group at 12 h; D: NAC Group at 6 h; E: NAC Group at 12 h.

4. Discussion

SAP can cause system damages associated with systemic disease progress fast, complications, with high case fatality rate around 17%[11-13]. Liver injury is the most serious complications of SAP[11-13], and the degree of liver injury was positively correlated with the severity of SAP. The basis of systemic inflammatory lesions in SAP is excessive inflammatory mediators[14]. This experiment aims to explore the effect of NAC on SAP liver damage.

Table 2
AST, ALT, MDA concentration at different time points (IU/L).

Group	AST		ALT		MDA	
	6 h	12 h	6 h	12 h	6 h	12 h
Sham group	58.2±3.0	60.5±2.8	168.2±8.5	176.1±8.3	3.4±0.6	3.6±0.3
SAP group	307.4±26.6$^{*\triangle}$	443.5±56.6$^{*\triangle}$	703.8±53.4$^{*\triangle}$	883.5±66.6$^{*\triangle}$	14.7±0.6$^{*\triangle}$	38.7±1.8$^{*\triangle}$
NACgroup	203.9±32.4*	254.6±22.5*	506.4±66.7*	602.7±67.1*	8.2±0.4*	26.1±1.3*

Compared with sham group*P<0.05; with NAC group $^{\triangle}P$<0.05.

Antioxidants NAC containing sulphur have shown that NAC can cause damages to multiple systems all over the body such as the lung, kidney and nervous system. Another study found that NAC can eliminateoxygen free radicals, inhibit nuclear factor-B activation, reduce calcium ion concentration in the gland cell and decrease the synthesis and release of cytokines, which can block the occurrence of acute pancreatitis development[18]. And also studies have shown that NAC can decrease the SAP TNF alpha in liver tissue, the expression of the nuclear factor-kappa B, thus improve liver function. NAC can inhibit AP damage, and also has a protective effect on SAP lung injury[19,20]. This study found that NAC can reduce serum MDA content, enhance the superoxide dismutase, thereby reduce apoptosis factor expression, so as to alleviate the liver injury. Serum amylase, AST, ALT and MDA content of rats in NAC group in the study at different time points were lower than thoses in the SPA group ($P<0.05$), and liver tissue pathologic damage was also significantly reduced in the SPA group. It showed that NAC has good protection of liver in SAP[21-23].

SAP is dangerous, and the case fatality rate is high. Its development depends on the dynamic balance of proinflammatory factor and anti-inflammatory factor. The experiments have shown that NAC can provide effective protection against liver injury doe to SAP.

Conflict of interest statement

We declare that we have no conflict of interest

References

[1] Sah RP, Garg P, Saluja AK. Pathogenic mechanisms of acute pancreatitis. C*urr Opin Gastroenterol* 2012; **28**: 507-515.

[2] Li RZ, Zeng HC, Mai ZL. Curative effect of Somatostatin in combination with ulinastatin treatment for severe acute pancreatitis. *China Med Guides* 2012; **9**(15): 89-92.

[3] Guo F, Liu JW, Xu YH, Lu KB, Xue XY, Liu RW, et al. Establishing pancreatic head GaBi method to treat acute necrotizing pancreatitis rats. *J Clin Lab Med* 2012; **11**: 1175-1177.

[4] Diao MX, He C, Shan HW, Lin ZF. Ulinastatin combined somatostatin in the treatment of severe acute pancreatitis system evaluation. *Chinese J Combine Trad Chin Western Med First Aid* 2012; **19**(2): 73-78.

[5] Alhan E, Turkyilmaz S, Ercin C. Does nuclear factor-kappa B in peripheral mononuclear cells have a prognostic role during acute necrotizing pancreatitis in rats. *Eur Surg Res* 2012; **48**: 34-39.

[6] Wang H, Liu JW, Li ZL, Xue XY, Zhao HY, Guo F, Xu YH, et al. Establishing lung injury model associated with severe acute pancreatitis in rats. *J World Chin Digest Mag* 2013; **21**(3): 211-219

[7] Yu WG, Xu G, Ren GJ. Preventive action of curcumin in experimental acute pancreatitis in mouse. *Indian J Med Res* 2011; **134**: 717-724.

[8] Zhang Y, Wang P, Yang YM, Yu YP, Meng XL, Chen XR, et al. Protection of Rhubarb anthraquinone on intestinal injury of severe acute pancreatitis on dogs at early stage. *Chin J Experimental Formulas Chin Med* 2013; **19**(5): 172-173.

[9] Wan H, Yuan Y, Liu J, Chen G. Pioglitazone, aPPAR-gamma activator, attenuates the severity of cerulein-induced acute pancreatitis by modulating early growth response-1 transcription factor. *Transl Res* 2012; **160**: 153-161.

[10] Tang LG, Lan LQ, Song SJ, An HM, Zhu H. The role of vascular endothelial growth factor in acute pancreatitis associated lung injury of rats. *J Pract Med* 2012; **28**(3): 380-382.

[11] Jin J, Liu B, Lei YZ, Hu JG, Wang GP, Li AJ, et al. Blood gas analysis and morphological study of animal models of acute lung injury in severe acute pancreatitis. *Clin Lung* 2013; **18**(2): 251-252.

[12] Chen H, Sun YP, Li Y, Hu PF, Liu WW, Xiang HG, et al. Hydrogen-rich saline ameliorates the severity of larginine-induced acute pancreati tisin rats. *Biochem Biophys Res Commun* 2010; **393**: 308-313.

[13] Lu CW, Liu LC, Hsieh YC, Yang LH, Chen RJ, Hsieh CH, et al. Increased admission serum estradiol level is correlated with high mortality in patients with severe acute pancreatitis. *J Gastroenterol* 2012; **25**: 25.

[14] Zhang H, Cai CZ, Zhang XQ, Li T, Jia XY, Li BL, et al. Breviscapine attenuates acute pancreatitis by inhibiting expression of PKCal-pha and NF-kappaB in pancreas. *World J Gastroenterol* 2011; **17**: 1825-1830.

[15] Jha RK, Ma Q, Lei Z, Sha H. Resveratrol ameliorates the deleterious effect of severe acute pancreatitis. *Cell Biochem Biophys* 2012; **62**: 397-402. [PMID: 22042732 DOI: 10.1007/s12013-011-9313-2]

[16] Werner J, Hartwig W, Hackert T, Kaiser H, Schmidt J, Gebhard MM, et al. Multidrug strategies are effective in the treatment of severe ex-perimental pancreatitis. *Surgery* 2012; **151**: 372-381. [PMID: 21982067 DOI: 10.1016/j.surg.2011.07.041]

[17] Nguyen-Khac E, Thevenot T, Piquet MA, Benferhat S, Goria O, Chatelain D, et al. Glucocorticoids plus N-acetylcysteine in severe alcoholic hepatitis. *N Engl J Med* 2011; **365**: 1781-1789. [PMID: 22070475 DOI: 10.1056/NEJMoa1101214]

[18] Tayman C, Tonbul A, Kosus A, Hirfanoglu IM, Uysal S, Haltas H, et al. N-acetylcysteine may prevent severe intestinal damage in necrotizing enterocolitis. *J Pediatr Surg* 2012; **47**: 540-550. [PMID: 22424351 DOI: 10.1016/j.jpedsurg.2011.09.051]

[19] Hou Y, Wang L, Zhang W, Yang Z, Ding B, Zhu H, et al. Protective effects of N-acetylcysteine on intestinal functions of piglets challenged with lipopolysaccharide. *Amino Acids* 2012; **43**: 1233-1242. [PMID: 22180025 DOI: 10.1007/s00726-011-1191-9]

[20] Wang SW, Chen DD, Li PJ, Li ZX, Gao N, Zhang L, et al. Protection of mycophenolate mofetil on cerebral injury of severe acute pancreatitis rats. *Emerg Med China* 2011; **31**: 421-425.

[21] Yuan Y, Liu RB, Wang D, Wang XD. Ulinastatin on endotoxin induced acute lung injury in rats serum hypersensitive C-reactive protein and the effects of calcitonin original. *Chin J Clin Physicians* 2011; **5**(22): 6771-6773.

[22] Zou XB, Li XY, Wang XY, Xin WQ, Chen Y, Yang Q. Protection of Ulinastatin on pulmonary vascular endothelial cells in severe sepsis serum. *Chin J Respiratory Critically Ill Guardianship* 2011; **10**(6): 547-550.

[23] Li N, You SY, Wang SL, Wang J. Ulinastatin on SP-A in severe acute pancreatitis rat lung injury. *Tianjin Med* 2013; **41**(3): 241-242.