Original Research Article

Investigation of non-pharmaceutical cures for acute pancreatitis induced by cerulein in rats

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Abstract

Purpose: To investigate the effect of N-acetylcysteine (NAC) alone or in combination with α-lipoic acid (ALA) on cerulein induced acute pancreatitis (AP) in rats.

Methods: AP was induced in 40 healthy male albino rats using a daily dose of cerulein (40 µg/kg) for three days. The rats were divided into four groups: control, recovery (pancreatitis rats left without treatment), NAC (pancreatitis rats injected intraperitoneal (i.p.) with 25 mg NAC/kg daily) and NAC+ALA (pancreatitis rats treated with 25 mg NAC+100mg ALA/kg daily). The levels of carcinoembryonic antigen (CEA), cancer antigen$_{242}$ (CA$_{242}$), tumor necrosis factor-α (TNF-α), interlukin-1β (IL-1β), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as well as the enzyme activities (amylase, lipase and trypsinogen activation peptide) were determined after 2- and 4-weeks of treatment.

Results: Treatment with NAC or NAC+ALA led to a marked decrease in the levels of tumor markers, inflammatory cytokines, enzymes activity, DNA and RNA. The best amelioration effect occurred in the NAC+ALA rats group.

Conclusion: NAC induces a significant improvement in all studied parameters. Incorporation of NAC and ALA results in preeminent effects and it is considered an ideal cure for acute pancreatitis.

Keywords: Acute pancreatitis, Cerulein, N-acetylcysteine, α-Lipoic acid

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease characterized by a wide clinical alteration, extending from a mild self-limiting to severe disease complicated by sepsis and remote organ failure, resulting in high death rates [1]. The release of digestive enzymes to the pancreatic interstitium and to the circulation is the manifestation of acute pancreatitis which leads to auto-digestion. Consequently, pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β act as mediators of the local and systemic indicators of acute pancreatitis [2]. Additionally, oxidative stress has noted in the advancement of acute pancreatitis [3].

There are still no specific medicines for AP. However, the concept that oxidative stress is one in all the precursors of acute pancreatitis drew the attention to address one of the nutraceuticals known to have the ability to resist oxidation. N-

acetylcysteine (NAC) is a thiol sulfhydryl containing compound comes from the sulfur-amino acid cysteine. It is a precursor of intracellular glutathione (GSH) and accordingly a ROS scavenger [4]. The anti-inflammatory effects of N-acetylcysteine have also been confirmed through diminishing the expression gene of cytokines and chemokines [5] and inhibiting nuclear factor kappa B (NF-kB) [6] in the pancreatic damage.

Alpha-lipoic acid (ALA) is a natural compound widely distributed in animals and plants. It is a powerful antioxidant with anti-inflammatory effect due to its property of oxidized (LA) and reduced (dihydrolipoic acid DHLA) forms. Thus, ALA is known as a universal antioxidant [7]. The significant activities of LA and its derivatives include the ability to increase glutathione content in the cells consequently scavenge free radical and to chelate toxic metals [8].

In brief, NAC and ALA are antioxidants that could be safely used in several diseases. Therefore, this work was planned to evaluate the synergistic effects of N-acetylcysteine and alpha-lipoic acid on some crucial indicators of inflammation and oxidative stress that escort acute pancreatitis in rats.

EXPERIMENTAL

Material

Cerulein, N-acetylcysteine (NAC) and α-lipoic acid (ALA) were obtained from Sigma Chemical Company (USA).

Experimental animals

Forty healthy adult male albino rats (150-180g) were used in the current study and were obtained from the vivarium of the Nuclear Research Center, Atomic Energy Authority, Egypt. The animals were maintained in the animal-holding room under normal conditions (12/12h light/dark cycle, 50-70% humidity and 30°C) and were allowed free access to a standard requirement diet and water *ad libitum*. Animal procedures were carried out in accordance with the Guide for the Ethics Committee for the Care and Use of Laboratory Animals published by the US National Council of Health [9]. The experimental procedures were approved by the Research Ethics Committee at the Faculty of Science, Ain Shams University, Egypt (REC-FS, ref no. 00033).

Experimental protocol

The present experiment involved two intervals; two and four weeks respectively. The rats were divided randomly into four groups (10 rats each) and they were treated as follows:

Control group: Animals were daily injected subcutaneous with normal saline for three days.

Recovery group: Animals were injected subcutaneous with 40µg cerulein/kg daily for three days (pancreatitis rats) and left without any further treatment for four weeks.

NAC group: Rats were treated with cerulein daily (pancreatitis rats) as mentioned before followed by intraperitoneal injection with 25mg NAC/kg daily for four weeks.

NAC+ALA group: Rats were injected daily with cerulein (pancreatitis rats) as mentioned before followed by intraperitoneal injection with NAC as described above plus 100mg ALA/kg daily for four weeks.

At the end of each experimental period (2- or 4weeks), the animals were anaesthetized and blood samples were collected by cardiac puncture. To obtain sera, blood samples were centrifuged at 3000rpm for 10 minutes. Sera were frozen until being used in biochemical assays.

Preparation of pancreatic homogenates

Pancreatic tissue (0.1-0.2g) was homogenized in 2mL of 0.2M Tris-HCl (pH 8.0) containing 0.05M $CaCl_2$ in a tissue mixer. The DNA, RNA and proteins were extracted from $HC1O_4$ precipitates of an aliquot of the homogenate. According to the methods of Burton [10] and Ceriotti [11], DNA and RNA content were measured respectively as well as the protein level was determined by using the method of Bradford [12].

Biochemical assay

Serum carcino-embryonic antigen (CEA) and the cancer antigen (CA_{242}) were assayed by radioimmunoassay (RIA) kits using a solid phase component system (Institute of Isotopes, Ltd., Budapest, Hungary). According to the manufacturer's instructions, commercial ELISA kits were used to estimate the levels of serum rat tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Kamiya Biomedical Company, USA), trypsinogen activation peptide (TAP) activity (Uscn, Life Science Inc., USA) and the level of

total nitric oxide (TNO) as well as α-amylase & lipase activities (Biovision, USA).

Statistical analysis

Using a computer program (Costate, Version 6.303), statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Duncan's multiple range test. $p < 0.05$ was considered statistically significant.

RESULTS

Effect of cerulein on tumor markers

As shown in Table 1, rats administrated cerulein revealed a significant ($p < 0.05$) increase in the levels of serum tumor marker (CEA and CA_{242}) when compared to the control group. On the other hand, treatment of pancreatitis rats group with NAC alone or in the presence of ALA led to a considerable amelioration in the tumor markers profile (CEA & CA_{242}) depending on the time of treatment (2 or 4weeks).

Effect of cerulein on α-amylase, lipase and trypsinogen activation peptide

Table 2 shows that the subcutaneous injection with cerulein (40µg/kg) for 3 days led to a significant ($p < 0.05$) increase in the activities of α-amylase, lipase and TAP when compared to corresponding control one. In addition, the results revealed that administration of NAC alone or in the presence of ALA led to a significant ($p < 0.05$) reduction in the activities of α-amylase

and lipase associated with a remarkable decrease in the concentration of TAP depending on time of treatment (2 or 4weeks).

Effect of cerulein on TNF-α, IL-1β and TNO

Injection of cerulein in rats (pancreatitis rats) induced a significant ($p < 0.05$) elevation in the levels of TNF-α and IL-1β accompanied with a significant ($p < 0.05$) increment in the total nitric oxide level when compared with control group (Table 3). While, treatment by NAC in pancreatitis rats group led to a remarkable amelioration in levels of TNF-α, IL-1β and TNO. The pancreatitis rats group which was treated with both NAC and ALA showed the best correction in the TNF-α, IL-1β and TNO levels depending on the time of treatment (2 or 4weeks).

Effect of cerulein on some physiological parameters

Body and pancreatic weights recorded a significant ($p < 0.05$) decrease in rats group which was treated with cerulin (Table 4). Moreover, DNA, RNA and protein levels were significantly ($p < 0.05$) decreased compared to their corresponding levels in the control group. In contrast, treatment with NAC alone or together with ALA led to a remarkable improvement in the body and pancreatic weights as well as a considerable correction in the levels of DNA, RNA and protein depending on the time of treatment (2 or 4 weeks).

Table 1: Effect of NAC alone or NAC plus ALA on tumor markers in pancreatitis rats induced by cerulein (mean ± SE)

Group		Control	Acute pancreatitis rats		
			Recovery	*NAC*	*NAC+ALA*
CEA	2 weeks	$0.18\pm0.004^{D}_{a}$	$2.54\pm0.040^{A}_{a}$	$1.35\pm0.045^{B}_{a}$	$1.02\pm0.049^{C}_{a}$
(ng/ml)	4 weeks	$0.19\pm0.004^{D}_{b}$	$3.21\pm0.063^{A}_{b}$	$1.01\pm0.044^{B}_{b}$	$0.79\pm0.031^{C}_{b}$
CA_{242}	2 weeks	$1.83\pm0.044^{D}_{a}$	$14.06\pm0.691^{A}_{a}$	$9.27\pm0.573^{B}_{a}$	$7.42\pm0.439^{C}_{a}$
(U/ml)	4 weeks	$1.86\pm0.048^{D}_{b}$	$17.21\pm0.775^{A}_{b}$	$7.46\pm0.495^{B}_{b}$	$4.79\pm0.312^{C}_{b}$

[A, B, C, D] Means with different superscript within a row are significantly different at $p < 0.05$ [a,b] Means with different subscript between two points (2 or 4 weeks) for the same parameters are significantly different at $p < 0.05$

Table 2: Effect of NAC alone or NAC plus ALA on α-amylase, lipase and trypsinogen activation peptide in pancreatitis rats induced by cerulein (mean ± SE)

Group		Control	Acute pancreatitis rats		
			Recovery	*NAC*	*NAC+ALA*
α-Amylase	2 weeks	$185.80\pm12.45^{D}_{a}$	$1405.64\pm16.11^{A}_{a}$	$1181.05\pm14.44^{B}_{a}$	$793.88\pm9.73^{C}_{a}$
(U/L)	4 weeks	$187.39\pm12.93^{D}_{b}$	$951.92\pm13.14^{A}_{b}$	$721.57\pm11.83^{B}_{b}$	$469.33\pm7.56^{C}_{b}$
Lipase	2 weeks	$107.95\pm1.01^{D}_{a}$	$425.39\pm7.43^{A}_{a}$	$356.75\pm5.67^{B}_{a}$	$238.89\pm5.86^{C}_{a}$
(U/L)	4 weeks	$109.94\pm1.12^{D}_{b}$	$390.58\pm6.88^{A}_{b}$	$259.31\pm4.35^{B}_{b}$	$148.32\pm1.51^{C}_{b}$
TAP	2 weeks	$10.15\pm0.269^{D}_{a}$	$26.85\pm0.929^{A}_{a}$	$19.31\pm0.794^{B}_{a}$	$16.12\pm0.60^{C}_{a}$
(ng/ml)	4 weeks	$9.96\pm0.259^{D}_{b}$	$30.88\pm0.991^{A}_{b}$	$15.13\pm0.417^{B}_{b}$	$11.78\pm0.821^{C}_{b}$

[A, B, C, D] Means with different superscript within a row are significantly different at $p < 0.05$ [a,b] Means with different subscript between two points (2 or 4 weeks) for the same parameters are significantly different at $p < 0.05$

Table 3: Effect of NAC alone or plus ALA on TNF-α, IL-1β and TNO in pancreatitis rats induced by cerulein (mean ± SE)

Group & exposure time		Control	Acute pancreatitis rats		
			Recovery	*NAC*	*NAC+ALA*
TNF-α	2 weeks	$5.40 \pm 0.109\,^D_a$	$40.13 \pm 1.507\,^A_a$	$26.99 \pm 1.59\,^B_a$	$19.34 \pm 1.251\,^C_a$
(pg/ml)	4 weeks	$5.45 \pm 0.109\,^D_b$	$51.72 \pm 1.891\,^A_b$	$18.76 \pm 1.049\,^B_b$	$10.83 \pm 0.858\,^C_b$
IL-1β	2 weeks	$9.52 \pm 0.279\,^D_a$	$33.26 \pm 1.774\,^A_a$	$25.01 \pm 1.291\,^B_a$	$21.65 \pm 1.104\,^C_a$
(pg/ml)	4 weeks	$9.52 \pm 0.279\,^D_b$	$39.98 \pm 1.812\,^A_b$	$16.24 \pm 2.916\,^B_b$	$14.55 \pm 0.863\,^C_b$
TNO	2 weeks	$19.53 \pm 0.402\,^D_a$	$87.44 \pm 2.664\,^A_a$	$56.18 \pm 1.884\,^B_a$	$43.45 \pm 1.423\,^C_a$
(μmol/L)	4 weeks	$19.68 \pm 0.405\,^D_b$	$100.38 \pm 3.291\,^A_b$	$35.21 \pm 1.095\,^B_b$	$26.18 \pm 0.914\,^C_b$

[A, B, C, D] Means with different superscript within a row are significantly different at p<0.05 [a,b] Means with different subscript between two points (2&4 wks) for the same parameters are significantly different at p<0.05

Table 4: Effect of NAC alone or NAC plus ALA on some physiological parameters in pancreatitis rats induced by cerulein (mean ± SE)

Group		Control	Acute pancreatitis rats		
			Recovery	*NAC*	*NAC+ALA*
Body weight	2 weeks	$186.14 \pm 9.387\,^A_a$	$141.58 \pm 6.939\,^D_a$	$153.97 \pm 7.174\,^C_a$	$175.33 \pm 8.625\,^B_a$
(g)	4 weeks	$217.48 \pm 11.541\,^A_b$	$172.02 \pm 8.953\,^D_b$	$192.36 \pm 10.251\,^C_b$	$210.14 \pm 11.263\,^B_b$
Pancreatic	2 weeks	$0.94 \pm 0.011\,^A_a$	$0.76 \pm 0.079\,^D_a$	$0.79 \pm 0.074\,^C_a$	$0.86 \pm 0.069\,^B_a$
weight (g)	4 weeks	$1.22 \pm 0.0296\,^A_b$	$0.81 \pm 0.0751\,^D_b$	$0.85 \pm 0.066\,^C_b$	$1.07 \pm 0.061\,^B_b$
DNA	2 weeks	$1.163 \pm 0.021\,^A_a$	$1.047 \pm 0.019\,^D_a$	$1.082 \pm 0.023\,^C_a$	$1.155 \pm 0.026\,^B_a$
(mg)	4 weeks	$1.318 \pm 0.026\,^A_b$	$1.088 \pm 0.022\,^D_b$	$1.117 \pm 0.026\,^C_b$	$1.276 \pm 0.028\,^B_b$
RNA	2 weeks	$5.612 \pm 0.074\,^A_a$	$4.139 \pm 0.048\,^D_a$	$4.707 \pm 0.052\,^C_a$	$5.258 \pm 0.063\,^B_a$
(mg)	4 weeks	$5.994 \pm 0.079\,^A_b$	$4.627 \pm 0.054\,^D_b$	$5.101 \pm 0.061\,^C_b$	$5.791 \pm 0.075\,^B_b$
Protein	2 weeks	$64.361 \pm 2.649\,^A_a$	$51.921 \pm 2.012\,^D_a$	$55.647 \pm 2.086\,^C_a$	$60.813 \pm 2.227\,^B_a$
(mg/g tissue)	4 weeks	$69.027 \pm 3.004\,^A_b$	$54.374 \pm 2.057\,^D_b$	$60.553 \pm 2.115\,^C_b$	$67.616 \pm 2.794\,^B_b$

[A, B, C, D] Means with different superscript within a row are significantly different at p<0.05 [a,b] Means with different subscript between two points (2 or 4 weeks) for the same parameters are significantly different at $p < 0.05$

DISCUSSION

AP is a complicated disorder in which detrimental effects may surpass the pancreas to multi-organ failure. The release of ROS and inflammatory cytokines within the pancreas are known to induce the evolution of pancreatitis with varying degrees of certainty [13]. Cerulein-induced pancreatitis in animal model has been considered the most relevant model that resembles the human phenotype in many aspects. In the current work, a significant elevation occurred in the tumor markers (CEA and CA$_{242}$) as a result of cerulein administration in rats. These results may be due to a defect in the immune system defense or the increment of free radical production and lipid peroxidation concentration. Several authors suggested that the elevation in the activities of phospholipase A2 and cyclooxygenase-2 may lead to pronounce acute pancreatitis in human [14] and in experimental animals [15].

Administration of cerulein in rats led to a significant elevation in the serum α-amylase, lipase activities and trypsinogen activation peptide (TAP) concentration. These data may be attributed to the release of ROS, phospholipase A2 and troubling inflammatory cytokines. The release of phospholipase A2 into blood is a result of decomposes the phospholipids component of cell membrane. Destruction of cell membrane stability and production of large leakage of lysosome enzymes generate bioactive free fatty acid followed by interstitial edema and inflammation within the acinar cells [16]. Furthermore, the production of a lot of ROS and stimulation of the oxidant-sensitive transcription factor kB (NF-kB) increase the release of inflammatory cytokines which can initiate the pancreatic inflammatory process [17].

The results of the current work revealed that cerulein caused elevation of total nitric oxide compared to the normal ones. Nitric oxide contributes to oxidative stress and mediates the cytotoxicity produced by activated neutrophils and macrophages in the inflammatory response [18]. It has been demonstrated that endogenous nitric oxide is an important inflammatory mediator that is essential for the early evolution of edema in cerulein pancreatitis [19].

The data showed a considerable depletion in both the body and pancreatic weights associated with significant decreases in the level of total protein and the concentrations of DNA and RNA in the pancreatitis rats. These data are in harmony with the results obtained by Sandeep and Veeresh [20]. Also, Tibullo *et al* [21] reported that free radicals when left free, they cause harm

to membranes and DNA. Since, these free radicals have the potential for such devastating damage, they are considered to be a major factor in the cancer and aging processes.

There exists a positive feedback between oxidative stress and cytokines which represent the most critical factors in the outcome of AP. In spite of the extensive researches in this field, there is no particular treatment against AP and therapy is fundamentally restricted to supportive care. Taken together, the main objective of the present investigation is to assess whether NAC alone or in combination with ALA could be viewed as a suitable treatment for acute pancreatitis.

NAC has a variety of pathological and biological processes because of its precursor of L-cysteine and glutathione reductase as well as its sulfhydryl groups which act as ROS scavenger in the cells. The current study showed that NAC is able to correct the changes occurred in all studied parameters attributing its beneficial effects on pancreatic tissue. NAC administration inhibits nuclear factor-kappa B activation and prevents overexpression genes of several inflammatory cytokines [5]. Chen and Zhou [22] reported that NAC can block the occurrence of AP development by reduced calcium ion concentration in the gland cell and decrease the release and synthesis of cytokines. Another study showed that NAC can decrease TNF-α, the expression of the nuclear factor-kappa B, reduce serum MDA content associated with enhancement of superoxide dismutase, thus mitigating the AP damage [23].

ALA is exceptional among natural antioxidants in its ability to achieve a lot of requirements, making it the most effective therapeutic agent for a number of pathological disorders implicated with oxidative injury. In this study, the pancreatitis rat group which was treated with both NAC and ALA recorded correction in the levels of all parameters studied herein. These results may be attributed to the presence of thiol group which increases glutathione content in tissues. It is well documented that ALA and its derivate DHLA may act as extra-and intracellular redox couples and potent free radical scavengers [24].

ROS-mediated inflammation induces activation of nuclear factor kappa B, activator protein 1 (AP-1) and mitogen-activated protein kinases. At the same time, NF-kB facilitates the production of inflammatory cytokines; tumor necrosis factor alpha, interleukin-6, cyclooxygenase-2 and inducible nitric oxide synthase [25]. Moreover, Tibullo *et al* [22] showed that ALA inhibits NF-kB

and decreases the production of tumor necrosis factor-α. The anti-inflammatory effects of ALA are attributed to suppression of inflammatory activity from NF-kB, TNF- α and IL-6 associated with correction of anti-inflammatory proteins [7].

CONCLUSION

The findings of this study shows that NAC exerts therapeutic activity by limiting pancreatic damage produced during AP. Moreover, supplementation of NAC in the presence of ALA leads to significant recovery due to the synergistic effects of both antioxidants. Therefore, this mixture can be used as an ideal therapy for acute pancreatitis.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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