N-acetylcysteine induces beneficial changes in the acinar cell cycle progression in the course of acute pancreatitis

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Abstract. Oxygen free radicals (OFR) are produced in the course of acute pancreatitis (AP). In addition to injurious oxidative effects, they are also involved in the regulation of cell growth. The aim of the present study was to examine the relationship between the effectiveness of N-acetyl-L-cysteine (NAC) to prevent the generation of OFR and the changes in the cell-cycle pattern of acinar cells in the course of AP induced in rats by pancreatic duct obstruction (PDO). NAC (50 mg/kg) was administered 1 h before and 1 h after PDO. Flow-cytometric measurement of OFR generation in acinar cells was carried out using dihydrorhodamine as fluorescent dye. Plasma amylase activity, pancreatic glutathione (GSH) content and TNF- α plasma levels were also measured. The distribution of acinar cells throughout the different cell-cycle phases was analysed at different AP stages by flow cytometry using propidium iodide staining. NAC administration reduced the depletion of pancreatic GSH content and prevented OFR generation in acinar cells of rats with PDO-induced acute pancreatitis. As a result, AP became less severe as reflected by the significant improvement of hyper-amylasaemia and maintenance of plasma TNF- α levels at values not significantly different from controls were found. NAC administration inhibited progression of cell-cycle phases, maintaining acinar cells in quiescent state at early PDO times. The protection from oxidative damage by NAC treatment during early AP, allows the pancreatic cell to enter S-phase actively at later stages, thereby allowing acinar cells to proliferate and preventing the pancreatic atrophy provoked by PDO-induced AP. The results provide evidence that OFR play a critical role in the progression of acinar cell-cycle phases. Prevention of OFR generation of acinar cells in rats with PDO-induced AP through NAC treatment, not only protects pancreas from oxidative damage but also promotes beneficial changes in the cell cycle progression which reduce the risk of pancreatic atrophy.

INTRODUCTION

Oxidative stress has been shown to be involved in the pathophysiology of acute pancreatitis (AP) (Schreck & Baeuerle 1991; Schoenberg *et al.* 1992; Rau *et al.* 2000; Weber & Adler 2001). The antioxidant capacity of cells is overwhelmed by large quantities of oxygen free radicals (OFR)

produced at early stages of AP. They play an important role as mediators in pancreatic damage (Schoenberg *et al.* 1992; Rau *et al.* 2000) as well as second messengers amplifying the inflammatory response to other organs (Schreck & Baeuerle 1991; Weber & Adler 2001), eventually leading to systemic complications. However, besides their injurious effects, an increasing body of evidence supports the involvement of OFR in transduction cascades acting as signalling molecules in the regulation of physiological processes such as cell arrest, proliferation, senescence and cell death (Thannickal & Fanburg 2000; Dröge 2002; Martindale & Holbrook 2002). The signalling pathways by which OFR regulate cell growth have not been clearly established, but a large family of serine/threonine kinases referred to as mitogen-activated protein kinases (MAPKs) has been identified as a key mediator in this regulation (Pagès *et al.* 1993; Dröge 2002; Torres 2003). Activation of MAPKs is elicited in response to several stimuli such as cytokines, growth factors, tumour promoters, hormones and oxidants (Dröge 2002; Torres 2003), and is required for cells to overcome the cell-cycle checkpoint in the transition from G₀/G₁ into S-phase (Pagès *et al.* 1993; Morisset *et al.* 1999). It is widely accepted that the exposure of mammalian cells to a pro-oxidant environment leads to mitogenic activation (Burdon 1995). OFR would exert their effect both by direct oxidative modification of signal transduction molecules and indirectly by altering the general redox state of the cell (Thannickal & Fanburg 2000).

In line with this, increased OFR generation in acinar cells and subsequent oxidative damage reported by Uruñuela *et al.* (2002a), as well as activated cellular proliferation (De Dios *et al.* 2000) have been found at early stages of AP induced by pancreatic duct obstruction (PDO). Antioxidant treatments, N-aceyl-L-cysteine (NAC) among them, have demonstrated their ability to inhibit cell growth (Kim *et al.* 2001; Kong *et al.* 2001; Kuruganti *et al.* 2002; Kyaw *et al.* 2002) and to exert a beneficial effect palliating AP symptoms, when administered at early stages (Schoenberg *et al.* 1992). NAC is an anti-inflammatory and antioxidant molecule, acting as a direct OFR scavenger and a precursor for glutathione (GSH) biosynthesis (Cotgreave 1997).

Because occlusion of the biliary tract by passage of gallstones is the most common cause of AP in humans, we have chosen PDO as an experimental AP model, with the aim of studying the relationship between the effectiveness of NAC treatment in preventing the generation of OFR and the changes in the cell-cycle pattern of pancreatic acinar cells throughout the different AP stages.

MATERIALS AND METHODS

Chemicals

NAC, collagenase type XI, soybean trypsin inhibitor (STI), amino acid mixture, bovine serum albumin (BSA), dihydrorhodamine-123 (DHR), N-2-hydroxyethyl piperazine-N'-(2-ethanesulphonic acid) (HEPES), glutathione, β -nicotinamide adenine dinucleotide phosphate reduced form (NADPH), glutathione reductase type III, ethylenediamine-tetraacetic acid (EDTA), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), buprenorphine, propidium iodide (PI) and RNase were supplied by Sigma Chemical Co. (Madrid, Spain). Pharmingen (San Diego, CA, USA) supplied the ELISA kit for TNF- α . Other standard analytical grade laboratory reagents were obtained from Merck (Darmstadt, Germany).

Animal groups

The animal experiments were performed according to the protocols approved by the Animal Care Committee of the University of Salamanca (Spain), in accordance with European Community guidelines on ethical animal research.

Male Wistar rats weighing 250–300 g were randomly divided into the following four groups, with four subgroups in each:

Group I Rats with pancreatic duct obstruction (PDO). After overnight fasting and under ether anaesthesia, a median laparotomy was performed and AP was induced by ligation of the common bile-pancreatic duct at its distal portion, close to the exit to the duodenum. The abdominal wall was immediately closed in a double layer and animals were put back in their cages with free access to food and water. Subcutaneous injections of buprenorphine (0.2 mg/kg/8 h) provided post-operative analgesia. PDO was maintained for 3 h ($n = 12$), 6 h ($n = 12$), 12 h ($n = 12$) and 24 h ($n = 12$).

Group II Sham-operated rats (Sham), surgically treated as those of Group I but without ligation of the pancreatic duct. These were studied at the same time points of 3 h ($n = 12$), 6 h ($n = 12$), 12 h ($n = 12$), and 24 h ($n = 12$) after surgery.

Group III Rats receiving intraperitoneally NAC resuspended in distilled water (50 mg/kg) 1 h before ligation of the bile-pancreatic duct followed by a new injection 1 h after ligating (NAC-PDO). Studies were carried out at 3 h ($n = 12$), 6 h ($n = 12$), 12 h ($n = 12$) and 24 h ($n = 12$) after PDO was performed.

Group IV Sham-operated rats receiving NAC as detailed for Group III (NAC-Sham) and studied 3 h ($n = 12$), 6 h ($n = 12$), 12 h ($n = 12$) and 24 h ($n = 12$) after surgical procedure.

Different animal pools were used either for flow-cytometric analysis or for the other assays.

Collection of samples

After overnight fasting, rats were anaesthetized with sodium pentobarbital (3 mg/100 g body weight, intraperitoneally) and median laparotomy was performed. Blood samples were obtained by cardiac puncture in order to determine plasma amylase activity and TNF- α levels. Finally, the entire pancreas was removed and freed of fat and lymph nodes, either to determine the glutathione (GSH) content, for isolation of pancreatic acinar cells and subsequent analysis of cellular DNA content and OFR generation by flow cytometry, or for histological analysis.

Assays

Amylase activity was determined in plasma according to the method of Hickson (Hickson 1970). Pancreatic GSH content was measured after homogenizing the pancreas in 5% 5-sulfosalicylic acid, using the specific technique described by Tietze (1969), based on the spectrophotometric monitoring of glutathione-mediated reduction of DTNB at 412 nm. Plasma TNF- α levels were measured using an enzyme-linked immunosorbent assay (ELISA), strictly following the recommendations of the manufacturer.

Preparation of isolated cells

Pancreatic acinar cells were isolated as previously described by Uruñuela *et al.* (2002b). Briefly, after ligating the bile duct close to the liver, HEPES solution pH 7.4 containing collagenase (40 U/ml), 0.1 mg/ml STI, 100 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 14 mM D-glucose, 2 mM glutamine, 2% (w/v) BSA and 2% (w/v) amino acid mixture was perfused into the main pancreatic duct. After digestion of the pancreas with collagenase and following gentle pipetting through tips of decreasing diameter (3–1 mm), cells were filtered through a double layer of muslin gauze and then centrifuged at 30 g for 30 s at 4 °C. The supernatant was

collected and centrifuged twice at 500 g, 5 min, 4 °C. The pellet was finally resuspended in HEPES buffer without collagenase at a concentration of 10^7 cells/ml. As previously shown by Uruñuela *et al.* (2002b), the labelling of isolated cells with specific antisera against digestive enzymes assessed the purity of the acinar cells as 99%.

Flow-cytometric analysis of OFR generation in acinar cells

Oxidative stress in individual acinar cells was measured by using dihydrorhodamine-123 (DHR), as previously reported by Uruñuela *et al.* (2002a). Briefly, a suspension of 100 µl of acinar cells was loaded with 25 mM DHR for 20 min at 4 °C in the dark. DHR easily crosses cell membranes and, upon oxidation by OFR, turns into rhodamine-123 and becomes fluorescent (Rothe *et al.* 1991). After washing twice with HEPES buffer, the intracellular green fluorescence (FI1) due to DHR dye was measured using the same FACScalibur dual laser flow cytometer described above. Leukocytes infiltrating the pancreas were excluded on the basis of their unique high light scatter properties (FSC/SSC). Cell Quest and Paint-a-Gate 3.0 software programs were used for data acquisition and analysis, respectively. A minimum of 10 000 cells were analysed in each experiment to obtain a mean value per individual cell. Cells from the different experimental groups were measured in parallel at each time point and changes in the intensity of fluorescence were expressed as percentages of values obtained each day in sham-operated rats.

Flow-cytometric analysis of DNA content in acinar cells

Cell DNA staining was performed as follows. A suspension of 100 µl of cells (10^7 /ml) in HEPES buffer was fixed with 70% cool ethanol for at least 30 min at 4 °C. Afterwards, cells were centrifuged at 500 g for 3 min. Immediately, the supernatant was removed and the cell pellet was resuspended in a solution containing RNase (0.1 mg/ml) and propidium iodide (50 µg/ml) and maintained with gentle stirring at 4 °C in the dark for at least 30 min before the flow-cytometric acquisition. For this, a dual laser FACScalibur flow cytometer (Becton-Dickinson, San José, CA, USA) equipped with a doublet discrimination module and an argon-ion laser emitting light at 488 nm and 15 mW was used. Calibration of the instrument was performed on a daily basis using Calibrite beads (Becton-Dickinson). Leukocytes infiltrating the pancreas were also excluded. Software programs CellQuest and Paint-A-Gate 3.0 (Becton-Dickinson) were used for data acquisition and analysis of the distribution of G_0/G_1 -, S-, G_2/M -, and sub G_1 -phase cells, respectively. This analysis was performed on gated single cells after excluding cell doublets using the RFIT mathematical calculation model by means of the CELLfit software programme.

Apoptosis determination by FITC-Annexin V/PI double staining

Cells undergoing apoptosis were detected by a double staining for FITC-Annexin V binding and for cellular DNA using propidium iodide according to the method of Vermes *et al.* (1995). One hundred microlitres of a suspension of cells (10^7 /ml) in HEPES buffer was incubated with FITC-Annexin V at a final concentration of 1 µg/µl for 20 min in the dark. After centrifuging at 500 g for 3 min, the cell pellet was resuspended in a propidium iodide solution at a final concentration of 1 µg/µl. After 10 min in the dark, cells were analysed by flow cytometry. Apoptosis was considered for positive FITC-Annexin V/negative PI cells.

Statistical analysis

Results are expressed as means $\pm$ SEM. Statistical analysis was carried out applying the analysis of variance (ANOVA) followed by Scheffé test in order to establish differences between the

experimental groups defined in each time period. *P*-values lower than 0.05 were considered to be statistically significant.

RESULTS

As shown in Table 1, PDO induced a significant increase in plasma amylase activity from 3 h after PDO onwards, with maximal values at 12 h in both NAC-treated and non-treated PDO rats. Nevertheless, administration of the antioxidant significantly reduced hyper-amylasaemia induced by PDO, displaying a greater effect within the first 6 h ($P < 0.001$).

NAC administration significantly ($P < 0.001$) increased the pancreatic GSH content in sham-operated rats. PDO-induced acute pancreatitis significantly decreased GSH levels from 3 h onwards, reaching the lowest values 6 h after PDO. However, NAC-treated PDO rats maintained control values 3 h after obstruction and, although GSH depletion at later AP stages was not completely prevented by NAC treatment, pancreatic GSH levels were found to be significantly higher ($P < 0.01$) in rats receiving NAC 6 h after PDO (Table 2).

Table 1. Plasma amylase activity

	3 h	6 h	12 h	24 h
Sham ($n = 12$)	5.16 ± 0.21	4.21 ± 0.28	4.80 ± 0.54	5.26 ± 0.40
NAC-Sham ($n = 12$)	4.71 ± 0.16	4.21 ± 0.36	4.22 ± 0.25	5.30 ± 0.47
PDO ($n = 12$)	10.39 ± 0.76 ^{ab} $P < 0.001$	13.81 ± 1.37 ^{ab} $P < 0.001$	15.94 ± 0.25 ^{ab} $P < 0.001$	14.96 ± 1.55 ^{ab} $P < 0.001$
NAC-PDO ($n = 12$)	7.19 ± 0.27 ^a $P < 0.01$ ^{bc} $P < 0.001$	7.45 ± 0.66 ^{ab} $P < 0.05$ ^c $P < 0.001$	12.15 ± 1.02 ^{ab} $P < 0.001$ ^c $P < 0.05$	10.61 ± 0.44 ^a $P < 0.001$ ^{bc} $P < 0.01$

Values are expressed in U/dl as means $\pm$ SEM. *n* represents the number of animals in each group: sham-operated rats (Sham), sham-operated rats treated with NAC (NAC-Sham), rats with PDO-induced AP (PDO) and PDO rats treated with NAC (NAC-PDO).

^aSignificant differences vs. Sham; ^bsignificant differences vs. NAC-Sham and ^csignificant differences vs. PDO rats.

Table 2. GSH content in pancreas

	3 h	6 h	12 h	24 h
Sham ($n = 6$)	1.58 ± 0.07	1.72 ± 0.07	1.63 ± 0.04	1.52 ± 0.04
NAC-Sham ($n = 6$)	1.82 ± 0.03	2.39 ± 0.12 ^a $P < 0.001$	2.46 ± 0.08 ^a $P < 0.001$	1.38 ± 0.06
PDO ($n = 6$)	1.14 ± 0.05 ^a $P < 0.05$ ^b $P < 0.01$	0.73 ± 0.05 ^{ab} $P < 0.001$	1.13 ± 0.03 ^{ab} $P < 0.001$	0.98 ± 0.04 ^a $P < 0.001$ ^b $P < 0.01$
NAC-PDO ($n = 6$)	1.71 ± 0.10 ^c $P < 0.001$	1.14 ± 0.05 ^{ab} $P < 0.001$ ^c $P < 0.01$	1.16 ± 0.08 ^{ab} $P < 0.001$	1.07 ± 0.10 ^a $P < 0.01$ ^b $P < 0.05$

Values are expressed in $\mu\text{mol/g}$ as means $\pm$ SEM. *n* represents the number of animals in each group: sham-operated rats (Sham), sham-operated rats treated with NAC (NAC-Sham), rats with PDO-induced AP (PDO) and PDO rats treated with NAC (NAC-PDO).

^aSignificant differences vs. Sham; ^bsignificant differences vs. NAC-Sham; ^csignificant differences vs. PDO rats.

Figure 1. Flow-cytometric measurement of OFR generation within pancreatic acinar cells, using DHR as fluorescent dye. Number of animals was six per group and experimental period. Results are expressed as percentages with respect to that obtained every day in sham-operated group. Values are means $\pm$ SEM. Scheffé test following ANOVA revealed significant differences vs. sham-operated rats ($\blacklozenge$, $P < 0.05$) as well as between NAC-treated and non-treated PDO rats ($P < 0.05$).

Figure 2. Plasma TNF- α levels found in sham-operated (Sham), NAC-treated sham-operated (NAC-Sham), pancreatic duct obstruction (PDO) and NAC-treated pancreatic duct obstruction (NAC-PDO) groups over the 24 h following surgery. Values are means $\pm$ SEM. Number of animals was 12 per group and experimental period. Application of ANOVA followed by Scheffé test showed statistically significant differences vs. Sham ($\blacklozenge\blacklozenge$, $P < 0.001$), vs. NAC-Sham ($\bullet\bullet\bullet$, $P < 0.001$) and vs. NAC-PDO groups ($P < 0.05$; $P < 0.001$).

Flow-cytometric analysis of acinar cells after DHR labelling (Fig. 1) showed early and progressive increase in the fluorescence intensity following AP induction, although statistical significance ($P < 0.05$) was only reached 6 h after PDO. NAC administration prevented this increase and consequently, control values were found throughout the 24 h of PDO.

Figure 2 shows the changes in TNF- α plasma levels observed over the 24 h following surgery. A slight increase was observed within the first 6 h after inducing AP by PDO, and statistical significance ($P < 0.001$) was reached 12 and 24 h after PDO. TNF- α plasma levels were maintained in control values within 12 h following PDO in rats receiving NAC. In this experimental group, only a small increase was observed at 24 h, but the values did not significantly differ from those found in the sham-operated group. Significantly lower TNF- α plasma levels were found from 6 to 24 h after PDO in rats treated with NAC.

As shown in Fig. 3, NAC treatment caused a significant increase in G_0/G_1 -phase from 6 to 12 h after surgery both in NAC-Sham and NAC-PDO rats. The comparison between NAC-treated and non-treated PDO rats revealed significantly ($P < 0.05$) higher number of G_0/G_1 -phase cells

Figure 3. Relative distribution of pancreatic acinar cells within the different phases of the cell cycle, established according to flow-cytometric analysis of cellular DNA content using PI staining. Application of ANOVA followed by Sheffè test showed significant differences vs. Sham (♦, $P < 0.05$; ♦♦, $P < 0.01$; ♦♦♦, $P < 0.001$), vs. NAC-Sham (◻◻, $P < 0.01$) and vs. PDO rats ($P < 0.05$).

in rats with AP receiving NAC. Concomitantly, significant decreases were observed in the percentage of G₂/M-phase cells of NAC-treated rats and, as a consequence, significant differences ($P < 0.05$) were found between NAC-treated and non-treated PDO rats. Regarding S-phase, no change was induced by NAC administration during early AP stages, but significant ($P < 0.01$) increases were found 24 h after PDO in rats receiving NAC up to values significantly higher than those of non-treated PDO rats.

No significant variation was found in Sub G₁-phase (Fig. 3) and Annexin V staining (Fig. 4), thus indicating that apoptosis was not induced during 24 h PDO either in NAC-treated or non-treated rats.

Figure 4. Percentage of apoptotic acinar cells measured as positive FITC-Annexin V/negative PI found in sham-operated (Sham), NAC-treated sham-operated (NAC-Sham), pancreatic duct obstruction (PDO) and NAC-treated pancreatic duct obstruction (NAC-PDO) groups over the 24 h following surgery. Values are means $\pm$ SEM. Number of animals was six per group and experimental period. Application of ANOVA followed by Scheffé test did not reveal statistical significant differences.

DISCUSSION

The deleterious effects of oxygen free radicals (OFR) at high and/or sustained levels in biological systems, and their involvement in a variety of diseases and in ageing have been widely studied (Schoenberg *et al.* 1992; Rau *et al.* 2000; Dröge 2002).

Recently, Uruñuela *et al.* (2002a) assessed the occurrence of excessive oxygen free radical production within pancreatic acinar cells, and the subsequent peroxidative damage to cell membranes in PDO-induced AP. With regard to DHR-dye, the present study has shown that NAC treatment prevented the enhanced acinar OFR generation after AP induction and, in view of the decreased plasma amylase activity, seems able to reduce the severity of the disease. Direct scavenging of OFR and effective L-cysteine supply for glutathione (GSH) synthesis might represent two different routes achieved by NAC administration, counteracting the excessive OFR generation and depletion of cellular GSH content, hallmarks in the progression of AP. In accordance with this, NAC effectively delayed and blunted the release of TNF- α into peripheral blood. TNF- α is one of the most important pro-inflammatory cytokines involved in the progression of local tissue damage during AP which contributes to the activation and amplification of the immune response (Norman 1998; Weber & Adler 2001; Frossard & Pastor 2002), thus leading to the appearance of systemic complications, usually responsible for AP-associated mortality in humans. Inhibition of cytokine production by NAC has been found in different experimental settings (Winyard & Blake 1997; Demols *et al.* 2000; Kim *et al.* 2000) and sometimes correlates with the suppressive effect on activation of the transcription factor nuclear factor (NF) κ B (Kim *et al.* 2000). NF- κ B regulates the expression of several genes, some encoding pro-inflammatory mediators, and its activation has been observed in pancreatic acinar cells in response to OFR *in vitro* (Kim *et al.* 2000) and *in vivo* (Steinle *et al.* 1999) after AP induced by cerulein supramaximal stimulation. In both cases, NF- κ B activation was down-regulated by NAC administration.

In addition to oxidative damage, OFR have demonstrated a regulatory role in cell proliferation. H_2O_2 and O_2^- were reported to be capable of increasing growth or growth response in different types of mammalian cells (Burdon 1995). However, the final outcome of OFR-mediated signalling pathways varied from one cell type to another, depending on the primary reactive

species generated and site of production, as well as the dosage and duration of treatment when OFR were exogenously added (Thannickal *et al.* 2000; Martindale & Holbrook 2002).

As regards the progression of pancreatic acinar cells throughout the different cell-cycle phases, increased levels of mitosis have previously been reported 12 h after PDO (De Dios *et al.* 2000). Because cholecystokinin (CCK) is one of the most important mitogenic stimuli for rat pancreatic acinar cells (Ohlsson *et al.* 1995), such results could be explained by the significant increase in plasma CCK levels as a result of deprivation of pancreatic enzyme secretion into the duodenal lumen, as has been reported following the obstruction of the biliary tract (Toriumi *et al.* 1993; Shirohara & Otsuki 1997), and/or by the increase in OFR generation within acinar cells reported by Uruñuela *et al.* (2002a). Given the implication of OFR in mitogenic signalling pathways, anti-proliferative effects should be expected after NAC administration. Accordingly, both in Sham and PDO rats, NAC treatment was seen to induce a significant increase in the proportion of cells in G₀/G₁-phase up to 12 h, and a simultaneous decrease in the relative number of cells in G₂/M-phase. NAC has been shown to provoke inhibition of MAPK cascades in several cell types, including pancreatic acinar cells (Dabrowski *et al.* 2000; Kuruganti *et al.* 2002). The activation of these pathways is a key regulatory point in the cell cycle, which is required for the cell to leave the quiescent state and enter S-phase (Pagès *et al.* 1993; Morisset *et al.* 1999). Accordingly, our results suggest that mitogenic stimulation due to both enhanced plasma CCK concentrations and OFR overproduction in acinar cells following AP might be inhibited by NAC administration, as both stimuli seem to make use of OFR-dependent MAPKs activation in order to achieve the proliferative response. As a result, NAC has been shown to suppress the proliferative response after AP induction and has promoted cell arrest at early PDO stages.

Cells were no longer actively proliferating 24 h after PDO induction, probably because they were progressively damaged in the course of PDO. Pancreatic atrophy has been reported 48 h after inducing AP by PDO, which develops with increased proportions of G₀/G₁-phase cells and apoptosis (Walker 1987; De Dios *et al.* 2000). By contrast, cells from NAC-treated PDO rats remained able to synthesize DNA, probably because they were effectively preserved from damage during early AP stages, when different pathological mechanisms involved in AP develop in this experimental model, as reported by Uruñuela *et al.* (2002a,b,c). Cells could be freed from the arrest asserted by NAC at earlier AP stages and they may actively enter S-phase at this PDO time point, probably in an attempt to restore the normal proliferation pattern. Mitosis might be expected at later stages when it would counteract the deletion of acinar cells found in rats with PDO-induced AP (De Dios *et al.* 2000). This event could be favoured by CCK, whose plasma levels have been found increased for at least 3 days after the onset of gallstones-induced acute pancreatitis (Shirohara & Otsuki 1997).

In this AP model, loss of acinar cells takes place mainly as a consequence of apoptotic processes, in which TNF- α is involved (Gukovskaya *et al.* 1997). A significant increase in apoptotic cells was found 48 h after PDO (Walker 1987; De Dios *et al.* 2000); however, apoptosis would not be expected throughout the 24 h of PDO in NAC-treated rats as TNF- α concentration is not increased and acinar cells are protected from oxidative damage at earlier AP stages.

In conclusion, the results provide evidence that OFR play a critical role in the progression of cell-cycle phases in pancreatic acinar cells. In parallel with the prevention of OFR generation, and therefore oxidative damage, NAC treatment maintained acinar cells in a quiescent state in early AP. These effects may combine together in the protection of the cells at early AP stages and consequently, would retain their ability to proliferate further in the course of AP. Furthermore, NAC reduced the inflammatory response during AP and, therefore, its severe consequences would also be delayed. As a result, administration of NAC could extend the time for effective removal of the obstruction and further recovery of the gland.

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