N-acetylcysteine attenuates lung injury in a rodent model of fracture

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Background **Neutrophil-mediated lung injury is a cause of significant morbidity and mortality in patients with multiple injuries. We have shown previously that fracture hematoma can activate neutrophils and is thus a putative mediator of the systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) in those patients with severe skeletal trauma.**

Our aim was to establish a rodent model of fracture which caused lung injury and subsequently to administer a drug following fracture to attenuate the lung injury. The drug we chose was N-acetylcysteine, a potent antioxidant.

Animals and methods **Adult Sprague-Dawley rats were assigned to 4 groups: (1) general anesthetic only, (2) general anesthetic with bilateral femur fractures and nailing, (3) general anesthetic and N-acetylcysteine, (4) general anesthetic with bilateral femur fractures and nailing and N-acetylcysteine after the injury (n = 6 in each group). The dose of N-acetylcysteine was 0.5 mg/kg which was given intraperitoneally after injury to the treated groups. The rats were killed 24 hours after injury and some parameters of lung injury were evaluated—i.e., bronchoalveolar lavage (BAL), lung tissue myeloperoxidase levels (MPO) and wet/dry ratios of lung tissue. The results were analyzed, using one-way analysis of variance.**

Results **Bilateral femur fracture produced a significant lung injury, measured by increases in MPO (25–43 μg/g tissue) and BAL protein (460–605 μg/mL). This effect was attenuated by treatment with N-acetylcysteine (MPO 43–9 μg/mL, BAL protein 605–198 μg/mL).**

Interpretation **N-acetyl cysteine, if given after skeletal trauma, is of potential therapeutic benefit, in preventing SIRS, ARDS and MOF.** ■

20% of late trauma-related deaths occur from late complications, such as acute respiratory distress syndrome (ARDS) (Clark et al. 1988). Trauma, including skeletal injury, is a significant cause of ARDS with a "biological gradient" increasing with the severity of the injury (Pepe et al. 1983). The incidence of ARDS following major trauma has been reported as between 5% and 8%, this indicates a significant morbidity and mortality (Pepe et al. 1982). Early fixation of long bone fractures is beneficial because it minimizes soft tissue injury associated with fracture fragments, permits rapid patient mobilization and reduces the complications associated with prolonged bed rest (Behrman et al. 1990). Although long bone fixation with intramedullary (IM) nailing facilitates early patient mobilization, there have been several reports of pulmonary dysfunction in patients with femoral fracture (Pape et al. 1983, Bone et al. 1989).

Neutrophil-mediated lung injury is implicated in ARDS. It has been suggested that lung capillary leak results from damage to the pulmonary vascular endothelium allowing subsequent neutrophil infiltration (Fonte and Hausberger 1971). In a concurrent in vitro study, we suggested that fracture hematoma is a cause of neutrophil activation, increasing respiratory burst, delaying apoptosis and increasing phagocytosis (Timlin 2002). Naturally these effects are of benefit in the area of injury, however, if large volumes of fracture hematoma are produced by trauma, this may cause a systemic inflammatory response syndrome which can subsequently cause a distant injury, such as ARDS.

N-acetylcysteine (NAC) is a potent antioxidant commonly used to treat an overdose of paracetamol. It acts as a source of cysteine for the synthesis of the tripeptide antioxidant glutathione and is also believed to have free radical scavenging properties (Lauterberg et al. 1983). It has been shown to reduce the neutrophil-mediated lung injury in an experimental model of endotoxin-induced lung injury (Davreux et al. 1997). In addition, our department has shown that NAC attenuates the muscle injury associated with ischemia reperfusion in an experimental model of compartment syndrome (Kearns et al. 2001). Previously, the administration of immune therapies before injury in experimental models and following injury in clinical trials has met with little success.

The aim of our study was first, to reproduce an inflammatory event which would result in lung injury. We chose a fracture model followed by intramedullary nailing because of its clinical relevance, and established that this model resulted in a reproducible lung injury. Secondly, we investigated the potential of NAC, administered after a fracture, to attenuate this lung injury. This novel approach may be applicable to clinical practice where a prophylactic therapy that could be administered after an injury would be most desirable.

Animals and methods

The animal experiments described were approved by the Department of Health and an animal experiment license was granted. The animal was fasted overnight and following the second general anesthetic, it was killled by thoracotomy and exsanguination.

Experimental groups

Adult male Sprague-Dawley rats (350–500 g) were assigned to four groups (n = 6 in each group).The first group was our control group, which were given a general anesthetic (GA), consisting of halothane and oxygen. The second group was the injury group and were given a GA and 18-gauge needles (Becton-Dickinson) were used as intramedullary nails introduced in a retrograde fashion through the knee into the femur. Both femurs are nailed and then fractured by using a pliers to hold the mid-shaft of the femur and the application of force to produce a fracture. This was checked by post-operative radiography in all cases (Figure 1). Group 3 were given a GA and NAC (0.5 mg/kg) one half hour following GA. Group 4 had GA and bilateral femur fractures with intramedullary nailing and NAC (0.5 g/kg) (Sigma) administered by intraperitoneal injection one half hour following injury. All animals were killled 24 hours after the injury.

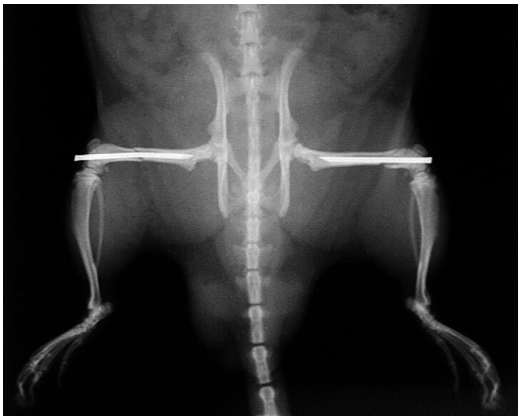

Figure 1. Bilateral rodent femur fractures with intramedullary nails in situ.

Parameters of lung injury

At 24 hours, GA was administered, a midline thoracotomy was performed, exposing the heart and lungs.

Bronchoalveolar lavage (BAL)

BAL was carried out by insertion of a 16-gauge cannula (Becton Dickenson) and clamping of the left main bronchus before infusion and aspiration of the lung with 1 ml normal saline. This was repeated three times; about 1.5 mL proteinaceous fluid was recovered. To separate cellular matter from the aspirate, samples were centrifuged at 1100 rpm for 10 minutes. Cell-free supernatant was collected and stored at –20 °C. BAL protein measurements were made by bicinchoninic acid assay (Pierce), using bovine serum albumin (BSA) as a standard. Briefly, 10 μL of sample were mixed with 200 μL of working solution and incubated at 37 °C for 30 minutes. The absorbance of the purple reaction product was measured by spectrophotometry at 560 nm.

Wet/dry (w/d) ratio

A sample of the right lung was taken, weighed and placed in a 37 °C oven for 72 hours, at which time it was reweighed. W/d ratio was calculated by dividing the final weight of the sample material into the initial, or wet, weight.

Myeloperoxidase (MPO) assay

Tissue MPO activity, a sensitive index of tissue neutrophil infiltration, was determined in the left lung by a modification of the method of Grisham et al. (1993), using the peroxidase catalyzed, hydrogen peroxidase-dependent oxidation of tetramethylbenzidene as a measure of enzymatic activity. Briefly, the clamp was removed from the left main bronchus and used to clamp the right main bronchus. 50 mL saline was injected through the right ventricle to flush blood from the lung. A sample of tissue was removed and weighed. Tissue was homogenized and frozen in a phosphate solution at –80 °C. In order to carry out the assay, tissue samples were subjected to two further freeze thaw cycles and centrifuged at 1100 rpm for 10 minutes. The supernatant was assayed spectrophotometrically for MPO activity by adding 0.1 mL sample to 2.9 mL working solution, which was 16 mM 3'3',5,5'-tetramethylbenzidene dissolved in N,N-dimethylformamide and 220 mM potassium phosphate buffer containing 110 mM NaCl. The reaction was initiated by the addition of 3 mM H_2O_2 and the change in absorbance during the first 3 minutes was measured.

Statistics

All data are reported as mean and standard error. Data points were distributed around the mean in a normal fashion and did not require transformation. Statistical analysis was performed using ANOVA to compare means between the 4 groups. Differences were judged as statistically significant when the p value was less than 0.05.

Results

NAC attenuates the injury-induced increase in BAL protein

BAL was performed as a measurement of macromolecular vascular permeability. The bilateral femur fracture model used in this study resulted in a statistically significant increase in BAL protein (Figure 2, p = 0.025). The administration of NAC after injury significantly attenuated this effect (Figure 2, p = 0.005). It was also noted that NAC in the absence of injury had no measurable effect on BAL protein (Figure 2).

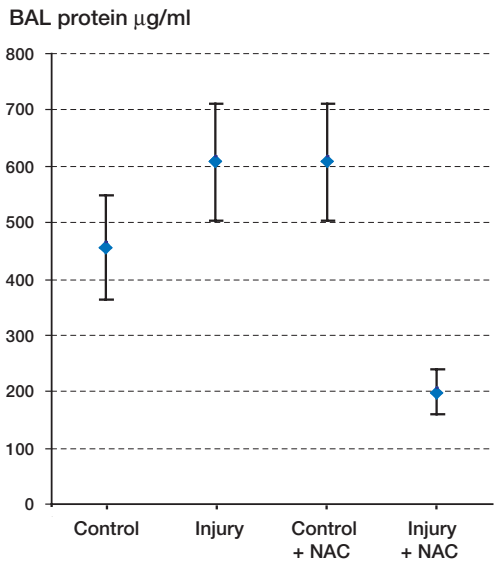

Figure 2. BAL protein measurements in the experimental groups. Data are expressed as mean ± SEM.

Lung W/D ratio is not affected by NAC

Lung w/d ratio is a crude method of measuring tissue edema, corresponding to a vascular leak. The fracture model did not cause an increase in lung tissue weight, and we found no effect on lung weight in the NAC treatment group (Figure 3).

NAC attenuates the injury-induced increase in tissue MPO activity

Tissue MPO activity is a reliable measurement of neutrophil infiltration. Bilateral femur fractures resulted in a significant increase in tissue MPO activity (Figure 4, p = 0.05). In the NAC treatment group, MPO activity was significantly less than in the injury alone group (Figure 4, p = 0.0011). Again it was noted that NAC had no effect on lung tissue MPO activity in the absence of an injury (Figure 4).

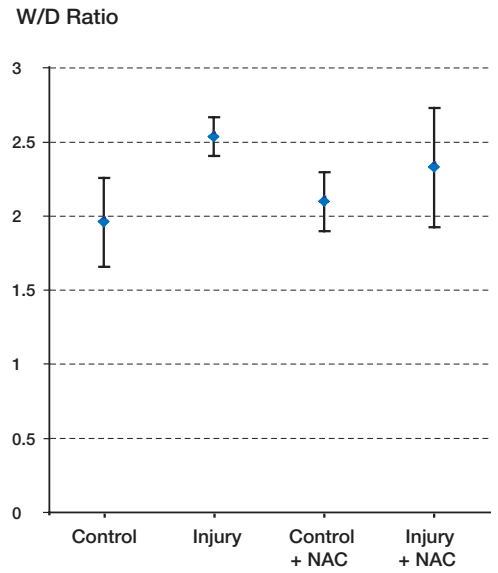

Figure 3. Lung wet/dry ratio in the experimental groups. Data are expressed as mean ± SEM.

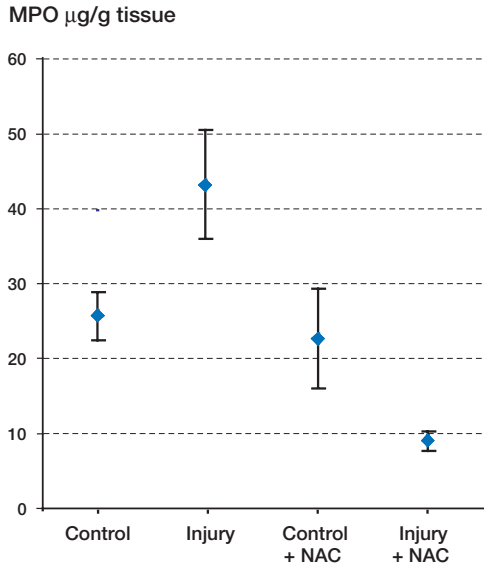

Figure 4. Lung tissue myeloperoxidase activity . Data are expressed as mean ± SEM.

Discussion

The incidence of ARDS after a major trauma is between 5% and 8% (Fowler et al. 1983). In the 1960s, fat embolus syndrome was considered the main cause of acute lung injury (Robinson 1969), but fat embolus has been found in 90% of patients after fracture (Gitin et al. 1993) and, invariably, after reamed IM nailing of fractures (Wenda et al. 1985, Christie et al. 1995). In vivo studies have shown that intravascular fat produces a negligible inflammatory response (Schemitsch et al. 1997, 1998). Other putative mechanisms underlying the development of ARDS include the local release of cytokines, the interactions between platelets and circulating neutrophils with vascular endothelial cells, transmigration of neutrophils into the interstitium and subsequent production of reactive oxygen species and activation of neuroendocrine, complement and fibrinolytic pathways (Robinson et al. 1969). The significance of the role of the neutrophil, a potent pro-inflammatory cell once activated, remains to be determined in the context of fracture-induced lung injury. Fracture hematoma (FH) is known to increase neutrophil respiratory burst (Hauser et al. 1999). We have recently completed an investigation into the pro-inflammatory effects of FH on neutrophil function. It was observed that FH not only increases neutrophil respiratory burst and phagocytosis, but also delays apoptosis (Timlin 2002). The neutrophil is the key effector of acute lung injury in ARDS resulting from trauma (Patrick et al. 1996).

Previous experimental models of femoral fracture and IM nailing have shown that this method results in a reproducible lung injury (Willis et al. 1999). Although in the present study the nail was introduced in a retrograde fashion through the knee before the fracture, a reproducible lung injury was achieved.

NAC is an antioxidant which has been used in the clinical setting to reduce the hepatic injury resulting from an overdose of paracetamol. In the experimental setting, NAC protects lung epithelial cells in vitro against oxidant injury mediated by neutrophils (Simon and Suttorp 1985). If indeed the acute lung injury described herein is mediated by the FHs effects on the neutrophil, the mechanism underlying the successful use of NAC in this model is very probably the result of its anti-oxidant properties and the reduction of oxidative stress. In clinical studies, patients with sepsis-related ARDS who were given NAC showed improvement in pulmonary vascular resistance, cardiac output, oxygen delivery and repletion of glutathione (Bernard 1991, Jepsen et al. 1992, Sutter et al. 1994).

The unique observation in our study is the finding that NAC has significant and beneficial effects on lung injury when given following a fracture. While a therapy which can be administered after injury is highly desirable, the effects of NAC on fracture healing remain to be investigated.

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No competing interests declared.

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