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Effects of early administration of dexamethasone, *N*-acetylcysteine and aprotinin on inflammatory and oxidant–antioxidant status after lung contusion in rats☆

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SUMMARY

Introduction: This experimental setting was undertaken to elucidate and confirm the role of inflammatory and oxidant–antioxidant mechanisms on blunt injury induced moderate pulmonary contusion (PC). We intended to determine the effects of dexamethasone (DXM), *N*-acetylcysteine (NAC) and aprotinin (APR) in terms of their ability to diminish the consequences of acute lung injury due to PC.

Methods: Rats were allocated to five subgroups. Except for the control, all subgroups had a moderate pulmonary contusion. Following 45 min of observation, animals in groups I and II received intraperitoneal saline, group III 10 mg/kg DXM, group IV 500 mg/kg NAC and group V 30,000 kIU/ml APR. After the procedure, 6 h after contusion, blood gas analysis, lung tissue nitric oxide (NO) and malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) activity, bronchoalveolar lavage (BAL) fluid and histopathological examination were performed.

Results: All PaO_2 values decreased significantly in contused rats as compared with the control group ($p < 0.05$). DXM, NAC and APR resulted in a slight increase in PaO_2 values compared with group II ($p < 0.05$). Lung tissue levels of MDA and NO were higher in the contusion group than in the control ($p < 0.05$). DXM, NAC and APR all decreased the levels of MDA and NO ($p < 0.05$), however the decrease in NO was not found to be significant with APR ($p > 0.05$). SOD and CAT activities increased significantly after contusion compared to control group ($p < 0.05$). There was no significant difference even though SOD levels were elevated in groups III, IV and V compared with contused animals ($p > 0.05$). Neutrophils in BAL fluid significantly increased in contused animals ($p < 0.05$). Only DXM significantly decreased neutrophil population in BAL fluid ($p < 0.05$). Scores for alveolar haemorrhage/oedema were higher in all contusion-performed rats than those in the control ($p < 0.05$). Compared with the other drugs, only APR significantly improved the haemorrhage/oedema scores compared to sham animals ($p = 0.024$).

Conclusions: Our findings demonstrate that moderate bilateral PC induced by blunt chest trauma leads to an early inflammatory process which is clearly associated with activation of the oxidant–antioxidant cascade. On this basis, early supportive treatment with DXM, NAC and APR may yield favourable results on pulmonary pathophysiological parameters which are adversely affected due to PC.

Introduction

Pulmonary contusion (PC), which may be severe or moderate, commonly accompanies blunt chest trauma although it is well known that any degree of PC associated with trauma adversely affects the patients' prognosis. Thus, resuscitative and supportive interventions play a vital role in patient outcome, especially when applied as early as possible. Although exact mechanisms are still poorly understood, blunt trauma related PC may lead to a variety of pathophysiological alterations which may manifest over a wide spectrum.[4,18,23,27,28] As there is still no widely accepted and standardised pharmacological therapeutic approach to blunt chest trauma related PC, treatment options are derived from empirical observations and clinical judgments.[9] Therapeutic choices in such patients are generally limited and include basic supportive tools

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such as supplemental oxygen, cardiopulmonary monitoring, analgesia and pulmonary hygiene.[14,15]

It has been elucidated that PC is associated with a progressive inflammatory response mediated by local and systemic immunological alterations.[11,14,16] Macrophages and neutrophils, which are potential inflammatory mediators, are activated after blunt trauma. Cytokines, reactive oxygen metabolites, and proteolytic enzymes are released by both leukocyte and macrophages leading to increased alveolocapillary membrane permeability and microvascular leakage associated with the formation of alveolar oedema fluid, proteolytic and lipolytic enzymes, and reactive oxygen species (ROS).[30] Recently, data are consistent with the findings that alveolar macrophages can produce potent ROS such as superoxide radicals and consequently peroxynitrite. Peroxynitrite can be produced by the reaction of NO with superoxide radicals and represents a highly oxidative species.[12] These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation. Various organs may control or prevent the damaging effects of the oxidant species by enzymatic and non-enzymatic antioxidant defense. These include enzymes like superoxide dismutase (SOD) and catalase (CAT).[19] Malondialdehyde (MDA) is a good indicator of free radical formation and its elevation shows increased lipid peroxidation due to the effects of these radicals.[25]

To date, several resuscitative agents have been tested to prevent the progressive process of lung injury induced by PC.[7,8,14,21] As the pathogenesis of lung injury's progression is mediated by inflammatory sources such as leukocytes and macrophages, and additionally oxidative stress is included this process, specific resuscitative drugs may ameliorate this course. The antioxidant and anti-inflammatory properties of *N*-acetylcysteine (NAC) has been widely documented in several experimental studies. NAC has also been reported to have anti-inflammatory effects in pulmonary oedema.[6,17] Dexamethasone (DXM) was previously shown to suppress the expression of proinflammatory cytokines and cell adhesion molecules involved in the migration of leukocytes into the extravascular space.[29] Aprotinin (APR) has the potential to block neutrophil elastase induced by neutrophil chemoattractants[1] and therefore may exert a potent combined protective effect on neutrophils, first by preventing their activation within the circulation and second by preventing secretion of histotoxic mediators within the tissues. Moreover, aprotinin can prevent neutrophil accumulation in the bronchial alveolar fluid by blocking leukocyte extravasation.[20]

In the current study, we firstly evaluated the effects of a moderate isolated bilateral lung contusion on various physiological parameters, as well as the resultant histopathological alterations and changes in oxidant–antioxidant status. Secondly, we intended to elucidate and determine the effects of DXM, NAC and APR in terms of their ability to diminish the consequences of acute lung injury due to PC.

Materials and methods

Animals

Thirty-five male Sprague–Dawley rats weighing 370–400 g each were obtained from the Experimental Research Centre of Kahramanmaras Sutcu Imam University Faculty of Medicine (Kahramanmaras, Turkey). The animals were acclimatised to the university's Animal Research Laboratory for 7 days before experiment and maintained on a 12-h light/12-h dark cycle at 21–22 °C. Rats were allowed free access to food and water *ad libitum*. The design of the study and the experimental procedures performed on rats were approved by the Ethics Committee of the Kahramanmaras Sutcu Imam University Faculty of Medicine. All animal procedures used were in strict accordance with the European Convention on Animal Care and National Institutes of Health Guidelines on the Care and Use of Laboratory Animals.

Blunt chest trauma

Lung contusion was induced using the model for isolated bilateral lung contusion described by Raghavendran et al.[27,28]. A hollow cylindrical weight (400 g) was dropped from a definite height (50 cm); it was encased in a vertical stainless steel tube which was positioned onto a lexon platform. This device was then suspended on Teflon guides in order to minimise friction and facilitate energy transfer. The platform was attached to a plastic protective shield which was in direct contact with the lateral aspect of the rat. This precordial shield was designed to protect the heart from contusion and thus directed the impact energy to the chest wall of the rats bilaterally. The impact energy created via this mechanism was calculated by using the equation $E = mgh$ (E: energy; g: gravity (9.8 m/s^2); h: height from the platform (50 cm); m: mass of the cylindrical weight (0.40 kg)). Total energy transferred to the chest wall of the rat was calculated as 1.96 J.

Experimental protocol

Animals were randomly allocated to five groups: group I, Control ($n = 7$); group II, Sham = Contusion ($n = 7$); group III, Contusion + DXM ($n = 7$); group IV, Contusion + NAC ($n = 7$); group V, Contusion + APR ($n = 7$). Animals were anaesthetised with intramuscular ketamine/xylazine 100/10 mg/kg. Blunt chest trauma was then performed. Analgesia was provided by morphine sulphate (0.05 mg/kg) administered intraperitoneally. Following the procedure, all subgroups were transferred to their cages. Rats were checked by observing breathing, nose bleeding, respiratory movements and cardiac rhythm. Following 45 min of observation, animals in groups I and II received intraperitoneal saline, group III was given 10 mg/kg dexamethasone, group IV was given 500 mg/kg NAC and group V was given 30,000 kIU/ml aprotinin. Animals in group I were used to obtain basal values. All subgroups were observed for 6 h in their cages. At the end of the observation period, rats breathed O_2 for 5 min before midsternotomy in order to assess the severity of pulmonary shunting. Thereafter, midsternotomy was performed on animals in all subgroups. Blood samples were collected from the descending aorta in a heparinised syringe, followed by analysis with a blood gas analyser. After the left main bronchus was clamped, BAL of the right lung was performed with 2 ml of normal saline through a tracheal cannula. This was repeated three times; 4 ml lavage fluid was obtained in total. At the end of the BAL, the right lung was harvested and the upper lobe was fixed in 10% formaldehyde for histopathological examination; the remaining portion of the lung was stored at −20 °C until further analysis.

Arterial blood gas measurements

Blood samples were collected from the descending aorta in a heparinised syringe during midsternotomy performed 6 h after blunt trauma. Analysis was performed by a blood gas analyser (Medica Easystat/USA) to assess and compare the effects of various drugs administered in the early period of blunt injury on pulmonary shunting.

Biochemical assay

Lung tissue samples were washed with sterile saline, weighed, and then homogenised by adding 2000 μL 0.01 molar phosphate