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Full Length Article

Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs

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ABSTRACT

Research in the area of oxidative stress in pneumonic pathology still requires attention in small ruminants especially with the use of bronchoalveolar lavage (BAL) which may be a more sensitive indicator of respiratory diseases than blood. This investigation evaluates the role of oxidative stress in the pathogenesis of caprine pneumonia using BAL fluid (BALf) from healthy and pneumonic goats. A BALf from 192 goats (whose pneumonic histopathology had been characterized using standard techniques) was biochemically assayed for anti-oxidants and pro-oxidants. Malondialdehyde (MDA), hydrogen peroxide generation (H_2O_2), myeloperoxidase (MPO) and reduced glutathione (GSH) contents were measured to assess free radical activity in the BALf. Superoxide dismutase (SOD), Glutathione transferase (GST) and Glutathione peroxidase (GPx) activity were also determined colourimetrically. There were significant increases in the BALf supernatant of MDA, H_2O_2 and MPO with decreases in GSH level and SOD activity in the pneumonic goats ($P < 0.05$). There was also significant correlation of BALf oxidative assay to the type and severity of pneumonia. The levels of MDA, H_2O_2, and MPO increased significantly ($P < 0.05$) in bronchopneumonia and bronchointerstitial pneumonia than other pneumonic conditions and normal lungs. The management of caprine pneumonia should often incorporate antioxidant supplementation to correct the imbalance in pro-oxidant and anti-oxidant levels.

1. Introduction

Respiratory diseases in sheep and goat have been established from exposure to stressors which may include long period of starvation and transportation, housing and weather followed by invasion of bacterial and viral infectious agents [1,2]. Exposure to these stressful conditions leads to excessive production of free radicals and reactive species with potential membrane damaging effects [3].

Oxidative stress involves oxidative modification by reactive oxygen species of biomolecules (proteins, nucleic acids, and lipids). It induces a variety of organ dysfunction as a result of imbalance between the pro-oxidant and anti-oxidant levels in cells and tissues [4,5].The pro-oxidant promotes oxidation while anti-oxidants checkmates the activities of these pro-oxidants. Oxidative stress may also result from defects in expression of the genes controlling anti-oxidant enzymes [6].

The respiratory pathogens survive insults from reactive species generated by the host through detoxification mechanisms using enzymatic and non-enzymatic processes. The anti-oxidant enzymes are involved in enzymatic detoxification mechanisms and other adaptive mechanism controlled by gene expression [5,7].

Research in the area of oxidative stress in pneumonic pathology still requires attention especially in small ruminants. Hitherto, evaluations of oxidative stress in animals require estimation of certain blood biomarkers that reflect the oxidative profile of affected cases [8,9], as was done in Peste des petits ruminant (PPR) virus infected sheep [10]. The assay of these markers in BAL may be a more sensitive indicator of respiratory diseases than blood. Hence, this study evaluates for the first time the oxidative stress parameters in bronchoalveolar lavage fluid of healthy and pneumonic goats.

2. Materials and methods

2.1. Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and also

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approved by University of Ibadan Animal Care Use and Research Ethics Committee (UI-ACUREC/17/0060).

2.2. Animals and bronchoalveolar lavage fluid

The source of animals and pattern of pneumonia have been previously described [11]. The bronchoalveolar lavage sampling was as described by Khin and Zamri-Saad [12]. Briefly, following slaughter, the trachea together with the lungs were resected and lavaged by introducing 40 mL of warm sterile phosphate buffered saline (PBS), pH 6.8 into the lungs. This was followed by gentle massage of the lungs before the fluid was re-collected into a measuring beaker. The colour and consistency of the BALf was noted before centrifuged at 3000 rfc for 15 min and the supernatant decanted. The BALf supernatant was stored at −20 °C. Out of 700 goats, 192 BALf were randomly selected for biochemical analysis of oxidative markers.

2.3. Oxidative biochemical assay of BALf

The total protein concentration was calculated using the biurets method [13]. Fifty μL of BALf supernatant were added to 100 μL of biuret reagent in the 96-well microtitre plate. The plate was left for 30 min at room temperature before read on the spectrophotometer at a wave length of 490 nm using distilled water as blank. The readings were extrapolated on the total protein standard curve.

The superoxide dismutase (SOD) activity was calculated according to Misra and Fridovich [14] and Omobowale et al. [7]. Twenty μL of the BALf were added to 250 μL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 μL of acidified reconstituted adrenaline. The change in absorbance was observed every 30 s for 180 s at 490 nm wave length.

The reduced glutathione (GSH) was determined as described by Jollow et al. [15]. Addition of 250 μL of 4% sulfosalicylic acid to 250 μL of BALf in test tube was carried out, the tube was centrifuged at 4000 rfc for 5 min. Twenty μL of the supernatant were aliquoted into wells of the 96-well microtitre plates, 180 μL of Ellman's reagent (containing 0.04g of DTNB in 100 mL of 0.1 M phosphate buffer, pH 7.4) was added to the well. The absorbance of the reaction was read on the spectrophotometer at a wavelength of 405 nm against distilled water as blank.

The glutathione peroxidase (GPX) was estimated as described by Beutler et al. [16]. The tube contained 250 μL 0.1 M phosphate buffer (pH, 7.4), 50 μL of Sodium azide, 100 μL of GSH solution, 100 μL of H_2O_2, 250 μL of BALf and 300 μL of distilled water. The tube was incubated at 37 °C for 5 min in water bath. Now 250 μL of TCA were added to the tube before centrifugation at 3000 rpm for 5 min. An amount of 50 μL of the supernatant was aliquoted into well of the 96-well microtitre plate, 100 μL of K_2PHO_4 and 50 μL of DTNB were added. The reaction absorbance was evaluated at a wavelength of 405 nm with distilled water as blank.

The glutathione transferase (GST) activity was estimated via the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione [17]. A unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25 °C. The changes in absorbance were monitored at a wavelength of 405 nm. The rate of the linear reaction was set at Δ405 nm. The absorbance Δ405 nm for the blank reaction was subtracted from the absorbance Δ405 nm for each BALf sample reaction. The molar extinction coefficient of CDNB was 0.0096 $\mu M^{-1}/cm$. Thus, GST activity = [(Adjusted Δ405 nm)/0. 0096 $\mu M^{-1}/cm$] × (1.0 mL /0.1 mL) × sample dilution = U/mL.

The malondialdehyde (MDA) level was as described by Varshney and Kale [18]. Each wells of the 96-well microtitre plate contained 400 μL of Tris-KCl, 125 μL of 30% TCA, 100 μL of BALf and 125 μL of 0.75% TBA prepared in 0.2 M HCl. The plate was incubated at 80 °C for 45 min in water bath. It was cooled on ice and centrifuged at 3000 rfc for 15 min. The reaction absorbance was read against distilled water as blank at wavelength of 490 nm. The level of lipid peroxidation (units/milligram protein) was valued on a molar extinction coefficient of $1.56 \times 10^5/M/cm$.

The hydrogen peroxide generation was evaluated according to Woff's [19]. Each wells of the 96-well microtitre plate contained 100 μL of 0.1 M phosphate buffer (pH 7.4), 50 μL of Ammonium ferrous sulphate, 20 μL of sorbitol, 10 μL of Xanthine Oxidase (XO), 25 μL of sulphuric acid and 50 μL of BALf. The plate was vortexed slightly change in colour (pink colour). The plate was then incubated at room temperature for 30 min. The reaction absorbance was read at 490 nm wavelength using distilled water as blank. The H_2O_2 standard curve was used for extrapolation of H_2O_2 level generated.

The myeloperoxidase (MPO) activity was evaluated as described by Xia and Zweier [20]. The well of the microtitre plate contained 200 μL O-dianisidine mixture and 10 μL of BALf. The reaction absorbance was monitored at 0, 30 and 60 s at 450 nm wavelengths.

2.4. Statistics

All statistical analyses were performed using Computer Software (SPSS version 16.0, Chicago, USA). Data were presented as Mean ± SEM, and compared using Pearson correlation and ANOVA at 5% significance.

3. Results

3.1. Pattern of lung lesion

Of the 192 BALf; 35 were of normal lungs, 29 of congestion and oedema, and 72 of bronchopneumonia, 29 of broncho-interstitial pneumonia, 22 of interstitial pneumonia and 3 of granulomatous pneumonia.

3.2. Pro-oxidant and anti-oxidant parameters

The total protein (TP) concentration in the BALf of goats with histologically normal lung was 9.56 ± 0.7 mg/ml. It was significantly lower than that in congestion and oedema (11.6 ± 1.1 mg/ml), bronchopneumonia (16.7 ± 1.7 mg/mL), broncho-interstitial pneumonia (13.5 ± 1.7 mg/ml), interstitial pneumonia (15.4 ± 3.5 mg/mL) and granulomatous pneumonia (15.2 ± 4.8 mg/ml).

The measure of Glutathione peroxidase (GPx) activity was 24.1 ± 1.5 units mg^{-1} protein in the BALf of normal goats. There were slight reduction in the GPx activity from congested and oedematous (21.4 ± 1.7 unit mg^{-1} protein), bronchopneumonia (18.9 ± 1.4 unit mg^{-1} protein), broncho-interstitial pneumonia (20.1 ± 1.8 unit mg^{-1} protein), interstitial pneumonia (22.3 ± 2.7 unit mg^{-1} protein) and granulomatous pneumonia (20.4 ± 4.9 unit mg^{-1} protein). The difference was significant in BALf from goats with bronchopneumonia ($P < 0.05$).

The level of reduced glutathione (GSH) was 9.9 ± 0.3 μg mL^{-1} form BALf of normal lungs. There were slight decrease to 9.4 ± 0.2 μg mL^{-1} in congested and oedematous lungs, 9.7 ± 0.10 μg mL^{-1} in bronchopneumonia, 9.5 ± 0.1 μg mL^{-1} in broncho-interstitial pneumonia, and similar level or slight increase in interstitial pneumonia (9.9 ± 0.3 μg mL^{-1}) and granulomatous pneumonia (10.2 ± 0.4 μg mL^{-1}).

The activity of Glutathione transferase (GST) was 8.0 ± 1.1 unit mg^{-1} protein in the BALf from normal lungs. It decreased in congested and oedematous lungs