The Role of Oxygen Toxicity in Peri-Anaesthetic Mortality

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Introduction

The unique avian respiratory functional anatomy and comparatively high metabolic rates results in birds having a significantly higher predicted lifetime oxygen consumption and hence oxidative burden than a comparatively sized mammal (Ogburn 1998). Birds have developed unknown mechanisms to reduce the impact of this oxidative burden, and demonstrate enhanced cell survival and reduced production of reactive oxygen species under conditions of hyperoxia in comparison with mammals, both in vitro and in vivo (Ogburn 1998, Ku 1993). Exposure of birds to normobaric hyperoxia during the provision of anaesthesia or supportive care has the potential to result in pulmonary oxygen toxicity. Significant pulmonary pathological changes have been previously reported in birds (Stauber 1991), however the antioxidant mechanisms utilised by birds have not been previously described.

Methods

Birds and exposure conditions

Sixty budgerigars (*Melopsittacus undulatus*) aged 12-36 months with no clinical evidence of respiratory disease were sourced from a single aviary flock. These birds were randomly divided into 4 equal groups of 15 birds which were allocated as control, acute, repeated acute and chronic exposure groups.

Oxygen exposure was performed in a 260 L clear perspex tank with a gas flow rate of 6 - 15 L/min as required to maintain outflowing oxygen concentrations at the required levels ie >95% or 21% for oxygen exposure or control birds respectively. Acute exposure birds, repeated acute exposure birds and chronic exposure birds were exposed to oxygen for a single 3 hour period, a 3 hour period daily for 3 sequential days and a single 72 hour period respectively. Control birds were exposed to 72 hours of compressed air (21% O_2) at 6 L/min. Following oxygen or air exposure, 5 randomly selected birds were euthanased for sample collection immediately following removal from the chamber, 5 birds were euthanased 24 hours after exposure and the remaining birds 96 hours after exposure.

Sample collection

Euthanasia was performed by briefly anaesthetising each bird with halothane (Halothane, Merial Australia Pty Ltd) in oxygen and exsanguination by jugular venepuncture. Aliquots of blood were collected into appropriate anticoagulants with or without antioxidants for determination of packed cell volume (PCV), blood gases, glutathione peroxidase (GSHPx), tocopherols, carotenoids, and 8-epi-isoprostane F2α concentrations. Following exsanguination, both lungs were collected and washed with ice cold sterile 0.9% sodium chloride solution. Samples of lung tissue were collected into buffered formalin and gluteraldehyde for processing for histology and transmission electron microscope

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evaluation respectively. The remaining lung tissue was further divided and homogenised in appropriate solutions. The supernatants were collected for determination of total protein, malonaldehyde (MDA), 4-hydroxyalkenals (4-HNE), superoxide dismutase (SOD), glutathione reductase (GSHR), GSHPx, uric acid, and reduced and oxidised glutathione (GSH and GSSG respectively).

Assay techniques

Blood gas analysis was performed on a ABL5 blood gas analyser (Radiometer, Copenhagen, Norway). Tocopherols and carotenoids were assayed by high performance liquid chromatography (HPLC) following deproteination and heptane extraction. Concentrations of 8-epi-isoprostane $F_{2\alpha}$ were determined by gas chromatography following solid state extraction, HPLC fractionation and derivation. MDA and 4-HNE were determined using a commercial assay kit (Bioxytech® LPO-586™, Sapphire Bioscience, NSW, Australia). GSHPx was determined a Se-GSHPx specific coupled enzyme assay in the presence of GSHR and hydrogen peroxide. GSHR was determined by the oxidation of NADPH in the presence of GSH and GSSG. GSH and GSSG were determined by assaying the recycling reaction of GSH with Ellman's reagent without or with 2-vinylpyridine respectively. UA was determined by the modified Trinder peroxide method and protein determinations were by the Biuret method.

Statistical analysis between groups was performed by analysis of variance (ANOVA) with comparison of means by Tukey tests, and between pairs of data by t-tests.

Discussion of Results

Evidence of pulmonary oxidative stress was apparent after both the acute and repeated acute oxygen exposures, while progression from pulmonary oxidative stress to pulmonary oxidative toxicity was evident following chronic exposure. Specifically, histological scoring demonstrated increased perivascular edema and bronchial pneumonia with increased duration of oxygen exposure. Venous blood gas analysis was consistent with increasing respiratory alkalosis with increased duration of oxygen exposure. Significant changes in enzymic antioxidants were limited to elevations of both blood and pulmonary glutathione peroxidase with repeated acute and chronic exposures. Changes in non-enzymic antioxidants included reduction in pulmonary non-enzymic antioxidants including uric acid, carotenoids, alpha tocopherol and oxidised glutathione. The reduction in concentrations of these non-enzymic antioxidants was massive, and is consistent with depletion of the tissue storage of these compounds, indicating exhaustion of the non-enzymic antioxidant protection of the pulmonary tissue.

References

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