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Corpuscular oxidative stress in desert sheep naturally deficient in copper

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ABSTRACT

Oxidative stress arises when there is an imbalance between radical-generating and radical-scavenging activity; it may therefore cause an increase in oxidation products and cell damage. This study aimed to determine antioxidant status, lipid peroxidation, and their relation to anemia of grazing sheep deficient in copper (Cu). For this purpose, 39 male lambs of native (Balady) breed, aged 6–7 months and reared in El-Dakhla oasis (in the western Egyptian desert), were divided according to plasma Cu (pCu) concentration into three groups, marginally deficient (MD, pCu = 4–8 μmol/l, $n = 12$), functionally deficient (FD, pCu < 3 μmol/l, $n = 12$) and control (pCu > 9 μmol/l, $n = 15$). Jugular blood was sampled for determination of red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), plasma ceruloplasmin activity (pCp), antioxidant activities of erythrocytic superoxide dismutase (eSOD), catalase (eCAT), glutathione peroxidase (eGSH-Px), and levels of erythrocytic malondialdehyde (eMDA, as a biomarker of lipid peroxidation). The Cu-deficient lambs were characterized by microcytic hypochronic anemia accompanied by decreased pCp, eSOD, eCAT and eGSH-Px activities and increased eMDA level when compared to the controls. The indices of anemia, pCp and eSOD were lower and eMDA was higher in FD compared to MD lambs. The enhanced eMDA was strongly correlated ($P < 0.01$) with the inhibited activity of pCu ($r = -0.79$), pCp ($r = -0.65$) and eSOD ($r = -0.71$) and to a lesser extent ($P < 0.05$) with eGSH-Px ($r = -0.38$) and eCAT ($r = -0.41$). In addition, eMDA was negatively correlated ($P < 0.01$) with RBC ($r = -0.75$), PCV ($r = -0.69$) and Hb ($r = -0.72$). This study suggests that Cu-deficient lambs incur an erythrocytic oxidative damage secondary to impaired oxidant defenses, which may be one of the mechanisms underlying Cu deficiency-induced anemia in grazing sheep.

1. Introduction

Copper (Cu) deficiency in grazing ruminants is a worldwide problem (Underwood and Suttle, 1999). In an oasis in the western Egyptian desert, copper deficiency was reported in soil, pasture, and grazing sheep (Yousef, 2006). Cu deficiency in sheep can occur by low Cu concentration in pastures (primary Cu deficiency) or high concentrations of molybdenum (Mo), sulfur (S), iron (Fe) and/or zinc (Zn) in forage (secondary or conditioned Cu deficiency) resulting in decreased bio-available Cu (Radostits et al., 2000). Marginal deficits of this element in sheep can contribute to disturbances in the immune system and a number of clinical manifestations such as anemia, growth retarda-

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tion, achromotrichia (lack of pigmentation of colored wool), neurological disorders (sway back), and infertility (Suttle and Jones, 2000).

Copper acts as a cofactor of several metalloenzymes and other metalloproteins such as ceruloplasmin (Cp), Cu–Zn superoxide dismutase (SOD), cytochrome *c* oxidase, lysyl oxidase, and metallothionein (Lee et al., 2002). The chemistry of Cu makes it an ideal participant in redox reactions because it cycles easily between the cuprous and cupric state (Johnson et al., 1992). With a normal body concentration of Cu, this element acts as an indirect antioxidant because of its contributory role of the antioxidation process (Johnson et al., 1992). Cp is the main cupremic determinant in plasma and acts as an extracellular scavenger of free radicals (Saenko et al., 1994). Also, the ferroxidase activity of Cp mediates oxidation of ferrous ions to the ferric state, thereby preventing ferrous ion-dependent formation of hydroxyl radicals ($OH^{\bullet}$) via the Fenton reaction (Gutteridge, 1995). The activity of SOD catalyzes the dismutation of O_2^- to H_2O_2 (Zelko et al., 2002). Under conditions of Cu deficiency, these antioxidant defense systems can be compromised (Prohaska, 1990). Predictably, the activities of Cp and SOD are sensitive to Cu status in sheep (Andrewartha and Caple, 1980; Blakley and Hamilton, 1985). In murine models, several non-cuproenzymatic antioxidants such as catalase (CAT) and glutathione peroxidase (GSH-Px), which catalyze the degradation of H_2O_2 to H_2O and O_2 (Gutteridge, 1995), are also influenced by Cu deficiency (Prohaska, 1991; Lai et al., 1995).

Oxidative stress arises when there is an imbalance between radical-generating and radical-scavenging activities; it may therefore cause an increase in the formation of oxidation products and tissue damage (Gutteridge, 1995). Lipid peroxidation is a general mechanism whereby free radicals induce toxic products and tissue damage, and is implicated under several diverse pathological conditions (Halliwell and Chirico, 1993). Malondialdehyde (MDA) concentration is used as an index of oxidative breakdown of lipids in membranes and an indirect biomarker of free radical generation (Gutteridge, 1995). Increased free radical generation and enhanced lipid peroxidation were demonstrated in whole animal and cell culture models of Cu deficiency (Uriu-Adams and Keen, 2005).

Anemia can be a feature of Cu inadequacy in livestock. Generally the anemia of Cu deficiency in sheep is classified as hypochromic microcytic in lambs (Suttle and Jones, 2000). This type of anemia is characteristic of anemia resulting from impaired utilization of iron for hemoglobin production (Underwood and Suttle, 1999). On the other hand, the membrane of erythrocytes is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals, and may cause erythrocytes to be vulnerable to oxidative damage (Jain et al., 1983). Also, erythrocytes are exposed to high concentrations of oxygen and contain heme iron that can be auto-oxidized, which results in O_2^- formation (Jain, 1993).

The aim of the present study was to determine the indices of oxidative stress including plasma Cp, erythrocytic antioxidant (SOD, GSH-Xp and CAT) activities, erythrocytic malondialdehyde (eMDA) levels, and their role as contributory factors of anemia of lambs grazing Cu-deficient pastures.

2. Materials and methods

2.1. The study area

An Egyptian oasis (El-Dakhla) is located in an arid tropical area in the western Egyptian desert. This oasis is a depression that extends between latitudes 25°15′ and 26°00′N and between longitudes 28°30′ and 29°47′E. No rivers or surface water are present and rainfall is negligible. Sheep in this area reared under unorganized farming with unsatisfactory standards of animal management and feeding. Sheep, in small flocks, are confined to limited areas for grazing pastures grown around groundwater wells without supplementation with concentrates or minerals. Alfalfa (*Medicago sativa*; called locally Berseem Hegazy) is the main food available for sheep in this area.

2.2. Animals

In this study, 39 male lambs of native (Balady) breed, reared in El-Dakhla oasis and 6–7 months of age, were used. Twenty-four lambs (23.2 ± 0.30 kg) were selected from flocks reared on a known Cu-deficient area (El-Hendaw) and divided according to plasma Cu concentration (Suttle, 1986) into two equal groups (12 lambs each). The first group had a plasma Cu concentration of 4–8 μmol/l, and was considered marginally deficient group (MD). The second group had a plasma Cu concentration of <3 μmol/l (functionally deficient group; FD). The rest (15 lambs, 21.3 ± 0.51 kg) were selected from flocks grazing an adjacent Cu-adequate area (Mout), with plasma Cu of >9 μmol/l, and used as a control group. All of the selected animals had been reared under similar management and feeding practices. On clinical examination, the control animals were apparently healthy, and the affected lambs were free from other diseases. Anemia was the only prominent clinical sign of the Cu-deficient lambs. Also, body weight of control and Cu-deficient lambs was different ($P < 0.05$).

2.3. Food, blood and fecal sampling

Representative samples (10 samples, each of ~100 g) of Berseem Hegazy (alfalfa) were collected from each of the Cu-deficient and Cu-adequate areas. These samples were clipped 2–3 cm above ground level at random from sites at which the lambs were grazing using stainless steel scissors. Care was taken to avoid contamination by soil when cutting the sample. Samples of each area were pooled into one sample, dried, ground and stored in air-tight containers for subsequent analysis. For hematological and biochemical investigations, 10 ml of jugular blood was drawn from each lamb in centrifuge tubes containing Na_2-EDTA as an anticoagulant. Fecal samples were taken from the rectum of each lamb, and examined by the standard flotation sedimentation technique (Coles, 1980). Cases harboring gastrointestinal parasites were not selected.

2.4. Hematological and biochemical analysis

2.4.1. Hematological investigations

The count of red blood cells (RBC) was determined using a hemocytometer, whereas packed cell volume (PCV) and hemoglobin concentration (Hb) were determined by microhematocrit and cyanomethemoglobin methods, respectively (Jain, 1986). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were calculated mathematically (Jain, 1986).

2.4.2. Preparation of erythrocyte hemolysate

Immediately after collection, blood samples were centrifuged at $1500 \times g$ for 15 min at 4 °C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate.

2.4.3. Mineral determinations

Concentrations of Cu, Fe, and Zn in the diet were determined with an atomic absorption spectrophotometer (GBC 932 AA; GBC Scientific