Oxidative stress in cows with acute puerperal metritis

O. KIZIL*, Y. AKAR2, M. YUKSEL2 AND N. SAAT2

1Department of Internal Disease, Faculty of Veterinary Medicine, University of Firat, 23 119 Elazig, TURKEY
2Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Firat, 23 119 Elazig, TURKEY.

SUMMARY

The aim of this study was to compare the oxidant / antioxidant balance between cows with acute puerperal metritis (n = 12) and healthy controls (n = 12) by measurement of MDA concentrations, antioxidant GSHPx (glutathione peroxidase) and catalase enzyme activities and concentrations of chain breaking antioxidant vitamins (A, E, C and β-carotene) in plasma determined by spectrophotometry methods. The acute puerperal metritis was clinically diagnosed and the mean MDA concentration was significantly higher in the diseased group than in controls whereas the mean enzyme activities and mean β-carotene and vitamin concentrations were markedly and significantly depressed. Moreover, 3 affected cows exhibited abnormal high values of MDA concentrations and at least, one antioxidant marker was altered in 9 cows. These results demonstrate the occurrence of an oxidative stress in cows with acute puerperal metritis which is exacerbated throughout antioxidant overutilization.

Keywords: Cow, puerperal metritis, lipid peroxidation, antioxidant, glutathione peroxidase, catalase, vitamin C, vitamin A, vitamin E, β-carotene.

Introduction

Acute puerperal metritis (APM), which is also called post-partum metritis, occurs within the first 10 days after parturition. It is characterized by foetid, watery, and reddish-brown to purulent vulvae discharge and fever. The palpation of the uterus per rectum reveals an enlarged and flaccid uterus [12, 28, 40]. A clinical or subclinical form of metritis may be present. Clinical metritis may be either acute, appearing quickly, and generally affecting the cow’s appetite and milk production, or chronic, persisting over a long period. The cow is extremely vulnerable to infection during this stressful period. The severity of metritis or endometritis in the stressful period depends on the infectious agent or agents involved the degree and duration of the infection, the nutritional status and the overall health of the individual animal [23, 27, 30, 40].

A free radical can be described as any atom or a group of atoms or molecules in which there is at least one unpaired electron in the outermost shell [32]. Usually, organism has sufficient antioxidant reserves to cope with the production of free radicals [6, 35] but the imbalance between the production of ROS (Radical Oxygen Species) and the availability of antioxidant molecules may result in oxidative stress [4, 35]. The components of the antioxidant system have been classified as preventive and chain breaking antioxidants. Glutathione peroxidase (GSHPx) and catalase (CAT) are anti-oxidant enzymes [9, 24, 35]. The chain-breaking antioxidants act after initiation of a chain reaction. This class of antioxidants includes reduced lipid soluble vitamin E [17], β-carotene, and water soluble ascorbate [32]. Vitamin A has been listed with lipid soluble antioxidant, although it does not have a major chain-breaking activity [35]. The β-carotene directly exert an anti-oxidant effect in dairy cattle which is independent of its role as provitamin A [7]. It can act as a quencher of singlet oxygen and has the ability to directly react with the peroxyl radicals involved in lipid peroxidation [5]. Whenever equilibrium between ROS and antioxidants is broken, progressive oxidation of other biological substrates (such as lipids, DNA and proteins) occurs, establishing an oxidative stress status that may impair health both directly and indirectly [9, 35]. Direct effects include peroxidation damage to important lipids and macromolecules. Indirect changes include effects on cellular membranes and components, modifying metabolic pathways and resulting in altered physiology [2].

RÉSUMÉ

Stress oxydatif chez des vaches atteintes de métrite aiguë post-partum

L’objectif de cette étude était d’évaluer l’équilibre oxydants / antioxydants chez des vaches présentant une métrite aiguë du post-partum, diagnostiquée cliniquement (n = 12) et chez des vaches en bonne santé (n = 12) en mesurant par spectrophotométrie les concentrations de MDA, les activités d’enzymes anti-oxydantes (glutathion peroxydase (GSHPx) et catalase), ainsi que les concentrations de vitamines anti-oxydantes qui interceptent les réactions radicales (vitamines A, E, C et β-carotène) dans le plasma. La concentration moyenne de MDA a été significativement plus élevée dans le groupe d’animaux malades que dans le groupe contrôle alors que les activités enzymatiques et les concentrations moyennes en β-carotène et en vitamines étaient nettement et significativement diminuées. En outre, 3 vaches malades ont présenté des concentrations de MDA anormalement élevées et au moins un des antioxydants étaient altérés chez 9 vaches. Ces résultats démontrent l’existence d’un stress oxydatif chez des vaches ayant une métrite du post-partum qui est exacerbé par une consommation importante des systèmes antioxydants.

The aim of this study was to determine and compare the oxidative status between cows with acute metritis and healthy controls on the basis of plasma lipid peroxidation intensity (MDA), antioxidant enzyme activities and chain breaking antioxidant system parameters such as vitamin A, E, C and β-carotene.

Materials and Methods

ANIMALS AND BLOOD SAMPLES

The study was performed on 12 cows with acute puerperal metritis and 12 healthy controls, 2-5 years old, with an average annual milk yield of 5400 kg, brought into the Clinic of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Firat between May and July 2008. The puerperal metritis was detected by rectal palpation with evidencing of an enlarged and flaccid uterus and vaginal examination. A watery, odour and reddish-brown to purulent vaginal discharge was observed in affected animals. Furthermore, all cows exhibited febrile syndrome with hyperthermia (rectal temperature above 39.5°C), lack of appetite and weakness.

Blood samples were taken by venipuncture of the jugular vein into heparinised vacutainer tubes. Plasma was separated by centrifugation (700 g, at +4°C, for 10 min) and stored at -20°C until biochemical analysis.

BIOCHEMICAL ANALYSIS

Plasma lipid peroxidation intensity (MDA)

The plasma lipid peroxidation intensity was measured throughout the concentration of malondialdehyde (MDA), which is a thiobarbituric acid reactive species [39]. Briefly, one volume of the test sample and two volume of stock reagent (15% w/v trichloroacetic acid in 0.25 N HCl and 0.375% w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated for 15 min in boiling water. After cooling, the precipitate was removed by centrifugation at 500 g 10 min and then absorbance of the supernatant was measured at 532 nm against a blank containing all the components except the enzyme on a spectrophotometer (Schimadzu UV-1208 UV-VIS, Japan). The catalase activity was expressed as kU/L.

Antioxidant enzyme determination

The plasma GSHPx activity was determined according to the method of LAWRANCE [26]. The reaction mixture contained potassium phosphate buffer (pH 7.0) 50 mM, EDTA 1 mM, sodium azide (NaN3) 1 mM, reduced nicotinamide adenine dinucleotide phosphate (NADPH) 0.2 mM, GSH 1 mM and glutathione reductase 1 U/mL. The enzyme source (0.1 mL) was added to 0.8 mL of the above mixture and incubated for 5 min at 25°C before the initiation of the reaction by the addition of 0.1 mL of hydroperoxide solution (H2O2 0.25 mM in final concentration). The absorbance at 340 nm was recorded for 5 min on a spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. The protein concentration was also measured by the method of LOWRY [31]. The results were expressed as U/g of proteins.

The plasma CAT activity was measured as previously described by GOTH [13]. Briefly, 0.2 mL of plasma samples was incubated in 1.0 mL substrate (65 µmol hydrogen peroxide per mL of a 50 mM phosphate buffer, pH 7.0) at 37°C for 60 s. The enzymatic reaction was terminated with 1.0 mL of a 32.4 mM ammonium molybdate solution. Hydrogen peroxide was measured at 405 nm against blank containing all the components except the enzyme on a spectrophotometer (Schimadzu UV-1208 UV-VIS spectrophotometer was used in all analysis).

STATISTICAL ANALYSES

Statistical analyses were performed using on SPSS Ms Windows Release 10.0 programme. Impaired t-test was used for evaluating data between groups. The data were expressed as mean ± standard error, and P<0.05 was taken as the level of significance.

Results

As shown in Table I, plasma MDA concentrations were markedly increased in the group of cows with acute puerperal metritis (P < 0.05) whereas enzyme GSHPx and catalase activities were significantly depressed (P < 0.01 and P < 0.05, respectively) as well as concentrations of vitamins A and E (P < 0.01), vitamin C (P < 0.05) and β-carotene (P < 0.001).

When considering the proportions of abnormal values for oxidative stress parameters defined as values outside the confidence intervals at 68% (mean ± standard deviation) and 95% (mean ± 1.96 x standard deviation) of the mean values obtained in healthy controls (Table II), abnormal elevated MDA concentrations were found in 25% (3/12) of diseased cows. Antioxidant enzyme GSHPx and catalase activities were markedly reduced (< mean - standard deviation) in 50% (6/12) of cases. In the same way, the chain breaking antioxidant concentrations were colorimetrically determined using a phosphotungstic acid method described by KYAW [25], and those of vitamin E were spectrophotometrically determined according to the MARTINEK’s method [33].
concentrations were also depressed in the majority of cows with acute puerperal metritis: vitamin C concentrations below 4.3 mg/L were observed in 4 cows, vitamin A concentrations below 315.6 mg/L in 6 cows, vitamin E concentrations below 1.3 μg/L in 9 cows and deeply depressed β-carotene concentrations (< 1.11 mg/L) in 7 cows. Moreover, 3 affected cows (25%) exhibited at least 3 abnormal values for oxidative stress markers, the most frequent combination being increased MDA concentrations coupled to decreased vitamin A and β-carotene concentrations. In addition, 25% of cows with acute puerperal metritis exhibited increased MDA concentrations coupled to decreased GSHPx and catalase activities.

**Discussion**

Susceptibility to certain infectious diseases also peaks at transition time [41]. Reported incidence rates for infections of the udder (mastitis) and of the uterus (metritis) are 1.7 to 54.6% and 10.1 to 65.5%, respectively [3, 19]. Infectious diseases such as metritis generally receive less attention than the metabolic diseases that are prevalent after calving [30]. Increased physiological demands during the transition period induce an inordinate metabolic demand for the organism [2]. These nutrient demands cannot be supported by a traditional grazing diet. According to a model for dairy cow response to metabolic stress described by KNIGHT et al. [23], cows may experience mild, moderate, or severe metabolic stress during transition.

Dietary antioxidants, notably vitamin E and selenium, are important for their contribution to ROS neutralization, thereby impeding the progression towards inflammation. Interestingly, plasma concentrations of α-tocopherol (vitamin E) decrease through the transition period [45], and low antioxidant status is associated with transition cow disorders [28, 37]. Beta carotene, a precursor of vitamin A, can also act as an antioxidant.
and concentrations of both vitamin A and \( \beta \)-carotene typically decrease during the transition period [28]. The supplementation of \( \beta \)-carotene during the transition period significantly decreased incidence of both metritis and retained placentia compared to vitamin A supplementation [34]. On the other hand, free radicals or reactive oxygen species is one of the major causes of many diseases including infertility [44]. The resultant antioxidant defence mechanisms against ROS, although activated, might be not enough efficient and clinical symptoms of illness may occur [14].

The determination of lipid peroxidation status (MDA concentrations) is among the most widely used methods for determination of oxidative stress. Increased plasma malondialdehyde (MDA) concentrations indicate lipid peroxidation [15, 20, 24, 36]. Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and leads to formation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes, and one of the most common intermediates is malondialdehyde (MDA) [8, 16]. In this study, mean plasma MDA concentration was found to be increased in the cows with puerperal metritis compared to the control group, while decreases of GSHPx and CAT activities were observed and alterations of these 3 markers were simultaneously evidenced in 25% of diseased cows. As GSHPx and CAT are involved in the conversion of radicals into less effective metabolites, these changes coupled to the increase of MDA concentrations confirmed the occurrence of an oxidative stress during metritis. These findings clearly indicate that GSHPx and CAT activities depressed when lipid peroxidation occurred, leading to an exacerbation of the oxidative stress.

Vitamin E (in the form of \( \alpha \)-tocopherol) is the major lipid-soluble antioxidant of lipoproteins and biomembranes [24, 29, 46]. Vitamin C (ascorbate) is as a potent water soluble chain breaking antioxidant in the biological fluids, but it cannot scavenge the radicals within the lipid region of the membranes [10]. Because ascorbate is produced in the liver of adult cattle, its biosynthesis capacity is believed to be sufficient for physiological requirement [11, 18, 21]. Nonetheless, ruminants can be prone to ascorbate deficiency due to rapid destruction by ruminal microflora [18]. This deficiency can lead to decreased resistance to inflammation [21, 22]. The synergism between ascorbic acid and \( \alpha \)-tocopherol in the inhibition of lipid peroxidation is well known. Vitamin C enhances the antioxidant activity of vitamin E by regenerating it as the reduced form and the depletion of the \( \alpha \)-tocopherol is markedly reduced [1, 29, 38]. In this study, the plasma vitamin A, E, C and \( \beta \)-carotene concentrations were found to be significantly lower in the puerperal metritis group than in the control group and at the individual scale, the respective antioxidant concentrations were depressed (below cut-off values) in 50%, 75%, 33% and 58% of diseased cows. In addition, associated decreases of vitamin E and C concentrations were observed in 25% of affected cows and associated decreases of vitamin A and \( \beta \)-carotene concentrations in 42%. The decrease of plasma chain breaking antioxidant concentrations may be induced firstly, by excessive utilization and intense consumption for neutralizing the ROS overproduction, and additionally by transient lack of appetite during the transition period and uterus inflammation reaction during puerperal metritis.

As a conclusion, this study has highlighted differences in oxidant / antioxidant balance as reflected by assessment of MDA concentrations, antioxidant enzymes, and chain breaking antioxidants between the cows with puerperal metritis and healthy controls. Results suggest that the antioxidant system is impaired and peroxidation reactions are accelerated in patients with puerperal metritis. Hence, it can be useful to add the antioxidant vitamins to the classical treatment procedures for get ride of the disease.

References


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