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Research Article

Evaluation of Some Antioxidant Enzymes in Cattle Infected with Foot and Mouth Virus

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ABSTRACT

The present study was conducted to evaluate the activities of superoxide dismutase, glutathione peroxidase, malondialdehyde, catalase and their correlation in cattle infected with foot and mouth virus (FMD). Forty cows were diagnosed and confirmed for FMD. Ten clinically healthy adult cattle were selected as control. Superoxide dismutase (SOD) glutathione peroxidase (GPx), malondialdehyde (MDA) and catalase (CAT) were measured by validated standard methods. SOD and GPx were significantly lower in the infected animals compared with the healthy group ($P<0.05$ in all cases). The mean concentration of malondialdehyde (MDA) was significantly higher in infected animals compared with healthy cows ($P<0.05$). There were no significant differences between the mean activities of catalase in control group compared with infected group. There were significant associations between (GPx) and catalase (CAT) in the control group. In the FMD group, significant associations were observed for MDA with catalase. The results of this study revealed that SOD, GPx decreased and MDA increased in FMD-infected cattle in response to viral infection. However, in the future more detailed future studies are required to characterize such responses and to improve the development of novel control strategies against FMD.

Key Words: Superoxide dismutase, glutathione peroxidase, malondialdehyde, catalase, Foot and mouth disease

ÖZET

ŞAP VİRÜSÜ İLE İNFEKTE SIĞIRLARDA BAZI ANTİOKSİDAN ENZİMLERİN DEĞERLENDİRİLMESİ

Bu çalışma şap virüsü (FMD) ile infekte sığırlarda süperoksit dismutaz, glutatyon peroksidaz, malondialdehit, katalaz aktivitelerini ve bunların bağıntılarını değerlendirmek için yapılmıştır. Kırk adet ineğin FMD yönünden tanısı ve konfirmasyonu yapıldı. Kontol grubu olarak on tane klinik olarak sağlıklı yetişkin inek belirlenmiştir. Süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), malondialdehit (MDA) ve katalaz (CAT) düzeyleri geçerli standart yöntemler ile ölçüldü. İnfekte hayvanlarda SOD ve GPx değerleri kontrol grubu ile karşılaştırıldığında ($P<0,05$ her vakada) anlamlı olarak düşük bulundu. İnfekte hayvanlarda malondialdehit (MDA)'in ortalama konsantrasyonu kontrol grubu ile karşılaştırıldığında ($P<0,05$) anlamlı olarak daha yüksek bulundu. İnfekte grubu ile kontrol grubu karşılaştırıldığında katalaz düzeylerinin ortalama değerleri arasında anlamlı bir değişiklik yoktu. Kontrol grubu içinde GPx ve katalaz (CAT) değerleri arasında anlamlı bir ilişki saptandı. İnfekte grubunda katalaz ile MDA değerleri arasında anlamlı bir ilişki gözlemlendi. Bu çalışmanın sonucunda, şap virüsü ile infekte sığırlarda viral infeksiyona

yanıt olarak SOD ve GPx değerlerinin azaldığı ve MDA değerlerinin arttığı belirlenmiştir. Ancak, gelecekte daha detaylı çalışmalar sayesinde bu gibi antioksidan enzimleri değişimlerinin karakterize edilmesi ve şap hastalığına karşı yeni kontrol stratejilerinin geliştirilmesi mümkün olacaktır.

Anahtar Kelimeler: Süperoksit dismutaz, glutatyon peroksidaz, malondialdehit, katalaz, Şap hastalığı

Introduction

Foot-and-mouth disease (FMD), an economical and a highly contagious acute vesicular disease of cloven-hoofed animals, in particular, cattle, sheep, goat and swine. The etiological agent, FMD virus, is associated with the Aphthovirus genus as a member of the Picornaviridae family (Belsham, 1993). Serologically, FMD virus can be divided into seven antigenically distinct serotypes: O, A, C, South African Territories (SAT) 1, SAT 2, SAT 3, and Asia1. There is no cross protection between serotypes immunologically. FMD virus infection produces a fairly acute pro-inflammatory reaction, which results in general depression/dullness, fever, vesicles in the mouth and on the muzzle, teats and feet, reduced feed intake, occasional inability to maintain body temperature, and even mortality. Persistent infection in ruminants, the so-called carrier state, is an important feature of FMD. This can occur in ruminants' convalescence from infection (Salt, 1993; 1998). These aspects of FMD although not well understood, are likely to result from virus–host interactions. This is an endemic disease in many countries in Asia, South America and Africa; nevertheless, an outbreak can induce high morbidity and mortality. In developing strategies to control the disease, it is essential to obtain a better perception of the virus–host interaction.

Antioxidants protect cells from the harmful effects of free radicals. Free radicals, molecules that contain an unshared electron, damage cells and may be a factor in the development of cardiovascular disease and cancer (Verhagen et al., 2006). Unshared electrons are particularly dynamic and react rapidly with oxygen to form reactive oxygen species (ROS). The body forms ROS endogenously when it converts food to energy, and it is also exposed to free radicals from environmental exposure, including cigarette smoke, air pollution, and ultraviolet radiation from the sun. ROS are part of signalling mechanisms among cells.

Selenium is an integral component of several selenoproteins including the glutathione peroxidases (GPx), which catalyze the reduction of harmful peroxides (Holben and Smith, 1999). Maintaining an optimum level of selenium and GPx is, therefore, important to protect the host from the development of diseases induced by oxidative damage such as cardiovascular disease (Salonen et al., 1982) and cancer (Combs and Gray, 1998).

There are no published reports relating to the changes of SOD, GPx, CAT and MDA in FMD-infected animals.

Therefore, the present study was conducted to evaluate the activities of SOD, GPx, CAT and MDA level in healthy cattle and cattle infected with FMDV. Furthermore, the correlation between the measured parameters was also investigated.

Materials and Methods

Animals

Forty cows from many dairy farms in Fars province of southern Iran with clinical signs of FMD were selected for the study. The presence of disease was assessed on the basis of clinical examination and laboratory findings. Ten clinically healthy adult cattle were selected as control group. A complete case history and owner complaints were recorded for each animal. All cows had a history of vaccination against brucellosis and anthrax about 5 months before the start of the study.

All samples were obtained before treatment began, in acute stage of disease. Barley, corn, and concentrates were added to the diet of dairy cows. Blood samples were collected from the jugular vein into two tubes with and without EDTA. The sera were separated by centrifugation at 750 g for 15 min and stored at

−20°C until used. The micro-serum neutralizing test described by Rweyemamu et al. (1978) was used to test serum samples for antibodies to FMD.

Measurements

Blood and Hemoglobin Preparation

The whole blood was centrifuged to remove plasma components. The packed red cells were washed three times in an isotonic saline solution (0.9% NaCl) and red cells were osmetically lysed with cold distilled water (2cc). Hemoglobin (Hb) was measured using cyanmethemoglobin method (Jain, 1986).

Superoxide dismutase (SOD) assay

SOD detection RANSOD kit (Randox lab, Crumlin, United Kingdom) was used to evaluate total SOD activity according to the manufacturer's instructions. The function of SOD is to hasten the dismutation of the toxic superoxide produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. In this methodxanthine and xanthine oxidase (XOD) are utilized to generate superoxide radicals which react with 2-(4-iodophenyl) -3 - (4-nitrophenol) -5-phenyltetra-zolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition in the rate of reduction of INT under the conditions of the assay. Using a standard curve SOD levels were recorded at 505 nm and were expressed as unit per gram of hemoglobin (U/g Hb).

Glutathione peroxidase (GPX) assay (GPx)

The activity of GPx was evaluated with GPx detection RANSEL kit (Randox lab. Crumlin United Kingdom) according to the manufacturer's instructions. GPx catalyze the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and nicotinamide adenine dinucleotid phosphate (NADPH), the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm against blank was measured spectrophotometrically. One unit (U) of GPX activity was defined as the amount of enzyme that converts 1 μmol of NADPH to NADP+ per minute. The GPX activity was expressed as unit per gram of hemoglobin (U/g).

Catalase (CAT) assay

Tissue catalase activity was assayed spectrophotometrically by monitoring the decomposition of H_2O_2 using the procedure of Aebi (1983). Briefly, 0.5 mL of 30 mmol/L H_2O_2 solution in 50 mmol/L phosphate buffer (pH= 7.0), 1 mL of 1:10 diluted erythrocyte lysates was added and the consumption of H_2O_2 was followed spectrophotometrically at 240 nm for 2 min at 25°C. The molar extinction coefficient was 43.6 L/mol per cm for H_2O_2. Catalase activity was expressed as the unit that is defined as μmol H_2O_2 consumed/min per gram hemoglobin.

Measurement of lipid peroxidation (MDA)

To evaluate lipid peroxidation in blood a modified HPLC method was used which is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a coloured MDA-TBA adduct (Lykkesfeldt, 2001). Briefly, 0.5 mL blood supernatant was added to 2 mL TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 mol/L HCl.The mixture was immediately heated (60 min at 95°C) and cooled with running water and thereafter butanol–pyridine (15:1, v/v) (1 mL) was added and the final volume was adjusted to 2 mL with distilled water. After vigorous mixing, the organic layer was separated by centrifugation (16 000 *g*, 3 minutes, at room temperature). The supernatant was analyzed on a UV-visible spectrophotometer fitted with an 80 μL flow cell (Hagar et al., 2006; Zal et al., 2007). The absorbance was measured at 532 nm (the mobile phase consisted of 300 mL/L methanol in 50 mM KH_2PO_4, pH: 7.0). 1, 1, 3, 3-tetraethoxypropane was used as a standard, and MDA-TBA reactive substances values were expressed as Unit per gram of hemoglobin (U/gHb). The HPLC system consisted of a

solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 mm × 4.6 mm, Phenomenex, CA, USA), and a UV–Vis detector (Jasco, UV-975, Tokyo, Japan) operated at 532 nm.

Statistical analysis

Descriptive statistics including mean, standard deviation and standard error mean were calculated for all variables. Data were evaluated for normality by Kolmogrov–Smirnov test. All variables did not show significant deviation from the normal distribution.

Association between studied variables was investigated using Pearson's and Spearman's correlations, and only statistically significant correlation coefficients were reported. Data were analyzed by SPSS software, version 11.5. P value <0.05 was considered as statistically significant.

Results

Descriptive statistics and results for comparison of diseased and healthy animals are presented in Table 1. As shown in the table, SOD and GPx were significantly lower in the diseased animals compared with the healthy group ($P<0.05$ in all cases). Mean MDA activity in the diseased group (5.06±0.66) was higher than the control group, significantly ($P<0.05$).

There was no significant difference between catalase activity in the diseased group in comparison to control group ($P>0.05$).

There was positive significant correlation between GPx and catalase ($r=0.732$, $P=0.016$) in the control group. In the FMD group, significant association was observed between MDA and catalase ($r=0.391$, $P=0.013$).

No statistically significant association was observed between the other variables.

Table 1. Summary statistics and comparison between parameters in FMD and control groups.

Tablo 1. FMD ve kontrol gruplarındaki parametreler arasındaki karşılaştırma ve istatistiksel özeti.

Mean ± SE							
Control group				FMD group			
MDA (nmol/gHb)	SOD (U/gHb)	GPX (U/g Hb)	CAT (U/gHb)	MDA (nmol/gHb)	SOD (U/gHb)	GPX (U/gHb)	CAT (U/gHb)
5.06± 0.1056	100.42± 3.2793	40.23± 1.7257	1833.80± 11.2997	4.00± 0.2291	125.08± 8.8803	55.26± 4.4071	1856.1± 21.4058

Discussion

In the present study SOD and GPx were significantly lower in the diseased animal compared with the healthy group ($P<0.05$), but the mean catalase activity showed no significant difference between the two studied groups. The results of this study shows FMD has oxidative stress effects that reduce SOD and GPx activities considerably, but the oxidative effect of this disease on catalase activity is low (It is possible that SOD and GPx are more sensitive than CAT, against oxidant substances). It seems FMD virus induces free oxygen radicals that cause enzymatic antioxidant usage, so it may be possible to reduce oxidative effects of this virus by antioxidant consumption. A number of studies indicate antioxidant compounds can increase individual defence, some of them are given below:

Selenium and α-tocopherol decrease the risk of suffering to prostatic neoplasm (Clark et al., 1996; Duffield-Lillico et al., 2002; Heinonen et al., 1998). Consumption of selenium, vitamin E, and beta carotene together decrease neoplasmic mortality (Blot et al., 1993).

Halliwell and Gutteridge (1999) described a number of defense mechanisms against reactive oxygen species in animals. Enzymes with

important antioxidant functions include: i) superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and iii) glutathione peroxidase (GPx), which facilitates the destruction of both hydrogen peroxide and organic peroxides. Reduced glutathione (GSH), a tri-peptide thiol, is both an important antioxidant and a co-factor for various antioxidant enzymes (Kidd, 1997). In this study considerable changes were observed in SOD but significant decrease in CAT activity was not observed. This may be due to the fact that SOD is the primary means of defense against ROS and is active in catalyzing detoxification of superoxide radical (Gonzales et al., 1984).The hydrogen peroxide generated in this reaction is returned to water in the presence of CAT and GPx. Perhaps FMD virus oxidative effects are not as extensive as the effect on CAT, which is considerable.

In the current study MDA level was increased in diseased animals considerably. This finding shows FMD increase levels of lipid peroxidation products. The main target substrates for oxygen radical activity is the polyunsaturated fatty acids that present in membrane phospholipids and result in disorganization of cell framework and function (Patterson and Leacke, 1998).

Lipid peroxidation is one indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA).

The quantization of MDA is commonly used as to indicate lipid peroxidation (Simsek et al., 2006). Increased levels of lipid peroxidation products like MDA have been reported in numerousdiseases including *Dicrocoelium dendriticum* infection in sheep (Simsek et al., 2006) and kidney diseases in dogs (Kargin and Fidanci, 2001). Superoxide radical and hydrogen peroxide have also been reported as a cause of brain injury (Kotos and Wel, 1986).

The antioxidant selenoenzyme, and glutathione peroxidase was found to be critically important in preventing viral mutations which could increase the viral pathogenicity (Beck, 2001).

Generation of free radicals is an primary feature of normal cellular function, while excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Reactive oxygen species (ROS) including superoxide radical, hydrogen peroxide and hydroxyl radical have a major impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen (Singh et al., 1999).

Conclusion

FMD virus affects antioxidant enzymes, and the reactive oxygen species (ROS) production may be one effect of this virus, so the usage of antioxidant agents can prevent the animals from FMD infection, but more research is necessary.

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