Praca oryginalna *Original paper*

Serum paraoxonase activity and total sialic acid in sheep with foot and mouth disease

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Received 22.05.2017 *Accepted 24.07.2017*

Deveci H. A., Kükürt A., Nur G., Alpay M., Merhan O., Bozukluhan K., Yilmaz V., Karapehlivan M.
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Summary

The aim of this study was to investigate paraoxonase (PON1) activity and total sialic acid to be measured for the first time in sheep infected naturally with foot and mouth disease, and their relationships with oxidative stress. A total of 30 Awassi sheep (aged between 2-4), which were healthy and infected with the foot and mouth disease virus (FMDV) were used in the study. Concentrations of paraoxonase activity (PON1), which is an important antioxidant against oxidative stress, high density lipoprotein (HDL) and total sialic acid (TSA), which has a critical role for immune system and is one of the significant indicators of cellular degeneration, were measured in serum samples drawn from animals. The total oxidant capacity (TOC) and total antioxidant capacity (TAC) were evaluated to determine the oxidative balance. It was observed that serum PON1 ($P < 0.001$) and HDL ($P < 0.01$) concentrations were significantly lower compared to the control group. TSA concentration was higher in the infected group ($P < 0.001$) compared to the healthy group. TOC was higher ($P < 0.001$) and TAC was lower ($P < 0.01$) in the infected group compared to the control group. Consequently, harmful effects of the infection of foot and mouth disease were shown as cause of oxidative cell damage and the high rate of sialic acid was produced depending on the degeneration during the infection.

Keywords: foot and mouth disease, paraoxonase activity, sialic acid, Awassi sheep

Foot and mouth disease (FMD) is a severe, highly contagious viral disease that affects cloven-hoofed domestic and wild ruminants (14). The disease caused by the Aphthovirus from the Picornaviridae family is characterized by high fever, lameness, and vesicular lesions in mouth, nose, tongue, feet, legs, and udders (14, 16). Cattle and sheep with FMD infection develop antibodies to structural (SP) and non-structural proteins (NSP). The presence of antibodies to NSPs indicate a previous FMD virus infection and animals with an unrecognized infection could represent a potential risk of FMD dissemination (2).

Although the disease has low mortality (2-5%), the mortality rate may increase (50-60%) depending on myocarditis in young and vulnerable animals. Even though there is plenty of information about the disease and the infecting virus, it is one of the leading diseases that are still frightening in a great part of the world and can spread effectively and rapidly (16, 31).

Paraoxonase (PON1) is a calcium-dependent ester hydrolase with a glycoprotein structure that possesses both arylesterase (E.C. 3.1.1.2) and paraoxonase (E.C.3.1.8.1) activity (8). PON1 is reported to have activity on HDL depending on apo A1 and to be protective against cellular oxidation of LDL (25). Free cysteine at position 284 is responsible for this antioxidant capacity of PON1 (3). As far as the autors know, there is no study on PON1 activity in sheep with FMD in the literature.

Sialic acid (SA) is an acetylated derivative of neuraminic acid. Because SA, an important structural component of biological membranes, is generally bound to glycoproteins, glycolipids, oligosaccharides, and polysaccharides, it is only free in the body in a small

amount (29). It is reported that sialic acid concentration increases rapidly following inflammation and injury processes (17). Previous studies have showed that SA concentrations increased during the course of numerous diseases (10, 33).

Intermediate metabolic products, called reactive oxygen species or free radicals, that cause oxidative damage in several tissues occur by reducing oxygen during biochemical reactions which are vital for living organisms. The resultant free radicals are inhibited by antioxidants (24). The total effect of all antioxidants found in plasma and body fluids reflects total antioxidant capacity (TAC); total effect of oxidants, on the other hand, reflects total oxidant capacity (TOC) (11, 12).

The aim of this study was to determine serum paraoxonase activity and also total sialic acid level, which is one of important indicators of inflammation and degeneration, in Awassi sheep naturally infected with foot and mouth disease virus, as well as their relationships with oxidative stress.

Material and methods

Experimental animals. A total of 30 adult Awassi sheep (aged 2-4 and without immunization against FMD virus), including 15 sheep with FMD and 15 healthy sheep, were used in this study. Serums were separated by centrifuging blood samples drawn from the vena jugularis of the animals. The samples were serologically tested for the presence of specific antibodies of the foot-and-mouth disease virus. Paraoxonase activity, high density lipoprotein, total sialic acid, total oxidant, and antioxidant levels were measured in serum samples.

Foot-and-mouth disease antibody test kit (FMD--3ABC bo-ov). Commercial ELISA kit (IDEXX Laboratories, Netherlands, FMD-3ABC bo-ov, Lieberfeld-bern Switzerland) was used to detect antibodies against FMDV in serum samples and the assay was carried out according to the manufacturer's instructions. The test detects antibodies against non-structural proteins of FMDV according to the following formula:

Value % = O.D samples – O.D negative ÷ O.D positive – O.D negative × 100

The value was considered positive if the OD of the sample was > 30; negative if it was < 20; and suspicious if it was between 20 and 30.

Biochemical assays. PON1 activity was measured according to the methods of Eckerson (9) and Gülcü and Gürsu (15). PON1 activity was determined by spectrophotometric measurement of absorbance at 25°C and 412 nm by color product yielded from 4-nitrophenol occurring as a result of enzymatic hydrolysis of paraoxonase (Sigma®) which was used as a substrate. For paraoxonase activity, the enzyme activity in 1 mL serum transforming 1 nmol paraoxonase into 4-nitrophenol in 1 min was identified as a unit and the results were given in U/L.

HDL was examined in autoanalyzer (Roche/Hitachi 917) using the Biotrol® commercial kit and the results were given in mg/dl.

TSA analysis was measured according to method of Sydow (32) at a spectrophotometer (PowerWave XS, BioTek®, USA) via colorimetric methods and the results were given in mg/dL.

TAC was measured by using the automatic measurement method of Erel (12) based on the decolorization of characteristic color, created by 2,2'azino-bis (3-etilbenzthiazoline-6-sulfonic acid) (ABTS) radical, via antioxidants in sample added into medium. The results were given in mmol Trolox equvalents/L.

TOC was measured by using the automatic measurement method of Erel (11). Oxidants in the sample transform ferrous ion complex into ferric ion (Fe^{3+}). Ferric ion (Fe^{3+}) occurring as a result of oxidation of iron (Fe^{2+}) into its more stabile form (Fe_2O_3) creates an orange color with xylenol in an acidic medium. The intensity of color measured spectrophotometrically was associated with total amount of oxidant molecules found in the sample. Measurement was calibrated with hydrogen peroxide (H_2O_2) and the results were given in micromolar H_2O_2 equivalent per liter (µmol H_2O_2 equiv./L).

Statistics analysis. The results were statistically evaluated by using SPSS packaged program (SPSS® 16.0, USA). The difference between the groups was determined by using student-*t* test and the results were expressed as a mean ± standard deviation ($\overline{x} \pm SD$).

Results and discussion

A commercial ELISA antibody test kit was used in this study to detect the presence of specific antibodies against the FMD virus in serum samples taken from 15 sheep clinically and macroscopic pathologically suspected of FMD infection. FMDV-specific antibodies were detected in all sheep in the study group. Animals which were healthy and did not have an antibody presence were used as the control group. Serum paraoxonase activity, high density lipoprotein, total sialic acid level, total oxidant and antioxidant capacity were examined in healthy (control) and FMD seropositive sheep. Serum PON1 activity ($P < 0.001$) and HDL concentration ($P < 0.01$) were significantly lower and TSA concentration ($P < 0.001$) was higher in infected sheep compared to the healthy group (Tab. 1).

It was determined that while TOC concentration was significantly higher in sheep infected by FMD virus compared to the control group ($P < 0.001$), TAC

Tab. 1. PON1 activity and HDL, TSA, TAC and TOC concentrations in control and FMD virus infected sheep

Parameters	Control (n = 15)	FMD (n = 15)	P value
PON1 (U/L)	197.12 ± 16.09	161.92 ± 16.15	< 0.001
HDL (mg/dL)	56.13 ± 6.71	51.34 ± 6.21	< 0.01
TSA (mg/dL)	68.23 ± 5.91	81.31 ± 9.72	< 0.001
TAC (mmol Trolox Eqv./L)	1.12 ± 0.09	0.92 ± 0.10	< 0.01
TOC (µmol H_2O_2 Eqv./L)	6.03 ± 0.79	10.24 ± 0.91	< 0.001

Explanations: FMD – foot and mouth disease; PON1 – paraoxonase activity; HDL – high density lipoprotein; TSA – total sialic acid; TAC – total antioxidant capacity; TOC – total oxidant capacity

concentration was lower in sheep infected by FMD virus compared to the control group ($P < 0.01$) (Tab. 1).

The condition of mouth lesions is one of the most important points focused on in sheep for the differential diagnosis of foot and mouth disease (35). In common with the academic studies revealing symptoms of FMD (1, 6), deterioration of the general condition, decreased feeding, saliva hanging in strings, and vesicular lesions in the mouth and between hoofs were determined in clinical examinations of the animals.

Free radicals continuously form in active sites of enzymes during enzymatic reactions for ordinary metabolic pathways in the cells and free oxygen radicals occur when free radicals infiltrate through the intermediate parts of enzymes and interact with molecular oxygen. Reactive oxygen species (ROS) are eliminated by mechanisms that are known as antioxidants in the organism. Free radicals and antioxidant mechanisms are balanced under physiological conditions. The increase of ROS causes cellular oxidative damage. In this case, inhibition of protein synthesis leads to occurrence of DNA damage and the formation of cellular death (7, 13, 23, 27, 28). In this study which was presented in parallel with results of Bozukluhan et al. (5), TOC was higher ($P < 0.001$) and TAC was lower in FMD group compared to control group. It was reported that nitric oxide (NO) with antimicrobial, anti-inflammatory, cell protective and cytotoxic functions was produced in extreme amounts in the organism in the case of a viral infection and this caused oxidative stress (36).

PON1 is an antioxidant enzyme that prevents lipid peroxidation of free radicals and metals and then the deformation of the structure of cell membranes and cell death. A decreased serum PON1 activity is assumed as an indicator of increased oxidative damage (19, 21, 22). In this study, PON1 activity in the FMD group was significantly lower in comparison to the control group ($P < 0.001$). Similarly, the HDL level (PON1 depends on this) also decreased ($P < 0.01$). The high TOC level and low TAC level indicated that the oxidative balance was disrupted and lipid peroxidation increased. Decreased PON1 activity may be associated with increased lipid peroxidation. This is because oxidized lipids were reported to inhibit PON1 activity (4).

Sialic acid (SA) is a 9-carbon acidic monosaccharide located in the terminal position of the glycan molecule found on a cell surface (18, 30). SA is reported to have a part in numerous physiological and pathological events, such as the attachment of pathogenic microorganisms, regulation of the immune system, and progression and metastasis of malignant cancers. They have important roles in the recognition of pathogens by acting as a ligant for selectin receptors revealed by leucocytes, platelets, and endothelium in immune systems and the formation of natural immunity (18, 34). In this study, TSA concentration was significantly higher ($P < 0.001$) in the FMD group as compared to the control. SA was reported to increase in a number of pathological conditions such as tissue damage, tissue proliferation or inflammation in humans and animals (20, 33). Under these circumstances, the increased TSA indicated that sialic acid was released from the cell membrane into blood circulation because it is abundant in all biological membranes (10, 26). The high sialic acid level found in this study could be associated with the fact that sialic acid was released from cell membrane glycoproteins due to oxidative cell damage.

In conclusion, it was observed in this study that serum PON1 activity, HDL, and TAC concentrations, which were measured for the first time in Awassi sheep with FMD, decreased; on the other hand, TSA and TOC concentrations increased. The authors believe that besides determining infectious factors by molecular diagnostic methods, supporting them with biochemical findings may contribute to both clinical diagnosis and literature.

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