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## Effect of *Corynebacterium cutis* Lysate on Serum Oxidative Stress and Plasma Prostaglandin F2 $\alpha$ Metabolite Levels

Ayşe Er<sup>1</sup>, Burak Dik<sup>1</sup> & Orhan Corum<sup>2</sup>

### ABSTRACT

**Background:** The *Corynebacterium cutis* lysate is commercial product. Unbalance between oxidants and antioxidants cause oxidative stress and lipid peroxidation in the cell. Macrophages phagocytose large pieces of bacteria and synthesize cytokines. In addition to the beneficial results of the drug have side effects. Since changes in biochemical parameters reflect structural dysfunction in the organism, monitoring changes of these parameters is a way to keep track of side effects. The aim of this study was to determine the effect of *Corynebacterium cutis* lysate on serum thiobarbituric acid-reactive substances (TBARS) and plasma 13,14-dihydro-15-keto-prostaglandinF2 $\alpha$  (PGM) levels in sheep.

**Materials, Methods & Results:** Six Merino crossbred ewes (aged >2 years, weight 40-60 kg) were used in this study. The procedures were approved by the Ethics Committee. A dose of 8 mg (0.4 mL) of commercial *Corynebacterium cutis* lysate was subcutaneously injected to each of the 6 Merino crossbred ewes. Blood specimens were taken from the sheep prior to injection (day 0, control) and after the injection on days 1, 2, 3, and 4. The levels of serum TBARS and plasma PGM were determined using an Enzyme Linked Immunosorbent Assay (ELISA) reader. The values of the hemogram [white blood cells (WBC), red blood cells (RBC), platelets (PLT), hematocrit (HTC), and hemoglobin (HBG)] were assessed using a blood cell count apparatus. The levels of plasma creatine kinase-MB (CK-MB), serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine, and cholesterol were determined on an autoanalyzer. The data obtained were analyzed using ANOVA and Scheffe's test as a post hoc test (SPSS 19.0). A  $P < 0.05$  value was taken as the cut-off value for statistical significance. An increase ( $P < 0.05$ ) in the levels of plasma PGM and serum cholesterol was detected when compared to the control samples, but there was no statistically significant ( $P > 0.05$ ) change in the other parameters.

**Discussion:** The *Corynebacterium cutis* lysate is a commercial product and used in cattle, newborn calves, sheep, and poultry as an immunostimulant against infections and to increase body resistance in times of stress. *Corynebacterium cutis* lysate increased ( $P < 0.05$ ) in plasma PGM and serum cholesterol levels compared to the control group. Detailed studies dealing with the effect of *Corynebacterium cutis* lysate on PGM and TBARS are not available in the literature. There is a balance between oxidants and antioxidants in the organism. Unbalance between oxidants and antioxidants caused by increased production of oxidizing species leads to oxidative stress and lipid peroxidation in the cells. The levels of TBARS or malondialdehyde are used in order to determine lipid peroxidation. The levels of serum TBARS, malondialdehyde and PGM increased in experimental infection models. Macrophages phagocytose large pieces of bacteria such as *Corynebacterium cutis* lysate and this case triggers the synthesis of cytokines by macrophages. Cholesterol metabolism may change in infections, and high levels of cholesterol were determined in test subjects after injection of LPS. Lipid metabolism may be affected by stimulants of the immune system, such as *Corynebacterium cutis* lysate. In conclusion, *Corynebacterium cutis* lysate has no effect on the oxidative status and number of blood cells and organ (heart, liver and kidney) damage markers in sheep and it may increase plasma PGM level by stimulating the immune system.

**Keywords:** *Corynebacterium cutis*, oxidative stress, 13,14-dihydro-15-keto-prostaglandinF2 $\alpha$ .

INTRODUCTION

*Corynebacterium cutis* lysate (CCL) is a commercial product that increases the nonspecific immune response. It is used in cattle, newborn calves, sheep, and poultry as an immunostimulant against infections and to increase body resistance in times of stress [20].

In the organism, there is a balance between oxidants and antioxidants. Unbalance caused by increased production of oxidizing species leads to oxidative stress and lipid peroxidation in the cells. The products of immune reactions in the organism are among the endogenous oxidants. In order to determine lipid peroxidation, the levels of thiobarbituric acid-reactive substances (TBARS) or malondialdehyde are measured [14]. Large pieces of bacteria such as CCL are readily phagocytosed by macrophages. This event triggers the synthesis of cytokines by macrophages as the first inflammatory response of the organism. Following the application of CCL, there is an increase in the secretion of tumor necrosis factor, interleukin-1, and interleukin-6. It is predicted that with increased immune response, mortality due to viral and bacterial infections may decrease [28]. Therapeutics also have side effects along with their beneficial consequences. Monitoring changes in the blood picture and/or enzyme levels in plasma/serum is a way to keep track of side effects, since the change in biochemical parameters is accepted as a messenger of structural dysfunction in the organism [4,9].

The main purpose of this study was to determine the effect of CCL on the levels of two inflammation mediators, serum TBARS and plasma PGM. An additional goal of the study was to evaluate the effect of CCL on hemogram and routine biochemical parameters.

MATERIALS AND METHODS

*Animals and experimental design*

Six Merino crossbred ewes were used (aged >2 years, weight 40-60 kg). Each sheep was subcutaneously injected with a single dose of 8 mg (0.4 mL) of *Corynebacterium cutis* lysate<sup>1</sup> as recommended by the manufacturer. Prior to the injection (day 0, control) and on days 1, 2, 3, and 4 following the injection, blood specimens were taken from the vena jugularis of each sheep. Plasma and serum were separated from each blood specimen. The serum levels of TBARS<sup>2</sup> and plasma PGM<sup>3</sup> were determined by using an ELISA reader<sup>4</sup>. Blood hemogram parameters (white blood cells, red blood cells, platelets, hematocrit, and hemoglobin) were determined by using a blood cell count apparatus<sup>5</sup>. The levels of plasma CK-MB, serum ALP, ALT, AST, GGT, total protein, albumin, BUN, creatinine, and cholesterol were measured on an autoanalyzer<sup>6</sup>.

*Statistical analysis*

The data obtained were analyzed using ANOVA and Scheffe's test as a post hoc test (SPSS 19.0). A *P* < 0.05 value was taken as the cut-off value for statistical significance.

RESULTS

The effect of CCL on serum TBARS and plasma PGM levels, hemogram values, and biochemical parameters are shown in Tables 1, 2, and 3, respectively. On day 4, following the injection of CCL, the levels of plasma PGM (Table 1) and serum cholesterol (Table 3) were found to be significantly higher (*P* < 0.05) than those on day 0 (control). No statistical difference was observed (*P* > 0.05) in other parameters.

**Table 1.** Effect of *Corynebacterium cutis* lysate on serum thiobarbituric acid reactive substances (TBARS) and plasma 13,14-dihydro-15-keto-prostaglandinF2 $\alpha$  (PGM) levels (mean  $\pm$  SE).

Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
TBARS ( $\mu$ M)	2.31 $\pm$ 0.48	2.64 $\pm$ 0.54	2.03 $\pm$ 0.24	1.35 $\pm$ 0.22	2.26 $\pm$ 0.74
PGM (ng/mL)	0.86 $\pm$ 0.16 <sup>b</sup>	0.42 $\pm$ 0.13 <sup>b</sup>	0.60 $\pm$ 0.21 <sup>b</sup>	0.72 $\pm$ 0.26 <sup>b</sup>	15.8 $\pm$ 1.19 <sup>a</sup>

<sup>a,b</sup>Different letters in the same line indicate statistically significant differences (*P* < 0.05).

**Table 2.** Effect of *Corynebacterium cutis* lysate on hemogram values (mean  $\pm$  SE).

Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
WBC ( $\times 10^9/L$ )	10.5 $\pm$ 1.44	10.6 $\pm$ 1.19	11.6 $\pm$ 0.99	11.8 $\pm$ 1.04	11.1 $\pm$ 0.77
RBC ( $\times 10^{12}/L$ )	8.37 $\pm$ 0.60	8.35 $\pm$ 0.82	9.00 $\pm$ 0.76	7.88 $\pm$ 0.57	8.03 $\pm$ 0.87
PLT ( $\times 10^9/L$ )	513 $\pm$ 51.5	438 $\pm$ 27.2	416 $\pm$ 85.3	576 $\pm$ 40.7	528 $\pm$ 90.9
HTC %	29.6 $\pm$ 2.04	29.4 $\pm$ 2.45	31.6 $\pm$ 2.11	28.2 $\pm$ 1.92	28.6 $\pm$ 3.30
HBG (g/L)	75.6 $\pm$ 10.2	85.3 $\pm$ 8.30	93.3 $\pm$ 8.09	84.6 $\pm$ 6.78	86.3 $\pm$ 10.1

WBC: white blood cell; RBC: red blood cell; PLT: platelet; HTC: hematocrit; HBG: hemoglobin. There was no statistical significance in the same line ( $P > 0.05$ ).

**Table 3.** Effect of *Corynebacterium cutis* lysate on biochemical parameters (mean  $\pm$  SE).

Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
CK-MB (U/L)	138 $\pm$ 17.1	128 $\pm$ 20.6	122 $\pm$ 17.5	117 $\pm$ 15.3	110 $\pm$ 12.9
ALP (U/L)	71.1 $\pm$ 21.2	41.6 $\pm$ 10.8	57.5 $\pm$ 20.5	49.5 $\pm$ 16.6	40.8 $\pm$ 14.2
ALT (U/L)	13.0 $\pm$ 2.94	15.5 $\pm$ 1.83	12.6 $\pm$ 2.80	12.3 $\pm$ 2.56	12.3 $\pm$ 2.67
AST (U/L)	63.6 $\pm$ 7.70	58.3 $\pm$ 7.42	74.3 $\pm$ 17.7	68.1 $\pm$ 14.7	64.5 $\pm$ 15.8
GGT (U/L)	62.1 $\pm$ 7.40	54.6 $\pm$ 4.92	47.5 $\pm$ 6.32	44.6 $\pm$ 4.89	37.0 $\pm$ 4.35
TP (g/dL)	7.31 $\pm$ 0.42	7.58 $\pm$ 0.47	7.83 $\pm$ 0.36	7.76 $\pm$ 0.32	7.66 $\pm$ 0.39
ALB (g/dL)	2.91 $\pm$ 0.18	2.91 $\pm$ 0.20	2.85 $\pm$ 0.24	2.80 $\pm$ 0.18	2.78 $\pm$ 0.20
CREA(mg/dL)	0.62 $\pm$ 0.03	0.65 $\pm$ 0.06	0.62 $\pm$ 0.04	0.63 $\pm$ 0.06	0.55 $\pm$ 0.04
BUN(mg/dL)	25.1 $\pm$ 4.16	19.6 $\pm$ 3.27	15.0 $\pm$ 3.29	18.8 $\pm$ 3.40	17.1 $\pm$ 2.68
CHOL(mg/dL)	64.1 $\pm$ 4.17 <sup>b</sup>	66.3 $\pm$ 4.76 <sup>b</sup>	68.8 $\pm$ 4.15 <sup>b</sup>	65.0 $\pm$ 4.41 <sup>b</sup>	107 $\pm$ 15.1 <sup>a</sup>

ALB: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CHOL: cholesterol; CK-MB: creatine kinase-MB; CREA: creatinine, GGT: gamma glutamyltransferase; TP: total protein; BUN: blood urea nitrogen. <sup>a,b</sup>Different letters in the same line indicate statistically significant differences ( $P < 0.05$ ).

### DISCUSSION

It has been reported that CCL may support the immune system in cattle with mastitis, and that the use of CCL in pregnant sheep and cattle leads to high birth weight and low birth fatalities. It has also been reported that when CCL is applied concomitantly with a vaccine, it stimulates the immune response, resulting in high titers of antibody, and that it increases resistance in calves against viral diseases, thus decreasing fatality rates [2,3,17,21,22,29]. Although commercial CCL is used as an ancillary drug in the treatment of various infectious diseases [22], there are no studies in the literature detailing the effect of CCL on PGM and TBARS.

Infections or application of lipopolysaccharides, cell-wall components of gram-negative bacteria

used in experimental infection models, may cause increased production of free oxygen radicals leading to oxidative damage [18,26]. In experimental infection models, an increase was observed in the levels of serum TBARS [16], malondialdehyde (another marker of oxidative stress), and PGM [26]. However, in the present study no significant change was found ( $P > 0.05$ ) in the level of serum TBARS after injection of CCL (Table 1). This result shows that CCL injected at the given dose may not cause oxidative stress.

In organisms challenged by bacterial components such as lipopolysaccharides, the production of prostaglandins and cytokines is also stimulated [5]. Prostaglandins (F, E, G, etc.) are synthesized from a fatty acid, arachidonic acid, by the mediation of the

enzyme cyclooxygenase. Different prostaglandins have different physiological functions in the organism. Nonsteroid anti-inflammatory drugs show their main effect by inhibiting prostaglandin synthesis [10]. It has been reported that 13,14-dihydro-15-keto-prostaglandin F2 $\alpha$  (PGM), a metabolite of prostaglandin F2 $\alpha$ , increases in inflammatory processes, and thus it may be used as a marker of inflammation [1,5]. On day 4, the level of PGM was markedly increased ( $P < 0.05$ ) when compared with that of the control (Table 1). Large bacterial fragments, such as CCL, used to stimulate the immune system, readily stimulate macrophages. As response to the stimulus, the activated macrophages synthesize cytokines [28]. Experimental studies showed that after injection of lipopolysaccharides into the organism, blood PGM and cytokine levels increase simultaneously together [6,7]. Furthermore, it has been reported that prostaglandin synthesis increases in natural infections [8] and that the PGM level is positively correlated with the degree of infection [15]. A study suggested that, since the level of PGM increased in test subjects following the injection of lipopolysaccharides, PGM assessment could be used as a marker in the diagnosis of endometritis [19]. In this study, after CCL treatment, increased level of the inflammation mediator PGM could be due to the stimulation of the immune system.

Dysfunction in bone marrow, some chronic diseases and infections cause changes in the blood picture. The routine examination of serum biochemical parameters can give information on organ functions and metabolism. Increased serum levels of creatine kinase-MB (CK-MB) is a marker of cardiac damage, increased serum alkaline phosphatase (ALP) is a marker of bile duct disorders, and increased serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) indicate hepatic cellular damage. The levels of serum total protein and albumin can give information about hepatic synthesis. The levels of blood urea nitrogen

(BUN) and creatinine are used as markers for renal function. Cholesterol gives information about lipid metabolism in the organism [11,12,23,24,25]. The level of serum cholesterol on day 4 was found to be significantly higher ( $P < 0.05$ ) than on other days (Table 3). Cholesterol metabolism may change during infections [13], and high levels of cholesterol were observed in test subjects after injection of LPS [6,27]. Stimulants of the immune system, such as CCL, may also affect lipid metabolism.

The use of CCL did not have an effect ( $P > 0.05$ ) on hemogram parameters (Table 2) nor on markers of blood, cardiac, hepatic, and renal damage (Table 3). These results indicate that the use of CCL has no effect on the number of blood cells and, hence, that it is presumably safe for the heart, liver, and kidneys.

In conclusion, the use of CCL does not cause oxidative stress and may promote plasma PGM synthesis by stimulating the immune system.

#### SOURCES AND MANUFACTURERS

<sup>1</sup>Corynebacterium cutis lysate-Ultra-corn® Inj. Susp. Virbac Animal Healthy, France.

<sup>2</sup>Cayman Chemical Company, ELISA Kit, USA.

<sup>3</sup>13,14-dihydro-15-keto prostaglandin F2 $\alpha$  EIA Kit Cayman Chemical Company, USA.

<sup>4</sup>MWgt Lambda Scan 200, Bio-Tek Instruments, USA.

<sup>5</sup>Shenzhen Mindray Bio-Medical Electronics, BC-2800 Auto Hematology Analyzer, China.

<sup>6</sup>ILab-300 BioMérieux Diagnostics, Milan, Italy.

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**Ethical approval.** The procedures were approved by the Ethics Committee of the Veterinary Faculty, Selcuk University (Approval No: 2013/047).

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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