Effects of Intramammary Infusion of *Escherichia coli* Endotoxin in Lactating She-camel Udder

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Abstract :

Intramammary infusion of 10ug *E. Coli* lipopolysacharide endotoxin to lactating camels has increased blood neutrophils and milk polymorphnurclear cells, California mastitis test score and N-Cetyl-B-D-glucosaminidase activity. The infusion of the endotoxin had also increased the level of lactoferrin in milk . It is suggested that administration of lactoferrin could be of value in the treatment of mastitis.

Keywords :

Camel, endotoxin, *E. coli* , udder, lactoferrin, polymorphnuclear cells .

Introduction

Recent experimental findings indicate that endotoxin interacts with specific membrane receptors localized on mononuclear phagocytic cells and neutrophils (Olson *et al* 1995). Binding of endotoxin to these cells together with endotoxin – induced activation of host vascular endothelium, initiates a series of signal transudation events that culminate in relase of numerous biochemical mediators (Retz 1993). Endotoxaemia and Gram – negative septicaemia are important clinical entities in animals (Brigham and Meyrick, 1986; Ziegler *et al* (1991).

Indeed, introduction of endotoxin into the circulation appears to be a key factor initiating the pathophysiology associated with clinical shock during experimental endotoxaemia (Morrison and Ryan, 1987; Naess *et al*. 1989). There are marked species differences both in the sensitivity of animals to endotoxin and the dose of endotoxin required to achieve 100% lethality (Schrauwen and Houvenaghel 1985). Recently, it was demonstrated (AL-Dughaym, 2004) that intravenous administration of endotoxin prepared form *E. coli* (serotype 055:B5, Sigma Chemicals,

U.K.) at a dose of 0.1 µg/kg body weight to calves and adult camels induced fever and increased haematocrit, triiodothyronine and cortisol values. The endotoxin – treated animals showed significantly decreased ($P<0.05$) total protein, urea, glucose and creatinine. A significant increase was seen in the activity of aspartate amino transaminase and creatinekinase . These results demonstrated a high sensitivity of camels to *E.coli* endotoxin.

Mastitis is the most costly infectious disease in modern dairy farms. In addition to the expenses for treatment and discarded milk , income is lost because inflammatory damage to the affected mammary gland may temporarily or permanently reduce milk production . Limiting inflammation in the affected mammary gland during a clinical episode of mastitis may also limit damage to the gland and preserve milk production.

Changes in the number and type of cells in milk occur during mastitis (Guidry *et al*., 1983). Characterization of cells and estimation of lactoferrin in milk is an important step in understanding the defensive mechanism and changes during physiological and pathological states of the udder of dairy animals. Lactoferrin an iron-binding glycoprotein in milk, serves as part of the defence system (Baggioloni *et al*., 1970) Milk from clinically healthy bacterian camels contain few cells and numerous cell-like structures characterized by complete absence of nuclei but with intact cytoplasmic organelles (Abdurahman *et al*., 1992.)

The objective of this study was to investigate the effect of *E. coli* endotoxin infused into the camel udder and monitor the pattern of changes in lactoferrin concentration and cell populations in milk and blood .

Materials and Methods :

Animals : Ten clinically healthy female camels aged 6-7 years were used. Animals were fed on barley and wheat straw and water ad libitum The camels were in their 3-4 month of lactation and the calves were separated from their dams. The camels were sedated with xylazine (Img/kg; Rumpun, Bayer, Germany) before endotoxin infusion.

Endotoxin infusion : Five camels were used for endotoxin infusion. Two posterior quarters of the udder were infused through the teat canal with a

dose of 10ug purified *E.coli* lipopolysacharide (055:B5, Sigma Chemicals UK) endotoxin in 3 ml of sterile isotonic saline solution (0.9% Nacl). The remaining five camels were used as control and the posterior quarters were infused with 3 ml of isotonic saline only. The udders were then massaged to ensure maximum distribution of endotoxin and saline .

Sample Collection : Oxytocin was administered to help milk let down. Quarter milk samples were collected before the endotoxin infusion (0 hour) and at post-infusion hours 3,6,22 and 24. Blood was obtained by jugular veinpuncture in vaccutainer tubes (Becton Dickson, Meulan, France) with sodium heparin as anticoagulant .

Clinical examination : The udder quarters were inspected and palpated and rectal temperature was taken at each sampling occasion .

Bacteriological examination : Milk samples (0.01 ml) from each quarter were streaked on blood agar plates and incubated for 48 hours at 37o C. The plates were examined for colony growth and morphology.

Milk somatic cell count : Quarter milk samples (0.01 ml) were spread over an area of l cm2 of a glass slide using a microsyringe. Four such squares were prepared form each sample. The smears were stained with methylene blue and examined under the microscope. Total cells/ml were counted according to Prescott and Breed (1990), and proportions of cells and cell fragments are estimated by counting 200 cells or particles .

Total and differential leukocyte count : The total blood leukocyte count was determined using Neubar haemocytometer after dilution . Blood was smeared on glass slides, air-dried and stained with Giemsa. And differential leukocyte count was made on 200 white blood cells .

California mastitis test (CMT) and milk N-acetyl-B-D-glucosaminidase (NAGase) activity determination:

The CMT and NAGse activity in milk were were determined and used as indicators of inflammation in the infused udder. The NAGase activity was analyzed using commercial kit (Roche Products, Herts, UK) based on the fluorgenic method of Kitchen *et al* (1978).

Lactoferrin determination in milk :

Lactoferrin concentration in milk samples was determined using dissociation-enhanced lanthanide fluoroimmunoassay (Kawai *et al*., 1999) as a comparative reaction and measured in a fluorometry (Biowhiltakar, MD, USA)

Statistical analysis:

Values were compared using student test. The probability value $P<0.05$ was considered significant .

Results :

The maximum increase in the rectal temperature of camels infused with endotoxin attained was 2.3^{o}C above control animals at 4 h post-infusion. No major pathogenic organisms were found in milk. Slight swelling of the teats was observed in endotoxin-treated animals .

A significant ($P<0.05$) increase in polymorph nuclear lenkocytes was observed in milk of endotoxin-treated camels compared to control camels (Table 1). Blood neutrophils were significantly ($P<0.05$) increased in endotoxin-treated camels (Table 2). The other cell types of the leukocytes series did not show any significant impressive change .

The milk NAGase activity has significantly ($P<0.05$) increased after endotoxin infusion . The highest value attained being 110 units/ml.

The CMT score has also significantly ($P<0.05$) increased 3 hours post infusion (Table 3).

The mean lactoferrin concentrations in milk is shown in (Table 4). Lactoferrin showed significant increase ($P<0.05$) at 3 hours post inoculation in endotoxin-treated camels and remained elevated reaching a level of 3.5 g/L at 24 hours post- inoculation . Basal levels were in the range of 0.12-0.15g/L .

Table (1)

Mean (± SD) Percentage of leukocytes in milk of endotoxin or saline treated camels

Time (hours)	PMNC %		MNC %		CF %	
	C	E	C	E	C	E
0	2.2 ± 0.2	2.1 ± 2.2	55. ± 3	57 ± 4	43.3. ± 2.1	41.2 ± 2.1
3	2.3 ± 0.2	21.6 ± 1.2*	60.2 ± 2.1	62.1 ± 2.3	37.5 ± 2.1	17.3 ± 0.6*
6	4.4 ± 0.3	68.3 ± 2.6*	26.1 ± 1.3	26.6 ± 1.4	69.5 ± 2.1	5.1 ± 0.2*
24	4.3 ± 0.2	78.3 ± 3.3*	30.2 ± 1.2	20.3 ± 1.3*	65.5 ± 2.1	1.4 ± 0.1*

C = Control (Saline treated) E= Endotoxin treated PMNC= Polymorphnuclear cells ,
MNC= Mononuclear cells ,
CF=Cell fragments ,
*P<0.05 significantly different from controls.

Table (2)

Mean (± SD) differential leukocyte counts in endotoxin treated camels.

Type of cells% Total	Time hours			
	0	3	6	24
WBC ($x10^a$/L)	22 ± 1	15.4 ± 1.2	13.4 ± 1.3	16.1 ± 0.2
Lymphocyte	20.1 ± 1.1	24.2 ± 1.6	25.4 ± 1.6	10.4 ± 0.6
Neutiophils	65.1 ± 2.1	64.9 ± 1.9	71.2 ± 2.1	80.1 ± 2.2
Eosinophils	6.8 ± 0.4	4.3	3.4	4.1 ± 0.6
Basophils	4.6 ± 0.3	3.2 ± 0.2	2.1	2.6 ± 0.8
Monocytes	3.4 ± 0.4	3.4 ± 0.3	2.3	2.8 ± 0.4

*P<0.05 , Significantly different from Control**s**

Table (3)

Mean (± SD) Lactoferrin concentrations in milk of endotoxin or control saline-treated camels

Time (hours)	Lactoferrin (g/L)	
	Control	Endotoxin treated
0	0.12 ± 0.05	0.13 ± 0.04
3	0.15 ± 0.04	0.9 ± 0.11 *
6	0.14 ± 0.03	2.1 ± 0.12 *
24	0.12 ± 0.04	3.5 ± 0.21 *

* P<0.05 , Significantly different from Controls .

Table (4)

Mean (± SD) California mastitis test (CMT) score and NAGase activity in milk of endotoxin – treated camels

Time (hours)	CMT		NAGase (Units/ml)	
	C	E	C	E
0	1.01 ± 0.1	1.1 ± 0.1	12.1 ± 2.6	10.6 ± 2.1
3	1.1 ± 0.1	2.2 ± 0.2 *	11.2 ± 2.1	24.1 ± 2.4 *
6	1.1 ± 0.1	3.6 ± 0.3 *	11.6 ± 1.6	84.6 ± 4.6 *
24	1.1 ± 0.1	4.5 ± 0.4 *	10.1 ± 2.1	110 ± 6 *

C = control
E = endotoxin-treated
* P<0.001 Significantly different from control

Discussion:

Intramammary infusion of *E.coli* endotoxin in the udder of lactating camels has produced fever and slight swelling of the teats.

The increase in the concentration of the cellular component of milk of endotoxin treated animals such as polymorphnuclear cells indicated that endotoxin infusion into camel udder provoked leukocyte migration into milk. Similar pattern of response was observed in cows (Jain et al; 1978), goats (Lengemann and Pitzrick, 1987) and guinea pig (Mckenzie and Anderson, 1981) Furthermore, the increase in CMT score and NAGase activity indicated inflammatory reaction of udder to endotoxin infusion.

Lactoferrin in milk of endotoxin treated camels has significantly increased over the control camels reaching a level of 3.5 g/L at 24 hours post-infusion. Lactoferrin concentrations in milk from dairy cows with clinical mastitis has been reported to be 0.3-2.3 g/L (Harmon *et al* 1975, Kawai *et al* 1999). Such levels might be due to the severity of infection since in subclinical mastitis lower levels 0.2-1.2g/L were reported (Kawai *et al* 1999). Lactoferrin has been reported to be secreted by neutrophiles (Harmon and Newbould, 1980). It has been shown to be bacteriostatic for a variety of microorganisms in vitro as well as in vivo (Oram & Reiter. 1968: Reiter. 1985: Zagulski *et al.,* 1989: Bhimani *et al*., 1999). The inhibitory activity is believed to result from the iron chelating ability of lactoferrin making iron unavailable to bacteria (Weinberg. 1984). In addition, specific portions of lactoferrin molecules are directly toxic to

bacteria, yeasts and moulds. The highly cationic bactericidal domain of lactoferrin located at the N-terminus of the protein increases bacterial cell membrane permeability and causes the release of lipoplysaccharides from Gram-negative bacteria (Ellison *et al*., 1988; Bellamy *et al*., 1992; Yamauchi *et al*., 1993). Besides bacteriostatic and bactericidal activities, lactoferrin may also exert antimicrobial activity through regulation of systemic immune responses by activated neutrophils . High-affinity Lactoferrin receptors have been identified on several cell types including lymphocytes, monocytes, macrophages, enterocytes and platelets (Brock, 1995). Furthermore lactoferrin has been found to affect production of antibody synthesis, production of interleukin (I L)-1, I L-2 and TNF-α, natural killer cell cytotoxicity, complement activation and lymphocyte proliferation (Sanchez *et al*; 1992, Brock, 1995) .

Antimicrobial agents are, however still used to treat mastitis despite lack of evidence for their therapeutic value (Jones and Word 1990, Erskine *et al*, 1991; Pyorala *et al*, 1994). If exogenous administration of lactroferrin could support clearance of bacteria from the udder, then laetoferrin could be of value in the treatment of mastitis. Such efficacy of lactoferrin needs to be further investigated in the camel and other dairy animals .

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تأثير حقن سموم الاندوتكسن في ضرع الجمال المرضعة

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الملخص :

لقد تسبب حقن ١٠ ميكروجرام من سموم الاندوتكسين لبكتيريا الاشريكا القولونية في ضرع الجمال المرضعه في زيادة خلايا النتروفيل في الدم وزيادة الخلايا المتعددة الانوية وكاشف التهاب الضرع وانزيم الاستايل جلكوز اميتريز واللاكتوفرين في الحليب . لقد اقترح بأنه ربما يكون حقن اللاكنوفرين ذا قيمة في علاج التهاب الضرع .