

Some immuno-suppressive trends: Effects Of Endotoxin on Camels (*Camelus dromedarius*)

A. M. Al-Dughaym and A. M. Homeida*

Dept. of Microbiology and Parasitology, College of Veterinary Medicine and Camel Research Center*,
P. O. Box: 1757, Al-Ahsa, 31982, King Faisal University, Saudi Arabia.

Abstract

Intravenous administration of a bolus of 0.05 µg/kg body weight endotoxin lipopolysaccharide (LPS) to camels has resulted in leukopenia, lymphopenia, neutropenia, proteinaemia, albunaemia, globulinaemia and reduced lysosomal enzyme activity. It is suggested that endotoxin at the dose of 0.05µg/kg body weight may produce immunosuppressive effects in camels.

Introduction

Typically endotoxin lipopolysaccharide is an extremely active biological agent, however, its activity is dependent on many factors, including bacterium of origin and hence chemical structure, hydrophobicity, solubility and sensitivity of the most animals (Berczi *et al* 1966, Schrauwen and Houvenaghel, 1985)

Recently, it was demonstrated (AL-Dughaym 2004) that intravenous administration of endotoxin lipopolysaccharide prepared from *E. coli* (serotype 055:B5) 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 total protein, urea, glucose and creatinine. A significant increase was seen in the activity of aspartat amino transaminase and creatinekinase. These results domenstrate a high sensitivity of camel to endotoxin.

Recent experimental findings indicate that endotoxin interacts with specific membrane receptors localized on mononuclear phagocytic cells and neutrophils biochemical mediators (Raetz 1993). This study was designed to investigate the effect of injection of small doses of endotoxin on some immune parameters in serum of camels.

Materials and Methods

Animals and treatments

Ten small camels (3-4 years old, 250-300 kg body weight) were used in the study. Animals were kept in an open yard with free access to feed and water. Animals were divided randomly into two equal groups: Group A animals were treated with saline and kept as controls, Group B animals were injected with endotoxin.

Endotoxin lipopolysaccharide (*E. coli* serotype 055:B5) was obtained from Sigma chemicals, UK and administered intravenously using methods of (AL-Dughaym 2004). The dose of 0.1 µg/kg bodyweight was considered lethal to the camel (AL-Dughaym 2004), therefore a bolus of 0.05 µg/kg was given to them. Blood samples were collected weekly for 10 days into heparinized or plain tubes. Heparinized blood was used for leukocyte series determination. Blood in plain tubes was used to prepare serum which was stored at 30°C until analysis.

White blood cell count was determined by a Counter Model B1 Counter (Coulter Electronics, Hialeah, FL, USA). The differential Leukocyte counts were carried out using blood smears stained with Giemsa and May-Gunwald solution. At least 200 cells were counted. Serum

protein and albumin concentrations were determined by refractometer (Bellman and Stanly Ltd, UK). Serum lysozyme concentrations were measured using *Micrococcus lysodieticus* as a substrate (lysozyme reagent kit, Worthington Biochemical, Co. Freehold, NJ) according to the manufacturer's recommendations (AL- Ankari and Homeida 1996). The percentage changes in transmission (at 510 nm) per min. were immediately recorded using a spectrophotometer (Hitachi, Japan). The values were compared to a standard curve simultaneously prepared using a known concentration of egg white lysozyme.

The data thus obtained was statistically analyzed using unpaired student test.

Results

Results of the effect of endotoxin on serum concentrations of proteins, lysozyme and counts of leukocytes series in camels are summarized in Table (1). A significant $p < 0.05$, decreased in serum protein, albumin and globulin have occurred in group B animals on the 5th day of endotoxin injection.

A significant ($p < 0.05$) reduction has also occurred in group B animals in leukocyte, neutrophil and lymphocyte count on the first, 5th and 10th day of endotoxin administration compared to control group A animals. Significant ($p < 0.05$) reduction in the activity of lysozyme enzymes also occurred on days 1, 5 and 10 of endotoxin injection in group B animals compared to group A animals. Saline injection in group A animals did not cause any changes in the estimated parameters.

Discussion

The serum protein got reduced in endotoxin treated animals. Serum albumin and globulin closely followed

the pattern of total protein due to impairment of liver or poor nutritional intake (Kaneko *et al*, 1997) as a result of endotoxin administration.

Earlier, Nagaraja *et al*, (1979) observed hypoproteinemia on *E. coli* endotoxin infusion in cow's calves while Southern and Thompson (1986) also reported a significant decrease in plasma total amino acids after *E. coli* endotoxin administration. Recently, Singh *et al* (2004), reported hypoproteinaemia and hypoalbuminaemia in cows infused with 5 µg/kg body weight endotoxin. However, the rising in total protein following endotoxin infusion has been reported by other investigators (Olsen and Brown 1986). These authors estimated the protein contents 3 hours following endotoxin injection and attributed that result to decrease in plasma volume.

Leukopenia, neutropenia and lymphopenia were observed shortly after endotoxin administration. Endotoxin caused an immediate accumulation, margination and activation of leukocytes in the microcirculation, particularly in the alveolar capillaries (Myerick and Brigham 1983, Deldar *et al* 1984). Lymphopenia has been attributed to release of endogenous corticosteroid (Deldar *et al* 1984, AL-Dughaym 2004), where as neutropenia is thought to result from the pulmonary sequestration of neutrophils (Warner *et al* 1988).

Serum lysozyme activity was significantly decreased in camels treated with endotoxin. Serum lysozyme activity is considered to be an index of macrophage function (Currie and Eccles, 1976). Similar studies showed that suppression of macrophage activity with methyl palmitate was associated with reduction of lysozyme enzyme release in serum (Koskoshis and Diluzio 1979).

Furthermore, endotoxin was able to reduce endotoxin lysozomal enzymes in domestic fowl (Bottler *et al* 1977).

Table 1. Effects of endotoxin on serum concentration of proteins, lysosome and counts of leukocytic series in camels.

Variable	Saline treated (Group 1) (N=5)	Endotoxin Treated (Group 2) (N=5)		
		Day 1	Day 5	Day 10
Total protein (g/dl)	7.51 ± 0.13	6.31 ± 0.13	5.4 ± 0.12*	7.1
Albumin (g/dl)	3.61 ± 0.05	3.10 ± 0.11	2.40 ± 0.13*	3.4 ± 0.16
Globulin (g/dl)	3.91 ± 0.16	3.40 ± 0.10	2.50 ± 0.14*	3.4 ± 0.15
Leukocytes (X 10 ⁹ /L)	9.3 ± 0.30	3.8 ± 0.60*	4.2 ± 0.31*	6.6 ± 0.45*
Neutrophils (X 10 ⁹ /L)	3.67 ± 0.11	1.2 ± 0.21*	1.60 ± 0.11*	2.1 ± 0.12*
Lymphocytes (X 10 ⁹ /L)	5.06 ± 0.21	2.1 ± 0.11*	2.4 ± 0.10	3.6 ± 0.12
Lysozyme activity (u/L)	8.5 ± 0.11	4.1 ± 0.10	5.1 ± 0.14*	6.8 ± 0.15*

* P < 0.05

These results suggest that endotoxin administration may produce immunosuppressive effects in camels.

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بعض اتجاهات تثبيط المناعة : تأثير سموم الأندوتكسن على الجمال

عبدالله محمد الدغيم و عبدالقادر موسى حميدة

قسم الأحياء الدقيقة والطب البيطري ، كلية الطب البيطري والثروة الحيوانية
جامعة الملك فيصل ، ص ب - ١٧٥٧ ، الأحساء - ٣١٩٨٢
المملكة العربية السعودية

المخلص

أدى الحقن الوريدي لسموم الأندوتوكسن بجرعة مقدارها ٠,٠٥ ميكروجرام للكيلوجرام في الجمال إلى نقصان في كريات الدم البيضاء والخلايا اللمفاوية والمتعادلة ونقصان في تركيز البروتين والالبيومين والجلوبيولين وكذلك في إنزيم الليسوسوم في سيرم الدم. لقد تم استنتاج أن سموم الأندوتوكسن بجرعة مقدارها ٠,٠٥ ميكروجرام للكيلوجرام قد تتسبب في تثبيط المناعة في الجمال.
