

PHYSIOLOGICAL EFFECTS OF *ESCHERICHIA COLI* HEAT-LABILE ENTEROTOXIN IN LAMBS

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SUMMARY

Three groups of five lambs each were given variable doses (45µg, 15µg and 5µg) of *Escherichia coli* heat-labile enterotoxin (LT) via a single intraperitoneal injection. A fourth group of five lambs served as sham-treated controls. One day prior to inoculation, the heart rate, respiration, rectal temperature, faecal consistency, haematological and serum biochemical parameters were monitored twice daily over a 3-day period and once daily over the next two days. Significant ($p < 0.05$) elevation of heart rate and increase in neutrophil:lymphocyte ratio were evident in lambs given 45µg of LT at 24 hours post-inoculation. Other clinical and haematological parameters remained unchanged. Similarly no changes were noted in the levels of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and blood urea nitrogen (BUN).

Keywords: *Escherichia coli* enterotoxin, lambs, physiological parameters

INTRODUCTION

Escherichia coli heat labile enterotoxin (LT) has been described to possess strong mucosal adjuvant properties when used with various antigens to immunise mice (Clements *et al.*, 1988). Other works suggest that LT is less toxic on a weight basis than cholera toxin (CT), an enterotoxin that shares partial structural homology, antigenicity and toxic mechanisms of adenosine di-phosphate ribosylation (Lycke and Holmgren, 1986; Lycke *et al.*, 1992; Walker and Clements, 1993). Intragastric administration of 5-10µg of CT into mice induces detectable net fluid secretion in the mouse intestine, while none is seen with LT at doses up to 100µg. Comparison between LT and CT following intraperitoneal challenge of Balb/c mice gave the LD₅₀ values of 12.6µg for CT and 79.4µg for LT. Staphylococcal enterotoxins have not been compared with LT but they have been shown to exhibit toxic effects at low doses when given intravenously to goats (Van Miert *et al.*, 1983, 1984). It is apparent that LT is comparatively less toxic and safer than the enterotoxins from *Vibrio* and *Staphylococcus*, which exert fatal effects in a few hours after administration.

We are particularly interested in the adjuvant property of LT and its usage as an immunomodulator in vaccine formulations against livestock mucosal pathogens. To evaluate its safety in lambs, clinical, haematological and serum biochemical parameters were analysed. This paper describes a dose-response trial conducted with the objective to ascertain whether LT would cause toxic reactions in young lambs. It is believed that this is the first report in an ovine model.

MATERIALS AND METHODS

Lambs

Female Malin lambs aged 3-4 months were group-housed and fed a commercial pelleted diet (150g of crude protein [CP] kg⁻¹ dry matter [DM]; 10.5 MJ metabolisable energy kg⁻¹ DM) at 5% body weight. Drinking water was offered *ad libitum*.

Experimental design

Lambs were segregated into 4 groups of 5 animals each with a balanced mean bodyweight. Groups 2, 3 and 4 each received 45, 15 and 5µg of the *E. coli* LT (Sigma Chemical Co.) in phosphate-buffered saline (pH 7.2) intraperitoneally through the right para lumbar fossa respectively. Lambs in group 1 were given PBS alone in a similar manner and served as sham-treated controls.

Clinical examination

Heart rate, respiration, rectal temperature and faecal consistency were monitored twice daily (8.00 a.m. and 4.00 p.m.) commencing 24 hours prior to the administration of LT until 48 hours post-inoculation after which they were monitored once daily (8.00 a.m.).

Blood collection

Following clinical examination, serum samples were collected for clinical biochemistry while whole blood was collected in ethylenediamine tetra-acetic acid (EDTA) for haematological analysis.

Haematology

The total white blood cell counts were determined electronically (Baker System 9120CP). Differential cell counts were done by a standard method described by Dacie and Lewis (1975) using Wright's stained blood smears.

Clinical biochemistry

Alkaline phosphatase (AP), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) concentrations were determined using standard diagnostic kits (Roche).

Statistical analysis

Due to high individual variation in all parameters measured, all data were transformed to \log_{10} prior to the analysis. Data were fitted into a general linear model and time series analysis was performed to determine evidence of significant alterations over time. A one-way ANOVA and Duncans Multiple Range Test were performed at all time points to determine whether the differences between groups were significant.

RESULTS

Clinical examinations

Significant ($p < 0.05$) increase in the heart rate was evident in group 2 at 24 hours post-inoculation. Faecal consistency, respiration and rectal temperature were not affected by the treatments (Fig. 1).

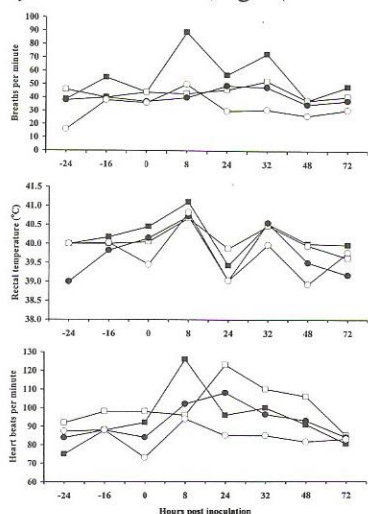


Fig. 1. The effect of a single intraperitoneal dose of LT on respiration, rectal temperature and heart rate of Malin lambs. ■ PBS only, □ 45µg LT, ● 15µg LT, ○ 5µg LT.

Haematology and serum biochemistry

The numbers of neutrophil increased significantly in group 2 and this was evident by a significant increase in the neutrophil:lymphocyte at 24 hours post-inoculation (Fig. 2). Other haematological parameters

were unaffected. The serum enzyme levels were not altered but the AST levels were decreasing (Fig. 3).

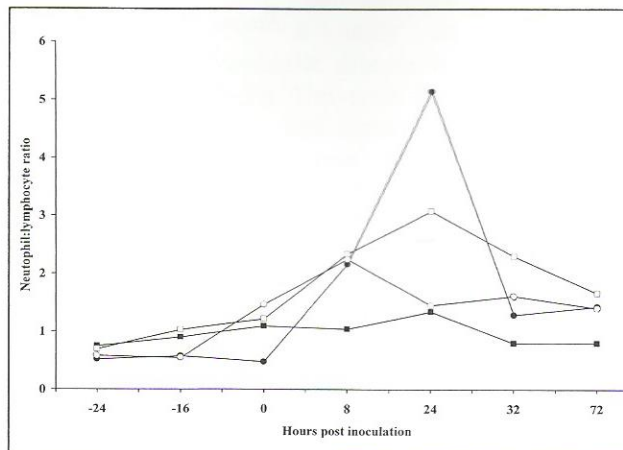


Fig. 2. The effect of a single intraperitoneal dose of LT on neutrophil:lymphocyte ratios of Malin lambs. ■ PBS only, □ 45µg LT, ● 15µg LT, ○ 5µg LT.

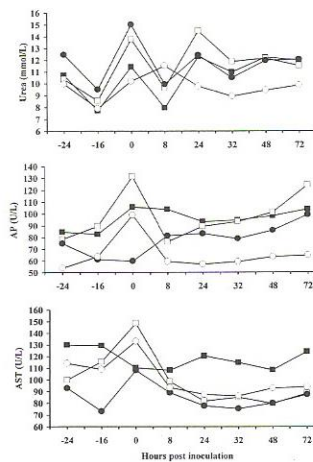


Fig. 3. The effect of a single intraperitoneal dose of LT upon blood urea nitrogen, alkaline phosphatase and aspartate aminotransferase levels in Malin lambs. ■ PBS only, □ 45µg LT, ● 15µg LT, ○ 5µg LT.

DISCUSSION

No signs of toxicity were observed in this study following administration of various doses of LT. The increase in the heart-rate and neutrophil:lymphocyte, however, were dose-dependent and transient in nature. There are no studies on induced changes in the clinical parameters after LT administration and therefore it is difficult to compare our findings with others. However Van Miert *et al.* (1984) demonstrated an increase in the heart rate of goats over the first 6 hours after an intravenous administration of staphylococcal enterotoxins. They suggested that tachycardia was due

to the induction of hypotension. There was also a significant reduction in circulating lymphocytes and an elevation of neutrophils over a 12-hour period post-inoculation. It has been established that neutrophils respond immediately to enterotoxins (Van Miert *et al.*, 1984) and was shown to be dose-dependent in our study. It has been suggested that fever induced by enterotoxins, which was not observed in this study, is mediated by endogenous proteins produced by the neutrophils, monocytes and other phagocytes (Dinarello, 1984). Since the doses of LT used in the present study did not induce fever, therefore, the induction of fever may be dose- and/or enterotoxin dependent. The significant increase in the neutrophil:lymphocyte could be due to a significant neutrophilia with no significant effect upon lymphocyte numbers, although studies with other enterotoxins and endotoxins have shown that lymphocytes are one of the most sensitive target cells (Van Miert *et al.*, 1983).

The reasons for unaltered serum enzyme levels and other parameters measured are uncertain. However, it is likely to be dose- and/or route-related. The route of administration used in this study may have reduced the potency of LT or the dose may have been too low to induce any serious effects. During infection, LT is in direct contact with the intestinal mucosa and requires proteolytic cleavage and reduction at specific sites on the A chain of the enterotoxin in order to express the toxic action (Sixma *et al.*, 1991). Therefore, LT administration via the intraperitoneal route would have precluded LT of proteolytic cleavage by intestinal proteases. It also may have facilitated a greater distribution of LT on other cell types through binding to its ligand galactose, which is widely distributed on the surface of all cells in the body (Clements *et al.*, 1980). This would possibly have reduced the amount able to bind to ganglioside GM₁, another ligand specifically located on epithelial cells of the gut, thereby not inducing diarrhoea.

On the other hand, it has been demonstrated that oral inoculation of LT in mice was less toxic than when given intraperitoneally (Walker and Clements, 1993). Since our aim was to use LT as an adjuvant, the intraperitoneal route of administration was chosen for two reasons: 1) oral administration of immunostimulants in ruminants has not been very successful probably owing to the complexity of the digestion and metabolism in the forestomach, 2) intraperitoneal administration is the second best alternative for induction of intestinal inducer and effector cells. With respect to serum biochemical parameters, it has been shown in goats that Staphylococcal enterotoxins do affect ALP and BUN. However, these studies employed intravenous administration and therefore more aggressive responses would be anticipated.

In conclusion we found that intraperitoneal administration of LT was safe at the highest dose of 45µg. However, it is possible that higher doses may cause severe toxicity. Future work will involve the use of LT as a mucosal immunomodulator in ruminants.

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intravenous administration of Staphylococcal enterotoxins B and F (Toxic shock syndrome toxin-1) in goats. *Infect. Immun.* **44**: 354-360.

RINGKASAN

KESAN FISILOGI PEMBERIAN ENTEROTOKSIN LABIL HABA ESCHERICHIA COLI DALAM ANAK BIRI-BIRI

Tiga kumpulan lima anak biri-biri setiap satu telah diberi pelbagai dos ($45\mu\text{g}$, $15\mu\text{g}$ dan $5\mu\text{g}$) enterotoksin labil haba *E. coli* (LT) melalui suntikan tunggal intraperitoneum. Kumpulan keempat lima ekor anak biri-biri pula bertindak sebagai kawalan pura-pura terperlaku. Bermula pada satu hari sebelum penginokulatan, kadar jantung, pernafasan, suhu rektum, kekonsistenan tinja, parameter hematologi dan biokimia serum telah dimantau dua kali sehari selama 3 hari dan disusuli dengan sekali sehari selama dua hari berikutnya. Peningkatan tererti ($p < 0.05$) dalam kadar jantung dan penokokan dalam nisbah neutrofil:limfosit jelas dalam anak diberi $45\mu\text{g}$ LT pada 24 jam pasca penginokulatan. Parameter klinikal dan hematologi lain tidak berubah. Begitu juga dengan aras alkaline fosfatase (AP), aspartat aminotransferase (AST) dan nitrogen urea darah (BUN), tiada perubahan yang dikesan.