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Research paper

Characterization of an intravenous lipopolysaccharide inflammation model in calves with respect to the acute-phase response

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ABSTRACT

Our objective was to develop a lipopolysaccharide (LPS) inflammation model in calves to evaluate the acute-phase response with respect to the release of pro-inflammatory cytokines and acute-phase proteins, fever development and sickness behaviour. Fourteen 4-week-old male Holstein Friesian calves were included and randomly assigned to a negative control group ($n=3$) and an LPS-challenged group ($n=11$). The latter received an intravenous bolus injection of 0.5 μg of LPS/kg body weight. Blood collection and clinical scoring were performed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 28, 32, 48, 54 and 72 h post LPS administration (p.a.). In the LPS group, the following clinical signs were observed successively: tachypnoea (on average 18 min p.a.), decubitus (29 min p.a.), general depression (1.75 h p.a.), fever (5 h p.a.) and tachycardia (5 h p.a.). Subsequent to the recovery from respiratory distress, general depression was prominent, which deteriorated when fever increased. One animal did not survive LPS administration, whereas the other animals recovered on average within 6.1 h p.a. Moreover, the challenge significantly increased plasma concentrations of tumour necrosis factor-α, interleukin 6, serum amyloid A and haptoglobin, with peaking levels at 1, 3.5, 24 and 18 h p.a., respectively. The present LPS model was practical and reproducible, caused obvious clinical signs related to endotoxemia and a marked change in the studied inflammatory mediators, making it a suitable model to study the immunomodulatory properties of drugs in future research.

1. Introduction

As a part of the outer membrane of Gram-negative bacteria, LPS or endotoxin is involved in many infectious bovine diseases (Radostitis et al., 2007). Particularly in calves, which are physiologically extremely sensitive to LPS, Gram-negative bacteria such as *Escherichia coli*, *Mannheimia haemolytica* and *Pasteurella multocida* play a crucial role in the most frequent and economically important calf diseases, namely neonatal diarrhoea and respiratory disease (Michaels and Banks, 1988; Constable,

Abbreviations: APP, acute-phase protein; APR, acute-phase response; BW, body weight; COX, cyclooxygenase; CV, coefficient of variation; HR, heart rate; Hp, haptoglobin; LOQ, limit of quantification; NF-κB, nuclear factor κB; NO, nitric oxide; PG, prostaglandin; PIM, pulmonary intravascular macrophage; RR, respiratory rate; RT, rectal body temperature; SAA, serum amyloid A; TLR4, Toll-like receptor 4.

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2004; Pardon et al., 2012). Also in septicaemia, which continues to be a life-threatening condition with high mortality risks, especially in calves with failure of passive transfer, the vast majority of isolates is Gram-negative (Fecteau et al., 2009).

Intravascular or peripheral exposure of host cells to LPS is a possible trigger of a complex series of non-specific, predetermined and well-orchestrated reactions, intending to control the Gram-negative bacterial infection (Conner et al., 1989; Baumann and Gauldie, 1994). This acute-phase response (APR) is initiated by the release of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, and other inflammatory mediators, including prostaglandins (PGs) (Werling et al., 1996). The APR is characterized by fever, tachycardia, leukocyte activation, mobilization of phagocytes, altered plasma concentrations of zinc, iron, calcium and copper, synthesis of acute-phase proteins (APPs) including serum amyloid A (SAA) and haptoglobin (Hp) by hepatocytes, and behavioural changes such as depression, anorexia and hyperalgesia (Watkins et al., 1994; van Miert, 1995; Zebeli et al., 2010). Nevertheless, the response can be excessive and subsequently result in detrimental effects to the host, like sepsis and septic shock (Peri et al., 2010).

As a consequence of the marked similarity between the systemic effects of Gram-negative bacterial infections and those provoked by an exogenous LPS challenge, endotoxin models have been widely applied in veterinary research to study diverse aspects of the APR (Lillie, 1974; Redl et al., 1993; Hodgson, 2006). More specifically, various experimental designs using different serotypes of LPS, ranging from high-dosed i.v. bolus injections to low-dose infusions over several hours, have been reported in calves (<6 months) (Adams et al., 1990; Kenison et al., 1991; Gerros et al., 1993; Semrad et al., 1993; Kinsbergen et al., 1994; Bieniek et al., 1998; Kushibiki et al., 2008). On the whole, data on the development of fever and the increased plasma concentrations of TNF-α are clear, emphasizing the impact of contact with LPS. The duration and magnitude of the response, on the other hand, has been described to be related to the amount of circulating LPS (Gerros et al., 1993). Remarkably, data on the release of IL-1β, IL-6 and APPs are either lacking or less frequently reported in young calves following experimental endotoxemia. Also regarding sickness behaviour in these animals, limited information is available (Borderas et al., 2008).

Therefore, our first objective was to develop an LPS inflammation model in 4-week-old calves, with inclusion of TNF-α, IL-1β, IL-6, SAA and Hp, which could be applied in further research regarding the immunomodulatory properties of (non)-steroidal anti-inflammatory drugs ((N)SAIDs) and antimicrobials. Secondly, through applying this model, the authors attempted to design a clinical score system for the early detection of illness.

2. Materials and methods

2.1. Experimental animals

Fourteen healthy male Holstein Friesian calves were conveniently selected from local farms shortly after birth. The calves were conventionally reared on the farm, generally through separation from the mother and subsequent housing in individual pens. All animals received an oral treatment with paromomycin sulphate (100 mg/kg body weight (BW), sid, 10 days) (Gabbrovet 70, Ceva Santé Animale, Brussels, Belgium) on the farm of origin, in order to reduce cryptosporidiosis-related symptoms (Grinberg et al., 2002). One week before the start of the experiment, at an average age of 22.6 ± 4.2 days, the calves were transferred to the Faculty of Veterinary Medicine. Upon arrival, the calves received a single treatment with 5 mg/kg BW enrofloxacin (Floxadil 50 mg/mL, Emdoka, Hoogstraten, Belgium) s.c., to limit the spread of respiratory infections. During the acclimatization period, the animals were housed in individual pens on straw with ad libitum access to hay and freshwater. The calves were fed milk replacer three times a day, receiving a total of 5 L daily. After the morning feeding, 50 g of starter mix was given to the calves.

The animals' clinical condition was evaluated twice a day, including the determination of the rectal body temperature (RT) and visual inspection of the faeces. Analysis of faecal samples (Easy-Digest, Bio K 151, Bio-X, Jemelle, Belgium) revealed the presence of antigen of rotavirus, *E. coli* and *Cryptosporidium parvum* in a number of calves (36, 21 and 29%, respectively). However, in order to avoid interference with the experiment, the calves were not treated pharmacologically during the week of acclimatization. In case of diarrhoea, the calves would receive electrolyte therapy. If illness would require an antimicrobial and/or an anti-inflammatory treatment, the calf would be excluded from the experiment.

At the end of the study, the calves were introduced in the teaching or experimental herd of our faculty.

2.2. Study protocol and sample collection

The day before the experiment, the calves were weighed (54.8 ± 7.0 kg), after which a 14 G indwelling catheter (Cavafix, B. Braun, Diegem, Belgium) was placed aseptically in the right jugular vein. A recovery period of at least 12 h was respected. After this period, the calves were randomly divided into two groups (average age 29.6 ± 4.2 days): a negative control group (CONTR; $n = 3$) and an LPS-treated group (LPS; $n = 11$). The clinical condition at 0 h was evaluated and a RT ≥ 40 °C was handled as an exclusion criterion at this time.

Reference venous blood samples (0 h) for cytokines and APPs were collected from the catheter and transferred into tubes containing EDTA. The calves in the LPS group were subsequently challenged i.v. with 0.5 μg/kg BW ultrapure LPS (500 units/kg BW, *E. coli* serotype O111:B4, LPS-EB Ultrapure, InvivoGen, Toulouse, France) via the catheter, while the CONTR calves received a similar volume of saline. Blood samples for cytokines and APPs analyses were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 28, 32, 48, 54 and 72 h post administration (p.a.). At all mentioned sampling points, RT, respiratory rate (RR) and heart rate (HR) were recorded. RR was assessed first, before entry of the pens. Subsequently, RT was measured using a digital thermometer, followed by HR determination. Animals were also clinically scored by a qualified veterinarian