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Pathophysiology of Experimental Bovine Endotoxicosis: Endotoxin Induced Synthesis of Prostaglandins and Thromboxane and the Modulatory Effect of Some Non-Steroidal Anti-Inflammatory Drugs

By *N. Jarløv, P. Haubro Andersen* and *M. Hesselholt*

Department of Clinical Studies, Section of Surgery, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Jarløv, N., P. Haubro Andersen and M. Hesselholt: Pathophysiology of experimental bovine endotoxicosis: Endotoxin induced synthesis of prostaglandins and thromboxane and the modulatory effect of some non-steroidal anti-inflammatory drugs. Acta vet. scand. 1992, 33, 1-8. – Endotoxin-induced synthesis of thromboxane A2 (TXA2), prostacyclin (PGI2) and prostaglandin E2 (PGE2) was studied in 3 cows after intravenous E. coli endotoxin (055:B5-0.025 mg/kg b.w.) administration. Blood sampling and monitoring of clinical signs were performed from 2 h prior to until 6 h after endotoxin challenge. Blood samples were analyzed for stable hydrolysis products of TXA2 (TXB2), PGI2 (6-keto PGF) and PGE2 (bicyclic PGE2), biochemical and haematological parameters. In a similar experimental design the efficacy of the non-steroidal anti-inflammatory drugs (NSAID) flunixin meglumine (FM) and phenylbutazone (PB) in suppressing eicosanoid synthesis and clinical signs in response to endotoxin challenge was investigated. Two groups of cows, each comprising 2 animals, were treated with FM and PB prior to endotoxin challenge. It was observed that plasma concentrations of TXB2, 6-keto PGF and bicyclic PGE2 increased rapidly after endotoxin challenge. Concentrations were significantly elevated for hours and were correlated to the severity of clinical signs of endotoxicosis. Pretreatment with NSAID suppressed mediator production and alleviated clinical signs. The experiments suggest a certain pathophysiological role of TXA2, PGI2 and PGE2 for the early systemic ill-effects of bovine endotoxicosis.

eicosanoids; prostacyclin; flunixin meglunine; phenylbutazone.

Introduction

Endotoxicosis is considered to be a serious complication to several diseases in domestic animals including cattle. The pathophysiology of endotoxicosis is not completely understood. The observations made by *Andersen et al.* 1988, that despite a rapid plasma endotoxin clearance in the bovine (minutes) the clinical signs of endotoxicosis persist for hours suggest the involvement of other pathogenetic factors. Findings in different species (dogs, pigs, horses, rats, rabbits and primates) have associated the vasoactive eicosanoids thromboxane A2 (TXA2) and prostacyclin (PGI2) with the systemic ill-effects of endotoxaemia (*Bottoms et al.* 1982, 1983, *Bult et al.* 1980, *Goto et al.* 1981, *Harris et al.* 1980, *Schrauwen et al.* 1983). The systemic production of these eicosanoids in experimental endotoxaemia is poorly investi-

gated in cattle. Studies in neonatal calves indicate that TXA2 and PGI2 are involved in experimental bovine endotoxicosis (Morris et al. 1986). Prostaglandin E2 (PGE2), a vasoactive eicosanoid, which is an important mediator of inflammation, has not yet been investigated in bovine experimental endotoxicosis.

Non-steroid anti-inflammatory drugs (NSAID) such as flunixin meglumine (FM) and phenylbutazone (PB) produce their anti-inflammatory effect through suppression of eicosanoid synthesis. The expanded clinical use of NSAID in disease conditions, where endotoxaemia is considered as a complication, is based on several experiments, where it is shown, that NSAID are able to suppress endotoxin-induced eicosanoid synthesis and attenuate clinical signs of experimental endotoxaemia. Thus suppression of endotoxin-induced vasoactive mediator synthesis by FM and reversal of cardiovascular changes are well documented in equine experimental endotoxaemia (*Bottoms et al.* 1982, *Dunkle et al.* 1985, *Moore et al.* 1986).

For the purpose of obtaining a further insight into the pathophysiology of bovine endotoxicosis it was the aim of the present study to monitor the synthesis of the eicosanoids TXA2, PGI2 and PGE2 in experimental bovine endotoxicosis. In addition it was the objective to elucidate the efficacy of pharmacological doses of FM and PB in suppressing synthesis of TXA2, PGI2 and PGE2 and clinical signs of experimental endotoxicosis.

Materials and methods

Animals

Endotoxin-induced synthesis of prostaglandins and thromboxane was studied in 3 Jersey cows (control group body weight: 243 to 260 kg). Suppression of endotoxin-induced eicosanoid synthesis by NSAID's was studied in 4 healthy Jersey cows (body weight: 320 to 360 kg) assigned to 1 of 2 groups (I: cows pretreated with FM; II: cows pretreated with PB). All the cows had indwelling 16 G catheters in the left jugular vein.

Experimental design

All cows received endotoxin at 0 min (*E. coli* endotoxin, 055:B5 Westphals extraction (SIGMA)) 0.025 mg/kg b.w. dissolved in sterile pyrogen-free water given intravenously as a bolus (20 sec).

Five min prior to endotoxin challenge cows in group I and II received a single intravenous dose of FM (2.2 mg/kg bw), and PB (10 mg/kg b.w.), respectively. Blood samples for eicosanoid analysis were drawn 120, 90, 60 and 30 min prior to and at 5, 15, 60, 120, 180, 240 and 300 min post endotoxin injection. Samples for determination of bicyclic PGE2 were not drawn 90 min prior to and 240 min post endotoxin injection. Blood samples for biochemical and haematological parameters were drawn 30 min prior to the challenge and at 15, 45, 120, 180, 240, 300 and 360 min after. All the cows were examined clinically at hourly intervals from 2 h before until 6 h after endotoxin injection. Signs and parameters monitored included rectal temperature, depression, rumen motility, fecal consistency, colic, heart rate, and respiratory rate.

Blood biochemistry and haematology

Concentrations of plasma glucose, zinc and leukocyte counts were determined according to routine clinical-chemical methods. Thrombocyte counts were made by phase contrast microscopy.

Eicosanoids

Plasma concentrations of the stable hydrolysis products TXB2, 6-keto PGF and bicyclic PGE2 were determined by use of commer-

cial Radio Immuno Assay (RIA) Kits (Amersham TRK code 780, 790 and 800) and managed as described by the manufacturer. The measured concentrations indirectly reflect the concentrations of TXA2, PGI2 and PGE2, respectively (*Granstrøm et al.* 1976, *Demers & Derck* 1980).To prevent spontaneous mediator release, blood samples were collected in tubes containing EDTA and indomethacin. Prior to sampling approximately 100 ml blood were allowed to flush the catheter in order to minimize catheter and trauma - induced arachidonic acid degradation. Immediately after collection the blood samples were centrifuged, and the plasma samples were stored below –30° C. Prior to the RIA, plasma extraction was performed at SepPak C-18 columns (Waters) as described elsewhere (*Powell* 1980).

Statistical analyses

Analysis of variance was performed on the results of the hematological and biochemical analyses using a computer statistical program (General Linear Models Procedure, Statistical Analysis Systems Inc, Cary, NC.). The model included the factors pretreatment vs control cows and time after endotoxin injection vs the base line period. The results of the eicosanoid analyses are given as mean ± 1 SD for each group without further statistical evaluation.

Results

Endotoxin-induced eicosanoid synthesis

Clinical signs of endotoxicosis (decreased rumen motility, diarrhea, tachycardia, tacypnoea) developed shortly after endotoxin administration.

The results of the leukocyte and thrombocyte counts and zinc and glucose determinations are given in Table 1. The statistical analyses revealed no statistically significant difference between Group I, Group II and the control group. The results are therefore presented as mean of all cows ± SD. The leukocyte and thrombocyte counts were significantly reduced 15 min after endotoxin administration and reached a minimum 3 h later (2.25 h $p<0.01$). A decrease in plasma zinc concentration was observed after 3 h ($p<0.001$). The glucose concentrations increased after endotoxin challenge (2.75 h $p<0.05$) and decreased after 4 h ($p<0.01$) (Table 1).

Table 1. Results of leukocyte and thrombocyte counts in blood and glucose and zinc determinations in plasma of control cows and cows pretreated with either flunixin meglumine or phenylbutazone presented as mean of all cows ± 1SD.

Time min	Leukocyte count mia/L (X±SD)	Thrombocyte count mia/L (X±SD)	Zinc conc. µmol/L (X±SD)	Glucose conc. mmol/L (X±SD)
–30	8.2±1.8	872±126	6.7±1.1	4.0±0.6
15	4.8±1.5	685±224	6.9±1.2	5.2±1.8
45	2.8±0.6	466±211	6.7±1.1	6.9±2.8
120	1.5±0.3	421±167	6.7±1.4	5.6±2.1
180	1.0±0.3	418±180	5.4±1.0	5.3±2.4
240	1.3±0.4	368±96	3.9±1.2	2.9±0.4
300	1.3±0.3	397±91	2.6±1.4	3.0±0.6
360	1.4±0.4	391±126	1.8±0.6	3.0±0.4

Plasma TXB2 concentrations increased rapidly reaching peak values 5 min after endotoxin injection followed by a gradual decrease towards the base-line level (Fig. 1). Plasma 6-keto PGF concentrations in all 3 cows increased from baseline values and reached peak values within 60 min after endotoxin injection. Plasma 6-keto PGF concentrations did not return to baseline values during the observation period (Fig. 2). Plasma bicyclic PGE2 concentrations reached peak values within 2 h after endotoxin administration, and did not return to baseline values during the observation period (Fig. 3).

Suppression of eicosanoid synthesis by NSAID

Clinical signs on endotoxicosis were considerably alleviated, especially in the FM pretreated group (group I) when compared with the control group.

In the FM pretreated group (group I) plasma concentrations of 6-keto PGF and bicyclic PGE2 did not increase above baseline values at any time. Plasma concentrations of TXB2 showed a minor increase during the first hour after endotoxin injection (Fig. 1, 2, 3).

In the PB pretreated group (group II) plasma concentrations of bicyclic PGE2 did not increase above baseline values during the 6 h study period (Fig. 1, 2, 3). A minor increase was observed in TXB2 concentration during the first h after endotoxin injection, while plasma concentrations of 6-keto PGF were considerably increased (Fig. 1, 2, 3).

Pretreatment with NSAID did not influence

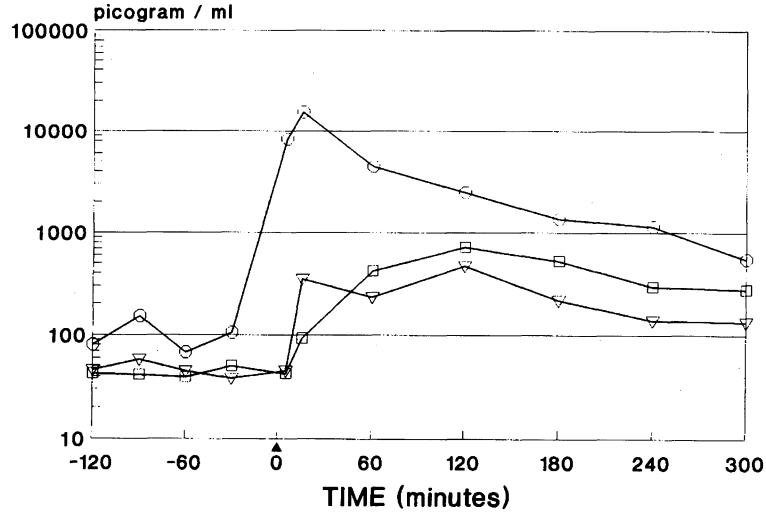

Figure 1. Plasma concentrations of TXB2 in the control group ○ (mean of 3 cows), cows pretreated with flunixin meglumine ▽ (mean of 2 cows) and cows pretreated with phenylbutazone □ (mean of 2 cows). Arrow indicates endotoxin challenge at 0 min. Y-axis is a logaritmic scale.

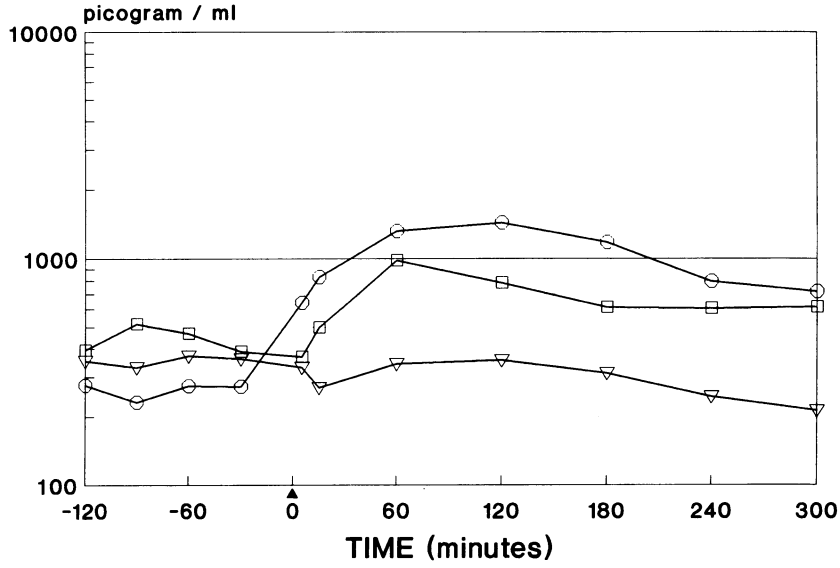

Figure 2. Plasma concentrations of 6-keto PGF in the control group ○ (mean of 3 cows), cows pretreated with flunixin meglumine ▽ (mean of 2 cows) and cows pretreated with phenylbutazone □ (mean of 2 cows). Arrow indicates endotoxin challenge at 0 min. Y-axis is a logaritmic scale.

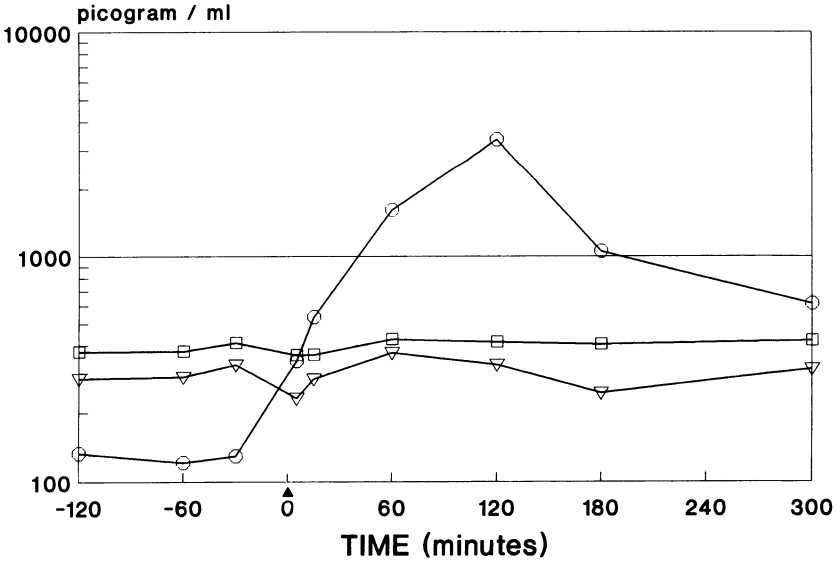

Figure 3. Plasma concentrations of bicyclic PGE2 in the control group ○ (mean of 3 cows), cows pretreated with flunixin meglumine ▽ (mean of 2 cows) and cows pretreated with phenylbutazone □ (mean of 2 cows). Arrow indicates endotoxin challenge at 0 min. Y-axis is a logaritmic scale.

leukopenia and thrombocytopenia or any of the other biochemical parameters investigated.

Discussion

Endotoxin-induced eicosanoid synthesis

The clinical and haematological changes observed after endotoxin challenge indicated an endotoxemic condition in the experimental animals. The increase in plasma TXB2 and 6-keto PGF after endotoxin challenge observed in the present study is in agreement with results from studies on experimental endotoxaemia in horses, dogs, pigs and primates (*Bottoms et al.* 1982, 1983, *Harris et al.* 1980, *Schrauwen et al.* 1983). In the present study an endotoxin-induced increase in plasma PGE2 was observed.

The peak plasma concentration ratio of TXB2 and 6-keto PGF observed in the present study differs from those obtained from similar studies in other animals (*Bottoms et al.* 1982, 1983, *Harris et al.* 1980), but is similar to the TXB2/6-keto PGF ratio observed in neonatal calves with experimental endotoxicosis (*Morris et al.* 1986). This apparent difference might be explained by species differences and/or variations in amounts of eicosanoids bound to protein (*Salmon & Flower* 1983).

The baseline values of 6-keto PGF observed in this study were higher than the corresponding values of TXB2. A physiological ratio of PGI2/TXA2 > 1 might reflect a decreased thrombocyte aggregation tendency of the bovine compared to e.g. the equine as discussed by *Dyerberg* (1987). We observed a negative correlation between thromboxane synthesis and thrombocyte count (Table 1 and Fig. 1), which also exists in other species (*Harris et al.* 1980). Whether this condition is responsible for the development of disseminated intravascular coagulopathy (DIC) or is a result of DIC, is questionable and the significance of other non-eicosanoid mediators has been suggested (*Hsush et al.* 1987). We did not observe clinical signs of DIC in control cows or NSAID pretreated cows.

Suppression of eicosanoid synthesis by NSAID

It appears from the present study that pretreatment with NSAID is able to suppress mediator production and alleviate clinical signs of bovine endotoxicosis. This effect was most pronounced in the FM pretreated group. A less pronounced inhibition was observed in the PB pretreated group. It is underlined that our observations are based upon comparison of plasma concentration values to baseline values in experimental groups comprising few animals. The efficacy of FM and PB in reducing eicosanoid generation and clinical signs in response to experimentally induced endotoxaemia observed in the present preliminary study confirms results from experiments in other animal species e.g. the equine and may justify the clinical application of FM and PB in disease conditions considered to be associated with endotoxaemia (*Anderson et al.* 1986).

Pretreatment with NSAID did not influence the endotoxin-induced reduction in leukocyte and thrombocyte counts, which may indicate the involvement of other mediators in bovine endotoxicosis.

The main objective of the present report was to study the possible role of the eicosanoids TXA2, PGI2 and PGE2 in the pathogenesis of bovine endotoxicosis. Our experiments showed that plasma concentrations of the stable metabolites TXB2, 6-keto PGF and bicyclic PGE2 increased rapidly after endotoxin challenge. The concentrations were significantly elevated for hours and correlated

to the severity of the clinical signs. Pretreatment with FM and PB leads to a suppression of mediator production and an alleviation of clinical signs of endotoxicosis. Even though the results are based on data from few animals our experiments suggest a certain pathophysiological role of the mediators especially for the early systemic ill-effects of bovine endotoxicosis.

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Sammendrag

Den eksperimentelle bovine endotoksikoses patofysiologi: Endotoksin-induceret eikosanoidsyntese og

den suppressive effekt af nogle non-steroide antiinflammatoriske stoffer.
Endotoksin-induceret syntese af thromboxan A2 (TXA2), prostacyklin (PGI2) og prostaglandin E2 (PGE2) blev undersøgt hos 3 køer efter intravenøs applikation af *E.coli* endotoksin (0.55:B5-0.025 mg/kg legemsvægt). Udtagning af blodprøver og registrering af kliniske symptomer blev foretaget fra 2 t før til 6 t efter endotoksinindgift. Blodprøverne blev analyseret for de stabile metabolitter af TXA2 (TXB2), PGI2 (6-keto PGF) og PGE2 (bicyklisk PGE2), biokemiske og hæmatologiske parametre. I en lignende eksperimentel undersøgelse belyses den suppressive effekt af de non-steroide antiinflammatoriske stoffer (NSAID) flunixin meglumin (FM) og fenylbutazon (PB) på eikosanoidsyntese og kliniske symptomer ved eksperimentel endotoksikose. To grupper køer, hver bestående af 2 dyr, blev behandlet med henholdsvis FM og PB inden endotoksinindgift. Forsøgene viste, at plasmakoncentrationerne af de stabile metabolitter steg hurtigt efter endotoksinindgift, var forhøjede i flere timer og korrelerede tidsmæssigt med graden af de kliniske symptomer på endotoksikose. Indgift af FM og PB hæmmede den endotoksininducerede eikosanoidsyntese og svækkede graden af de kliniske symptomer. Resultaterne peger på, at eikosanoiderne TXA2, PGI2 og PGE2 har patofysiologisk betydning for de systemiske manifestationer af bovin eksperimentel endotoksikose.

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Reprints may be requested from: Pia Haubro Andersen, Department of Clinical Studies, Section of Surgery, Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Denmark.