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Brief Communication

Portal Infusion of Low Dosage Endotoxin: A Model Simulating Translocation of Ruminal Endotoxin in Cattle

Translocation of bacteria and endotoxin from the gastro-intestinal tract to the portal blood is described to occur in healthy humans and animals, and is probably facilitated by ruminal epithelium damage in cattle *(Berg* 1992). Controversy exists regarding the possible role of endotoxin in the pathogenesis of ruminal acidosis. Systemic disease during ruminal acidosis is clinically characterized by forestomach stasis, anorexia, depression, tachycardia, tachypnea and fever. It has been shown that blood concentrations of arachidonic acid metabolites increase during ruminal acidosis, which may explain many of these clinical signs *(Andersen et al.* 1994). At the same time, we found that only few cows with experimentally induced rumen acidosis had endotoxin in the systemic blood *(Andersen et al.* 1990, 1994), while other authors describe systemic endotoxaemia as an occasional finding in similar or milder cases of grain-engorgement *(Boosman et al.* 1990, *Aiumlamai et al.* 1992). Arachidonic acid metabolites are readily produced in the presence of endotoxin, but might also be expected to be produced during a chemical inflammation process of ruminal epithelium, damaged by a low pH and high osmolar concentration. The purpose of the present study was to evaluate the role of low grade portal endotoxaemia for pre-hepatic release of inflammatory mediators 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-PGF) and thromboxane B_2 (TXB) and the relation to systemic disease. Four healthy cows were surgically equipped with chronic catheters in the portal vein, in a mesenteric vein 20 cm distally to portae hepatis and in a hepatic vein. After recovery, the cows received at maximum 3 different treatments at monthly intervals in a randomized design. Treatments were saline solution infused into the mesenteric vein at 2.5 µL / kg body weight per min (control), *Escherichia coli* endotoxin (055:B5 Westphals extraction, Sigma) at 0.025, 0.25 and 2.5 ng/kg body weight per min (Model I, Model II and Model III, respectively, Table 1). Infusions were continued for 180 min, or until respiratory distress (respiration rate > 40 per min) occurred. One h before a session, a jugular catheter was inserted, and blood samples were collected from the portal, hepatic and jugular vein for determination of clinical-chemical parameters (acid-base balance, packed cell volume (PCV), leukocyte and thrombocyte counts), endotoxin, TXB and 6-keto-PGF. Methods are described elsewhere *(Andersen et al.* 1994). After initiation of the experimental infusion, sampling was continued for 330 min at intervals of 30 min. Clinical parameters (rectal temperature, pulse and respiratory rates and ruminal movements) were determined hourly.

The results of the experiment are given in Figs. 1 & 2 (Leukocyte and thrombocyte

Tabel 1. Occurrence of peak values (mın after initiation of pre-hepatic endotoxın administration) and relative peak values (% of base lıne values) for TXB_2 and 6-keto-PGF.

		Endotoxın ınfusıon rate ng/kg/mın	Cumulatıve dosage ng/kg	TXB_2 peak occurence, mınutes	TXB_2 peak sıze, % of baselıne value	6-keto-PGF peak occurrence, mınutes	6-keto-PGF peak sıze, % of baselıne value
			4.5	180	283	–	–
Model I	(n=3)	0.025	4.5	180	256	–	–
			4.5	180	312	–	–
			22.5	60	2222	180	131
Model II	(n=3)	0.25	22.5	120	556	180	122
			45	150	228	–	–
Model III	(n=1)	2.5	225	30	1067	180	162
Control	(n=2)	0	0	–	–	–	–

–: no peak occurred.

counts, respectively) and in Table 1 (TXB and 6-keto-PGF). The response to the endotoxin infusions showed marked differences between models and between individuals. Administration of endotoxin at 0.025 ng/kg/min (Model I) did not cause clinical effects or alteration on leukocyte and thrombocyte counts. However, TXB synthesis was enhanced and peaked when infusion was stopped. The concentration rapidly declined and was at baseline level 60 min later. Increased concentrations of 6-keto-PGF was not observed. When endotoxin was infused at 0.25 ng/kg/min (Model II), 2 of the cows developed transient respiratory distress and rumen atony, and the infusions were stopped after 90 min. These cows developed leukopenia in various degrees after 60 min and a slight thrombopenia was noted after 150 min. TXB2 synthesis was enhanced, the peaks were higher and occurred earlier, when compared to the cows in Model I. Enhanced synthesis of 6-keto-PGF was observed only in the 2 cows, who showed the earliest and strongest TXB response. Administration of endotoxin at 2.5 ng/kg/min caused clinical signs as depression, tremor, fever, respiratory distress and rumen atony. A marked and rapid onset of leukopenia and thrombopenia was observed in this case. The TXB and 6-keto-PGF concentrations peaked at 30 and 180 min, respectively. These findings confirm the observations of *Eades* (1993), who concluded that the biochemical pathways for thromboxane are very sensitive to the effects of endotoxin. The concentrations of 6-keto-PGF was significantly lower in the hepatic blood compared to portal blood concentrations, suggesting a clearing of this mediator in the liver. The TXB concentrations showed a similar, but less clear, tendency. There was no difference in leukocyte and thrombocyte counts between the different vessels. The present results indicate that the number of leukocytes is more sensitive to low dosage endotoxin, than the number of thrombocytes.

Endotoxins injected intravenously in healthy cows disappear rapidly from the blood, even when injected in doses at 25 µg/kg body weight *(Andersen et al.* 1988), and as expected endotoxin was not detected in any of the cows in this study. If the portal blood flow was e.g. 25 l/min, the concentration of endotoxin in the portal vein theoretically would be below or

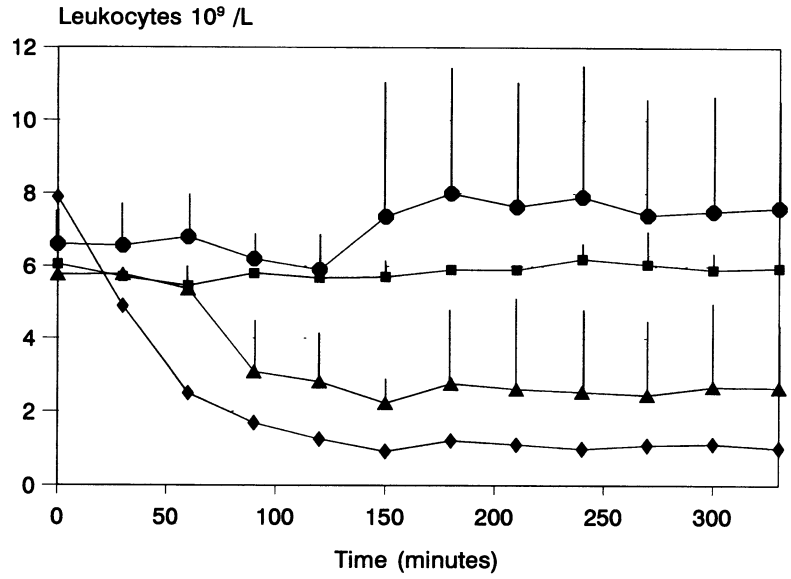

Figure 1. Blood leukocyte count before, during and after infusion of saline (■) or endotoxin at 0.025 (●), 0.25 (▲) and 2.5 (◆) ng/kg body weight/min. Bars indicate S.D.

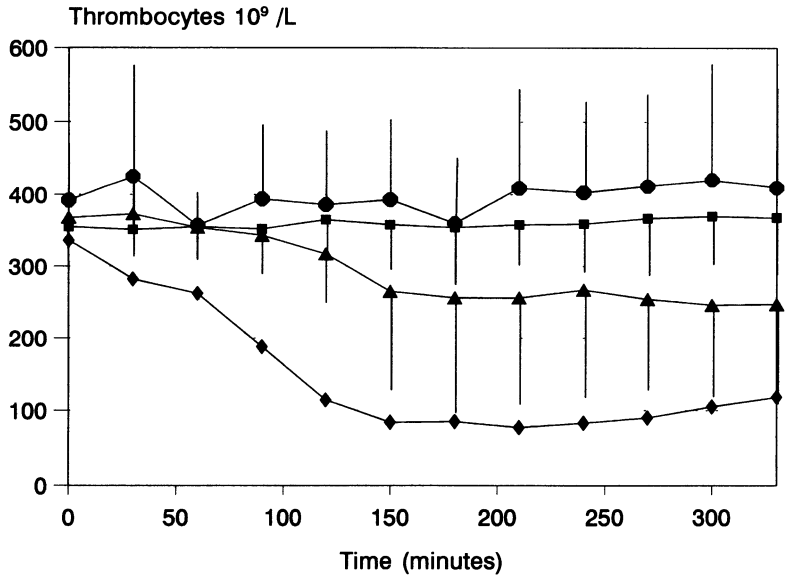

Figure 2. Blood thrombocyte count before, during and after infusion of saline (■) or endotoxin at 0.025 (●), 0.25 (▲) and 2.5 (◆) ng/kg body weight/min. Bars indicate S.D.

close to the detection limit of the *Limulus* Amoebocyte Lysate (LAL) assay on 3 pg *Escherichia coli* endotoxin 055:B5/ml in water, which is much lower in whole blood. These results show, that endotoxaemia, as defined by positive LAL results, not necessarily is related to the clinical signs on endotoxicosis. This is in accordance with endotoxin investigations in cases of ruminal acidosis, where absence of endotoxaemia is an occasional finding, despite signs of endotoxicosis *(Andersen & Jarløv* 1990, *Aiumlamai et al.* 1992, *Andersen et al.* 1994). It is also interesting that the cows in Model I seemed to be unaffected by the endotoxin infusion, but still had increased concentrations of TXB.

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