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Brief Communication

Liberation of $PGF_{2\alpha}$ from Whole Blood as an Assay to Detect Inflammatory Mediators

The aim of this study was to evaluate a simple method of monitoring an inflammatory process based on the reaction of normal whole blood to plasma from animals suffering from an induced acute inflammation. There is a strong need to find relevant methods to be able to screen for mediators of inflammatory reactions thereby facilitating diagnosis of inflammatory processes. In e.g. induced endotoxaemia, the endotoxin disappears very quickly from the circulation and is not a suitable substance to analyse in plasma *(Aiumlamai et al.* 1990).

In order to induce an acute inflammatory reaction, 2 healthy Swedish Red and White calves were iv injected with a low dose of endotoxin (100 ng/kg body weight) extracted from *Salmonella typhimurium* by the hot phenol method as described by *Lindberg et al.* (1983). Blood samples were collected for 26 h, starting 2 h before the injection, and collected in heparinized or plain acid washed tubes (Vacutainer®, Becton and Dickinson Vacutainer Systems Europe, Meylan-Cedex, France). Plasma was separated by centrifugation at 3000 g for 15 min. The acid washed tubes were kept at room temperature for 3 to 4 h and centrifuged to separate the serum. Whole blood was collected from a healthy Swedish Red and White heifer in EDTA tubes (Vacutainer). The procedure of managing the blood of the heifer was first determined in a series of factorial designed experiments in which different types of vacuum tubes (EDTA, citrate or heparin), additions to the blood (plasma or serum of calves at different times after the induced endotoxaemia and saline for comparison), quantities of plasma to be monitored and time of incubation, were tried. The blood was divided in fractions of 1 ml in inert plastic tubes. The best procedure was the following: in each tube, 0.1 ml of plasma was added from the endotoxaemic calves; the blood mixed with the plasma was incubated at room temperature for 10 min in a gentle mixing machine; in order to stop the incubation process, the tubes were centrifuged for 15 min at 3000 g at 18°C and the supernatant was separated and frozen. Later on, it was analyzed by radioimmunoassay to determine the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations *(Lindgren et al.* 1974). The original plasma from the calves were also analyzed by the same method to determine the original levels of $PGF_{2\alpha}$. In order to compare the $PGF_{2\alpha}$ concentrations of the original plasma and of the plasma mixed with blood, the basal $PGF_{2\alpha}$ levels of the heifer were also analysed. The $PGF_{2\alpha}$ concentrations before the induction of the endotoxaemia were similar in their basal levels. The basal $PGF_{2\alpha}$ levels of the heifer ("EDTA"-plasma) tended to be lower than those of the calves ("heparin"-plasma) before induction of endotoxaemia. This difference probably reflects that EDTA used as anticoagulant causes less artifactual formation of $PGF_{2\alpha}$ when the blood is collected. Another reason might be that the heparin

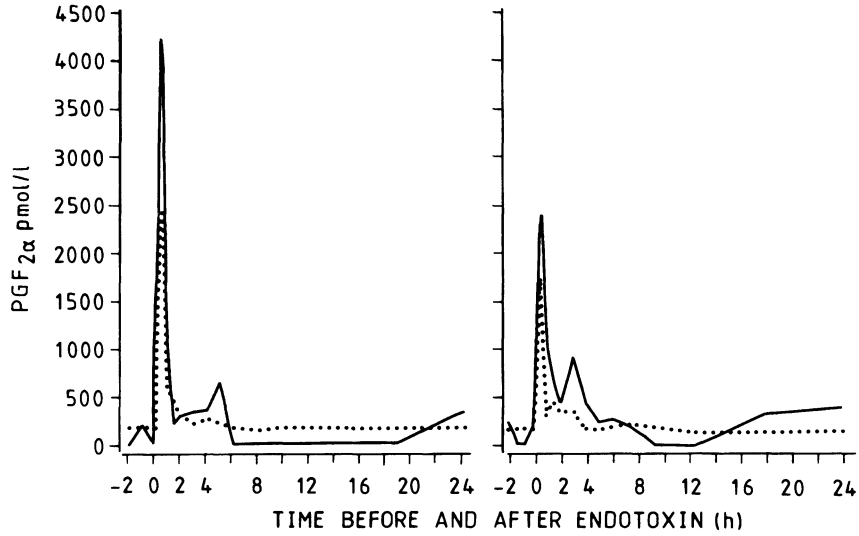

Figure 1: Concentrations of $PGF_{2\alpha}$ in original plasma from 2 calves injected with endotoxin (•••) and in plasma obtained from the combination of blood from a healthy heifer and plasma from the same endotoxaemic calves (——).

contains trace amounts of endotoxin stimulating blood cells to increased $PGF_{2\alpha}$ production. It can be advisable to use EDTA as anticoagulant in all blood collections. In both calves, a peak of $PGF_{2\alpha}$ was observed 30 min after the induction of the endotoxaemia. In the 2 cases, the peak reached higher values (around 80% higher in one calf and 30% in the other) in the plasma from the calves mixed with the blood of the heifer compared with the original concentrations. After the peak, the $PGF_{2\alpha}$ from the original plasma decreased to normal or basal levels. In contrast, the $PGF_{2\alpha}$ levels in the plasma obtained after mixing with blood first decreased, but then showed a second peak. This peak was of lower amplitude and more sustained than the first one, and occurred 5 and 3 h after the induction of the endotoxaemia in the 2 calves, respectively. Afterwards, the levels of $PGF_{2\alpha}$ returned to normal values (Fig. 1).

These results show that the blood responded to the addition of plasma from endotoxin injected animals because, in the period following the injection of the endotoxin, important differences between the levels of $PGF_{2\alpha}$ in the original plasma from the calves and in the plasma mixed with blood were seen. First, the higher initial peak of $PGF_{2\alpha}$ in plasma mixed with blood and then the second peak occurring. This response is likely to be due to some inflammatory mediators remaining in the plasma from the calves that can activate some cells or particles of the blood from the heifer and induce a liberation of $PGF_{2\alpha}$. If the whole blood had not responded, the levels of the original plasma and the ones of the blood mixed with plasma from endotoxaemic calves should have been in the same order of magnitude, as seen in the samples before the induction of the endotoxaemia. This was also the case about 6 h after the injection of the endotoxin. In this pilot study it was neither planned to identify the bioactive substance(s) in the

endotoxaemic calves causing liberation of $PGF_{2\alpha}$ from the whole blood, nor the reacting cell. It might also be different bioactive substances responsible for the 2 peaks seen in the plasma from the calves. Unfortunately, this assay was only capable of detecting bioactive substances in the endotoxaemic calves for a period of 6 h, which might be a too short time when screening for inflammatory mediators. However, the fact that this relatively simple assay system is recognizing bioactive substances is encouraging and merits further work to make the system more sensitive e.g. by separating the blood cells and using concentrated cell populations of the active cells. The problem is however to match simplicity of an assay with a more advanced, but complicated system.

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Bárbara Zentilli del Campo[1] *and Hans Kindahl.*

Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

References

Aiumlamai S, Fredriksson G, Kindahl H, Edqvist L-E, Kulander L, Eriksson O: Endotoxin concentrations in the blood following intravenous injection and effect on prostaglandin $F_{2\alpha}$ release, calcium and bile acids in goats. Res. vet. Sci. 1990, *48,* 190-195.

Lindberg AA, Greisman SE, Svenson SB: Induction of endotoxin tolerance with nonpyrogenic O-antigenic oligosaccharide-protein conjugates. Infect. Immun. 1983, *41,* 888-895.

Lindgren J-Å, Kindahl H, Hammarstrom S. Radioimmunoassay of prostaglandins E_2 and $F_{2\alpha}$ in cell culture media utilizing antisera against prostaglandins $F_{2\beta}$ and $F_{2\alpha}$. FEBS Lett. 1974, *48,* 22-25.

[1] On leave from the Universidad de Chile, Facultad de Ciencias Veterinarias, Santiago, Chile.

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Reprints may be requested from: H. Kindahl, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden.