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Endotoxin Interaction with Canine Platelets Fails to Stimulate Thromboxane Production[1] (41364)

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Abstract. Endotoxin (0.2–20 μg/ml) induced a limited, dose-dependent change in light transmission when incubated with canine platelet-rich plasma. This response differed from that induced by collagen and ADP in its magnitude (ca. 25%) and by its insensitivity to a wide variety of inhibitors. However, endotoxin (20–1000 μg/ml) did not induce detectable aggregation of canine platelets in citrated whole blood under conditions where collagen and ADP were effective. In addition, thromboxane production was not stimulated. These data are consistent with the occurrence of an immune adherence reaction between endotoxin and canine platelets, as has been previously described for other species, but without platelet activation (aggregation, mediator, and thromboxane release).

During experimental endotoxin shock, circulating prostaglandin levels are elevated in dogs (1–4). In baboons, a dramatic increase in thromboxane B_2 (TXB_2) occurs during the acute phase of the shock state and there is a concurrent drop in circulating platelets and leukocytes and an increase in pulmonary artery pressure (5). Pretreatment of either dogs or baboons with indomethacin blocks the endotoxin-induced increase in circulating prostaglandins and the rise in pulmonary artery pressure but not the reduction in circulating platelets (3, 4, 6). Because the primary product of arachidonate metabolism in the platelet is thromboxane A_2 (TXA_2), a potent vasoconstrictor, a causal relationship between these three early events in endotoxin shock is suggested. The present study examines the direct effects of endotoxin on canine platelets in whole blood and platelet-rich plasma (PRP).

Materials and Methods. Blood, collected from adult mongrel dogs into tubes containing 3.2% trisodium citrate (9:1, v/v), was tested directly in an impedance aggregometer (7). This device measures changes in electrical impedance caused by aggregation of platelets on its electrodes.

PRP was prepared by centrifuging the anticoagulated blood at 120*g* for 5 min. Platelet aggregation in PRP was assessed by the light transmittance method (8) in a Beckman DU spectrophotometer with a water-jacketed cuvette holder (37°) and a magnetic stirrer modification (900 rpm). The sensitivity of this instrument was decidedly better than conventional instruments and permitted the reproducible measurement of small transmittance changes.

Following assessment of aggregation in the impedance aggregometer, blood samples were spun in a Beckman microfuge for 1 min and aliquots of plasma were diluted with a Tris buffer and incubated overnight at 4° with [^{3}H]TXB_2 (ca. 6000 cpm, New England Nuclear) and a specific antibody elicited in rabbits to a TXB_2–bovine serum albumin conjugate. Prostaglandin D_2 was the only major prostaglandin which significantly cross-reacted (8%) with this antibody (9, 10). Antibody-bound radioactivity in the supernatant was counted in a scintillation counter following treatment of the sample with a dextran-coated charcoal suspension and centrifugation.

The purified endotoxin used in these experiments was prepared from *Serratia marcescens* (08, No. 591) by published methods

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(11). A second *Escherichia coli* preparation was purchased from Difco (LPS B 0111:B4) and used for comparative purposes.

Results. Neither of the endotoxin preparations induced detectable platelet aggregation in canine whole blood anticoagulated with citrate. Incubations with endotoxin (1 mg/ml) for as long as 15 min produced no change in the impedance signal (Fig. 1). In contrast, collagen (5 μg/ml) and ADP (10 μg/ml) were maximally effective under the same conditions.

Significant stimulation of TXB_2 production was found in samples incubated with collagen (10 μg/ml) or arachidonic acid (200 μg/ml), but not with endotoxin (20 μg/ml) or ADP (10 μg/ml) (Table I).

In contrast, endotoxin (0.2–20 μg/ml) induced a dose-dependent increase in light transmittance when incubated with canine PRP (Fig. 2). The lag period preceding this change was independent of the endotoxin concentration. The maximum effect was generally observed with 10–20 μg/ml endotoxin and amounted to only 20–25% of the transmittance change normally produced by other platelet stimuli.

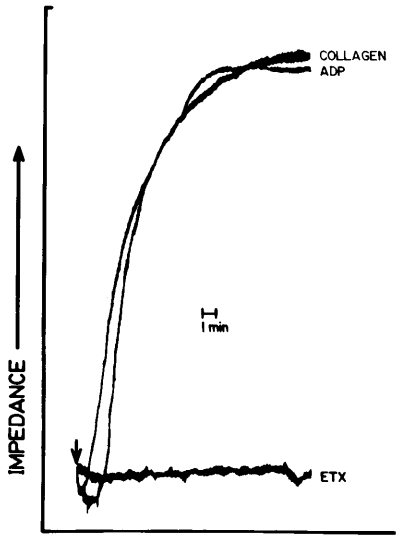

FIG. 1. Comparative effects of endotoxin (*Serratia marcescens*, 1 mg/ml), collagen (5 μg/ml), and ADP (10 μg/ml) on canine platelet aggregation as measured in a whole blood aggregometer. Similar results were obtained with an endotoxin preparation from *E. coli* (1 mg/ml).

The response to endotoxin was unaffected by several classes of platelet aggregation inhibitors. These included stimulants of platelet adenylate cyclase (prostacyclin (2.7 μ*M*) and adenosine (74 μ*M*)), inhibitors of arachidonate metabolism (indomethacin (112 μ*M*) and 5,8,11,14-eicosatetraynoic acid (100 μM)), neutralizers of released intracellular mediators (creatine phosphate/creatine phosphokinase (4.2 m*M*/16 U/ml) and methysergide (20 μ*M*)), and the local anesthetic lidocaine (8 m*M*).

Ethylenediaminetetraacetic acid (EDTA, 1 m*M*), a chelator of divalent metal ions, abolished the endotoxin effect. Increasing the concentration of citrate, whose anticoagulant activity is dependent on its ability to chelate calcium, also attenuated the effect. The concentration of citrate used in these experiments was selected because it maximized the response to endotoxin but prevented spontaneous platelet aggregation, which occurred at lower citrate concentrations. The low platelet yields achieved when heparin was tried as anticoagulant precluded its use.

Discussion. The present study demonstrates that the interaction between endotoxin and canine platelets differs from the aggregation produced by other platelet stimuli. Although transmittance changes were observed when endotoxin was incubated with PRP in a light aggregometer, the degree of the changes and size of the resultant aggregates differed from classical aggregation patterns. In the impedance aggregometer, endotoxin failed to induce a measurable response even at concentrations up to 100-fold greater than those which produced a maximal effect in the light aggregometer. In addition, several classes of platelet aggregation inhibitors did not materially alter the endotoxin effects. Thromboxane production, which generally serves as a measure of platelet activation, was not stimulated by endotoxin. Thus these studies suggest that endotoxin does not induce an aggregation of canine platelets.

Previous electron microscopic studies

TABLE I. EFFECT OF SELECTED STIMULI ON CANINE PLATELET THROMBOXANE PRODUCTION

Stimulus	Dose (μg/ml)	TXB_2 (ng/ml)	Aggregation
Saline	—	1.2	−
ADP	10	1.4	+
Collagen	10	31.5	+
Endotoxin[a]	20	1.4	−
Sodium arachidonate	200	44.4	+

Note. Citrated whole blood was stirred with the listed stimulus for 5 min in the whole blood aggregometer. The samples were treated and assayed for TXB_2 by the methods described. A 15-min incubation gave similar results.

[a] *Serratia marcescens* preparation.

have demonstrated that particulate endotoxin interacts with canine platelets at concentrations similar to those producing transmittance changes (12, 13). This interaction was judged to be an immune adherence phenomenon because the removal of plasma complement eliminated it (12). Additionally, the thrombocytopenia, which normally occurs upon administration of endotoxin to dogs, is not observed in decomplemented animals (14).

In contrast to its effect on canine platelets, endotoxin induces platelet aggregation of other immune-adherent-positive species like the rabbit and rat (15, 16). It is much less effective with guinea pig platelets except when the animals have been presensitized (17). Aggregation in these species generally occurs in two phases (16). The secondary phase is accompanied by granule secretion, is abolished by PGE_1 in combination with theophylline, and is blocked by inhibitors of prostaglandin biosynthesis. The primary phase of aggregation is not affected by these inhibitors but is inhibited by EDTA. In this respect the endotoxin interaction with canine platelets is strikingly similar to the primary aggregation observed for other species. In the dog, however, this interaction does not stimulate platelet thromboxane production, nor has it been found to induce platelet degranulation (12).

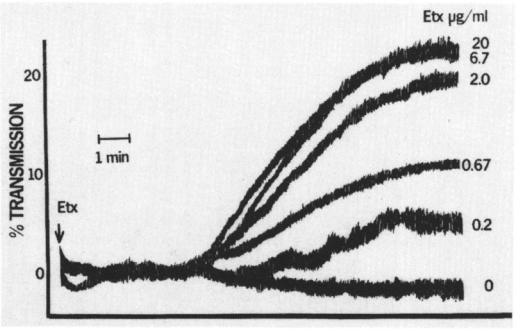

FIG. 2. Light transmission changes produced in canine platelet-rich plasma by graded doses of endotoxin (*Serratia marcescens*). Larger doses produced no greater response.

Platelets play an important role in the early cardiovascular complications associated with endotoxin shock. Intravenous endotoxin produces a dramatic reduction in circulating platelets in dogs (13, 18, 19). This is accompanied by the appearance of platelet microemboli in the lung and increases in pulmonary artery pressure. In thrombocytopenic dogs these sequelae are not observed (20, 21). Although the argument has been advanced that the mechanical obstruction produced by the microemboli is responsible for the pressure changes (22), this explanation has been largely refuted (23). Thus a humoral mechanism is suggested.

Of the platelet-derived vasoactive products, TXA_2 appears to be the best candidate as mediator of the pulmonary effects. Antagonists to histamine and serotonin do not significantly reduce the pressor response to endotoxin (24, 25), whereas indomethacin, an inhibitor of prostaglandin and thromboxane biosynthesis, prevents it (1, 4, 26). Additionally, elevations in circulating TXB_2 levels coincident with the pulmonary vascular changes have been observed in baboon endotoxin shock (5).

The source of the increased thromboxane

and the mechanism for its production remain unknown. The present studies demonstrate that it is not produced in stirred citrated samples and suggest that it is not the result of a direct action of endotoxin on platelets or other formed elements of the blood even though an interaction does occur. One possibility is that the entrapment of these platelet microaggregates in the pulmonary circulation produces a physical perturbation sufficient to activate the arachidonic acid cascade, thereby liberating TXA_2. Such a mechanism would permit the concentrated release of this potent vasoactive species near its postulated site of action. This is especially attractive because this metabolite is extremely short lived and therefore would be expected to produce its effects only over a very limited range. This hypothesis is currently under investigation.

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