PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 206–210 (1987)

Erythrocyte Deformability in Canine Septic Shock and the Efficacy of Pentoxifylline and a Leukotriene Antagonist (42536)

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Abstract. Decreased microcirculatory flow in the case of endotoxic shock has been extensively reported. The flexibility of the red blood cell plays an important role in tissue perfusion. The present study with a canine shock model was designed to investigate the changes in erythrocyte flow properties during septic shock. Deformabilities of canine erythrocytes were found to decrease considerably 6 hr after infusion of *Escherichia coli* organisms ($P < 0.05$). Pretreatment of dogs with pentoxifylline reduced the effect by improving erythrocyte deformability significantly and by increasing animal survival times. The leukotriene antagonist LY171883 also increased survival time, but did not change red cell filterability. The mortality of the dogs was not affected by either pentoxifylline or the leukotriene antagonist. Both drugs aided the concentration recovery of circulating leukocytes by 6 hr post-*E. coli* infusion.

Circulatory responses in dogs to endotoxin provocation are multifaceted. Complement activation, platelet and white cell aggregation, hypercoagulation, release of vasoactive agents, and altered cardiac output lead to poor tissue perfusion and maldistribution of flow, to organ dysfunction and death (1–5). Goslinga *et al.* (6), following the work of Pries and Gaethgens, suggest that the maldistribution of flow in shock not only results from changes in flow rate, hematocrit, and capillary diameter, but is also influenced by rheological factors (e.g., blood viscosity and erythrocyte aggregation). Blood viscosity is known to increase in *Escherichia coli* endotoxemia over shear rates ranging from 0.05 to 50 s^{-1} (5). While blood viscosity is sensitive to many factors, it reflects flow resistance when blood is considered a pseudo-homogeneous material (i.e., a pure fluid). This is not applicable to the microcirculation where the deformation properties of individual blood components or combinations of blood components must be considered.

It has been shown that erythrocyte deformability in rabbits decreases with endotoxic shock, using a microsieving technique (7); moreover, this change is countered by pentoxifylline, a drug which may have multiple effects but seems to increase red cell flexibility and lower fibrinogen concentrations (8). In a recent study by Bjornson, it was found that administration of pentoxifylline significantly decreases mortality during intraabdominal sepsis in the rat (9). Using a rat model of peritonitis, Chalkiadakis and co-workers observed increased survival rates with administration of pentoxifylline (10). Schade and Schonharting investigated the effects of pentoxifylline in endotoxin-induced shock in mice and they found that the survival rate was improved by 40% (11). In another study, Sugiura observed alleviation of shock induced flow disturbances in the mesocecum microcirculation of rats with the drug, but there was no reduction in the lethality of the toxin (7).

Much recent interest has focused on the mediators of shock, such as the eicosanoids which include prostaglandins and leukotrienes (29). Several of these agents are known to affect red cell rheology *in vitro*. The prostaglandin PGE_2, with pronounced increases in intravascular concentration during shock, reduces erythrocyte filterability (30). That prostacyclin is considered by some groups to be beneficial in shock coupled with a report of improved *in vitro* red cell flow characteristics by PGI_2 (31) warrants investigating the role of the red cell

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0037-9727/87 $1.50

and eicosanoids in contributing to hypoperfusion during shock.

We are reporting the effects of an LD_{100} dose of *E. coli* bacteria on erythrocyte deformability using a well-established canine shock model, in the presence and absence of pentoxifylline and a leukotriene antagonist. We have hypothesized that the red blood cell (RBC) contributes to the poor peripheral perfusion during shock because of changes in its deformability characteristics and have examined these changes during *E. coli*-induced shock. These findings are reported, together with deformability and survival rates following intravenous administration of pentoxifylline and a leukotriene antagonist, LY171883 in dogs (14).

Materials and Methods. Red cells were separated from whole-blood samples, washed with saline and Ringers solution (147.5 mmole/liter NaCl, 4 mmole/liter KCl, and 2.25 mmole/liter $CaCl_2$), and adjusted to a given concentration with Ringers solution using an electronic counter prior to determination of erythrocyte deformability. After the washings leukocyte concentrations were typically 200–500 WBC/mm^3. A constant volumetric flow rate filtration test of the cell suspension was used with 3 μm pore-size Nuclepore membranes (15). Pressure was monitored as an indicator of flow resistance. The pressure versus flow time curve had an initial transient region followed by a linear region with a small positive slope. Extrapolation of this linear portion back to zero time yielded the zero time pressure drop P_0. The transient portion of the curve resulted from a dilution effect of a small cavity in the filter holder, the response of the electronics and increasing shear rate due to pore plugging. The linear region resulted from the flow of the sample through the membrane and the plugging by leukocytes and large red cells. Packed red cell volume was measured in duplicate by microhematocrit determinations. Hemoglobin concentrations were determined by spectrophotometry.

The Student's t test was used to analyze the data and a paired test was used when the sample sizes were the same. A one-way analysis of the sample variances was done using the F statistic for each of the experimental series below.

In vivo studies. Fourteen mongrel dogs of either sex weighing an average of 26.4 kg were preconditioned over several weeks in the animal holding facility. Animals were fasted overnight before the experiment; in the morning, they were intravenously anesthesized with sodium pentobarbital (25 mg/kg) and intubated orally. Femoral arteries and veins were catheterized aseptically to sample blood, to infuse bacteria and drugs, and to monitor blood pressure and heart rate. After blood pressure stabilized, the dog was infused for 1 hr with live *E. coli* at a volume of 1 ml/kg (LD_{100}) (16, 17). At 60 min following the onset of *E. coli* infusion, all dogs were infused with the antibiotic gentamicin sulfate intravenously for 75 min at a dosage of 0.5 mg/kg with additional treatments at 3 and 6 hr at dosages of 1 mg/kg infused for 30 min. In the first group (control), saline solutions of volumes equal to the drug solutions were infused. Blood samples for the deformability test were collected before anesthesia, prior to infusion of *E. coli,* and at 1 and 6 hr after infusion of *E. coli.* A second group was pretreated with pentoxifylline, (supplied by Hoechst–Roussel Pharmaceuticals) at 24, 12, and $\frac{1}{2}$ hr before infusion of *E. coli* with a dosage of 5 mg/kg. In a third group of animals, the leukotriene antagonist LY171883(Eli Lilly (14)) was pre- and postinfused in several variations at dosages of 10–30 mg/kg. When one-fourth of the *E. coli* was infused (15 min), a 345-min infusion of the antagonist was initiated. Following postmortem examination any animal with adult heart worms was eliminated from the study. Deformability data were analyzed using the t test at the 95% confidence level.

Results. Decreased filterability of erythrocytes from dogs with time after infusion of bacteria was observed and confirmed by analysis of variances (Table I). This conclusion is not valid for animals treated with pentoxifylline. Further, the administration of pentoxifylline reduced or eliminated the effects of *E. coli* on the altered flow properties after 6 hr ($P < 0.05$). No significant difference was observed between samples drawn 30 min before infusion and at time of infusion, indicating that animals were indeed stabilized and that there was no measurable effect of the anesthetic on deformability for this time period.

TABLE I. RESULTS OF RBC FILTRATION STUDIES, INITIAL PRESSURE DROPS[a]

Time	*E. coli* control	Pentoxifylline + *E. coli*	LY171883 + *E. coli*	LY171883 only
−30 min	27.7 ± 4.7 (3)[b]	27.0 ± 3.5 (4)[b]	26.8 ± 2.6 (4)[b]	28.5 (1)
0	29.1 ± 3.6 (3)	28.9 ± 3.0 (4)	25.6 ± 5.5 (6)	28.5 (1)
1 hr	30.7 ± 4.7 (3)[b]	33.0 (2)	33.9 ± 6.4 (5)[b]	28.0 (1)
6 hr	37.8 ± 4.7 (3)[c]	31.4 ± 5.1 (4)[b]	38.2 ± 2.6 (6)[c]	29.0 (1)

[a] All values are means (mm Hg) ± SD with the number of independent runs in parentheses. The *P* values are obtained from the Student's *t* test, in a comparison with zero time.
[b] Values are not statistically different from control values, $P > 0.05$.
[c] Values are statistically different from control values, $P < 0.05$

All animals died whether given pentoxifylline or not, but survival times increased from 7 to 10 hr for dogs given only the antibiotic, to 10 to 40 hr for dogs given antibiotic plus pentoxifylline. Moreover, survival times in the limited number of studies appeared to correlate with deformability changes ($r = 0.91$, $P < 0.05$).

In a single study, LY171883 given alone did not affect erythrocyte deformability, nor in a set of six experiments did it lessen the effects of sepsis on red cell deformability (see Table I). Survival times generally increased in the antagonist studies to 8, 9, 12, 36, and 42 hr with one additional animal surviving and being sacrificed at 7 days. There was no correlation of survival time and erythrocyte deformability for the leukotriene antagonist.

Platelet and white cell counts in dogs decrease markedly with *E. coli* sepsis (18); however, with pentoxifylline and LY171883, the initially lowered average concentrations of leukocytes seemed to recover to a much greater degree after 6 hr (Table II), though not in a statistically significant way. Mean cell volumes (MCV) and mean cell hemoglobin concentrations (MCHC) were constant during the course of the deformability experiments; hematocrits rose from an initial value of 39% to values of 50–55% for *E. coli* only and for *E. coli* and pentoxifylline or leukotriene antagonist.

Discussion. The diminished filterability of canine red cells in endotoxin shock is interesting in that the altered flow properties of the erythrocyte may exacerbate many known effects of shock and should further contribute to the maldistribution of blood flow and poor peripheral perfusion. An observation from preliminary studies in our laboratories that the addition of endotoxin to citrated human whole blood, and incubation at 37°C, do not change the filterability of isolated erythrocytes relative to a matched control intimates that the *in vivo* phenomenon of reduced flexibility is secondary to another mechanism. Endotoxin is known to interact with the red cell membrane (12, 13).

Pentoxifylline has been widely tested primarily for its action in improving blood flow (19–25). The drug clearly countered the altered deformability in canine endotoxin shock. Though survival rates were similar to that of

TABLE II. WHITE BLOOD CELL CONCENTRATIONS[a] (THOUSANDS/mm^3) FROM DOGS INFUSED WITH *E. coli*

	Time from *E. coli* infusion (min)			
Sample	−30	0	60	360
E. coli	12.6 ± 2.3 (3)	8.6 ± 0.7 (3)	1.0 ± 0.4 (3)	2.9 ± 1.2 (3)
+Pentoxifylline	11.2 ± 3.1 (4)	11.2 ± 2.8 (4)	1.9 ± 0.9 (4)	6.0 ± 4.6 (4)
+LY171883	10.9 ± 3.4 (6)	7.7 ± 3.1 (5)	1.2 ± 0.9 (5)	5.7 ± 4.5 (6)

[a] Errors are standard deviations with the number of independent runs in parentheses. LY171883 refers to the leukotriene antagonist.

an LD_{100} dose of *E. coli,* survival times were lengthened. Earlier studies in rats and mice in which survival rates were improved were carried out at a lower endotoxin dose which may explain the difference in our results. Our findings are consistent, but not conclusive, with the concept that pentoxifylline should improve tissue perfusion in shock. Pentoxifylline also causes a rapid recovery of previously sequestered white blood cells; previous work from this laboratory has suggested a possible relationship between numbers of white cells, particularly neutrophils, and survivability (18).

The role of leukocytes also extends to the microcirculation in shock. With a feline model of hemorrhagic shock, Bagge and co-workers observed capillary plugging by leukocytes which was attributed to lower perfusion pressure coupled with the normal stiffness of white cells (26). White cell losses in the canine septic shock model might also indicate capillary trapping. The observation of decreasing concentration of leukocytes is also important because the presence of white blood cells is a known complication in filtration studies of red cells (27). Nonetheless, increasing filtration pressure during endotoxic shock runs counter to decreasing white cell concentrations in the dog. This seems to support our interpretation of impaired red cell deformability. These factors are relevant because the filterability of monocytes and polymorphonuclear neutrophils (PMNs) has been shown to increase with pentoxifylline (28) so that it cannot be stated unequivocally that red cell deformability was improved by the drug. Consequently, extended survival times may be due to the effect of pentoxifylline on erythrocytes or leukocytes or both.

Solely on the basis of the deformability measurements with the leukotriene antagonist alone, the eicosanoid leukotriene D_4 would appear not to be a mediator of shock. LY171883 did extend survival times variably; however, for the antagonist, there was no relation between survival time and the filtration measurement. Hence, if survival times increased with pentoxifylline because of its effects on the red cell, its mode of action differs from that of the antagonist. The mechanism of altered filterability may not be uniquely identified with the eicosanoids. Catecholamines also rise in shock and, among these, epinephrine extends filtration times for erythrocytes (30). Further, Torsellini *et al.* has found that an agent released from activated platelets reduces red blood cell flexibility (32). The mediator(s) of decreased erythrocyte flow properties remains to be identified.

In conclusion, red cell deformability decreases in canine *E. coli*-induced shock which may contribute to poor tissue perfusion. Pentoxifylline acts to counter the effect but the leukotriene inhibitor LY171883 does not. Both agents increase animal survival times and improve circulatory white blood cell recovery but do not increase survival rates in shock at LD_{100} challenges with *E. coli.*

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Received September 25, 1986. P.S.E.B.M. 1987, Vol 185.
Accepted March 4, 1987.