Interspecies Variation in the Cellular Phase of Blood Fibrinolytic Activity (43187)

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Abstract. **In normal humans, whole blood fibrinolytic activity is three to six times greater than that of companion plasma. This additional activity derives from neutrophil enzymes, with possible contributions from other cell types. Rats and dogs are frequently used to study fibrinolysis in animal models of human disease. Compared with humans, rats are relatively neutropenic, whereas dogs have a relative neutrophilic leukocytosis. Interspecies variation in cellular phase fibrinolytic activity has not been examined. We therefore determined whole blood, plasma, and cellular phase fibrinolytic activity in 27 rats and 6 dogs, using a ^{125}I-fibrin solid phase assay. Whole blood and plasma activities were similar in rats, consistent with very low cellular activity. Dogs, however, had high cellular phase activity, making up an average 91% of whole blood activity. These results suggest that blood fibrinolytic mechanisms in rats differ from those in humans and dogs, and that this difference should be considered when studying fibrinolysis in models of human disease.** [P.S.E.B.M. 1991, Vol 196]

When fibrinolytic activity is determined in normal humans by methods capable of distinguishing activities of whole blood and plasma, the activity measured in blood is in considerable excess of that found in companion plasma, indicating the existence of a large cellular phase component in addition to the well-known plasma component (1). This cellular component, representing approximately 70–80% of the activity measurable in blood (2–6), appears to derive largely from neutrophils, a rich source of the fibrinolytic enzymes, elastase, and cathepsin G (1).

Rats are commonly employed in animal models of human disease, many of which involve disturbances in fibrinolysis (7–10). It is therefore relevant to determine whether the pattern of cellular and plasma phase activity in rats is similar to that seen in humans. Interspecies variation in fibrinolytic activity has been studied in humans, rats, rabbits, and dogs, but such studies have been restricted to examination of plasma activity (11–16), with no reported studies of the relative contribution of cells and plasma to blood fibrinolytic activity in species other than humans.

In rats, lymphocytes are the predominant leukocyte in blood, contrasting with the preponderance of neutrophils in the blood of normal humans. Because of the neutropenia of normal rats relative to humans, and the importance of neutrophils in blood activity in humans, we have determined the contributions of cellular and plasma phases to blood fibrinolysis in normal rats, using a solid phase radiofibrin assay (6, 17) capable of discriminating these phases, and have examined the relationship between total and differential leukocyte counts and cellular phase activity. We have also determined the relative contributions of cells and plasma to fibrinolytic activity in mongrel dogs.

Materials and Methods

Subjects. Twenty-seven male Sprague-Dawley rats (CD-VAF; Charles River, Lacolle, Quebec, Canada) were studied. Their weight ranged from 220 to 440 g (mean, 331 g). In addition, venous blood samples were available from six adult mongrel dogs. All animals were given food and water *ad libitum* and handled in accordance with the guidelines of the Canadian Council on Animal Care.

Measurement of Fibrinolytic Activity. The rats were anesthetized with an intraperitoneal injection of pentobarbital. While breathing but deeply comatose,

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their abdomens were opened and 4–7 ml of blood were withdrawn from the inferior vena cava into a plastic syringe containing heparin (10 units/ml blood; Leo Laboratories, Pickering, Ontario, Canada). In the dogs, blood was drawn from a forepaw vein into a plastic syringe containing heparin, with use of a tourniquet for less than 10 sec. Aliquots of this blood were immediately transferred to EDTA containing evacuated tubes (Vacutainer; Becton Dickinson, Mississauga, Ontario, Canada) for subsequent cell counting. With the remaining heparinized blood, fibrinolytic activities of the whole blood and platelet-poor plasma (centrifugation, 800*g* for 15 min) were measured, using ^{125}I-fibrin solid phase assay, as described elsewhere (6, 17). Results of quadruplicate measurements were expressed as ng of fibrin lysed/hr. Cellular phase activity was calculated as the difference between whole blood and plasma activities. All assays were begun within 20 min of blood sampling.

Cell Counts. Total and differential leukocyte counts, and platelet counts and hematocrit, were measured using the Coulter model S Plus counter (Coulter Electronics, Hialeah, FL). Differential leukocyte counts were also confirmed on random samples by manual counting of blood smears. The rats were killed by exsanguination through the inferior vena cava, immediately after blood sampling.

Statistical Analysis. Results for the group are presented as mean ± SD. To examine for relationships between cell counts and fibrinolytic activities, least squares linear regression was performed.

Results

Cell Counts. The mean leukocyte count for the rats was 7.9 × 10^9/liter ± 2.9 SD, and the mean granulocyte count was 0.52 × 10^9/liter ± 0.4 SD (Fig. 1). Three rats had granulocyte counts greater than 1.0 × 10^9/liter. They were not all of the same age or weight and were otherwise indistinguishable from their littermates. The mean leukocyte count for the dogs was 11.4 × 10^9/liter ± 3.1 SD, and their mean granulocyte count was 6.1 × 10^9/liter ± 2.1 SD.

Fibrinolytic Activities. The mean whole blood activity for the rats was 108.9 ± 21.9 (SD) ng of fibrin lysed/hr and the mean plasma activity was very similar to that of whole blood, at 106.9 ± 15.2 ng of fibrin lysed/hr (Fig. 2). Mean calculated cellular phase activity was 2.0 ± 23.0. Thirteen rats had calculated cellular phase activities greater than zero, with six having greater than 20 ng of fibrin lysed/hr. This cellular activity, however, did not represent more than 30% of whole blood activity in any of the six rats. Fourteen of the calculated cellular phase activities were less than zero, indicating lower activity in whole blood as compared with that in an equal volume of companion plasma. The mean ratio of whole blood to plasma activity was 1.0 ± 0.2 (Fig. 3), with four rats having a ratio <0.8 and six rats having a ratio >1.2.

The mean whole blood activity in the dogs was 136 ± 16 (SD) ng of fibrin lysed/hr, with a much lower mean plasma activity of 12 ± 1 SD (Fig. 2). The mean calculated cellular phase activity made up 91% of whole

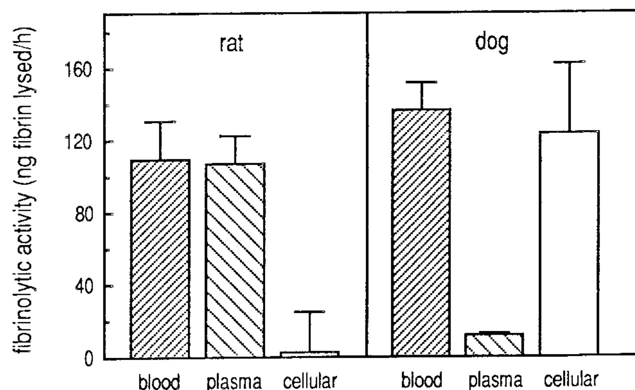

Figure 2. Whole blood, plasma, and calculated cellular phase fibrinolytic activities of 27 normal rats and 6 normal dogs, expressed as ng of fibrin lysed/hr. Bars are mean ± SD.

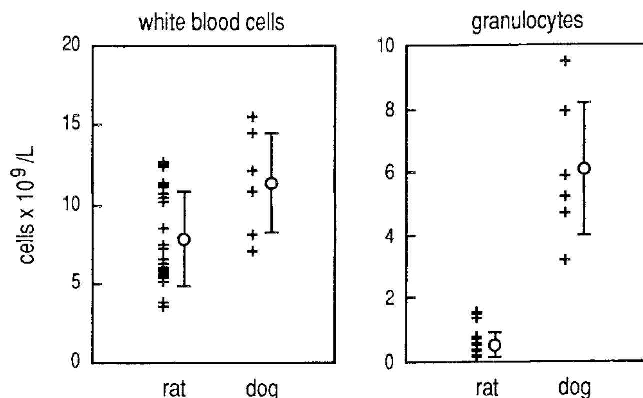

Figure 1. Leukocyte (left) and granulocyte (right) counts in the blood of 27 normal rats and 6 normal dogs. Bars are mean ± SD.

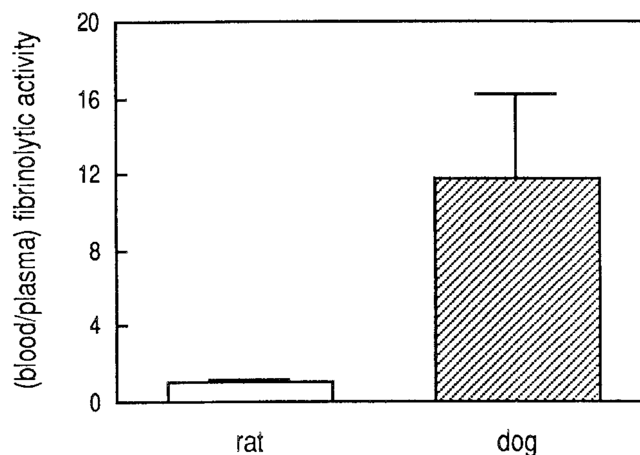

Figure 3. Ratio of blood to plasma fibrinolytic activity in normal rats and dogs. Bars are mean ± SD.

blood activity in the dogs, with a mean blood to plasma ratio of 11.7 ± 4.5 SD (Fig. 3).

There were poor correlations between white blood cell counts (total or neutrophil) and fibrinolytic activities (blood, plasma, or cellular), with r values <0.30 in all comparisons.

Discussion

These data show that rats, unlike humans and dogs, have very low cellular phase fibrinolytic activity in blood, since the majority of the activity measurable in rat blood (over 66% in all animals studied) could be accounted for by activity in the plasma phase. In view of the importance of neutrophils in cellular phase activity (1), this undoubtedly reflects the relative neutropenia of rats compared with humans. We did not examine the fibrinolytic activity of purified neutrophils, and it is possible that low activity of individual neutrophils may also contribute, although interpretation of such a study would have to be tempered by consideration of the functional activation of neutrophils resulting from their manipulation during purification. The poor correlation between cellular phase activity and neutrophil count may indicate that variable degrees of neutrophil activation may occur. However, even in the rat with the highest cellular activity, cellular phase activity represented only 33% of blood activity.

Elastase is the major fibrinolytic enzyme released from neutrophils (1). Our studies of cellular fibrinolytic activity were performed in relatively unmanipulated whole blood, which encompasses plasma, and its individually variable concentration of α_1-proteinase inhibitor (α_1-antitrypsin), which is the primary inhibitor of elastase. We did not measure this inhibitor directly, and it is possible that high levels in rat blood could also contribute to the low measured cellular phase activity. Regardless of the mechanisms involved, it is evident that rats have low cellular phase fibrinolytic activity in their blood.

We have also examined another species, the dog, which has a white blood cell differential count pattern closer to that of humans than does the rat. The dogs had predominantly cellular phase activity, as do humans. The extreme differences in granulocyte levels and in cellular activity between rats and dogs suggests that, at least in healthy animals, the granulocyte count is the major determinant of cellular activity.

The results of this study suggest that blood fibrinolytic mechanisms are different in rats and humans and that these differences must be considered when using rats to examine fibrinolysis in models of human disease.

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