**NOTE** *Pharmacology*

# Comparison of the Blood Coagulation Profiles of Ferrets and Rats

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ABSTRACT. The aim of this study was to examine the blood coagulation profiles of ferrets and compare them with those of rats. The ferret activated partial thromboplastin time (aPTT) was slightly longer than the rat aPTT. In contrast, the ferret prothrombin time and thrombin time were profoundly shorter than the corresponding rat values. The fibrinogen level in ferret plasma was 2 times higher than that in rats. Heparin prolonged all blood coagulation times in a concentration-dependent manner in both ferret and rat plasma. A significantly ($P$<0.01) higher concentration of heparin was required to double the aPTT in ferrets than rats. These blood coagulation data for ferrets will be useful in experimental animal studies.

KEY WORDS: blood coagulation, ferret, fibrinogen, heparin, rat.

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The ferret has been used as an experimental animal in a wide variety of studies including bacteriology, virology, physiology, toxicology, and pharmacology studies [2]. In particular, the ferret is used as a gold standard animal model of nausea and vomiting [6]. In addition, ferrets have also become a very popular mammalian pet species. In veterinary clinical settings, ferrets are susceptible to a number of diseases that may be associated with hemostatic disorders, including hepatic disease, endocrine disorders, and neoplasia [5, 10, 16]. Determination of the blood coagulation profiles of healthy ferrets would facilitate the detection and monitoring of coagulopathies and drug effects in this species. However, there is limited information available about the blood coagulation values of ferrets [1, 8].

Prothrombin time (PT), activated partial prothrombin time (aPTT), and thrombin time (TT) are the most commonly used clotting time assays in mammals. PT, aPTT, and TT assess the function of the extrinsic pathway, the intrinsic pathway, and the common pathway, respectively [15]. The aim of our study was to examine the blood coagulation profiles of ferrets. We compared the blood coagulation times of ferrets with those of rats, one of the most widely used experimental animals. We also compared the anticoagulant activity of unfractionated heparin (heparin), which is currently the most widely used anticoagulant in experimental and clinical settings, between the two species.

All study protocols were approved by the Animal Research Committee of Azabu University. Ferrets (*Mustela putorius furo*, 6 females, 1 year of age) and rats (Sprague-Dawley strain, 5 females and 5 males, 10 weeks of age) from Japan SLC Inc. (Shizuoka, Japan) were utilized. The animals were housed in climate controlled rooms under a 12 hr dark and light periods and allowed free access to food and water.

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Blood samples were collected from the cranial vena cava of the ferrets after they had been anesthetized with ketamine (20 mg/kg, *im*, Fujita Pharmaceutical Co., Ltd., Tokyo, Japan) and the abdominal aorta of the rats after they had been anesthetized with pentobarbital (50 mg/kg, *ip*, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). The blood samples were collected in a syringe containing 0.129 M sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant. The samples were centrifuged at 2,000 × $g$ for 15 min, and the platelet poor plasma was immediately separated from the cells and frozen at –80°C until the assays. Plasma coagulation times were determined within 4 weeks of collection. Our previous studies confirmed that plasma clotting times were stable (mean difference, < 5%) at least until 4 weeks after the plasma collection when the plasma was stored at –80°C.

Coagulation assays were performed with citrated plasma obtained from the ferrets and rats using a STart4 analyzer/reagent combination (Diagnostica Stago, France, distributed by Roche Diagnostics KK, Tokyo, Japan) according to the manufacturer's instructions. PT was measured by mixing plasma (50 $\mu l$) that had been preincubated at 37°C for 2 min with PT reagent supplemented with calcium (Neoplastin® Cl plus; 100 $\mu l$) at 37°C. aPTT was measured by mixing the plasma (50 $\mu l$) with reagent 1 (PD®; 50 $\mu l$) at 37°C for 3 min. Clot formation was initiated by adding 50 $\mu l$ of 0.025 M $CaCl_2$ solution. TT was measured by diluting plasma (100 $\mu l$) with Owren's diluent buffer (Fibri-Prest®) at a ratio of 1:19, incubating it at 37°C for 1 min, and then adding thrombin reagent (STA®-Thrombin; 50 $\mu l$, 50 NIH concentration). Fibrinogen was calibrated from the TT assay (i.e., the Clauss method) [3] using 1:10, 1:20, and 1:40 dilutions of control plasma (Coag Control N+P®). All assays were performed in duplicate, and mean values were calculated.

We determined the responses of ferret and rat plasma to heparin (heparin sodium, Mitsubishi Tanabe Pharma Corp., Tokyo, Japan) according to the method of Leblond *et al*. [7]. Briefly, plasma (80 $\mu l$) that had been preincubated with var-

Table 1. Plasma clotting times and fibrinogen concentrations of ferrets and rats

| Species | PT (sec) | aPTT (sec) | TT (sec) | Fibrinogen (mg/d*l*) |
|---|---|---|---|---|
| Ferrets | 11.3 ± 0.4** (10.9–12.0) | 17.0 ± 1.2 (15.3–18.7) | 13.7 ± 2.7** (9.4–16.9) | 486.7 ± 97.9** (382.5– 658.3) |
| Rats | 18.3 ± 1.0 (17.1– 20.0) | 15.8 ± 0.5 (15.2–16.7) | 27.9 ± 4.8 (18.8– 34.0) | 247.3 ± 45.9 (200.6– 346.5) |

Results are expressed as the mean ± SD (minimum—maximum) for 6 ferrets (all females) and 10 rats (5 females and 5 males). **, indicates $P<0.01$ versus rats.

ious concentrations of heparin (20 $\mu l$) or saline (control) for 1 min at 37°C was used for the coagulation assays. PT and aPTT were measured as described above. To measure TT, coagulation was induced with the addition of thrombin at a lower concentration (9 NIH units/m*l*) to determine the anti-thrombin activity of heparin. The anticoagulant activity of heparin was evaluated by determining the concentrations required to double each plasma clotting time ($PT_2$, $aPTT_2$, and $TT_2$).

Data are expressed as the mean ± SD and minimum–maximum values. Statistical analysis was performed using the Student's *t*-test. Differences were considered significant at $P<0.05$.

We obtained the following data (mean ± SD) from the ferrets: prothrombin time (PT): 11.3 ± 0.4 sec, activated partial thromboplastin time (aPTT): 17.0 ± 1.2 sec, thrombin time (TT): 13.7 ± 2.7 sec, and fibrinogen concentration: 486.7 ± 97.9 mg/d*l*, as shown in Table 1. Compared with the rat values, the ferret aPTT was slightly ($P>0.05$) longer, and the ferret PT and TT were significantly ($P<0.01$) shorter. The fibrinogen level in ferret plasma was two times higher than that present in rat plasma ($P<0.01$, Table 1). No statistically significant difference was found in PT, aPTT, or fibrinogen values between female and male rats.

To determine the anticoagulant activity of heparin, plasma samples from ferrets and rats were preincubated with various concentrations of heparin before clotting was initiated. Heparin prolonged all coagulation times tested in a concentration dependent manner (Fig. 1). The aPTT assay was most sensitive to the effects of heparin, followed by the TT and PT assays in both ferrets and rats. $PT_2$, $aPTT_2$, and $TT_2$ were measured as the heparin concentration required to double each baseline clotting time. As shown in Table 2, the $PT_2$ and $TT_2$ values of ferrets and rats were comparable, but the $aPTT_2$ of the ferrets (0.14 ± 0.01 IU/m*l*) was slightly but significantly ($P<0.01$) higher than that of the rats (0.10 ± 0.02 IU/ m*l*). No statistically significant difference was found in $PT_2$, $aPTT_2$, or $TT_2$ values between female and male rats.

Blood coagulation testing is a common laboratory assay, and a number of different coagulation analyzers are currently commercially available. However, the clotting time assay is known to be sensitive to changes in individual reagents and analyzers [9, 11, 13]. There are two main types of clot detection systems: photo-optical clot detection systems and electro-mechanical clot detection systems. We

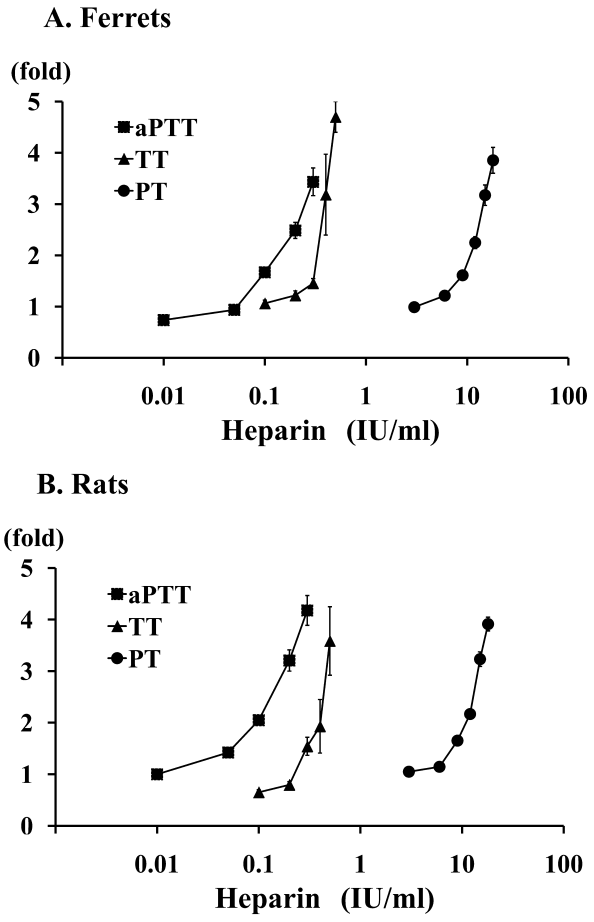


Fig. 1. Effects of heparin on the plasma clotting times (PT, aPTT, and TT) of ferrets (A) and rats (B). Results are expressed as fold values compared with the basal plasma clotting times (PT, aPTT, and TT), and represent the mean ± SD for 6 ferrets (all females) and 10 rats (5 females and 5 males).

used an electro-mechanical coagulation analyzer in our study, which measures the clot induced reduction in the movement of a steel ball immersed in a plasma sample and subjected to an alternating electromagnetic field. Thus, our data is unlikely to be affected by variables that affect light transmission, such as hyperbilirubinemia or lipemia. As far as we know, this is the first report about the measurement of ferret blood coagulation times to use an electro-mechanical

Table 2. Comparison of $PT_2$, $aPTT_2$, and $TT_2$ between ferrets and rats

| Species | $PT_2$ (IU/m*l*) | $aPTT_2$ (IU/m*l*) | $TT_2$ (IU/m*l*) |
|---|---|---|---|
| Ferrets | 11.1 ± 0.9 (10.1–12.6) | 0.14 ± 0.01** (0.13– 0.15) | 0.35 ± 0.03 (0.32–0.40) |
| Rats | 11.1 ± 0.5 (10.3–12.0) | 0.10 ± 0.02 (0.07–0.13) | 0.38 ± 0.08 (0.26–0.48) |

Results are expressed as the mean ± SD (minimum—maximum) for 6 ferrets (all females) and 10 rats (5 females and 5 males). **, indicates $P<0.01$ versus rats. $PT_2$, $aPTT_2$, and $TT_2$ is the heparin concentration required to double the basal value of PT, aPTT, and TT, respectively.

system.

The ferret PT and aPTT values found in our study were similar to the previously reported reference values for ferrets [1, 8]. However, the ferret fibrinogen value (486.7 ± 97.9 mg/d*l*) detected in our study was quite different from the previously reported value (107.4 ± 19.8 mg/d*l*) [1]. This discrepancy might have been due to the different assays used because the fibrinogen measurement was performed with the PT-fibrinogen method using a photo-optic analyzer in the previous report [1] and by the Clauss method with an electro-mechanical analyzer in our study. Although fibrinogen is an acute phase protein whose levels are elevated in infectious and other inflammatory diseases, pregnancy, and myeloproliferative disorders [4], the ferrets used in the present study were clinically healthy and non-pregnant. Thus, the high fibrinogen level found for ferrets in our study is unlikely to be due to pathophysiological factors. The fibrinogen levels of the Sprague-Dawley rats used in our study were similar to the previously reported reference values for Sprague-Dawley rats [14], which were measured by the Clauss method in a photo-optic analyzer. Therefore, it is noteworthy that the fibrinogen levels of the ferrets were higher than those of Sprague-Dawley rats assayed using the same method.

Our study provides new data about the *in vitro* anticoagulant activity of heparin in ferrets and rats. In the present study, heparin prolonged all clotting times tested, with a sensitivity order of aPTT>TT>>PT, which is in line with the concept that the aPTT is highly sensitive to heparin, and is thus suitable for monitoring the anticoagulant effects of heparin in clinical settings [12]. The concentration of heparin required to double the baseline value of aPTT ($aPTT_2$) in ferrets was slightly but significantly ($P<0.01$) higher than that in rats (Table 2). A slightly higher dose of heparin may be required to exert anticoagulant effects in ferrets. The reason why the $aPTT_2$ values differed significantly between ferrets and rats is unclear, but one possible explanation is a difference in the affinity of heparin for antithrombin or the intrinsic factor tenase complex between the two species.

This study describes the blood coagulation profiles of female ferrets and compares it with those of rats. These blood coagulation data for ferrets will be useful in experimental animal studies. In a further study, it should be examined whether there is a sex difference in the coagulation profiles of ferrets.

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