Gender and inter-species influence on coagulation tests of rats and mice

Cristina Lemini⁎, Ruth Jaimez, Yanira Franco

Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, Apartado Postal 70-297, CP 04510, D. F., Mexico

Received 21 June 2005; received in revised form 19 October 2006; accepted 23 October 2006
Available online 5 December 2006

Abstract

Introduction: Rats and mice have been used to evaluate effects of natural and synthetic oestrogens. However, data about oestrogen's effects on haemostasis in rodents is very limited. The aim of this work was to standardize blood coagulation screening tests in adult male, female, and ovariectomized (Ovx) Wistar rats and CD1 mice in an effort to evaluate the influence of gender and species differences on haemostasis.

Materials and methods: Values were obtained for the following haemostatic parameters: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin clotting time (TT), and fibrinogen (FIB), through modifications of the conventional techniques used for human blood coagulation analysis.

Results: Both rats and mice showed gender intra-species and inter-species differences of high significance in PT, aPTT, TT, and FIB values. Intra-species differences were found in TT (+10% \( p<0.01 \)) and FIB concentration (−21% \( p<0.001 \)) between male and Ovx rats. Male vs. Ovx mice showed a TT difference of −20% (\( p<0.001 \)). The main inter-species differences found were PT values of male rats vs. male mice (−39%) and female rats vs. female mice (−35%, both \( p<0.001 \)). Female rats and mice aPTT values vs. those corresponding to Ovx animals showed differences of +15% and +32% (\( p<0.001 \), respectively.

Conclusions: These data reveal the great importance of gender intra- and inter-species differences on the values of haemostatic screening tests, which should be taken into consideration when evaluating the effects of oestrogens and other drugs on the coagulation system.

© 2006 Elsevier Ltd. All rights reserved.

KEYWORDS
Gender; Coagulation tests; Inter-species; Rats; Mice

Introduction

Epidemiological studies have found important evidence of increased risk in thromboembolic diseases among women consuming oestrogens for...
contraception or hormonal replacement therapy [1–4], and only few clinical methods and appropriate markers are available to accurately diagnose and prevent embolism. Human prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen concentrations (FIB) are some of the most common screening parameters assayed allowing to explore the status of the main coagulation pathways [5]. Research of the in vivo and in vitro models to understand the mechanisms in which oestrogens are involved to activate blood coagulation is still insufficient [6–14]. In this work we studied the screening tests, PT, aPTT, TT, and FIB, in male, female, and ovariectomized (Ovx) rats and mice in an effort to evaluate the influence of gender and species differences on haemostasis.

Materials and methods

Animals

All experimental studies were conducted in accordance to the Mexican National Protection Laws of Animal Welfare (NOM-062-Z00-1999). Adult male and female Wistar rats (200 to 300 g), adult CD1 male and female mice (25–30 g) were bred in our animal housing facilities. The animals were kept at constant temperature (20–22 °C) in a room with 12 h–12 h light–dark cycle and maintained on standard chow (Nutricubos, Purina) and water ad libitum.

Ovariectomy

Female Wistar rats and CD1 mice were ovariectomized (Ovx) under chloral hydrate anaesthesia and the haemostatic parameters were evaluated 21 days after ovariectomy.

Experiments

Blood collection

Different groups (N=10–15 animals per group) of male and female rats and mice and Ovx animals were caged according to each condition until blood samples were drawn. Animals were anaesthetized with chloral hydrate (4% solution, 7 ml/kg) prior to blood withdrawal. Each animal was placed on its back on a cork surgery table and restrained with string fixed at the corners. Arterial blood was collected by suctioning from the iliac bifurcation with a one way plastic syringe and disposable-gauge needles (rats, 21 × 32 mm; mice, 27 × 13 mm), which provided free of haemolysis blood samples for rats (4–7 mL) or mice (0.5–0.6 mL). Blood was immediately drawn out into plastic tubes containing 0.11 M sodium citrate (1:9, v:v). The samples were gently mixed and centrifuged at 2500 xg for 10 min at 4 °C. Plasma samples were separated, frozen and stored at −80 °C until assays were performed.

Haemostatic parameters

The coagulation screening tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen concentrations (FIB), were performed by modifications of the conventional clinical procedures. For all clotting tests, Dade® Behring reagents were used and assayed according to instructions in the package insert. Reference curves and accuracy controls were set up using the corresponding control plasma of the manufacturer. PT, aPTT, and TT clotting times were recorded using a Behring Fibrintimer II (Dade® Behring). Plasma samples were warmed at 37 °C for 60 s before the addition of the indicated reagent. Clot formation was the end point of the reactions and results are reported in seconds (s).

PT determination was performed according to the technique described by Quick [15] using rabbit brain thromboplastin C plus (Dade® Behring). Plasma samples (50 μl) were pipetted into cuvettes and then 100 μl of thromboplastin C was added to activate the reaction. aPTT was assessed following Proctor’s method [16] using the Actin FS reagent (Dade® Behring) containing purified soy phosphatides. Actin FS (100 μl) was added to plasma samples (50 μl). The mixture was incubated at 37 °C for 120 s and 50 μl of 0.02 M CaCl2 (37 °C) was added to activate the reaction. TT was determined by a modification of Rampling’s techniques [17] using Bovine thrombin (Dade® Behring) in a concentration of 5 IU/ml. Plasma samples (50 μl) were treated with 50 μl of bovine thrombin to activate the reaction. FIB concentration was evaluated by Clauss’ coagulometric technique [18] The mechanical fibrometer Fibrosystem Becton-Dickinson Mod 5–117 V was used for the determination. Bovine thrombin (100 IU/ml; Dade® Behring) was added to plasma samples (50 μl) to induce clot formation. The FIB concentration was obtained from a reference curve calibrated with human plasma fibrinogen and reported in milligrams per deciliter (mg/dL).

Statistical analysis

Statistical significance among groups was assessed by variance analysis (ANOVA). The significance of the differences among groups was estimated by Mann–Whitney test [19]. Analysis between Wistar and CD1 values was performed using the Sigma Stat
3.1 program. Results were expressed in means ± standard error (SEM). Values of \( p < 0.05 \) were considered statistically significant.

**Results**

Information in Table 1 shows the mean ± SEM; the minimal–maximal values and the coefficient of variation of the data obtained for PT, aPTT, TT, and FIB from rats, mice (male and female), and Ovx animals.

**Gender differences of the screening tests**

The comparisons of PT, aPTT, TT, and FIB values obtained in rats and mice are depicted in Table 2. Male and female rats showed very close PT values, although higher in females (+8.9%; \( p < 0.05 \)). The PT values difference between male and female mice was higher and more significant than that of rats (+16%; \( p < 0.01 \)). In contrast, the aPTT values for female rats and mice were 12% and 27.6% lower with respect to males, respectively (\( p < 0.001 \)). TT values were less affected, those from female rats were 4.7% higher (\( p < 0.05 \)) than those from males. Female mice showed a 7.7% decrease with respect to males (\( p < 0.05 \)). The FIB concentration was also different among genders. Female rats and mice depicted 12.8% and 6.9% lower values than males (\( p < 0.05 \)).

**Inter-species differences of the screening tests**

Inter-species differences were found in all the comparisons of PT, aPTT, TT and FIB between rats and mice. Highly significant differences of PT values in both species were found. Male rats vs. male mice and

---

**Table 1** Haemostatic coagulation tests values

<table>
<thead>
<tr>
<th>Gender</th>
<th>Species</th>
<th>Test (s)</th>
<th>Animals tested</th>
<th>Mean ± SEM</th>
<th>Minimal–maximal (values)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Wistar</td>
<td>PT 46</td>
<td>16.9 ±0.30</td>
<td>13.9–21.1</td>
<td>11.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male CD1 mice</td>
<td>aPTT 45</td>
<td>22.5 ±0.60</td>
<td>15.2–33.9</td>
<td>18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male CD1 mice</td>
<td>TT 51</td>
<td>11.5 ±0.20</td>
<td>9.6–15.3</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Wistar</td>
<td>PT 47</td>
<td>10.3 ±0.20</td>
<td>7.9–14.5</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Wistar</td>
<td>aPTT 44</td>
<td>31.1 ±0.80</td>
<td>23.9–43.0</td>
<td>17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Wistar</td>
<td>TT 23</td>
<td>13.9 ±0.20</td>
<td>12.7–15.2</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female CD1 mice</td>
<td>PT 29</td>
<td>202 ±4.0</td>
<td>175–230</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female CD1 mice</td>
<td>aPTT 25</td>
<td>19.7 ±0.40</td>
<td>15.5–23.1</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female CD1 mice</td>
<td>TT 30</td>
<td>12.0 ±0.20</td>
<td>10.4–14.1</td>
<td>8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female CD1 mice</td>
<td>FIB (mg/dl)</td>
<td>242 ±4.0</td>
<td>200–305</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Ovx</td>
<td>CD1 mice</td>
<td>10.3 ±0.20</td>
<td>7.9–14.5</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Ovx</td>
<td>aPTT 20</td>
<td>22.5 ±0.60</td>
<td>15.2–33.9</td>
<td>18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Ovx</td>
<td>TT 23</td>
<td>12.8 ±0.40</td>
<td>8.7–17.2</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Ovx</td>
<td>FIB (mg/dl)</td>
<td>242 ±4.0</td>
<td>200–305</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 2** Gender intra and inter-species differences of rats and mice on the coagulation tests: PT, aPTT, TT, and FIB

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Gender</th>
<th>Inter-species</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT mean</td>
<td>16.9–18.4</td>
<td>10.3–11.9</td>
</tr>
<tr>
<td>( \Delta % )</td>
<td>+8.9*</td>
<td>+16**</td>
</tr>
<tr>
<td>Male rats vs.</td>
<td>Male mice vs.</td>
<td>Male rats vs. Male</td>
</tr>
<tr>
<td>female rats</td>
<td>female mice</td>
<td>mice</td>
</tr>
<tr>
<td>aPTT mean</td>
<td>22.5–19.7</td>
<td>31.1–22.5</td>
</tr>
<tr>
<td>( \Delta % )</td>
<td>−12*</td>
<td>−27.6***</td>
</tr>
<tr>
<td>Male rats vs.</td>
<td>Male mice vs.</td>
<td>Male rats vs. Male</td>
</tr>
<tr>
<td>female mice</td>
<td>female mice</td>
<td>mice</td>
</tr>
<tr>
<td>TT mean</td>
<td>11.5–12.0</td>
<td>13.9–12.8</td>
</tr>
<tr>
<td>( \Delta % )</td>
<td>+4.3*</td>
<td>−7.7*</td>
</tr>
<tr>
<td>Male rats vs.</td>
<td>Male mice vs.</td>
<td>Male rats vs. Male</td>
</tr>
<tr>
<td>female mice</td>
<td>female mice</td>
<td>mice</td>
</tr>
<tr>
<td>FIB mean</td>
<td>242–211</td>
<td>202–188</td>
</tr>
<tr>
<td>( \Delta % )</td>
<td>−12.8**</td>
<td>−6.9*</td>
</tr>
</tbody>
</table>

Obtained by Mann–Whitney test. *\( p < 0.05 \); **\( p < 0.01 \); ***\( p < 0.001 \).

\( \Delta \% = \frac{\text{Percent difference}}{\text{Mean of the second group} \times 100} - 100 \)
female rats vs. female mice yielded differences of ~39% and ~35% respectively. The aPTT values of rats and mice also showed significant differences of 38% and 14%. ATT difference of 20.9% (p < 0.001) between male rats and male mice was also found. The FIB concentration was significantly lower in mice than in rats (Table 2).

Ovariectomy effect on PT, aPTT, TT, and FIB in rats and mice

Ovariectomy induces changes in blood coagulation altering some of the screening coagulation tests. The differences between the haemostatic parameters obtained from male and female rats and mice and their corresponding Ovx are described in Table 3. Comparison between male rats and Ovx rats showed significant differences in TT values (+10.4%; p < 0.01) and FIB concentration (−20.6%; p < 0.001). The same comparison among mice revealed a different behaviour. In them, PT, aPTT, and FIB concentration were not altered; however TT values decreased 20.1%. When comparing female rats against their corresponding Ovx animals, only aPTT was affected (+15%; p < 0.001). In female mice, ovariectomy modified all the parameters in a greater proportion than in female rats. The aPTT values increased 31.5% and TT was significantly reduced by 13.2% and to a lesser extent PT and FIB concentration (Table 3).

Discussion

Modulation of reactions in the blood coagulation system is the result of a balance between natural anticoagulants (antithrombin III, heparin cofactor II, protein C, protein S, and tissue factor pathway inhibitor) and indicators of procoagulant function (prothrombin fragment 1 + 2, fibrinopeptide A, the thrombin–antithrombin III complex, and D-dimer) [20]. Many factors influence haemostatic changes, and gender plays an important role with the influence due to the steroid sex hormones [21]. Experimental studies indicate that 17β-oestradiol acts as a regulator for blood coagulation factor genes, inducing transcription of factors, such as FXII (Hageman factor), involved in fibrinolysis, kinin production, and the inflammation process, playing an essential role in the activation of proteolytic pathways of the coagulation cascade [22,23]. Administration of synthetic oestrogens to female rats decreases the vitamin K-dependent blood clotting factors, inducing changes in blood clotting [9]. Gender differences in anticoagulant responses have been observed in castrated adult male and female rats treated with testosterone or 17β-oestradiol [10]. However, experimental information regarding the influence of gender and the influence of ovariectomy on the most common blood clotting screening tests used for the haemostatic parameters has not been analysed yet. A report by Karges et al. on the clotting factors and fibrinolytic parameters of laboratory and domestic animals showed substantial differences in protein coagulation reactivity and the fibrinolytic system among different species, including humans [12]. Our results show lower values than those obtained by Karges et al. in the male rat [12], which might be due to the different experimental conditions (reagents used, anticoagulant concentration, sample collection methods, and equipment’s sensitivity). However, our PT, aPTT, TT, and FIB concentration data, from rats and mice, clearly demonstrate gender intra-species and inter-species differences in all the haemostatic parameters been expressed, especially in PT and aPTT values. Gender influences the intrinsic, extrinsic, and common pathways of plasmatic coagulation, indicating inequalities among blood haemostatic parameters in the two species.

Rodent ovariectomy induces suppression of the main source of oestrogens, producing uterus involu- lution that can be restored by the administration of oestrogens. These models have been used widely to understand clinical conditions (hysterectomy, ovariecto- my, or menopause), where a drop of oestrogen levels occurs. Our results demonstrate changes in PT and TT haemostatic parameters in Ovx animals, indicating that ovariectomy leads to changes that can influence blood clotting.

The gender, intra and inter-species differences observed in PT, aPTT, TT, and FIB data emphasise the importance and implications in establishing inter-laboratory comparisons of coagulation tests to make inferences and draw conclusions. In the clinical
practice, coagulation varies among men, women, pregnant women, and menopausal women. Recent reports have demonstrated differences in the action of heparin on the coagulation status among men, women, and pregnant women [24]. Gender differences in haemostasis of patients with ischemic heart disease revealed that women, when compared with men, had higher baseline levels of fibrinogen anti-thrombin III, protein C, and plasminogen [25]. Clinical blood screening tests are always expressed in ranges, indicating the need to develop separate studies considering gender, reproductive condition, and, perhaps, different pathologies in order to improve the knowledge on the actual haemostatic clinical status and, consequently, leading to better diagnoses.

Values presented here from rats and mice will be useful tools to evaluate the effects of oestrogens in our laboratory and might also help to investigate the pharmacological behaviour of other compounds on the coagulation system.

Acknowledgements

The authors are grateful to Ingrid Mascher for reading the manuscript and valuable suggestions. To Biol. Maria Estela Avila from our Department for her technical assistance.

References


