PROLONGATION OF PT AND APTT UNDER EXCESSIVE ANTICOAGULANT IN PLASMA FROM RATS AND DOGS

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ABSTRACT — Prothrombin time (PT) and activated partial thromboplastin time (APTT) were studied under excessive sodium citrate using the plasma from rats and beagle dogs. Addition of sodium citrate into the plasma caused a prolongation of PT and APTT. The prolongation was dependent on the concentration of sodium citrate or calculated hematocrit. The degree of prolongation was more severe in rats than in dogs, and in APTT than in PT. These results suggest that an artificial prolongation of PT and APTT occurs under excessive sodium citrate (e.g., elevated hematocrit), and that the degree differs between species and between parameters.

KEY WORDS: Prothrombin time, Activated partial thromboplastin time, Anticoagulant, Sodium citrate

INTRODUCTION

Plasma for tests on blood coagulation is usually prepared by mixing 9 vol. of blood and 1 vol. of anticoagulant (sodium citrate solution). It has been common knowledge in a routine test for humans that excessive sodium citrate (e.g., in a case of elevated hematocrit) causes a prolongation in prothrombin time (PT) and/or activated partial thromboplastin time (APTT) (Hardisty and Ingram, 1965; Koepke et al., 1975; Ingram and Hills, 1976; Harms, 1982). In contrast, this information is extremely limited in laboratory animals (Whitfield and Levy, 1983; O'Brien et al., 1995). Since such knowledge is of importance in toxicological and pharmacological study of new chemical entities, we have therefore investigated the prolongation of PT and APTT under excessive sodium citrate in two species of laboratory animals, - rats and beagle dogs.

MATERIALS AND METHODS

Reagent

Sodium citrate (trisodium citrate dihydrate; Na₃C₆H₅O₇ · 2H₂O) was purchased from Nacalai Tesque (Kyoto City, Japan), and was dissolved in distilled water to give concentrations of 3.2% (3.2g trisodium citrate dihydrate / 100ml H₂O) and 3.8% (3.8g/100ml). The PT and APTT reagents were Dade Thromboplastin C (Dade Diagnostics, Aguada, USA) and Dade Actin Activated Cephaloplastin Reagent (Dade Diagnostics, Aguada, USA), respectively.

Animals

Rats (Rattus norvegicus, strain, Slc: SD) were purchased from Japan SLC Inc. (Hamamatsu City, Japan), and beagle dogs (Canis familiaris, strain, Beagle/CSK) were from CSK Research Park (Suwa City, Japan). They were kept in rooms controlled for temperature (23 ±2°C), relative humidity (55 ±10%), light-dark period (5:00 - 19:00) and fresh air changes (14 - 16 times/hr). Rats and dogs were fed with pellets (CE-2, Crea, Tokyo, Japan) and dog food (CD-5, Crea), respectively, and allowed to access tap water ad libitum. The animals were fasted overnight prior to experiments, and were treated in accordance with Chugai Pharmaceutical's ethical guidelines for animal care, handling and termination.

Experiment 1: PT and APTT in plasma mixed with sodium citrate solution

Four male 10-week-age rats weighing 295 - 310g and four male 16 - 25 month-age beagle dogs weighing

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10.4 - 11.8 kg were used. Rats were anesthetized by inhalation of ether, and 9 vol. (7.2 ml) of blood was taken from the abdominal aorta into a syringe containing 1 vol. (0.8 ml) of 3.2% sodium citrate solution. For beagle dogs, the blood sample was collected from the cephalic vein without anesthesia. Hematocrit of the citrate blood was measured, and then centrifuged (1870 g, 4°C, 10 min) to separate plasma. The plasma was mixed with the 3.2% sodium citrate (solution) following 5 different ratios: (i) plasma only, (ii) 0.58 ml plasma + 0.02 ml solution, (iii) 0.55 ml plasma + 0.05 ml solution, (iv) 0.50 ml plasma + 0.10 ml solution, and (v) 0.40 ml plasma + 0.20 ml solution.

Experiment 2: PT and APTT under different mixture ratio with anticoagulant in blood sampling

Four male rats of 10 week-age weighing 290 - 308 g were used. After rats were anesthetized by ether inhalation, blood was drawn from the abdominal aorta into three syringes to give different ratios of blood-to-sodium citrate solution: (i) 1.8 ml blood + 0.2 ml of 3.2% sodium citrate solution; (ii) 1.7 ml blood + 0.3 ml solution; and (iii) 1.6 ml blood + 0.4 ml solution. The hematocrit was measured, and the plasma for measurement of PT and APTT was separated by centrifugation (1870 g, 4°C, 10 min).

Experiment 3: Effect of concentration of sodium citrate on PT and APTT

Eight female 10-week-age rats weighing 177 - 242 g were used. These rats were divided into two groups. In one group, 3.2% sodium citrate solution was used as an anticoagulant (4.5 ml blood + 0.5 ml sodium citrate), whereas in another group 3.8% sodium citrate solution was used. The plasma with 3.2% or 3.8% sodium citrate solution was further mixed with 3.2% or 3.8% sodium citrate solution, respectively. Ratio of the plasma and sodium citrate solution was as follows: (i) plasma only, (ii) 0.387 ml plasma + 0.013 ml solution, (iii) 0.367 ml plasma + 0.033 ml solution, (iv) 0.333 ml plasma + 0.067 ml solution, and (v) 0.267 ml plasma + 0.133 ml solution. Other procedures were the same as those in experiment 1 described above.

Measurements

PT and APTT were measured by means of an automatic analyzer (Amelung KC-10A, Hynrich Amelung, USA). Hematocrit value was determined by the standard laboratory method using a micro-hematocrit centrifuge.

Calculation

Equation (1)

\[ \text{Sodium citrate initial (vol. %)} = 100 \times \frac{x}{T} \times Hc \times 100 \times \frac{100}{T(T-x)} \]

Sodium citrate initial (vol. %): Volume of sodium citrate solution in the plasma just after blood collection

\[ Hc : \text{Hematocrit in citrate blood} \]

\[ T : \text{ml of total sample volume (blood plus sodium citrate solution)} \]

\[ x : \text{ml of sodium citrate solution} \]

Equation (2)

\[ \text{Sodium citrate (vol. %)} = 100 \times \frac{b + a \times \text{Sodium citrate initial (100)}}{a+b} \]

Sodium citrate (vol. %): Volume of sodium citrate solution after mixing plasma with sodium citrate solution

\[ a : \text{ml of plasma} \]

\[ b : \text{ml of added sodium citrate solution} \]

Equation (3)

\[ \text{Sodium citrate (conc. %)} = 3.2 \times (\text{Sodium citrate (vol. %)}) \]

Sodium citrate (conc. %): Concentration of sodium citrate in plasma

Equation (4)

\[ Hc(\%) = \frac{(1000 - 10000 \times \text{Sodium citrate (vol. %)})}{9} \]

Hc(%) : Calculated hematocrit

Statistical analysis

The Dunnett test was applied to the statistical analysis. Probability (P) less than 0.05 was considered to be significant.

RESULTS

Experiment 1

As shown in Table 1, addition of sodium citrate solution into the plasma from rats and beagle dogs significantly prolonged PT and APTT. A significant elevation was also found in hematocrit which was theoretically calculated. The degree of prolongation in PT and APTT was more severe in a higher concentration of sodium citrate or in a higher value of the calculated hematocrit. Judged from values of PT (%) and APTT (%) shown in this table, the most severe effect was found in rat APTT, followed by canine APTT, rat PT and canine PT. The canine PT was quite resistant with excessive sodium citrate solution.

Experiment 2

Similarly, an increasing ratio of sodium citrate solution in blood sampling caused prolongation of PT and APTT (Fig. 1).
Table 1. Effect of sodium citrate solution on PT and APTT in the plasma from rats and beagle dogs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citrated plasma (ml)</th>
<th>Citrate sol. (ml)</th>
<th>Sodium citrate</th>
<th>Calculated Ht (%)</th>
<th>PT (sec)</th>
<th>PT (%)</th>
<th>APTT (sec)</th>
<th>APTT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.600</td>
<td>0.000</td>
<td>17.7±0.2</td>
<td>0.57±0.00</td>
<td>48.3±0.6</td>
<td>18.1±0.6</td>
<td>100.0</td>
<td>22.2±1.2</td>
</tr>
<tr>
<td>B</td>
<td>0.580</td>
<td>0.020</td>
<td>20.4±0.1</td>
<td>0.66±0.00</td>
<td>56.7±0.3</td>
<td>18.6±0.8</td>
<td>103.0</td>
<td>23.9±1.5</td>
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<tr>
<td>C</td>
<td>0.550</td>
<td>0.050</td>
<td>24.5±0.1</td>
<td>0.79±0.00</td>
<td>65.8±0.2</td>
<td>19.7±0.9</td>
<td>108.9</td>
<td>25.5±1.5</td>
</tr>
<tr>
<td>D</td>
<td>0.500</td>
<td>0.100</td>
<td>31.4±0.1</td>
<td>1.01±0.00</td>
<td>75.8±0.2*</td>
<td>24.6±1.8</td>
<td>135.3</td>
<td>37.2±3.2*</td>
</tr>
<tr>
<td>E</td>
<td>0.400</td>
<td>0.200</td>
<td>45.1±0.1</td>
<td>1.45±0.00</td>
<td>86.5±0.1**</td>
<td>110.5±38.7**</td>
<td>596.6</td>
<td>387.7±63.4**</td>
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</table>

Beagle dog

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citrated plasma (ml)</th>
<th>Citrate sol. (ml)</th>
<th>Sodium citrate</th>
<th>Calculated Ht (%)</th>
<th>PT (sec)</th>
<th>PT (%)</th>
<th>APTT (sec)</th>
<th>APTT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.600</td>
<td>0.000</td>
<td>18.0±0.4</td>
<td>0.58±0.01</td>
<td>49.3±1.3</td>
<td>7.4±0.4</td>
<td>100.0</td>
<td>11.3±0.3</td>
</tr>
<tr>
<td>B</td>
<td>0.580</td>
<td>0.020</td>
<td>20.7±0.4</td>
<td>0.66±0.01</td>
<td>57.5±1.0</td>
<td>7.4±0.3</td>
<td>100.4</td>
<td>11.5±0.3</td>
</tr>
<tr>
<td>C</td>
<td>0.550</td>
<td>0.050</td>
<td>24.8±0.4</td>
<td>0.79±0.01</td>
<td>66.3±0.7</td>
<td>7.4±0.4</td>
<td>100.0</td>
<td>12.4±0.4</td>
</tr>
<tr>
<td>D</td>
<td>0.500</td>
<td>0.100</td>
<td>31.7±0.3</td>
<td>1.02±0.01</td>
<td>76.0±0.4*</td>
<td>7.6±0.4</td>
<td>102.7</td>
<td>15.3±0.6</td>
</tr>
<tr>
<td>E</td>
<td>0.400</td>
<td>0.200</td>
<td>45.3±0.2</td>
<td>1.45±0.01</td>
<td>86.6±0.1**</td>
<td>10.7±0.9**</td>
<td>143.3</td>
<td>130.7±20.5**</td>
</tr>
</tbody>
</table>

Vol. % means the volume of 3.2% sodium citrate solution occupying the plasma.
Conc. % means the final concentration of sodium citrate in the plasma.
Each value represents the mean ±SE (n=4) except with % of PT or APTT, in which the value represents the mean of 4 data.
*P<0.05, **P<0.01, significantly different from A (Dunnett, parametric or non-parametric).
Experiment 3

On the basis of volume of sodium citrate solution, more severe prolongation was found in the plasma with 3.8% sodium citrate solution than with 3.2% solution. However, the difference disappeared upon identifying a final concentration of sodium citrate (Fig. 2).

DISCUSSION

The plasma for measurement of blood coagulation tests is usually prepared by mixing 9 vol. of blood and 1 vol. of sodium citrate solution. An increasing hematocrit must heighten the ratio of sodium citrate occupying the plasma segment (see the Equation 1).

An increased ratio of sodium citrate to blood prolonged PT and APTT in both species tested. Similar results were observed in two different experiments (Experiment 1 and 2), confirming the relationship between the anticoagulant concentration and the prolongation. The degree of prolongation was more severe in a higher concentration of sodium citrate or in a higher value of "calculated" hematocrit, supporting the expectation derived from Equation 1.

Two contributing factors, (1) an increment of sodium citrate concentration, and/or (2) dilution of plasma, are expected to cause the prolongation of PT and APTT under excessive sodium citrate. In order to clarify this point, we carried out an experiment using two standard concentrations (3.2% or 3.8%) of sodium citrate. When sodium citrate solution was added to the plasma, more severe prolongation was observed in the plasma plus 3.8% sodium citrate solution. But the difference was not found on the basis of final concentration of sodium citrate in plasma, indicating that the prolongation of PT and APTT is caused by increasing concentration of sodium citrate, and not by dilution of plasma.

Our results indicate that 3.2% sodium citrate solution rather than 3.8% solution should be recommended so that artificial prolongation by excessive sodium citrate may be prevented.

The prolongation appeared more severely in APTT than in PT, and more in rats than in dogs. These differences may be attributed to different amounts of coagulation factors or different sensitivity to calcium depletion between the two species and between the two parameters. Further studies may clarify this issue.

It is noteworthy that the canine PT was quite stable under excessive sodium citrate. This supports a recent

![Fig. 1](image1.png)

**Fig. 1.** Comparison of two different experimental data on the prolongation of PT and APTT under excessive sodium citrate. Symbols are as follows: PT in experiment 1 (open circle), PT in exp. 2 (closed circle), APTT in exp. 1 (open triangle), and APTT in exp. 2 (closed triangle). Each value represents the mean ± SE (n=4).

![Fig. 2](image2.png)

**Fig. 2.** Contributing factor in prolongation of PT and APTT under excessive sodium citrate. Vol. % means the volume of sodium citrate solution occupied in the plasma. Conc. % means the final concentration of sodium citrate in the plasma. Symbols are as follows: 3.8% sodium citrate solution (closed circle) and 3.2% sodium citrate solution (open circle). The value represents the mean of 4 experiments.
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report by O'Brien et al. (1995), showing that hematocrit elevation prolongs APTT, but not PT.

The present results indicate a relationship among PT/APTT and sodium citrate and hematocrit in the blood from rats and beagle dogs, and suggest that an artificial prolongation of PT and APTT occurs when the sodium citrate is in excess in plasma from experimental animals, and that the degree of prolongation differs between the species and between the parameters.

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