Original Article

Phenobarbital (PB)-induced changes in blood coagulation-related parameters in pregnant rats, lactating rats and pups

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ABSTRACT — Effects of repeated administration of phenobarbital (PB) on blood coagulation-related parameters were examined in non-pregnant, pregnant and lactating rats, and also in pups born to PB-treated lactating dams. PB was orally administered at a dose level of 80 mg/kg/day to pregnant (from gestation day (GD) 13), postpartum (from postpartum day (PPD) 7) and non-pregnant rats (from 13 weeks of age) for 7 days. Blood was collected on GD20 or PPD14 to perform blood coagulation examination. Concurrently, the blood coagulation parameters were examined in the pups. Increases in liver weight and/or hepatic cytochrome P450 content were observed in the PB-treated non-pregnant, pregnant and lactating rats. Activated partial thromboplastin time (APTT) was prolonged and anti-thrombin III (ATIII) concentration was increased in the lactating rats, while there were no changes in prothrombin time (PT) or APTT in the non-pregnant and pregnant rats. Moreover, prolongation of PT and APTT and decreases in factors VII and IX activities were observed in their pups. Thus, prolongation of blood coagulation time was confirmed in both dams and their pups following PB-administration to lactating dams. Effects of vitamin K2 (VK2) on PB-induced changes in blood coagulation-related parameters of both dams and their pups were examined by co-administration with PB and VK2 to lactating dams. PT and APTT were comparable to the control and PB-induced prolongation of blood coagulation time was improved in the pups while APTT was prolonged in dams, suggesting that VK2 was beneficial to pups but not to dams.

Key words: PB, Vitamin K, APTT, Pregnancy, Lactation, Pup

INTRODUCTION

Factors II, VII, IX and X are synthesized in the liver as inactive pro-proteins (Bloom and Brandt, 2001). In order to activate these coagulation factors, the posttranslational modification that converts glutamate residues in pro-proteins to gamma-carboxyglutamate residues is required, and it is catalyzed by vitamin K-dependent carboxylation reaction (Marcus and Coulston, 1996). Vitamin K is commonly found in plant and animal tissues which are the sources of vitamin K for human beings. Additionally, intestinal bacteria can synthesize vitamin K, and thus, vitamin K-deficiency is extremely rare in adults (Sakurakawa, 1985). On the other hand, newborns are vulnerable to vitamin K-deficiency bleeding, because vitamin K is poorly delivered to the fetus via the placenta during pregnancy and the intestinal flora in newborns lacks microorganisms that synthesize the vitamin (Marcus and Coulston, 1996). Moslet and Hansen (1992) reported an occurrence of neonatal hemorrhage in infants born to mothers treated with a barbiturate antiepileptic drug, phenobarbital (PB), a CYP2B inducer, during pregnancy, suggesting a connection of PB-induced P450 isoenzymes to vitamin K metabolism. It was also reported that a single injection of barbiturates to rats prolonged blood coagulation time (Lox and Frederick, 1983). Furthermore, Wilson and Park (1984) reported that metabolism of vitamin K was accelerated by PB administration in rabbits as well as in rats.

Recently we reported that prolongation of blood coagulation time was observed in both male Sprague-Dawley rats and male Japanese white rabbits which were treated with PB (Mochizuki et al., 2008, 2009). However, there are almost no reports of effects of PB on blood coagulation time in pregnant or lactating dams and their pups, although they are widely used in reproduction studies. In addition,
during pregnancy, it is well known that significant changes are observed in blood parameters in rats and rabbits as well as in women (Mizoguchi et al., 2009; Urasoko et al., 2009; Honda et al., 2008; De Rijk et al., 2002; Katoh et al., 1992; Wells et al., 1999). Therefore, this study was performed to clarify the effects of PB administration on blood coagulation-related parameters in non-pregnant or pregnant rats and in lactating rats and their pups. In addition, the participation of the intrinsic or the extrinsic system on blood coagulation-related parameters in these animals was examined in view of changes in activities of factors VII, IX and X (vitamin K-dependent coagulation factors). Furthermore, modification of PB-induced changes in blood coagulation-related parameters by vitamin K$_2$ (VK$_2$) was investigated in lactating dams and their pups after co-administration of PB and VK$_2$ to dams.

All procedures on this study were conducted in compliance with “Guidelines for Proper Conduct of Animal Experiments” (Science Council of Japan, June 1, 2006) and according to the Protocol approved by the Animal Care and Use Committee at BOZO Research Center Inc. All efforts were made to minimize animal suffering.

MATERIALS AND METHODS

Animals

Male and female specific pathogen-free rats of the Sprague-Dawley strain (Crl:CD(SD)) were obtained from Charles River Japan Inc. (Kanagawa, Japan) at 10 weeks of age. They were mated on a one-to-one basis at 11 weeks of age in our laboratory, and 30 pregnant rats were obtained. In addition, 10 non-pregnant female rats (13 weeks old) of the same strain were also used. The animals were kept individually in cages in an air-conditioned animal room (temperature: 23 ± 3°C; humidity: 50 ± 20%; air ventilation: 10-15 times/hr; lighting: 12hr/12hr light/dark cycle). Animals from mating to gestation day (GD) 17 were housed individually in stainless steel wire mesh cages, and the animals from GD17 to postpartum day (PPD) 14 were housed individually with their litters in plastic cages with bedding. On PPD4, litter size was reduced to 8 pups (4 males and 4 females) by random selection. The animals were allowed free access to pelleted diet (NMF, Oriental Yeast, Co., Ltd., Tokyo, Japan) and tap water ad libitum during the experimental period.

Chemicals

Phenobarbital sodium and corn oil were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and menaquinone 4 (VK$_2$) from Sigma Chemical Co. (St. Louis, MO, USA). PB was dissolved in sterilized water for injection (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to achieve a final concentration of 16 mg/ml, and VK$_2$ was prepared with corn oil to achieve a final concentration of 6 mg/ml immediately before use.

Experimental designs

Effects on dams

Twenty pregnant and 10 non-pregnant rats at 13 weeks of age were used. In a previous study in which PB was administered to dams for 7 days from PPD7 (daily dose levels: 50, 100 and 150 mg/kg/day), a small number of dams of the 100 and 150 mg/kg/day groups did not nurse their pups. Therefore, the dose level was set at 80 mg/kg/day in the present study, and 5 pregnant and 5 lactating rats were given PB by oral gavage for 7 days from GD13 and PPD7, respectively. In addition, 5 non-pregnant rats were also given PB in the same way from the day of 13 weeks of age. On the day following the last administration, they were subjected to blood sampling via the abdominal aorta under ether anesthesia and then to necropsy. As the non-treated control groups, 5 non-pregnant, 5 pregnant (GD20) and 5 lactating rats (PPD14) were prepared, and they were subjected to blood sampling and necropsy in the same way.

Effects on pups

Forty male and 40 female pups were used. On PPD14, 4 male and 4 female pups each of 5 PB-treated lactating dams were subjected to blood sampling in the same way. They were divided evenly into 2 subgroups; one for measuring activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen values and the other for measuring factors VII, IX and X activities. In addition, 4 male and 4 female pups each of 5 non-treated lactating dams were prepared and subjected to blood sampling in the same way on PPD14.

Effects of VK$_2$, co-administration

A total of 10 lactating dams and their pups (2 males and 2 females of each dam) were used. Five lactating dams were treated orally with both PB (80 mg/kg/day) and VK$_2$ (30 mg/kg/day) for 7 days from PPD7. The dose level of VK$_2$ was selected according to Iwamoto et al. (2005), and VK$_2$ was given to the animals 3 hr after PB-treatment (at the expected Tmax of PB, Kubota et al., 2004). On the day following the last administration (PPD14), they were subjected to blood sampling via the abdominal aorta under ether anesthesia. Two male and two female pups each of 5 dams were subjected to blood sampling in the same way on PPD14. They were divided evenly into 2
subgroups; one for measuring APTT, PT and fibrinogen values and the other for measuring factors VII, IX and X activities. In addition, 5 non-treated lactating dams and their pups (2 males and 2 females of each dam) were also subjected to blood sampling in the same way.

**Clinical observations**

In all experiments, all animals (including pups) were observed for clinical signs. Furthermore, dams were also observed for nursing behavior.

**Measurement of liver weight**

Except for pups, the liver was excised from each animal at necropsy and weighed. The liver weight per 100 g body weight (relative liver weight) was calculated from the terminal body weight and the absolute liver weight. Each values in Fig. 1 indicates a percentage of change against the mean control value.

**Preparation of hepatic microsomes**

At necropsy, the liver was excised from 3 pregnant, 3 lactating and 3 non-pregnant rats each of the non-treated and PB-treated groups. The liver was perfused with 1.15% KCl solution and frozen at -80°C prior to microsomal preparation. Then, the liver was thawed and homogenized with 1.15% KCl-1M EDTA Na solution to achieve 25% homogenate. The homogenate was centrifuged at 9,000 x g for 20 min. S9 was also prepared and centrifuged at 105,000 x g for 60 min to prepare a liver microsomal solution.

**Determination of total hepatic cytochrome P450 content**

Total hepatic cytochrome P450 content was determined according to the reduced-CO difference spectrum method of Omura and Sato (1964). Hepatic microsomes prepared at about 1 mg protein/ml were subjected to measurement of CO difference spectra on a spectrophotometer U-3900 (Hitachi High-Technologies Corporation, Tokyo, Japan). Protein content was determined according to the method described by Lowry et al. (1951) using a spectrophotometer U-3900 to calculate hepatic cytochrome P450 content.

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**Fig. 1.** Effects of 7-day PB-administration on the liver weight and the hepatic cytochrome P450 content. The PB level was set at 80 mg/kg/day. Each value of the liver weight represents the mean of 5 dams, and that of the hepatic cytochrome P450 content represents the mean of 3 dams.

* < 0.05, ** < 0.01, Significantly different from each control group (Student’s t-test). Control: non-treated rats, NP: non-pregnant rats, P: pregnant rats on GD20, L: lactating rats on PPD14.

Each value in the figure indicates a percentage of change against the control mean value, although the measured values were used for the statistical analysis.
per microsomal protein weight (n mol/mg protein). Each value in Fig. 1 indicates a percentage of change against the mean control value.

Blood coagulation tests

Blood collected via the abdominal aorta under ether anesthesia as scheduled was put into test tubes containing 3.8% sodium citrate solution (1 volume to 9 volumes of blood). The samples were centrifuged (approximately 1,600 × g, 10 min) to obtain plasma. PT, APTT and fibrinogen were measured by a coagulometer, ACL Elite (Instrumentation Laboratory, MA, USA), while anti-thrombin III (ATIII) concentration was measured by a clinical chemistry autoanalyzer, TBA-120FR (Toshiba Corporation, Tokyo, Japan). In addition, Thrombotest (TBT), factors VII, IX and X activities were measured using HemosIL Procomplex, HemosIL Factors VII, IX and X (Instrumentation Laboratory) by a coagulometer, ACL Elite. In order to measure the activities of coagulation factors, the blood coagulation time was measured using blood samples after adding plasma lacking each coagulation factor and thromboplastin reagent. Each coagulation factor activity was calculated through the use of the calibration curve from measured blood coagulation time.

### Statistical analysis

All parameters are expressed as mean ± standard deviation (S.D.). Each mean difference from each control was analyzed by Student's t-test (level of significance: 1 and 5%, two-tailed) (Snedecor and Cochran, 1989).

### RESULTS

#### Effects on dams

There were no abnormal clinical signs observed in any of the PB-treated non-pregnant, pregnant or lactating rats throughout the experimental period. Lactating rats showed normal nursing behavior.

Changes in liver weights and hepatic cytochrome P450 contents are shown in Fig. 1. When compared with those in the non-treated control rats, both the liver weight and the hepatic cytochrome P450 content increased in the PB-treated non-pregnant and lactating rats (the former: 17 to 28% higher, the latter: about 60% higher). In addition, the hepatic cytochrome P450 content increased in the PB-treated pregnant rats (64% higher).

Changes in blood coagulation parameters are shown in Table 1. As compared with the non-treated rats, in the PB-treated non-pregnant rats, there were no changes in blood coagulation time. In addition, ATIII concentration

### Table 1. Effects of 7-day PB-administration on coagulation parameters in pregnant and lactating rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NP</th>
<th>P</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>Control</td>
<td>13.2 ± 0.7</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>13.0 ± 0.8</td>
<td>13.3 ± 0.5</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>Control</td>
<td>18.1 ± 3.0</td>
<td>23.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>19.2 ± 2.6</td>
<td>21.4 ± 2.0</td>
</tr>
<tr>
<td>TBT (sec)</td>
<td>Control</td>
<td>24.2 ± 1.3</td>
<td>21.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>23.2 ± 1.3</td>
<td>21.3 ± 0.3</td>
</tr>
<tr>
<td>ATIII (%)</td>
<td>Control</td>
<td>146.6 ± 3.6</td>
<td>146.7 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>161.8 ± 10.8*</td>
<td>132.5 ± 7.0</td>
</tr>
<tr>
<td>FIB (mg/dl)</td>
<td>Control</td>
<td>233.6 ± 20.3</td>
<td>505.8 ± 71.7</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>247.0 ± 53.4</td>
<td>440.6 ± 36.6</td>
</tr>
<tr>
<td>Factor VII (%)*</td>
<td>Control</td>
<td>285.6 ± 43.2</td>
<td>313.4 ± 29.6</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>358.4 ± 30.5*</td>
<td>297.8 ± 29.7</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>Control</td>
<td>40.1 ± 17.8</td>
<td>33.9 ± 21.6</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>19.3 ± 18.1</td>
<td>52.3 ± 21.3</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>Control</td>
<td>48.1 ± 10.7</td>
<td>84.9 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>73.9 ± 15.8*</td>
<td>82.0 ± 5.4</td>
</tr>
</tbody>
</table>

The PB level was set at 80 mg/kg/day. Each value represents the mean ± S.D. of 5 dams.

*: < 0.05, Significantly different from each control group (Student's t-test).


*: Unit (%) = coagulation time in calibration plasma (HemosIL3%) (s) / coagulation time in sample (s) × 100.
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and factors VII and X activities increased, and factor IX activity showed a tendency to decrease in the PB-treated rats. However, they were considered not to be noteworthy since there were no changes observed in PT and APTT. In the PB-treated pregnant rats, there were no PB treatment-related changes in blood coagulation parameters. In the PB-treated lactating rats, APTT was prolonged (14% longer) and ATIII concentration increased (21% higher).

Effects on pups

There were no abnormal clinical signs observed in any pups of the PB-treated dams, all of which showed normal nursing behavior.

Changes in blood coagulation parameters are shown in Table 2. As compared with the pups of the non-treated dams, PT and APTT were prolonged in the pups of both sexes of the PB-treated dams (7 to 14% longer). In addition, a decrease in factor VII activity (14% lower) in males and a decrease in factor IX activity (31% lower) in females were also observed.

Effects of VK co-administration

Changes in blood coagulation parameters of dams are shown in Table 3. As compared with the non-treated dams, APTT was prolonged (20% longer) and factor IX activity tended to decrease (50% lower) in the dams co-administered PB and VK$_2$. In addition, the activities of factors VII and X showed an increase (11 or 16% higher) in the dams co-administered PB and VK$_2$, however, such changes were considered not to be noteworthy since shortening of PT or APTT was not observed.

Changes in blood coagulation parameters of pups are shown in Table 4. As compared with the pups of non-treated dams, no significant changes were noted in any parameter.

**DISCUSSION**

In this study, the effects of repeated administration of PB on blood coagulation-related parameters were examined in pregnant and lactating dams and pups obtained from PB-treated lactating dams. In addition, changes in blood coagulation-related parameters were examined in lactating dams and their pups after PB and VK$_2$ co-administration to dams.

Increases in the liver weights and/or the hepatic cytochrome P450 contents were observed in the PB-treated non-pregnant, pregnant and lactating rats. PB is a well-known hepatic CYP2B inducer and Ejiri et al. (2005a, 2005b) reported that PB induces hepatic CYP2B1 in pregnant rats. Therefore, PB-induced increase in hepatic cytochrome P450 content observed in the present study is considered to reflect the induction of hepatic CYP2B. Among blood coagulation-related parameters, APTT was prolonged in PB-treated lactating dams, indicating a participation of the endogenous pathway. Moreover, in pups, APTT and PT were prolonged and activities of factors VII and IX, vitamin K-dependent blood coagulation fac-

### Table 2. Effects of 7-day PB-administration to dams on coagulation parameters in their pups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PB</th>
<th>Control</th>
<th>PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>10.7 ± 0.9</td>
<td>11.4 ± 0.3*</td>
<td>10.6 ± 0.3</td>
<td>11.5 ± 0.6*</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>10.3 ± 1.1</td>
<td>11.4 ± 0.9*</td>
<td>9.7 ± 0.8</td>
<td>11.1 ± 1.7*</td>
</tr>
<tr>
<td>FIB (mg/dl)</td>
<td>159.5 ± 11.1</td>
<td>158.3 ± 7.6</td>
<td>160.0 ± 14.0</td>
<td>166.6 ± 33.9</td>
</tr>
<tr>
<td>Factor VII (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>348.2 ± 37.8</td>
<td>298.5 ± 41.0*</td>
<td>347.0 ± 40.9</td>
<td>316.5 ± 40.9</td>
</tr>
<tr>
<td>Factor IX (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>233.3 ± 210.5</td>
<td>135.2 ± 93.6</td>
<td>270.0 ± 76.7</td>
<td>187.6 ± 79.8*</td>
</tr>
<tr>
<td>Factor X (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.3 ± 10.9</td>
<td>52.1 ± 10.3</td>
<td>45.1 ± 5.5</td>
<td>48.6 ± 11.8</td>
</tr>
</tbody>
</table>

The PB level was set at 80 mg/kg/day.  
Each value represents the mean ± S.D. of 10 pups.  
*: < 0.05, **: < 0.01, Significantly different from the control group (Student's t-test).  
Control: pups of non-treated dams, PB: pups of PB-treated dams.  
<sup>a</sup> Unit (%) = coagulation time in calibration plasma (HemosIL<sup>TM</sup>) (s) / coagulation time in sample (s) × 100.
tors, were decreased, suggesting a participation of the exogenous pathway as well as the endogenous pathway. Effects of PB on blood coagulation-related parameters were more severe in pups than in their dams. VK is supplied to pups through dams’ milk, and therefore, during the lactation period the dams need large amounts of VK. The metabolism of vitamin K is accelerated by PB administration (Wilson and Park, 1984). In addition, Bouwman et al. (1992, 1999) reported that administration of HxCB, a CYP2B inducer, to germfree rats of the WAG/Rij strain resulted in increases in vitamin K-dependent carboxylase and KO-reductase activities, which are related to the hepatic vitamin K cycle, as well as in decrease in factor VII activity. PB increased activities of DT-diaphorase and its coenzyme, NADPH cytochrome P450 reductase (Utley and Mehendale, 1989). Moreover, decreased plasma vitamin K levels have been observed in pregnant women receiving anticonvulsants (Cornelissen et al., 1993). Thus, PB administration induced CYP2B, increased vitamin K turnover and accelerated vitamin K metabolism. Therefore, prolongation of blood coagulation time observed in this study seems to be caused by decreases in vitamin K-dependent coagulation factors. Moreover, prolongation of blood coagulation time of pups by PB administration to dams was improved by VK2 co-administration to lactating dams. This also supports the idea that the PB-induced prolongation of coagulation time is related to vitamin K deficiency.

PB is able to be transferred to pups via dams’ milk (Atkinson et al., 1988), and this may suggest a direct effect of PB on prolongation of blood coagulation time in pups. However, such direct effect was considered to be minimal, because pups are immature to metabolize drugs and, as mentioned above, prolongation of blood coagulation time in pups was improved by VK2 co-administration to lactating dams.

The prolongation of APTT was still observed in lactating dams.

### Table 3. Effects of co-administration of PB and VK2 on coagulation parameters in dams

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PB + VK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>12.7 ± 1.0</td>
<td>13.0 ± 0.3</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>18.4 ± 2.5</td>
<td>22.0 ± 2.3*</td>
</tr>
<tr>
<td>FIB (mg/dl)</td>
<td>259.6 ± 37.2</td>
<td>267.2 ± 37.3</td>
</tr>
<tr>
<td>TBT (sec)</td>
<td>23.7 ± 1.1</td>
<td>23.0 ± 0.6</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>348.8 ± 27.9</td>
<td>385.8 ± 20.4*</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>11.9 ± 8.9</td>
<td>5.9 ± 3.6</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>47.9 ± 4.5</td>
<td>55.9 ± 5.4*</td>
</tr>
</tbody>
</table>

The PB and VK2 levels were set at 80 and 30 mg/kg/day, respectively. Each value represents the mean ± S.D. of 5 dams.

* <0.05, Significantly different from the control group (Student’s t-test).

Control: non-treated dams, PB + VK2: dams co-administered with PB and VK2.

= Unit (%) = coagulation time in calibration plasma (HemosIL™) (s) / coagulation time in sample (s) × 100.

### Table 4. Effects of co-administration of PB and VK2 to dams on coagulation parameters in their pups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>Control</td>
<td>8.1 ± 1.0</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>7.4 ± 0.3</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>Control</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>7.8 ± 0.4</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>FIB (mg/dl)</td>
<td>Control</td>
<td>154.4 ± 10.5</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>150.4 ± 17.2</td>
<td>162.2 ± 9.7</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>Control</td>
<td>374.8 ± 147.7</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>493.0 ± 48.9</td>
<td>324.1 ± 251.8</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>Control</td>
<td>937.7 ± 402.1</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>1,512.0 ± 559.6</td>
<td>1,291.7 ± 534.7</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>Control</td>
<td>65.3 ± 7.4</td>
</tr>
<tr>
<td>PB + VK2</td>
<td>67.7 ± 16.9</td>
<td>71.3 ± 13.4</td>
</tr>
</tbody>
</table>

The PB and VK2 levels were set at 80 and 30 mg/kg, respectively. Each value represents the mean ± S.D. of 5 pups.

No significant difference in PB and VK2 co-administration group from the control group (Student’s t-test).

Control: pups of non-treated dams, PB + VK2: pups of dams co-administered with PB and VK2.

Unit (%) = coagulation time in calibration plasma (HemosIL™) (s) / coagulation time in sample (s) × 100.
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tating dams after co-administration of PB and VK₂, suggesting that VK-supply to pups may take precedence over vitamin K-use by dams themselves.

It is reported that the risk of vitamin K-deficient neonatal hemorrhage in humans is higher in boys than girls (Hanawa et al., 1988). However, there was no significant sex difference in PB-induced changes in coagulation-related parameters in this study. PB is commonly administered at a dose level of 50 to 200 mg/man/day in humans, and the dose used in this study (80 mg/kg/day) is approximately 24-100 times higher than that used in humans. Such a high dose may induce no sex difference in the response of the coagulation system to PB-administration. Moreover, prolongation of APTTT was observed in lactating rats while no prolongation of blood coagulation time was observed in non-pregnant or pregnant rats. During the lactation period, dams supply vitamin K to pups via milk, and therefore, it is considered that the requirement of vitamin K is larger in lactating rats than in non-pregnant and pregnant rats. This suggests that a vitamin K deficiency may occur more easily in lactating rats than in non-pregnant and pregnant rats. We recently reported that prolongation of APTTT was observed from Day 1 of repeated administration of PB to male rats (Mochizuki et al., 2008). Prolongation of blood coagulation time by single administration of HxCB was more severe in males than that in females (Bouwman et al., 1999). In addition, Uchida and Komeno (1988) reported that vitamin K deficiency occurred more easily in male rats than in female rats. Taking these findings into consideration, it is reasonable to consider that there is a sex difference in PB-induced prolongation of coagulation time.

In conclusion, this study clarified the effect of PB administration on blood coagulation-related parameters in pregnant rats, lactating rats and their pups and the effects of VK on PB-induced changes in coagulation-related parameters in lactating dams and their pups.

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