Effects of DDAVP Administered Subcutaneously in Dogs with Aspirin-induced Platelet Dysfunction and Hemostatic Impairment due to Chronic Liver Diseases

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ABSTRACT. To evaluate the hemostatic effects of desmopressin (DDAVP) in dogs with aspirin-induced platelet dysfunction and hemostatic impairment in chronic liver diseases, 3 µg/kg DDAVP was administered subcutaneously. In aspirin-induced platelet dysfunction dogs (n=5), prolonged BMBT (buccal mucosal bleeding time) was shortened significantly after DDAVP injection (2.2 ± 1.2 min, P<0.05). In dogs with chronic liver diseases (n=4), activated partial thromboplastin time (APTT) tended to shorten by 0.9 to 3.0 sec, and prolonged BMBT was shortened in two cases for 4.2 and 1.7 min after DDAVP injection. Therefore, the present results indicated that DDAVP shortened the prolonged BMBT in dogs with aspirin-induced platelet dysfunction and chronic liver disease. DDAVP might be helpful in hemostasis under invasive procedures such as biopsy or surgery for dogs with hemostatic impairment.

KEY WORDS: aspirin-induced platelet dysfunction, canine, chronic liver disease, DDAVP, hemostasis.


MATERIALS AND METHODS

Animals: Fourteen dogs were used in this study, ten healthy adult beagle dogs (5 female and 5 male weighing 9–12 kg, 1–2 years old) as experimental study and four clinical cases with chronic liver diseases. The healthy dogs of experimental study were randomly divided into two groups; group 1 was a control without treatment (n=5), and group 2 was treated with aspirin (20 mg/kg) orally twice every 11 hr for platelet dysfunction model (n=5). Four clinical cases with chronic liver disease (group 3) had hemostatic impairment in coagulation profiles and required liver biopsy for definitive diagnosis (Table 1).

Anesthesia: The dogs were premedicated with atropine sulfate (0.05 mg/kg, SC), midazolam (0.1 mg/kg, IV) and butorphanol (0.2 mg/kg, IV). Inhalation anesthesia with isoflurane was used following induction with isoflurane via face mask and intratracheal intubation.

DDAVP: A 0.01% solution of DDAVP (100 µg/ml) intended for intranasal administration (Ferring AB, Malmo, Sweden) was adjusted for subcutaneous use. DDAVP diluted with 0.9% sodium chloride solution and sterilized by 0.45 µm filter (Millipore Co., Bedford, MA, U.S.A.) was injected subcutaneously at the dorsal thoracic area (3 µg/kg of body weight).

Blood collection and bleeding time: Blood collection and examination of bleeding time were performed under anesthesia. In group 1, blood collection and examination of bleeding time were performed immediately before administration and 30, 60, 90, 120 min after treatment of DDAVP. In group 2, they were performed before and after aspirin administration, and 60 min after DDAVP. In group 3, blood was collected from the dogs before and 60 min after DDAVP injection, and bleeding time was examined in two
dogs (Case 1 and Case 2).

Blood was drawn into 0.13 M trisodium citrate (9 parts blood to 1 part citrate, v/v), and the samples were centrifuged (2,000 × g for 15 min), the plasma was frozen at −80°C and stored until analysis. In group 2, the blood samples were also centrifuged to obtain platelet rich plasma (800 × g for 15 min) for evaluation of platelet aggregation.

Bleeding time was measured by using a Simplate device (Organon Teknika Co., North Carolina, U.S.A.), as was previously described in the report of buccal mucosal bleeding time (BMBT) [5].

Laboratory methods: The following coagulation profiles were evaluated: prothrombin time (PT; Dade Behring, Marburg, Germany), activated partial thromboplastin time (APTT; Dade Behring, Marburg, Germany) and coagulant activity of clotting factor VIII (F VIII:C). In this study, F VIII:C was used normal human plasma and factor VIII deficient human plasma (George King Bio-Medical, Overland Park, KS, U.S.A.). Platelet aggregation was examined by a lumi-aggregometer (NBS HEMA TRACER 601, NIKO BIOSCIENCE, Tokyo, Japan) using 10 µM of adenosin diphosphate (ADP; MC Medical, Tokyo, Japan) and maximum platelet aggregation was measured.

Laparoscopy: Laparoscopy was performed in group 3. After anesthesia, each dog was positioned in left lateral recumbency, and the abdominal cavity was insufflated with carbon dioxide. A 5 mm trocar (Struz, German) was placed, and the liver was visualized and explored by 5 mm laparoscope (Struz, German). Liver biopsy was performed with disposable biopsy needle (14-gauge, 170 mm, HAKKO, Tokyo, Japan) from 60 to 90 min after DDAVP administration. After biopsy, clotting at the biopsy site was assured by laparoscopy.

Statistical analysis: All data were analyzed with a one-way ANOVA appropriate. When a significant effect was observed, the differences were located with a post hoc Bonferroni test. P<0.05 was considered significant. All data are reported as mean ± SD.

RESULTS

Subcutaneous injection of DDAVP (3 µg/kg) in healthy dogs (group 1) induced significant increases (P<0.05) in plasma concentrations of F VIII:C (Fig. 1). This increase continued from 60 to 120 min. However, there were no sig-

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Table 1. Canine cases with chronic liver disease (group 3)

<table>
<thead>
<tr>
<th>Breed</th>
<th>Case 1 (LC)</th>
<th>Case 2 (CAH)</th>
<th>Case 3 (CPH)</th>
<th>Case 4 (CC)</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7</td>
<td>12</td>
<td>9</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>–</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>23.6</td>
<td>3.8</td>
<td>6.3</td>
<td>4.2</td>
<td>–</td>
</tr>
<tr>
<td>Clinical history</td>
<td>Vomiting, Icterus</td>
<td>Vomiting, Icterus</td>
<td>Depression, Anorexia</td>
<td>Vomiting, Anorexia</td>
<td>–</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>753</td>
<td>2268</td>
<td>418</td>
<td>22825</td>
<td>23–212</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>502</td>
<td>665</td>
<td>243</td>
<td>1701</td>
<td>10–100</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>209</td>
<td>321</td>
<td>163</td>
<td>148</td>
<td>0–50</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>9.9</td>
<td>10.5</td>
<td>7.3</td>
<td>5.5</td>
<td>6.0–8.0</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>19.9</td>
<td>39.0</td>
<td>16.0</td>
<td>17.0</td>
<td>12.0–18.0</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>73</td>
<td>119</td>
<td>98</td>
<td>271</td>
<td>200–400</td>
</tr>
<tr>
<td>Antithrombin III (%)</td>
<td>61</td>
<td>33</td>
<td>62</td>
<td>89</td>
<td>90–150</td>
</tr>
<tr>
<td>Platelet count (× 10^3/mℓ)</td>
<td>341</td>
<td>364</td>
<td>391</td>
<td>300</td>
<td>250–500</td>
</tr>
</tbody>
</table>

LC: Liver cirrhosis, CAH: Chronic active hepatitis, CPH: Chronic persistent hepatitis, CC: Chronic cholangitis. PT: Prothrombin time, APTT: Activated partial thromboplastin time.

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Fig. 1. Change in PT, APTT, F VIII:C, platelet count and BMBT in healthy dogs (group 1) after subcutaneous administration of DDAVP (3 µg/kg).
EFFECTS OF DDAVP IN DOGS

Table 2. Effects of the subcutaneous administration of DDAVP on the PT, APTT, BMBT, platelet count and maximum platelet aggregation in five dogs with aspirin-induced platelet dysfunction (group 2)

<table>
<thead>
<tr>
<th>Case</th>
<th>Subcutaneous administration of DDAVP</th>
<th>PT (sec)</th>
<th>APTT (sec)</th>
<th>BMBT (min)</th>
<th>Platelet (× 10³/µl)</th>
<th>Maximum platelet aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before Aspirin</td>
<td>8.4 ± 0.5</td>
<td>12.4 ± 0.8</td>
<td>2.7 ± 0.6</td>
<td>317 ± 62</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>After Aspirin</td>
<td>8.3 ± 0.2</td>
<td>12.8 ± 1.1</td>
<td>4.6 ± 1.3</td>
<td>334 ± 60</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>3</td>
<td>After DDAVP</td>
<td>8.2 ± 0.4</td>
<td>12.5 ± 0.7</td>
<td>2.4 ± 0.3</td>
<td>310 ± 58</td>
<td>28 ± 9</td>
</tr>
</tbody>
</table>

PT: Prothrombin time, APTT: Activated partial thromboplastin time, BMBT: Buccal mucosal bleeding time.
Figures indicate mean ± SD, †: P<0.05, NS: not significant.

Table 3. Effects of the subcutaneous administration of DDAVP on the PT, APTT, BMBT and F VIII:C in dogs with chronic liver disease (group 3)

<table>
<thead>
<tr>
<th>Case</th>
<th>Subcutaneous administration of DDAVP</th>
<th>PT (sec)</th>
<th>APTT (sec)</th>
<th>BMBT (min)</th>
<th>F VIII:C (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before After</td>
<td>9.8</td>
<td>9.6</td>
<td>20.2</td>
<td>10.2</td>
</tr>
<tr>
<td>2</td>
<td>Before After</td>
<td>7.8</td>
<td>8.4</td>
<td>24.0</td>
<td>23.0</td>
</tr>
<tr>
<td>3</td>
<td>Before After</td>
<td>8.4</td>
<td>8.2</td>
<td>19.6</td>
<td>18.1</td>
</tr>
<tr>
<td>4</td>
<td>Before After</td>
<td>5.5</td>
<td>5.4</td>
<td>17.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>


In this study, subcutaneous injection of DDAVP (3 µg/kg) in healthy dogs induced rapid increases in plasma concentrations of F VIII:C, and this increase continued from 60 to 120 min after DDAVP administration. The prolonged BMBT in dogs with aspirin-induced platelet dysfunction was shortened significantly after DDAVP injection. Prolonged BMBT and APTT in dogs with chronic liver diseases tended to shorten by DDAVP administration.

Intravenous and subcutaneous injection of DDAVP is known to increase factor VIII and von Willebrand factor plasma levels dose-dependently in normal dogs [6, 10]. This effect would be resulted by the release of von Willebrand factor from vascular endothelial cells. Therefore, DDAVP has been used to treat hemophilia A and von Willebrand disease in dogs [15, 17]. In dogs with congenital factor deficiencies, DDAVP has been used by IV or SC injection at the dosage of 1 to 5 µg/kg diluted in saline, and increase of F VIII:C peaked within 120 min [6]. In this study, DDAVP diluted in saline (3 µg/kg) was injected and evaluate the effects of it until 120 min in healthy dogs (group 1). F VIII:C rapidly increased after DDAVP administration and the effect continued from 60 to 120 min after DDAVP injection. Their effects were nearly same as other investigators [6]. Thus it was suggested that DDAVP might be effective, when given approximately 60 min before biopsy or surgery.

It is known that DDAVP infusion shortens the bleeding time in human patients treated with aspirin [13]. DDAVP has been used in controlling aspirin-induced coagulopathy after cardiac surgery [8]. Aspirin prolongs bleeding time also in dogs by inhibiting platelet aggregation [4, 5, 18]. In group 2, prolonged BMBT due to aspirin was shortened significantly after DDAVP injection. However, the increased maximum platelet aggregation showed no significant changes. The results suggested that DDAVP could shorten the prolonged BMBT by aspirin without relation to platelet aggregation and von Willebrand factor increased with F VIII:C might enhance platelet adhesion and potentiate primary hemostasis.

Diagnosis of chronic liver diseases in dogs frequently requires acquisition of a liver biopsy [7]. However, these cases are suffering from bleeding problems because of the integral role of the liver in hemostasis [2]. Thus, it is impor-
tant to examine the coagulation such as PT and APTT before liver biopsy. Furthermore, BMBT is a safe, easy and helpful test in evaluating primary hemostatic disorders [16]. In group 3, BMBT was prolonged in two dogs examined (case 1 and case 2). APTT and BMBT tended to be shortened at 60 min after DDAVP injection. Though this change was also observed in human [9, 13], the mechanisms of these beneficial effects are not understood. In any way, all dogs in which liver biopsy was performed after DDAVP injection were good at hemostasis in biopsy sites when observed by laparoscopy after liver biopsy.

In human with aspirin-induced platelet dysfunction and chronic liver diseases, DDAVP infusion induces a significant shortening in bleeding time with the increase of plasma F VIII:C and von Willebrand factor [10]. This effect may be due to an increase in plasma level of von Willebrand factor which facilitates platelet adhesion to subendothelium [19]. A shortening of APTT in dogs with chronic liver diseases is probably due to the included F VIII:C.

In conclusion, subcutaneous injection of DDAVP shortens the prolonged BMBT in dogs with aspirin-induced problems under invasive procedures such as liver biopsy or surgery.

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REFERENCES