Spectrofluorophotometric Determination of Quinidine Sulfate in Equine Plasma After Oral Administration

Sachiko Oh-ISHI*, Tetsuo SENTA*, Akio AMADA** and Katsuyoshi KUBO*

Spectrofluorophotometric determination of quinidine sulfate in horse plasma was established, using deproteinization with 20% metaphosphoric acid. The plasma levels of quinidine sulfate after a single oral administration with 40 or 60 mg/kg were determined. A peak value of 10 µg/ml was found 3 hours after administration with 60 mg/kg. The substance was detectable even 30 hours after administration. A single oral dose of 40 mg/kg showed no significant side effects, but one of 60 mg/kg induced colic or uterine pain in one horse as side effects.

Cardiovascular disorder in racehorses may not only be injurious to the affected animal itself but also involve other horses in fatal accident during the race.

The authors have been keeping watch on heart diseases of racehorses and intending to treat those troubles, including atrial fibrillation1. Atrial fibrillation in the horse and its treatment with quinidine is precisely described by DETWEILER and PATTERSON2 in the textbook. Although quinidine sulfate, one of the most commonly used antiarrhythmic drugs, is generally recognized to be effective against atrial fibrillation, one should use it with a continuous monitoring on its blood level and clinical changes due to its significant side effects. DETWEILER reported the dose schedule of quinidine sulfate for horses, but there is little information about the blood level. In man the critical level of quinidine which prevents arrhythmia is reported as 4 to 6 µg/ml, and the toxic level as over 10 µg/ml3.

The present paper deals with the establishment of a method to determine the presence of quinidine in equine blood and measure its blood level after oral administration.

Materials and Methods

Animals: Three gelding and female horses of a light breed 3 to 5 years old weighing 400 to 500 kg each were used (Table. 1). One to 0.5% quinidine sulfate solution in water was administered via a stomach tube. Prior to and following the administration with the drug solution, additional water was given so that a total amount of water might reach 3 liters. Blood samples were collected from the external jugu-
Determination of Quinidine Sulfate in Equine Plasma

Table 1. Experimental animals

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Age* (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trotter</td>
<td>Female</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Thoroughbred</td>
<td>Gelding</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Anglo-Arabian</td>
<td>Female</td>
<td>4</td>
</tr>
</tbody>
</table>

* Counted in the Japanese manner; that is, any fraction of a year is counted as one full year.

Quinidine determination: One-half ml of heparinized equine plasma (3-5 U/ml of heparine) was diluted in 19.5 ml of distilled water and mixed with 20 ml of freshly prepared 20% metaphosphoric acid solution on an electric shaker for 15 minutes and centrifuged at 4,500 g for 15 minutes. The resulting supernatant solution was examined for fluorescence at the wave length of 350 mp (excitation) or 450 mp (emission) with each slit 10 mp wide by a Hitachi fluorescence spectrophotometer (MPF-2A). A standard curve was obtained with each solution containing 0.5 ml of control equine plasma in 19.5 ml distilled water. Then, 0, 3, 5, or 8 µg of quinidine sulfate was treated by the same procedure as the plasma samples. The plasma levels of quinidine sulfate were calculated from the standard curve.

Hematocrit value: Each blood sample was subjected to the measurement of hematocrit value by the capillary method (11,000 rpm for 5 minutes).

Electrocardiography (ECG): Animals were kept on watch by ECG and examined for clinical signs throughout the experimental period of 3 minutes before the collection of each blood sample. A precise manual for the A-B lead using a portable electrocardiograph was cited in reference 4.

Results and Discussion

The deproteinization of equine plasma is not so easy as that of human plasma. An effort was made to modify the concentration of the solution of metaphosphoric acid and the dilution of plasma by the method by Brodie5,6). In it, 0.5 ml of plasma was diluted with 9.5 or 19.5 ml of distilled water and mixed with 10 or 20% metaphosphoric acid solution. Of four combinations of the above-mentioned dilution

![Fig. 1. Plasma concentration of quinidine sulfate in horses after single oral administration](image1)

Circles show the mean values of 3 (40 mg/kg) and 2 (60 mg/kg) horses. The drug level is expressed in terms of µg/ml of plasma, as determined by the spectrofluorophotometric method.

![Fig. 2. Plasma concentration of quinidine sulfate in Horse-1 after single oral administration](image2)

The three curves show the plasma level determined after administration with 20, 40, and 60 mg/kg, respectively.
and metaphosphoric acid, the dilution with 19.5 ml of water using 20% metaphosphoric acid exhibited the highest recovery rate of 95-100%. When this combination of deproteinization solution was used, spectrophotometric determination of quinidine in horse plasma was successful and standard quinidine sulfate (between 0-10 µg) showed a linear increase in fluorescence in proportion to the concentration.

By means of the method mentioned above, the plasma concentration of quinidine sulfate was determined after a single oral administration in horses. Fig.1 shows the plasma concentrations which are mean values of 3 (40 mg/kg) and 2 (60 mg/kg) horses after a single oral administration with 40 or 60 mg/kg of quinidine sulfate. Peak plasma levels, 10 (60mg/kg) and 7.5 (40mg/kg) µg/ml, were found 3 hours after administration, respectively. Even after 30 hours a detectable amount was found in a dose of 40 mg/kg.

Horse-1, a 3-year-old female, showed a gradual increase in plasma level in proportion to the dose, as indicated in Fig. 2. In this case, a side effect, periodical pain resembling colic or labor pain, was found 3 hours after administration with a dose of 60 mg/kg and maintained for 3 hours, as shown in Fig. 3. No other side effects were found on heart rate, hematocrit value, or ECG. After administration with 40 mg/kg, no significant side effects were observed in any horse tested, except a 5-year-old female suffering from mild diarrhea and inflammation around the anus and vaginal opening. Quinidine contained in feces might have induced the inflammation. Such inflammation was prevented in the next series of experiments by local application of vaselin prior to the administration of quinidine. As shown in Fig. 4, another horse, a 4-year-old male, was free from any side effect. Further investigation is under way on the blood level of
Determination of Quinidine Sulfate in Equine Plasma

quinidine sulfate in the course of treat-
ment of various cardiac diseases in
horses.

Acknowledgments

The authors wish to thank Misses Mieko SEKITA and Keiko NISHIMOTO for their technical assistance.

Reference

2) DETWEILER, D. K. and D. F. PATTERSON (1963): Diseases of the blood and cardiovas-

ウマ血漿中キニジンの蛍光分光定量法について

大石幸子**・千田哲生*・天田明男***・久保勝義*

馬血漿中のキニジン定量法を確立し、実験馬3頭を用
いて硫酸キニジン経口投与後の血漿中キニジン濃度を
測定した。除蛋白剤として20％ノマ酸を、測定法と
して蛍光分光光度法を用い良好な成績を得た。馬体重
1kgあたり40mgおよび60mgの割合の硫酸キニジンを0.5
ないし1％水溶液として1回投与したところ
いずれも30分後からキニジンが血漿中出現し始め3
時間後には最高濃度（40mg/kg投与では7.5μg/ml、
60mg/kg投与では10μg/ml）に達した。その後漸減
したが、30時間後においても極く微量のキニジンが検
出された。副作用としては、60mg/kg投与3時間後
から約3時間後にわたって筋痛様症状を示した牝馬1頭
を除き他に異常がみられなかった。