In vitro and in vivo assessment of platelet function in healthy dogs during administration of a low-dose aspirin regimen

Jillian M. Haines DVM, MS
John M. Thomason DVM, MS
Eileen C. Seage VMD
Robert W. Wills DVM, PhD
Camilo Bulla DVM, PhD
Kari V. Lunsford DVM
Andrew J. Mackin BVMS, DVSc

Received October 23, 2014. Accepted April 20, 2015.

From the Departments of Clinical Sciences (Haines, Thomason, Seage, Lunsford, Mackin) and Pathobiology and Population Medicine (Wills, Bulla), College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762. Dr. Haines’ present address is Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164. Dr. Seage’s present address is School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Address correspondence to Dr. Haines (jmhaines@vetmed.wsu.edu).

OBJECTIVE
To assess the in vitro and in vivo platelet function of healthy dogs during administration of a low-dose aspirin regimen.

ANIMALS
16 dogs.

PROCEDURES
Dogs received aspirin (1 mg/kg, PO, q 24 h) for 7 days. Blood and urine samples were collected before (day 1; baseline) and on days 3 and 7 of the low-dose aspirin regimen. Platelet function was evaluated by use of turbidimetric and conventional impedance aggregometry, multiple-electrode impedance aggregometry, a platelet function analyzer (PFA), and determination of urine 11-dehydro-thromboxane B2 concentration. Turbidimetric aggregometry results were compared with the results obtained by the other 4 methods. Fourteen days after cessation of aspirin, platelet-rich plasma was incubated with acetylsalicylic acid and platelet function was assessed by turbidimetric aggregometry to determine whether this technique could accurately identify dogs that responded to the low-dose aspirin regimen.

RESULTS
Of the 16 dogs, 13 had turbidimetric and conventional impedance aggregometry results that were decreased by > 25% from baseline on days 3 and 7, and 4 and 7 dogs had PFA closure times > 300 seconds on days 3 and 7, respectively. The median urine 11-dehydro-thromboxane B2 concentration-to-creatinine concentration ratio decreased by 49% between days 1 and 7. Turbidimetric aggregometry results were correlated with conventional impedance aggregometry results. There was poor agreement between the turbidimetric aggregometry and PFA results. The multiple-electrode impedance aggregometry protocol failed to reliably detect aspirin-induced platelet dysfunction. In vitro incubation of platelet-rich plasma with acetylsalicylic acid followed by turbidimetric aggregometry did not predict whether dogs responded to the low-dose aspirin regimen.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that the response to a low-dose aspirin regimen varied among healthy dogs. (Am J Vet Res 2016;77:174–185)
with a low-dose aspirin regimen, whereas laboratory aspirin resistance is defined as inadequate inhibition of platelet function as determined by in vitro methods in an individual who is being treated with a low-dose aspirin regimen.\textsuperscript{21-25}

The purpose of the study reported here was to assess the in vitro and in vivo platelet function of healthy dogs during administration of a low-dose aspirin regimen. We assessed platelet function in healthy dogs that were receiving a low-dose aspirin regimen by use of turbidimetric aggregometry (the established method for identification of human patients with laboratory aspirin resistance), impedance aggregometry, a point-of-care PFA, and measurement of urine 11-dTXB\textsubscript{2} concentration in the hopes of determining which method was the best for identifying dogs with laboratory aspirin resistance and elucidating the underlying mechanisms of that condition. Ideally, identification of laboratory aspirin resistance would accurately predict clinical aspirin resistance; however, human patients with laboratory aspirin resistance do not always develop clinical aspirin resistance.\textsuperscript{19} In human medicine, a pretreatment protocol has been developed to determine whether a patient is likely to respond to the antiplatelet effects of aspirin. That protocol involves in vitro exposure of platelets to acetylsalicylic acid followed by platelet aggregometry.\textsuperscript{24} We hypothesized that the same in vitro protocol could be used for platelet samples obtained from dogs prior to initiation of and during a low-dose aspirin regimen to predict whether those dogs will respond to aspirin treatment and estimate the extent of aspirin-induced platelet dysfunction.

**Materials and Methods**

**Animals**

All study protocols were reviewed and approved by the Mississippi State University Institutional Animal Care and Use Committee. Clinically normal adult dogs owned by veterinary students, staff, faculty, and clients associated with the College of Veterinary Medicine at the University of Mississippi were recruited for the study. Owner consent was obtained for each dog prior to its enrollment in the study. To be considered for the study, dogs could not receive any medications or vaccines for at least 2 weeks prior to initiation of the study and had to be healthy as determined by results of a physical examination, CBC, serum biochemical analysis, heartworm and tick-borne disease assay,\textsuperscript{8} urinalysis, and platelet function analysis\textsuperscript{9} performed by a point-of-care PFA.\textsuperscript{5} Dogs that had any abnormal physical examination or diagnostic test results or that required routine medication were excluded from the study.

**Study design**

Prior to study initiation, a power analysis was performed with the use of information obtained from a previous study.\textsuperscript{17} Results of the power analysis indi-
icated that a sample size of 7 aspirin responders and 7 aspirin nonresponders was necessary for detection of aspirin-induced inhibition of platelet function with 80% power.

Each dog was administered aspirin\(^4\) (1 mg/kg, PO) once daily in the morning for 7 days. The aspirin dose for each dog was calculated on the basis of the dog’s body weight (rounded to the nearest kilogram) at study enrollment, and the appropriate dose was compounded into capsules by the College of Veterinary Medicine Pharmacy at Mississippi State University. No medications other than aspirin were administered to the dogs during the study.

**Sample collection**

From each dog, a blood sample (22.5 mL) for platelet aggregometry and PFA was obtained by jugular venipuncture with a 20-gauge needle and collected directly into evacuated 4.5-mL blood collection tubes that contained either 3.2% sodium citrate\(^6\) or hirudin\(^6\) immediately before initiation of the low-dose aspirin regimen on day 1 (baseline) and again on days 3 and 7. On days 3 and 7, the blood samples were collected between 2 and 5 hours after aspirin administration. An additional blood sample (18 mL) from each dog was collected into a blood collection tube containing 3.2% sodium citrate at least 14 days after the last dose of aspirin was administered for in vitro incubation with acetylsalicylic acid.\(^25\) During each sample collection, a sufficient amount of blood was obtained to achieve an anticoagulant-to-blood ratio of approximately 1:9 to standardize the extent of anticoagulation among samples.

A urine sample for determination of urine 11-dTXB\(_2\) concentration was also obtained from each dog on days 1, 3, and 7. On days 3 and 7, the urine samples were collected between 2 and 8 hours after aspirin administration. On each sample collection day, attempts were made to obtain the sample by free catch; however, if that was not possible, cystocentesis was performed with a 22-gauge, 1.5-inch needle attached to a 12-mL syringe. The volume of urine obtained on each sample collection day varied between 3 and 10 mL. Urine samples were stored frozen at -80°C until analysis.

**Platelet aggregation**

Platelet aggregation was determined for each blood sample within 4 hours after collection by the use of 3 methods (turbidimetric and impedance aggregometry performed by a conventional aggregometer and impedance aggregometry performed by an MEIA). For each aggregometric method, 4 replicates of each blood sample were analyzed, and the mean platelet aggregation for those 4 replicates was calculated and used for analysis.

The Hct and platelet count were within the respective reference ranges for all blood samples. To perform turbidimetric aggregometry, PRP and PPP had to be harvested from each blood sample. Blood samples that were collected into tubes that contained 3.2% sodium citrate were centrifuged at 1,200 X g for 3 minutes at room temperature (23°C). The PRP supernatant was decanted and collected, and the remaining blood sample was centrifuged at 1,800 X g for 8 minutes at room temperature to obtain PPP.

Turbidimetric aggregometry was then performed with a conventional, combined turbidimetric and impedance 2-channel platelet aggregometer\(^6\) in accordance with the manufacturer’s guidelines.\(^20\) Briefly, 450 µL of PRP was transferred to a glass cuvette that contained a siliconized magnetic stir bar, and 500 µL of PPP was transferred to a glass cuvette without a stir bar. The samples in the cuvettes were incubated at 37°C for 5 minutes and then placed into the aggregometer. Stable baseline values for minimal (obtained from PPP sample) and maximal (obtained from PPP sample) platelet aggregation were determined and assigned values of 0% and 100%, respectively. Prior to analysis, the platelet count in each PRP sample was not adjusted to a standardized count by dilution with PPP on the basis of recommendations by the International Society of Thrombosis and Haemostasis Platelet Physiology and Scientific and Standardization Committee.\(^27\) Once the baseline values were established, 5 µL of collagen (concentration, 10 µg/mL) was added to the PRP sample, and the sample was held at a temperature of 37°C and stirred at a speed of 1,200 revolutions/min for 12 minutes. Then, the maximal percentage of platelet aggregation was calculated by use of a software program\(^3\) that was interfaced with the aggregometer.

Impedance aggregometry was also performed with a conventional, combined turbidimetric and impedance 2-channel platelet aggregometer\(^6\) in accordance with the manufacturer’s guidelines.\(^20\) Briefly, 450 µL of saline (0.9% NaCl) solution and then 450 µL of citrated whole blood were transferred to a plastic cuvette containing a magnetic stir bar and incubated at 37°C for 5 minutes. The cuvette was placed into the aggregometer, a reusable impedance probe was inserted into the cuvette, and a stable baseline measurement for platelet aggregation was obtained. Then, 10 µL of collagen (concentration, 10 µg/mL) was added to the sample, and the sample was held at a temperature of 37°C and stirred at a speed of 1,200 revolutions/min for 12 minutes. The maximal amplitude of the sample, measured in ohms, was calculated and recorded as an indicator of maximal platelet aggregation.

Additionally, impedance aggregometry was performed with an MEIA\(^7\) in accordance with the manufacturer’s recommendations.\(^31\) Briefly, each whole blood sample that was collected into a tube that contained hirudin was transferred to a single-use test cell that contained a dual-sensor unit, Teflon-coated magnetic stir bar, and warmed saline solution. The volume of each sample or solution used in the analysis was controlled by an automated pipette provided with the aggregometer. The electrical resistance between the sensor wires of the dual-sensor unit was recorded.
Then, 10 µL of collagen (concentration, 10 µg/mL) was added to the sample, and the sample was held at a temperature of 37°C and stirred at a speed of 1,200 revolutions/min for 12 minutes. Platelet aggregation was measured and recorded as the AUC.

For each dog and aggregometric method, platelet aggregation results for blood samples obtained on days 3 and 7 were compared with those for blood samples obtained on day 1 (baseline). A dog was considered an aspirin responder if the maximal platelet aggregation on day 3 or 7 was decreased by > 25%, compared with that at baseline.32

**Platelet function analysis**

Platelet function was analyzed by the use of a commercially available point-of-care PFA that has been validated for use in dogs.18,33–35 The PFA is an in vitro method that mimics the exertion of high shear force over an area of vascular damage. Briefly, whole blood samples collected into tubes that contained 3.2% sodium citrate were gently mixed, but not agitated, and maintained at room temperature until analysis. Then, 800 µL of each sample was aspirated at a high shear rate through a disposable cartridge that contained an aperture within a membrane coated with either collagen (equine type 1 collagen, 2 µg) and ADP (50 µg; used during pretrial screening to determine whether potential study dogs had abnormal platelet function) or collagen (equine type 1 collagen, 2 µg) and epinephrine (10 µg; used during the study to assess aspirin-induced platelet dysfunction).1 The sample was aspirated through the cartridge for 300 seconds (cutoff time for the instrument) or until a platelet plug formed and blood flow was inhibited. The time required for a sample to form a platelet plug and inhibit blood flow (ie, closure time) was measured in seconds. All cartridges were stored at 4°C and warmed to room temperature prior to use. For each citrated blood sample collected, platelet function was determined for 2 replicates, and the mean was calculated and used for analysis purposes. All blood samples were analyzed within 4 hours after collection.

On days 3 and 7, a dog was considered an aspirin responder if the closure time was > 300 seconds for both replicates.36 If a dog had 1 replicate with a closure time < 300 seconds and 1 replicate with a closure time > 300 seconds, a third replicate was analyzed as a tie-breaker. In those instances, a dog was considered an aspirin responder if the closure time was > 300 seconds for 2 of the 3 replicates.

**In vitro aspirin incubation**

In vitro incubation of platelets with acetylsalicylic acid (aspirin) was performed as described.24 Briefly, PRP was obtained from the citrated whole blood samples collected at least 14 days after cessation of aspirin as described for samples used for turbidimetric aggregometry. Acetylsalicylic acid was dissolved in 100% ethanol to create a 400mM solution. Then, 0.25 µL (100µM) of that acetylsalicylic acid solution was added to 1 mL of PRP and incubated at room temperature for 15 minutes. Following incubation, turbidimetric aggregometry was performed to assess platelet aggregation. Four replicates of each sample were analyzed, and the mean maximal percentage amplitude of platelet aggregation was calculated and used for analysis purposes. A dog was considered an aspirin responder if the maximal platelet aggregation after in vitro incubation had decreased by > 25%, compared with baseline.32

**Urine 11-dTXB₂ and creatinine concentrations**

Urine 11-dTXB₂ concentration was determined by the use of a commercially available ELISA that has been validated for use with canine urine.37 Prior to analysis, urine samples were thawed and allowed to warm to room temperature. The ELISA was performed in accordance with the manufacturer’s instructions, and 3 replicates of each sample were analyzed. Briefly, each sample was diluted with assay buffer solution and thoroughly vortexed. Then, 50 µL of each sample was added to a well of a 96-well plate, followed by 50 µL of 11-dTXB₂ monoclonal acetylcholinesterase tracer and 50 µL of 11-dTXB₂ monoclonal antibody. The plate was incubated at room temperature in the dark on an orbital shaker for 2 hours. Following incubation, 200 µL of Ellman reagent was added to each well, and the plate was incubated at room temperature in the dark on an orbital shaker for 1 hour. The plate was read with an automated plate reader at a wavelength of 412 nm. A correction factor was then applied to each reading to account for the dilution of the urine with the assay buffer solution, and the results were reported in picograms per milliliter.

The creatinine concentration of each urine sample was determined by use of an automated biochemical analyzer.40 Then, the ratio of 11-dTXB₂ concentration to creatinine concentration in each urine sample was calculated.38,39

**Statistical analysis**

Histograms were used to visually assess whether the platelet aggregation data for each aggregometric method were normally distributed. The distributions of the platelet function data and aggregometry results for the in vitro aspirin incubation assay were assessed for normality by use of a commercially available statistical software program.4 Data were not normally distributed for any of those outcomes; therefore, a Spearman rank test was used to assess the correlation of results among diagnostic tests (turbidimetric and impedance aggregometry, PFA, and in vitro aspirin incubation assay). Values of $P \leq 0.05$ were considered significant for all analyses.

For each diagnostic test, a method similar to the nonparametric Friedman test was used to evaluate differences in results for each dog over time. The data for each test were ranked within each dog. Then, ANOVA was performed with dog and sample day included as
fixed effects in the model. When the sample day effect was significant ($P \leq 0.05$), the least squares mean values were compared among the sample days by use of a Bonferroni adjustment to control for multiple comparisons.

Dogs were classified as aspirin responders or nonresponders on the basis of the respective results of the 3 aggregometric methods and PFA for blood samples collected on days 3 and 7 and results of the in vitro aspirin incubation assay. Kappa statistics were calculated to assess the extent of agreement beyond chance between the dichotomized turbidimetric aggregometry results and the dichotomized results for each of the other diagnostic tests and between the dichotomized impedance aggregometry and PFA results on days 3 and 7. Because some cells contained small numbers, exact methods were used to calculate the $\kappa$ statistics. The strength of agreement was classified in accordance with a scale proposed by Landis and Koch. Briefly, $\kappa$ values $< 0$ were classified as poor agreement, between 0.00 and 0.20 were classified as slight agreement, between 0.21 and 0.40 were classified as fair agreement, between 0.41 to 0.60 were classified as moderate agreement, between 0.61 and 0.80 were classified as substantial agreement, and $> 0.81$ were classified as almost perfect agreement.

Results

Dogs

Initially, 36 dogs were screened for inclusion in the study. Eighteen dogs did not meet the inclusion criteria for the study because they had positive results for infectious diseases, thrombocytopenia, or persistent lipemia; required unexpected surgery; or were going to be unavailable during part of the study period. Two dogs were excluded from the study because the closure time for the initial PFA test was $> 300$ seconds. Consequently, only 16 dogs (2 sexually intact males, 6 neutered males, 1 sexually intact female, and 7 spayed females) were enrolled in the study. The median age for the study population was 4.5 years (range, 1 to 9 years), and the median weight was 26.2 kg (range, 14.2 to 50.5 kg). Breeds represented included mixed breed ($n = 4$), Australian Shepherd (2), Labrador Retriever (2), American Pit Bull Terrier (2), and Alapaha Blue-Blood Bulldog, German Shorthaired Pointer, Great Dane, Greyhound, Pembroke Welsh Corgi, and Standard Poodle (1 each). Aspirin was administered to each dog for 7 days, and no adverse effects were reported.

Conventional turbidimetric aggregometry

For all 16 dogs, the median platelet aggregation amplitude as determined by turbidimetric aggregometry was 53.4% (range, 28.8% to 71.5%) on day 1 (immediately before initiation of low-dose aspirin regimen; baseline), 29.5% (range, 0.8% to 62.8%) on day 3, and 18.3% (range, 0.8% to 55.8%) on day 7. The median platelet aggregation amplitudes for days 3 and 7 did not differ significantly ($P = 0.414$) but were significantly ($P < 0.001$ for both) decreased from baseline. Thirteen dogs were classified as aspirin responders (platelet aggregation amplitude was decreased $> 25\%$, compared with that at baseline) on both days 3 and 7. Two dogs were classified as aspirin nonresponders on both days 3 and 7. Two dogs had conflicting results between days 3 and 7; 1 dog was classified as a responder on day 3 and a nonresponder on day 7, whereas the other dog was classified as a nonresponder on day 3 and a responder on day 7.

Conventional impedance aggregometry

Blood samples were only analyzed in triplicate instead of quadruplicate for 5 dogs (2 dogs on day 1, 2 dogs on day 3, and 1 dog on day 7) because of an unexpected machine error or insufficient sample volume; thus, for those dogs, the mean was calculated for the results of the 3 replicates and used for analysis. The median impedance aggregometry amplitude for all dogs was 5.6 $\Omega$ (range, 0.8 to 13.8 $\Omega$) at baseline, 0.9 $\Omega$ (range, 0 to 13.8 $\Omega$) on day 3, and 0.9 $\Omega$ (range, 0 to 13 $\Omega$) on day 7. The median impedance aggregometry amplitudes on days 3 and 7 did not differ significantly ($P = 1.000$) but were significantly ($P < 0.001$ for both) decreased from baseline.

Thirteen dogs were classified as aspirin responders on both days 3 and 7. One dog was classified as an aspirin nonresponder on both days 3 and 7. Four dogs had conflicting results between days 3 and 7; 2 dogs were classified as responders on day 3 and nonresponders on day 7, whereas the other 2 dogs were classified as nonresponders on day 3 and responders on day 7.

When the aspirin responsiveness as determined by conventional impedance aggregometry was compared with the aspirin responsiveness as determined by conventional turbidimetric aggregometry on the same blood samples, the results were in perfect agreement on day 3. On day 7, 10 dogs were classified as aspirin responders by both methods, 3 dogs were classified as responders by impedance aggregometry and nonresponders by turbidimetric aggregometry, and the remaining 3 dogs were classified as nonresponders by impedance aggregometry and responders by turbidimetric aggregometry. Thus, there was fair agreement between the conventional turbidimetric and impedance aggregometry results on day 7 (Table 1); however, the extent of that agreement did not differ significantly ($P = 0.582$) from that expected by chance.

Multiple-electrode impedance aggregometry

Multiple-electrode impedance aggregometry results were available for only 9 of the 16 dogs because the instrument became unavailable for use during the study period. For the 9 dogs that were evaluated, the median AUC was 1,656 U (range, 1,043 to 2,304 U) on day 1, 1,524 U (range, 1,280 to 2,945 U)
Table 1—Number of dogs classified as aspirin responders and nonresponders on days 3 and 7 of a low-dose aspirin regimen (1 mg/kg, PO, q 24 h) as determined by each of 4 methods for evaluation of platelet function.

<table>
<thead>
<tr>
<th>Method</th>
<th>Responders</th>
<th>Nonresponders</th>
<th>( \kappa ) (95% confidence interval)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional turbidimetric aggregometry</td>
<td>13</td>
<td>3</td>
<td>Referent</td>
<td>—</td>
</tr>
<tr>
<td>Conventional impedance aggregometry</td>
<td>13</td>
<td>3</td>
<td>1.00 (1.00 to 1.00)</td>
<td>0.002</td>
</tr>
<tr>
<td>Multiple-electrode aggregometry†</td>
<td>1</td>
<td>8</td>
<td>–0.25 (–0.74 to 0.25)</td>
<td>0.111</td>
</tr>
<tr>
<td>PFA</td>
<td>4</td>
<td>12</td>
<td>0.14 (0.05 to 0.33)</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3</td>
<td>Referent</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3</td>
<td>–0.23 (–0.41 to –0.05)</td>
<td>0.582</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>9</td>
<td>–0.16 (–0.53 to 0.21)</td>
<td>0.550</td>
</tr>
</tbody>
</table>

The study population included 16 healthy adult (> 1-year-old) dogs of various breeds. For each aggregometry method, an aspirin responder was defined as a dog in which there was > 25% reduction in maximal aggregation from baseline (day 1; immediately prior to initiation of aspirin administration), and an aspirin nonresponder was defined as a dog in which there was < 25% reduction in maximal aggregation from baseline. For PFA, a dog was defined as an aspirin responder if the closure time was > 300 seconds. Values of \( \kappa \) < 0 were classified as poor agreement, between 0.00 and 0.20 were classified as slight agreement, between 0.21 and 0.40 were classified as fair agreement, between 0.41 to 0.60 were classified as moderate agreement, between 0.61 and 0.80 were classified as substantial agreement, and > 0.81 were classified as almost perfect agreement.

* The \( P \) value for \( \kappa \) represents the probability that the results obtained were caused by chance. †Results available for only 9 of 16 dogs because the aggregometer became unavailable for use during the study period.

= Not calculated.

on day 3, and 1,989 U (range, 1,107 to 2,908 U) on day 7. The AUC did not vary significantly (\( P = 0.485 \)) by study day.

Of the 9 dogs evaluated by multiple-electrode impedance aggregometry, 8 were classified as aspirin nonresponders on day 3 and all 9 were classified as nonresponders on day 7. The only dog that was classified as an aspirin responder by multiple-electrode impedance aggregometry on day 3 was classified as a nonresponder by turbidimetric aggregometry. Thus, on day 3, there was poor agreement between the results obtained by multiple-electrode aggregometry and turbidimetric aggregometry, and that agreement did not differ significantly (\( P = 0.111 \)) from that expected by chance (Table 1). On day 7, only 1 of the 9 dogs classified as a nonresponder by multiple-electrode impedance aggregometry was also classified as a nonresponder by conventional turbidimetric aggregometry, and a \( \kappa \) value was not calculated.

PFA

For all 16 dogs, the median closure time was 118.5 seconds (range, 90 to 163 seconds) on day 1, 229 seconds (range, 112 to 300 seconds) on day 3, and 236.7 seconds (range, 157.5 to 300 seconds) on day 7. The median closure times for days 3 and 7 did not differ significantly (\( P = 0.634 \)) but were significantly (\( P < 0.001 \)) longer than that at baseline.

Four dogs were classified as aspirin responders (closure time > 300 seconds) on day 3, and 7 dogs were classified as aspirin responders on day 7. Only 3 dogs were classified as aspirin responders on both days 3 and 7. On day 7, the blood samples for 5 dogs had to be analyzed by PFA in triplicate before the dogs could be classified as responders or nonresponders. On day 3, all 4 dogs classified as responders and 3 dogs classified as nonresponders by the PFA were likewise classified by conventional turbidimetric aggregometry. However, there was disparate agreement between the classification results for 9 dogs, which suggested there was only slight agreement between the results of PFA and conventional turbidimetric aggregometry on day 3, and that agreement did not differ significantly (\( P = 0.529 \)) from that expected by chance (Table 1). On day 7, 5 of the 7 dogs classified as responders and 1 of 9 dogs classified as nonresponders by PFA were likewise classified on the basis of results of conventional turbidimetric aggregometry. Thus, there was poor agreement between the PFA and conventional turbidimetric aggregometry results on day 7, and that agreement did not differ significantly (\( P = 0.550 \)) from that expected by chance. When the PFA classification results were compared with the conventional impedance aggregometry classification results, there was slight agreement (\( \kappa = 0.14; 95\% \) confidence interval, –0.05 to 0.33; \( P = 0.529 \)) on day 3 and poor agreement (\( \kappa = 0.16; 95\% \) confidence interval, –0.53 to 0.21; \( P = 0.550 \)) on day 7.

In vitro aspirin incubation

The median platelet aggregation amplitude as determined by conventional turbidimetric aggregometry for blood that was incubated with acetylsalicylic acid in vitro was 2.3% (range, 1% to 10%). Compared with baseline results, the mean percentage reduction in amplitude was 91.9% (range, 66.7% to 98.0%). Although results of the in vitro aspirin incubation test indicated that all 16 dogs had marked suppression of platelet aggregation amplitude, 3 dogs had evidence of weak platelet aggregation, which suggested that those dogs still had some residual platelet function. Nevertheless, the decrease in platelet aggregation amplitude was > 25% from baseline for all dogs, which resulted in them being classified as aspirin responders.

AJVR • Vol 77 • No. 2 • February 2016 179

Unauthenticated | Downloaded 01/08/24 03:32 AM UTC
Urine 11-dTXB₂ concentration

Urine 11-dTXB₂ concentration results were unavailable for 3 dogs on day 1 and 2 dogs on day 7 because the concentrations were below the detection limit for the assay. The median ratio for urine 11-dTXB₂ concentration to urine creatinine concentration was 4.9 (range: 2.0 to 9.1) on day 1, 3.2 (range: 0.8 to 8.1) on day 3, and 2.5 (range: 0.8 to 13.5) on day 7. Overall, the median ratio for urine 11-dTXB₂ concentration to urine creatinine concentration decreased by 34.7% between days 1 and 3, by 49% between days 1 and 7, and by 21.9% between days 3 and 7. The ratio of urine 11-dTXB₂ concentration to urine creatinine concentration did not change significantly between days 1 and 3 (P = 0.315) or between days 3 and 7 (P = 0.664) but decreased significantly (P = 0.028) between days 1 and 7.

Correlation of aggregometry and PFA results

When the data from all 3 sample days were combined, the conventional turbidimetric aggregometry results were positively correlated with the conventional impedance aggregometry results (r = 0.63; P < 0.001) and negatively correlated with the PFA results (r = -0.56; P < 0.001). The conventional impedance aggregometry results were also negatively correlated with the PFA results (r = -0.55; P < 0.001). When the data for each sample day were analyzed separately, the results for the 3 different test methods were not significantly correlated on days 1 and 7. On day 3, the conventional turbidimetric aggregometry results were positively correlated with the conventional impedance aggregometry results (r = 0.66; P = 0.005), and the impedance aggregometry results appeared to be negatively correlated with the PFA results (r = -0.053), although that correlation did not quite reach significance (P = 0.053).

Discussion

Turbidimetric aggregometry is considered the gold-standard method for assessing aspirin-induced platelet dysfunction in humans on a low-dose aspirin regimen. In the present study, turbidimetric aggregometry was more consistent than conventional or multiple-electrode impedance aggregometry or PFA for identification of aspirin-induced platelet dysfunction in healthy dogs after 3 and 7 days of administration of a low-dose aspirin regimen (1.0 mg/kg, PO, q 24 h). Only 2 of the 16 dogs of the present study were inconsistently classified as either an aspirin responder or nonresponder on the basis of turbidimetric aggregometry results on days 3 and 7, whereas 4 and 5 dogs were inconsistently classified as aspirin responders or nonresponders on days 3 and 7 on the basis of results of conventional impedance aggregometry and PFA, respectively. The results of the present study were comparable to the results of another study in which dogs that received a low-dose aspirin regimen had inconsistent aspirin-induced platelet dysfunction detected during the observation period. In human patients, aspirin-induced platelet dysfunction is typically observed within minutes after ingestion of the drug; therefore, the results of platelet function tests performed 3 and 7 days after initiation of a low-dose aspirin regimen should be comparable. The inconsistent platelet function test results obtained for some dogs of the present study suggested that the tests evaluated did not reliably detect aspirin response in individual dogs on a day-to-day basis. Alternatively, the discrepancy in the results for platelet function observed between days 3 and 7 might be an indication that, in some dogs, aspirin-induced platelet dysfunction might be delayed and not fully evident until 7 days after initiation of a low-dose aspirin regimen or that the platelet response to aspirin may change over time. In the present study, 1 dog as determined by turbidimetric aggregometry results and 2 dogs as determined by impedance aggregometry results were classified as aspirin nonresponders on day 3 and aspirin responders on day 7. Those findings suggested that platelet function was progressively inhibited during aspirin administration. However, 1 dog as determined by turbidimetric aggregometry results and 2 dogs as determined by impedance aggregometry results were classified as aspirin responders on day 3 and aspirin nonresponders on day 7. Possible explanations for those findings include variable bioavailability of aspirin in some dogs, poor owner compliance with aspirin administration, an abnormally increased rate of platelet turnover resulting in the release of platelets that have not been exposed to aspirin into the circulation, or expression of a COX-1 enzyme variant that is less sensitive to aspirin inhibition than the normally expressed COX-1 enzyme.

Because turbidimetric aggregometry is considered the gold standard for assessment of platelet function in patients receiving a low-dose aspirin regimen, we chose to compare the results of all other platelet function tests evaluated in the present study with the turbidimetric aggregometry results. Although there was perfect agreement between the turbidimetric aggregometry and conventional impedance aggregometry results on day 3, the agreement between the results of those 2 tests was not perfect on day 7 (ie, each test identified 3 different dogs as aspirin nonresponders). Results of a study in which 6 platelet function tests were used to assess platelet function and the prevalence of aspirin resistance in human patients administered a low-dose aspirin regimen indicated that impedance aggregometry results had the highest correlation and the second highest agreement with turbidimetric aggregometry results for classification of patients with aspirin resistance (ie, nonresponders). Interestingly, on the basis of impedance aggregometry results, the prevalence of aspirin resistance in the human patients of that study (18% [36/200]) was similar to that in the dogs of the present study (19% [3/16]). Results of the present study suggested that impedance aggregometry may be a useful method for evaluation of aspirin-induced platelet dysfunction in dogs when
turbidimetric aggregometry is not feasible or available, although impedance aggregometry results may vary from turbidimetric aggregometry results in some dogs on some days.

Turbidimetric aggregometry is considered a non-physiologic method because it is performed on PRP, the platelets are mixed under low shear conditions, and aggregation occurs only after the addition of platelet agonists, which does not mimic the normal physiologic process for platelet adhesion, activation, and aggregation adjacent to damaged endothelium. An MEIA has been validated for use in dogs, which is why we chose to evaluate that type of aggregometer in the present study.The MEIA analyzes platelets within whole blood samples, which is a more natural environment than the PRP that is used for turbidimetric aggregometry because no blood components have been removed from the sample. Also, whole blood samples are not centrifuged, which decreases the risk for the loss of platelet subpopulations or activation of platelets prior to aggregometric analysis; however, they do need to be diluted, and that might affect the analysis.

Unfortunately, the MEIA was not available for use for the entire duration of the present study, and blood samples of only 9 of the 16 study dogs were evaluated with that method. The number of dogs evaluated by the MEIA resulted in insufficient power to allow for a true comparison of those results with the results of the other platelet function tests evaluated. Of the 9 dogs evaluated by the MEIA, only 1 was classified as an aspirin responder on day 3 and all 9 dogs were classified as aspirin nonresponders on day 7. On the basis of visual assessment of the curves generated by the MEIA, the protocol used in the present study did not reliably detect aspirin-induced platelet dysfunction, a finding that was discrepant with the results of other platelet function tests evaluated, and it is unlikely that evaluation of blood samples from additional dogs with multiple-electrode impedance aggregometry would have changed this conclusion. Although the MEIA is more user-friendly than the conventional aggregometer, the results of the present study suggested that the MEIA protocol, in which collagen was used as a platelet agonist, was not a reliable or accurate method for assessment of aspirin-induced platelet dysfunction in dogs receiving a low-dose aspirin regimen.

Calcium plays a critical role in platelet signaling, and calcium mobilization leads to the activation of platelet integrins, shape change, and granule secretion. The various anticoagulants used during collection of whole blood samples have different mechanisms of action, which could affect the exposure of platelets in those samples to calcium and result in artificial changes in platelet function. Citrate chelates divalent cations such as calcium; therefore, it is suggested that whole blood samples in which citrate was used as the anticoagulant be diluted with a calcium-containing solution prior to analysis with an MEIA. The use of hirudin or heparin as an anticoagulant preserves the ionized calcium concentration in whole blood samples, and those samples do not require dilution with a calcium-containing solution prior to analysis with an MEIA. Results of multiple studies indicate that citrate is a poor choice of anticoagulant for whole blood samples in which platelet function is to be evaluated with an MEIA, and in fact, the manufacturer of the MEIA used in the present study recommends that citrate not be used as the anticoagulant for whole blood samples analyzed by that instrument. Investigators of 1 study concluded the heparin or hirudin should be used as the anticoagulant in whole blood samples that are going to be analyzed with an MEIA, although the use of hirudin could result in some spontaneous platelet aggregation. On the basis of the results of preliminary research conducted by our laboratory group, the results of multiple-electrode impedance aggregometry have not been affected by spontaneous platelet aggregation in whole blood samples in which hirudin was used as the anticoagulant.

In the present study, we used collagen as a platelet agonist in the multiple-electrode impedance aggregometry protocol at a concentration that was similar to that used in the conventional aggregometry protocols and aggregometry protocols used to evaluate aspirin responsiveness in human patients. The use of a different platelet agonist such as arachidonic acid might improve the assessment of aspirin-induced platelet dysfunction by multiple-electrode impedance aggregometry.

Similar to the findings of other studies, results of the present study indicated that aspirin-induced platelet dysfunction in dogs could be detected by the use of a point-of-care PFA and a collagen and epinephrine cartridge and that PFA closure times were significantly correlated with the results of both turbidimetric and conventional impedance aggregometry. However, when the dichotomized (responders or nonresponders) PFA results were compared with the dichotomized turbidimetric aggregometry results, there was only slight agreement ($\kappa = 0.15$) between the 2 methods on day 3 and poor agreement ($\kappa = -0.16$) between the 2 methods on day 7. On the basis of the PFA results of the present study, the prevalence of aspirin resistance (nonresponders; 75% [12/16] on day 3; 56% [9/16] on day 7) for dogs administered a low-dose aspirin regimen was substantially greater than that (33% [8/24]) for dogs of another study that used the same PFA protocol. In a study of human patients, the prevalence of aspirin resistance (59.5% [119/200]) was similar to that on day 7 for the dogs of the present study; the same PFA protocol was used for both studies. Collectively, the results of the present study and those other studies indicate that, although the point-of-care PFA is user-friendly and is frequently used to evaluate platelet function in individuals receiving a low-dose aspirin regimen, it markedly overestimates the prevalence of aspirin resistance, compared with the results of turbidimetric aggregometry, and might not be the ideal method for use to determine whether a dog is definitively aspirin resistant.
In 2002, Weber et al. described an in vitro technique that involves the incubation of PRP with acetylsalicylic acid followed by turbidimetric aggregometry for diagnosis and categorization of aspirin resistance in human patients. In human patients, aspirin resistance is categorized into different types on the basis of results of a panel of tests that include evaluation of platelet function, measurement of the extent of thromboxane suppression after aspirin administration, and evaluation of platelet function after in vitro incubation of patient platelets with acetylsalicylic acid. This in vitro aspirin incubation technique is typically performed for human patients during periods of aspirin administration; however, the goal for evaluation of this technique for the dogs of the present study was to determine whether aspirin responsiveness could be predicted prior to initiation of a low-dose aspirin regimen. Results of the present study indicated that in vitro incubation of PRP with aspirin consistently inhibited platelet function, and the dichotomous turbidimetric aggregometry results following in vitro aspirin incubation were in poor agreement with the dichotomous results obtained by conventional turbidimetric and impedance aggregometry and PFA. These findings suggested that the in vitro aspirin incubation protocol used in the present study was not an effective method for predicting whether dogs will respond to a low-dose aspirin regimen. The in vitro aspirin incubation protocol used in the present study was exactly the same as that described by Weber et al. The pharmacokinetic profile of aspirin for dogs following oral administration approximates that for humans, although the aspirin doses evaluated in those studies were higher than the aspirin dose (1.0 mg/kg) administered to the dogs of the present study. The pharmacokinetic profile has not been established for the low-dose aspirin regimen that was administered to the dogs of the present study, and it is unknown whether the concentration of acetylsalicylic acid (100µM) used in the in vitro aspirin incubation protocol was similar to the blood concentration of acetylsalicylic acid achieved in dogs during administration of a low-dose aspirin regimen. It is possible that the concentration of acetylsalicylic acid used for the in vitro aspirin incubation protocol of the present study was too high, which might have accounted for the high prevalence of aspirin responders, and mimicked the blood concentration of acetylsalicylic acid achieved by administration of high doses of doses of aspirin, which consistently inhibit platelet function in dogs. A study to determine the pharmacokinetic profile for aspirin in dogs that will properly dissolve acetylsalicylic acid. Prior to initiation of the present study, we incubated canine PRP samples with only 100% ethanol to ensure that the ethanol did not alter platelet function. Following incubation, all of those samples had strong platelet aggregation as determined by turbidimetric aggregation, which suggested that 100% ethanol did not adversely affect platelet function.

In human patients, the stable thromboxane A2 metabolite 11-dTXB2 is used as an indicator of aspirin-induced COX inhibition. Measurements of thromboxane metabolite concentrations in plasma or serum can be artificially increased because of platelet activation during blood collection, storage, or processing. However, measurements of thromboxane metabolite concentrations in urine are not so affected, and urine thromboxane metabolite concentrations are considered more reliable indicators of thromboxane synthesis than are serum and plasma thromboxane concentrations. The 2 thromboxane metabolites most frequently evaluated are 11-dTXB2 and 2,3-dinorTXB2, with 2,3-dinorTXB2 thought to be the primary metabolite. Results of another study indicate that urine 2,3-dinorTXB2 concentration is a more sensitive indicator of aspirin-induced thromboxane inhibition in dogs than is urine 11-dTXB2 concentration. However, results of a study conducted by our laboratory group indicate that the urine 11-dTXB2 assay used in the present study was sufficiently sensitive to detect aspirin-induced COX inhibition in dogs receiving a low-dose aspirin regimen. One proposed mechanism of aspirin resistance in human patients is variable responsiveness of platelets to thromboxane, and it appears that thromboxane may not be a consistent or necessary platelet agonist in some dogs. Approximately 70% of canine platelets are insensitive to stimulation by thromboxane because they contain impaired thromboxane A2 receptor–linked G proteins. In the present study, 2 dogs were inconsistently classified as aspirin responders between days 3 and 7 on the basis of turbidimetric aggregometry results despite the fact that the ratio of urine 11-dTXB2 concentration to urine creatinine concentration in both dogs decreased between days 1 and 7. Interestingly, 3 of the dogs that were classified as aspirin responders as determined by turbidimetric aggregometry results on both days 3 and 7 had an increase in the ratio of urine 11-dTXB2 concentration to urine creatinine concentration between days 1 and 7. The fact that there was no clear agreement or correlation between the turbidimetric aggregometry results and the urine 11-dTXB2 concentration–to–urine creatinine concentration ratios for the dogs of the present study suggested that aspirin resistance in some dogs, as in some humans, is not simply the result of failure to suppress COX enzymes. Studies in which urine 2,3-dinorTXB2 concentration is measured in dogs receiving a low-dose aspirin regimen are warranted to elucidate the role of thromboxane in aspirin resistance. Unfortunately, an assay to measure 2,3-dinorTXB2 concentration in canine urine is not
currently commercially available. Causes of aspirin resistance other than failure of thromboxane inhibition in dogs as well as human patients include variable aspirin bioavailability in some individuals, poor compliance with the low-dose aspirin regimen, and platelet activation by agonists other than thromboxane such as ADP. On the basis of the results of the present study, aspirin resistance in dogs appears to be multifactorial, and the precise cause may vary among patients.

The most commonly reported low-dose aspirin regimens for platelet inhibition in dogs range from 0.5 to 1 mg/kg, PO, once daily.13,17,37,55–57 We chose to use an aspirin dosage of 1 mg/kg, PO, once daily for 7 days in the present study, because that dose was low enough to minimize the risk of adverse effects and higher than the 0.5 mg/kg dose, which is associated with inconsistent inhibition of platelet function in dogs.16,57 Interestingly, high doses of aspirin (10 mg/kg, PO, q 12 h) consistently inhibit platelet function in dogs, which suggests that aspirin-associated platelet dysfunction is dose-dependent.13 Therefore, one potential cause of aspirin resistance in dogs that receive a low-dose (0.5 to 1 mg/kg, PO, q 24 h) aspirin regimen is underdosing, and simply increasing the aspirin dose administered might result in the desired effect in some dogs. Additional studies are warranted to determine the optimal aspirin dose range that maximizes inhibition of thromboxane synthesis and platelet function and minimizes the risk of adverse effects.

Two dogs were excluded from the study during the initial screening because they had PFA closure times > 300 seconds. It is unknown whether those dogs had underlying intrinsic or acquired platelet function abnormalities. Acquired platelet dysfunction can result from ingestion of an NSAID that was intentionally administered by the owner and not disclosed or consumed without the owner’s knowledge. Conversely, those findings might have been caused by PFA measurement error. However, the PFA did not indicate any functional errors at the time the samples were analyzed, and duplicate samples yielded the same results. The PFA was calibrated and cleaned regularly, and similar discrepancies were not detected in any of the other blood samples evaluated on that day; therefore, we do not believe that the results for those 2 dogs were caused by PFA measurement error or malfunction.

In some dogs with normal platelet function, the use of cartridges that contain collagen and epinephrine in the PFA results in abnormally prolonged closure times.34,35 Although the reason for those abnormally prolonged closure times is unknown, it is possible that the platelet agonists inside the cartridges were not capable of stimulating platelet aggregation. Because we wanted to ensure that all dogs enrolled in the present study had normal platelet function prior to aspirin administration, the initial screening of dogs for the study involved assessment of the platelet function of all dogs with standard techniques such as evaluation of closure time by a PFA with a collagen and epinephrine cartridge. Dogs that had abnormally prolonged closure times were excluded from the study to prevent any potential confounding of PFA results following initiation of aspirin administration. Although it would have been interesting to determine why 2 apparently healthy dogs had abnormally prolonged PFA closure times during the initial screening for this study, that was beyond the scope of the study.

One limitation of the present study was the use of client-owned dogs, which prevented us from precisely controlling all aspects of the environment of each dog. For example, some dogs might have been inadvertently exposed to medications other than aspirin during the study period (e.g., consumed medication intended for another pet in the household) or owners might not have fully complied with the low-dose aspirin regimen resulting in intermittent dosing, which might have contributed to the inconsistent responses to aspirin that were observed. However, evaluation of dogs of various breeds rather than a relatively homogenous research dog population allowed for a more clinically relevant assessment of aspirin resistance in the general dog population.

Another limitation of the present study was that urine was not obtained from each dog at the same time on each sample collection day. Although we attempted to collect urine at the same time each day, some dogs urinated or had a minimal amount of urine in the bladder prior to entering the hospital for sample collection. Variability in the duration of time after aspirin administration that urine samples were collected may have affected urine thromboxane concentrations. Additionally, the dogs evaluated in the present study were all healthy, and the results may not be directly applicable to diseased dogs. Further research is needed to determine the effect of a low-dose aspirin regimen on the platelet function of dogs with specific disorders.

Results of the present study indicated that administration of a low-dose aspirin regimen (1 mg/kg, PO, q 24 h) to healthy dogs resulted in aspirin-induced platelet dysfunction as determined by turbidimetric aggregometry in most, but not all, dogs. Conventional impedance aggregometry results were significantly correlated and had reasonable, albeit not perfect, agreement with turbidimetric aggregometry results. However, the MEIA protocol used in the present study did not perform well, and we do not recommend the use of that method or protocol to assess platelet function in dogs following aspirin administration. Point-of-care PFA results had poor agreement with turbidimetric aggregometry results and tended to overestimate the prevalence of aspirin resistance in the study population, which suggested that the PFA might not provide reliable measurement of aspirin-induced platelet dysfunction in dogs. In vitro incubation of PRP with acetylsalicylic acid followed by turbidimetric aggregometry did not accurately predict whether dogs responded to a low-dose aspirin regimen. Additional studies are warranted to determine whether evidence of aspirin resistance as determined by laboratory

Acknowledgments

This manuscript represents a portion of a thesis submitted by Dr. Haines to the Mississippi State University Department of Veterinary Clinical Sciences as partial fulfillment of the requirements for a Master of Science degree. Supported by the Mississippi State University College of Veterinary Medicine Internal Competitive Research Grant and Dr. Hugh G. Ward Endowment. Presented in part in abstract form at the 2014 American College of Veterinary Internal Medicine Forum, Nashville, Tenn, June 2014.

The authors thank Lydia Shafer, Aimee Daniel, Matthew Raby, Leslie Reed, and Lisa Pritchard for technical assistance.

Footnotes

a. SNAP 4DX Plus Test, Idexx Laboratories Inc, Westbrook, Me.
b. Dade PFA-100 Collagen/ADP Test Cartridge, Siemens Healthcare Diagnostics Inc,Tarrytown, NY.
c. PFA-100 System, Siemens Healthcare Diagnostics Inc, Tarrytown, NY.
e. Vacutainer tube, Becton Dickinson, Franklin Lakes, NJ.
f. Hirudin vacuum blood collection tube, Verum Diagnostica GmbH, Munich, Germany.
h. AGGRO-LINK 8, Chrono-Log Corp, Haverton, Pa.
i. Multiplate Analyzer, Verum Diagnostica GmbH, Munich, Germany.
j. Dade PFA-100 Collagen/EPI Test Cartridge, Siemens Healthcare Diagnostics Inc,Tarrytown, NY.
l. SpectraMax M5 multi-mode microplate reader, Molecular Devices, Sunnyvale, Calif.
m. ACE Alera clinical chemistry system, Alfa Wasserman Inc, West Caldwell, NJ.
n. SAS software, version 9.2, SAS Institute, Cary, NC.

References

30. Cattaneo M, Lecchi A, Zighetti ML, et al. Platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize stud