Platelet Storage Pool Deficiency in Pigs

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We report a new bleeding disease—storage pool deficiency (SPD) of platelets—in pigs from the Mayo swine colony of homozygous von Willebrand’s disease (vWD) and of heterozygous carriers of vWD. Levels of factor VIII, von Willebrand factor antigen (vWF:Ag), and ristocetin cofactor (RCof) were similar in the vWD carriers and SPD pigs. The latter pigs, however, had bleeding times of 15 minutes or more and were severe bleeders, in contrast to clinically normal vWD carriers. Platelet aggregation in response to collagen was reduced in most SPD pigs. Total platelet content of ADP, ATP, and serotonin was less than that of normal pigs. While the initial uptake of \(^{14}C\)-labeled serotonin into platelets was similar in SPD and normal pigs, retention of serotonin was reduced in platelets of SPD pigs. Transmission electron microscopy showed a large decrease of dense bodies in the platelets of SPD pigs. These findings support a diagnosis of SPD. Genetic analyses suggest an autosomal recessive mode of inheritance. A breeding program is under way to produce pigs affected only at the SPD gene, thus allowing further characterization of SPD and SPD-carrier pigs.

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mmol/L NaH₂PO₄ • 7H₂O, 3.8 mmol/L KH₂PO₄, and phenol red (0.1 g/L). After 15 minutes at 37 °C, the samples were sedimented to pellets, and the supernatant was discarded and replaced with 3% glutaraldehyde in the same buffer. Fixation was continued at 24 °C for 60 minutes. The cells were then washed in buffer and combined with 1% osmic acid in barbital acetate buffer (0.02 N HCl, a 20% stock solution containing 0.14 mol/L sodium barbital and 0.145 mol/L sodium acetate, and a 6.8% solution of a stock solution containing 1.7 mol/L NaCl and 18 mmol/L CaCl₂). After exposure to the second fixative for 1 hour, the cells were dehydrated in a graded series of alcohol and embedded in an epoxy resin (Epon 812). Contrast of thin sections cut from plastic blocks on an ultramicrotome was enhanced with uranyl acetate and lead citrate. Observations were made with an electron microscope (Philips 301).

**Infusion studies.** Two SPD swine were placed on their backs in wooden cradles and restrained by ropes; pigs lie quietly in this position. One 136-kg pig received 1,400 mL of normal pig cryoprecipitate prepared by plateletphoresis in a cell processor (Haemonetics model 30 blood cell processor). The total platelet content was 10.7 × 10¹¹, which was calculated to result in an increase of 3.0 × 10¹⁰ platelets/mL. Blood was removed at intervals for hemostatic tests, and ear bleeding times were determined.

**RESULTS**

**Aggregation studies.** Collagen-induced aggregation in citrated PRP was absent or decreased in 10 of the 11 SPD pigs (Fig 1). The response to collagen of platelets from vWD and vWD-carrier pigs was varied (two of eight vWD pigs and two of six vWD-carrier pigs having absent or decreased collagen-induced aggregation). A likely explanation for this finding is that some vWD-carrier pigs were heterozygous at the SPD gene and the vWD pigs were either homozygous or heterozygous at the SPD gene. A breeding program is under way to breed separate strains of SPD and vWD pigs and to characterize pigs of each of the nine possible genotypes involving the SPD and vWD genes.

Results of studies using resuspended gel-filtered platelets are plotted in Fig 2. The maximal recorder pen deflection was expressed as ΔT. SPD platelets were less responsive to collagen whether resuspended in normal, vWD, or autologous PPP. In contrast, normal platelets aggregated in response to collagen when resuspended in PPP from pigs with SPD.

**ADP, ATP, and serotonin contents of platelets.** The total content of adenine nucleotides, as well as serotonin, in SPD platelets was less than that of normal pigs (Fig 3). Mean values in SPD pigs studied were 31% for ADP, 34% for ATP, and 23% for serotonin of the mean values in normal pigs. To test whether the low values in SPD pigs were
Fig 4. Release of \(^{14}\)C-labeled serotonin from SPD and unrelated normal pig platelets. \(^{14}\)C-labeled serotonin (0.29 \(\mu\)mol/L final concentration) was added to 2.5 mL of citrated PRP (2.5 \(\times\) 10\(^8\) platelets/mL). After 15 minutes at 37°C, imipramine was added to a final concentration of 10 \(\mu\)mol/L, and incubation at 37°C was continued. Aliquots (300 \(\mu\)L) were removed at intervals and centrifuged, and supernatant radioactivity was determined in a liquid scintillation counter.

characteristic of swine in our closed herd, ADP and ATP contents were determined in three vWD pigs and serotonin content was determined in one vWD pig. Pigs with vWD whose platelets aggregated normally in response to collagen were used for these determinations. Mean values were ADP 109.8%, ATP 104.7%, and serotonin 91.8% of the mean values for unrelated normal pigs.

Uptake and retention of \(^{14}\)C-labeled serotonin. Uptake of \(^{14}\)C-labeled serotonin after 15 minutes was similar in the two groups of pigs (normal 76.2%, SPD 70.7%). At 15 minutes, imipramine was added to inhibit the reuptake of any serotonin released by the platelet. Release of \(^{14}\)C-labeled serotonin from SPD platelets was evident 15 minutes after the imipramine was added; and at 4 hours, SPD platelets had released approximately one third of the \(^{14}\)C-labeled serotonin originally taken up (Fig 4). Normal pig platelets retained the amine for 4 hours.

Electron microscopic findings. Thin sections of the platelet preparation from a normal and a SPD pig were examined by transmission electron microscopy. There were 57 dense bodies in thin sections of 125 platelets in the normal pig and 15 dense bodies in the same number of cells from the SPD pig (Fig 5).

Infusion studies. Enough normal porcine cryoprecipitate was infused into a SPD pig to raise the levels of vWF:Ag from 26 to 97 U/dL and of RCof from 29 to 103 U/dL 30 minutes after infusion. The bleeding time remained abnormal (over 15 minutes) through 24 hours (Fig 6). Such an infusion corrects the bleeding time in vWD pigs. Another SPD pig received platelet concentrate from a normal pig, which raised its platelet concentration by a maximum of 2.1 \(\times\) 10\(^8\) platelets/mL 1 hour after infusion. Bleeding time was shortened and transiently corrected to normal (Fig 6). Increased collagen-induced platelet aggregation was apparent after platelet infusion (Fig 6). Before infusion, the response curve to collagen had a slope of 1.5 and a zero \(\Delta T\). (This can occur because SPD platelets undergo a transient change in shape, resulting in a measurable slope; however, \(\Delta T\) does not exceed zero—the original baseline—in some instances.)

DISCUSSION

The pigs in this investigation that we identified as having SPD were all derived from a herd of vWD and vWD-carrier swine. Our studies have shown that the platelets of these SPD pigs have a decreased number of dense granules and decreased amounts of serotonin, ADP, and ATP. Furthermore, platelet retention of serotonin after the addition of imipramine was impaired.

Pigs with homozygous vWD are characterized in part by extremely low vWF:Ag and RCof values (less than 3 U/dL), bleeding times of more than 15 minutes, and a life-threatening bleeding diathesis. Pigs heterozygous for vWD have higher, but not normal, mean levels of vWD-Ag and RCof (26 and 30 U/dL, respectively), normal bleeding times, and no bleeding tendency. Pigs from our herd with SPD have vWF:Ag and RCof levels similar to those in pigs heterozygous for vWD but, in contrast, have prolonged bleeding times—often more than 15 minutes—and a life-threatening bleeding tendency.

The observation that the SPD pigs originated in a vWD herd prompted us to investigate the possibility that they were
Pig. Platelet count increased by a maximum of 2.1 x 10^4 times before and after infusion of 410 ml of platelet concentrate normal pig into SPD pigs. (A) Solid circles represent bleeding determinations per time point per pig. In the pig, mean bleeding times by 37%, 273%, and 255% over preinfusion levels, respectively. FVIII, vWF:Ag, and RCof increased normal porcine cryoprecipitate. (B) T was used; "rebleeding," a phenomenon common in SPD pigs, is not indicated in the graph. (B) T was maximal slope of collagen-induced platelet aggregation curve before and after infusion of platelet concentrate. Pre, preinfusion; Imm, immediately after infusion; 30, 30 minutes after infusion; and Nor, unrelated normal pig control.

affected by a variant form of vWD similar to that found in humans because they had long bleeding times and mean values of vWF:Ag and RCof of 25 and 35 U/dL, respectively. Examination of the von Willebrand factor multimers, however, revealed no abnormality, and our experience with porcine vWD suggested that the plasma level of von Willebrand factor was not low enough to cause bleeding without some additional abnormality being present. This led us to investigate other causes of the prolonged bleeding time and to the discovery of platelet SPD.

Like vWD, SPD is inherited as a simple autosomal recessive locus segregating independently of the similarly autosomal recessive von Willebrand locus. The SPD pigs in our herd, therefore, are homozygous, heterozygous, or normal at the SPD locus. Those with homozygous SPD display impaired collagen-induced platelet aggregation; all others have normal collagen-induced aggregation and nucleotide content. The vWD carriers also are either heterozygous or normal at the SPD locus. The vWD carriers have normal bleeding times and therefore cannot be homozygous at the SPD locus. Pigs with prolonged bleeding times, low platelet nucleotide content, decreased collagen-induced aggregation, and mean levels of vWF:Ag and RCof of 25 and 35 U/mL, respectively, are SPD pigs that also happen to be carriers of vWD. We have not been able to identify SPD pigs that definitely do not carry vWD. The lower values of vWF:Ag and RCof in normals overlap the higher values in carriers.

Because our breeding program was focused on producing vWD and vWD-carrier pigs, it can be expected that most of the SPD pigs identified were carriers of vWD. In fact, pedigree analysis shows that all of the SPD pigs in this study may be carriers of vWD (eg, some SPD pigs were the offspring of one vWD pig, and the others were offspring of vWD carriers). We have initiated a backcross breeding program to produce pigs affected only at the vWD locus or the SPD locus. It will then be possible to determine if the decreased levels of vWF:Ag and RCof are due to heterozygosity at the vWD locus or are linked to the expression of SPD.

Like platelets in the human disease, platelets in porcine SPD show abnormal aggregation with collagen. The secondary response of platelet aggregation to epinephrine and ADP is also abnormal in human SPD. Using our aggregation procedure, however, platelets of normal pigs do not aggregate in response to epinephrine, and there is only primary (reversible) aggregation with ADP. Thus, abnormal collagen-induced aggregation is the only detectable abnormality of porcine platelet aggregation that resembles SPD in humans.

Platelet storage pool deficiency in humans can be categorized on the basis of a decrease of substances stored in the dense granules (SPD or δ-SPD), rarely the α-granules (α-SPD or gray platelet syndrome), or both dense and α-granules (αδ-SPD). SPD involving dense granules often accompanies other clinical abnormalities, such as the Chediak-Higashi syndrome, Hermansky-Pudlak syndrome, Wiskott-Aldrich syndrome, and thrombocytopenia absent radii syndrome. SPD has been found in association with the Chediak-Higashi syndrome in mink, cattle, and cats. Several pigment mutations in mice have been associated with SPD. Recently, it has been shown that SPD in fawn-colored rats is a pleiotropic effect of the red-eyed dilution gene.

The only additional abnormality that we have thus far detected in our SPD pigs is reduced vWF:Ag and RCof. We believe that the original breeding strategy would have produced many SPD pigs that were also carriers of vWD, so that pigs produced by the current breeding program and affected only at the SPD gene may have normal levels of vWF:Ag and RCof. If this is true, these pigs may be an animal model for the idiopathic inherited SPD found in humans.

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REFERENCES


27. Pnieur DI, Meyers KM: Genetics of the fawn-hooded rat strain: The coat color dilution and platelet storage pool deficiency are pleiotropic effects of the autosomal recessive red-eyed dilution gene. J Hered 75:349, 1984


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