Early treatment with Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes (H12-(ADP)-liposomes) ameliorates post-partum hemorrhage with coagulopathy caused by amniotic fluid embolism in rabbits

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BACKGROUND: Amniotic fluid embolism is an unpredictable and sometimes lethal complication of childbirth. Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes (H12-(ADP)-liposomes), which were developed as a platelet substitute, may be useful to control postpartum hemorrhage with consumptive coagulopathy.

OBJECTIVE: This study aimed to establish a hemodynamically stable amniotic fluid embolism animal model and evaluate the efficacy of H12-ADP-liposome infusion in the initial management of postpartum hemorrhage complicated with amniotic fluid embolism—involved coagulopathy.

STUDY DESIGN: Pregnant New Zealand white rabbits (28th day of pregnancy or normal gestation period of 29−35 days) underwent cesarean delivery, followed by intravenous administration of amniotic fluid (a total of 3.0 mL administered in 4 doses over 9 minutes). Thereafter, uncontrolled postpartum hemorrhage was induced by transecting the right midartery and concomitant vein in the myometrium. After initial bleeding for 5 minutes, rabbits received isovolemic fluid resuscitation through the femoral vein with an equivalent volume of blood loss every 5 minutes for 60 minutes. The transfusion regimens included platelet-rich plasma, platelet-poor plasma, and a bolus administration of H12-ADP-liposomes followed by platelet-poor plasma transfusion (8 rabbits per group). Moreover, 60 minutes after initiation of bleeding, rabbits received surgical hemostasis by ligation of bleeding vessels, except in cases with spontaneous hemostasis.

RESULTS: The administration of amniotic fluid caused thrombocytopenia ($56\pm3 \times 10^3/\mu L$) and prolonged both clotting time (before administration: 130.0±3.0 to 171.0±5.0 seconds) and prothrombin time (4.5±0.1 to 4.7±0.1 seconds). After the initial 5-minute bleeding in the rabbits, the mean arterial pressure fell to 43±2 mm Hg. Platelet-poor plasma transfusion alone further prolonged clotting time and prothrombin time at 60 minutes (192.0±10.0 and 5.2±0.1 seconds, respectively) with decreasing mean arterial pressure to <40 mm Hg. By contrast, the administration of H12-ADP-liposomes followed by platelet-poor plasma transfusion reduced the prolonged clotting time (153.0±5.0 seconds) and prothrombin time (4.9±0.1 seconds) similar to platelet-rich plasma transfusion (154.0±11.0 and 4.9±0.1 seconds, respectively) at 60 minutes. These rabbits maintained a mean arterial pressure of >45 mm Hg throughout the experiment. H12-ADP-liposome infusion and platelet-poor plasma transfusion and platelet-rich plasma transfusion yielded spontaneous hemostasis in 4 of 8 rabbits, whereas platelet-poor plasma transfusion did not stop bleeding in any of the rabbits. The total blood loss was 59±17 mL in the H12-ADP-liposomes and platelet-poor plasma group, which was half of that in the platelet-poor plasma group (124±10 mL).

CONCLUSION: H12-ADP-liposome infusion may be effective in the initial management of postpartum hemorrhage complicated with amniotic fluid embolism, resulting in mitigation of consumptive coagulopathy.

Key words: amniotic fluid embolism, artificial platelet substitute, disseminated intravascular coagulation, postpartum hemorrhage

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Why was this study conducted?

Amniotic fluid embolism (AFE) is an unpredictable complication of childbirth and occurs suddenly even in low-risk pregnancies. As such, initial fluid resuscitation with fibrinogen γ-chain peptide-coated, ADP-encapsulated liposomes (H12-(ADP)-liposomes), an artificial substitute for platelets, has been proposed to prevent consumptive coagulopathy in hemorrhagic animal models with AFE.

Key findings

Animals treated with H12-ADP-liposomes and platelet-poor plasma (PPP) infusion or platelet-rich plasma (PRP) transfusion showed reduced total blood loss compared with animals treated with PPP infusion. H12-ADP-liposomes and PPP infusion achieved spontaneous hemostasis of arterial bleeding in half of the animals, similar to PRP transfusion.

What does this add to what is known?

This study established a new rabbit hemorrhagic model simulating AFE that showed moderate consumptive coagulopathy at late pregnancy. Early H12-ADP-liposome treatment may prevent exacerbating coagulopathy (overt disseminated intravascular coagulation).

Introduction

The incidence of amniotic fluid embolism (AFE) is approximately 6 cases per 100,000 deliveries, and patients with AFE are more likely to have coagulopathy (adjusted odds ratio: 24.68). The overall failure-to-rescue rate after AFE is 17%.[1] In a representative case study, the episode of coagulopathy was found to start within 1 hour after delivery.[2] However, AFE remains an unpredictable and sometimes lethal complication of childbirth.

Blood transfusion composed of packed red blood cells (RBCs), fresh frozen plasma (FFP), and platelets (PLTs) is recommended in hospitals when patients are experiencing uncontrolled hemorrhage and hypotension because of AFE.[3–5] Unlike large hospitals, small maternity clinics or midwife-managed birth centers sometimes cannot provide effective blood transfusion. Therefore, in such small medical facilities, patients with postpartum hemorrhage (PPH) should be immediately referred or transferred to secondary care hospitals and treated initially with resuscitative fluid. In addition, even at large hospitals, PLT transfusion is usually delayed for patients with AFE for several reasons. In particular, PLT concentrate has a relatively short shelf life, and its preparation and storage need additional equipment. Therefore, PLT substitutes may be useful as the initial resuscitative fluid for treating patients with AFE.

To overcome these problems, we have developed liposome-based hemostatic nanoparticles (mean diameter: 210 nm) bearing synthetic HHLGGAKQAGDV (H12) peptides corresponding to the carboxyl terminus of the fibrinogen g-chain on the surface and carrying the physiological PLT agonist adenosine 5′-diphosphate (ADP).[6] According to the preparation protocol, the process of manufacturing and quality control for H12-ADP-liposomes takes 5 days, and the product can be safely stored in the refrigerator for 7 years.[7,8]

We have published several animal studies showing the effectiveness of H12-ADP-liposome treatment to halt lethal bleeding in cases of traumatic pulmonary hemorrhage and dilutional coagulopathy because of liver injury or iliac vessel injury.[9–11] In the current study, we focused on consumptive coagulopathy induced by AFE.

Despite the potential benefit of PLT substitutes, there are few appropriate animal models of PPH with AFE for evaluating their efficacy and safety. Moreover, animal AFE models show moderate anemia and mild coagulopathy, and the acute reaction of amniotic fluid (AF) administration into the maternal body occurs without uncontrolled hemorrhage.[12–15]

Here, a new rabbit model of AFE was developed that showed thrombocytopenia and coagulopathy followed by lethal hemorrhagic shock at late pregnancy. Using this model, we evaluated the efficacy of H12-ADP-liposomes as an alternative to PLT transfusion to rescue pregnant animals from disseminated intravascular coagulation (DIC) and hemorrhagic shock. We hypothesized that early treatment with H12-ADP-liposome infusion could prevent consumptive coagulopathy. As a primary outcome, we selected blood loss after 60 minutes.

Materials and Methods

Rabbits

This study was conducted according to the guidelines of the institutional review board (IRB) for the Care of Animal Subjects of the National Defense Medical College. The IRB approved this study (ethical approval number: 21032). Female New Zealand white rabbits in late pregnancy (3.7±0.1 kg, 28th day of pregnancy; Japan SLC, Hamamatsu, Japan) were used for the current study. In addition, male New Zealand white rabbits (2.5±0.1 kg; Japan SLC) were used as donors of platelet-rich plasma (PRP) or platelet-poor plasma (PPP).

Preparation of fibrinogen γ-chain peptide-coated, ADP-encapsulated liposomes (H12-(ADP)-liposomes)

H12-ADP-liposomes were prepared using an ethanol administration method. Briefly, 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (1.652 g, 2.25 mmol), cholesterol (870 mg, 2.25 mmol), 1,5-dihexadecyl-*N*-succinyl-L-glutamate (312 mg, 450 mmol), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-amino(polyethylene glycol) (89 mg, 15 mmol), and H12-PEG-Glu2C18 (77 mg, 15 mmol (Nippon Fine Chemical Co Ltd, Osaka, Japan) were dissolved in ethanol (10 mL). The ethanol solution was administered into phosphate-buffered saline containing 5 mM ADP (23.3 mL) while stirring. Liposome size was controlled by

extrusion using Nuclepore (pore size: 0.20 mm; Cytiva, Tokyo, Japan) to prepare H12-ADP-liposomes. The remaining ethanol and ADP were removed with crossflow filtration (Vivaflow 200, polyether sulfone, molecular weight cutoff: 100 kDa; Sartorius, Göttingen, Germany). The diameter of H12-ADP-liposomes was 216±58 nm.

Preparation of platelet-rich plasma and platelet-poor plasma

Donor rabbits (2.5±0.1 kg) were anesthetized using intramuscular administrations of ketamine (25 mg/kg) and xylazine (10 mg/kg). A local anesthetic (1% lidocaine) was administered subcutaneously into the left inguinal area. As described previously,[10,11] blood samples withdrawn with a 10% volume of 3.8% (weight in volume) sodium citrate were centrifuged at 980 m/sec^2 for 15 minutes, and the supernatant was used as PRP. The remaining sample was further centrifuged at 4900 m/sec^2 for 10 minutes, and the supernatant was used as PPP. The allogeneic PRP was slowly shaken to avoid coagulation at 22°C until use, and the PPP was stored at 4°C until use.

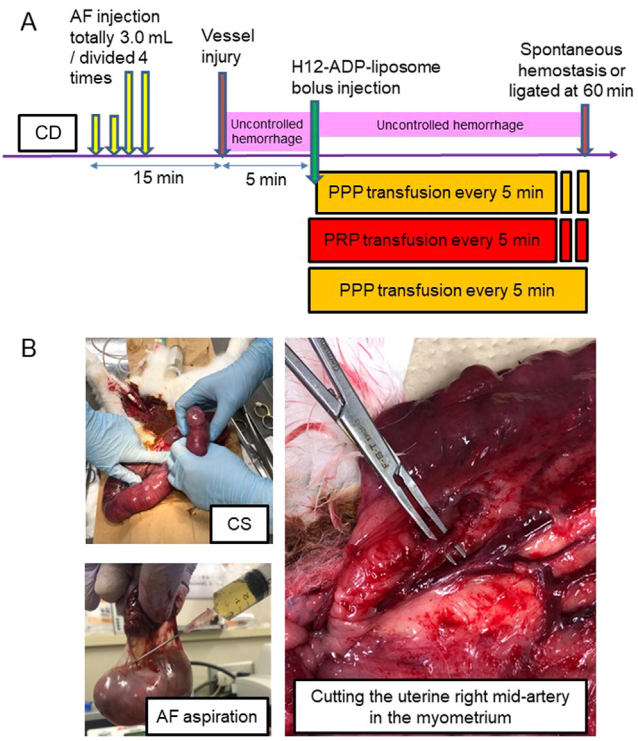
Administration of amniotic fluid after cesarean delivery

All rabbits were anesthetized with ketamine and xylazine followed by intravenous administrations of pentobarbital (15 mg/kg) every 30 minutes during the experiment. A local anesthetic (1% lidocaine) was administered subcutaneously into the left inguinal area and midabdominal region. The adequacy of general anesthesia was monitored by the loss of the ear pinch reflex. Anesthetized rabbits were placed on warming plates to maintain the body temperature at 37°C. Aseptic techniques were adopted for all surgical procedures. Surgical catheters (polyethylene indwelling needle 18G; Terumo Corporation, Tokyo, Japan) were inserted into the femoral artery and vein in each rabbit. After lower midline laparotomy, we performed a cesarean delivery and delivered all fetuses from the uterus. AF was aspirated from the amniotic sac and was centrifuged at 4900 m/sec^2 for 10 minutes to remove the fetal blood and debris. Subsequently, AF was administered via the femoral vein in the following protocol. Briefly, the initial administration was 0.5 mL; the second administration was 0.5 mL, performed 3 minutes later; the third administration was 1.0 mL, performed 3 minutes later; and the fourth administration was 1.0 mL, performed 3 minutes later (overall, 3.0 mL was administered over 9 minutes) (Figure 1).

Induction of postpartum hemorrhage and isovolemic fluid resuscitation for postpartum hemorrhage

Of note, 15 minutes after the initial AF administration, uncontrolled hemorrhage was induced in rabbits by transecting the right midartery and concomitant vein in the myometrium (Figure 1). The animals were randomly divided into 3 groups composed of the following fluid regimens: (1) PRP, (2) PPP, and (3) single-shot bolus administration of H12-ADP-liposomes (20 mg/mL/kg) followed by PPP (8 rabbits per group). Isovolemic infusion of PRP (1) or PPP (2 and 3) was repeated to equal the volume of blood loss every 5 minutes in each rabbit group (Figure 1). The volume of blood loss was measured by the weight of gauzes absorbing the hemorrhage from the injured site. Moreover, 60 minutes after the start of bleeding, all animals underwent surgical hemostasis by ligation of the bleeding vessels, except in cases with spontaneous hemostasis.

FIGURE 1
Experimental design and surgical procedures



A, Rabbits were randomly divided into 3 groups in which isovolemic resuscitation fluids were given through the femoral vein every 5 minutes. All animals except those showing spontaneous hemostasis underwent surgical hemostasis (vessel ligation) after 60 minutes of bleeding. **B,** After laparotomy, CD was performed. AF was aspirated from the amniotic sac. Animals underwent uncontrolled hemorrhagic shock by transection of the right midartery in the myometrium.

AF, amniotic fluid; *CD*, cesarean delivery; *H12-ADP-liposome*, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma.

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TABLE
Changes in hemodynamic and hematological parameters before and after AF infusion

Variable	Before experiment	15 min after AF infusion
MAP (mm Hg) (n=24)	58±2	56±2
WBC ($\times 10^3/\mu$L) (n=24)	3.6±0.3	2.2±0.2[a]
RBC ($\times 10^5/\mu$L) (n=24)	6.1±0.1	5.9±0.1[a]
Hb concentration (g/dL) (n=24)	11.7±0.2	11.7±0.2
Hct concentration (%) (n=24)	40±1	39±1[a]
PLT ($\times 10^3/\mu$L) (n=24)	175±8	56±3[a]
CT (s) (n=24)	130±3	171±5[a]
MP-COL (AUC) (n=12)	36±4	24±4[a]
MP-ADP (AUC) (n=14)	25±4	11±2[a]
PT (s) (n=18)	4.5±0.1	4.7±0.1[a]
AT3 (%) (n=20)	103±2	99±2[a]
Fibrinogen (mg/dL) (n=14)	194±12	153±10[a]
FDP (mg/mL) (n=13)	0.05±0.03	0.04±0.03
TF (pg/mL) (n=15)	216±39	217±38

Data are presented as mean±standard error of the means.

AF, amniotic fluid; *AT3*, antithrombin 3; *AUC*, area under the curve; *CT*, clotting time; *FDP*, fibrinogen degradation product; *Hb*, hemoglobin; *Hct*, hematocrit; *MAP*, mean arterial pressure; *MP-ADP*, multiplate adenosine 5′-diphosphate test; *MP-COL*, multiplate collagen test; *PLT*, platelet; *PT*, prothrombin time; *RBC*, red blood cell; *TF*, tissue factor; *WBC*, white blood cell.

[a] $P<.05$ vs before the experiment.

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To clarify the effects of H12-ADP-liposomes on AFE itself, we added groups of AFE animals, which also showed mild coagulopathy after similar administration of AF followed by administration of H12-ADP-liposomes alone (n=4) or not (n=3) without induction of vessel injury.

Blood sample collection

Arterial blood samples (6 mL) were withdrawn for measurement of blood cell counts and blood gas analyses before the experiment, at 15 minutes after AF administration, and at 15, 30, and 60 min after the onset of vessel injury.

Blood cell counts and blood gas analyses

Blood cell counts and hemoglobin (Hb) concentrations were measured using an Erma PCE 170 hematology analyzer (Erma, Tokyo, Japan). Blood gas analyses and plasma lactate levels were measured using an ABL 80 blood gas analyzer (Radiometer Medical, Copenhagen, Denmark).

Analyses of whole blood coagulation activity and platelet function

Coagulation activity was evaluated using a Sonoclot coagulation analyzer (model SCP2; Sienco Inc, Morrison, CO). The Sonoclot signal typically describes coagulation parameters, including "clotting time (CT)," which indicates the period up to the beginning of fibrin formation.[16]

To test PLT function, multiple electrode impedance PLT aggregometry was performed using a multiplate analyzer (Roche Diagnostics, Mannheim, Germany), and PLT aggregation was assessed (ADP and collagen tests).[17] Blood and reagents were pipetted using an electric pipette into test cells containing 2 independent sensor units. The adhesion and aggregation of PLTs were measured by the change in electrical resistance between 2 sensors for 6 minutes. The impedance change was plotted against time, and the area under the curve (AUC) value was recorded.

To measure prothrombin time and plasma antithrombin 3 (AT3) activity, blood samples were collected into tubes filled with 10% sodium citrate (volume per volume) and centrifuged at 4900 m/sec^2 at 4°C for 10 minutes, and the aforementioned parameters were measured at the Sanritsu Zelkova Laboratory (Tokyo, Japan).

Enzyme-linked immunosorbent assay

Plasma samples were collected at the indicated time points and stored at −80°C until enzyme-linked immunosorbent assay (ELISA). A commercially available rabbit fibrinogen ELISA kit (category number: LS-F10457; Lifespan Biosciences, Seattle, WA), rabbit fibrinogen degradation product (FDP) and D-dimer ELISA kit (category number: LS-F54269; Lifespan Biosciences), and rabbit Tissue Factor ELISA kit (category number: LS-F23306; Lifespan Biosciences) were used, and measurements were calculated according to the manufacturer's instructions.

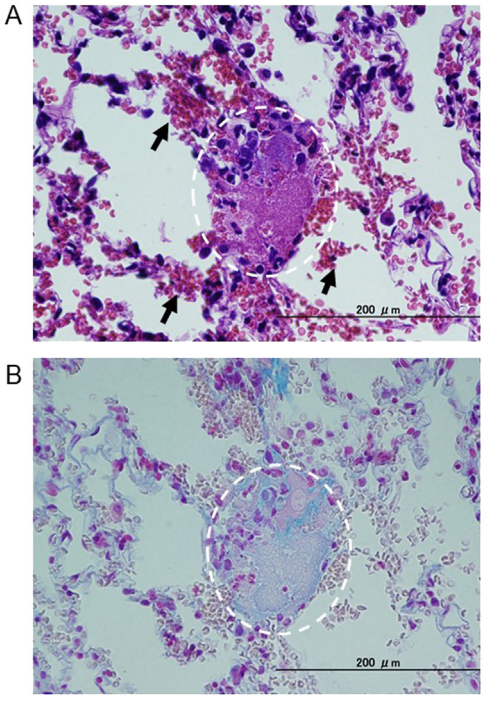
Histologic examination of lung

After the administration of AFs (just before inducing vessel injury), several rabbits were subjected to humane euthanasia, and specimens were harvested from the lungs. All tissues used in this study were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 5-mm-thick serial sections for hematoxylin-eosin (H&E) staining and Alcian blue staining.

Statistical analyses

Statistical analyses were performed using the Stat View 4.02J software package (Abacus Concepts, Berkeley, CA). Statistical comparisons between 2 groups were performed using Mann-Whitney *U* tests, and statistical comparisons among 3 groups were performed using 1-way analysis of variance, followed by Bonferroni posthoc tests. Data are presented as means±standard

FIGURE 2
Histologic findings



A, AF embolization (*dotted circle*) was observed, and alveolar hemorrhage (*arrows*) was detected by hematoxylin-eosin staining. **B,** Thrombosis was associated with precipitates of AF-derived mucin detected by Alcian blue staining (*dotted circle*). Representative data from similar results are shown.

AF, amniotic fluid.

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errors, and results with P values of <.05 were considered statistically significant.

Results

Coagulopathy with amniotic fluid embolism

AF administration caused thrombocytopenia ($56\pm3\times10^3/\mu$L), low PLT aggregation (collagen stimulation: 25±4; ADP stimulation: 12±2 AUC), and significantly prolonged CT (170.0±5.0 seconds) and PT (4.7±0.1 seconds) compared with the baselines (Table). Histologically, thromboses were observed in the lung by H&E staining and were associated with the precipitates of AF-derived mucin detected by Alcian blue staining (Figure 2). These findings were consistent with the pathophysiology for AFE diagnosis.[18]

Data for the AFE animals administered H12-ADP-liposomes alone without induction of bleeding injury indicated that H12-ADP-liposomes alone helped to restore the PLT counts for the initial 30 minutes. The PLT aggregating capacity shown in MP-ADP promptly recovered, but this effect gradually decreased with AFE until the end of the experiment (Supplemental Table).

Hemodynamics and blood loss

At 5 minutes after initiation of bleeding, the mean arterial pressure fell to 43±2 mm Hg (Figure 3). H12-ADP-liposome infusion followed by PPP infusion and PRP transfusion alone yielded spontaneous hemostasis in 4 of 8 animals, whereas PPP transfusion did not stop bleeding in any rabbits. In the H12-ADP-liposomes and PPP group, the total blood loss was 59±17 mL (Figure 3, A), and mean arterial pressure was maintained at >45 mm Hg throughout the experiment (Figure 3, B). In the PRP group, total blood loss was 73±20 mL (Figure 3, A), and mean arterial pressure gradually decreased to 36 mm Hg at 60 minutes (Figure 3, B). In the PPP group, total blood loss was 124±10 mL (Figure 3, A), which was significantly higher than in the other groups (P<.05). In addition, the mean arterial pressure decreased to 35 mm Hg at 60 minutes (although no significant difference was found among the 3 groups) (Figure 3, B).

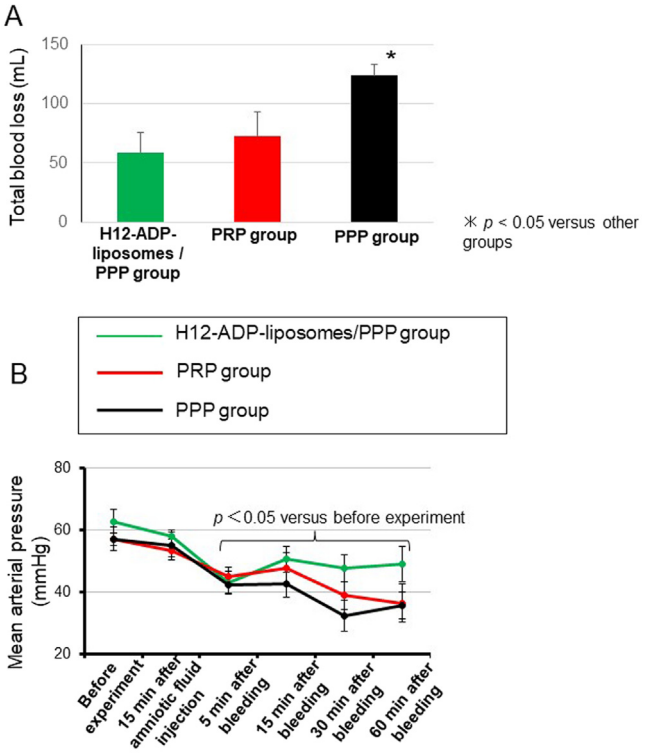
Changes in blood cell counts

Although the PLT concentration was restored to $80\times10^3/\mu$L to $100\times10^3/\mu$L after PRP transfusion, the H12-ADP-liposomes and PPP and PPP groups showed similar decreases in PLT count at 60 minutes after bleeding because H12-ADP-liposomes did not affect PLT counts (Figure 4, A). Hb concentrations were decreased to 6 to 7 g/dL in the H12-ADP-liposomes and PPP and PRP groups but reduced to 4 g/dL in the PPP group at 60 minutes (Figure 4, B). This was significantly lower than in the other groups (P<.05). In addition, changes in hematocrit (Hct) concentration were similar to those of Hb concentration (Figure 4, C). Both the H12-ADP-liposomes and PPP and PRP groups showed significantly higher Hct concentration than the PPP group at 60 minutes after initiation of bleeding.

Changes in whole blood coagulation activity and platelet aggregation activity

CT was significantly prolonged in the PPP group at 60 minutes compared with that in the H12-ADP-liposomes and PPP and PRP groups (Figure 5, A). Multiplate examinations showed that the AUC observed in ADP and collagen tests was similar; aggregation function tended to be restored in the H12-ADP-liposomes and PPP and PRP groups after treatment but was reduced again at 60 minutes in the PPP group (Figure 5, B and C). In addition, prothrombin time was significantly prolonged in the PPP group at 60 minutes (5.2±0.1 vs 4.6±0.1 seconds [baseline]) and was maintained within 120% of the baseline value in the H12-ADP-liposomes and PPP group (4.9±0.1 vs 4.5±

FIGURE 3
Changes in hemodynamics and total blood loss



A, Total blood loss was significantly higher in the PPP group than in the other groups. **B,** Mean arterial pressure decreased to 36 mm Hg at the end of the experiment in the PRP and PPP groups but remained at approximately 50 mm Hg in the H12-ADP-liposomes group. Data are presented as means±standard errors of the means (8 rabbits per group).

H12-ADP-liposome, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma.

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0.1 seconds [baseline]) and in the PRP group (4.9±0.1 vs 4.6±0.1 seconds [baseline]) (Figure 5, D).

Changes in coagulation factors

Plasma fibrinogen levels in the H12-ADP-liposomes and PPP group and in the PRP group were maintained at 144±21 and 132±10 mg/dL, respectively, even at 60 minutes (Figure 6, A). In contrast, hypofibrinogenemia occurred in the PPP group at 60 minutes, with fibrinogen levels of 107±10 mg/dL (P<.05 vs other groups) (Figure 6, A). In addition, AT3 levels were maintained at 87%±2% and 86%±5% at 60 minutes in the H12-ADP-liposomes and PPP infusion and PRP transfusion groups, respectively, but were significantly decreased to 74%±8% in the PPP group at 60 minutes (Figure 6, B). Neither significant changes in FDP nor tissue factor (TF) levels were observed by transfusion of H12-ADP-liposomes and PPP, PRP, or PPP, and no difference in these parameters was found among the 3 groups (Figure 6, C and D).

Blood gas analyses

All rabbits maintained a pH of >7.3 by respiratory compensation throughout the experiment in the H12-ADP-liposomes and PPP and PRP groups but not in the PPP group (Figure 7, A). Plasma lactate levels were significantly increased at 60 minutes in the PPP group; however, the H12-ADP-liposomes and PPP and PRP groups did not show such elevation of plasma lactate (Figure 7, B).

Comment

Principal findings

Our current study demonstrated the effectiveness of H12-ADP-liposome infusion in a new rabbit model in which we established PPH with coagulopathy because of AFE, describing the details of PLT function and coagulopathy status.

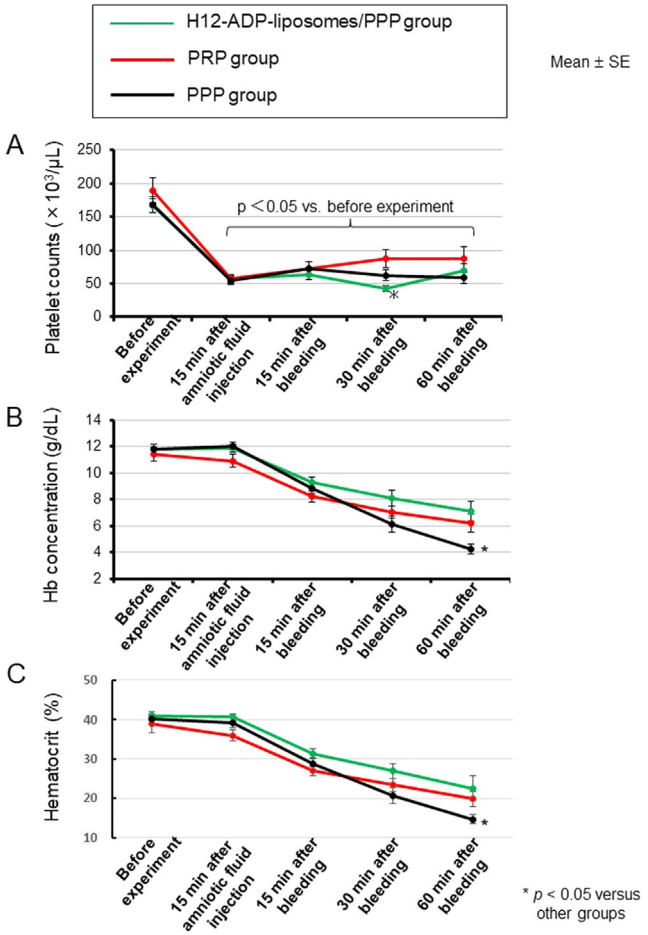
Results in the context of what is known

First, we identified the appropriate AF infusion protocol causing coagulopathy without significant hemodynamic change. Briefly, a total AF infusion volume >5 mL caused hypotension and sometimes death (data not shown). The administration of a total of 3 mL (0.8 mL/kg) divided into 4 doses assured the onset of moderate coagulopathy with stable hemodynamics. This procedure certainly induced thrombocytopenia and CT prolongation, causing pathologic microthrombi in lung capillaries. However, the volume of AF infusion was relatively lower than those in previous reports (1.8−8.2 mL/kg).[12−15] Hence, critically consumptive coagulopathy was not yet induced according to AT3 or FDP levels.

To examine the infusion of H12-ADP-liposomes as a substitute for PLT transfusion, we aimed to develop an appropriate PPH animal model for replication of the clinical PPH scenario, in which prompt interventions for patients with PPH enable maintenance of hemodynamics and transfer to higher-level emergency hospitals, thereby controlling bleeding to some extent. Thus, we adjusted the bleeding status to the initial bleeding speed (60 mL/kg/h) and the postpartum treatment time as 60 minutes. Surgical ligation was applied to injured vessels 60 minutes after bleeding, based on practical clinical situations.[19] During the initial 60 minutes, most patients with PPH are transferred to higher-level emergency hospitals to surgically stop bleeding.[20,21]

In addition to establishing AFE conditions, transecting the midartery in the uterine membrane caused uncontrolled hemorrhage. In this model, the average bleeding speed reached 0.9 mL/kg/min during the first 5 minutes, which was

FIGURE 4
Changes in hematological parameters



A, PLT counts were gradually restored during the experiment in the PRP group. The H12-ADP-liposomes and PPP and PPP groups showed similar decreases in PLT counts. **B,** Hb concentrations were significantly decreased at 60 minutes after the initiation of bleeding in the PPP group compared with those in the other 2 groups. **C,** Hematocrit concentration was gradually decreased in all groups over time. Data are presented as means±SEs of the means (8 per group).

H12-ADP-liposome, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *Hb*, hemoglobin; *PLT*, platelet; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma; *SE*, standard error.

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similar to clinical findings for critical PPH.[19,22] Total blood loss was more than 40% of the total blood volume, which was severe enough to cause death within a few hours in the absence of any fluid resuscitation.[23] Therefore, PPP transfusion alone caused severe anemia and hemorrhagic shock, decreased fibrinogen levels to approximately 100 mg/dL at 60 minutes after bleeding and AT levels to 73%, and prolonged prothrombin time to 1.15 times that at baseline, which satisfied the criteria for obstetrical DIC.[24] In contrast, H12-ADP-liposomes and PPP infusion and PRP transfusion prevented the acceleration of bleeding speed by up to 1.6 mL/kg/min from 5 to 60 minutes, even in cases in which hemostasis was not induced, resulting in a total blood loss that was <40% of the total blood volume.

Such reducing effects on blood loss were explained by the following coagulation findings. Essentially, PLT aggregation ability was restored by H12-ADP-liposome infusion and by PRP transfusion. Furthermore, infusion of H12-ADP-liposomes attenuated hypofibrinogenemia, prothrombin time, and CT prolongation compared with PPP alone.

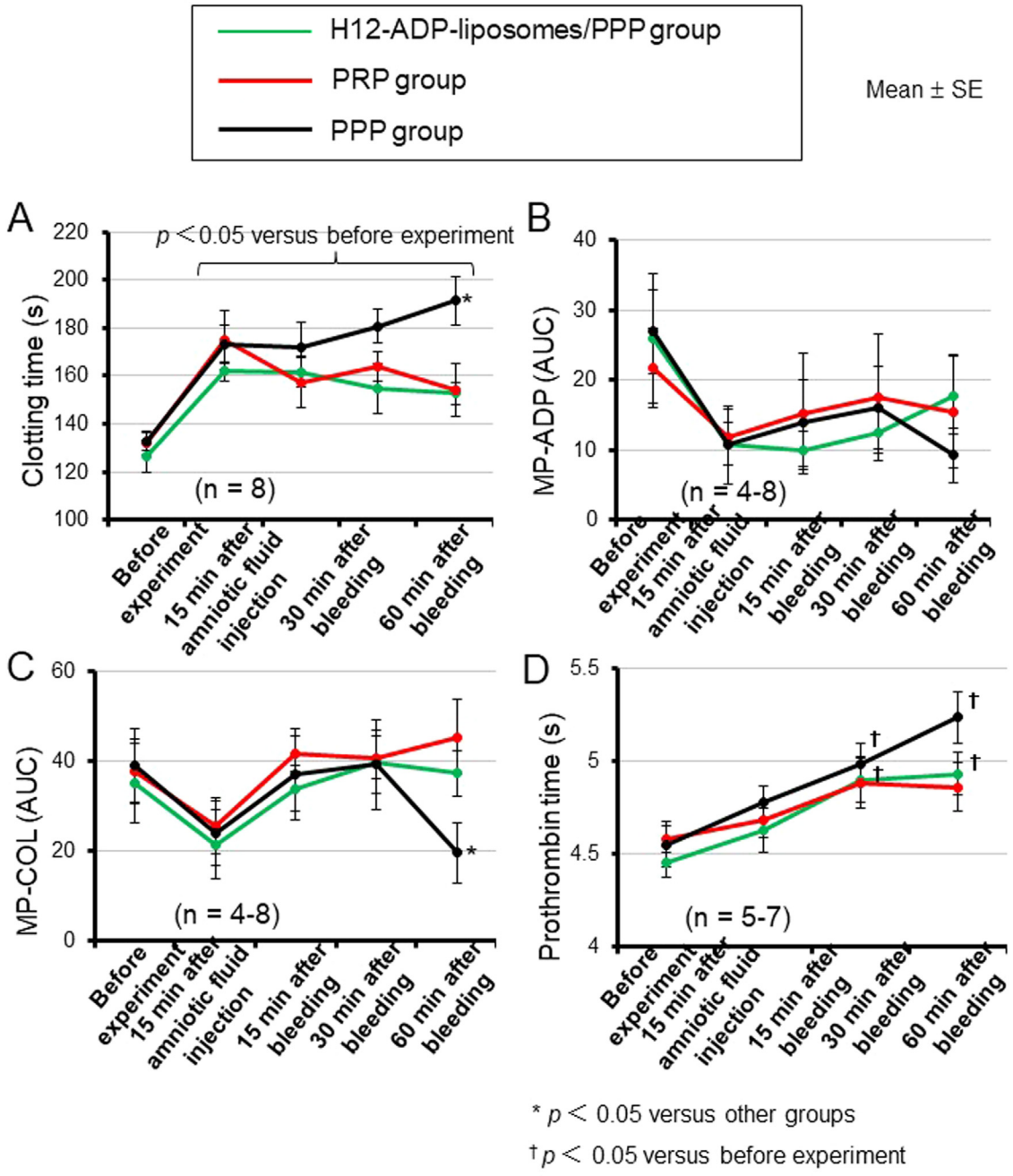
Clinical implications

Currently, hemostatic support in patients with PPH typically involves the administration of FFP during the early phase.[5,25] From the viewpoint of trauma care, prompt PLT transfusion ensures that patient outcomes will be improved. Oda et al[26] reported that early detection and intervention for AFE-related consumptive coagulopathy is crucial. Consistent with this, rapid treatment with H12-ADP-liposome infusion may result in better hemostatic outcomes. According to the results of our current study, H12-ADP-liposome infusion may prevent the development of DIC without adverse effects; H12-ADP-liposome infusion did not increase FDP or TF values, suggesting that the administration of H12-ADP-liposomes did not affect PLT overactivation. Moreover, H12-ADP-liposome infusion permitted minimum volume loading (1 mL/kg infusion volume), which has advantages for treatment in critical care situations to prevent hemodilution.

Preemptive treatment is an attractive strategy for ultrarapid progression of AFE pathophysiology; unfortunately, The FIB-PPH trial: fibrinogen concentrate as initial treatment for postpartum haemorrhage: study protocol for a randomised controlled trial did not determine the efficiency of fibrinogen concentrate treatment.[27] Consistent with this, the VIIa agent is not recommended as a first-line treatment for AFE.[28]

Our current study suggested that preemptive H12-ADP-liposome infusion may be an alternative option in situations in which PLT transfusion is not available for the initial treatment of AFE. AFE itself sustained thrombocytopenia, low PLT aggregation, and CT prolongation for 60 minutes even if not bleeding. Against that, H12-ADP-liposome alone infusion modestly shortened CT prolongation (Supplemental Table). These results revealed that H12-ADP-liposomes with PPP infusion contributed to hemostasis at injured vessels.

FIGURE 5
Changes in whole blood coagulation



A, Clotting time was significantly increased at 60 minutes in the PPP group but was maintained at a normal level in the H12-ADP-liposomes and PPP and PRP groups. **B and C,** Multiplate signal levels were periodically restored after transfusion but decreased again in the PPP group. **D,** Prothrombin time gradually increased, reaching 1.15 times that at baseline in the PPP group. Data are presented as means±SEs of the means (**A**–**C**: 8 per group; **D**: 5 per group).

AUC, area under the curve; *H12-ADP-liposome*, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *MP-ADP*, multiplate adenosine 5′-diphosphate test; *MP-COL*, multiplate collagen test; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma; *SE*, standard error.

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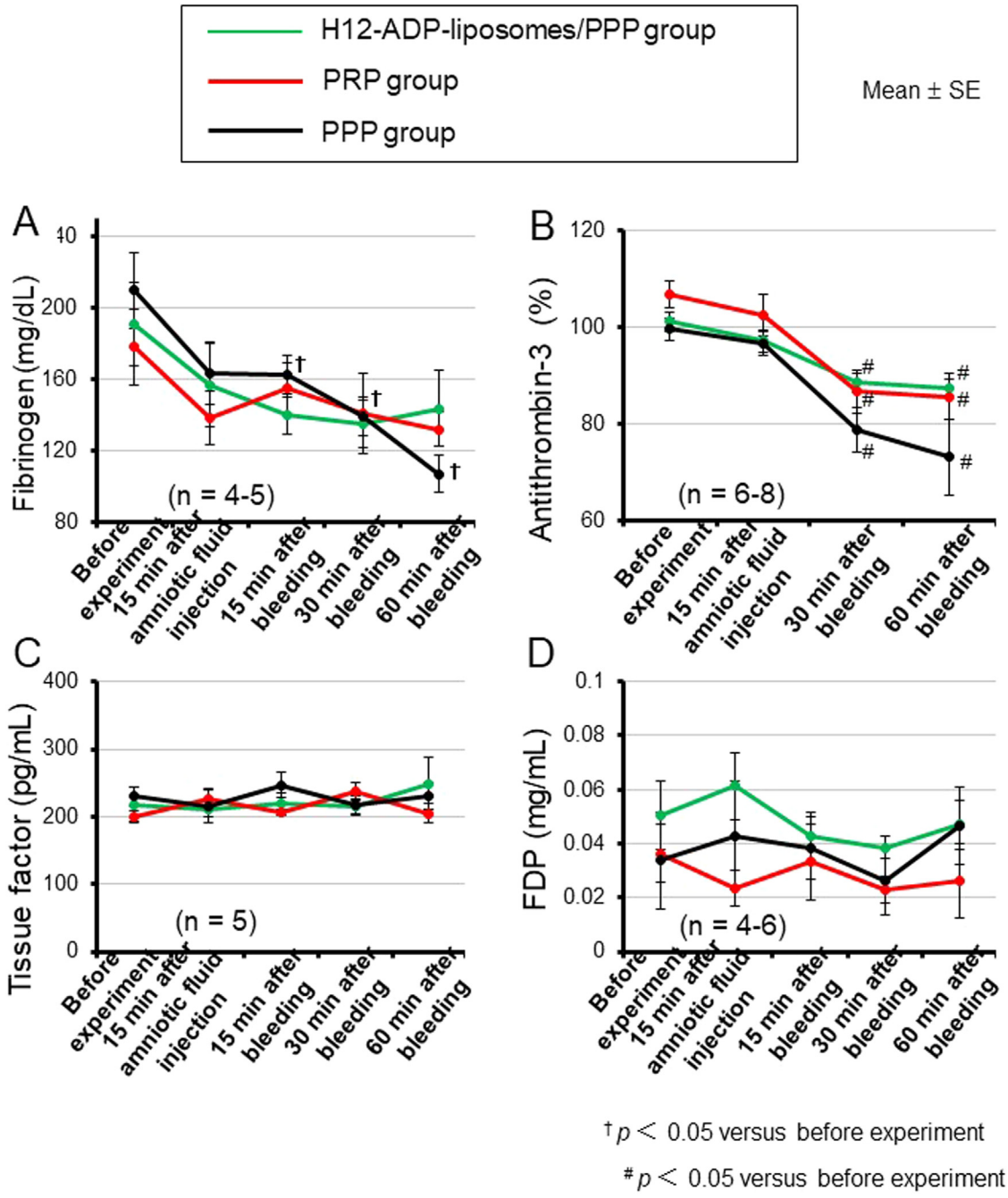
Research implications

Although RBC transfusion is essential for hemorrhage in the clinical setting, we only examined the hemostatic effects of each transfusion (PRP, PPP, and bolus administration of H12-ADP-liposomes followed by PPP); we did not apply RBC transfusion in our current study. Hence,

FIGURE 6
Changes in coagulation factors



A, Plasma fibrinogen levels gradually decreased to 70% of baseline in the H12-ADP-liposomes and PPP and PRP groups but were halved to 100 mg/dL in the PPP group. **B,** Plasma antithrombin 3 levels gradually decreased to 90% in the H12-ADP-liposomes and PPP and PRP groups and were 70% in the PPP group. **C and D,** Tissue factor and FDP showed normal levels during the experiment. Data are presented as means±SEs of the means (5 per group).

FDP, fibrinogen degradation product; *H12-ADP-liposome*, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma; *SE*, standard error.

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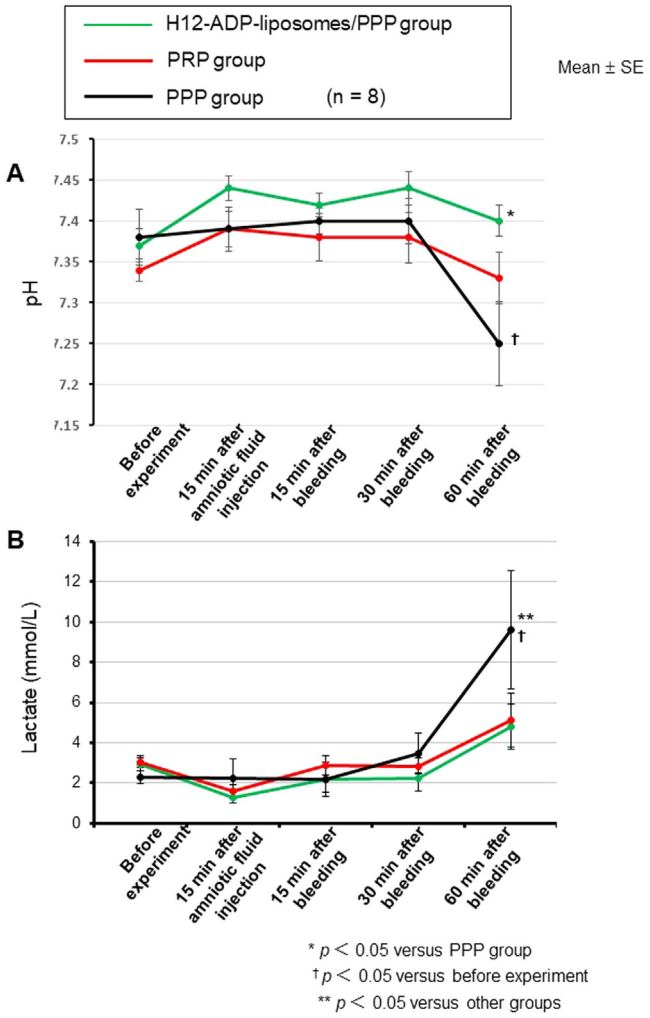
controlling anemia will be the target of our next studies using RBC substitutes.[29] In addition, combination therapy with an RBC substitute and PLT substitute should be examined in future studies.

Strengths and limitations

In our current study, we mimicked coagulopathy alone because of AFE;

FIGURE 7
Blood gas analyses



A, pH decreased to <7.3 in the PPP group. **B,** Plasma lactate levels were significantly elevated at 60 minutes after the initiation of bleeding in the PPP group. Data are presented as means±SEs of the means (8 per group).

H12-ADP-liposome, Fibrinogen γ-chain peptide-coated, ADP-encapsulated Liposomes; *PPP*, platelet-poor plasma; *PRP*, platelet-rich plasma; *SE*, standard error.

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however, coagulopathy was moderate owing to the limitation of available AF volume, which was at most 5 mL in each animal. We found that the speed and volume of AF administration affected the severity of AFE. In our preliminary study, a single bolus administration of whole AF, including meconium, caused severe hypotension and fatality in half of the animals within a few minutes. Therefore, we administered centrifuged AF to avoid cardiopulmonary collapse and induce pure coagulopathy. Therefore, we selected the administration of 4 doses of AF in a total volume of 3.0 mL in 9 minutes. In addition, we centrifuged the collected AF to remove fetal blood and debris; however, meconium is essential for reproducing the severe pathophysiology of AFE.[13] In the future, we plan to use whole AF administration (>5 mL) to establish cardiopulmonary collapsed AFE.

In the clinical setting, PLT concentrates are applied for PPH. However, we applied PRP as an alternative to PLT transfusion in our current study because PLT concentrates are not applied in animal experiments. In addition, PPP was applied in animals rather than FFP transfusion. In our previous studies,[6,9–11] the appropriate H12-ADP-liposome concentration for hemostatic support was found to be 1 mL/kg. Hence, infusion of H12-ADP-liposomes alone was not sufficient to compensate for blood loss. Furthermore, colloid or crystalloid fluid infusion reduced the hemostasis effect caused by H12-ADP-liposomes in our previous study.[11] Therefore, PPP transfusion (equivalent to blood loss) was applied to maintain colloid pressure and to correct coagulopathy, followed by H12-ADP-liposome infusion.

Conclusions

H12-ADP-liposome infusion may be effective in the initial management of PPH complicated with AFE to prevent consumptive coagulopathy. ■

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xagr.2023.100280.

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