MHSRS 2026. August 4th 13:00~15:00

Development of Temperature-Stable Whole Blood Analog with Extended Shelf-Life Using Synthetic Components in Japan

National Defense Medical College: Manabu Kinoshita, Kohsuke Hagisawa, Osamu Ishida, Makoto Aoki, Morihiro Hotta

U.S. Army Japan ESEP/Iowa State University: MAJ Bradley M. Kearney

Nara Medical University: Hiromi Sakai

Waseda University: Shinji Takeoka

Non-Disclosure

MHSRS 2026
Military Health System Research Symposium

The authors have no financial conflicts of interest to disclose concerning the presentation.

Background

The Japan Self-Defense Forces (JSDF) and the U.S. military have great interest in the Okinawa AO, which has unique geographic characteristics. It is far from the Japanese main islands and consists of several small, remote islands scattered throughout the East China Sea. Prolonged field care (PFC) capabilities are essential for this AO.

Our team has developed a room-temperature stable whole-blood analog with an extended shelf life consisting of liposome-based red blood cell (RBC) and platelet (PLT) substitutes and freeze-dried plasma (FDP) in a soft-bag formulation for use in emergency settings when cold-chain logistics cannot be guaranteed.

Prepositioning of PLT substitutes and FDP for prolonged casualty care is critical prior to armed conflict or disasters.

It's my personal opinion, not official (maybe)

Whole Blood Analog in Japan

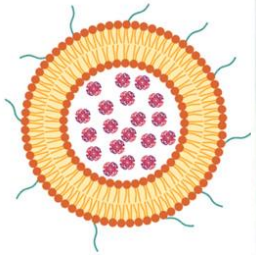
Transfusion of blood components for massive hemorrhage

Red Blood Cells (RBC)

Delivers oxygen

RBC substitutes

Hemoglobin vesicles (HbVs)

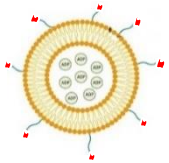


Platelets (PLT)

Stops bleeding

PLT substitutes

H12-(ADP)-liposomes



Fresh Frozen Plasma (FFP)

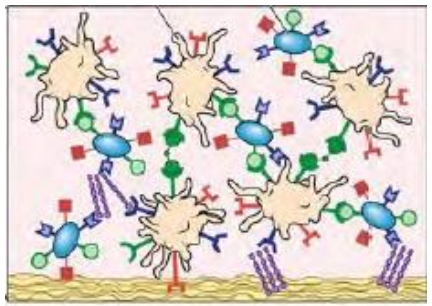
Replaces circulation volume

Stops bleeding

Freeze-dried plasma

FSHARP or RAPIID program

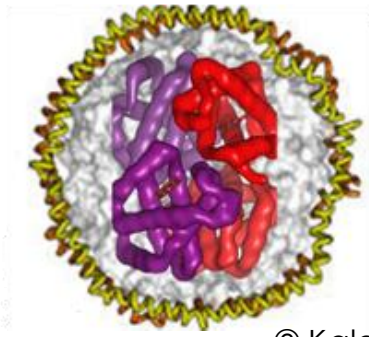
Stops bleeding



© Haima Therapeutics

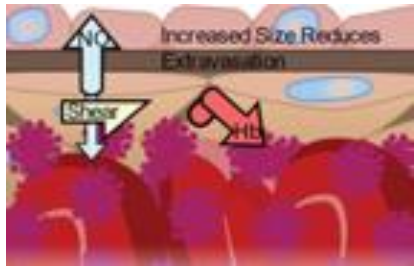
- Nanoparticle-based synthetic platelet Synthoplate

Delivers oxygen



© Kalocyte

- Encapsulated hemoglobin construct ErythroMer



© Palmer Lab, OSU

- Polymerized hemoglobin PolyHb

Replaces circulation volume

Stops bleeding

© Teleflex

- Freeze-dried plasma

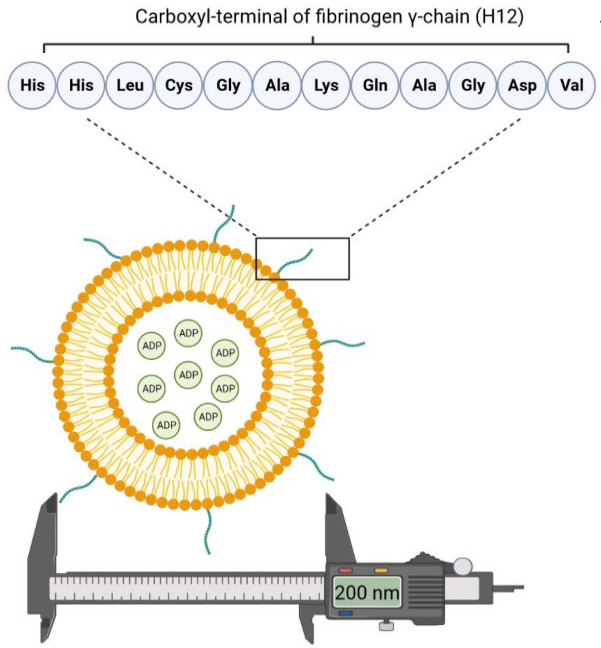
PLT Substitute: H12-(ADP)-Liposomes

H12-(ADP)-liposomes are hemostatic nanoparticles and have several attractive advantages to conventional PLT concentrates.

	H12-(ADP)-liposomes	PLT concentrates
Storage condition	No agitation required	Requires continuous agitation
Shelf life	12 months at 4°C 1 month at 25°C	6 days at 20-24°C Requires 2 days for bacterial screening
Contamination risk	No risk (fully synthetic product)	Vulnerable

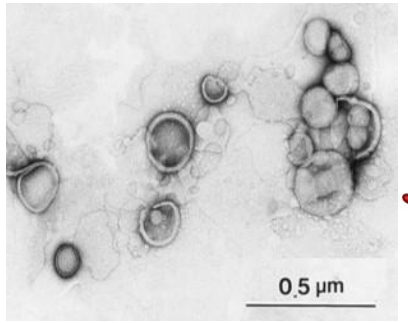
H12-(ADP)-Liposome Structure and Properties

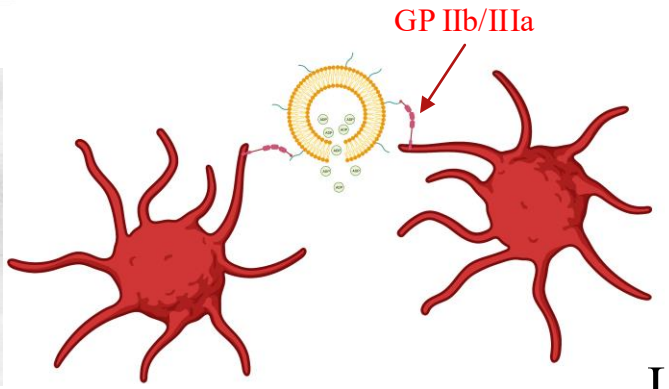
Structure



Liposome-based PLT substitutes studded with synthetic dodecapeptides (H12, HHLCGAKQAGDV) corresponding to the C-terminus of fibrinogen γ-chain on the surface. H12 is the active site of fibrinogen.

The liposome encapsulates a PLT agonist, adenosine 5'-diphosphate (ADP).





Activated PLT

H12 binds to GP IIb/IIIa (fibrinogen receptor) expressed on the surface of activated PLTs that accumulate at the bleeding site.

↓

Liposome shearing releases ADP at bleeding site

↓

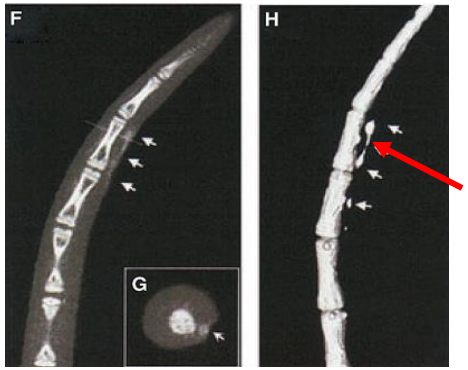
Localized, synergistic enhancement of PLT activation

(BR)

H12-(ADP)-Liposome Activity *in vivo*

Accumulation at the bleeding site, using H12-GP IIb/IIIa interaction

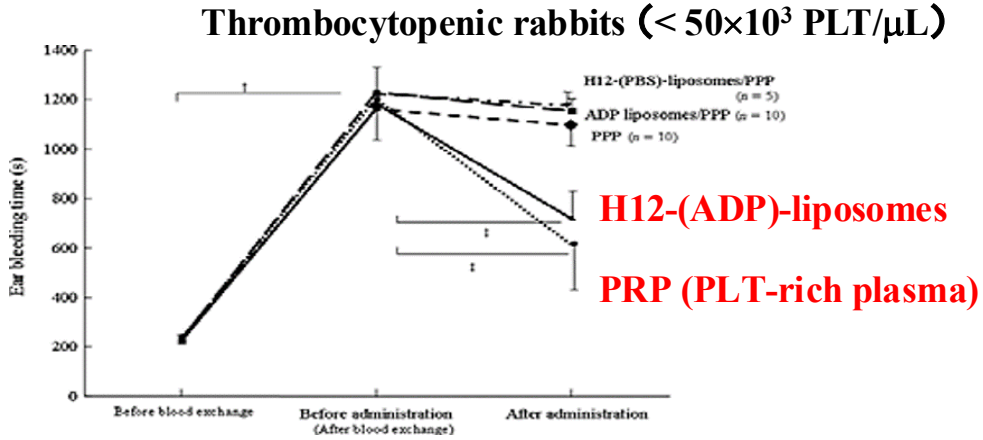
Rat tail injury

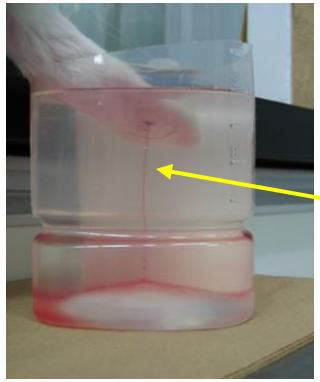


Accumulation of H12 liposomes containing contrast agent

J Thromb Haemost 2009; 7: 470-7.

Reduction of prolonged bleeding in thrombocytopenia





Bleeding from the ear of a thrombocytopenic rabbit

J Thromb Haemost 2012; 10: 2137-48.

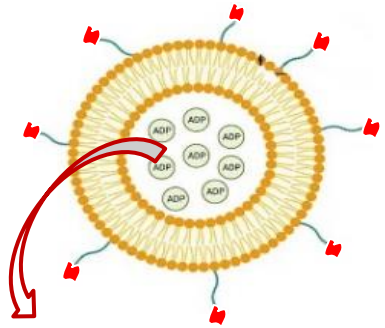
Shelf Life and Fibrinolytic Effects of H12-(ADP)-Liposomes

Shelf Life (at least)

4°C for 1 year

25°C for 1 month

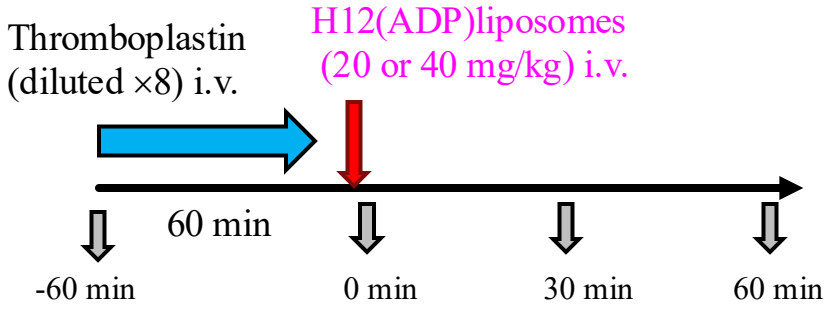
40°C for 1 week

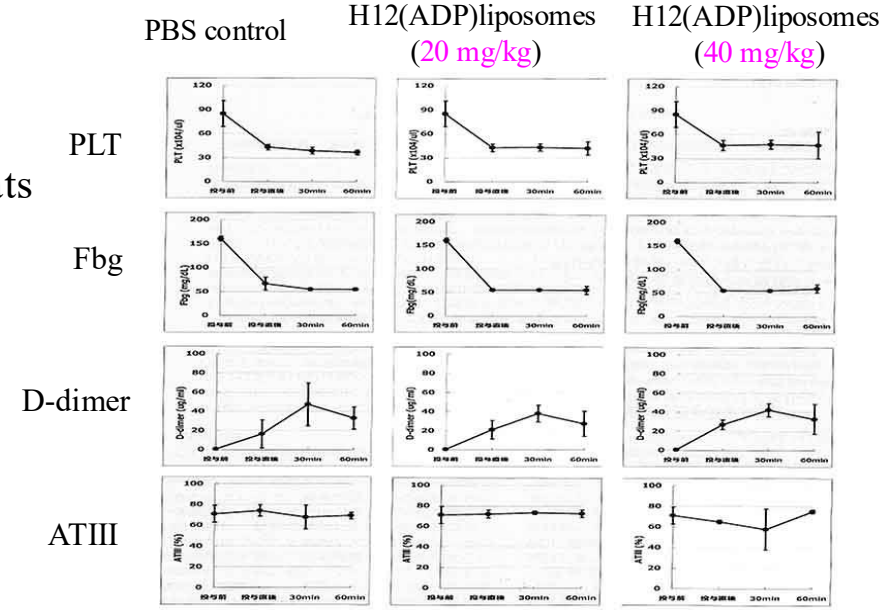


Encapsulated ADP is easily converted to AMP.

Fibrinolytic effect

The effect of H12-(ADP)-liposomes on thromboplastin-induced hypercoagulability in rats



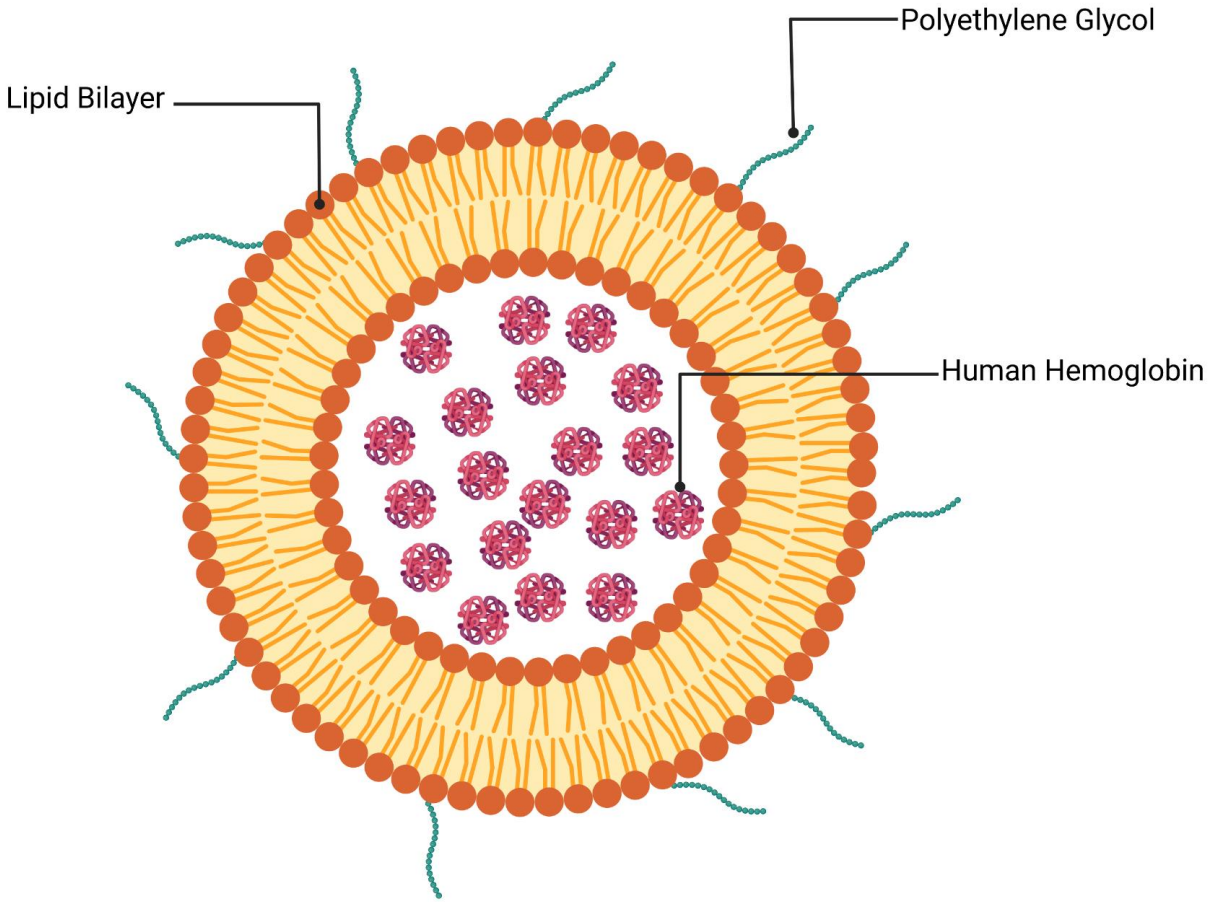


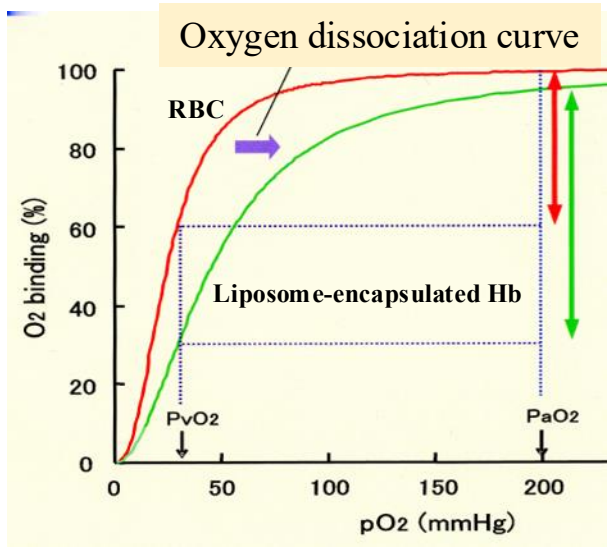
RBC Substitute: Hemoglobin Vesicle (HbV)

	HbVs	RBCs
Blood Typing	Not required	Required
Shelf life	2 years at room temperature	21 days at 4° C
Contamination risk	Low risk (virus inactivation)	Vulnerable

Hemoglobin Vesicle Structure

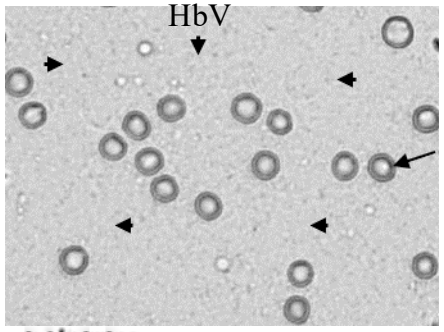
Structure - Hemoglobin encapsulated by a lipid bilayer membrane





Hemoglobin isolated from expired human RBCs provided by Japanese Red Cross Society (BR)

Hemoglobin Vesicle Size



RBC

HbVs

Polymerized Hbs

Hemoglobin

Capillary vessels

Red Blood Cells

5 nm 20 nm 250 nm >6000 nm

(BR)

Combination therapy using H12-(ADP)-liposomes and HbVs

ORIGINAL RESEARCH

Combination therapy using fibrinogen γ-chain peptide-coated, ADP-encapsulated liposomes and hemoglobin vesicles for trauma-induced massive hemorrhage in thrombocytopenic rabbits

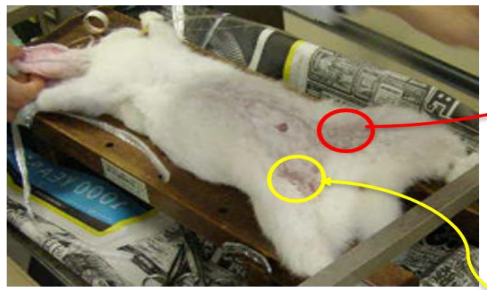
Kohsuke Hagisawa,[1] Manabu Kinoshita,[2] Masato Takikawa,[3] Shinji Takeoka,[4] Daizoh Saitoh,[5] Shuhji Seki,[2] and Hiromi Sakai[6]

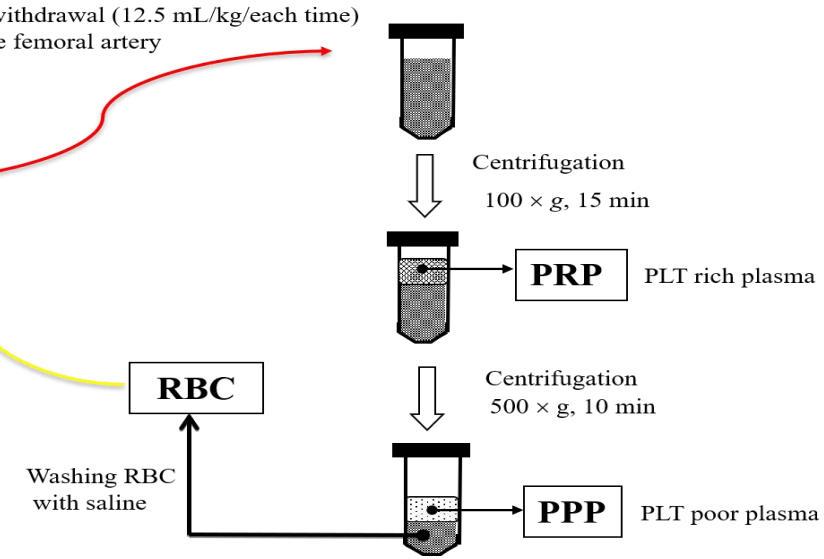
H12-(ADP)-liposomes

Transfusion 59; 3186-96, 2019.

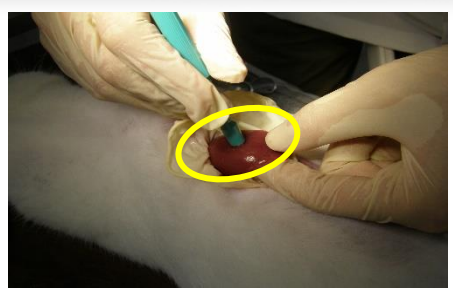
Thrombocytopenic Rabbit Model

Thrombocytopenia (PLTs < 50,000 /μL) was induced in rabbits by repeated blood withdrawal from the femoral artery and isovolumic transfusion of autologous washed RBCs via the femoral vein.





Experimental Protocol



Rabbit weight 2.5 kg

PLT < 50,000/μL

Mean arterial blood pressure < 50 mmHg

Blood withdrawal 25 mL/kg × 8

Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8

Penetrating liver injury

Tamponade

for 5 min

Measure blood loss

for 10 min

Development of fatal anemia

2 hours later

Survival monitoring

Dextran 40
25 mL/kg

RBC transfusion 25 mL/kg × 8

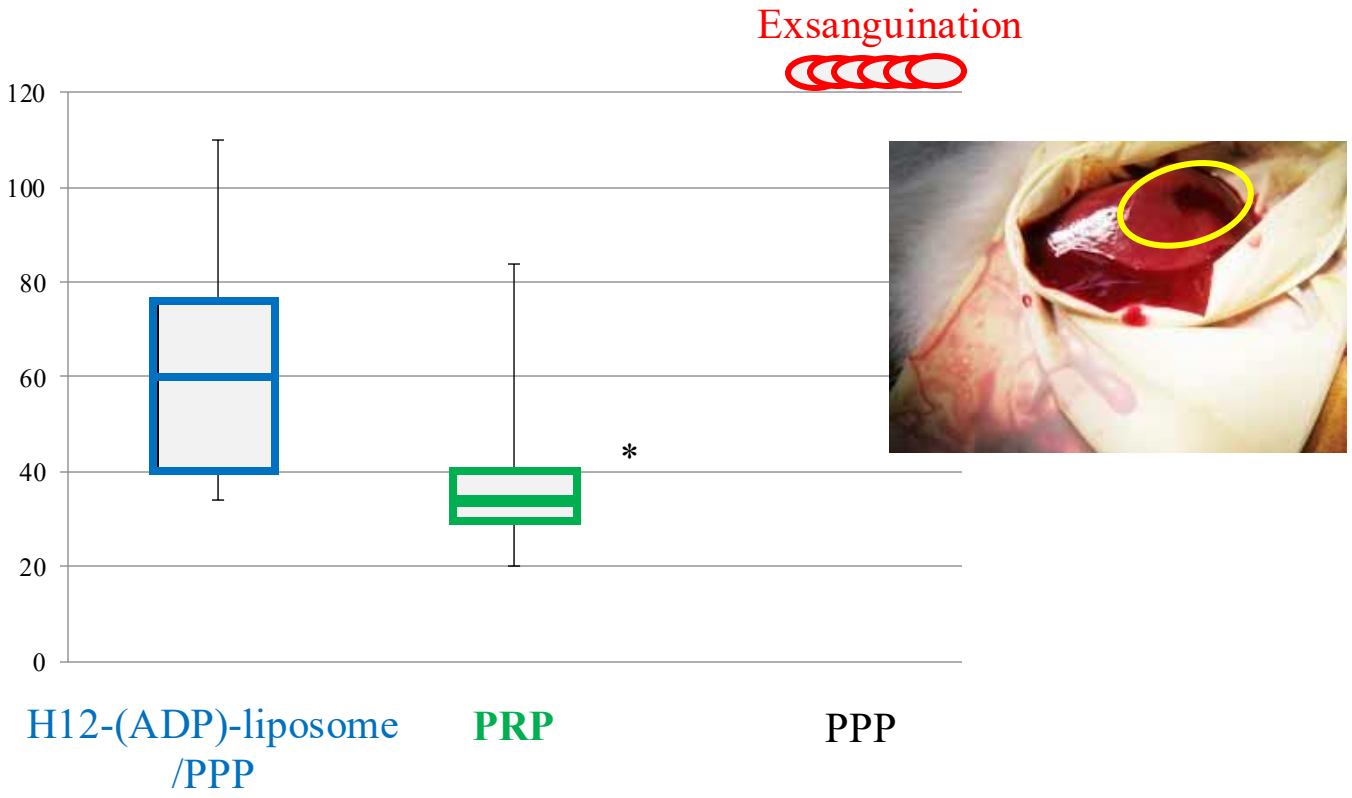
Intravenous administration with 15 mL/kg

Tamponade the bleeding site with a urethral catheter balloon for 5 min

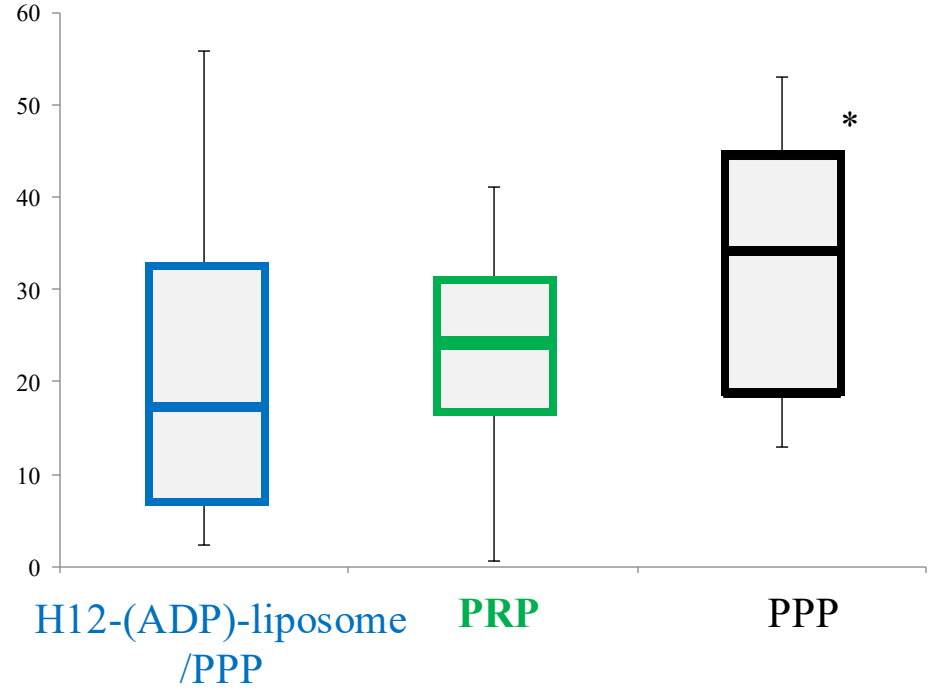
H12-(ADP)-liposome (20 mg/kg) + PPP	*Hemostasis*	HbV infusion	Group 1
		RBC transfusion	Group 2
PRP (PLT Rich Plasma) Conventional therapy	*Hemostasis*	HbV infusion	Group 3
		RBC transfusion	Group 4
		Albumin infusion	Group 5
PPP (PLT Poor Plasma) alone	*Exsanguination*		Group 6

Results: H12-(ADP)-Liposomes Achieve Hemostasis

Bleeding time at liver injury site (min)



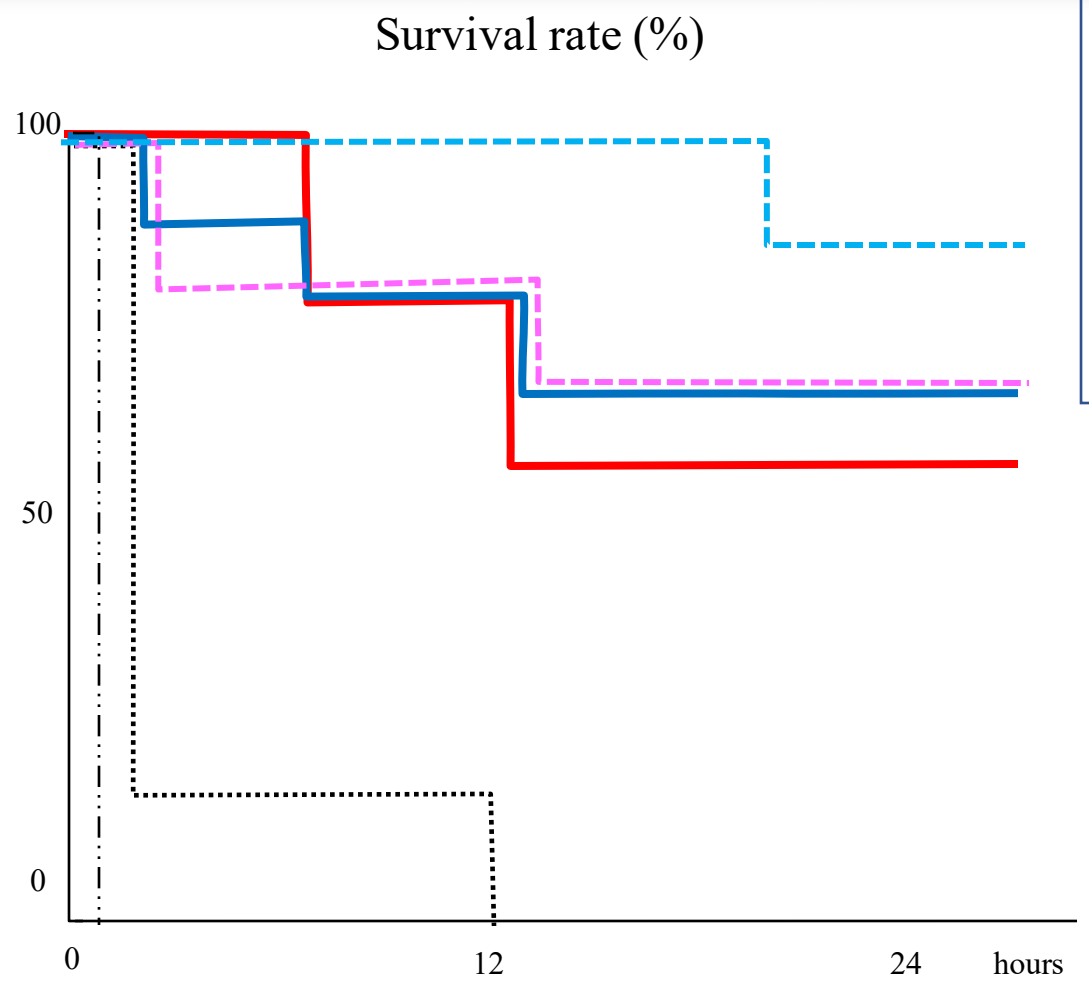
Blood loss during initial 10 min (mL)



All rabbits treated with H12-(ADP)-liposomes/PPP or PRP achieved hemostasis

* $p < 0.05$ vs H12-(ADP)-liposome/PPP

Results: HbVs Mitigate Fatal Anemia



Group 1: H12-(ADP)-liposome/PPP + HbV (n=10)

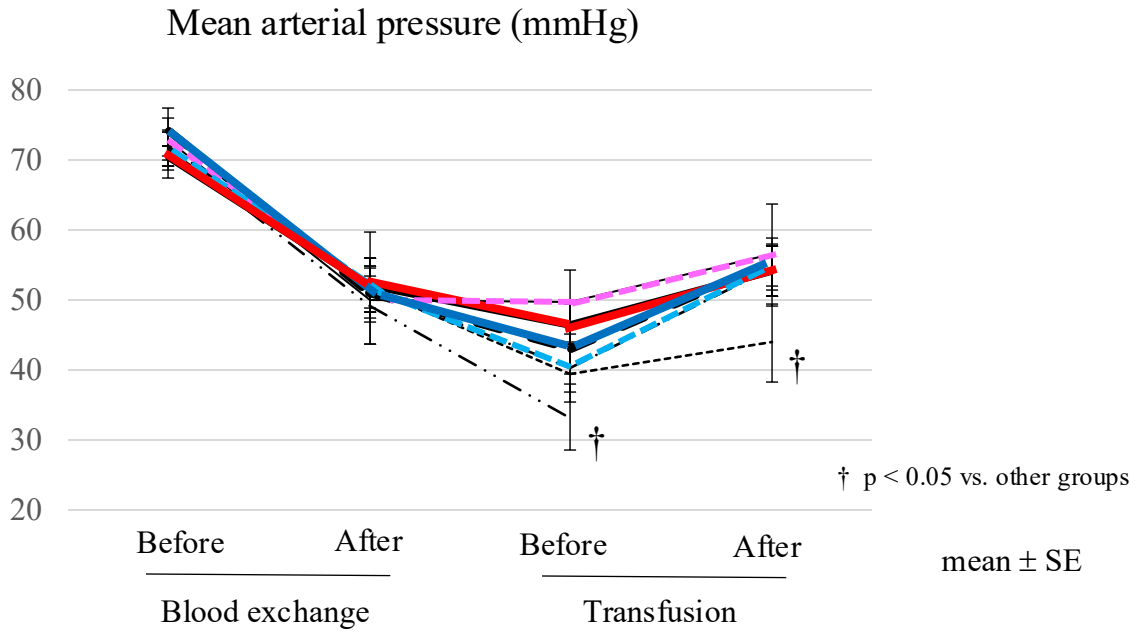
Group 2: H12-(ADP)-liposome/PPP + stored RBCs (n=10)

Group 3: PRP + HbV (n=10)

Group 4: PRP + stored RBCs (n=10)

Group 5: PRP + Albumin (n=6)

Group 6: PPP (n=5)



Human Phase I Study of HbVs

RESEARCH LETTER

blood advances

Check for updates

TO THE EDITOR:

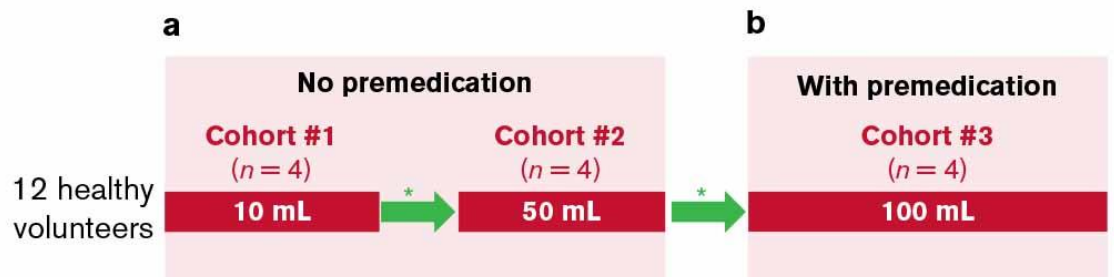
First-in-human phase 1 trial of hemoglobin vesicles as artificial red blood cells developed for use as a transfusion alternative

Hiroshi Azuma,[1] Toraji Amano,[2] Naoya Kamiyama,[3] Naofumi Takehara,[3] Maki Jingu,[2] Harumi Takagi,[2] Osamu Sugita,[2] Naoko Kobayashi,[4] Tomoko Kure,[4] Taro Shimizu,[5] Tatsuhiro Ishida,[5] Masanori Matsumoto,[6,7] and Hiromi Sakai[4]

Blood Advances, 6, 5711-5, 2022.

Professor H. Sakai, PhD, Nara Medical University

Protocol



Dexamethasone 6.6 mg I.V.
(with famotidine 20 mg P.O.)

Acetaminophen 500 mg P.O.
1 h before HbV administration

HbV solution - Hb content of 8.5-11.6 g/dL

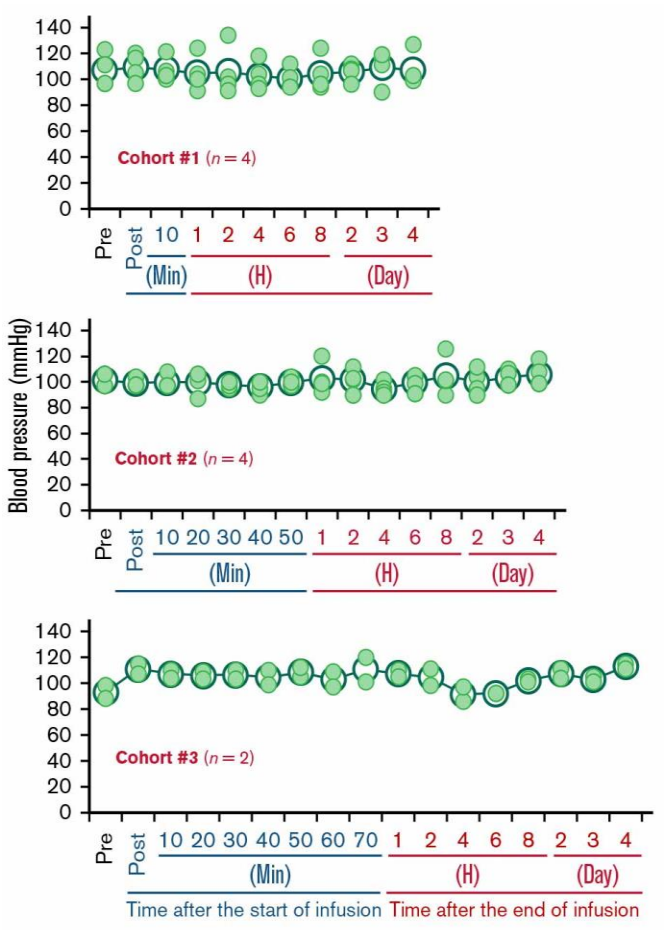
Adverse effects

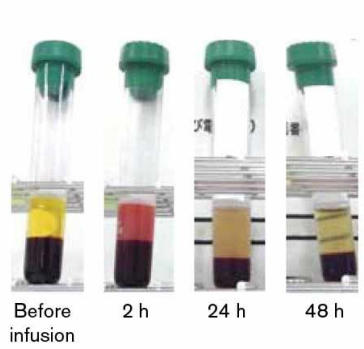
Summary of adverse events associated with infusion of HbV

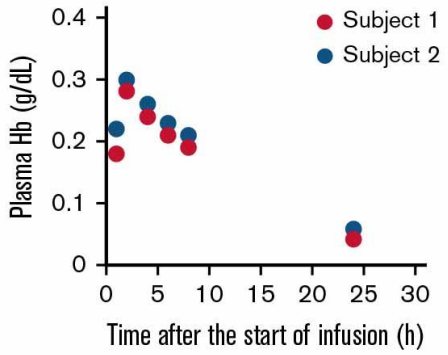
	Cohort #1 (n = 4)	Cohort #2 (n = 4)	Cohort #3 (n = 3)
(Premedication)	–	–	+
Serious adverse event	0/4	0/4	0/3
Infusion reaction	3/4 Burning sensation (3) Flushing (1) Head dullness (1) Fatigue (1), Dizziness (1) Feeling of strangulation (1)	0/4	1/3 Sense of discomfort in the lower back (1) Rash and wheal (1)
Fever (37–38°C)	2/4	1/4	0/3
Fever (>38°C)	1/4	1*/4	0/3
Conjunctivitis	0/4	1*/4	0/3

Human Phase I Study of HbVs

No elevation of blood pressure after administration of HbVs, suggesting that HbVs do not have a NO scavenging effect.





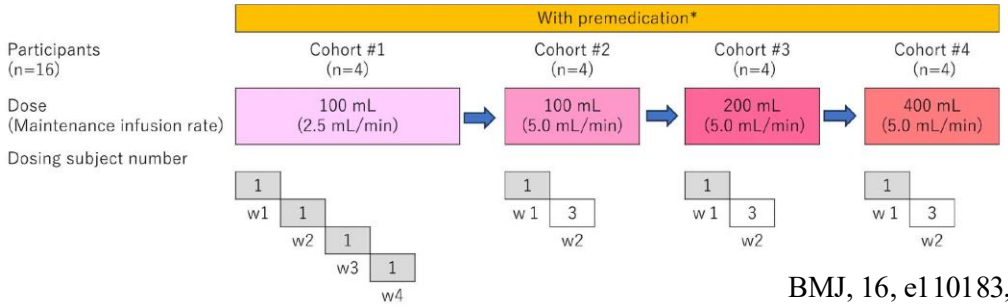


Pharmacokinetic parameters

Subject	AUC_t mg·h/dL	AUC_∞ mg.h/dL	C_{max} mg/dL	T_{max} h	Kel 1/h	No. of trace point	Correlation coefficient	$T_{1/2}$ h	MRT_t h	CL/F dL/h
Subject 1	4030.0	3980.3	280.0	2.0	0.0927	3	0.9989	7.5	9.13	2.51
Subject 2	4700.0	4747.4	300.0	2.0	0.0780	3	0.9991	8.9	9.91	2.11
Mean	4365.0	4363.8	290.0	2.0	0.0854	3	0.9990	8.2	9.52	2.31
S.D.	473.8	542.4	14.1	0.0	0.0104	0	0.0001	1.0	0.55	0.29

Half-life: approximately 8 hours

Late Phase I trial currently underway (up to 400 mL i.v.)



BMJ, 16, e110183, 2026.

Freeze-dried Plasma (FDP) in a Soft-Bag Formulation (Terumo)

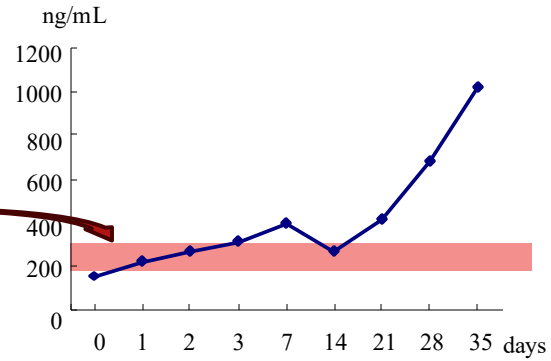
	Production	Storage/Shipping	Thawing/Reconstitution
FFP Fresh frozen plasma	Quick freezing	< -20°C	30 min
			
FDP Freeze-dried plasma	Freeze-drying	**Room temperature**	**3 min**
			

TERUMO

Freeze-dried Plasma (FDP) in a Soft-Bag Formulation (Terumo)

Quality parameters	FFP (n=6)	FDP (n=59)	% difference	*p* value
Coagulation factors				
Factor II (U/ml)	0.81 ± 0.03	0.78 ± 0.04	−4.9	NS[c]
Factor V (U/ml)	0.92 ± 0.06	0.83 ± 0.08	−9.8	.03[c]
Factor VII (U/ml)	0.99 ± 0.04	0.92 ± 0.04	−7.1	.0007[d]
Factor VIII (U/ml)	0.7 ± 0.2	0.6 ± 0.2	−14.3	.0015[d]
Factor IX (U/ml)	0.77 ± 0.05	0.73 ± 0.05	−5.2	.0035[d]
Factor XI (U/ml)	1.03 ± 0.07	1.00 ± 0.08	−2.2	NS[d]
Factor XIII (U/ml)	1.2 ± 0.2	1.1 ± 0.02	−6.9	.018[d]
Fibrinogen (g/L)	2.99 ± 0.06	2.9 ± 0.1	−3.0	NS[d]
Coagulation- and fibrinolysis-related proteins				
Protein C (U/ml)	0.93 ± 0.06	0.91 ± 0.07	−1.6	NS[d]
Protein S (U/ml)	0.91 ± 0.03	0.79 ± 0.07	−12.1	.013[d]
Antithrombin (U/ml)	0.92 ± 0.02	0.84 ± 0.05	−8.7	.04[d]
α_2-Antiplasmin (U/ml)	0.7 ± 0.1	0.6 ± 0.1	−14.3	.0093[d]
VWF (U/ml)	1.1 ± 0.4	1.0 ± 0.4	−9.1	.011[d]
ADAMTS-13 (U/ml)	1.4 ± 0.2	1.2 ± 0.1	−14.3	NS[d]
D-Dimer (μg/ml)	0.30 ± 0.09	0.29 ± 0.08	−3.3	NS[d]
TAT (ng/ml)	7 ± 1	7 ± 2	0.0	NS[d]
Hemostasis screening tests				
PT (s)	12.9 ± 0.2	13.6 ± 0.3	+5.9	.007[d]
APTT (s)	34 ± 1	36 ± 2	+6.9	.021[d]
Complement-related factors				
C3a (ng/ml)	140 ± 20	230 ± 50	+64	.002[d]
C5a (ng/ml)	5.7 ± 0.6	6.2 ± 0.6	+9.0	NS[d]
Physical characteristics				
pH	7.25 ± 0.08	7.53 ± 0.06	Not applicable	.0006[d]
Osmolality (mmol/kg)	311 ± 3	298 ± 5	−4.3	NS[d]
Residual moisture (%)	Not applicable	1.6 ± 0.3	Not applicable	Not applicable

C3a levels in whole blood during storage



Transfusion, 37, 264-8, 1997.

Mean ± SD

Transfusion, 62, 418-8, 2022.

Summary

- Combination therapy using synthetic platelet and RBC substitutes demonstrated efficacy *in vivo* comparable to transfusion of natural platelets and RBCs.
- These substitutes are expected to be effective tools in pre-hospital resuscitation for severe hemorrhagic shock with coagulopathy.
- Extended shelf life of synthetic blood products will be useful for medical treatment of casualties in multidomain operations (*e.g.*, during prolonged field care or interrupted logistical support).
- Freeze-dried plasma (FDP) in a soft-bag formulation is useful on the battlefield and is expected to be clinically available soon.

Acknowledgements

National Defense Medical College, Tokorozawa, Japan

Kohsuke Hagisawa, MD, PhD; Osamu Ishida MD, PhD; Luka Sasa MD

Nara Medical University, Nara, Japan

Hiromi Sakai, PhD

Waseda University, Tokyo, Japan

Shinji Takeoka, PhD

Some figures created using BioRender.com (BR)