Research on pharmacokinetic analysis and safety evaluation for clinical development of artificial blood

(人工血液の臨床開発に向けた動態特性解析及び安全性評価に関する研究)

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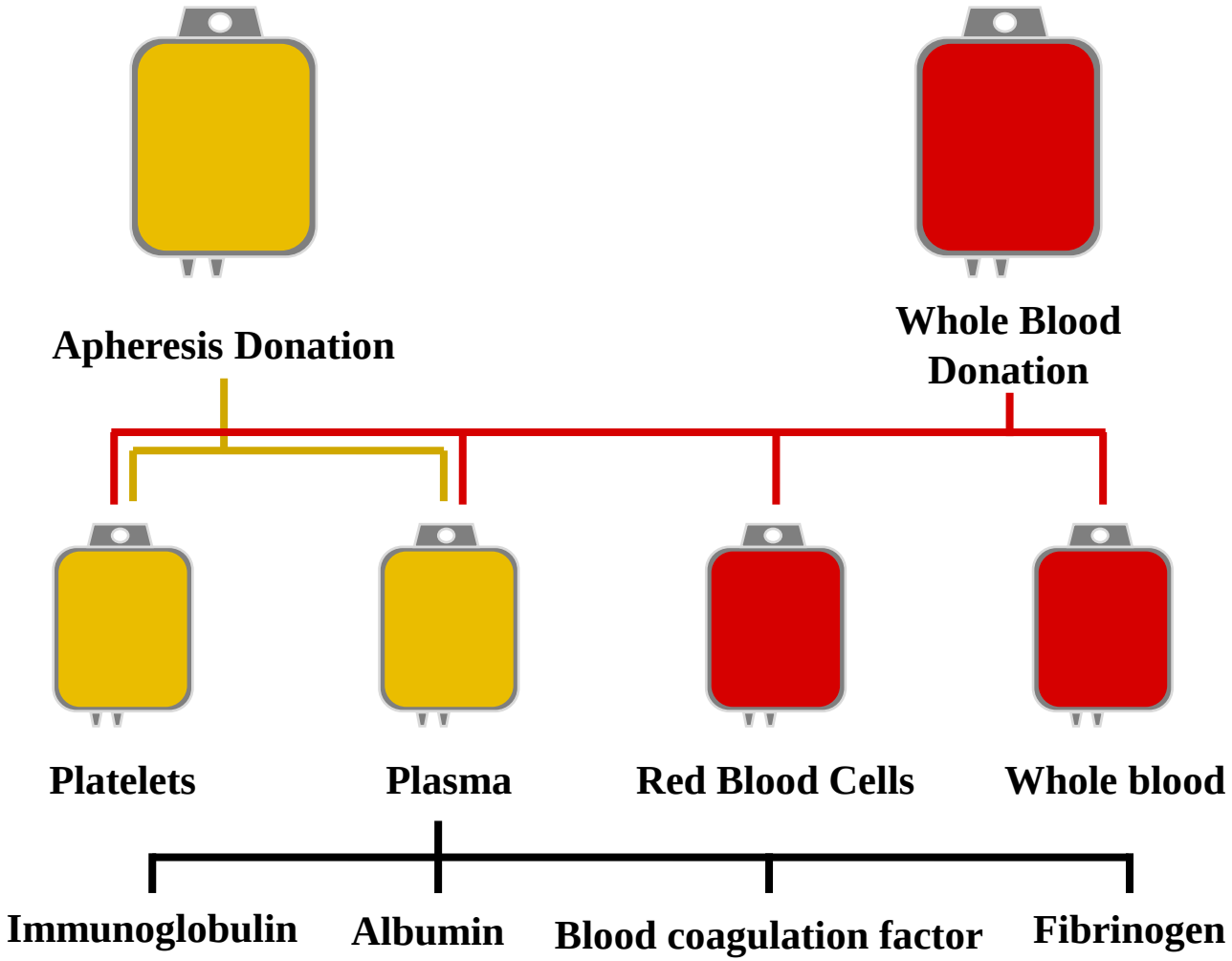
Blood Transfusion System Problems in Japan



~ Safety ~

- **Viral contamination**

  Nucleic acid Amplification Test (NAT) improves the safety by screening for many types of virus, such as HIV and hepatitis.

  ➡ Threats of unrecognized virus

- **Medical malpractice**

  ABO-incompatible blood transfusion

~ Stable supply~

- Decrease in future population in Japan
- Aging Japanese society with fewer children

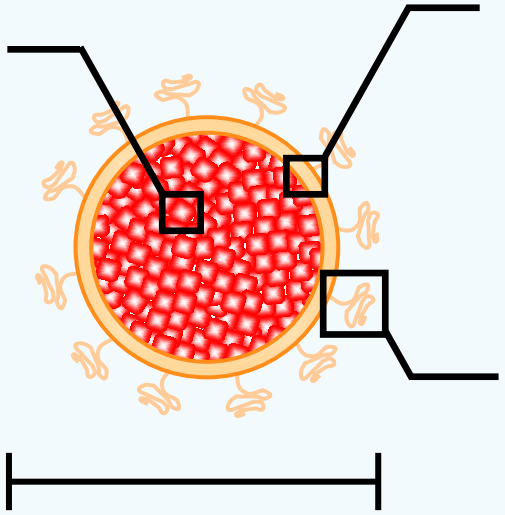
  ➡ Decrease in donated blood

A stable supply of safe blood products remains a potent concern.

Development of Artificial Blood in Japan

Hemoglobin-vesicles [Hb-V]

Human hemoglobin (ca. 35 g/dL)



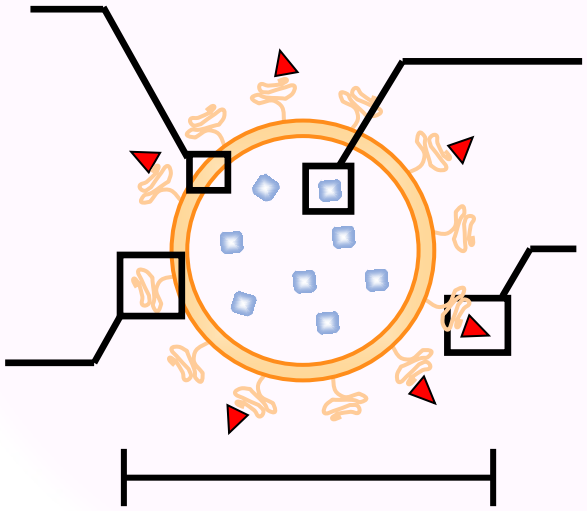
250 - 280 nm

Lipid membrane

- DPPC
- DHSG
- cholesterol

Polyethylene glycol (PEG)

Fibrinogen γ-chain–coated, ADP-encapsulated liposomes [H12-(ADP)-liposomes]



adenosine diphosphate (ADP)

Dodecapeptide (H12) (HHLGGAKQAGDV)

250 ± 50 nm

Ministry of Health, Labour and Welfare

Japan Agency for Medical Research and Development

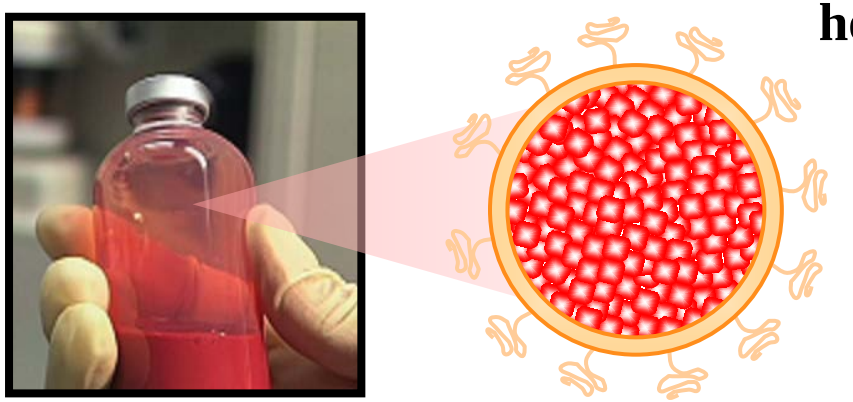
AMED

I have clarified the pharmacokinetic properties of these artificial bloods during the pre-clinical studies.

DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine DHSG: 1,5-bis-*O*-hexadecyl-*N*-succinyl-L-glutamate

Hemoglobin-vesicles (Hb-V)

Hb-V



Artificial red blood cell that encapsulates highly concentrated human hemoglobin into a phospholipid bilayer membrane (liposomes).

The evidence for positive characteristics of Hb-V as a red blood cell transfusion alternative have been accumulated:

- **the absence of viral contamination and cross-matching test**
- **a long-term storage period (over 2 years at room temperature)**
- **comparable oxygen carrying capable to red blood cell**

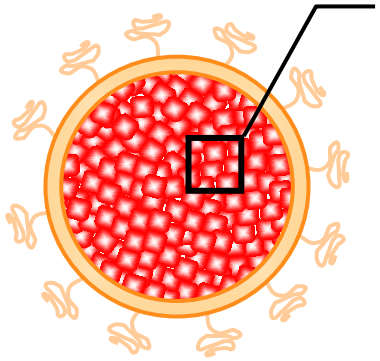
The pharmacokinetic profiles of Hb-V have not been well characterized !

	Hb-V	Doxil®	Ambisome®
Principal-agent	20000 mg/200 mL	20 mg/vial (80 mg/1.72 m^2)	50 mg/vial (420 mg/70 kg)
lipid	20000 mg/200 mL	159.9 mg/vial (640 mg/1.72 m^2)	265.64 mg/vial (2940 mg/70 kg)

Hb-V preparation contains massive amounts of principal-agent (hemoglobin) and lipid components compared to other liposome preparations.

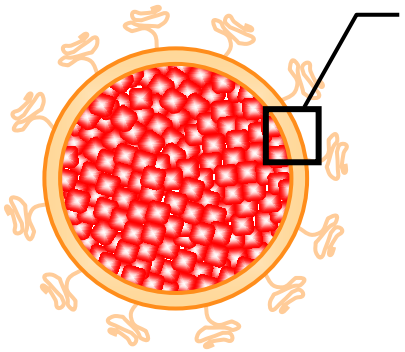
Pharmacokinetic Evaluations of Hb-V and its Components in Rodent

^{125}I-Hb-V



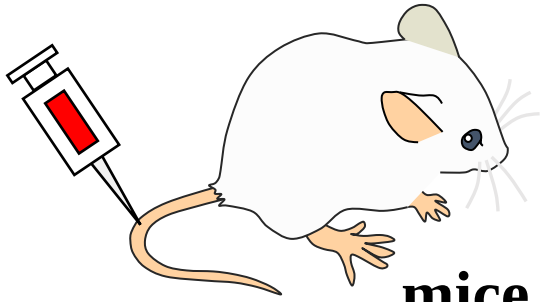
Hemoglobin
labeled with ^{125}I

^{3}H-Hb-V



Lipid membrane
labeled with ^{3}H

^{125}I-Hb-V or ^{3}H-Hb-V
(1400 mg/kg)



mice and rats

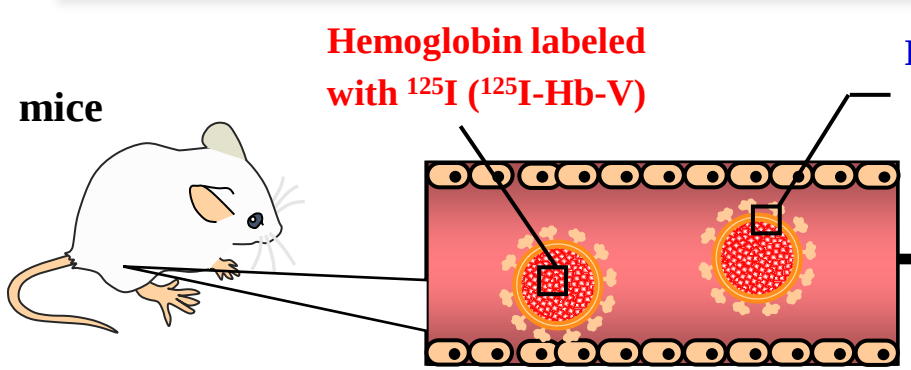
Blood retention

Biodistribution

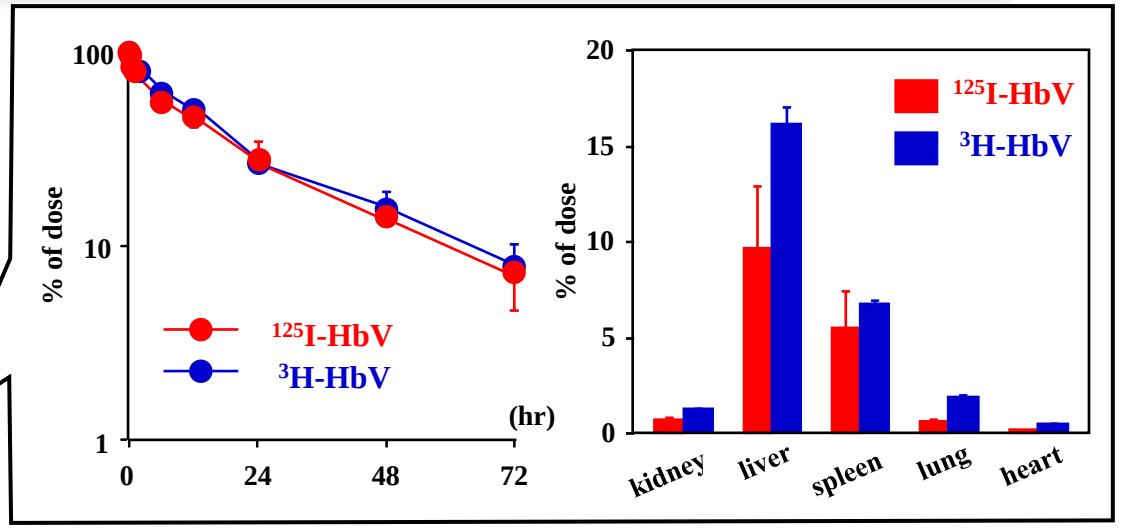
Metabolism

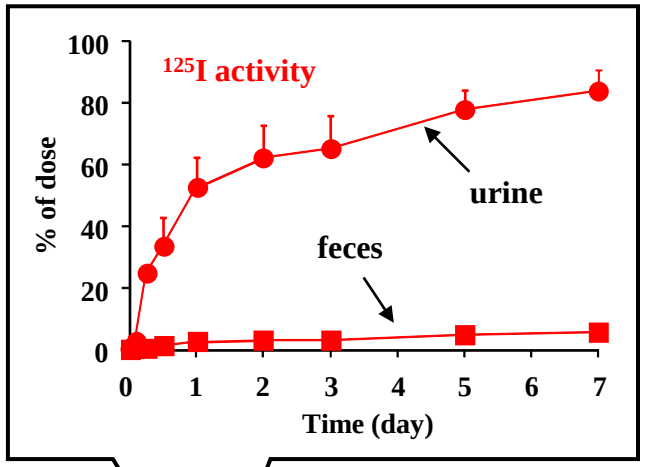
Excretion
(in urine and feces)

Disposition of Hb-V in Rodent

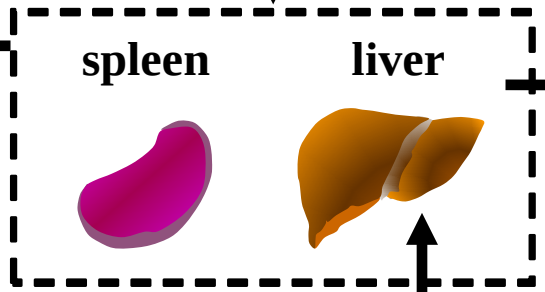


Circulating in the form of stable Hb-V

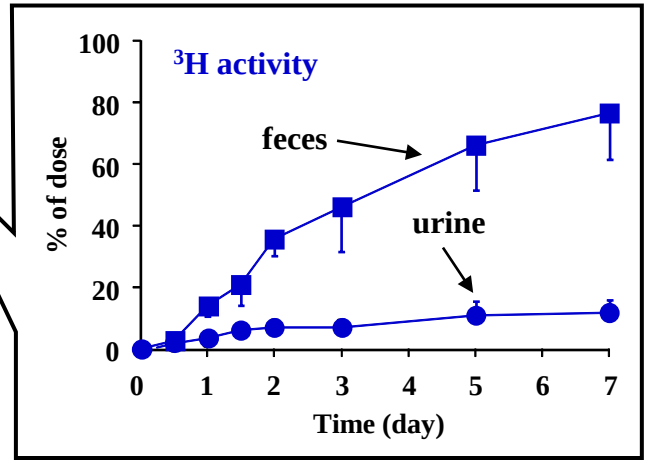


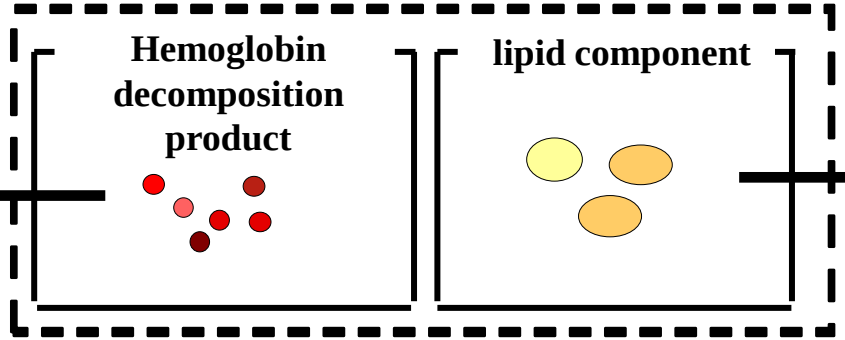


Degraded by MPS



Eliminate in the feces



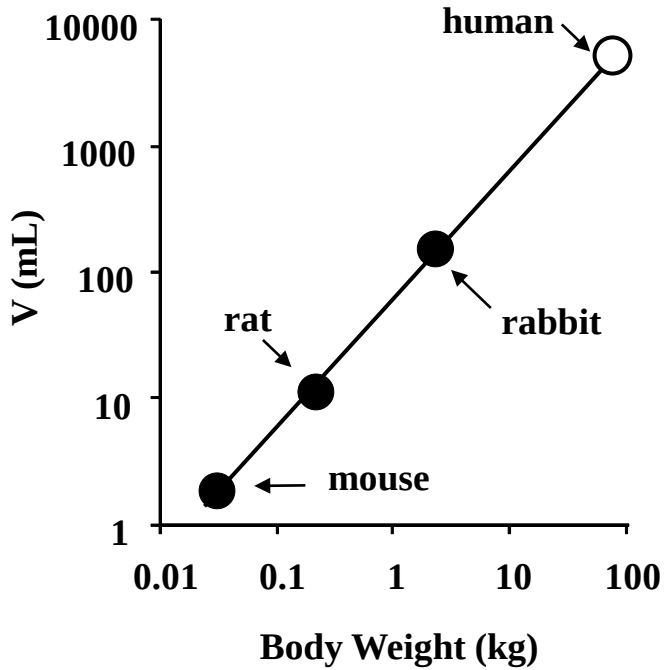


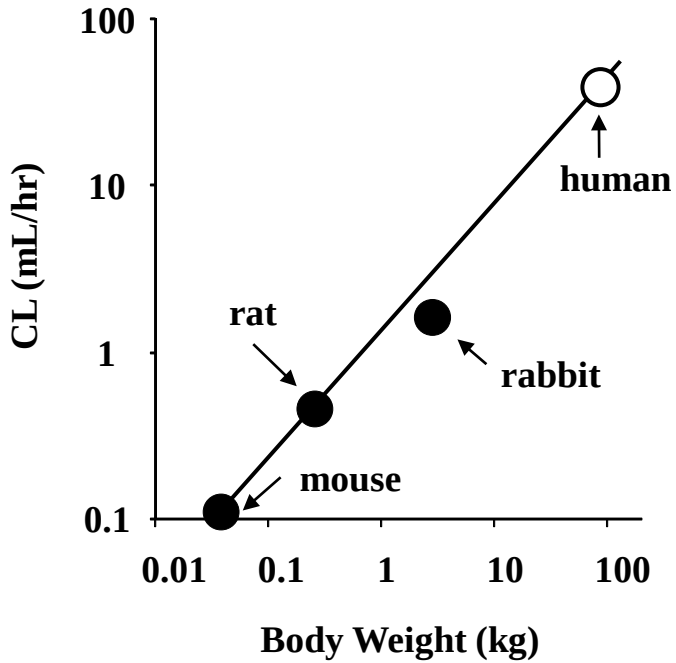
Eliminate in the urine

Based on the pharmacokinetic studies, the disposition of Hb-V and its components occurs as shown in this slide.

Drug Metab. Dispos. 37: 1456-63. (2009)

Prediction of Human Pharmacokinetics





	mouse	rat	rabbit
Half-life	19 hr	30 hr	62 hr
metabolism	liver, spleen		
Excretion	Same manner as the endogenous substances		

The half-life of Hb-V in humans was estimated using an allometric equation

The half-life of Hb-V in humans was extrapolated to be approximately 96 hr.

J. Control. Release 136: 232-9. (2009)

Pharmacokinetic evaluations of Hb-V in the conditions of poor metabolism and excretion

Hb-V and its components have favorable metabolic and excretion profiles.

However, the Hb-V pharmacokinetic properties under hepatic disease and abnormal lipid metabolism require further investigation.

Because……..

(i) Hb-V is mainly metabolized in the liver

(ii) Massive amount of lipid are infused when Hb-V is administered.

Since Hb-V will be used in emergencies, Hb-V needs to have good metabolic and excretion profiles even in patients with poor metabolism and excretion.

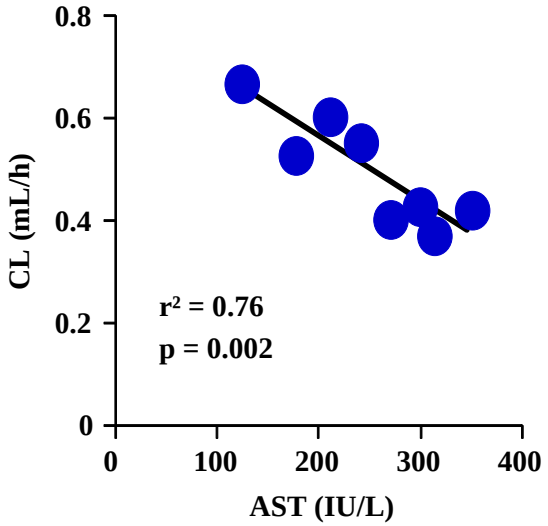
Disorders that suspected of poor metabolism and excretion of Hb-V

★ **Hepatic disease** → **Hepatic cirrhosis model rats**

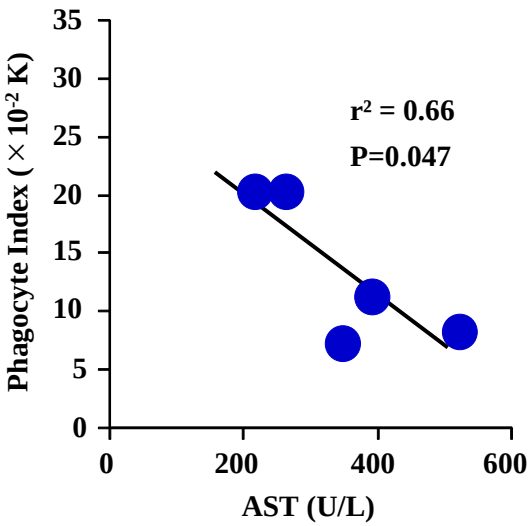
★ **Abnormal lipid metabolism** → **Hyperlipidemia model mice**

Pharmacokinetics of Hb-V in Hepatic Cirrhosis Model Rats

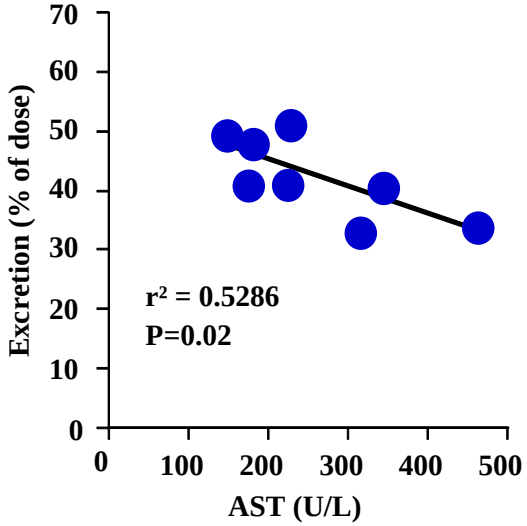
Plasma retention

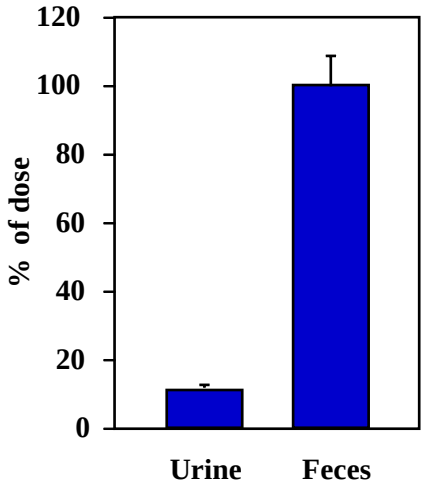


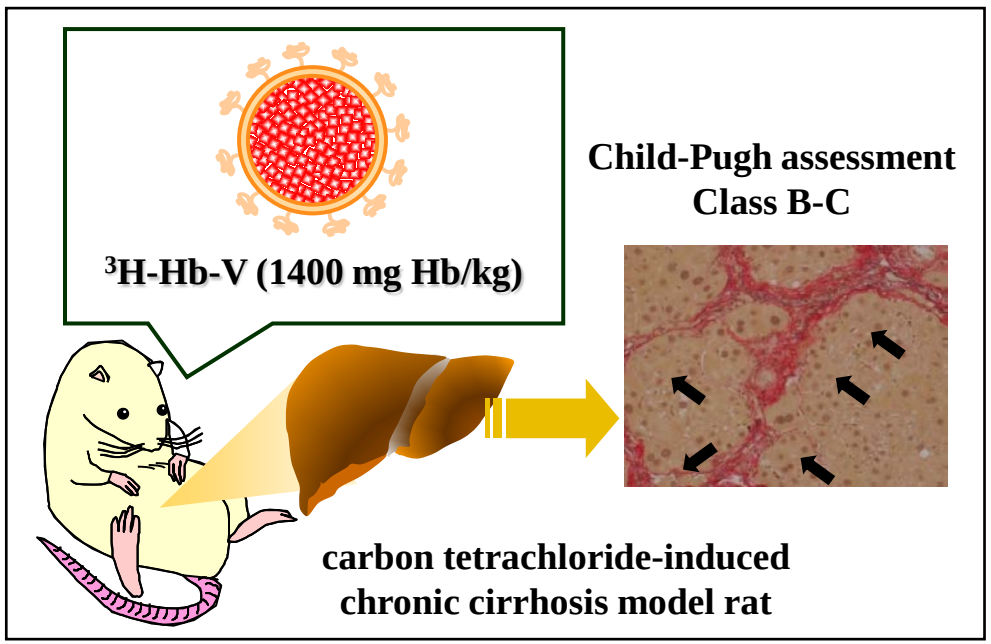
Metabolism



Excretion



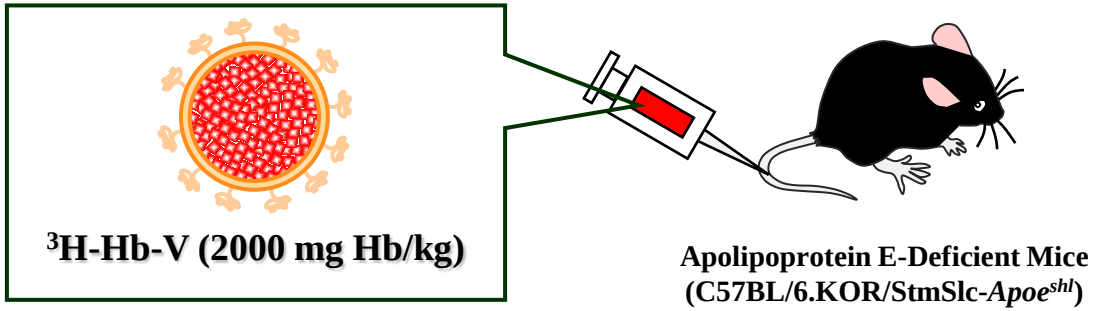


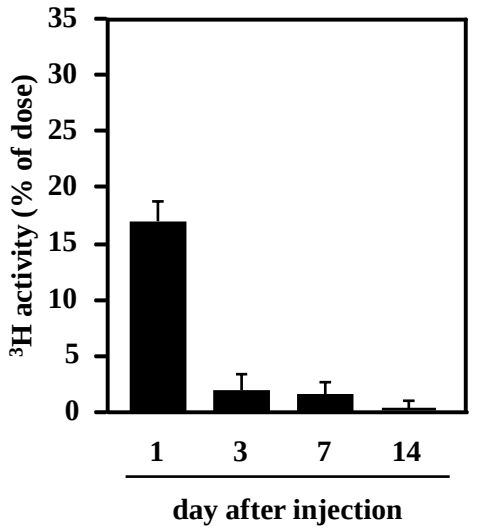


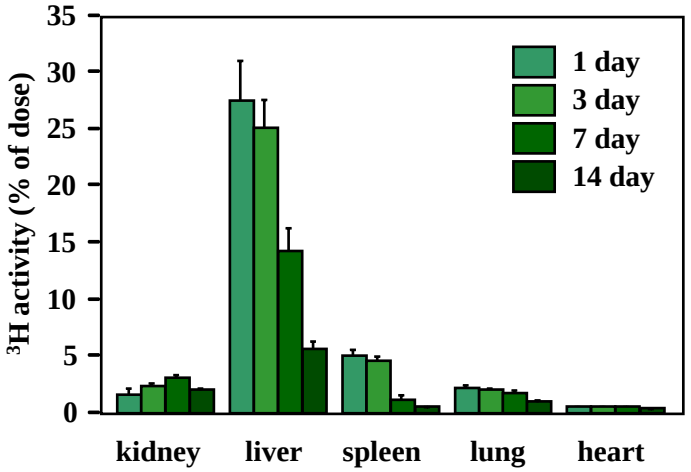
- ✓ **The pharmacokinetics of Hb-V is altered by the extent of hepatic impairment due to the decrease in metabolic activity in the liver.**
- ✓ **Most of Hb-V was excreted from the body within 7 days.**

Toxicol. Appl. Pharmacol., 248: 234-241 (2010) *J Pharm. Sci.* 100:775-83. (2011)

Pharmacokinetic Properties of Hb-V in Hyperlipidemia Model Mice







✓ **Hb-V was mainly distributed in the liver and spleen and disappeared from the body within 14 days.**

Hb-V exhibits a good metabolic and excretion profile even under pathological conditions of poor metabolism and excretion.

Biol. Pharm. Bull. 38: 1606-16. (2015)

Pharmacokinetic evaluations of Hb-V in the condition of expected clinical application

『Guideline for the Development of Liposome Drug Products』

4. 2 Nonclinical pharmacokinetics

4. 2. 2 Pharmacokinetics

When the *in vivo* pharmacokinetics and active substance release are investigated, the selection of animal species and animal model should be justified, with careful consideration of the following points: the expected clinical application of the liposome drug product, liposome composition, the properties of the active substance, and blood concentration and tissue distribution including the accumulation and retention in the target organ and/or tissue of both the active substance and liposome drug product.

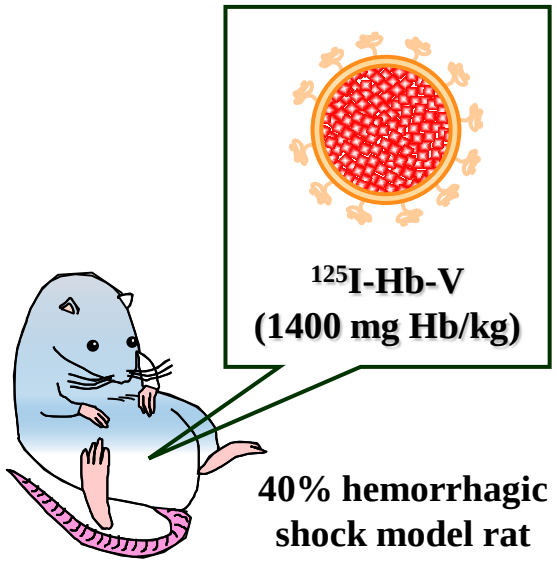
~ The expected clinical patients who Hb-V are administered ~

★ **Patients with massive bleeding**

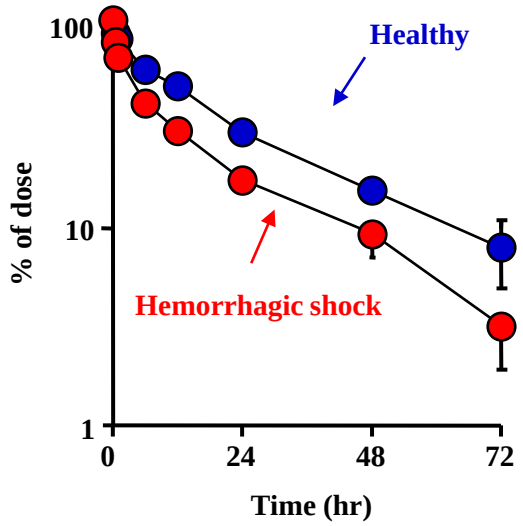
Injury, surgical operation etc.

➡ **Hemorrhagic shock model rats**

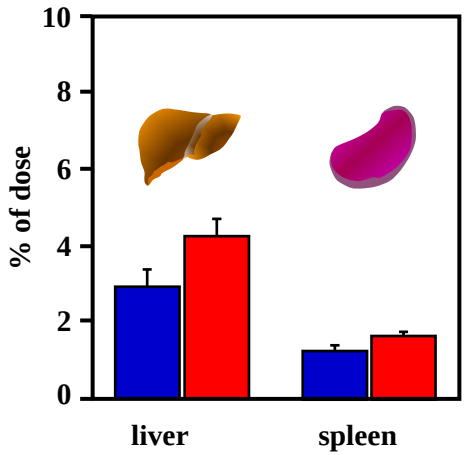
Pharmacokinetics of Hb-V in Hemorrhagic Shock Model Rats



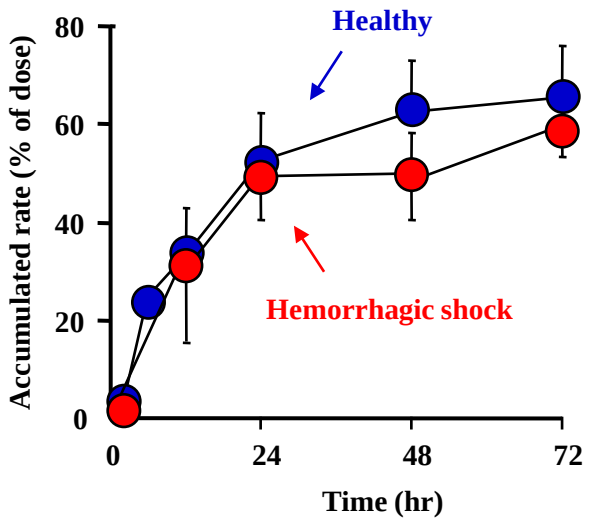
Plasma concentration



Biodistribution



Excretion (in urine)



- ✓ The plasma clearance of Hb-V in the hemorrhagic shock model rats was faster than that of healthy rats.
- ✓ Hb-V distribution in the liver and spleen was not different between the healthy and hemorrhagic shock model rats.
- ✓ Urinary excretion of Hb-V was not different between the healthy and hemorrhagic shock model rats.

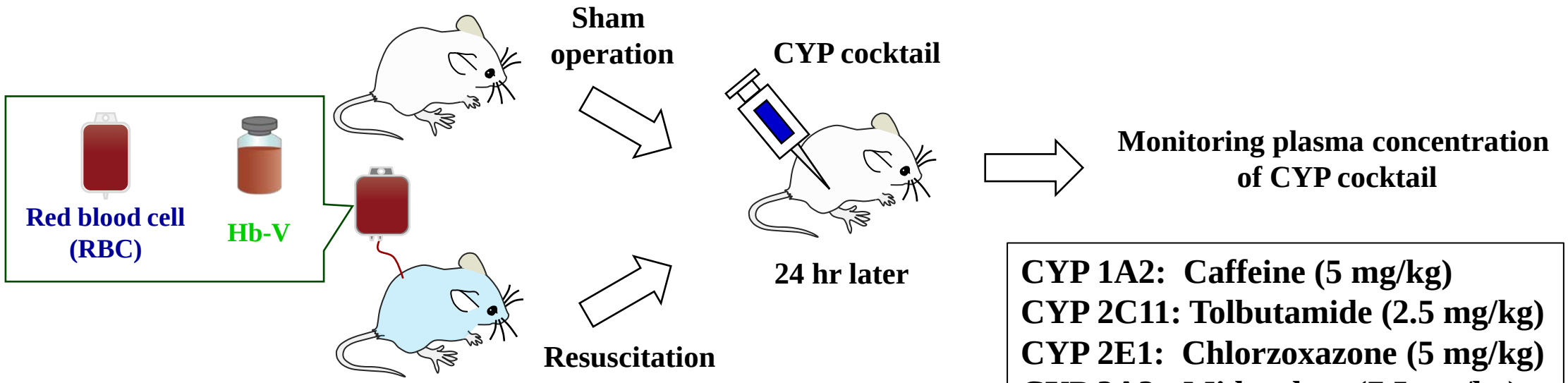
➡ The shorter half-life in hemorrhagic shock rats resulted in an apparent reduction in Hb-V in circulation.

J. Control. Release 136: 232-9. (2009)

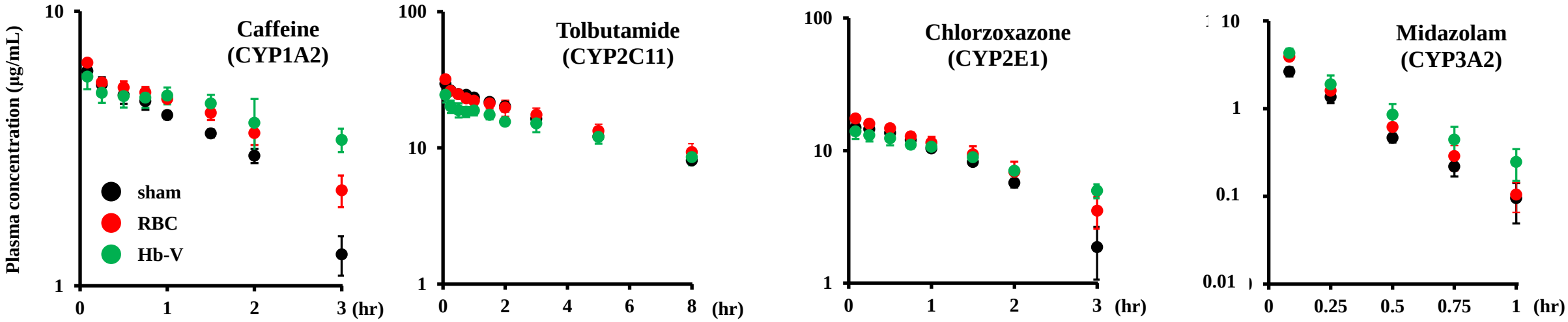
Cytochrome P450 (CYP)-based Drug-Drug Interactions of Hb-V

Patients given Hb-V are mostly co-administered with medications, such as a narcotic, an analgesic, or a steroid, with the aim of controlling their general medical condition.

The pharmacokinetics of CYP-metabolizing drugs after Hb-V resuscitation from a massive hemorrhage were investigated in hemorrhagic shock model rats.



Effects of Hb-V on hepatic CYP in hemorrhagic shock model rats



The CYP-metabolizing drugs in the Hb-V resuscitation rats were retained compared to sham rats and red blood cell resuscitation rats.

	CYP protein expression	CYP activity
CYP 1A2	Sham>>RBC>Hb-V	Sham>RBC>>Hb-V
CYP 2C11	Sham>RBC=Hb-V	Sham>RBC>Hb-V
CYP 2E1	Sham>RBC=Hb-V	Sham>RBC>Hb-V
CYP 3A2	Sham>RBC>Hb-V	Sham>RBC>Hb-V

Protein expression and activity of hepatic CYP were lower in the Hb-V resuscitation rats than those in sham rats and red blood cell resuscitation rats.

These changes may not have clinical relevance!

Drug Metab Pharmacokinet. **35:417-424. (2020)**

Summary

The pharmacokinetic properties of Hb-V and its components under various pathophysiological conditions for potential clinical applications of Hb-V were clarified.

- ✓ Healthy animals (mice, rats, monkeys)
- ✓ Hepatic cirrhosis model rats
- ✓ Hyperlipidemia model mice
- ✓ Hemorrhagic shock model rats

In the winter of 2020, Phase Ia clinical trial of Hb-V as artificial red blood cells commenced!

I would like to translate artificial blood into a practical application by conducting pharmacokinetics research.

Acknowledgement

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