that HbV is pharmacologically efficacious in a rat model of HS induced by massive hemorrhage (Sakai et al., 2004b, 2009) is retained for a sufficiently long period to meet oxygen-delivery demands until autologous blood volume and oxygen-carrying capacity are restored.

Pharmacokinetic properties of HbV in the condition with chronic liver failure

As mentioned above, the liver is the determinant for the pharmacokinetic properties of HbV, because HbV is mainly degraded by Kupffer cells, and the lipid components of HbV, especially cholesterol, are excreted to the feces via biliary excretion (Sakai et al., 2001; Taguchi et al., 2009b). Consequently, HbV can be classified as a hepatically cleared and excreted drug. In the case of other hepatically cleared and excreted drugs, some are contraindicated for a person with a hepatic injury. Because hepatic impairment affects the pharmacokinetics of drugs, including their metabolism and excretion (Okumura et al., 2007), these changes have the potential to induce toxicity and accumulate in the body, subsequently causing unexpected adverse effects. Thus, if HbV and its components show the changes of pharmacokinetic properties under conditions of liver failure, it may also be contraindicated for a person with liver impairment under such conditions. Therefore, we investigated the pharmacokinetic properties of HbV using a chronic cirrhosis rat model with fibrosis induced by the administration of carbon tetracholoride, which is categorized as Child-Pugh grade B (Taguchi et al., 2011b).

After the administration of HbV to chronic cirrhosis rats, the plasma concentration of HbV varied widely among individuals, similar to their liver function. To clarify the effect of hepatic impairment on the plasma concentration of HbV, the clearance and the area under the concentration-time curve values for HbV, as calculated from the plasma concentration curve, were plotted against plasma aspartate aminotransferase (AST) levels. As a result, a good, negative correlation was found for the clearance of HbV with changes in plasma AST levels. In addition, the hepatic distribution of HbV was negatively

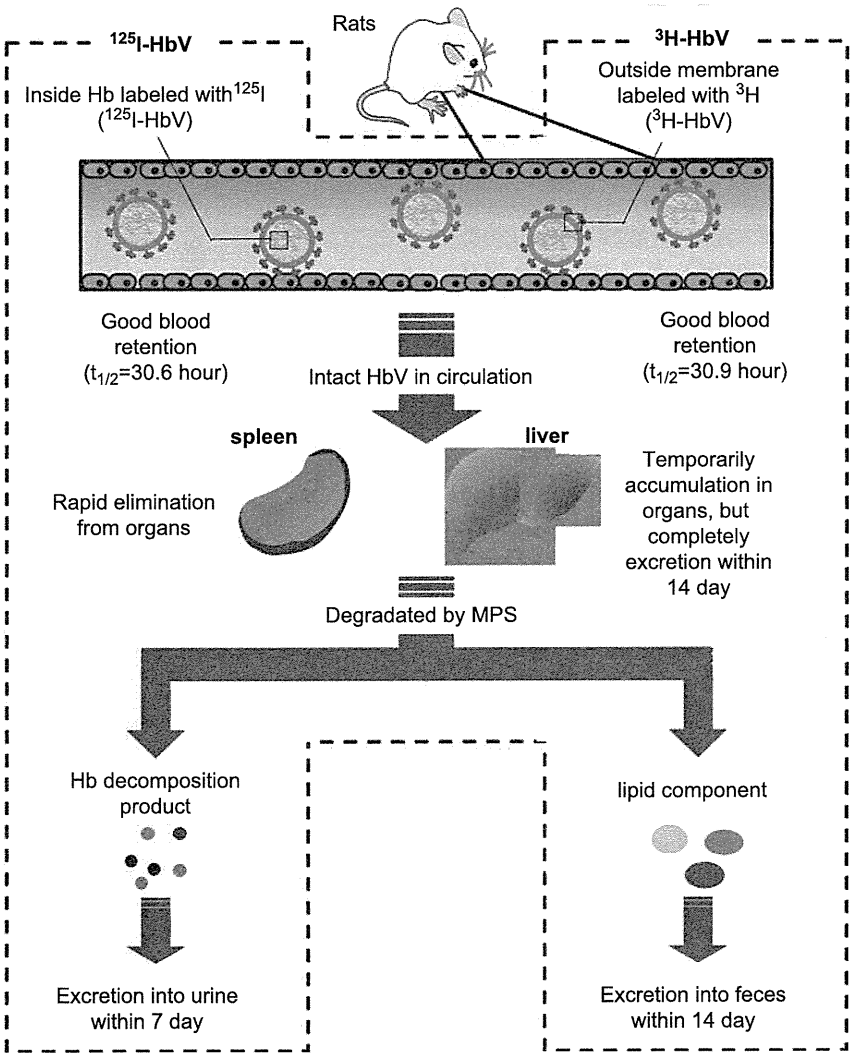

Figure 4. Representation of a sequence of HbV disposition, metabolism, and excretion from pharmacokinetic examinations, using ^{125}I-HbV and ^{3}H-HbV. After circulating in the form of stable HbV, it is distributed to the liver and spleen, where it is degraded by MPS. Finally, the enclosed Hb and outer lipid components are mainly eliminated to the urine and feces, respectively.

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correlated with plasma AST levels, but this was not found for the spleen. Moreover, carbon clearance, which serves as a measure of phagocyte activity in Kupffer cells (Zweifach and Benacerraf, 1958), was also negatively correlated with plasma AST levels. Therefore, the changes in HbV pharmacokinetic properties were significantly influenced by a reduction in liver function and were especially dependent on a decrease in phagocyte activity by Kupffer cells in the chronic cirrhosis rat.

In addition, the excretion of lipid components (e.g., cholesterol) in feces was also negatively correlated with plasma AST levels. The cholesterol of the vesicles should reappear in the blood mainly as lipoprotein cholesterol after entrapment by Kupffer cells and should then be excreted in the bile after entrapment of the lipoprotein cholesterol by the hepatocytes (Kuipers et al., 1986). Therefore, the extent of damage to parenchymal cells also affects the pharmacokinetic properties of HbV components. Such a suppressed elimination of HbV components may have an impact on their tissue accumulation. However, the lipid components, especially cholesterol, nearly completely disappeared from organs after 7 days in the chronic cirrhosis rat. Further, our recent study showed that the plasma levels of other lipid components, such as phospholipids, was temporarily increased after the administration of HbV at a dose of 1,400 mg Hb/kg in the chronic cirrhosis rat, but recovered to baseline levels within 14 days (Taguchi et al., 2010). In addition, if the metabolic and excretion performance of HbV were reduced by chronic cirrhosis, tissue damage could be induced, resulting in a change in blood biochemical parameters. However, the morpholgical changes in organs were minimal (Figure 6), and only negligible changes in plasma biochemical parameters were observed after an HbV injection at a dose of 1,400 mg Hb/kg in the chronic cirrhosis rats. Based on these findings, it can be concluded that the pharmacokinetics of HbV were altered by hepatic impairment, and these changes can be attributed to a decrease in Kupffer-cell phagocyte activity (Figure 7). However, HbV and its components were completely metabolized and excreted within 14 days, and a temporary accumulation did not cause any obvious adverse effects.

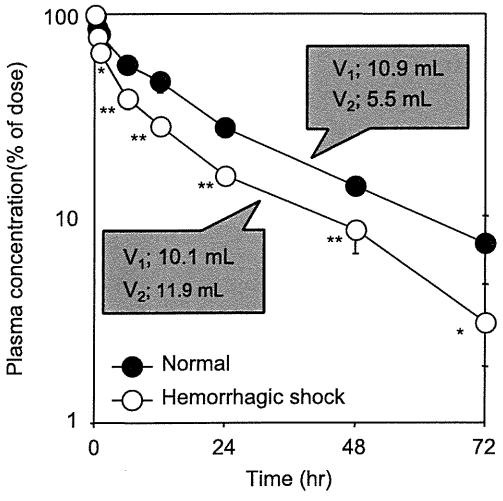

Figure 5. Relative plasma concentration of ^{125}I-HbV after administration of 1,400 mg Hb/kg via injection of normal (filled circles) or hemorrhagic shock rats (open circles). After inserting polyethylene catheters into the left femoral artery, SD rats received a single injection of ^{125}I-HbV to the left femoral artery at a dose of 1,400 mg Hb /kg. Blood was collected from the tail vein under ether anesthesia, and a plasma sample was obtained. Each point represents the mean ± SD (n=5).

Pharmacokinetic properties of HbV after repeated administration in mice

HbV is modified by PEG to prolong its half-life and prevent aggregation during long-term storage, etc., as well

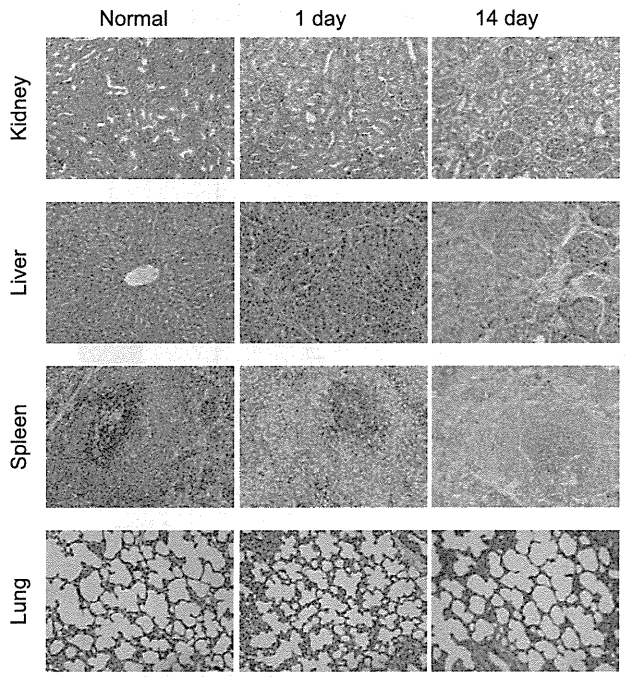

Figure 6. Light micrographs of kidney, liver, spleen, lung, and heart in CCl_4-treated rats after an HbV injection stained with hematoxylin and eosin (X100). Chronic cirrhosis model rats received a single injection of HbV at a dose of 1,400 mg Hb/kg. No noticeable changes were observed in all organs after HbV injection.

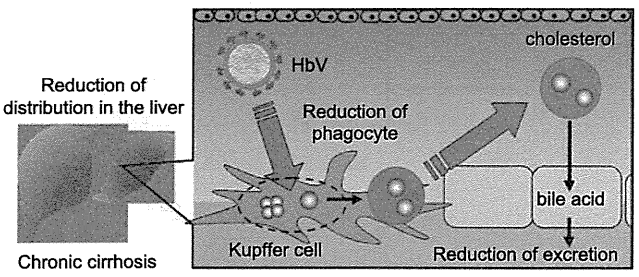

Figure 7. Representation of the pharmacokinetic properties of HbV in a rat model of chronic cirrhosis. Hepatic impairment altered the pharmacokinetic properties of HbV, such as blood retention, hepatic distribution, and fecal excretion, by a reduction in Kupffer cell phagocyte activity and damage to parenchymal cells.

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as other liposome preparations. However, it was reported that repeated intravenous injection of PEGylated liposomes causes the second dose of liposomes to lose their long-circulating characteristics and accumulate extensively in the liver, when they are administrated at the same dose for the second time to the same animal within a several-day interval [referred to as the accelerated blood clearance (ABC) phenomenon] (Dams et al., 2000; Ishida et al., 2003a). The time frame between administration of the first and second dose for this to occur depends on the experimental animal, for example, 4–5□days for the rat and 7–10 days for the mouse. Repeated HbV injections of high doses would be routinely used in clinical practice for an RBC substitute. Therefore, the possibility remains that repeated injections of HbV could induce the ABC phenomenon in a clinical situation. If the ABC phenomenon were induced by repeated injections, then the pharmacological action of HbV could be influenced. Therefore, we investigated the issue of whether HbV induces the ABC phenomenon in mice at a low dose (0.1 mg Hb/kg), a dose that is generally known to induce the ABC phenomenon (Ishida et al., 2003a), or a high dose (1,400 mg Hb/kg), the putative dose for clinical use.

At 7 days, in which the ABC phenomenon in mice is typically observed the most strongly (Ishida et al., 2003b), after the first injection of nonlabeled HbV (0.1 or 1,400 mg Hb/kg), the mice received ^{125}I-HbV. At a low dose (0.1 mg Hb/kg), plasma HbV in the second injection was rapidly cleared, compared to that in the first injection. In contrast, at a high dose (1,400 mg Hb/kg), the pharmacokinetics of HbV were negligibly affected by repeated injections (Taguchi et al., 2009c). The liver and spleen are the major distribution organs for HbV (Taguchi et al., 2009b) and are related to the induction of

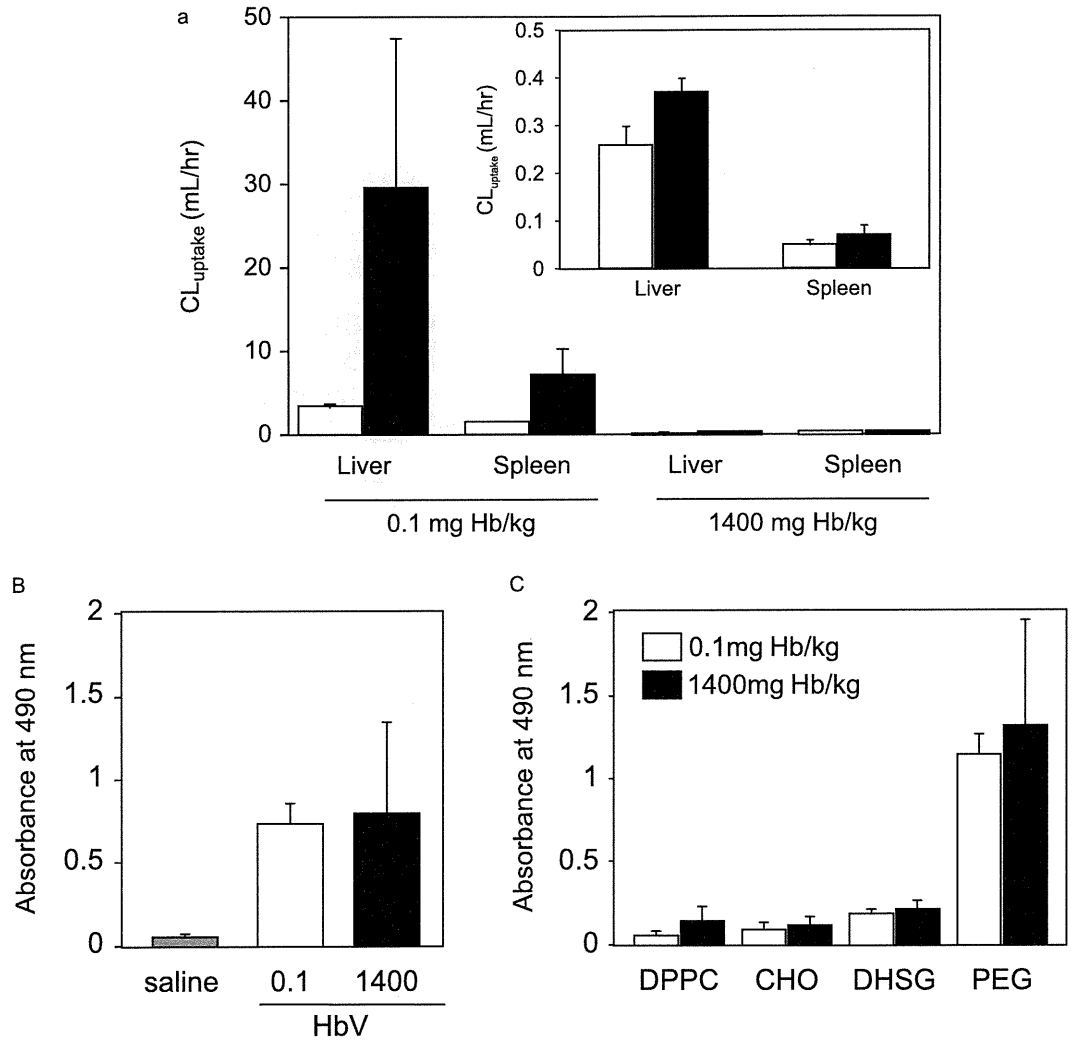

Figure 8. (**A**) Uptake clearance of HbV in the liver and spleen after 1 or 2 injections of ^{125}I-HbV. Uptake clearance for each organ was calculated by integration plot analysis at designated times from 1 to 30 minutes after injection. Each bar represents the mean ± SD (n=4). (**B**) Determination of IgM against HbV after a single intravenous injection of saline (gray bars), HbV at a dose of 0.1 mg Hb/kg (open bars), or 1,400 mg Hb/kg (closed bars) in mice. (C) Determination of the specific recognition site of IgM against HbV after a single intravenous injection of HbV at a dose of 0.1 mg Hb/kg (open bars) or 1,400 mg Hb/kg (closed bars) in mice. DdY mice were injected with saline or HbV (0.1 or 1,400 mg Hb/kg) containing 5% rHSA to the tail vein. At 7 days after an injection of saline or HbV, blood was collected from the inferior vena cava, and plasma was obtained. IgM against HbV and each lipid component were detected by ELISA. Each bar represents the mean ± SD (n=4).

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the ABC phenomenon (Ishida et al., 2008). At a low dose, the hepatic and splenic CL_{uptake} for the second injection was 8.5 and 4.5 times higher than that for the first injection, respectively (Figure 8A), whereas at a high dose, the hepatic and splenic CL_{uptake} for the second injection was little changed, compared to that for the first injection (Figure 8A, insert). In addition, Ishida et al. proposed a mechanism for the ABC phenomenon as follows: Immunoglobulin M (IgM), produced in the spleen by the first injection with PEGylated liposomes, selectively binds to the PEG on the second injected PEGylated liposome, and subsequent complement activation by IgM results in an accelerated clearance and enhanced hepatic uptake of the second injected PEGylated liposome (Ishida et al., 2006a, 2006b). Therefore, we examined whether IgM against HbV is elicited by an initial injection of saline or HbV at a low or high dose. At 7 days after the HbV injection, IgM against HbV appeared at both the low and the high dose (Figure 8B). Moreover, the specific recognition site of IgM against HbV strongly bound to DSPE-PEG, and other lipid components (DPPC, cholesterol, and DHSG) were negligible at both the low and high dose (Figure 8C). These results indicate that repeated injections of HbV to mice at a dose of 1,400 mg Hb/kg did not appear to induce the ABC phenomenon, even though the plasma levels of IgM against HbV are elevated. Therefore, these data suggest that a clinical dose of HbV is not likely to induce the ABC phenomenon due to the saturation of phagocytic processing by the MPS.

Pharmacokinetic properties of HbV after repeated administration in hemorrhagic shock model rats

Because there are limited data available for the ABC phenomenon under various disease conditions, we also investigated whether the ABC phenomenon would be induced in the rat model of hemorrhagic shock induced by a massive hemorrhage, when HbV is injected at a dose of 1,400 mg Hb/kg at hourly intervals, typical conditions for transfusions of patients with massive hemorrhage.

The plasma concentration of HbV was prolonged in the second injection, compared with the first injection, and it was recovered to that in normal rats (Figure 9A). As mentioned above, Ishida et al. reported that a dosing interval of approximately 5 days induced the ABC phenomenon in rats, accompanied by the production of antiliposome IgM, which elicits a response by the spleen (Ishida et al., 2006b; Wang et al., 2007). Therefore, the inhibition of anti-HbV IgM production by short intervals appears to prevent induction of the ABC phenomenon. In fact, anti-HbV IgM was detected at 5 days after the administration of HbV to normal rats at a dose of 0.1 mg Hb/kg, but was not detected at 1 hour after HbV administration to hemorrhagic shock rats at a dose of 1,400 mg Hb/kg (Figure 9B). Therefore, it appears that the repeated administration of HbV under conditions of hemorrhagic shock has negligible effect on the pharmacokinetics of HbV, when short dosing intervals are involved. However, our recent study showed that the repeated injection of HbV induced the ABC phenomenon in the case of a longer dosing interval (4 and 7 days) accompanied by the production of antiliposome IgM and increased phagocyte activity (Taguchi et al., 2011a). Therefore, in a clinical setting, it would be necessary to consider the dosing regimen and interval for patients with hemorrhagic shock in the base where a longer dosing interval was used.

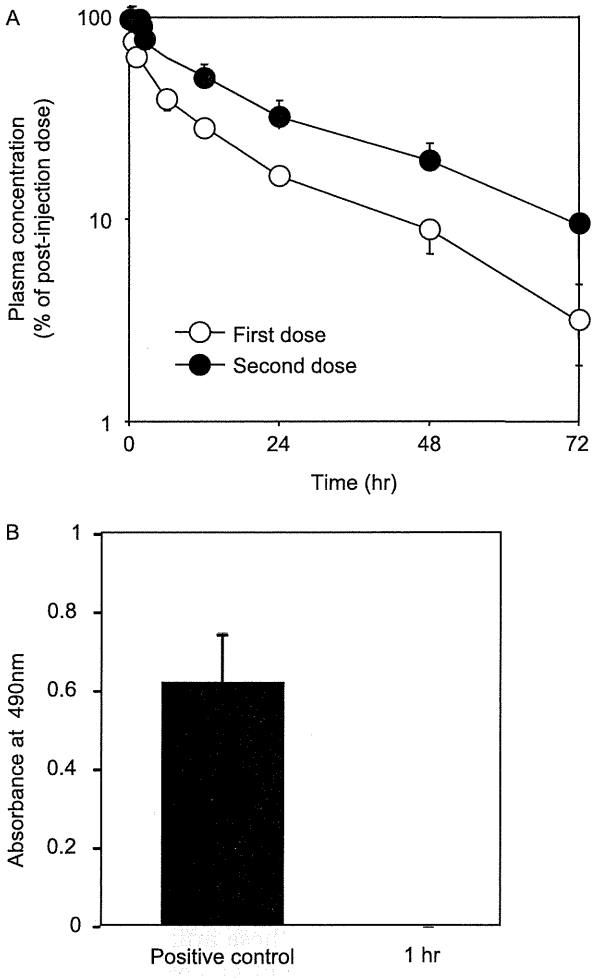

Figure 9. (**A**), Plasma concentration of ^{125}I-HbV as the percent of postinjection dose after the first (open symbol) or second dose (filled symbol) of ^{125}I-HbV to hemorrhagic shock rats at a dose of 1,400 mg Hb/kg for each injection. Each point represents the mean ± SD (n=5). Plasma concentration percentage profile for the first dose (○) was obtained from injection of a dose of ^{125}I-HbV administered after hemorrhagic shock. The profile for the second dose (•) was obtained from the injection of a dose of ^{125}I-HbV 1 hour after injection of the first dose of nonradiolabeled HbV administered after hemorrhagic shock. (**B**) Determination of IgM against HbV 5 days after a single intravenous injection of HbV to normal rats at a dose of 0.1 mg Hb/kg (closed bars) or 1 hour after a single intravenous injection of HbV to hemorrhagic shock rats at a dose of 1,400 mg Hb/kg (open bars) in mice. IgM against HbV were detected by ELISA. Each bar represents the mean ± SD (n=3–5).

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Extrapolation to human subjects

From the viewpoint of future clinical applications, predictions of human pharmacokinetics based on data obtained from animal studies—so called, "animal scale-up"—is important for the determination of optimal doses and intervals (Izumi et al., 1996). Thus, we attempted to predict the half-life of HbV in humans using an allometric equation that is generally used in animal scale-up studies. Using the relationships observed for mice (Taguchi et al., 2009b), rats (Taguchi et al., 2009a), and rabbits (Sou et al., 2005), the half-life of HbV in healthy humans was predicted to be approximately 96 hours. In addition, based on half-life data and percent of injected dose values obtained from pharmacokinetic studies of HbV in rats and rabbits, Sou et al. also predicted that the half-life of HbV in healthy humans would be approximately 72 hours (Sou et al., 2005). Further, the half-life of liposomal preparations is empirically 2–3-fold greater in humans than in rats (Gabizon et al., 2003). In fact, the half-life of liposomal doxorubicin (Doxil formulation) in rats and humans is 35 and 56–90 hours, respectively (Gabizon et al., 2003). Therefore, the half-life of HbV in humans would be predicted to be 3–4 days (Sou et al., 2005). For HbV to function as an artificial oxygen carrier, it is desirable that intravascular persistence be at least equal to the time required to regenerate RBCs (Sehgal et al., 1984). Following a massive hemorrhage, the lost blood volume and oxygen-carrying capacity is replaced within approximately 5 days (Hughes et al., 1995; Awasthi et al., 2007). Because the half-life of HbV in humans was estimated to be approximately 3–4 days, HbV would function as a temporary oxygen carrier until a blood transfusion is available or until autologous blood is recovered after a massive hemorrhage.

Conclusion

Like other drugs, a pharmacokinetic evaluation is an important issue for the development of HbV as a substitute of RBC. In fact, though the perfluorocarbon-based oxygen carriers and acellular-type HBOCs were moved into the clinical trial stages, these artificial oxygen carriers dropped from further clinical development due to severe and unexpected side effects, which might have been predicted from pharmacokinetic analysis data. Therefore, it is also necessary to conduct an in-depth pharmacokinetic study of HbV before moving on to the clinical trial stage.

Our recent preclinical study of HbV clearly demonstrated five major findings on pharmacokinetic profiles. First, HbV and its components have favorable metabolic and excretion profiles in mammalian species, similar to endogeneous substances. Second, HbV is safe and useful under conditions of a massive hemorrhage. Third, HbV did not show any toxicity and accumulation in the body, even under conditions of hypometabolism and excretion (i.e., hepatic cirrhosis). Fourth, HbV has the potential to induce the ABC phenomenon, but the repeated use of HbV at a putative dose would not be expected to induce the ABC phenomenon in a clinical situation. Finally, HbV has a good retention in the blood circulation, and the half-life of HbV in humans was estimated to be approximately 3–4 days, which is sufficient for it to function as an oxygen carrier. These findings support previous views related to the pharmacological efficacy and safety of HbV in normal and hemorrhagic shock model rats from the view point of pharmacokinetics.

In addition to functioning as a substitute for RBCs, HbV would be expected to have a variety of other applications, based on its oxygen transport characteristics, such as in cardiopulmonary bypass priming solutions (Yamazaki et al., 2006), wound healing in critically ischemic skin (Plock et al., 2009), and as a radiation therapy agent (Yamamoto et al., 2009). Therefore, this issue deserves to be studied further, with further data collected in preclinical pharmacokinetic studies for future applications of HbV in the clinic.

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Declaration of interest

The authors declare no financial conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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人工血小板

半田　誠

要　旨：人工血小板は，凍結乾燥させたヒト血小板由来産物と生体適合性に優れたアルブミンなどの微粒子表面にヒトフィブリノゲンもしくはそのペプチドを結合させた人工物がある。人工血小板は残存した血小板の機能を補助することで，血小板輸血に代わって血小板減少症の止血や出血予防を行う。すでに，いくつかの試験物が初期臨床試験に供されているが未だ実用化には至っていない。

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Key words: artificial platelet, activated platelet, fibrinogen, albumin particle, liposome

はじめに

血小板の量的・質的異常に起因した出血の予防や治療に使用される血小板濃厚液は，有効期限が短くしかも厳密な保存条件が必要なため，緊急時の輸血に対応することは極めて困難である。生きた細胞である血小板濃厚液の欠点を克服し，医療現場で常備できる人工血小板の開発が，主に軍事目的で，米国で始まってからすでに50年以上が経過した[1,2]。残念ながら，未だ実用化には至っていないが，いくつかの有望な試験物が報告され，一部は初期臨床試験に供され，将来の製剤化への取組みが継続されている(**Fig. 1**)[3]。血小板の止血機能と対比して，人工血小板の開発の現状を概説する。

人工血小板の種類

人工血小板(artificial platelets/ 広義)は二つに大別される(**Fig. 1**)。第一は血小板由来産物で，凍結乾燥処理した固定化ヒト血小板(fixed, lyophilized platelet, Stasix™)そのものや，あるいはすでに初期臨床研究に供された血小板膜断片(infusible platelet membrane; IPM, Cyplex™)がある。第二は狭義の人工血小板で，生体適合性のある担体(赤血球，アルブミン微粒子，リン脂質小胞体：リポソームなど)を用い，その表面に血小板受容体のリガンドを結合させたもの(リガンド結合微粒子)で，90年代初めにフィブリノゲンやその合成ペプチド(RGD)をコートした赤血球(thromboerythrocyte)が先駆けとなり，いくつかの微粒子が開発された[1]。まず最初に，ヒトフィブリノゲンを表面固定したアルブミン微粒子(Synthocyte™やFibrinoplate-S™)が初期臨床研究に供された。これらはフィブリノゲンやアルブミンなどの生物由来物質をそのまま使用した生物製剤(biological products)であり，それに続いて，いわば第二世代といえるすべてが人工物で構成された製剤(synthetic products)が開発されてきた。

人工血小板の機能

止血機構における血小板の機能は，止血部位への特異的な粘着，アデノシンニリン酸(ADP)などの刺激物質の放出と細胞の活性化反応，細胞同士の凝集反応による一次血栓の形成，活性化した細胞上での凝固反応の促進(凝固活性)に分けられる。しかし，血小板のすべての機能を代替することは不可能である。したがって，人工血小板は残存した血小板を介してこれらの機能を増強する。

1)血小板由来産物

期限切れとなったヒト血小板を破壊した後，加熱して凍結乾燥した血小板断片製剤(IPM)は止血局所を特異的に認識する血小板受容体GPIb/IXがインタクトに保存されていることに加えて，凝固活性を発現するフォスファチジルコリン等の陰性荷電リン脂質が豊富に表現されており，その血小板代替機能は主にフィブリン血栓の生成

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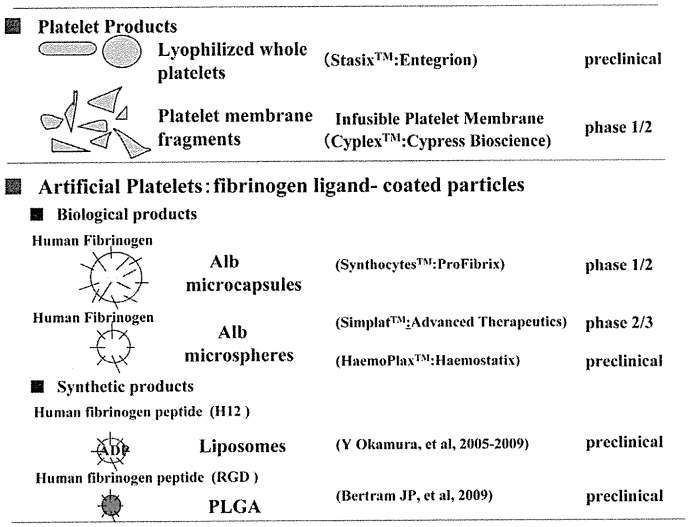

Figure 1 Artificial platelets.
Artificial platelets are classified into platelet products and artificial particles coated with fibrinogen or related peptides. The latter (artificial platelets by narrow definition) are produced using components derived from human or totally synthesized.

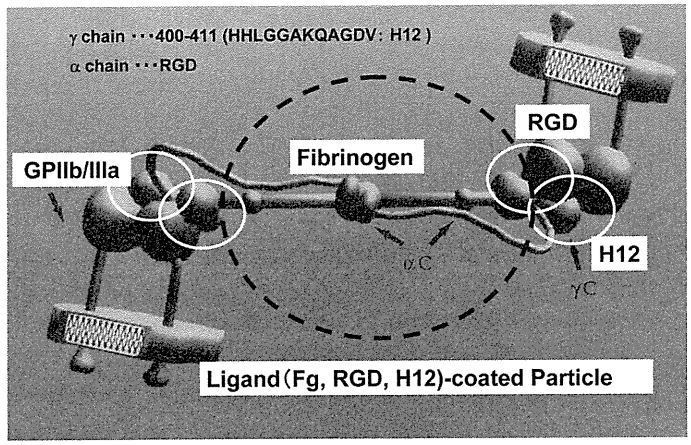

Figure 2 Platelet aggregation and artificial platelets.
Platelet aggregation is induced by bridging adjacent platelets with binding of plasma fibrinogen to activated GPIIb-IIIa complex on the cell surface.[5] The binding sites on the ligand for the receptor are localized at sequence of H12 or RGD. Fibrinogen or the synthetic peptide-coated particles reinforce platelet aggregation by receptor-ligand interactions being more multivalent than fibrinogen itself.

補助に起因するとされる[4]。一方，ヒト血小板をインタクトのままマイルドに固定化し，その後凍結乾燥して粉末状にし，使用時に再水溶化できる製剤(Stasix™)が開発されてきた[2]。本製剤は長い改良の歴史を持ち，特徴は止血局所集積性を体現するGPIb/IXとともにフィブリノゲンの受容体であるGPIIb/IIIaの機能も一部保持されており，IPMと類似の凝固活性を発揮することに加えて，血小板凝集を補助する作用を有すると報告されている。

2)人工血小板(狭義)

血小板凝集は，活性化した血小板膜GPIIb/IIIa複合体(受容体)への結合を介して，血漿中のフィブリノゲン(リガンド)が細胞同士の架橋となって引き起こされる(**Fig. 2**)[5]。フィブリノゲン分子のGPIIb/IIIa複合体との結合部位は，3カ所あり，そのうち2カ所はαサブユニット上のRGD配列に，後の1カ所はγサブユニットのカルボキシ末端を構成する12個のアミノ酸(^{400}HHLGGAKQAGDV^{411}：H12)に限定される。人工血小板は，フィブリノゲンもしくはその合成ペプチドを表面にコートさせた生体適合性を有する微粒子(アルブミン，リポソーム，乳酸・グリコール酸ポリマー)として，フィブリノゲンに代わり残存した血小板の凝集反応を増強するとされる。人工血小板は，その表面にリガンドを高密度に固相化することにより，血漿中のフィブリノゲンでは実現できない多点結合を介した血小板凝集を引き起こ

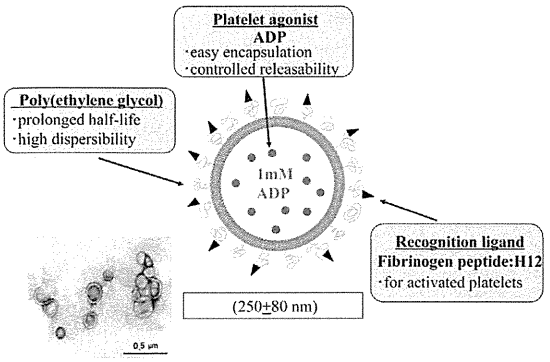

Figure 3　H12-(ADP) liposome.
H12-(ADP) liposome, one of the developing artificial platelets is surface-coated with polyethylene glycol to ensure its blood compatibility and dispersibility. H12 peptide is covalently conjugated to the tip of polyethylene glycol moiety as a target molecule of activated platelets. ADP, a physiologically important platelet agonist stored in platelet alpha granules is stably encapsulated into the inner space of the liposome.

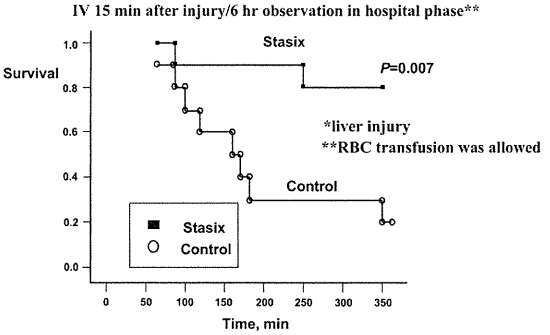

Figure 4　Evaluation of lyophilized fixed platelets (Stasix®) as an infusible hemostatic agent in experimental non-compressible hemorrhages* in swine.[8]
Using a non-compressible liver hemorrhage swine model, the effect of lyophilized fixed platelets (Stasix®) on survival was compared with normal saline. As a result, the survival rate in the Stasix group (80%) was significantly higher than that in the control (20%).

す。さらに，リポソーム製剤では，内水相に加えたADPの血小板凝集に伴う放出機能を付与させた(**Fig. 3**)。

人工血小板の開発状況

1)血小板由来産物

IPMは初期臨床試験に供され，1995年の米国でのフェーズ1臨床研究では，健常人22名への単回投与では急性毒性や免疫原性は認められず，8例の血小板減少患者(血小板数5万/mm³以下)のうち6例(75%)において，出血時間の有意な短縮効果が認められた[6]。1997年の出血症状を有する40例の血小板減少症患者を対象としたフェーズ2試験では，27例(65%)において症状の改善や止血効果等の有効性が示された[7]。しかしながら，その後，理由が明らかにされないまま開発は中止された。

一方，固定化凍結乾燥血小板であるStasixは，その強い凝固活性と極めて短い血中滞留性(半減期：約10分)を活かして，戦場や災害時の緊急避難的な止血剤としての利用が，米国国防省の援助のもと模索されている[8](**Fig. 4**)。

2)人工血小板/アルブミン微粒子

1995年に，平均径が1.2ミクロンのアルブミン・マイクロスフェア(Fibrinoplate-S™)が，1999年には，より大型の平均径3.5～4.5ミクロンのアルブミン・マイクロカプセル(Synthocyte™)が報告され，前者はフェーズIII，後者は少なくともフェーズIIまでの臨床試験が行われた。Fibrinoplate-Sは，63,214人の白血病や再生不良性貧血等の血小板減少患者(血小板数3万/mm³以下)への二重盲検比較対照試験で，出血時間の短縮効果が投与後24時間後でも有意に持続することが報告された(4th Asian Pacific Congress on Thrombosis & Haemostasis, 2006; Advanced Therapeutics社のホームページ)。一方，Synthocyteは，抗がん剤により惹起された血小板減少ウサギに投与することで耳介出血時間の短縮や腹部手術モデルでも術創からの出血量の減少効果が一定時間(3時間)持続し，止血局所への集積も形態的に証明され，有望な前臨床試験結果が公表された[9]。しかしながら，いずれもその後の経過の詳細は公表されておらず，未だ実用化に至っていない。さらに，第3の有望な人工物(HaemoPlax™)が開発されている。このHaemoPlax™は，アルブミン・マイクロスフェアの表面に，ヒトフィブリノゲンと高い親和性を有する合成ペプチドが固相化されたもので，血中に投与すると，その表面にフィブリノゲンが速やかに吸着されることで，上記の人工物と同様の血小板代替機能を発揮する。前臨床試験はすでに終了して，早期の臨床試験への移行を表明しているが，詳細は明らかでない(Haemostatix社ホームページ)。

3)人工血小板/リポソーム

フィブリノゲンのGPIIb/IIIaへの結合部位はそのγ鎖のカルボキシ末端を構成する12個のアミノ酸配列(^{400}HHLGGAKQAGDV^{411}：H12)である(**Fig. 2**)。そこ

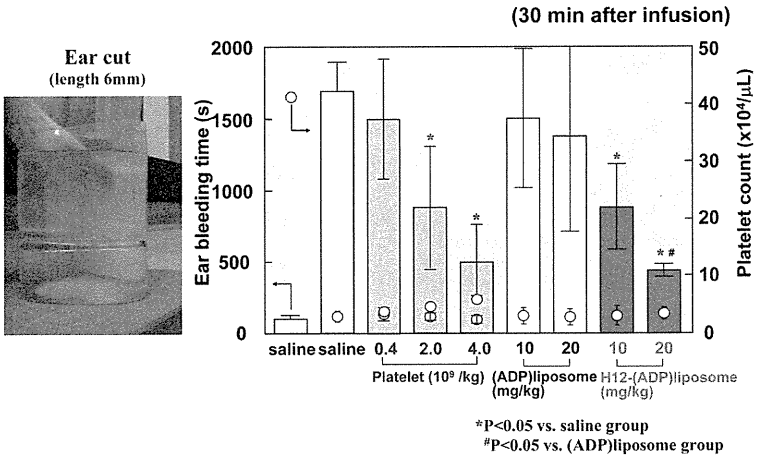

Figure 5 Comparison of hemostatic effects of H12-(ADP) liposomes with those of platelet-rich plasma in severely thrombocytopenic rabbits.[9]
The hemostatic effect of H12-(ADP) liposome was compared with that of platelet rich plasma in severely thrombocytopenic rabbit models induced by busulphan. Ear bleeding time of busulphan-induced thrombocytopenic rabbits was measured 30 min after infusion of platelet preparation or the liposome. Indeed, platelet transfusion effectively corrected the prolonged bleeding time in a dose-dependent fashion. Similar correcting effect was also observed with H12-(ADP)-liposomes in a dose-dependent fashion. The extent of correction by the liposome was just comparable to that obtained by platelet transfusion, indicating that H12-(ADP)-liposome exhibits in vivo hemostatic ability as efficiently as platelet transfusion.

で，表面結合リガンドとしてヒトフィブリノゲンの代わりにH12合成ペプチドを，担体として血液適合性に優れかつすでに臨床応用がなされているリポソームを使用した平均直径250ナノメータの(H12(ADP)リポソーム)が開発された。この微粒子の特徴は，止血作用を強化する目的でADPを内包化させたことである(**Fig. 3**)[10]。血小板凝集を増強するとともに，凝集依存性に内包化されたADPを放出することで，血小板に匹敵する止血効果(出血時間短縮効果)を発揮することが抗がん剤惹起ウサギ血小板減少症モデルで報告された(**Fig. 5**)。H12(ADP)リポソームの利点は，その表面をポリエチレングリコールで修飾することでその血中滞留時間を長く(平均6時間)できることである。したがって，血小板輸血を代替して出血の予防投与への適応が期待されている。

4)人工血小板/乳酸・グリコール酸ポリマー

H12(ADP)リポソームと同様に完全合成型の人工血小板である。フィブリノゲンのGPIIb/IIIaへの結合部位は，血小板に特異的なH12以外に，受容体ファミリーに非特異的に認識されるRGDがあるα鎖に局在する(**Fig. 2**)。この人工物は，生体吸収性に優れて多方面の医療材料に利用されている乳酸/グリコール酸共重合体(poly(lactic-co-glycolic acid)：PLGA)を担体として用い，その表面をPEGで修飾し，その先端にRGD配列を含んだペプチド(GRGDS)を結合させた，粒径およそ150ナノメータの微粒子である。本微粒子は，活性化した血小板にのみ結合して血小板の凝集を増強し，健常ラットの大腿動脈からの出血を陰性対照物に比して有意により短時間で止める機能がある(**Fig. 6**)[10]。しかし，投与量を多くすると血栓傾向が増強されるとされ，また血中半減期も短いことから，止血剤としての適応を目的としているようである。すくなくとも，緊急避難的に止血剤として欧米では標準的に使用されているリコンビナント活性化凝固第VII因子(Novoseven™)をはるかに凌駕する止血効果を示した。

人工血小板の課題

1)適応

人工血小板の適応は，内科的な出血予防を目的とした持続的な使用と外科的な止血を目的とした急性使用に分けられる。予防薬として必須の要件は，効果の持続性で

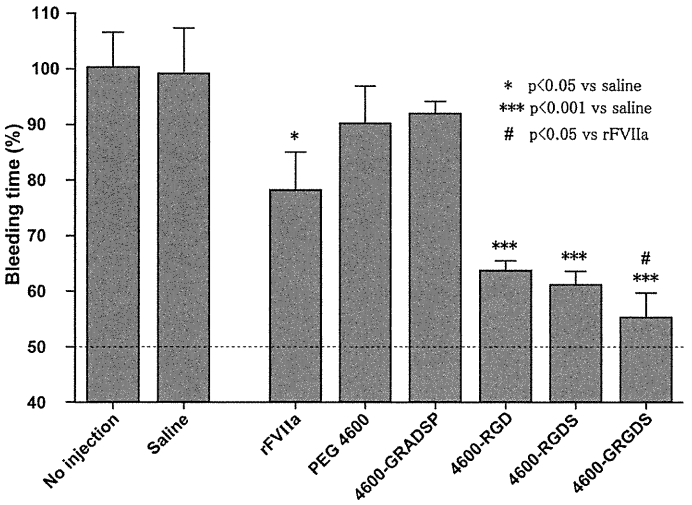

Figure 6　Effect of RGD-PLGA particles on bleeding time in rat femoral artery injury model.[10]
Using a rat femoral artery injury model, the effect of RGD peptide-coated PLGA particles (4600-RGD, 4600-RGDS and 4600-GRGDS) on bleeding time was evaluated by comparison with control treatments (Normal saline: Saline, recombinant coagulation factor VIIa: rFVIIa, RGD-uncoated particles; PEG 4600 and RGD reverse peptide-coated particles: 4600-GRADSP). The hemostatic ability of these RGD-PLGA particles was found to be significant as compared with saline control. In addition, the effect of RGD-PLGA particles was significantly higher than a clinically useful hemostaic agent rFVIIa.

ある。現在開発中の微粒子は血中半減期を延ばすためにその表面をポリエチレングリコールで処理している[9-11]。しかしながら，血小板に匹敵する体内寿命を獲得することは現在の技術では不可能であり，せいぜい一日2〜3回投与が限界である。さらに，生体の異物排除機構が働いて微粒子に対する抗体が惹起されることが危惧される。一方，緊急性の高い止血薬への適応は大いに有望である[8, 11]。血小板数の補正が生死を分ける状況下での緊急使用は意義の高いものである。そして，単回使用のため血中半減期は短くても構わないし，抗体の惹起は気にする必要がない。

2)副作用

人工血小板の働きは残存した血小板を介するものとそのものが血小板の代わりとなる場合がある。いずれの場合も，血栓症が最も危惧される副作用である。実際，Stasixを使用したブタモデルでの検討では心内膜や肺動脈の血栓が1例で観察されており，PLGA微粒子でも高用量の投与で血栓症と思われる心肺症状が高頻度に出現した[8, 11]。いずれの場合でも，血小板数の低下は明らかでなく，試験薬が血小板輸血の代替としての使用でないことを注意すべきである。あくまでも血小板減少症を対象とした人工血小板としての役割を評価すべきであろう。

おわりに

薬剤としての人工血小板は未だに実現されていない。長い歴史があるにもかかわらず，欧米を中心にその開発はベンチャー企業を主体に継続されているためデータがほとんど公表されていない。そのため，本稿では限られた情報に基づいた推測を多く取り混ぜた。実用化に向け，薬剤の開発目的を止血治療に限定するのか，あるいは適応範囲が広く採算性も高い出血予防に拡大するかが今後の鍵となろう。

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Artificial Platelets

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Key words: artificial platelet, activated platelet, fibrinogen, albumin particle, liposome

Artificial platelets are classified into lyophilized products derived from human platelets and fibrinogen or related peptide-coated microparticles made from blood-compatible materials such as albumin. The artificial platelets are substituted for platelets in transfusions for treatment or prevention of bleeding in patients with thrombocytopenia; they reinforce the hemostatic abilities of residual platelets. Although some of the products have been tested in early phase clinical trials, none of them have yet been approved for clinical use.

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