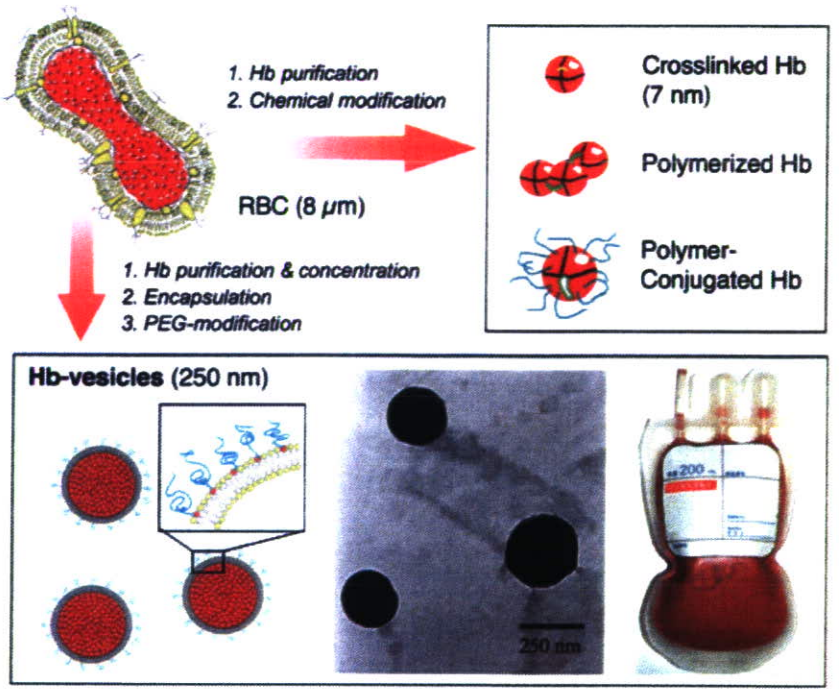

Figure 1. Schematic representation of a series of Hb-based oxygen carriers. In the case of Hb-vesicles (Hbv), the purified and concentrated Hb solution (35 g/dL) is encapsulated in phospholipid vesicles and the surface is modified using PEG chains. The particle size is well regulated at 250 nm, which is apparent from the transmission electron micrograph. The color of HbV is like a mixture of red wine and milk, due to the light scattering effect of small particles. Hb, hemoglobin; PEG, polyethyleneglycol; RBC, red blood cell.

cells, such as the concentration of phosphates [2,3-diphosphoglycerate (2,3-DPG), adenosine triphosphate, etc.] and other electrolytes; (v) RBCs are the major component that renders blood as non-Newtonian and viscous, which is necessary to pressurize the peripheral artery for homogeneous blood distribution and for maintenance of blood circulation[10]; and (vi) the cellular structure of RBCs retards oxygen release in comparison to acellular Hb solutions,[11,12] thereby retaining oxygen to peripheral tissues where oxygen is required.

For those reasons, the optimal structure of HBOCs might be to mimic the RBC cellular structure. The pioneering work of Hb encapsulation to mimic the cellular structure of RBCs was performed in 1957 by Chang,[13] who prepared microcapsules (5 μm) made of nylon, collodion and other materials. Toyoda[14] in 1965 and the Kambara-Kimoto group[15] in 1968 also investigated encapsulation of Hbs with gelatin, gum arabic, silicone, etc. Nevertheless, results emphasized the extreme difficulty in regulating the particle size to be appropriate for blood flow in the capillaries and to obtain sufficient biocompatibility. After Bangham and Horne[16] reported in 1964 that phospholipids assemble to form vesicles in aqueous media, and that they encapsulate water-soluble materials in their inner aqueous interior, it seemed reasonable to use such vesicles for Hb encapsulation. Djordjevich and Miller[17] in 1977 prepared liposome-encapsulated Hb (LEH) composed of phospholipids, cholesterol, fatty acids, etc. The US Naval Research Laboratories showed remarkable progress in the use of LEH.[18–20] Terumo Corporation (Tokyo) developed a different LEH called Neo Red Cells (Table 1).[21,22]

However, some intrinsic issues of encapsulated Hbs remained, which were mainly related to the nature of molecular assembly and particle dispersion. What we call HbV, with their high-efficiency production processes and improved properties, were established by our group based on technologies of molecular assembly in concert with precise analyses of pharmacological and physiological aspects (Table 2).[23–25] We use stable carbonylhemoglobin (HbCO) for purification with pasteurization at 60°C for 10 hours. The purity of the obtained Hb solution is extremely high.[26,27] Utilization of the stable and purified HbCO enables higher concentrations than 40 g/dL using ultrafiltration and easy handling of encapsulation by the extrusion method without causing protein denaturation. It has been confirmed that HbV encapsulates nearly 35 g/dL with a thin bilayer membrane. In the final process, HbCO in HbV is photodissociated by irradiation of visible light under an oxygen atmosphere to convert HbO_2.[28]

Table 1. A list of representative LEH extensively studied aiming at industrialization

Product Name	Group	Characteristics	Current status
Hb-vesicles (HbV)	Waseda University and Keio University	1. Pasteurization of HbCO at 60°C for virus inactivation, and high purity and concentration of encapsulated Hb 2. Lipid composition to improve blood compatibility 3. PEG modification and deoxygenation for 2 years storage 4. (Hb) = 10 g/dL	Preclinical
Neo Red Cells (NRC)	Terumo Corporation	1. Inositol hexaphosphate to regulate P_{50} (= 40–50 torr) 2. Lipids: HSPC/cholesterol/fatty acid/PEG-lipid 3. Storage in a refrigerator for 6 months 4. (Hb) = 6 g/dL	Preclinical
Artificial Red Cells (ARC)	NOF Corporation and Waseda University	1. Polymerized lipids (DODPC) for stabilization 2. Storage in powdered or frozen state 3. Difficulty in degradation in RES	Suspended
LEH	US Naval Research Laboratory	1. Freeze-dried powder with trehalose 2. Low Hb encapsulation efficiency 3. Thrombocytopenia, complement activation	Suspended
Synthetic erythrocytes	Rush-Presbyterian-St. Luke's Medical Center, University Illinois	1. The first attempt of LEH	Suspended

DODPC, 1,2-dioctadecadienoyl-*sn*-glycero-3-phosphatidylcholine; Hb, hemoglobin; HbCO, carbonylhemoglobin; HSPC, hydrogenated soy phosphatidylcholine; LEH, liposome-encapsulated Hbs.

Table 2. Characteristics of Hb-vesicles developed in Waseda University

Parameter	
Particle diameter	240–280 nm
P_{50}	25–28 torr
(Hb)	10 g/dL
Suspending medium	Physiologic saline solution (0.9% NaCl)
Colloid osmotic pressure	0 torr
Intracellular Hb concentration	ca. 35 g/dL
Lipid composition	DPPC/cholesterol/DHSG/DSPE-PEG_{5000}
Weight ratio of Hb to lipids	1.6–1.9 (w/w)
Stability for storage at room temperature	2 years
Circulation half-life	32 hours (rats)

DHSG, 1,5-*O*-dihexadecyl-N-succinyl-L-glutamate; DPPC, 1,2.dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphatidyethanolamine-*N*-polyethyleneglycol; Hb, hemoglobin.

The oxygen-bound HbV can provide oxygen-transport capacity that is both sufficient and comparable to that of RBCs in experiments related to extreme blood exchange[29-34] and fluid resuscitation from hemorrhagic shock.[35-38] A recent experiment of HbV as a priming solution for cardiopulmonary bypass in a rat model showed that HbV protects neurocognitive function by transporting oxygen to brain tissue even when the hematocrit is markedly reduced.[39] Other studies investigating HbV suspension as a possible perfusate for organ transplantation are also underway for the heart, liver, intestine, etc.

In fact, Hb encapsulation provides a unique opportunity to add new functions to particles. Other regulators, such as antioxidants and enzymes, can be embedded on the capsule or coencapsulated to reduce methemoglobin (metHb),[40-42] as can allosteric effectors to modulate oxygen affinity (P_{50}).[33,43] The P_{50} of HbV is regulated by coencapsulation of pyridoxal 5′-phosphate (PLP) in place of 2,3-DPG. The present HbV, being developed by Waseda University, contains PLP at PLP/Hb = 2.5 by mol; the resulting P_{50} is about 25–28 torr, which shows sufficient oxygen transporting capacity as a transfusion alternative. The P_{50} of HbV without PLP and Cl^- is 8–9 torr. This formulation is effective for targeted oxygen delivery to anoxic tissues caused by reduced blood flow.[34,44,45]

In addition to HbV, new encapsulated Hbs without liposomes have emerged with the use of recent advanced nanotechnologies, such as polymersome,[46] polyethyleneglycol (PEG)-poly(ε-caprolactone) copolymer nanoparticles,[47] and *in vivo* evaluation of oxygen-carrying capacities of these new materials is anticipated. Encapsulation of Hb can reduce the toxicity of cell-free Hbs. However, many hurdles must be surmounted to realize encapsulated Hbs because of the components of the capsules themselves and their structural complexity as a molecular assembly. It is also important to consider the larger dosage requirement of encapsulated Hb for blood substitution in comparison with those available with conventional drug delivery systems, which require no large dosage.

STRUCTURAL STABILIZATION OF ENCAPSULATED HEMOGLOBIN FOR STOCKPILING

Hb autoxidizes to form metHb and loses its oxygen-binding ability during storage, as well as during blood circulation. Therefore, prevention of metHb formation is necessary. A method exists to preserve deoxygenated Hbs in a liquid state using well-known intrinsic characteristics of Hb: the Hb oxidation rate in a solution is dependent on the oxygen partial pressure; also, deoxyHb is not autoxidized at ambient temperatures.[48] In the case of HbV, not only the encapsulated Hb but also the capsular structure (liposome) must be physically stabilized to prevent irreversible intervesicular aggregation, fusion and leakage of the encapsulated Hb.

Liposomes, as molecular assemblies, have been generally inferred to be structurally unstable. The US Naval Research Laboratory tested the addition of cryoprotectants and lyoprotectants, such as trehalose, to LEH for its preservation as a powder without causing hemolysis after rehydration.[49,50] In addition, many researchers have developed stabilization methods for liposomes that use polymer chains.[51-54] Polymerization of phospholipids that contain two dienoyl groups (DODPC) was studied extensively in our group. For example, gamma-ray irradiation induces radiolysis of water molecules and generates OH radicals that initiate intermolecular polymerization of dienoyl groups in DODPC. This method produces enormously stable liposomes, resembling rubber balls, which are resistant to freeze-thawing, freeze-drying and rehydration.[55,56] However, the polymerized liposomes were so stable that they were not degraded easily in the macrophages, even 30 days after injection.[57] It became widely believed that polymerized lipids are inappropriate for intravenous injection. Subsequently, it was clarified that the selection of appropriate lipids (phospholipid/cholesterol/negatively charged lipid/PEG-lipid) and their composition are important to enhance the stability of nonpolymerized liposomes.[31,58] Surface modification of liposomes with PEG chains is sufficient for dispersion stability.[32] In fact, in comparison to RBCs, HbV is highly resistant to hypotonic shock, freeze-thawing and enzymatic attack by phospholipase A_2.

We investigated the possibility of long-term preservation of HbV during storage for 2 years through a combination of two techniques: deoxygenation and PEG modification.[59] The PEG chains on the vesicular surface stabilize the dispersion state and prevent aggregation and fusion for 2 years because of their steric hindrance.[60] The original metHb content (ca. 3%) before preservation decreased gradually to less than 1% in all samples after 1 month because of the presence of a

reductant, such as homocysteine, inside the vesicles that consumed the residual oxygen and gradually reduced the trace amount of metHb. The rate of metHb formation was strongly dependent on the oxygen partial pressure: a lack of increase in the metHb formation was observed because of the intrinsic stability of the deoxygenated Hb. In fact, the metHb content did not increase for 2 years. These results indicate the possibility that the HbV suspension can be stored at room temperature for at least 2 years, which would enable stockpiling of HbV for any emergency.

BLOOD COMPATIBILITY OF LIPOSOMES AND HEMOGLOBIN VESICLES

Liposome is not a solute but a particle in a suspension. The surface of the particle may be recognized, interact with blood components, including complements. The so-called *injection reaction*, or pseudoallergy, is caused by complement activation, giving rise to anaphylatoxins that trigger various hypersensitivity reactions. This reaction is sometimes observed not only with liposomal products,[61] but also with fat emulsions[62] and a perfluorocarbon emulsion.[63] Therefore, the examination of blood compatibility of encapsulated Hbs is important for clinical use. Transient thrombocytopenia and pulmonary hypertension in relation to complement activation is an extremely important hematologic effect observed in rodent models after infusion of LEH (containing DPPG: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl glycerol) developed by the US Naval Research Laboratory[64,65] and of other products. In our group, exchange transfusion with the proto-type HbV (containing DPPG, no PEG modification) in anesthetized rats engendered transient thrombocytopenia and slight hypertension.[30] Similar effects were also observed for administration of negatively charged liposomes.[66,67] The transient reduction in platelet counts caused by complement-bound liposomes was also associated with sequestration of platelets in the lung and liver. Such nonphysiological platelet activation probably leads to initiation and modulation of inflammatory responses as platelets contain an array of potent proinflammatory substances. However, it must be emphasized that the present HbV formulation apparently does not induce thrombocytopenia in animal experiments, probably because the present HbV contains PEG-modification and a different type of negatively charged lipid (DHSG: 1,5-*O*-dihexadecyl-*N*-succinyl-L-glutamate), not DPPG or a fatty acid.[68-70]

Detailed blood compatibility of HbV in relation to negatively charged lipid was examined by Dr H. Ikeda at the Hokkaido Red Cross Blood Center (Sapporo) and his colleagues.[69-72] The present PEG-modified HbV containing DHSG did not affect the extrinsic or intrinsic coagulation activities of human plasma, although HbV containing DPPG and no PEG modification tended to shorten the intrinsic coagulation time. The kallikrein-kinin cascade of the plasma was activated slightly by the proto-type DPPG-HbV, but not by the present PEG-DHSG-HbV. Moreover, the complement consumption in the plasma was detected by incubation with DPPG-HbV, but not with the present PEG-DHSG-HbV.[71] The exposure of human platelets to high concentrations of the present HbV (up to 40%) *in vitro* did not cause platelet activation and did not adversely affect the formation and secretion of prothrombotic substances or proinflammatory substances that are triggered by platelet agonists. These results imply that HbV, at concentrations of up to 40%, has no aberrant interactions with either unstimulated or agonist-induced platelets. It can be concluded that the present PEG-DHSG-HbV has a higher blood compatibility.

BIODISTRIBUTION, METABOLISM AND EXCRETION OF HEMOGLOBIN VESICLES

The dosage of blood substitutes should be considerably larger than those of other drugs, while their circulation time is considerably shorter than that of RBCs. Therefore, their biodistribution, metabolism, excretion and side effects must be characterized in detail, especially in relation to the reticuloendothelial system, RES (or termed the mononuclear phagocytic system).

Normally, free Hb released from RBC is bound rapidly to haptoglobin and is consequently removed from circulation by hepatocytes. However, when the Hb concentration is greater than the haptoglobin binding capacity, unbound Hb is filtered through the kidney, where it is actively absorbed. Hemoglobinuria and eventual renal failure occur when the reabsorption capacity of the kidney is exceeded. The encapsulation of Hb in vesicles completely suppresses renal excretion. However, HbV in the bloodstream is ultimately captured by phagocytes in the RES in much the same manner as senescent RBCs are, as confirmed by radioisotope ^{99m}Tc-labelled HbV

injection.[19,68] The HbV are finally distributed mainly in the liver, spleen and bone marrow. The circulation half-life is dose-dependent; when the dose rate was 14 mL/kg, the circulation half-life was 32 hours in rats. The circulation time in the case of the human body can be estimated as two or three times longer; or about 2 or 3 days at the same dose rate.

It is generally accepted that the liposome clearance by RES at a small dosage is accelerated by opsonization (absorption of plasma proteins such as antibodies and complements on the liposomal surface); PEG-modification prevents opsonization for prolonged circulation times.[73] However, considering the condition that the dosage of HbV is extremely high and requires a considerable amount of opsonins, and that HbV does not induce complement activation,[71] then the opsonin-dependent phagocytosis would not be a major component in the case of HbV with a large dosage. Actually, opsonin-independent phagocytosis, a direct recognition by macrophages, has been clarified in some studies.[74,75]

Transmission electron microscopic analysis of the spleen 1 day after infusion of HbV revealed the presence of HbV particles in the phagosomes of macrophages.[76] However, after 7 days, the HbV structure cannot be observed. We confirmed transient splenomegaly with no irreversible damage to the organs and complete metabolism within a week. Immunochemical staining with a polyclonal antihuman Hb antibody was used as the marker of Hb in the HbV, clarifying that HbV almost disappeared after 7 days in both the spleen and liver.

Bilirubin and iron are believed to be released during metabolism of Hb, but our animal experiments of topload infusion, daily repeated infusions and 40% blood exchange showed that neither of those products increased in the plasma within 14 days.[77–79] The released heme from Hb in HbV might be metabolized by the inducible form of heme oxygenase-1 in the Kupffer cells of the liver and the spleen macrophages. Bilirubin would normally be excreted in the bile as a normal pathway; no obstruction or stasis of the bile is expected to occur in the biliary tree. Berlin blue staining revealed considerable deposition of hemosiderin in the liver and spleen, even after 14 days. Hemosiderosis often occurs in patients who have received repeated blood transfusions because of the shorter half-life of the stored RBCs. Moderate splenomegaly and hemosiderin deposition were also confirmed in the spleen after injection of stored RBCs, partly because of the accumulation and degradation of stored RBCs with lowered membrane deformability and shortened circulation half-life.[79]

As for membrane components of Hb-vesicles, phospholipids are metabolized and reused as a component of the cell membrane, or excreted in bile, especially as fatty acids and CO_2 in exhaled air. The plasma cholesterol level elevated transiently 3 days after injection, that was released from macrophages after degradation of HbV in the phagosomes.[77,79] However, the plasma phospholipid level did not increase significantly. It was recently clarified using ^{3}H-cholesterol that the cholesterol of HbV is released from macrophages to blood; it is ultimately excreted in the feces. The PEG chain is widely used for surface modification of liposomal products. The chemical crosslinker of PEG-lipid is susceptible to hydrolysis to release PEG chains during metabolism. The released PEG chains, which are known as inert macromolecules, should be excreted in urine through the kidneys.[80]

In order to know the physiological capacity of RES for degradation of HbV, we tested massive intravenous doses by daily repeated infusion of 10 mL/kg/day into Wistar rats for 14 days. The cumulative dosage was 140 mL/kg (Hb and lipids, 20,689 mg/kg). The total volume was equal to 2.5 times of whole blood volume (56 mL/kg).[78] Even though the splenohepatomegaly was significant, all rats tolerated the infusions, and the body weight increased until the intentional sacrifice for the succeeding 14 days. The phagocyted HbV disappeared though significant hemosiderin deposition and was confirmed in the spleen, liver, kidney, adrenal grand and bone marrow. We could not define a lethal dose of HbV in this experiment.

The profile of liposome clearance is species-dependent. More precise data are necessary to extrapolate the phenomena observed in animal experiments to humans. However, these results imply that the metabolism and excretion of HbV are within the physiological capacity that has been well characterized for the metabolism of senescent RBCs and conventional liposomal products.

UNIQUE RHEOLOGICAL PROPERTY OF HEMOGLOBIN VESICLES SUSPENSION

The extremely high concentration of the HbV suspension [(Hb) = 10 g/dL; (lipids) = 6 g/dL, volume fraction, ca. 40 vol%] imparts an oxygen-carrying capacity that is comparable to that of blood. The HbV suspension does

not possess a colloid osmotic pressure (COP) because one HbV particle (ca. 250 nm diameter) contains about 30,000 Hb molecules, and HbV acts as a particle, not as a solute. Therefore, HbV must be suspended in or coinjected with an aqueous solution of a plasma substitute. This requirement is identical to that for emulsified perfluorocarbon, which does not possess COP;[81,82] it contrasts to characteristics of other HBOCs, intramolecular crosslinked Hbs, polymerized Hbs and polymer conjugated Hbs, which all possess very high COP as protein solutions.[8,83]

Animal tests of HbV suspended in plasma-derived human serum albumin (HSA) or recombinant HSA (rHSA) showed an oxygen-transporting capacity that is comparable to that of blood.[36,39] We reported previously that HbV suspended in plasma-derived HSA or rHSA was almost Newtonian: no aggregation was detected microscopically.[31,32] In Japan, rHSA will be approved for clinical use in 2007,[84] but various plasma substitutes are used worldwide, such as hydroxylethyl starch (HES), dextran (DEX), and modified fluid gelatin (MFG). The selection among these plasma substitutes should be determined not only according to their safety and efficacy, but also by the related price, experience of clinicians and customs of respective countries. Water-soluble polymers generally interact with particles such as polystyrene beads, liposomes and RBCs to induce aggregation or flocculation.[85,86] For that reason, it is important to determine the compatibility of HbV with these plasma substitutes. With that background, we studied rheological properties of HbV suspended in these plasma substitute solutions using a complex rheometer and a microchannel array.[87] The rheological property of an HBOC is important because the infusion amount should be considerably large, which might affect the blood viscosity and hemodynamics.

The HbV suspended in rHSA was nearly Newtonian. Its viscosity was similar to that of blood, and the mixtures with RBCs at various mixing ratios showed viscosities of 3–4 cP. Other polymers, HES, DEX and MFG, induced flocculation of HbV, possibly by depletion interaction, and rendered the suspensions as non-Newtonian with the *shear-thinning* profile.[87] These HbV suspensions showed a high viscosity and a high storage modulus (G') because of the presence of flocculated HbV. On the other hand, HbV suspended in rHSA exhibited a very low G'. The viscosities of HbV suspended in DEX, MFG and high molecular weight HES solutions responded quickly to rapid step changes of shear rates of 0.1–100 s^{-1} and a return to 0.1 s^{-1}, indicating that flocculation formation is both rapid and reversible. Microscopically, the flow pattern of the flocculated HbV perfused through microchannels (4.5 μm deep, 7 μm wide, 20 cmH_2O applied pressure) showed no plugging. Furthermore, the time required for passage was simply proportional to the viscosity.

It has been regarded that lower blood viscosity after hemodilution is effective for tissue perfusion. However, microcirculatory observation shows that, in some cases, lower viscosity decreases shear stress on the vascular wall, causing vasoconstriction and reduced functional capillary density.[88] Therefore, an appropriate viscosity might exist, which maintains the normal tissue perfusion level. The large molecular dimension of HbV result in a transfusion fluid with high viscosity. A large molecular dimension is also effective to reduce vascular permeability and to minimize the reaction with NO and CO as vasorelaxation factors. These new concepts suggest reconsideration of the design of artificial oxygen carriers.[89] Actually, new products are appearing, although they are in the preclinical stage, not only HbV, but also zero-link polymerized Hb[90] and others with larger molecular dimensions and higher oxygen affinities.[91] Erni *et al.* clarified that HbV with a high O_2 affinity (low P_{50}, such as 8–15 torr) and high viscosity (such as 11 cP) suspended in a high-molecular-weight HES solution was effective for oxygenation of an ischemic skin flap.[45,92,93] That study showed that HbV would retain O_2 in the upper arterioles, then perfuse through collateral arteries and deliver oxygen to the targeted ischemic tissues, a concept of targeted oxygen delivery by an HBOC.[44] Some plasma substitutes cause flocculation of HbV and hyperviscosity. However, reports show that hyperviscosity would not necessarily be deteriorative and might be, in some cases, advantageous in the body.[10] The combination of HbV and plasma substitute solutions provides a unique opportunity to manipulate the suspension rheology, not only as a transfusion alternative, but also for other clinical applications, such as oxygenation of ischemic tissues and *ex vivo* perfusion systems.

CONCLUSION

Other related issues for HbV in a clinical situation include the interference effect of HbV on spectrophotometric measurements in routine clinical laboratory tests

and noninvasive pulse oximetry monitoring of arterial blood oxygen saturation. Such interference is caused by strong light scattering resulting from the small HbV particles in blood.[94] We clarified that HbV can be removed easily from a blood specimen by the addition of high molecular weight dextran and centrifugation. Pulse oximetry can be improved by some modifications of the detection wavelength and software.

Encapsulation of Hb was initiated with the simple idea of duplicating the structure and function of RBCs. However, we are convinced that obstacles remain for the approach to realize the sophisticated function of RBCs; for example, it is impossible to mimic the flexibility of the unfilled biconcave structure of RBCs. The present HbV lacks ionophores in the bilayer membrane which facilitate the transport of small functional molecules from the outer medium, such as ascorbic acid or glutathione, to reduce metHb in HbV that does not contain enzymatic metHb reducing system, because the unstable enzymes are removed during the virus inactivation process of Hb purification.[26,27] On the other hand, clear advantages of simplified HBOCs exist, such as the absence of blood-type antigens and infectious viruses, along with stability for a long-term storage at room temperature for any emergency, all of which might overwhelm the functions of RBCs. The shorter half-life of the HBOCs in the bloodstream (2–3 days) limits their use, but they are applicable as a transfusion alternative for shorter periods of use. Easy manipulation of physicochemical properties of HbV such as P_{50} and viscosity supports the possible development of tailor-made oxygen carriers that suit various clinical indications. The achievements of ongoing HbV research described above give us confidence in advancing further development of HbV, with the expectation of its eventual realization.

ACKNOWLEDGMENTS

The authors acknowledge Professor Marcos Intaglietta (University of California, San Diego) for cooperation in this work and inviting us to participate in this special issue of *Transfusion Alternatives in Transfusion Medicine*. The authors acknowledge Professor Koichi Kobayashi (Keio Univ.), Professor S. Takeoka (Waseda Univ.), Dr H. Ikeda (Hokkaido Red Cross Blood Center), Dr M. Takaori (Higashitakarazuka Satoh Hospital), Professor D. Erni (Inselspital Hospital, University of Berne), Professor W.T. Phillips (University of Texas, San Antonio), Professor M. Otagiri (Kumamoto University), and their active colleagues for meaningful discussions and contributions to this research.

DISCLOSURE

This work was partly supported by Health Sciences Research Grants (Research on Regulatory Science) from the Ministry of Health, Labour and Welfare, Japan. The authors are the holders of patents related to the production and utilization of HbV.

REFERENCES

1 de Figueiredo LF, Mathru M, Solanki D, Macdonald VW, Hess J, Kramer GC. Pulmonary hypertension and systemic vasoconstriction may offset the benefits of acellular hemoglobin blood substitutes. *J Trauma* 1997; 42: 847–54.

2 Tsai AG, Kerger H, Intaglietta M. Microcirculatory consequences of blood substitution with αα-hemoglobin. In: Winslow RM, Vandegriff K, Intaglietta M (eds). *Blood Substitutes: Physiological Basis of Efficacy*, Birkhauser: Boston, MA, 1995, pp. 155–74.

3 Goda N, Suzuki K, Naito M, *et al.* Distribution of heme oxygenase isoforms in rat liver. Topographic basis for carbon monoxide-mediated microvascular relaxation. *J Clin Invest* 1998; 101: 604–12.

4 Sakai H, Hara H, Yuasa M, *et al.* Molecular dimensions of Hb-based O_2 carriers determine constriction of resistance arteries and hypertension. *Am J Physiol Heart Circ Physiol* 2000; 279: H908–15.

5 Murray JA, Ledlow A, Launspach J, Evans D, Loveday M, Conklin JL. The effects of recombinant human hemoglobin on esophageal motor function in humans. *Gastroenterology* 1995; 109: 1241–8.

6 Burhop K, Gordon D, Estep T. Review of hemoglobin-induced myocardial lesions. *Artif Cells Blood Substit Immobil Biotechnol* 2004; 32: 353–74.

7 Neragi-Miandoab S, Vlahakes GJ. Elevated troponin I level with hemoglobin based oxygen carrying solutions (HBOCs) as a priming solution despite improved left ventricular function. *Interact Cardiovasc Thorac Surg* 2006; 5: 135–8.

8 Sakai H, Yuasa M, Onuma H, Takeoka S, Tsuchida E. Synthesis and physicochemical characterization of a series of hemoglobin-based oxygen carriers: objective comparison between cellular and acellular types. *Bioconjugate Chem* 2000; 11: 56–64.

9 Balla J, Jacob HS, Balla G, Nath K, Eaton JW, Vercellotti GM. Endothelial-cell heme uptake from heme proteins: induction of sensitization and desensitization to oxidant damage. *Proc Natl Acad Sci U S A* 1993; 90: 9285–9.

10 Martini J, Cabrales P, Tsai AG, Intaglietta M. Mechanotransduction and the homeostatic significance of maintaining blood viscosity in hypotension, hypertension and haemorrhage. *J Intern Med* 2006; 259: 364–72.

11 Sakai H, Suzuki Y, Kinoshita M, Takeoka S, Maeda N, Tsuchdia E. O_2 release from Hb vesicles evaluated using an artificial, narrow O_2-permaeble tube: comparison with RBCs and acellular Hbs. *Am J Physiol Heart Circ Physiol* 2003; 285: H2543–55.

12 Vandegriff KD, Olson JS. The kinetics of O_2 release by human red blood cells in the presence of external sodium dithionite. *J Biol Chem* 1984; 259: 12609–18.

13 Chang TMS. Therapeutic applications of polymeric artificial cells. *Nature Rev Drug Discov* 2005; 4: 221–35.

14 Toyoda T. Artificial blood. *Kagaku* 1965; 35: 7–13. (in Japanese)

15 Kimoto S, Hori M, Toyoda T, Sekiguchi W. Artificial red cells. *Gekachiryou (Surgical Therapy)* 1968; 19: 324–32. (in Japanese)

16 Bangham AD, Horne RW. Negative staining of phospholipids and their structure modification by surface-active agents as observed in the electron microscope. *J Mol Biol* 1964; 8: 660–8.

17 Djordjevich L, Miller IF. Lipid encapsulated hemoglobin as a synthetic erythrocyte. *Fed Proc* 1977; 36: 567.

18 Farmer MC, Rudolph AS, Vandegriff KD, Hayre MD, Bayne SA, Johnson SA. Liposome-encapsulated hemoglobin: oxygen binding properties and respiratory function. *Biomater Artif Cells Artif Organs* 1988; 16: 289–99.

19 Rudolph AS, Klipper RW, Goins B, Phillips WT. In vivo biodistribution of a radiolabeled blood substitute: ^{99m}Tc-labeled liposome-encapsulated hemoglobin in an anesthetized rabbit. *Proc Natl Acad Sci USA* 1991; 88: 10976–80.

20 Phillips WT, Klipper RW, Awasthi VD, *et al.* Polyethylene glycol-modified liposome-encapsulated hemoglobin: a long circulating red cell substitute. *J Pharmacol Exp Ther* 1999; 288: 665–70.

21 Usuba A, Osuka F, Kimura T, *et al.* Effect of liposome-encapsulated hemoglobin, neo red cells, on hemorrhagic shock. *Surg Today* 1998; 28: 1027–35.

22 Matsumoto T, Asano T, Mano K, *et al.* Regional myocardial perfusion under exchange transfusion with liposomal hemoglobin: in vivo and in vitro studies using rat hearts. *Am J Physiol Heart Circ Physiol* 2005; 288: H1909–14.

23 Sakai H, Hamada K, Takeoka S, Nishide H, Tsuchida E. Physical properties of hemoglobin vesicles as red cell substitutes. *Biotechnol Progress* 1996; 12: 119–25.

24 Takeoka S, Ohgushi T, Terase K, Ohmori T, Tsuchida E. Layer-controlled hemoglobin vesicles by interaction of hemoglobin with a phospholipid assembly. *Langmuir* 1996; 12: 1755–9.

25 Sou K, Naito Y, Endo T, Takeoka S, Tsuchida E. Effective encapsulation of proteins into size-controlled phospholipid vesicles using freeze-thawing and extrusion. *Biotechnol Progress* 2003; 19: 1547–52.

26 Sakai H, Takeoka S, Yokohama H, Seino Y, Nishide H, Tsuchida E. Purification of concentrated Hb using organic solvent and heat treatment. *Protein Expression Purif* 1993; 4: 563–9.

27 Abe H, Ikebuchi K, Hirayama J, *et al.* Virus inactivation in hemoglobin solution by heat treatment. *Artif Cells Blood Substit Immobil Biotechnol* 2001; 29: 381–8.

28 Chung J, Hamada K, Sakai H, *et al.* Ligand-exchange reaction of carbonylhemoglobin to oxyhemoglobin in a hemoglobin liquid membrane. *Nippon Kagaku Kaishi* 1995; 2: 123–7.

29 Izumi Y, Sakai H, Hamada K, *et al.* Physiologic responses to exchange transfusion with hemoglobin-vesicles as an artificial oxygen carrier in anesthetized rats: changes in mean arterial pressure and renal cortical oxygen tension. *Crit Care Med* 1996; 24: 1869–73.

30 Izumi Y, Sakai H, Takeoka S, *et al.* Evaluation of the capabilities of a hemoglobin vesicle as an artificial oxygen carrier in a rat exchange transfusion model. *ASAIO J* 1997; 43: 289–97.

31 Sakai H, Takeoka S, Park SI, *et al.* Surface-modification of hemoglobin vesicles with poly(ethylene glycol) and effects on aggregation, viscosity, and blood flow during 90%-exchange transfusion in anesthetized rats. *Bioconjugate Chem* 1997; 8: 23–30.

32 Sakai H, Tsai AG, Kerger H, *et al.* Subcutaneous microvascular responses to hemodilution with red cell substitutes consisting of polyethylene glycol-modified vesicles encapsulating hemoglobin. *J Biomed Mater Res* 1998; 40: 66–78.

33 Sakai H, Tsai AG, Rohlfs RJ, *et al.* Microvascular responses to hemodilution with Hb-vesicles as red cell substitutes: influences of O_2 affinity. *Am J Physiol Heart Circ Physiol* 1999; 276: H553–62.

34 Cabrales P, Sakai H, Tsai AG, Takeoka S, Tsuchida E, Intaglietta M. Oxygen transport by low and normal oxygen affinity hemoglobin vesicles in extreme hemodilution. *Am J Physiol Heart Circ Physiol* 2005; 288: H1885–92.

35 Sakai H, Takeoka S, Wettstein R, Tsai AG, Intaglietta M, Tsuchida E. Systemic and microvascular responses to the hemorrhagic shock and resuscitation with Hb-vesicles. *Am J Physiol Heart Circ Physiol* 2002; 283: H1191–9.

36 Sakai H, Horinouchi H, Masada Y, *et al.* Hemoglobin-vesicles suspended in recombinant human serum albumin for resuscitation from hemorrhagic shock in anesthetized rats. *Crit Care Med* 2004; 32: 539–45.

37 Yoshizu A, Izumi Y, Park SI, *et al.* Hemorrhagic shock resuscitation with an artificial oxygen carrier hemoglobin-vesicle (HbV) maintains intestinal perfusion and suppresses the increase in plasma necrosis factor alpha (TNFα). *ASAIO J* 2004; 50: 458–63.

38 Terajima K, Tsueshita T, Sakamoto A, Ogawa R. Fluid resuscitation with hemoglobin vesicles in a rabbit model of acute hemorrhagic shock. *Shock* 2006; 25: 184–9.

39 Yamazaki M, Aeba R, Yozu R, Kobayashi K. Use of hemoglobin vesicles during cardiopulmonary bypass priming prevents neurocognitive decline in rats. *Circulation* 2006; 114(Suppl. 1): I220–5.

40 Atoji T, Aihara M, Sakai H, Tsuchida E, Takeoka S. Hemoglobin vesicles containing methemoglobin and L-tyrosine to suppress methemoglobin formation in vitro and in vivo. *Bioconjug Chem* 2006; 17: 1241–5.

41 Teramura Y, Kanazawa H, Sakai H, Takeoka S, Tsuchida E. Prolonged oxygen-carrying ability of hemoglobin vesicles by coencapsulation of catalase in vivo. *Bioconjug Chem* 2003; 14: 1171–6.

42 Ohki N, Kimura T, Ogata Y. The reduction of methemoglobin in Neo Red Cell. *Artif Cells Blood Substit Immobil Biotechnol* 1998; 26: 477–85.

43 Wang L, Morizawa K, Tokuyama S, Satoh T, Tsuchida E. Modulation of oxygen-carrying capacity of artificial red cells (ARC). *Polymer Adv Technol* 1992; 4: 8–11.

44 Tsai AG, Vandegriff KD, Intaglietta M, Winslow RM. Targeted O_2 delivery by low-P_{50} hemoglobin: a new basis for O_2 therapeutics. *Am J Physiol Heart Circ Physiol* 2003; 285: H1411–9.

45 Plock JA, Tromp AEContaldo C, *et al.* Hemoglobin vesicles reduce hypoxia-related inflammation in critically ischemic hamster flap tissue. *Crit Care Med* 2007; 35: 899–905.

46 Arifin DR, Palmer AF. Polymersome encapsulated hemoglobin: a novel type of oxygen carrier. *Biomacromolecules* 2005; 6: 2172–81.

47 Zhao J, Liu CS, Yuan Y, *et al.* Preparation of hemoglobin-loaded nano-sized particles with porous structure as oxygen carriers. *Biomaterials* 2007; 28: 1414–22.

48 Sakai H, Takeoka S, Seino Y, Tsuchida E. Suppression of methemoglobin fomation by glutathione in a concentrated hemoglobin solution and in a hemoglobin vesicle. *Bull Chem Soc Jpn* 1994; 67: 1120–5.

49 Rudolph AS. The freeze-dried preservation of liposome encapsulated hemoglobin: a potential blood substitute. *Cryobiology* 1988; 25: 277–84.

50 Rabinovici R, Rudolph AS, Vernick J, Feuerstein G. Lyophilized liposome encapsulated hemoglobin: evaluation of hemodynamic, biochemical, and hematologic responses. *Crit Care Med* 1994; 22: 480–5.

51 Ringsdorf H, Schlarb B, Venzmer J. Molecular architecture and function of polymeric oriented systems – models for the study of organization, surface recognition, and dynamics of biomembranes. *Angew Chem Int Ed* 1988; 27: 113–58.

52 Kato A, Arakawa M, Kondo T. Preparation and stability of liposome-type artificial red blood cells stabilized with carboxymethylchitin. *J Microencapsul* 1984; 1: 105–12.

53 Mobed M, Nishiya T, Chang TM. Preparation of carboxymethylchitin-incorporated submicron bilayer-lipid membrane artificial cells (liposomes) encapsulating hemoglobin. *Biomater Artif Cells Immobilization Biotechnol* 1992; 20: 365–8.

54 Li S, Nickels J, Palmer AF. Liposome-encapsulated actin-hemoglobin (LEAcHb) artificial blood substitutes. *Biomaterials* 2005; 26: 3759–69.

55 Tsuchida E, Hasegawa E, Kimura N, Hatashita M, Makino C. Polymerization of unsaturated phospholipids as large unilamellar liposomes at low-temperature. *Macromolecules* 1992; 25: 207–212.

56 Sakai H, Takeoka S, Yokohama H, Nishide H, Tsuchida E. Encapsulation of Hb into unsaturated lipid vesicles and gamma-ray polymerization. *Polymer Adv Technol* 1992; 3: 389–94.

57 Akama K, Awai K, Yano Y, Tokuyama S, Nakano Y. In vitro and in vivo stability of polymerized mixed liposomes composed of 2,4-octadecadienoyl groups of phospholipids. *Polymer Adv Technol* 2000; 11: 280–7.

58 Yoshioka H. Surface modification of haemoglobin-containing liposomes with polyethylene glycol prevents liposome aggregation in blood plasma. *Biomaterials* 1991; 12: 861–4.

59 Sakai H, Tomiyama K, Sou K, Takeoka S, Tsuchida E. Poly(ethylene glycol)-conjugation and deoxygenation enable long-term preservation of hemoglobin-vesicles as oxygen carriers in a liquid state. *Bioconjugate Chem* 2000; 11: 425–32.

60 Sato T, Sakai H, Sou K, Buchner R, Tsuchida E. Poly(ethylene glycol)-conjugated phospholipids in aqueous micellar solutions: hydration, static structure, and interparticle interactions. *J Phys Chem B* 2007; 111: 1393–401.

61 Szebeni J. Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity. *Toxicology* 2005; 216: 106–21.

62 Van de Velde M, Wouters PF, Rolf N, Van Aken H, Vandermeersch E. Comparative hemodynamic effects of three different parenterally administered lipid emulsions in conscious dogs. *Crit Care Med* 1998; 26: 132–7.

63 Vercellotti GM, Hammerschmidt DE, Craddock PR, Jacob HS. Activation of plasma complement by perfluorocarbon artificial blood: probable mechanism of adverse pulmonary reactions in treated patients and rationale for corticosteroids prophylaxis. *Blood* 1982; 59: 1299–304.

64 Phillips WT, Klipper R, Fresne D, Rudolph AS, Javors M, Goins B. Platelet reactivity with liposome-encapsulated hemoglobin in the rat. *Exp Hematol* 1997; 25: 1347–56.

65 Szebeni J, Fontana JLWassef NM, *et al.* Hemodynamic changes induced by liposomes and liposome-encapsulated hemoglobin in pigs: a model for pseudoallergic cardiopulmonary reactions to liposomes. Role of complement and inhibition by soluble CR1 and anti-C5a antibody. *Circulation* 1999; 99: 2302–9.

66 Loughrey HC, Bally MB, Reinish LW, Cullis PR. The binding of phosphatidylglycerol liposomes to rat platelets is mediated by complement. *Thromb Haemost* 1990; 64: 172–6.

67 Chonn A, Cullis PR, Devine DV. The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes. *J Immunol* 1991; 146: 4234–41.

68 Sou K, Klipper R, Goins B, Tsuchida E, Phillips WT. Circulation kinetics and organ distribution of Hb-vesicles developed as a red blood cell substitute. *J Pharmacol Exp Ther* 2005; 312: 702–9.

69 Abe H, Fujihara M, Azuma H, *et al.* Interaction of hemoglobin vesicles, a cellular-type artificial oxygen carrier, with human plasma: effects on coagulation, kallikrein-kinin, and complement systems. *Artif Cells Blood Substit Immobil Biotechnol* 2006; 34: 1–10.

70 Wakamoto S, Fujihara M, Abe H, *et al.* Effects of hemoglobin vesicles on resting and agonist-stimulated human platelets in vitro. *Artif Cells Blood Substit Immobil Biotechnol* 2005; 33: 101–11.

71 Abe H, Azuma H, Yamaguchi M, *et al.* Effects of hemoglobin-vesicles, a liposomal artificial oxygen carrier, on hematological responses, complement and anaphylactic reactions in rats. *Artif Cells Blood Substitutes Biotechnol* 2007; 35: 157–72.

72 Wakamoto S, Fujihara M, Abe H, *et al.* Effects of poly(ethyleneglycol)-modified hemoglobin vesicles on agonist-induced platelet aggregation and RANTES release in vitro. *Artif Cells Blood Substit Immobil Biotechnol* 2001; 29: 191–201.

73 Bradley AJ, Devine DV, Ansell SM, Janzen J, Brooks DE. Inhibition of liposome-induced complement activation by incorporated poly(ethylene glycol)-lipids. *Arch Biochem Biophys* 1998; 357: 185–94.

74 Hu Q, Liu D. Co-existence of serum-dependent and serum-independent mechanisms for liposome clearance and involvement of non-Kupffer cells in liposome uptake by mouse liver. *Biochim Biophys Acta* 1996; 1284: 153–61.

75 Shibuya-Fujiwara N, Hirayama F, Ogata Y, Ikeda H, Ikebuchi K. Phagocytosis in vitro of polyethylene glycol-modified liposome-encapsulated hemoglobin by human peripheral blood monocytes plus macrophages through scavenger receptors. *Life Sci* 2001; 70: 291–300.

76 Sakai H, Horinouchi H, Tomiyama K, *et al.* Hemoglobin-vesicles as oxygen carriers: influence on phagocytic activity and histopathological changes in reticuloendothelial system. *Am J Pathol* 2001; 159: 1079–88.

77 Sakai H, Horinouchi H, Masada Y, Takeoka S, Kobayashi K, Tsuchida E. Metabolism of hemoglobin-vesicles (artificial oxygen carriers) and their influence on organ functions in a rat model. *Biomaterials* 2004; 25: 4317–25.

78 Sakai H, Masada Y, Horinouchi H, *et al.* Physiologic capacity of reticuloendothelial system for degradation of hemoglobin-vesicles (artificial oxygen carriers) after massive intravenous doses by daily repeated infusions for 14 days. *J Pharmacol Exp Ther* 2004; 311: 874–84.

79 Sakai H, Horinouchi H, Yamamoto M, *et al.* Acute 40 percent exchange-transfusion with hemoglobin-vesicles (HbV) suspended in recombinant human serum albumin solution: degradation of HbV and erythropoiesis in a rat spleen for 2 weeks. *Transfusion* 2006; 46: 339–47.

80 Yamaoka T, Tabata Y, Ikada Y. Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. *J Pharm Sci* 1994; 83: 601–6.

81 Nolte D, Pickelmann S, Lang M, Keipert P, Messmer K. Compatibility of different colloid plasma expanders with perflubron emulsion: an intravital microscopic study in the hamster. *Anesthesiology* 2000; 93: 1261–70.

82 Jouan-Hureaux V, Audonnet-Blaise S, Lacatusu D, *et al.* Effects of a new perfluorocarbon emulsion on human plasma and whole-blood viscosity in the presence of albumin, hydroxyethyl starch, or modified fluid gelatin: an in vitro rheologic approach. *Transfusion* 2006; 46: 1892–8.

83 Vandegriff KD, McCarthy M, Rohlfs RJ, Winslow RM. Colloid osmotic properties of modified hemoglobins: chemically cross-linked versus polyethylene glycol surface-conjugated. *Biophys Chem* 1997; 69: 23–30.

84 Kobayashi K. Summary of recombinant human serum albumin development. *Biologicals* 2006; 34: 55–9.

85 Meyuhas D, Nir S, Lichtenberg D. Aggregation of phospholipid vesicles by water-soluble polymers. *Biophys J* 1996; 71: 2602–12.

86 Neu B, Meiselman HJ. Depletion-mediated red blood cell aggregation in polymer solutions. *Biophys J* 2002; 83: 2482–90.

87 Sakai H, Sato A, Takeoka S, Tsuchida E. Rheological property of hemoglobin-vesicles (artificial oxygen carriers) suspended in a series of plasma substitute aqueous solutions. *Langmuir* 2007; 23: 8121–8.

88 Tsai AG, Friesenecker B, McCarthy M, Sakai H, Intaglietta M. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model. *Am J Physiol Heart Circ Physiol* 1998; 275: H2170–80.

89 Intaglietta M, Cabrales P, Tsai AG. Microvascular perspective of oxygen-carrying and – noncarrying blood substitutes. *Annu Rev Biomed Eng* 2006; 8: 289–321.

90 Rebel A, Ulatowski JA, Kwansa H, Bucci E, Koehler RC. Cerebrovascular response to decreased hematocrit: effect of cell-free hemoglobin, plasma viscosity, and CO_2. *Am J Physiol Heart Circ Physiol* 2003; 285: H1600–8.

91 Dimino ML, Palmer AF. High O_2 affinity hemoglobin-based oxygen carriers synthesized via polymerization of hemoglobin with ring-opened 2-chloroethyl-β-D-fructopyranoside and 1-o-octyl-β-D-glucopyranoside. *Biotechnol Bioeng* 2007; 97: 462–72.

92 Plock JA, Contaldo C, Sakai H, *et al.* Is the Hb in Hb vesicles infused for isovolemic hemodilution necessary to improve oxygenation in critically ischemic hamster skin? *Am J Physiol Heart Circ Physiol* 2005; 289: H2624–31.

93 Contaldo C, Plock J, Sakai H, *et al.* New generation of hemoglobin-based oxygen carriers evaluated for oxygenation of critically ischemic hamster flap tissue. *Crit Care Med* 2005; 33: 806–12.

94 Sakai H, Tomiyama K, Masada Y, *et al.* Pretreatment of serum containing hemoglobin vesicles (oxygen carriers) to prevent their interference in laboratory tests. *Clin Chem Lab Med* 2003; 41: 222–31.

日経バイオテク Biotechnology Japan

2007年06月15日

日本血液代替物学会、早大、血液由来のヘモグロビンを使わない次世代人工酸素運搬体を提案

2007年6月14日、慶応大学で日本血液代替物学会年次大会が開催され、人工血液研究の最前線と題するシンポジウムの中で、早稲田大学理工学術院の小松晃之准教授は、アルブミンにヘムを結合させたアルブミン-ヘムについて報告した。

ヘムは2価の鉄を中心に持つポルフィリン化合物。ヘモグロビンは4つのヘムを持つ分子量6万4000のたんぱく質だ。現在、オキシジェニックス（東京・港、大村孝男社長）やテルモが人工酸素運搬体を開発しているが（関連記事1、関連記事2）、これらは日本赤十字社から提供された期限切れの赤血球製剤から精製したヘモグロビンをリポソームに封入して作ったもの。血液型に関係なく利用でき、長期間保存できるなどの利点があるが、ヘモグロビンの安定性の問題や、赤血球製剤からヘモグロビンを精製する過程で病原体が混入する可能性がゼロにはならない、などの課題がある。

一方、アルブミンについては、三菱ウェルファーマや化学及血清療法研究所が遺伝子組み換え技術で製造されたものを医薬品として開発しており、血液由来ではないものがもうすぐ実用化されようとしている。

小松准教授らはアルブミンが脂肪酸と結合することに着目し、アルブミンと結合するヘムを30種類程度化学合成した。ヘムによって酸素親和性が高いものから低いものまでさまざまあり、「用途に応じて使い分けられる」と小松准教授は説明した。ヘムを解離しにくいようにアルブミン-ヘムの表面をPEGで修飾したものを作成し、貧血状態にしたラットに投与した結果、赤血球を投与したのと同様の効果が得られた。これらのことから、血液由来の成分を全く使わない完全合成のアルブミン-ヘムを次世代の人工酸素運搬体として利用できる可能性を示した。さらに小松准教授は、アルブミンのアミノ酸を遺伝子組換えにより3つだけ改変すれば、天然のヘムでも結合し酸素を運搬できるようになることを明らかにした。

小松准教授は、「アルブミン-ヘムは、たんぱく質製剤として利用されているアルブミンを酸素の運搬に使おうというもので、ヘモグロビンを封入したリポソームとはコンセプトが違う。また、リポソームの直径は200nmから250nmだが、アルブミンは8nm程度なので、脳梗塞などの虚血部位への酸素供給など、用途も広がる」と語った。ちなみに、小松准教授はアルブミン-ヘムの適応として、出血ショックの蘇生液、救急車内での酸素供給液、虚血部位への酸素供給液のほか、移植用臓器の灌流液、体外循環回路の補填液などを挙げている。また、天然のヘムを利用しても、「ヘモグロビンを利用する場合と違って感染因子が入り込む可能性はほとんどない」（小松准教授）とのことだ。（橋本宗明）