Review
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Evaluation of Safety and Efficacy of Hemoglobin-Vesicles and Albumin-Hemes

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Since the discovery of a red-colored saline solution of a heme derivative that reversibly binds and releases O_2 (1983), significant efforts have been made to realize an O_2 infusion as a red cell substitute based on the sciences of both molecular assembling phenomena and macromolecular metal complexes. We have specified that hemoglobin (Hb)-vesicles (HbV) and recombinant human serum albumin -hemes (rHSA-hemes) would be the best systems that meet the clinical requirements. The HbV encapsulates ultrapure conc. Hb solution, that is free of any infectious elements, with a phospholipid bimolecular membrane (diameter, 250 nmϕ), and its solution properties can be adjusted comparable with blood. Surface modification of HbV with a water-soluble polymer ensures stable dispersion state and storage over a year at 20℃. *In vivo* tests have clarified the efficacy for extreme hemodilution and resuscitation from hemorrhagic shock, and safety in terms of biodistribution, metabolism in RES, clinical chemistry, blood coagulation, etc.. The HbV does not induce vasoconstriction thus maintains blood flow and tissue oxygenation. The rHSA-heme is a totally synthetic O_2 carrier that incorporates 8 heme derivatives (axial base substituted hemes) as O_2 binding sites in the hydrophobic pockets of rHSA, which is now manufactured in Japan as a plasma-expander. Hb binds endothelium-derived relaxation factor, NO, and induces vasoconstriction. The rHSA-heme binds NO as Hb does, however, it does not induce vasoconstriction due to its low *pI* (4.8) and the resulting low permeability across the vascular wall (1/100 of Hb). A 5%-albumin solution possesses a physiologic oncotic pressure. Therefore, to increase the O_2-transporting capacity, albumin dimer is effective. Albumin dimer can incorporate totally 16 hemes with a regulated oncotic pressure. The rHSA-heme is effective not only as a red cell substitute but also for O_2 therapeutics (e.g., oxygenation for tumor). Significant efforts have been made to produce HbV and rHSA-hemes with a facility of GMP standard, and to start preclinical and finally clinical trials.

Key Words : Blood Substitutes, Oxygen Carriers, Hemoglobin-vesicles, Albumin-hemes,

1. Introduction

For human beings to survive, it is necessary to continuously deliver O_2 that is needed for the respiration of all tissue cells. Blood, a so-called moving internal-organ, reversibly binds and releases O_2 under physiological conditions. From this point of view, realization of red blood cell (RBC) substitutes, or O_2-Infusions, would contribute significantly to human health and welfare. In this research field, the basic sciences for macromolecular complexes, molecular assemblies, and nano-molecular sciences play fundamental roles. We have systematically studied the metal complexes (synthetic heme derivatives) embedded into a hydrophobic cluster in aqueous medium, and clarified that the electronic processes of the active sites are controlled by the surrounding molecular environment. As a result, the reaction activity is observed as cooperative phenomena with the properties of the molecular atmosphere. In other words, the development of our O_2-Infusion has been based on "the Regulation of the Electronic Process on Macromolecular Metal Complexes" [1,2].

The study to reproduce the O_2-binding ability of RBCs, that is, the development of a synthetic O_2-carrier that does not need hemoglobin (Hb), was the starting point of our idea. In general, central ferric iron of a heme is immediately oxidized by O_2 in water, preventing the O_2 coordination process from being observed. Therefore, the electron transfer must be prevented. Fortunately, we could detect the formation of the O_2-adduct complex but for only several nano-seconds by utilizing the molecular atmosphere and controlling the electron density in the iron center. Based on this finding, we succeeded in reversible and stable O_2-coordination in 1983 and preparing phospholipid vesicles embedded amphiphilic-heme, known as lipidheme/phospholipids vesicles [3-5]. This was the first example of reversible O_2-binding taking place under physiological conditions. For example, human blood can dissolve about 27 mL of O_2 per dL, however a 10 mM lipidheme-phospholipid vesicle solution can dissolve 29 mL of O_2 per dL. This material is suitable for "O_2-Infusion". We have synthesized over hundred types of heme derivatives, and recently

synthesized new lipidheme bearing phospholipid groups, which completes self-organization in water to form stable vesicles [6].

In 1985, Dr. Sekiguchi at Hokkaido Red Cross Blood Center proposed Waseda group to consider the utilization of Hb in outdated RBCs. Thus the research of Hb-vesicles (HbV) based on molecular assembly technologies has been started (Fig. 1). In the latter 1990's, a mass-production system for recombinant human serum albumin (rHSA) was established and we then prepared albumin-heme hybrids (rHSA-heme) using its non-specific binding ability, which is now considered to be one promising synthetic material. Based on our effective integration of nano-molecular science and technologies for functional materials developed by Waseda University, and the outstanding evaluation system of safety and efficacy developed by Keio University using animal experiments, we have made strong progress on our research on the O_2-Infusion Project. In the near future, mass production and clinical tests of O_2-Infusion will be started by the pharmaceutical industry.

2. Development of Hb-based O_2 carriers and the characteristics of HbV.

Historically, the first attempt of Hb-based O_2-carrier in this area was to simply use stroma-free Hb (Fig. 2). However, several problems became apparent, including dissociation into dimers that have a short circulation time, renal toxicity, high oncotic pressure and high O_2-affinity. Since the 1970s, various approaches were developed to overcome these problems [7,8]. This includes intra-molecular crosslinking, polymerization and polymer-conjugation. However, in some cases the significantly different structure in comparison with RBCs resulted in side effects such as vasoconstriction [9].

Another idea is to encapsulate Hb with a lipid bilayer membrane to solve all the problems of molecular Hb [10]. RBCs have a biconcave structure with a diameter of about 8000 nm. RBCs can deform to a parachute-like configuration to pass through narrow capillaries. The possibility of infection and blood-type mismatching, and short shelf life are the main problems. The idea of Hb encapsulation with a polymer membrane mimicking the structure of RBC is originated from Dr. Chang at McGill University [7]. After that, the encapsulation of Hb within a phospholipid vesicle was studied by Dr. Djordjevici at the University of Illinois in the 1970s [11]. However, it was not so easy to make HbV with a regulated diameter and adequate O_2-transport capacity, we made a breakthrough in routinely producing HbV by using fundamental knowledge of macromolecular and supramolecular sciences [12-19]. Several liters of HbV are routinely prepared in a completely sterile condition. Hb is purified from outdated RBCs, and concentrated to 40 g/dl. Virus removal is performed using a combination of pasteurization at 60 degrees and filtration with a virus removal filter. The Hb encapsulation with phospholipids bilayer membrane and size regulation was performed with an extrusion method.

The particle diameter of HbV is regulated to about 250 nm, therefore, the bottle of HbV is turbid. One vesicle contains about 30,000 Hb molecules so that it does not show oncotic pressure. There is no chemical modification of Hb. O_2-affinity is

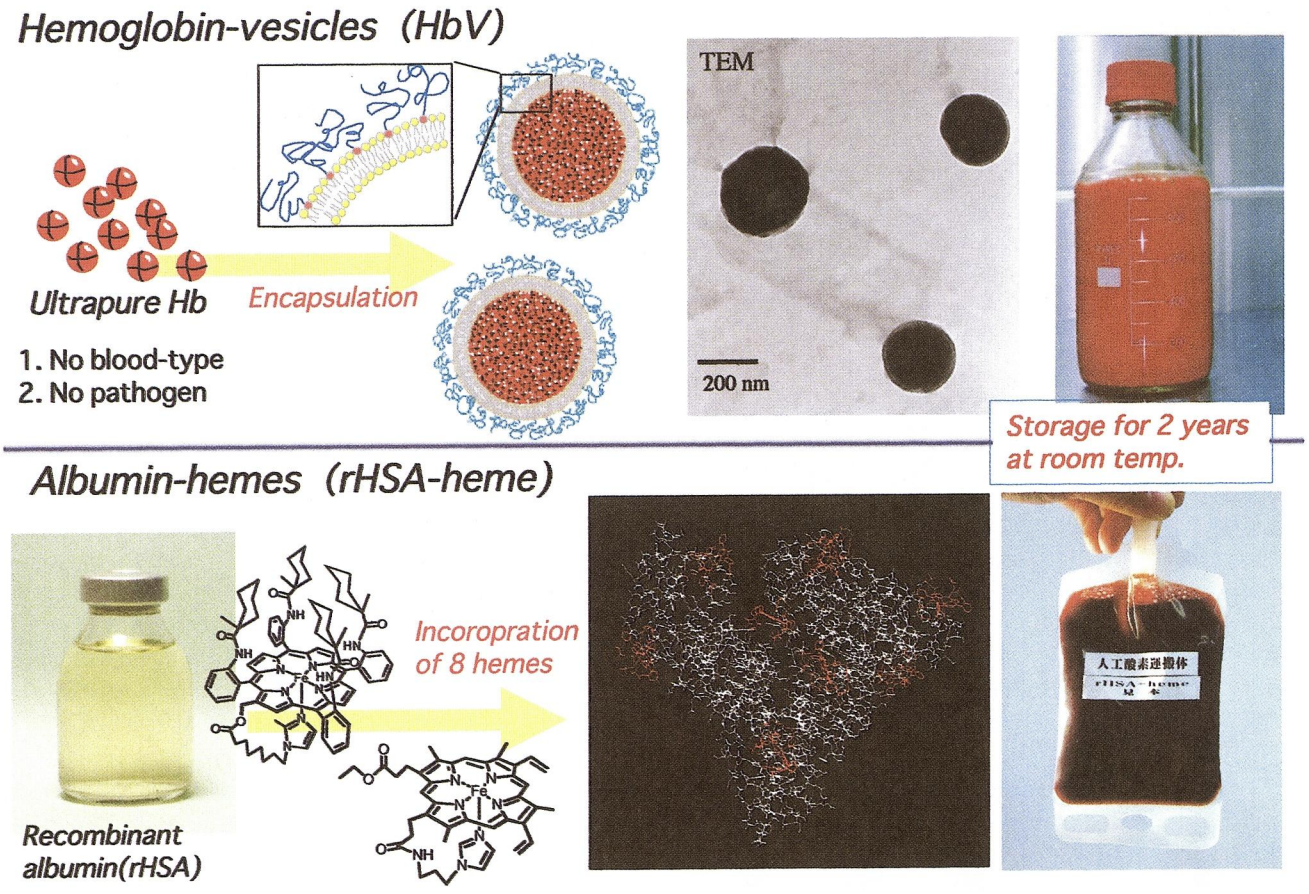

Figure 1 Hemoglobin-vesicles (HbV) and and albumin-hemes (rHSA-heme) as potential artificial O_2 carriers.

controllable with an appropriate amount of allosteric effectors, pyridoxal 5-phosphate. Hb concentration is regulated to 10 g/dL, and the weight ratio of Hb to total lipid approaches 2.0 by using an ultra pure and concentrated Hb solution of 40 g/dL, which is covered with a thin lipid bilayer membrane. The surface is modified with 0.3 mol% of PEG- lipid. Viscosity, osmolarity, and oncotic pressure are regulated according to the physiological conditions.

HbV can be stored for over two years in a liquid state at room temperature [18]. There is little change in turbidity, diameter, and P_{50}. MetHb content decreases due to the presence of reductant inside the HbV, which reduces the trace amount of metHb during storage. This excellent stability is obtained by deoxygenation and PEG-modification. Deoxygenation prevents metHb formation. The surface modification of HbV, with PEG chains prevents vesicular aggregation and leakage of Hb and other reagents inside the vesicles. Liquid state storage is convenient for emergency infusion compared to freeze-dried powder or the frozen state.

3. In vivo Efficacy of HbV

The efficacy of HbV has been confirmed mainly with isovolemic hemodilution and resuscitation from hemorrhagic shock [20-28]. In this review two important cases are described. One is isovolemic hemodilution with 90% blood exchange in a rat model. The other is resuscitation from hemorrhagic shock in a hamster model.

To confirm the O_2-transporting ability of HbV, extreme hemodilution was performed with HbV suspended in human serum albumin (HSA) [21,23] (Fig. 3). The final level of blood exchange reached 90%. Needle-type O_2 electrodes were inserted into the renal cortex, and the blood flow rate in the abdominal aorta was measured with the pulsed Doppler method. Hemodilution with albumin alone resulted in significant reductions in mean arterial pressure and renal cortical O_2 tension, and finally all the rats died of anemia. On the other hand, hemodilution with HbV suspended in HSA sustained both blood pressure and renal cortical O_2 tension, and all the rats survived. These results clearly demonstrate that HbV has sufficient O_2 transporting capability.

To observe the microcirculatory response to the infusion of Hb products, the intravital microscopy was used that equipped with all the units to measure blood flow rates, vascular diameter, O_2 tension, and so on, in collaboration with Dr. Intaglietta at Univ. California, San Diego. The hamster dorsal-skin fold preparation allows observation of blood vessels from small arteries down to capillaries. We evaluated the HbV suspension, as a resuscitative fluid for hemorrhagic-shocked hamsters [26]. About 50% of the blood was withdrawn, and the blood pressure was maintained at around 40 mmHg for 1 hr, and the hamsters either received HbV suspended in HSA (HbV/HSA), HSA alone, or shed blood (Fig. 4). Immediately after infusion, all the groups showed increases in mean arterial pressure. However, only the HSA infusion resulted in incomplete recovery. On the other hand, the HbV/HSA group showed the same recovery with the shed autologous blood infusion. During the shock period, all the groups showed significant hyperventilation that was evident from the significant increase in arterial O_2 tension. Simultaneously, base excess and pH decreased significantly. Immediately after resuscitation, all the groups tended to recover. However, only the HSA group showed sustained hyperventilation. Base excess for the HSA group remained at a significantly lower value one hour after resuscitation. Blood flow decreased significantly in arterioles to 11% of basal value during shock. The HbV/HSA and shed autologous blood groups immediately showed significant increases in blood flow rate after resuscitation, while the albumin group showed the lowest recovery.

4. Safety evaluation of HbV

We further examined the safety profile of HbV such as cardiovascular responses, pharmacokinetics, influence on RES, influence on clinical measurements and daily repeated infusion [29-37].

The microvascular responses to the infusion of intra-

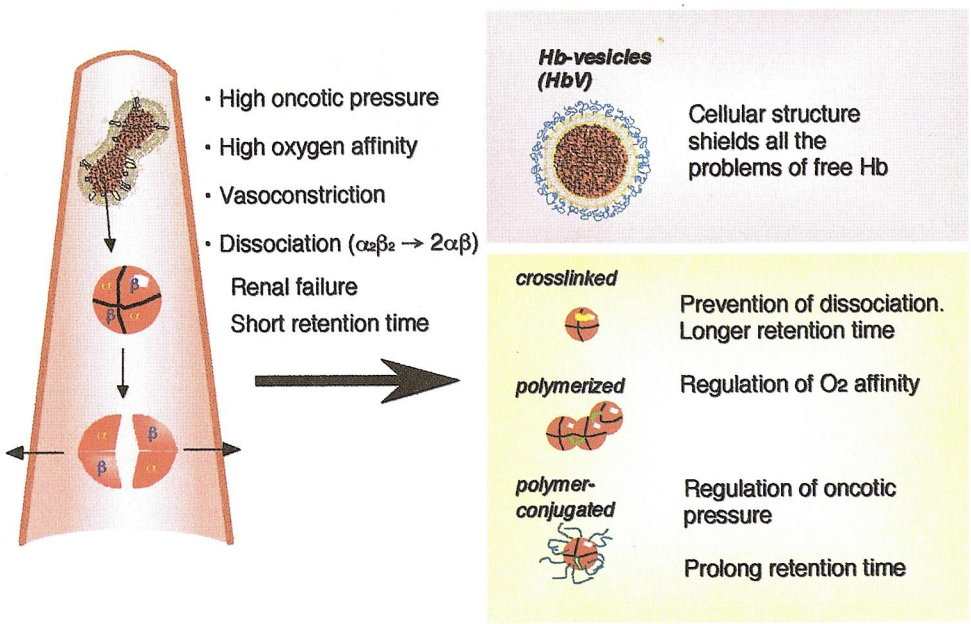

Figure 2 Approaches to solve the problems of utilization of Hb as an O_2 carrier, chemical modification or encapsulation of Hb.

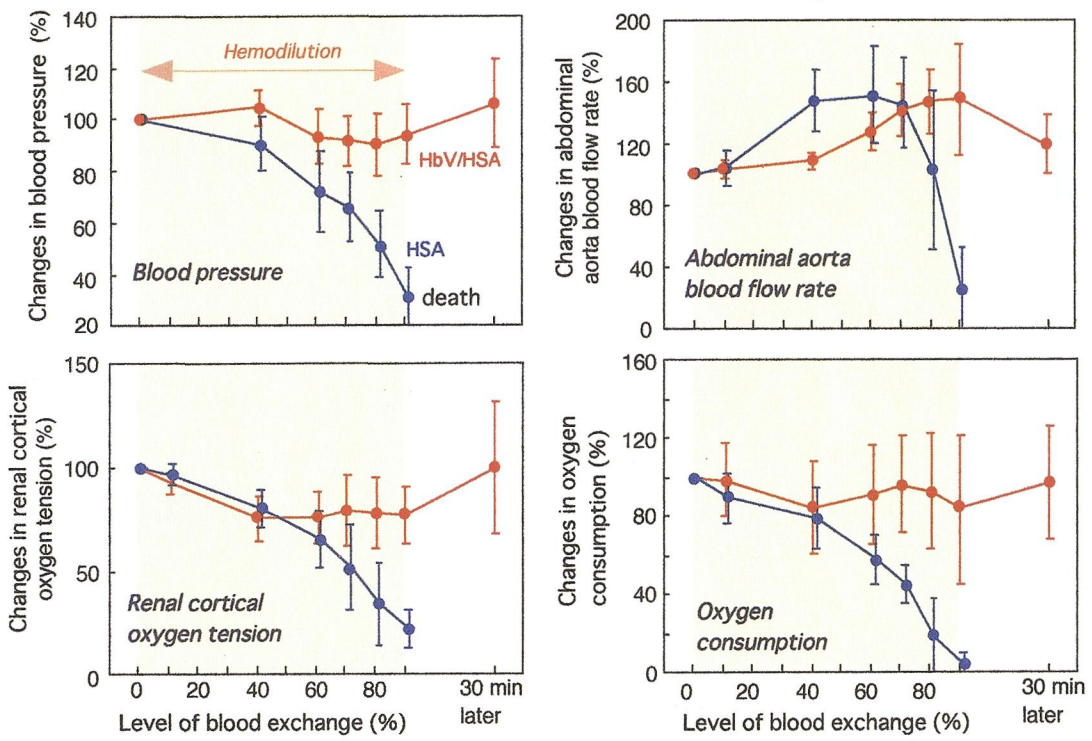

Figure 3 Ninety percent exchange-transfusion with HbV suspended in HSA (HbV/HSA), or HSA alone. Mean arterial pressure, renal cortical O_2 tension, abdominal aortic blood flow rates, and oxygen consumption were monitored. Mean ± SD.

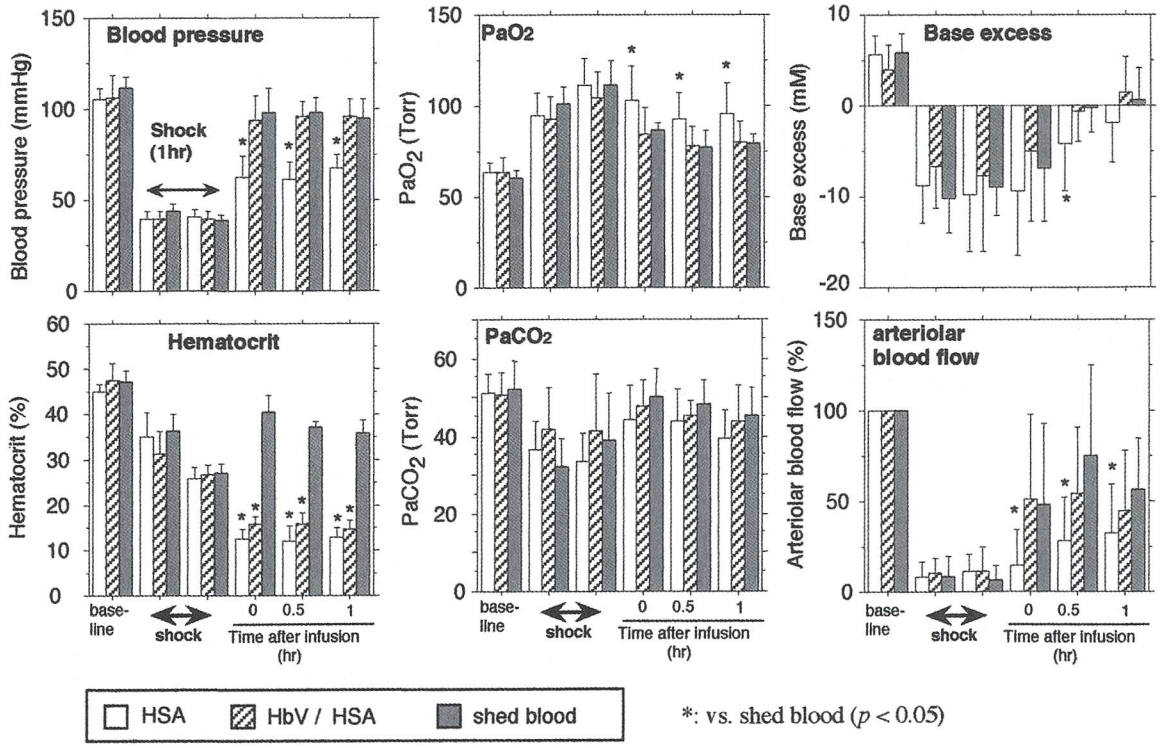

Figure 4 Resuscitation from hemorrhagic shock with HbV suspended in HSA (HbV/HSA) in hamster dorsal skinfold model. Mean ± SD.

molecularly cross-linked Hb (XLHb) and HbV were studied using conscious hamsters. XLHb (7 nm in diameter) showed a significant increase in hypertension equal to 35 mmHg, and simultaneous vasoconstriction of the resistance artery equal to 75% of the baseline levels [30] (Fig. 5). On the other hand, HbV with diameter of 250 nm showed minimal changes. The small acellular XLHb is homogeneously dispersed in the plasma, and it diffuses through the endothelium layer of the vascular wall and reaches the smooth muscle. XLHb traps nitric oxide (NO) as an endothelium-derived relaxation factor, and induces vasoconstriction, and hypertension. On the other hand, the large HbV stay in the lumen and does not induce vasoconstriction. Several mechanisms are proposed for Hb-induced vasoconstriction. These include NO-binding, excess O_2 supply, reduced shear stress, or the presence of Hb recognition site on the endothelium. But it is clear that Hb-encapsulation shields against the side effects of acellular Hbs.

Prof. Suematsu at Keio University has revealed the effects of Hb-based O_2 carriers in hepatic microcirculation [29,32] (Fig. 6). On the vascular wall of the sinusoid in hepatic microcirculation, there are many pores, called fenestration, with a diameter of about 100 nm. The small Hb molecules with a diameter of only 7 nm extravasate through the fenestrated endothelium and reach the space of Disse. On the other hand, HbV particles, which are larger than the pores, do not extravasate. Heme of extravasated Hb is excessively metabolized by hemeoxygenase-2 in hepatocyte to produce CO and bilirubin. Even though CO acts as a vasorelaxation factor in the liver, the excess amount of Hb rapidly binds CO, resulting in the vasoconstriction and an increase in vascular resistance. On the other hand, HbV (250 nm in diameter) is large enough to remain in the sinusoid, and the vascular resistance is maintained.

From these results, we can speculate the optimal molecular dimension of Hb-based O_2 carriers. The upper limitation is below the capillary diameter to prevent capillary plugging, and for sterilization by membrane filters (Fig. 7). On the other hand, smaller sizes exhibit a higher rate of vascular wall permeability with side effects such as hypertension and neurological

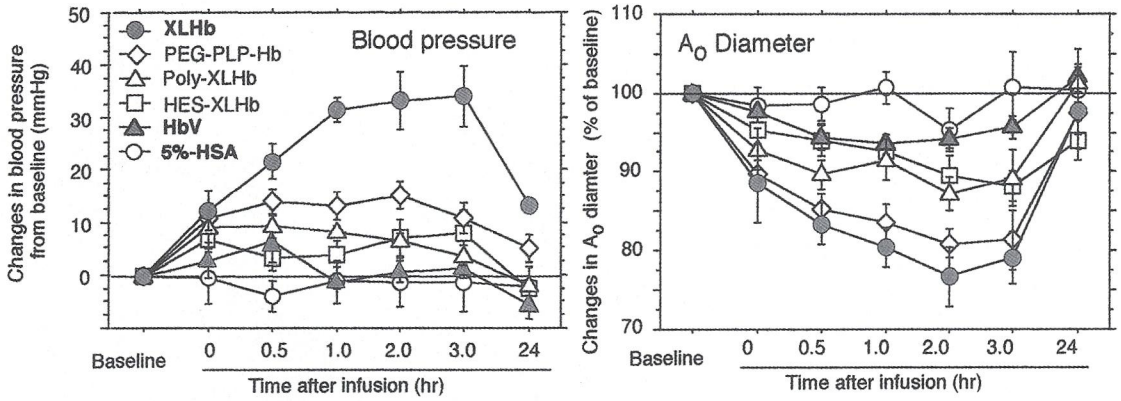

Figure 5 Changes in mean arterial pressure and the diameters of the resistance artery in hamster dorsal skin microcirculation after the bolus infusion of Hb-based O_2 carriers. Mean ± SD

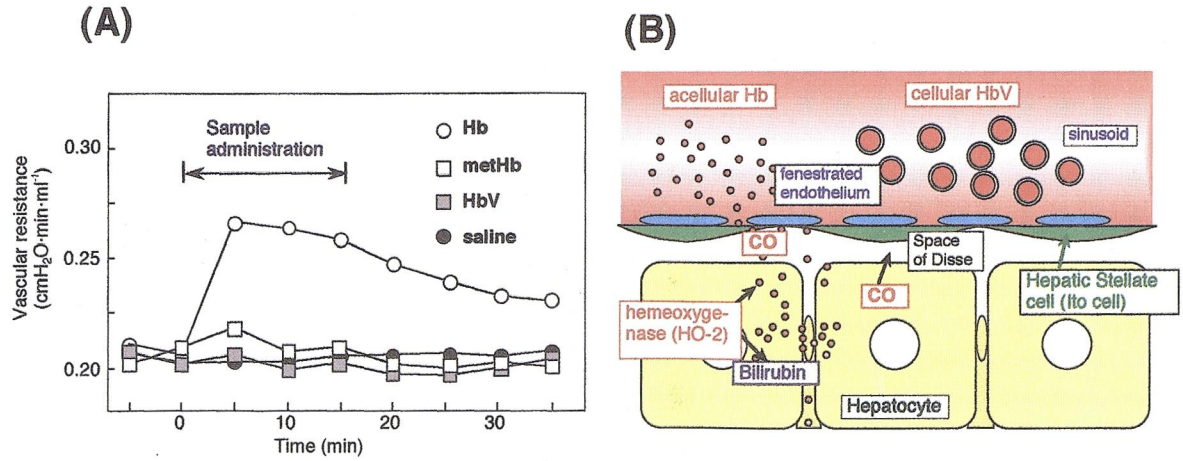

Figure 6. (A) Changes in vascular resistance during perfusion of exteriorized rat liver with HbV, Hb, metHb, or saline. (B) Schematic representation of hepatic microcirculation : The small Hb molecule extravasate across the fenestrated endothelium to reach to the space of Disse, where heme of Hb is catabolised by hemeoxygenase-2 (HO-2) and CO is released as a vasorelaxation factor. However, the excess amount of the extravasated Hb traps CO and induces vasoconstriction and the resulting higher vascular resistance. On the other hand, the larger HbV retains in the sinusoid and there is no extravasation and vasoconstriction.

disturbances. HbV exhibits a very low level of vascular wall permeability. Therefore, the HbV appears to be appropriate from the viewpoint of hemodynamics. We have clarified the influence of HbV on the RES, because the fate of HbV is RES trapping.

Circulation persistence was measured by monitoring the concentration of radioisotope-labeled HbV in collaboration with Dr. Phillips at the University of Texas at San Antonio [37]. The circulation half-life is dose dependent, and when the dose rate was 14 ml/kg, the circulation half-life was 35 hrs in rats. The circulation time in the case of the human body can be estimated to be twice longer ; or about 3 days at the same dose rate. Gamma camera images of radioisotope-labeled HbV showed the time course of biodistribution. After HbV finished playing its role in O_2-transport, a total of 35% of HbV are finally distributed mainly in the liver, spleen and bone marrow. The transmission electron microscopy (TEM) of the spleen 1 day after infusion of HbV clearly demonstrated the presence of HbV particles in macrophages, where HbV particles that appear as black dots are captured by the phagosomes [34] (Fig. 8). RBCs and HbV contain a lot of ferric ion with a high electron density, so that they

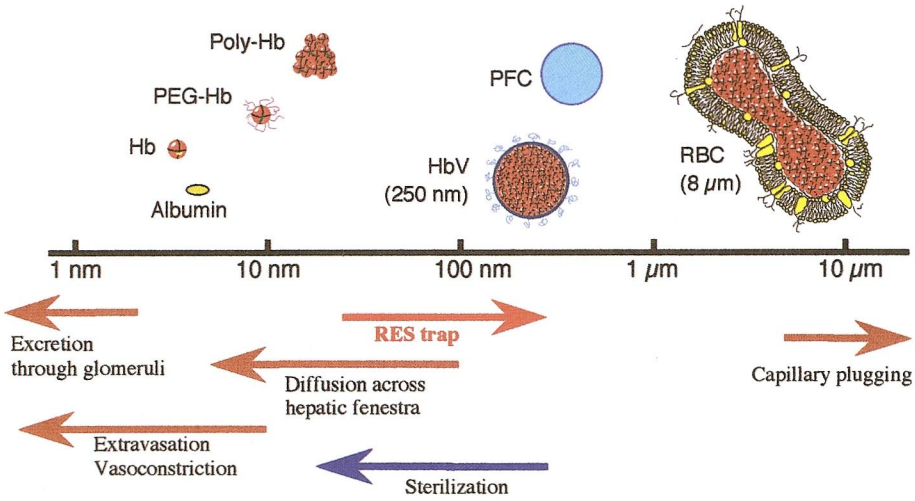

Figure 7 Optimal diameter of Hb-based O_2 carriers from the view point of physiological response and production process.

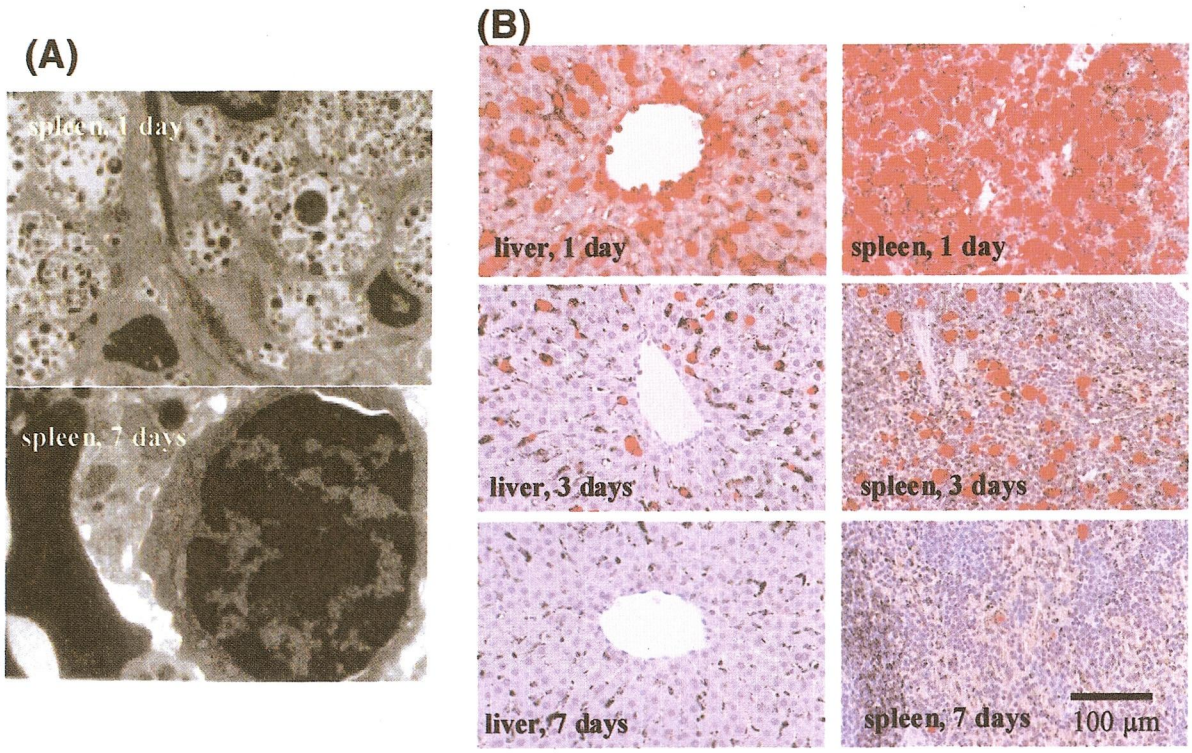

Figure 8. (A) Transmission electron microscopy of rat spleen one day after the infusion of HbV (20 mL/kg) and after 7 days. Black dots are HbV particles captured in phagosomes in the spleen macrophages, and they disappeared at 7 days. (B) Staining with anti-human Hb antibody showed the presence of HbV in spleen and liver. HbV particles disappeared within 7 days.

show strong contrast in TEM. However, after 7 days, the HbV structure cannot be observed. There were no abnormalities in the tissues and no irreversible damages to the organs. A Polyclonal anti-human Hb antibody was used as the marker of Hb in the HbV. This antibody does not recognize rat Hb. The red colored parts indicate the presence of Hb in HbV, and they have almost disappeared after 7 days in both the spleen and liver. Therefore, this shows that HbV can be metabolized quite promptly.

One issue of the Hb-based O_2-carriers is that they have a significant influence on clinical laboratory tests. They remain in the plasma phase in a blood collecting tube after centrifugation of blood samples, and interfere with the colorimetric and turbidimetric measurements. However, HbV can be simply removed from blood plasma either by ultracentrifugation or centrifugation in the presence of a high-molecular-weight dextran to enhance precipitation. We can obtain a very clear supernatant for accurate analyses [35]. This is one advantage of HbV in comparison with acellular Hb solutions. Accordingly we examined the influence on organ functions by serum clinical laboratory tests after the bolus infusion of HbV at a dose rate of 20 mL/kg. Albumin, ALT, AST, and LDH, which reflect the liver function, moves their values within normal range [36]. Concentrations of bilirubin and ferric ion are maintained at a low level. The concentration of lipids transiently changed. In particular, the cholesterols increased significantly. And phospholipids slightly increased, however, they returned to the original level after 7 days. These results indicate that the membrane components of HbV, once they reappear from RES, are metabolized on the physiological pathway.

A test of daily repeated infusion is required to evaluate the safety of a new drug. We tested the daily repeated infusion of HbV in Wistar rats at a dose rate of 10 mL/kg/day for 14 days, everyday. The total infusion volume (140 mL/kg) was 2.5 times as much as the volume of the whole blood (56 mL/kg), however, all rats well tolerated and survived. The body weight showed a monotonous but slightly depressed increase in comparison with the saline. However, after 2 weeks there was no significant difference with the saline control group. All the rats seemed very healthy and active. Histopathological examination one day after the final infusion of HbV showed significant accumulation of HbV in spleen macrophages, and liver Kupffer cells, and they mostly disappeared after 14 days. There were no irreversible other morphological abnormalities, and the serum clinical chemistry indicated transient but reversible increases in lipid components. AST and ALT were within the normal range. From these results we are confident with the safety of HbV.

5. Design and physicochemical properties of rHSA-heme

On the other hand, we have been conducting research on totally synthetic O_2-carriers, or so-called albumin-heme that does not require Hb. Human serum albumin is the most abundant plasma protein in our blood stream, but its crystal structure has not been elucidated for long time. In 1998, Dr. Stephen Curry of the Imperial College London first elucidated the crystal structure of the human serum albumin complexed with seven molecules of myristic acids [38]. He found that the dynamic conformational changes of albumin take place by the binding of fatty acid. On the other hand, in Japan, recombinant human serum albumin (rHSA) is now manufactured on a large scale by expression in the yeast Pichia pastoris, and it will appear on the market soon [39]. A large-scale plant, which can produce one million vials per year, has been already established. From the viewpoint of clinical application, O_2-carrying albumin is quite exciting and may be of extreme medical importance. With this background, we have found that synthetic heme derivative is efficiently incorporated into rHSA, creating a red-colored rHSA-heme hybrid (Fig. 1). This rHSA-heme can reversibly bind and release O_2 molecules under physiological conditions in the same manner as Hb. In other words, our rHSA-heme hybrid is a synthetic O_2-carrying hemoprotein, and we believe that its saline solution will become a new class of RBC substitute [40-51]. The maximal binding numbers of heme to one albumin are eight, and the magnitude of the binding constants ranged from 10^6 to 10^4 (M^{-1}). The isoelectric point of rHSA-heme was found to be 4.8, independent of the binding numbers of heme. This value is exactly the same as that of albumin itself. Furthermore, the viscosity and density did not change after the incorporation of heme molecules, and the obtained solution showed a long shelf life of almost two years at room temperature. Since the O_2-binding sites of rHSA-heme are iron-porphyrin, the color of the solution changed in a similar way to Hb. Upon addition of O_2 gas through this solution, the visible absorption pattern immediately changed to that of the O_2-adduct complex. Moreover, after bubbling carbon monoxide gas, rHSA-heme formed a very stable carbonyl complex.

Figure 9 shows the O_2-binding equilibrium curve of rHSA-heme. The O_2-binding affinity of rHSA-heme is always constant independent of the number of heme, and the O_2-binding profile does not show cooperativity. However, the O_2-transporting efficiency of rHSA-heme between the lungs measuring 110 Torr and muscle tissue measuring 40 Torr increases to 22%, which is identical to the 22% efficiency for RBCs. The O_2-binding property of rHSA-heme can be controlled by changing the chemical structure of heme derivatives incorporated. More recently, we have found that a protoheme derivative is also incorporated into albumin and can bind and release O_2 as well [52].

6. *In vivo* safety and efficacy of rHSA-heme

Based on these findings, we can say that rHSA-heme can become an entirely synthetic O_2-carrier, and satisfy the initial clinical requirements for a RBC substitute. However, we have

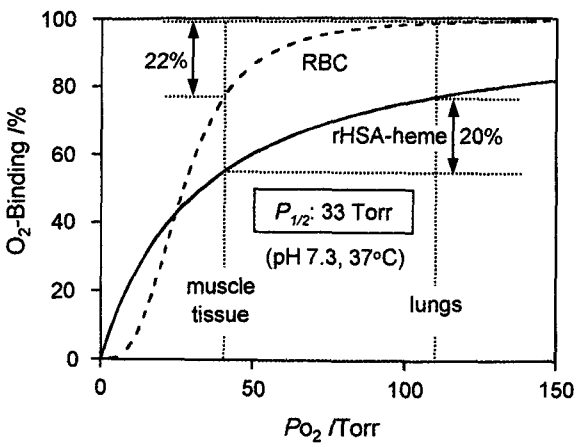

Figure 9 O_2-binding equilibrium curve of albumin-heme.

another problem to solve before we can use this material as an O_2-carrier in the circulatory system. This problem is NO scavenging. Of course, rHSA-heme can bind NO, and it may be anticipated that the injection of rHSA-heme also induce hypertensive action. We have evaluated the efficacy and safety of this rHSA-heme solution with animal experiments.

As described before, small Hb molecules extravasate through the vascular endothelium and react with NO, thus inducing vasoconstriction and acute increases in systemic blood pressure. Contrary to the expectations, the observation of the intestinal microcirculation after the infusion of rHSA-heme into an anesthetized rat revealed that the diameters of the venules and arterioles were not deformed at all [53]. Indeed, only a small change in the mean arterial pressure was observed after the administration of the rHSA-heme solution (Fig. 10). In contrast, the infusion of Hb elicited an acute increase in blood pressure. Why does rHSA-heme not induce vasoconstriction or hypertension? The answer probably lies in the negatively charged molecular surface of albumin. One of the unique characteristics of serum albumin is its low permeability through the muscle capillary pore, which is less than 1/100 that for Hb due to the electrostatic repulsion between the albumin surface and the glomerular basement membrane around the endothelial cells.

We are now evaluating the O_2-transporting ability of this rHSA-heme molecule in the circulatory system with further animal experiments [54]. First, we determined the physiological responses to exchange transfusion with rHSA-heme solution into rats after 70% hemodilution and 40% hemorrhage (Fig. 11). The declined mean arterial pressure and blood flow after a 70% exchange with albumin and further 40% bleeding of blood showed a significant recovery of up to 90% of the baseline values by the infusion of the rHSA-heme solution. On the other hand, all rats in the control group only injected with albumin died within 30 min. Furthermore, muscle tissue O_2-tension significantly increased. These responses indicate the *in vivo* O_2-delivery of the rHSA-heme solution. More recently, we have synthesized human serum albumin dimer, which can incorporate sixteen hemes in its hydrophobic domain [55]. The human serum rHSA-heme dimer solution dissolves 1.3-times more O_2 compared to that of RBC and keeps its colloid osmotic pressure at the same level as the

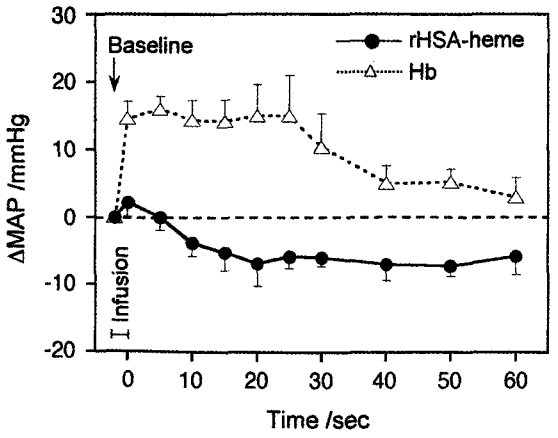

Figure 10 Change of MAP after the administration of rHSA-heme solution in the anesthetized rats (n = 5). All data are shown as changes from the basal values (DMAP) just before the infusion and expressed as mean ± S.E. Basal value is 90.1 ± 3.0 mmHg.

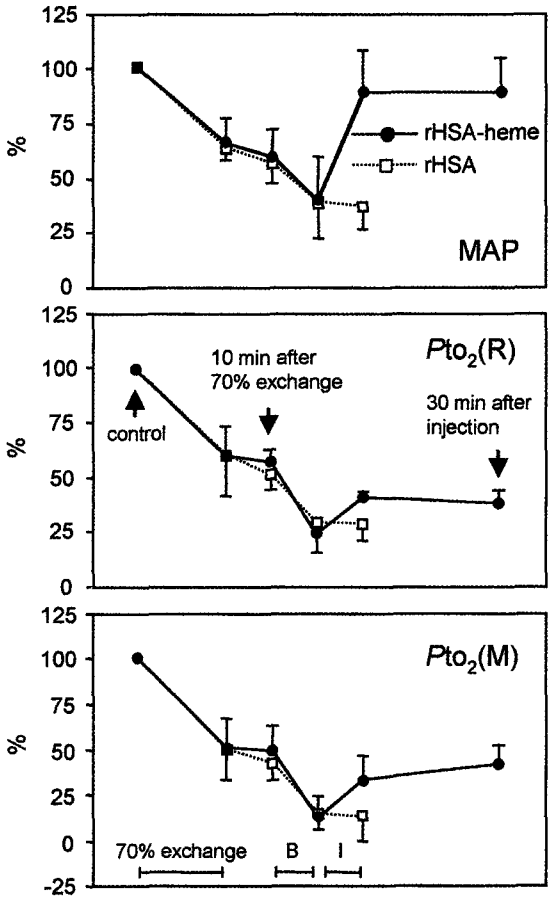

Figure 11 Change of (a) MAP and (b) O_2-tension in renal cortex during the 70% hemodilution with 5 wt% rHSA and further 40% exchange transfusion with rHSA-heme in anesthetized rats (n = 5). All data are shown as changes from the basal values and expressed as mean ± S.E.

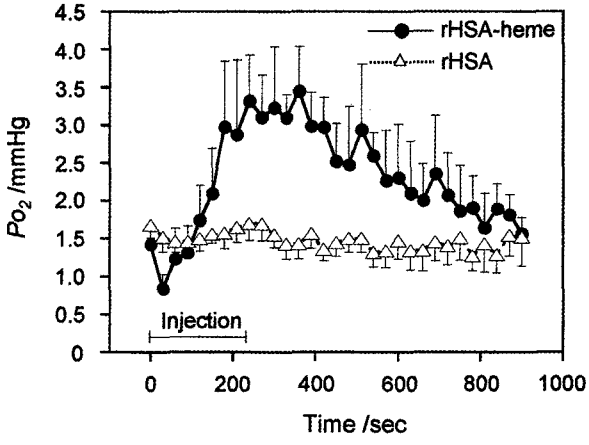

Figure 12 Changes in the O_2 tension of the hypoxic region of the ascites hepatoma LY80 solid tumor after the administration of the O_2 saturated rHSA-heme or rHSA solutions in the anesthetized rats (n = 4 each). All data are shown as changes from the basal values (Po_2) just before the infusion and expressed as mean ± S.E.

physiological value.

Unlike vessels in normal tissues, the development of a vasculature in a tumor lacks regulation and is hence, highly heterogeneous. Consequently, areas of hypoxia are quite common in tumors. In these hypoxic regions, it can be added that tumor cells acquire resistance to treatments such as chemotherapy and radiation. Our rHSA-heme was injected into the responsible artery that supplies circulation to an implanted tumor (Fig. 12) [56]. O_2-tension of the tumor rises immediately after intra-arterial infusion of albumin heme up to 2.4 times that of the baseline value. Our findings in animals indicate that tumor tissue O_2-levels can be elevated by the administration of artificial O_2-carriers due to the difference in O_2-transporting properties from RBCs. Whether this increase in tissue O_2 can potentiate cancer treatment is currently under investigation.

7. Potential Applications of Artificial O_2 Carriers and future scope

As described above, the primary application of artificial O_2 carriers would be the resuscitative fluid for hemorrhage. Since some of the characteristics of artificial O_2 carriers overwhelm those of donated blood, there are many potential applications other than blood substitutes, such as oxygenation of ischemic tissues [27,28]., organ preservation, or extracorporeal circulation fluid [57].

The research field of the red cell substitutes is moving forward very rapidly, and the paradigm in this field is expanding from red cell substitutes to "O_2 therapeutics". The sufficient safety and efficacy of our O_2 carriers encourage us to make significant efforts aiming at producing HbV and albumin-heme with a facility of GMP standard, and preclinical and finally clinical trials.

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