The lifetime and $p_{1/2}(O_2)$ values of the oxygenated synthetic hemes in an organic solvent generally depend on their molecular structure and the properties of the solvent used, e.g. polarity [24]. Especially in protic solutions, the apolar cavity around the O_2-binding site is significantly effective in inhibiting an oxidation through a proton-driven process.

Although the LH molecules are incorporated into HSA by hydrophobic interaction, the micro-environment around the heme does not contribute much to the protection of the coordinated oxygen, because of its relatively short lifetime. The oxidation reaction is probably prevented mainly by its own hydrophobic substituents besides the O_2-binding site, i.e. the four pivalamide groups on the porphyrin ring. In fact, the half-life of the HSA hybrid with a double-sided lipidheme (DLH, Fig. 1) ($\tau_{1/2}$: 15 h at 25°C, 2.3 h at 37°C) was obviously longer than that of HSA-LH [18]. Consequently, the O_2-binding ability of the HSA-LH system could be controllable by modifying the molecular structure of the incorporated LH.

The O_2-binding equilibrium curves of the HSA-LH (LH/HSA: 4) were obtained from its saturated spectrum at each O_2-partial pressure (Fig. 5). Because the $p_{1/2}(O_2)$ value was ca 30 mmHg, it is expected that the HSA-LH would release 22% of the bound oxygen, if it is circulated between the lungs (pO_2: 110 mmHg) and the mixed venous system (pO_2: 40 mmHg).

The thermodynamic parameters for the O_2-binding, enthalpy changes (ΔH) and entropy changes (ΔS) for HSA-LH were estimated to be $-59\,kJ\,mol^{-1}$ and $-109\,J\,K^{-1}\,mol^{-1}$, respectively. The Hill coefficient was 1.0; no allostericity was therefore observed.

The O_2- and CO-binding parameters are summarized in Table 2. The high O_2-binding affinity of HSA-LH in an aqueous solution [$p_{1/2}(O_2)$: 14 mmHg at 25°C] compared to that in toluene (38 mmHg) mainly arises from the small O_2-dissociation constant. The CO binding parameters of HSA-LH, on the other hand, were almost the same as those in toluene. We considered that a highly polar amide-environment

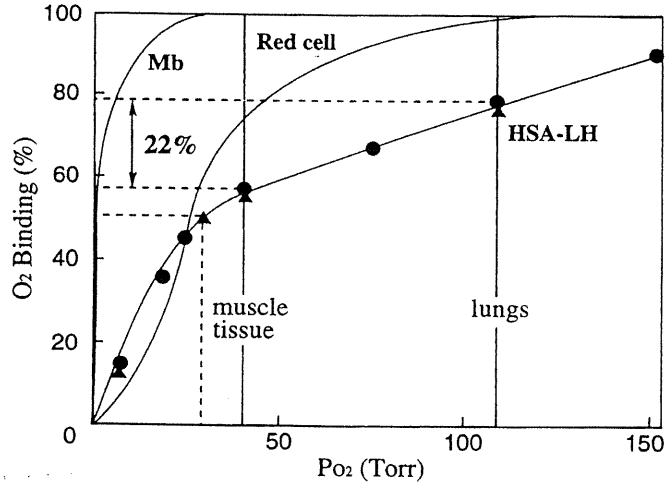

Fig. 5. O_2-equilibrium curve of HSA-LH solution at 37°C.

Table 2

O_2- and CO-binding parameters of HSA-LH solution at 25°C

System	Solution	O_2			CO		
		$p_{1/2}$ (mmHg)	$10^{-7} k_{on}$ ($M^{-1} s^{-1}$)	$10^{-2} k_{off}$ (s^{-1})	$10^{2} p_{1/2}$ (mmHg)	$10^{-6} k_{on}$ ($M^{-1} s^{-1}$)	$10^{2} k_{off}$ (s^{-1})
HSA-LH	pb[b]	14	1.9	4.3	1.4	3.9	8
r-HSA-LH[a]	pb[b]	13	1.9	4.3	1.7	3.5	9
LH	toluene	38	16	46	0.6	2.9	17
Hb (R-state)	pb[c]	0.22	3.3	0.13	0.14	4.6	0.9
Red cell	pb[b]	8.8	0.0011	0.0016	57	0.014	1

[a] r-HSA: recombinant HSA.
[b] pb: phosphate buffer (pH 7.4).
[c] pb: phosphate buffer (pH 7.0).

surrounds the LH moieties constructed by polypeptides and causes the decreasing $k_{off}(O_2)$ value. This assumption was supported by the result of a high O_2-binding affinity of LH in amide solution, e.g. $p_{1/2}(O_2)$: 0.8 mmHg in DMF.

The k_{on} and k_{off} are significantly high relative to those of red cells and hemoglobin. The experiments on the CO-binding kinetics of HSA-LH on a nanosecond time scale exhibited a rapid geminate recombination reaction, which is often observed in hemoglobin and also in the HSA-protoheme-CO complex [13].

As described above, it is quite remarkable that the molar absorption coefficient, the O_2- and CO-binding affinities, and the half-life of oxygenated species of HSA-LH are independent of the binding numbers of LH from one to eight.

From the O_2-transporting efficiency (22%) in Fig. 3, the transporting amount of oxygen was calculated as shown in Table 3. The concentration of HSA was adjusted to that in blood (5 wt%; 0.75 mM) to control the colloidal osmotic pressure. The 5 wt% of HSA-LH solution (LH/HSA: 8) can transport 3.4 ml dl^{-1} of oxygen during the circulation between the lungs (pO_2: 110 mmHg) and the mixed venouse system

Table 3

O_2-Transport by HSA-LH solution at 37°C

System	[HSA] (mM)	Heme/HSA (molar ratio)	[Heme] (mM)	O_2-transport (ml dl^{-1})
HSA-LH	0.75	1	0.75	0.42
	0.5	4	2.0	1.1
	0.75	4	3.0	1.7
	0.75	8	6.0	3.4
human blood	—	4	9.2	5.9

(pO_2: 40 mmHg). This corresponds to about 60% of the O_2-transporting amount of human blood (5.9 ml dl^{-1}), because the heme concentration of the HSA-LH solution (6.0 mM) is lower than that of blood (9.2 mM) ([hemoglobin]: 15 g dl^{-1}). The OTE value can, however, be increased by using other heme derivatives with a low $p_{1/2}(O_2)$, and the transporting amount of oxygen can be improved by increasing the HSA concentration for in vivo use.

In vivo O_2-transporting capabilities of HSA-LH solution

To evaluate the O_2-transporting capabilities of the HSA-LH, the physiological responses on exchange transfusions with HSA-LH solution in hemorrhagic shocked rats were observed. Ten male Wister rats (male, 337 ± 12 g) were anesthetized with an intraperitoneal injection of pentobarbital (50 mg kg^{-1}) and catheters (outer diameter 0.8 mmϕ), inner 0.5 mmϕ) were introduced into the right jugular vein for infusion and into the right common carotid artery for blood withdrawal. Abdominal aortic blood flow was measured as an indicator of cardiac output.

70% of the estimated total blood volume (56 ml kg^{-1}) was first exchanged with 5 g dl^{-1} HSA solution by withdrawal-infusion cycles (1 ml min^{-1}), leading to a certain decrease in the hematocrit value (Hct) of 14%. After 10 min, 40% of the circulatory volume was shed via the arterial line, affording excessively hemorrhagic rats with an Hct of only 8%. The same volume of HSA-LH solution (5 g dl^{-1}, LH/HSA: 4) was then intravenously injected, where the HSA solution was also used for relative data. Blood samples for arterial and venous blood gas analyses were taken at (i) before exchange, (ii) after 70% exchange, (iii) 10 min later, (iv) after 40% shedding, (v) after injection of HSA-LH (or HSA) and (vi) 30 min after injection. The mean arterial pressure (MAP) and renal cortical tissue O_2-tension [ptO_2(R)] were measured according to our previously reported procedures [29,30].

The MAP declined to 60% of the baseline level after 70% exchange with HSA solution and decreased to 30% immediately after shedding. The low MAP value could not be recovered simply by injection of the same amount of HSA, and all the rats died within 30 min. In contrast, the HSA-LH group showed a significant increase in MAP up to 95% of the baseline which was sustained even after 30 min (Fig. 6(a)).

Aortic blood flow was slightly increased by the initial 70% exchanging but definitely decreased to 50% after shedding. Although HSA injection tended to enhance the blood flow, the HSA-LH injection demonstrated much larger efficacy; complete recovery to the value before injection (Fig. 6(b)). The pH was somewhat decreased in the HSA-LH group but dramatically declined in the HSA group. paO_2 tended to increase even in the HSA-LH group during the experiment (max. ca 140% of the baseline), probably due to hyperventilation induced by slight acidosis.

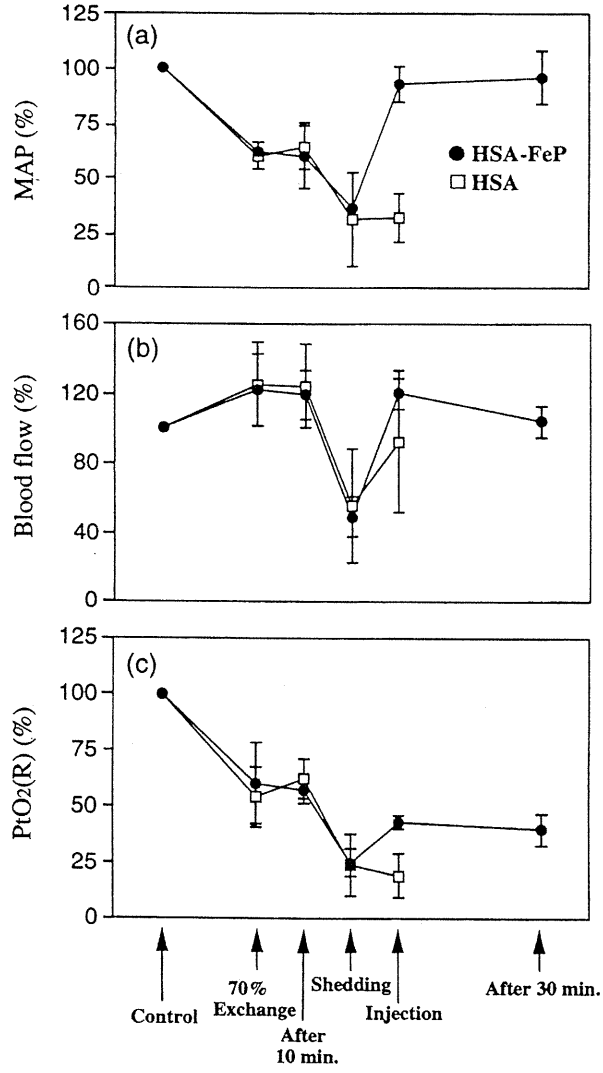

Fig. 6. Change in hemodynamic parameters during the exchange transfusion with HSA-LH solution. (a) MAP, (b) blood flow, (c) $ptO_2(R)$ shown as percentages of the basal values.

$ptO_2(R)$ generally correlates with systemic O_2-delivery, which is considered to be the product of arterial O_2-content and cardiac output. Because renal perfusion is controlled in response to a change in systemic hemodynamics, it first decreases under systemic hypoperfusion due to redistribution of systemic blood flow. Consequently, $ptO_2(R)$ is relatively sensitive to a subtle change in the systemic circulation. The monitored $ptO_2(R)$ was also obviously increased by injection of the HSA-LH solution up to 50% of the baseline (Fig. 6(c)). After the exchange transfusion, the HSA-LH group survived for more than 12 h until sacrifice, while there were significant differences in life time from the HSA group. These results are preliminary but clearly show that HSA-LH solution transports oxygen in vivo and is useful to save life at least from the hemorrhagic state.

Totally synthetic O_2-carrier with recombinant human serum albumin

Moreover, there is much current interest in recombinant HSA (rHSA) which has been recently manufactured by gene cloning and expression in *Phichia pastoris*, etc. [31]. We have already obtained several results for a rHSA-FeP complex as a totally synthetic O_2-carrier (Table 2), and a more detailed study is now being undertaken. These compounds should act as new O_2-carrying hemoprotein molecules instead of the hemoglobin in the bloodstream.

Acknowledgment

This work was partially supported by the Health Science Research Grants (Artificial Blood Project), the Ministry of Health and Welfare, Japan.

References

[1] U. Kragh-Hansen, Molecular aspects of ligand binding to serum albumin. Pharmacological Reviews, 33 (1981) 17–53.

[2] J.R. Brown, Serum albumin: amino acid sequence. In: Albumin structure, function and uses, V.M. Rosenoer, M. Oratz, M.A. Rothschild (Eds.), Pergamon Press, New York, pp. 27–51, 1976.

[3] T. Peters Jr., Serum albumin, The plasma proteins. Structure, functions and genetic control, 2nd Ed, F.W. Putman (Ed.), Academic Press (New York), pp. 133–181, 1975.

[4] T. Peters Jr., Serum albumin. Advances in Protein Chemistry, 37 (1985) 161–245.

[5] T. Peters Jr., All about albumin. Academic Press, New York, 1996.

[6] H.N. Little, J.B. Nelands, Binding of hematin by human serum albumin. Nature, 170 (1960) 913–915.

[7] G.H. Beaven, S.-H. Chen, A. D'Albis, W.B. Gratzer, A spectroscopic study of the haemin-human-serum-albumin system. Eur. J. Biochem., 41 (1974) 539–546.

[8] U. Muller-Eberhand, W.T. Morgan, Porphyrin-binding proteins in plasma. Ann. NY Acad. Sci., 244 (1975) 625–650.

[9] P.A. Adams, M.C. Berman, Kinetics and mechanism of the interaction between human serum albumin and monomeric haemin. Biochem. J., 191 (1980) 95–102.

[10] W.T. Morgan, A. Smith, P. Koskelo, The interaction of human serum albumin and hemopexin with porphyrins. Biochim. Biophys. Acta, 624 (1980) 271–285.

[11] A.A. Lamola, I. Asher, U. Muller-Eberhard, M. Poh-Fitzpatrick, Fluorimetric study of binding of protoporphyrin to haemopexin and albumin. Biochem. J., 196 (1981) 693–698.

[12] L. Casella, M. Gullotti, S. Poli, L. Gioia, Haem-protein interactions. The binding of haem complexes to serum albumin, Gazzetta Chmica. Italiana, 123 (1993) 149-154.

[13] M.C. Marden, E.S. Hazard, L. Leclerc, Q.H. Gibson, Flash photolysis of the serum albumin-heme-CO complex. Biochemistry, 28 (1989) 4422–4426.

[14] J. Bonaventura, M. Brouwer, T. Brouwer, B. Cashon, S. Cameron, Presented in part at the 11th Congress ISABI, Boston, July, 1994.

[15] E. Tsuchida, T. Komatsu, S. Kumamoto, K. Ando, H. Nishide, Synthesis and O_2-binding properties of tetraphenylporphyrinatoiron(II) derivatives bearing a proximal imidazole covalently bound at the β-pyrrolic position, J. Chem. Soc., Perkin Trans., 2, 1995 (1995) 747–753.

[16] T. Komatsu, K. Ando, N. Kawai, H. Nishide, E. Tsuchida, O_2-Transport albumin: a new hybrid-haemoprotein incorporating tetraphenylporphyrinatoiron(II) derivative. Chem. Lett., 1995 (1995) 813–814.

[17] E. Tsuchida, K. Ando, H. Maejima, N. Kawai, T. Komatsu, S. Tekeoka, H. Nishide, Properties of and oxygen binding by albumin-tetraphenylporphinato-iron(II) derivative complexes. Bioconjugate Chem., 8 (1997) 534–538.

[18] T. Komatsu, K. Hamamatsu, S. Takeoka, H. Nishide, E. Tsuchida, Human serum albumin bound synthetic hemes as an oxygen carrier: determination of equilibrium constants for heme binding to host albumin. Art. Cells, Blood Subs. Immob. Biotechnol. 1998, in press.

[19] D.C. Carter, X.-M. He, S.H. Munson, P.D. Twigg, K.M. Gernert, M.B. Broom, T.Y. Miller, Three-dimensional structure of human serum albumin. Science, 244, (1989) 1195–1198.

[20] T.Y. Miller, X.-M. He, D.C. Carter, J. Cryst. Growth, 122 (1992) 306–309.

[21] X.M. He, D.C. Carter, Atomic structure and chemistry of human serum albumin. Nature, 356 (1992) 209–215.

[22] D.C. Carter, J.X. Ho, Structure of serum albumin. Advances in Protein Chemistry, 45 (1994) 153–203.

[23] Z. Hrkal, S. Klementova, Bilirubin and haem binding to human serum albumin studied by spectroscopy methods. J. Biochem., 16 (1984) 799–804.

[24] M. Momenteau, C.A. Reed, Synthetic heme dioxygen complexes. Chem. Rev., 94 (1994) 659–698.

[25] E. Tsuchida, T. Komatsu, K. Arai, H. Nishide, Synthesis and dioxygen-binding properties of double-sided porphyrinatoiron(II) complexes bearing covalently bound axial imidazole. J. Chem. Soc., Dalton Trans., 1993 (1993) 2465–2469.

[26] J.E. Fletcher, A.A. Sector, J.D. Ashbrook, Analysis of macromolecule-ligand binding by determination of stepwise equilibrium constants. Biochemistry, 9, (1970) 4580–4587.

[27] J.D. Ashbrook, A.A. Sector, E.C. Santos, J.E. Fletcher, Long chain fatty acid binding to human plasma albumin. J. Biological Chem., 250 (1975) 2333–2338.

[28] H. Sakai, S. Takeoka, Y. Seino, E. Tsuchida, Suppression of methemoglobin formation by glutathione in a concentrated hemoglobin solution and in a hemoglobin vesicle. Bull. Chem. Soc. Jpn., 67 (1994) 1120–1125.

[29] Y. Izumi, H. Sakai, K. Hamada, S. Takeoka, T. Yamahata, R. Kato, H. Nishide, E. Tsuchida, K. Kobayashi, Physiologic responses to exchange transfusion with hemoglobin vesicles as an artificial oxygen carrier in anesthetized rats: changes in mean arterial pressure and renal cortical tissue oxygen tension. Crit. Care Med., 24 (1996) 1869–1873.

[30] H. Sakai, S. Takeoka, S.-I. Park, T. Kose, H. Nishide, Y. Izumi, A. Yoshizu, K. Kobayashi, E. Tsuchida, Surface modification of hemoglobin vesicles with poly(ethyleneglycol) and effects on aggregation, viscosity, and blood flow during 90% exchange transfusion in anesthetized rats. Bioconjugate Chem., 8 (1997) 23–30.

[31] A. Sumi, W. Ohtani, K. Kobayashi, T. Ohmura, K. Yokoyama, M. Nishida, T. Suyama, Purification and physicochemical properties of recombinant human serum albumin. Biotechnology of Blood Proteins, Vol. 227, C. Rivat, J.F. Stolts (Eds.), John Libbey Eurotext, 1993, pp. 293–298.

Blood Substitutes — Present and Future Perspectives
E. Tsuchida (Editor)

CHAPTER 29

The Impact of Blood Substitutes on the Blood Program

S. Sekiguchi

Hokkaido Red Cross Blood Center, Sapporo, Japan

Introduction

The major tasks of the national blood program are the establishment of transfusion safety and the self-sufficiency of blood products. The elimination of untoward transfusion-associated side effects, especially allosensitization and transfusion-transmitted infectious diseases are important goal in transfusion medicine. The accomplishment of domestic self-sufficiency in blood and plasma component products are also strongly requested by WHO, since Japan has been consuming approximately 30% of worldwide used albumin. Very recently, coagulation factor VIII has become available in a recombinant form. In addition, recombinant human albumin will be artificially produced within several years. Artificial oxygen carriers are also expected to be applicable as substitutes for red blood cell transfusion. These substitutes will greatly modify the ongoing transfusion medicine by reducing the chance of allogeneic transfusion. As well, critically ill patients suffering from massive hemorrhage at an emergency condition will be rescued by use of artificial oxygen carriers. The potentials and the roles of these blood substitutes in transfusion medicine should be addressed.

Demand and supply of blood products in Japan

Whole blood and blood component products

Japan has already established self-sufficiency in whole blood and blood components for transfusion [1]. The changes in the number of blood donors are shown in Fig. 1. In 1986, 400-ml whole blood donation and apheresis donation were introduced. After their introduction, the number of 200-ml whole blood donation has been decreasing gradually, in contrast, the number of 400-ml donation has increased. In 1996, percentages of 400-ml and 200-ml donations were 44.1% and 35.7%, respectively. The number of apheresis donors has been relatively constant in these several years, consisting of 20.2% of donors in 1996. Majority of apheresis donors are donating platelet concentrates. Figure 2 shows a yearly profile of the platelet units supplied and composition of the unit number. It is obvious that platelet supply derived from 200-ml

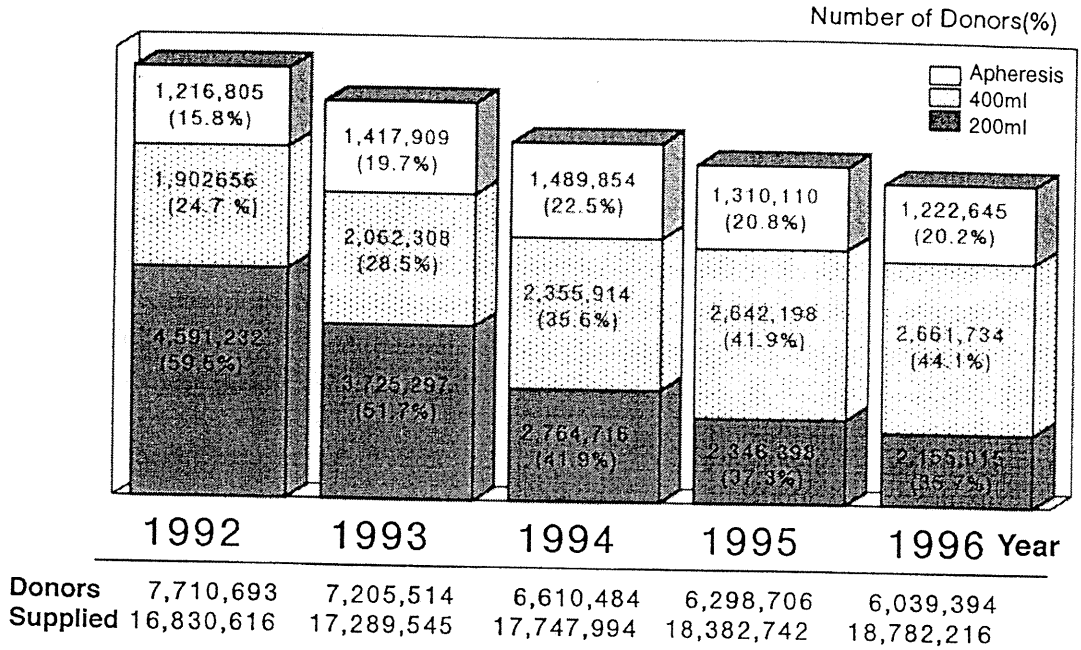

Fig. 1. Annual collection of blood.

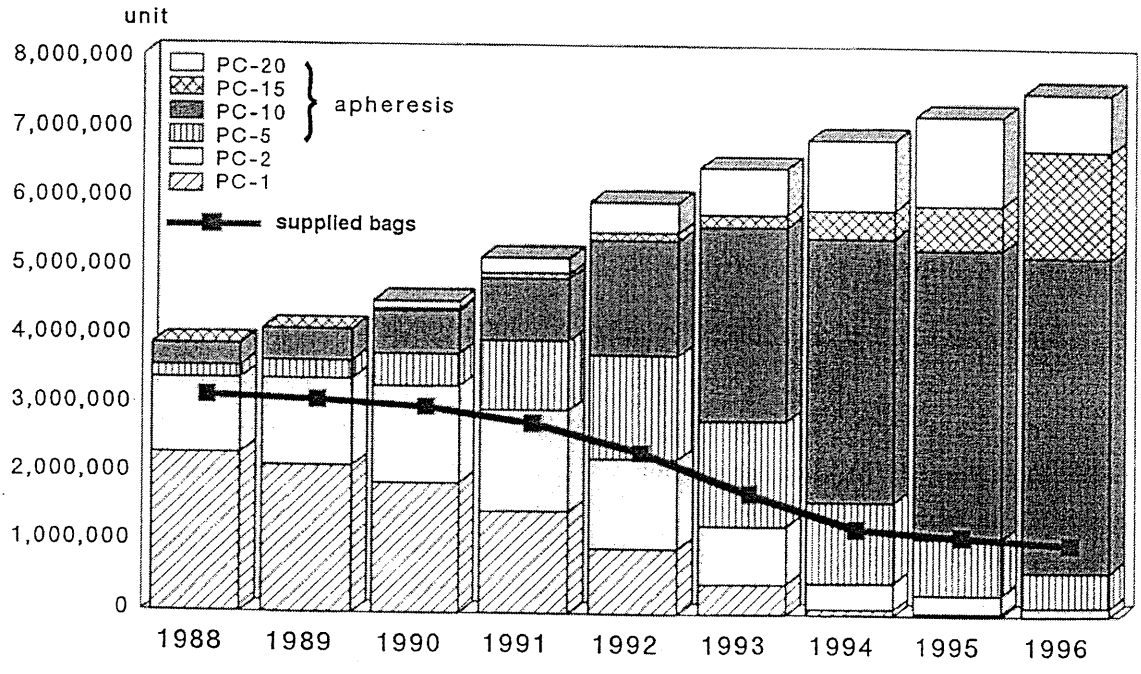

Fig. 2. Yearly profile of platelet supply classified by unit number of products.

and 400-ml donation (PC-1 and PC-2) has diminished yearly. At present the majority of platelet supply is from apheresis PC-10, PC-15 and PC-20. PC-1 or PC-2 is now rarely used in the pediatric cases. In contrast with the increasing number of platelet units, the donor number has decreased, because higher unit apheresis products occupy the majority.

The yearly supply of blood products is again shown in Fig. 3, including fresh frozen plasma. As already mentioned, the number of platelet supply markedly increased, but those of red cell concentrates and fresh frozen plasma show almost horizontal line. The usage of whole blood has decreased. Recent advance in chemotherapies and bone marrow transplantation in the oncology and hematology medicine, the necessity of platelet transfusion remarkably increased. In order to meet the demand, the introduction of apheresis protocol must have been necessary.

Plasma derivatives

This term consists of blood coagulation factors, serum albumin, and globulin. The use of plasma derivatives once increased around in 1975 in Japan, and in those periods the most of the products or their source plasma were then imported from other countries through commercial pharmaceutical manufactures. For this reason the percentage of the self-sufficiency of plasma products reduced to less than 10%. Thereafter, Japanese Red Cross Society established the plasma fractionation center to achieve self-sufficiency in plasma products in Japan. Recent self-sufficiency ratios of plasma products are listed in Table 1. Self-sufficiency for factor VIII is 66% in 1996,

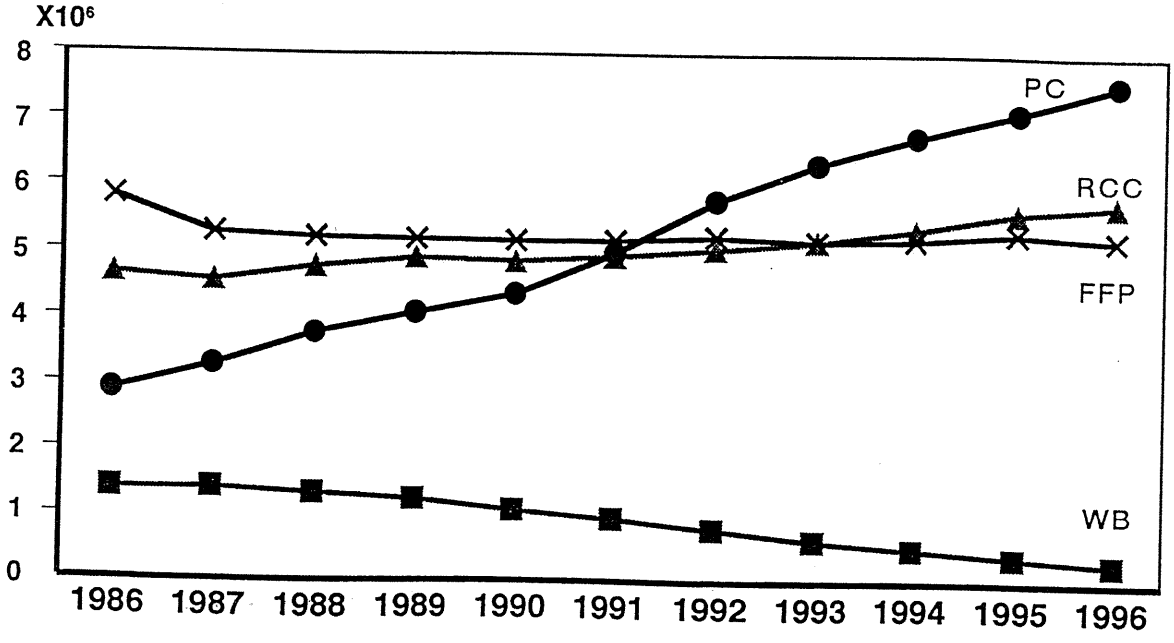

Fig. 3. Distribution of blood and blood components in units.

Table 1

Self-sufficiency of plasma products (1996)

Source plasma	74×10^4 l	Self-sufficiency
Factor VIII	$12{,}800 \times 10^4$ U	66%
Factor IX	250×10^4 U	76%
Albumin	18,800 Kg	25%
IV Globulin	1600 Kg	48%

but another 34% of factor VIII is supplied by recombinant factor VIII product also in Japan. The recent advance of molecular biological technology enables us to produce recombinant factor VIII. So at present the sum of plasma-derived factor VIII reaches 100% of the demand. During processing blood coagulation factors, factor VIII is first extracted from the source plasma, then factor IX is purified. Owing to this process, the problem of collecting source plasma for the production of factor IX will not separated exist. Unfortunately, both albumin and globulin still remain to be self-satisfied. Especially for albumin, its consumption in Japan consists of 20–30% of worldwide products. Some regulation must be introduced for the clinical usuage of albumin, but there are still attempts for clinicians to administer a large amount of albumin for edematous patients and patients with ascites.

Unused blood products

Due to the change of balance between the demand and supply of blood products and the presence of disqualified donors, a part of blood products will remain unused. The ratios are listed in Table 2. The ratios of outdated products from 400-ml and 200-ml

Table 2

Unused red blood cell products in Japan of 1996

Bags	400 ml	200 ml
Donations	2,660,000	2,110,000
Supplied Products	2,173,000	1,635,000
Unused Products	487,000	475,000
% of Donations	(18%)	(22%)
Disqualified Products	313,000	211,000
% of Donations	(12%)	(10%)
Outdated Products	174,000	264,000
% of Donations	(6%)	(12%)

Finally, total volume of outdated red cell products is estimated to be 84,680 l.

donations are 6% and 12%, respectively. Finally, total volume of outdated red cell products is estimated to be 84,680 l. Unless specially considered, these outdated products will be discarded. In order to maintain voluntary donations, it is desirable to apply these products for another usage. For example, from outdated red blood cells, we can recover hemoglobin and use it as a source material for hemoglobin-based substitutes. This is one of the reasons why we are attempting to develop red cell substitutes.

Transfusion-associated adverse effects and their prevention

The recent advancements in the donor screening tests mean that blood products have become increasingly safer in terms of transfusion-related viral transmission. Figure 4 shows the number and detail of transfusion-associated adverse effects reported to the Japanese Red Cross Central Blood Center. Now it has become mandatory for hospitals to report cases to the corresponding Red Cross Blood Center when adverse reactions occur. In 1996, about 700 cases of adverse reactions were reported. The majority of cases are non-hemolytic adverse reactions such as skin reactions, febrile reactions and anaphylaxis reactions. Seventy-four suspected cases of post-transfusion hepatitis were reported, but the precise analyses and comfirmative tests identified 2 cases to be highly probable of post-transfusion hepatitis. Another critical adverse reaction is graft-versus-host disease (GVHD). Sixty suspected GVHD cases were reported, 11 cases of which were confirmed to be definite GVHD cases by use of microsatellite-DNA examination. Figure 5 shows the number of reported transfusion-associated GVHD cases. Since information concerning GVHD has become common in the clinical field, the number of suspected cases has been increasingly

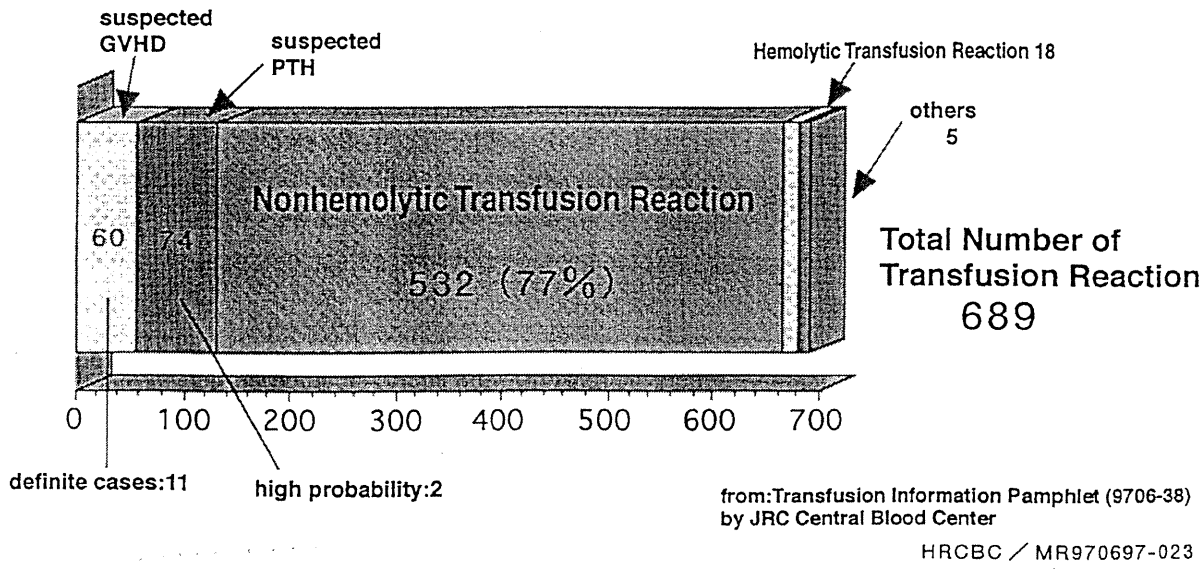

Fig. 4. The number of transfusion reactions in Japan (1996).

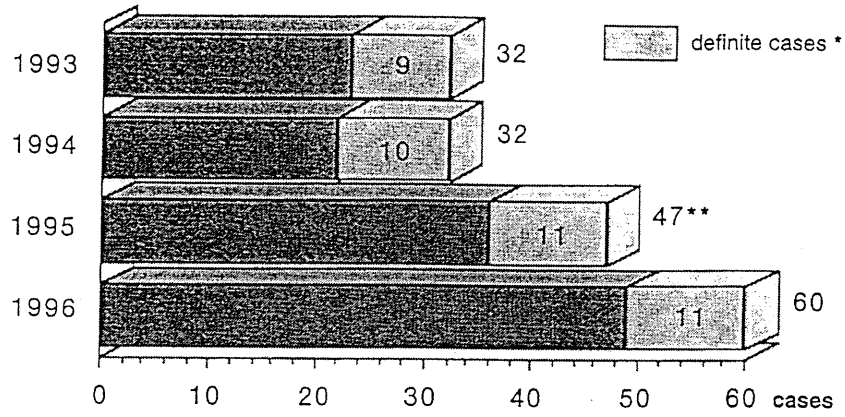

Fig. 5. The number of TA-GVHD cases reported to Japanese Red Cross Blood Centers.

reported. In contrast, however, around 10 cases still occur each year and the complete avoidance of GVHD has not been achieved yet [2].

Transfusion-associated viral transmission is still a critical problem. There still exists the risks of acquiring HIV and viral hepatitis. After the introduction of an anti-HIV test into the donor screening test in Japan in 1996, the first HIV infection case was reported early in 1997. Table 3 shows the probability of HIV transmission through the blood transfusion in the USA and Japan. Compared with the USA, the probability of transfusion-associated HIV transmission is fairly low, but still zero-risk has not been achieved. According to the statistical estimation, 1 bag out of 4.3 million bags may escape from the current screening test which can not detect donors in the window period of viral infection. The calculation indicates that 1 or 2 recipients will be transmitted HIV infection through blood transfusion annually in Japan. One remarkable point is the significantly higher risk in the metropolitan area in Japan. The HIV-positive ratio in that area is around 3 times higher than average ratio.

Table 3

Probability of HIV transmission by blood transfusion

	HIV positive rate of voluntary donors	Risk of HIV transmission by blood transfusion
USA (1994)	9.7 : 100,000 people	1 : 340,000 unit
JAPAN (1996)	0.76 : 100,000 people	1 : 4,320,000 bag
	(2.1 : 100,000 people)	(1 : 156,000 bag)

( ): The Metropolitan area.

Table 4

Estimated risk of major transfusion reaction

Major transfusion reactions	Reported transfusion reactions from hospitals to blood centers		
	No. (follow-up completed)	Cases of high-probability caused by transfusion	Incidence
Hepatitis B	69(22)	3	1/1,590,000
Hepatitis C	81(41)	1	1/4,770,000
HIV	1	1	1/12,000,000
HTLV-I	0	0	0
GVHD	139	30	1/160,000
Shock	239	—	1/20,000

From: Transfusion Information Pamphlet (9705-37), by JRC Central Blood Center.

The summary of major transfusion-associated adverse reactions and their frequencies is listed in Table 4. Since a great deal of effort has been made to reinforce the virus screening tests, the incidence of adverse reactions has decreased in Japan. With regard to hepatitis B, after the introduction of the combination analysis system using anti-HBc antibody and HBs antigen, the transfusion-associated hepatitis B has remarkably reduced in number and now the risk is about 1 case out of 1.5 million transfusion cases in Japan. A similar situation is also made possible for hepatitis C and the risk is about 1 case out of 4.7 million transfusion cases. There has been no report relating to transfusion-associated HTLV-1 transmission. In order to reduce the transfusion-associated risks to "zero", the development of blood substitutes should be necessary.

Future aspect of transfusion medicine

It is necessary to establish the safer transfusion or "zero" risk blood transfusion. The reduction of homologous blood transfusion is one of the main strategies and the prevailing of the autologous blood transfusion should also be the point. Again the efforts to reinforce the laboratory screening tests should be conducted further. The strategy to remove viruses or virus inactivation of the blood products should be further applied. In order to reduce the adverse reactions relating to contaminating leukocytes, the system to remove leukocytes or inactivate leukocytes must be prevailed in the clinical field. The development of blood substitutes will cover the functions of plasma, red cells and platelets. In addition, recent advancements in molecular biology will enable us to apply several hematopoietic cytokines to regulate the hematopoiesis. If we can expand hematopoietic stem cells, progenitor cells and mature functioning cells in a mega-culture system, it will become applicable to transfuse or transplant cultured cells into recipients (Fig. 6).

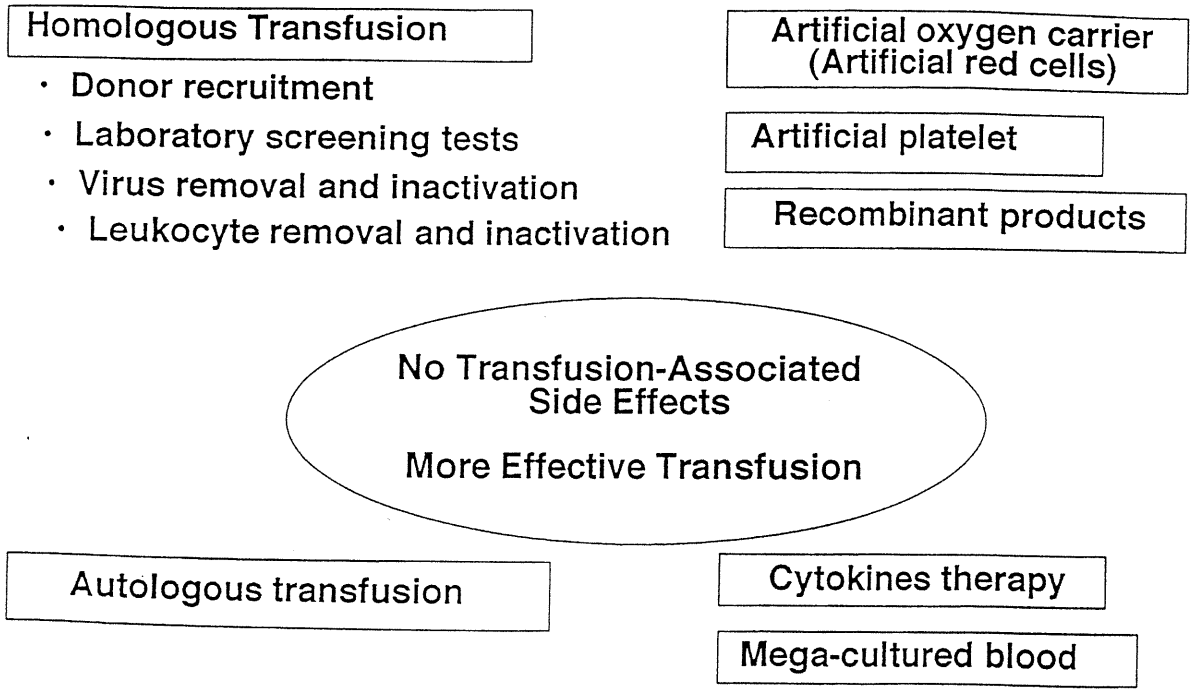

Fig. 6. Blood transfusion in the future.

Among them, the development of red cell substitutes have become near to practical usage. It is my opinion that the red cell substitutes will be used for the blood loss below 1200 ml and a further blood loss will be rescued by autologous blood or combined use of erythropoietin. The application protocol of artificial red cells should be discussed officially in early convenience [3].

Summary

The final goal of the blood program is to create a transfusion system which completes self-sufficiency by non-remunerated voluntary donors and supplys safer blood and blood products with "zero" transfusion-associated adverse reactions. To reach this goal, blood substitutes and substitute therapies can contribute a great deal in the near future.

References

[1] S. Sekiguchi, Donor apheresis: recent advances and future development, Vox Sang., 70 (Suppl 3) (1996) 126–134.

[2] S. Sekiguchi, M. Fujihara, Recent transfusion reaction and prevention. Journal of Clin. Anesth. (in Japanese), 21 (1997) 7–17.

[3] S. Sekiguchi, The impact of red cell substitutes on the blood service in Japan. Artif. Cells Blood Substit. Immobil. Biotechnol., 25 (1997) 53–60.

F. 人工血液

Key words：人工血液，赤血球代替物，ヘモグロビン小胞体，リピドヘム，cellular，acellular

1．人工血液の分類と開発の歴史

血液成分のうち，赤血球の役割（酸素運搬）以外については現在何らかの代替物が完成しているので，赤血球に代わる酸素輸送体が実現すれば現行の輸液利用技術と併せて，いわゆる人工血液が誕生する（**表 1**）．長期保存可能な人工血液が棚置きで常備されており，血液型に関係なく，緊急時に即応していつでもどこでも，必要量の人工血液を供給できる体制の確立は，近未来医療に期待される姿である．災害時や各種事故の際必ず必要となる緊急輸血に備えて，感染の心配がなく血液型に無関係で投与できる人工血液の常備は，国家的見地からも重要な危機管理施策であり，現在，パーフルオロケミカル乳剤，ヘモグロビン利用系（cellular 型，acellular 型），それに全合成系の 3 種類が臨床応用を目指して開発されてきている（**表 2**）[1]．

人工血液開発の歴史は，輸血技術確立の歴史そのものといえる[2]．貧血患者の治療に精製ヘモグロビン溶液を投与した von Stark らの実験（1898 年）以来，同様の試みが多く検討されるようになり，具体的には 1960 年代後半にあったいくつかの重要な発見（Hb 精製工程での膜成分除去率の向上が毒性低減につながる，架橋 Hb では腎排泄が抑制され滞留時間の延長が認

表 1　血液の成分とその代謝物

	血漿（55%）			血球（45%）		
血液	血漿タンパク アルブミン グロブリン フィブリノーゲン その他	電解質 (Na^+,K^+,Ca^{2+}, Mg^{2+},Cl^-,HCO_3^-, HPO_4^{2-})	栄養	赤血球 ヘモグロビン	白血球	血小板
代替物	血漿増量剤 デキストラン 修飾デンプン 抗生物質	電解質 輸液	脂肪乳剤	人工酸素運搬体	抗生物質 化学療法剤	凝固促進剤

表 2　赤血球代替物の分類と開発状況

分　類	製剤（企業または研究グループ）
PFC 乳剤	*Oxygent™ (Alliance), Oxyfluor™ (HemaGen)*
ヘモグロビン系	
非細胞（Acellular）型	
架橋型	*DCLHb™ (Baxter Healthcare)*
重合型	*PolyHeme™ (Northfield), HemoLink™ (Hemosol), Hemopure™ (Biopure)*
高分子結合型	*PEG-Hb (Enzon), PHP (Apex Bioscience)*
遺伝子組換型	*Optro™ (Somatogen)*
細胞（Cellular）型	Hemoglobin Yesicle (Waseda), NRC (Terumo)
リピドヘム系	Lipidheme Vesicles, Lipidheme-Microsphere, Albumin-Heme (Waseda)

斜字は臨床試験中

められる，など）が端緒となって，この領域の展開が飛躍的に進展した．この流れは今日，修飾 Hb（accelular 型）として継続されている．

まず，投与したストローマフリーHb(SFHb)が速やかに腎排泄されるのを防止する目的で分子量を大きくする方法が検討され，グルタルアルデヒドで架橋した，いわゆる重合 Hb が合成された（図 1）．この場合，分子数が低減するため，膠質浸透圧を低く（Hb 15 g/d*l* で 20 Torr）保つことができる[3]．そのほか，ピリドキサール-5′-リン酸（PLP）を結合させて酸素親和度を調節したヒト SFHb に，ポリオキシエチレン（POE）などの水溶性高分子の複数個を共有結合させて得た高分子結合 Hb も開発された[4]．また，サブユニット間を結合させた架橋 Hb では二量体への解離が抑制されるため，血中滞留時間が延長される．ジアスピリン分子内架橋 Hb は構造の明確さの点で優れており，酸素親和度（P_{50}：28 Torr）も良好である[5]．最近では，Hb 遺伝子を用いて培養菌体に産生させたリコンビナント Hb も注目を集めている[6]．

他方，Hb が小胞に包まれている赤血球の生化学的意義に着目し，早くから人工細胞の展開に尽力してきた Chang ら（1957 年）の知見をもとに，Toyota ら（1965 年）が取り組んだ cellular 型 Hb 小胞体の構成は，当時膜成分に適当な材料が得られず，造粒工程や粒径調整に困難が伴い，具体的展開には至らなかった．1977 年になってようやく，生体成分であるリン脂質を利用した Hb 小胞体（HbV）の検討が始まった[7]．これは 2, 3-ジホスホグリセリン酸を内包させ，P_{50} と Hill 係数を適当な値に調節したもので，ここに初めて血液と同じ赤色の人工赤血球が誕生した．その後，造粒法や成分組成を調整して，Hb の高濃度化，さらに被覆層数をできるだけ少なくする技術が確立され，現在赤血球代替物としての機能を十分に備えた HbV 製剤となっている．

また，輸血に代わる完全人工物投与の検討例は，酸素の物理的高溶解量を利用したパーフルオロケミカル乳剤（Fluosol-DA：ミドリ十字：1978 年）が最初で，医薬品認可も得られたが 1993 年以降生産されていない．一方，Hb の活性部位であるプロトヘムはグロビンから脱離すると酸素結合能を失うことはよく知られており，タンパク質の構築する立体枠組みの役割の重要性は広く認識されていた．1983 年，リン脂質類似構造の置換基をもつ鉄リピドポルフィリン誘導体（リピドヘム：Lh）を小胞体膜の二分子層間に分散配向させると，生理条件下で可逆的に酸素吸脱着できる全合成系人工赤血球（リピドヘム小胞体（LhV））が開発された[8]．ま

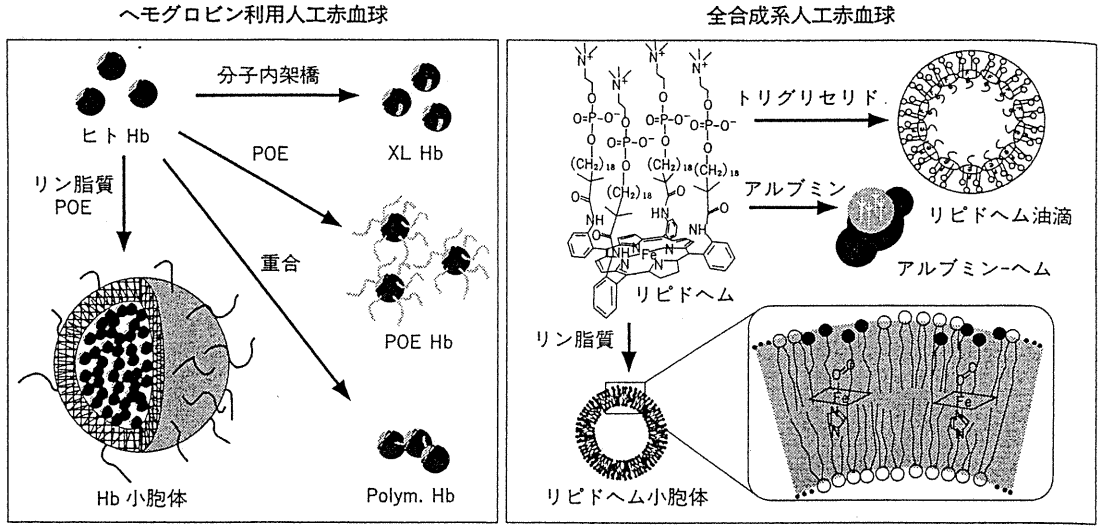

図 1　人工赤血球の構造

た，このリピドヘムの界面活性を利用して大豆油を乳化して得たリピドヘム油滴小球（LhM：粒径 200 nmϕ）は，栄養輸液と同様の構造をもつ[9]．ごく最近，ヒト血清アルブミン（HSA）に疎水性のリピドヘムを効率よく結合させた複合体（アルブミン-ヘム：AlbH）が，生理条件下で，酸素を可逆的に運搬できることも明らかにされた[10]．アルブミン 1 分子当たりヘム 8 分子までが結合する．さらに，リコンビナント HSA（r-HSA）にヘムを結合させた系の酸素運搬能力も HSA 系に比較して遜色なく，新しい全合成系酸素輸送体（酸素輸液：oxygen infusion）として期待がもたれている．

2．材料学的特性

修飾 Hb 系は優れた性能と保存安定度が確認されており，臨床第 III 相試験までが終了している例（米国）もあるが，膠質浸透圧や粘度の増大に起因する投与限界量の存在，低い酸素輸送量，化学修飾に伴う Hb の変性，血流中での比較的早いメト化など，膜構造を有する赤血球とは裏腹の問題が生じてくる．

高濃度 Hb（約 38 g/dl）をリン脂質小胞体の内相へ封入し，毛管流動と細網内皮系捕捉を考慮して粒径 200 nmϕ に揃えた Hb 小胞体については，すでに（一酸化炭素結合 Hb の物理化学的安定度がきわめて高くなる特徴を利用した）大量調製法が確立されている[11]．HbV は長期に保存した場合の形態安定度に問題を残していたが，改良が進み現在ではトレハロースを共存させた系を乾燥粉末化する技術など，注目すべき進歩もみられる[12]．

HbV をアルブミン溶液に分散させると，アルブミンとの相互作用により凝集を惹起する場合がある．しかし，これは表面をポリオキシエチレン（POE）鎖で修飾して完全に抑制されている[13]．表面修飾法は容易で，たとえば HbV 分散液に合成糖脂質や POE 脂質を添加するだけで自発的に外表面に取り込まれ，小胞体の表面修飾が完了する．

LhV の場合，最小粒子径は 30 nm まで調整が可能[7]，凍結乾燥した粉末は適量の水を添加して容易に再生できる．この分散液は非タンパ

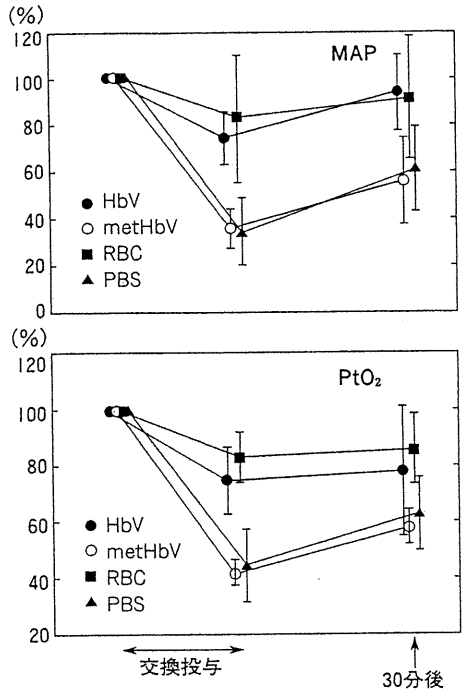

図 2 麻酔ラットに交換輸血後の末梢組織酸素分圧と平均動脈圧の推移

ク質の純人工物であるので，将来的には無菌雰囲気の化学プラントでの大量生産と安価供給が可能である．さらに興味深いことは，これらリピドヘムが水中で自己集合して，分子集合体（小胞体型：粒径 200 nmϕ）を形成することである[14]．もちろん，この場合も酸素の可逆的吸脱着が可能で，粒子一つ当たり 1.5×10^5 モル（ヘムと同モル数）の酸素分子を結合できる．

3．機能と臨床応用

HbV 系の場合，PLP を Hb とともに包み込むと，酸素親和度と Hill 係数のどちらも適切な値（P_{50}：27～39 Torr, n：1.7～2.7）に調節できる．前臨床評価はすでに最終段階にきている．リン酸緩衝生理塩水溶液（PBS）に分散させた HbV［Hb 濃度（10 g/dl），酸素親和度（P_{50}）：32 Torr］を麻酔ラットに交換輸血（40%）し，末梢組織（腎皮質）の酸素分圧や平均動脈圧（MAP）の推移を観測すると，計算上だけでなく実際にヒト血液と同等の酸素輸送量を示す（図 2）．PBS 群や metHbV 群では交換後の MAP は交換前の 35%まで低下，左腎皮質酸素分圧にも同様の傾向を認めているのに対し，HbV 群では 75%値を維持，同 Hb 濃度の洗浄赤血球（RBC）群とほぼ同様な結果となっている[15]．

また，HbV を用いた交換輸血 90%以上の試験では，血圧，末梢酸素分圧，心拍出量ともに RBC 群と同様に安定した推移を示し，生存率は 100%である[13]．現在，細網内皮系捕捉や代謝の影響，血中滞留時間の延長とメト化率抑制が検討されている．

全合成系リピドヘムの場合も，酸素親和度（P_{50}：30～40 Torr (37°C)）は赤血球の値（P_{50}：27 Torr）とほぼ同等で，たとえば肺-末梢組織間の酸素運搬効率は約 25 %となる．酸素結合速度（K_{on}：$10^7M^{-1}s^{-1}$）は，ヘム部自体が膜成分として機能するため赤血球の値に比べて 10^3 倍速い．各種の物理化学測定から，配位酸素の電子構造は Hb と同様であることが示されている．30%脱血ショック犬への LhV の投与試験では，いったん低下した混合静脈酸素分圧が投与後確実に上昇するので，リピドヘムが有効な酸素運搬体として作動していることが確認されている．

4．体内環境における安定度と体内動態

HbV を 50%交換輸血（ラット）した例については 30 日後の臓器病理所見は正常，血栓形成も全く認められず，95%交換輸血でも全例の生存が確かめられている．肝臓の網内皮系に一過性の貯留を認めるが，安全性はきわめて高い．しかし，最終的には網内皮系への取り込みが飽

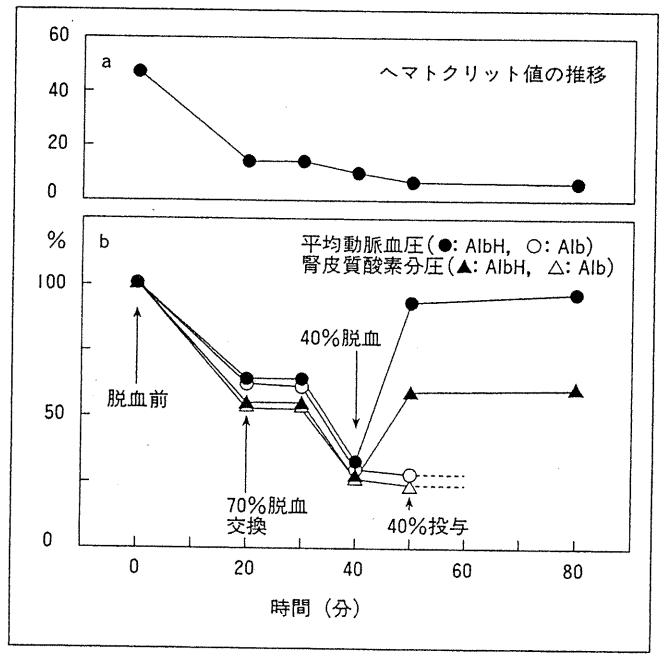

図 3　脱血交換試験における(a)ヘマトクリット値と(b)平均動脈血圧と腎皮質酸素分圧の変化

和された場合，エンドトキシンや感染に対して免疫機能が十分に作動するかの心配に対し，現在検討が進められている．肝脾機能は重要で，投与後脾臓における貪食能亢進は認められるものの，肝機能の異常例は少ない．血中滞留時間（半減期）は 16 時間程度，代謝は 48～72 時間以内で，各種データは 1～2 週間で正常範囲に戻っている．

リピドヘムの場合，血中滞留時間は 12 時間，急性毒性は認められず，ヘム濃度に対応した酸素運搬量が実測されている[16]．LhM 分散液を投与すると，血中脂質濃度は投与直後に上昇するが，24 時間後にはほぼもとの値に回復しており，注入された脂肪が体内で円滑に処理されていることが確認されている．脱血ショックラットへの AlbH 投与による回復試験では，確実に酸素輸送されており救命効果が明らかである（図 3）．

5．副作用

修飾 Hb 投与の場合には，投与後の血圧異常亢進が避けられない問題として残る．これは小さな Hb 分子が血管内皮細胞の接合部位を通過した後，平滑筋近傍にまで接近し，血管弛緩因子である一酸化窒素（NO）を捕捉するためである[15]．HbV では，このような投与直後の急激な血圧上昇は全く認められていない．

経皮透視法による皮下組織血管の微小循環動態の顕微観察からは，微小血管径の変化，血流速度，組織の酸素分布との相関が明らかにされている[17]．HbV 投与に際しては，修飾 Hb 系で観測される血管収縮は全く認められない．

6. 今後の動向

米国では，比較的加工調製が容易な分子内架橋型 Hb の開発に集中した推進を図っており，一部はすでに臨床第 III 相試験を終了，製造プラント完成を待って 1998 年中に上市を予定している．リコンビナント Hb 系，分子間架橋型 Hb 系でも臨床試験が進行しており，医療体制が十分整わない地域までを含めた世界的需要を睨んだ量産設備の具体化が進められている．わが国でも上述したように，HbV 系について前臨床最終評価試験成績をまとめる段階にある．

一方では，自己血輸血や erythropoietin 投与による赤血球増殖などの進展に伴い，現行輸血体制の変革も議論されるようになりつつあるが，当面は Hb 利用系赤血球代替物の促進が優先することになろう．しかし，将来はこれらヒト由来の Hb を用いる代替物展開よりも，赤血球に近い酸素輸送能力を十分発揮できる全合成系が重要であるとの認識が強まってきており，現在その推進が加速されている．赤血球代替物に併せて，いわゆる血液代替物として r-HSA, Factor-VIII, 人工血小板，人工免疫など，安全度の高い血液成分代替物の研究展開も急速で，人工血液が安定供給される日も間近いと考えてよい．

これらの話題に関連して，FDA がイヌ用の人工血液を認可した報道[18]がある．これはウシ血液からの製剤（オクシグロビン "Oxyglobin", Biopure 社）ですでに上市されており，いずれまとまった評価が明らかになろう．

1997 年 9 月 7 日～10 日（4 日間），第 7 回血液代替物国際会議（International Symposium on Blood Substitutes-Tokyo 1997）が東京で開催された．現在急進展している Hb 利用系人工赤血球の臨床試験結果も含めた最新の話題を中心に議論が集中，この領域に国際的関心が集まっていることが明らかになった．

（土田英俊）

文 献

1) Tsuchida E (ed)：Artificial Red Cells, Materials, Performances and Clinical Study as Blood Substitutes, John Wiley & Sons, Chichester (1995)
2) 土田英俊，酒井宏水：血液代替物の展開．医学のあゆみ **172**：99-104 (1995)
3) Sehgal LR, Moss GS：Preparation and *in vitro* Characterization of Polymerized Pyridoxalted Hemoglobin. *Transfusion* **23**：158-162 (1983)
4) 岩下研二：酸素運搬機能を発現．化学 **43**：440-441 (1981)
5) Malcolm DS：Characterization of the Hemodynamic Response to Intravenous Disaspirin Crosslinked Hemoglobin Solution in Rats. *Artif Cells Blood Substit Immobil Biotechnol* **22**：91-107 (1994)
6) Gerber MJ, Stetler GL, Templeton D：Recombinant Human Hemoglobin Designed for Use as an Oxygen Delivering Therapeutic. Artificial Red Cells, Materials, Performances and Clinical Study as Blood Substitutes, Tsuchida E (ed), pp187-197, John Wiley & Sons, Chichester (1995)
7) Djordjevich L, Miller IF：Lipid Encapsulated Hemoglobin as a Synthetic Erythrocyte. *FASEB Proceedings* **36**：567 (1977)
8) Tsuchida E, Nishide H：Hemoglobin Model-Artificial Oxygen Carrier Composed of Porphinatoiron Complexes. *Top Curr Chem* **132**：64-99 (1986)
9) Komatsu T, Matsubuchi E, Nishide H, Tsuchida E：Lipidheme/Microsphere. A New