- Eliminate COI by separation of industrial sponsor from investigators.
- Share HBOC products developed by consortium members with other member investigators.

29.4.4 Structure

In the early stage of HBOC development, a company's research collaboration is usually limited to one or two close collaborators. But as the company grows with substantial research revenue, the collaboration expands to multiple academic investigators (a traditional one industry–multiple investigator model; see Figure 29.1). In this industry-centered model, an academia–industry relationship is generally driven by the sponsoring industry, as the company selects projects/investigators based on its needs. The nature of developmental work and research results are generally shared only within the circle of collaborators, especially when problems are encountered or study outcomes are negative. Thus, unless specifically requested, there is virtually no possibility that outside investigators can contribute to the resolution of any issues associated with a particular product.

To help resolve the issues associated with the traditional collaboration model in HBOC development, we propose a new academia–industry collaboration model that is, at least in concept, designed to be more objective and transparent. We envision an HBOC research consortium consisting of multiple HBOC producers and multiple academic investigators

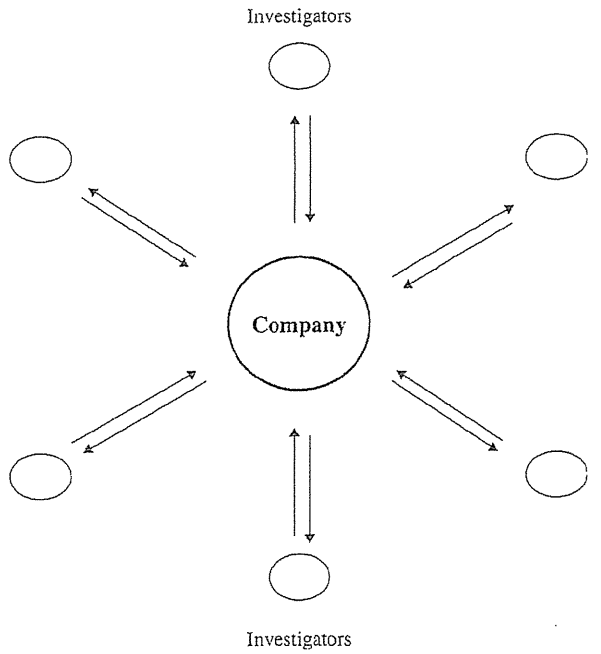

Figure 29.1 *A traditional model of academia–industry collaboration.*

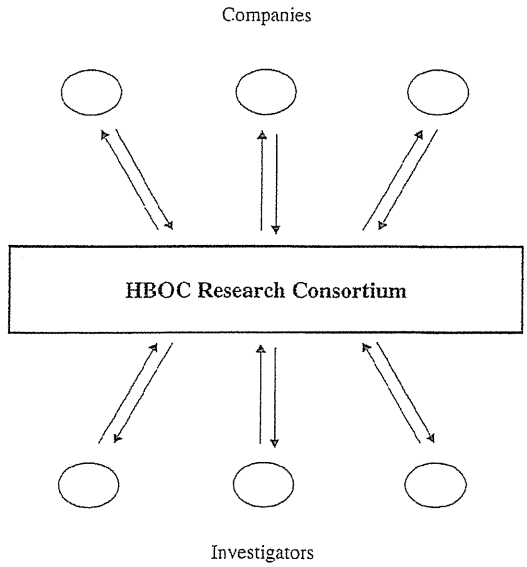

Figure 29.2 *A new model of academia–industry collaboration in HBOC development.*

in the field, each with distinct expertise (Figure 29.2). This approach will allow pooling of the expertise and resources needed to resolve issues associated with current HBOCs and to develop a new generation of improved products for eventual regulatory approval. In addition, the joint effort will enhance the chances of securing the research funds necessary for such work. The involvement of multiple HBOC companies which produce distinct HBOC products will enhance the chances of developing a successful product(s). As presented in the guiding principles, while honoring HBOC producers' IPs and other agreed-upon rights, the consortium will adopt an unrestricted publication policy for all study results, whether positive or negative in outcome. This will facilitate open discussion of any problems/issues and thereby ensure quicker resolution. In this model, HBOC producers do not have direct sponsor–investigator relationship with researchers. Rather, they are separated by a 'buffer', the consortium. This separation of sponsors and investigators will minimize potential COI and bias issues, thus increasing objectivity in the conduct of research studies and interpretation of results. If practical, the industrial developers and research investigators could be blinded to each other by the consortium by removing or codifying any words/phrases that would reveal their identity in the study protocol, product description, and communications.

29.4.5 Operation

The consortium may be based on an academic institution or it could be operated as a standalone nonprofit entity established for the purpose in a location convenient to its members and staff. The consortium will be run by a director under an operational policy/set of directives generated by a steering/executive committee. The consortium director must not have any COI issues with any of the industrial and academic participants. The executive

committee will be composed of participating academic and industrial members, as well as some independent members as appropriate (e.g. financial sponsors, selected independent investigators, concerned public). The executive committee may authorize establishment of specific task groups (e.g. a study review committee) as necessary to achieve the stated goals. A small full- or part-time support staff may assist in day-to-day operation. Ideally, the operating funds should come primarily from grants from government agencies and nonprofit foundations, but genuinely 'no-strings-attached' nonrestricted gifts/grants from industries may also be considered.

29.5 Discussion

Today, blood transfusion is generally safe and is a routine clinical therapy in developed countries. However, in many third-world countries, safe blood is scarce for transfusion because of the prevalence of AIDS, hepatitis, and other transfusion-transmittable infectious diseases in the donor pool and inadequate donor blood-screening due to limited resources [18]. Therefore, there is a need to develop alternatives to standard allogeneic donor blood, including safe and effective HBOCs [19]. The purpose of this chapter is to initiate discussion on how academia and industry may collaborate to facilitate development of viable HBOC products in the foreseeable future.

There have been many successful new product-development cases in engineering, biotechnology, pharmaceutical, and other fields as a result of successful academia–industry collaboration. However, although there are many similarities, there seems to be no standard pathway/formula that guaranties success; every case is different, depending on such factors as technical field, the nature of the product, and the goals of the parties involved. As in most other fields, there are some generic barriers to effective academia–industry collaboration in HBOC development, including disparities in cultures, time lines, missions/goals, management, and issues related to proprietary rights and ethics. The success of academia–industry collaboration will depend on surpassing these barriers [20].

Resolving issues related to COI and IP rights may be among the most complicated and difficult tasks. Recently, COI issues in biomedical research have been brought to public scrutiny as many prominent scientists and administrators have been reported to receive research/training funds from industry or to serve as paid consultants for companies. Results published by the investigators of industry-sponsored studies may be perceived as biased even when their COI statuses are clearly disclosed. In the proposed consortium model, such perceived or real bias/COI would be substantially reduced or eliminated, since the investigators and industrial sponsors are 'buffered' through a consortium. When an HBOC producer contracts the consortium for a specific study, the consortium will select an appropriate investigator to conduct the study. In addition, an investigator will not receive funds directly from the industrial sponsor, eliminating a key element of potential COI. Consequently, studies conducted in this way should be perceived as more objective. This is beneficial for the public good, as both positive and negative results will be reported without bias.

However, there are challenges with this model. It may be too idealistic and there may be difficulty in recruiting industrial members, for a couple of reasons. First, HBOC

producers will have virtually no influence or control over the conduct of the study or the interpretation of results, even though they may still be paying for the study. This could be perceived as too high a risk for a company run with funds from private and public investments. To alleviate this situation, it would be best that the consortium be operated with funds from government or nonprofit foundation grants, rather than with fees from industrial sponsors.

Another area of difficulty is IP rights resulting from this approach. Every institution has different IP policies and may require individual negotiations for each of the consortium members. This could lead to long and difficult negotiations for some consortium participants. Therefore, implementation of a standardized IP agreement for all members would be essential for success. The HBOC research consortium can be implemented with members across national borders, as successfully demonstrated by the Waseda–Keio and EuroBloodSubstitutes consortia, although their approaches are quite different. Relative to a domestic consortium, an international consortium consisting of members from multiple nations will be much more complicated as there are intercountry disparities in laws covering product approval/regulation, IP, material-transfer agreements, and other issues. However, with proper arrangements, it should be feasible to overcome these difficulties, as numerous preclinical and clinical trials of pharmaceuticals are currently being conducted across national borders, often involving sites in multiple countries.

Clearly, there are many issues that must be overcome before a workable model of an HBOC research consortium could be constructed. Of note, the proposed HBOC research consortium is designed primarily for academia–industry collaborations in early to mid stages of HBOC development, involving mostly product characterization and preclinical safety and efficacy studies. The intent of this chapter is not to present a blueprint of a perfected model but to present a concept from which debate and discussion will generate a new framework that can be transformed into a productive structure.

29.6 Conclusions

Observations of some serious AEs in recent HBOC clinical trials and failure to gain regulatory approval are hampering further development of HBOCs as viable oxygen therapeutics. HBOC developmental efforts to date are largely based on a traditional industry-centered research model. However, this traditional approach so far has not been successful in producing accepted HBOC products that are considered safe and effective by regulatory agencies in the USA and Europe. To facilitate progress of HBOC development, we propose an HBOC research consortium consisting of key academic laboratories and HBOC producers. In this model, 'sponsors' and 'investigators' are buffered by the consortium, thus minimizing potential COI while encouraging open communications and increased transparency and objectivity in the conduct of research. If properly structured and operationalized, an HBOC research consortium would greatly facilitate development of viable HBOC products through pooled expertise and resources. In addition, a research consortium approach would enhance the chances of securing research funds in this time of limited resources and increased competition. Finally, this concerted research effort may also reveal clinical niches for currently available HBOC products.

Appendix: Successful Academia–Industry Collaboration Cases in HBOC Development

Case A: Waseda–Keio–Industry Research Collaboration

Waseda University and Keio University have worked in close cooperation on artificial O_2-carrier research for over 25 years. Here is a brief summary of how this began and how the academic research group has been established with domestic and overseas research institutes.

Professor Tsuchida first started the study of synthetic hemes embedded in a hydrophobic cluster, and clarified that the electronic processes of the active sites are controlled by the surrounding molecular environment. He tried to reproduce the O_2-binding ability of red blood cells (RBCs); that is, the development of a synthetic O_2 carrier without using Hb. In general, the central ferrous iron of a heme is immediately oxidized by O_2 in water, preventing the O_2 coordination process from being observed. In 1983, Professor Tsuchida succeeded in producing reversible and stable O_2 coordination and preparing phospholipid vesicles embedded with amphiphilic-heme, known as lipidheme/phospholipids vesicles [21].

Soon after this invention, Professor Kobayashi of Keio University asked Professor Tsuchida for a chance to evaluate the lipidheme solution with *in vivo* experiments. Since that time, the joint research and collaboration on lipidheme/phospholipids vesicles has continued. In 1985, Dr Sekiguchi, the former director of Hokkaido Red Cross Blood Center (Sapporo, Japan), proposed that Professor Tsuchida consider the utilization of outdated RBCs and Hbs, because the totally synthetic system was definitely promising, but it appeared that it would take considerable time to arrive at a social consensus. Production of HbVs was started using purified Hbs and molecular-assembly technologies [22]. In the late 1990s, a mass-production system for recombinant human serum albumin was established by a Japanese pharmaceutical company. Albumin–heme hybrids using the nonspecific binding ability of hemes have been studied as an O_2-carrying plasma expander [23].

Based on the variety of new functional materials developed by Waseda University, and the evaluation system by Keio University using animal experiments, strong progress has been made in the scientific research of artificial oxygen carriers. During this period, the project has received grants from the Japanese government (Ministry of Health, Labor and Welfare, MHLW; Ministry of Education, Culture, Sports, Science and Technology, MEXT). In particular, since 1997 this research has continuously received a Health Sciences Grant from the MHLW (Principal Investigators: Professor Tsuchida, 1997–2002; Professor Kobayashi, 2003–2007; Associate Professor Horinouchi, 2008–present). This was a driving force in establishing the present interdisciplinary academic consortium, comprising researchers not only from Waseda University, Keio University, and Hokkaido Red Cross, but also from Kumamoto University, Higashitakarazuka-Sato Hospital, and National Defense Medical College. With the governmental support, some overseas experts were invited to Japan, and some young Japanese researchers were given opportunities to study in universities in the USA. Since then, collaborations have expanded to multiple international partners (University of California, San Diego; University of Texas, San Antonio; University of Berne; McMaster University; Cornell

University College of Medicine; and Massachusetts General Hospital) to undertake safety and efficacy evaluations of artificial oxygen carriers using various animal models.

Aiming at the industrialization of HbV, in the early 1990s Waseda collaborated with NOF Corp. (Tokyo, Japan). At that time it was believed that liposomes were unstable corpuscles. A polymerizable phospholipid bearing two dienoyl groups was utilized as a main membrane component, and the HbV obtained was irradiated with gamma rays to polymerize the membrane. The resulting particles were so stable that they were resistant to freeze-thawing and freeze-drying. However, the particles were not degraded easily and remained in the reticuloendothelial system for several months. Due to this problem, the collaboration was terminated. After a period of trial and error, the present optimal lipid composition (without polymerization) was found to enable long-term storage and prompt degradation in the reticuloendothelial system. A bioventure company, Oxygenix Corp. (Tokyo, Japan), was established in 2003, and production was transferred from Waseda University to Oxygenix. However, due to financial problems and its eventual bankruptcy, Oxygenix terminated development in 2008 before the product could enter clinical trials. Currently, Nipro Corp. (Osaka, Japan) is continuously developing HbV in order to establish an efficient production method.

The HbV is categorized as a molecular assembly and its production method is complicated in comparison to cell-free HBOCs. Moreover, its dosage as a blood substitute is estimated to be considerably larger than that of conventional liposomal drugs and it is difficult to design animal experiments for safety and efficacy evaluation. Therefore, the academic professionals in this field, including the material inventors and clinicians, have supported the initial industrial development of HbVs of pre-clinical stage. Waseda and Keio Universities individually contracted with Oxygenix and Nipro for technology transfer, license, or consultancy. The companies have participated in our consortium, supported by the MHLW. Recently, as concerns about COI have been heightened in Japan, all these relationships have been disclosed. All members signed collaborative research agreements, specifying confidentiality, material transfer, new IP filing, and submission of academic papers and presentations. Because the research is supported by grants from the MHLW, all results from the consortium are submitted to the Ministry. In the future, when this research enters the clinical-trial stage, it will be even more important to consider COI. Clinical trials will be conducted by appropriate investigators who are in a neutral position and are free of COI.

Apart from Waseda–Keio activity, there is a long history of R&D of artificial oxygen carriers in Japan, beginning in the 1950s. The perfluorocarbon emulsion of Green Cross Corp. was the first US FDA-approved oxygen carrier (though the production was terminated due to side effects and limited clinical usage). The pyridoxalated-Hb-poly(oxyethylene) conjugate (PHP) of Ajinomoto Corp. was the first HBOC approved for clinical trials in the USA, by the FDA. The Japanese researchers who are studying or interested in artificial blood (substitutes for all the components of blood) established the Society of Blood Substitutes Japan (SBSJ) in 1993 (Former President, Professor Tsuchida; Current President, Professor Kobayashi) [24]. Since then, annual meetings have been held, inviting researchers from both engineering and medical fields, industries, and governmental agencies. Joint symposia with the International Society of Blood Substitutes were held in 1997 and 2003.

After the experiences of an HIV epidemic due to unpasteurized blood products, the disaster of the Great Hanshin–Awaji Earthquake, and the global trend for artificial blood, the government decided to promote R&D of artificial blood, aiming at industrialization, as clearly stated in the Revised Pharmaceutical Affairs Act of 2002.

The development of HbV as a cellular-type HBOC goes far behind that of the cell-free HBOCs; however, it is quite interesting to note that the side effects of molecular Hb and the physiological importance of the cellular structure of RBCs have been recognized through R&D of artificial oxygen carriers. Due to the current economic depression and concerns over development of new drugs, especially those categorized as a biologic drugs, Japanese pharmaceutical companies seem reluctant to develop artificial red cells. Despite some failures for industrialization, the academic consortium in Japan continues the research with a strong will and the support of the government, aiming at the realization of artificial oxygen carriers, which will eventually benefit human health and welfare.

Case B: EuroBloodSubstitutes Consortium

In 2004, Dr Kenneth C. Lowe, University of Nottingham, led the organization of the EuroBloodSubstitutes consortium, a multicenter consortium of 13 European academic and industrial teams [25], most with a consolidated experience in hemoglobin research and very limited previous activities in blood substitutes. This was the first time that a European coordinated action towards the development of blood substitutes took place. The EuroBloodSubstitutes project was funded by a grant from the European Union's 6th Framework Programme. As stated in the final publishable activity report of the project, there were six key scientific and technological objectives (end results) of the EuroBloodSubstitutes project, which were to:

(i) Identify, using quantitative and qualitative approaches to data collection, the perceived benefits and risks among stakeholders (e.g. public, patient groups, health care professionals, regulatory agencies) of blood and blood-substitute use in European society.
(ii) Develop a technological platform and genomic basis for generating prototype native, modified, and variant heme proteins for improved formulations in blood substitutes to replace some blood uses.
(iii) Identify and overcome bottlenecks in the efficient production of prototype heme proteins using fungi and plants as expression systems.
(iv) Characterize, using appropriate biochemical and biophysical techniques, the prototype heme proteins.
(v) Develop and optimize standard assays for the biological characterization of the prototype heme proteins.
(vi) Disseminate, through reports, publications, and patents, the new knowledge and understanding on issues associated with the generation and stakeholder perception of blood-substitute materials.

The implementation of the activities within the EuroBloodSubstitutes consortium was carried out via eight Workpackages, which led to the following main results:

(i) Workpackage 1 *(Blood and blood substitutes in European Society)*: Definitive survey materials identifying prevailing attitudes and opinions of the UK public to the societal use of blood (e.g. in transfusion) and potential clinical use of different types of blood substitute were piloted, refined, and fully developed. The materials were distributed to 12 000 members of the UK public and in the Netherlands [26].

(ii) Workpackages 2, 3, and 7 *(Protein design, protein expression, fermentation and scale-up)*: Genes for native hemoglobin were obtained, expression of α- and β-chains of hemoglobin in the bacterium *Escherichia coli* was achieved, and new mutant hemoglobin subunits were synthesized and expressed, carrying modifications at α42, β37, α C-terminus and β C-terminus. The synthesis and characterization of a two-subunit, four-domain hemoglobin derivative was completed [27]. Recombinant hemoglobin was expressed in yeasts, optimizing the yields, by exploiting microarray technology.

(iii) Workpackages 4, 5, and 6 *(Protein modification and purification, biochemical, biophysical, and physiological characterization)*: Chemical procedures for the production under anaerobic conditions of PEGylated-hemoglobin were developed. Robust procedures for the biochemical, biophysical, and *in vitro* and *in vivo* physiological characterization of PEG hemoglobin derivatives were carried out [28–35].

(iv) Workpackage 8 *(Management and dissemination of information)*: Achievements throughout the project include several internal meetings and the organization of the International Visions on Blood Substitutes Conference – Hemoglobin-Based Oxygen Carriers: From Chemistry to Clinic in Parma, Italy, September 17–20, 2006. Based on the success of this conference, the XII International Symposium on Blood Substitutes was organized for the first time in Europe, in Parma, Italy, August 25–28, 2009.

The EuroBloodSubstitutes consortium was managed by a committee composed of workpackage leaders and by a committee composed of selected academic and industrial partners dealing with IP-related issues, such as data publications. Partners were bound to work toward achieving objectives, milestones, and deliverables defined at the time of proposal submission at the European FP6 commission. Partner meetings, held every six months, allowed monitoring of achievements, discussion of emerging issues, and definition of strategies to overcome difficulties. In parallel, scientific and financial reports were collected and reviewed by the EU commission. This thorough and time-consuming documentation made the consortium activities very transparent and verifiable at any stage. Consortium partners were also bound by the Consortium Agreement, which dictated rules on all issues associated with the project, including IP protection and data dissemination. Patents generated from activities carried out within the consortium belong to the universities involved, not to all partners. Before submission of a publication, authors were asked to receive an approval by the consortium committee. Products generated from consortium activities were freely shared among partners. For example, a novel PEGylated HBOC, called EuroPEG-Hb [29], developed by one partner was characterized at the biochemical and physiological levels by others partners.

The key feature of the consortium was the coordination of different laboratories with distinct expertises towards a common goal. This scheme has recently been proposed at the international level as a possible strategy to overcome the present fragmentation in blood-substitute research activities, shortage of funding, and unnecessary competition. A key lesson from the EuroBloodSubstitutes consortium is that a curiosity-driven research is much more rewarding and, in the long run, more likely to be successful than a market-driven research in solving basic research issues.

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REVIEW ARTICLE

Pharmacokinetic properties of hemoglobin vesicles as a substitute for red blood cells

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Abstract

The development of artificial oxygen carriers has attracted considerable recent interest because of the increasing cost of collecting and processing blood, public concerns about the safety of blood products, complications from blood transfusions, military requirements for increased volumes of blood during military conflicts, and a decrease in the number of new donors. To overcome these problems, perfluorocarbon-based oxygen carriers as well as acellular- and cellular-type, hemoglobin-based oxygen carriers have been developed for use as artificial oxygen carriers. Despite their extensive evaluation, including formulation and pharmacology, they have not been extensively used in clinical settings. One of the reasons for this is that their pharmacokinetics have not been well characterized. Artificial oxygen carriers require not only an acceptable level of physicochemical activity, but also clinical efficacy, as reflected by their retention in the circulation, and the absence of measurable accumulation in the body, if unexpected adverse effects are to be avoided. In this review, the pharmacokinetic properties of artificial oxygen carriers are discussed, with a focus on recent developments of our research related to the pharmacokinetic properties a cellular type of hemoglobin-based oxygen carrier.

Keywords: Artificial oxygen carrier, disposition, liposome, mononuclear phagocyte system, hemorrhagic shock, hepatic chronic cirrhosis, accelerated blood clearance phenomenon

Introduction

In modern medical care, there is now little doubt that the transfusion of red blood cells (RBCs) is the gold standard for treatment of patients with massive hemorrhages and is currently in widespread use. Nevertheless, the potential for mismatching exists and infections by unrecognized pathogens, hepatitis, HIV, or West Nile virus, etc., are always a possibility. In addition, ensuring a steady supply of RBCs at a time of a disaster and during military conflicts could be difficult, because the lifetime of donated RBCs is limited to a short period. Further, a decrease in donors and an increase in recipients in some developed countries is also a problem. To overcome these problems, various artificial oxygen carriers have been under development worldwide. They can be divided into three major classes of materials, as follows: perfluorocarbon-based oxygen carriers, acellular-type, hemoglobin-based oxygen carriers (HBOCs), and cellular-type HBOCs (Figure 1). Despite the many efforts to develop artificial oxygen carriers during the past several decades, some of them were, unfortunately, rejected for use as the result of preclinical and clinical trials. It is noteworthy that perfluorocarbon-based oxygen carriers and acellular-type HBOCs were excluded as possible candidates for artificial oxygen carriers, even though they proceeded to the stage of clinical trials.

One of the reasons that induced these adverse effects was due to the insufficient characterization of pharmacokinetics of these artificial oxygen carriers under various situations. The desirable features of artificial oxygen carriers as a substitute for RBCs is not only a long retention in the circulation to sustain its pharmacological effects, but also no bioaccumulation, which could lead to adverse effects. Unlike other drugs, because the dosage volume of

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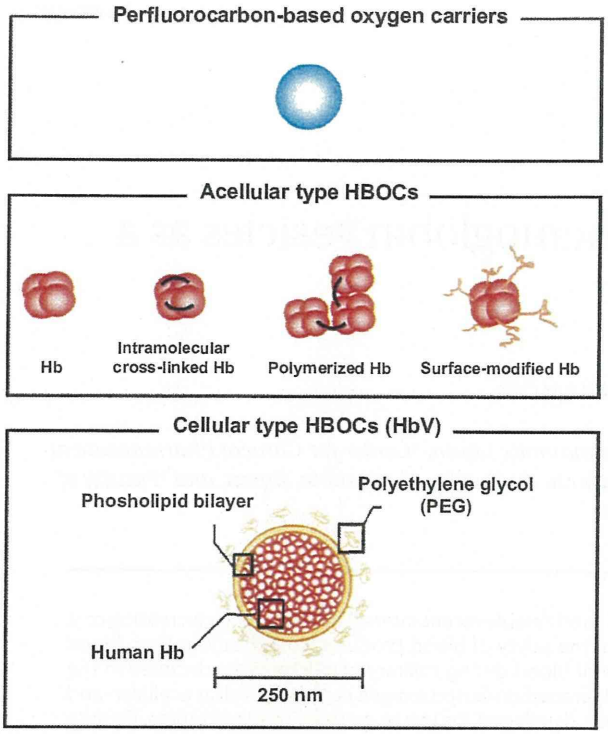

Figure 1. Schematic representation of perfluorocarbon-based oxygen carriers, acellular-type hemoglobin-based oxygen carriers (HBOCs), and cellular-type HBOCs (HbV). In the case of HbV, the surface is modified with polyethylene glycol (PEG) chains, and one HbV particle contains approximately 30,000 human Hb molecules obtained from outdated donated blood. The encapsulated Hb contains pyridoxal 5'-phosphate as an allosteric effector to regulate P_{50} to 25–28 torr. The lipid bilayer was comprised of a mixture of DPPC, cholesterol, and DHSG at a molar ratio of 5:5:1, and DSPE-PEG_{5000} (0.3 mol%). The average particle diameter was regulated to approximately 250 nm.

an artificial oxygen carrier as an RBC substitute is more than a hundred times higher than that of other drugs, detailed information regarding the fate of an artificial oxygen carrier, including its constituent components, is needed, in order to predict unexpected adverse effects.

In this review, the pharmacokinetic properties of artificial oxygen carriers are discussed, with a focus on hemoglobin vesicles (HbVs), in which, among the current artificial oxygen carriers, its pharmacokinetic properties have been extensively characterized.

Perfluorocarbon-based oxygen carriers

The perfluorocarbon-based oxygen carriers are characterized by a high gas-dissolving capacity, low viscosity, and chemical and biological inertness (Spahn and Kocian, 2003). They are molecules that are constructed from cyclic or straight-chain hydrocarbons, in which the hydrogen atoms are replaced by halogens, and are virtually immiscible with water and, therefore, must be emulsified prior to their use in intravenous applications (Pape and Habler, 2007). When perfluorocarbon emulsion droplets are injected into an organism, they are rapidly taken up and slowly broken down by the mononuclear phagocyte system (MPS). After being degraded, the emulsion droplets are again taken up by the blood and transported to the lungs, where any unaltered molecules are excreted via exhalation (Spahn and Kocian, 2003; Jahr et al., 2007; Pape and Habler, 2007). However, perfluorocarbon-based oxygen carriers induced chronic pneumonitis due to their inefficient excretion from the body and their accumulation in the lung, a condition that persists for more than 1 year (Nose, 2004) (Table 1).

Acellular-type HBOCs

The stroma-free hemoglobin (Hb) was developed for use as artificial oxygen carriers, but their systemic half-lives were too short (~0.5–1.5 hours) for them to effectively function as an optimal oxygen carrier (Savitsky et al., 1978). In addition, the Hb tetramers dissociate into their component αβ dimers, which are then eliminated by the kidneys, and induce renal toxicity (Creteur and Vincent, 2003). In an attempt to increase their systemic half-life and stability, the following three groups of chemically modified acellular-type HBOCs were developed: surface-modified Hb (Smani, 2008), intramolecularly cross-linked Hb (Chen et al., 2009), and polymerized Hb (Jahr et al., 2008) (Figure 1). These acellular HBOCs have improved systemic half-lives, in the range of 18–24 hours, and show decreased renal failure (Stowell, 2005) (Table 1). The polymerized bovine-derived Hb has been approved for limited use in South Africa (Lok, 2001). However, it was recently reported that the use of some acellular-type HBOCs leads to the development of myocardial lesions, as the result of decreasing nitric-oxide levels 24–48 hours after a single topload infusion (Burhop et al., 2004), leading to an increase in mortality rates in humans (Natanson et al., 2008).

Hemoglobin vesicles

The hemoglobin vesicle (HbV) is a cellular-type HBOC that contains polyethylene glycol (PEG), in which phospholipid vesicles encapsulating highly concentrated human Hb are imbedded (Sakai et al., 2008) (Figure 1). The cellular structure of HbV (particle diameter: approximately 250 nm) most closely mimics the characteristics of a natural RBC, such as the cell-membrane function, which physically prevents the direct contact of Hb with the components of blood and the vasculature during its circulation. The characteristics of HbV are superior to donated RBCs in the following ways: the absence of viral contamination (Sakai et al., 1993; Abe et al., 2001), a long-term storage period of over 2 years at room temperature, and no blood-type antigens (Sakai et al., 2000; Sou et al., 2000) (Table 2). In addition, HbVs have the ability to transport oxygen equivalent to RBCs and also show improved survival in hemorrhagic shock animal models (Sakai et al., 2004b; Terajima et al., 2006; Sakai et al., 2009). Further, HbVs can control the release of oxygen by

adjusting the amount of allosteric effector and regulate rheological properties (e.g., viscosity and colloid osmotic pressure) to added human serum albumin (Sakai and Tsuchida, 2007). Therefore, HbV has attracted considerable attention as a possible new artificial oxygen carrier and has considerable promise for use in clinical settings.

We recently characterized the pharmacokinetic properties of HbV to clarify its efficacy and safety under conditions that mimic a clinical setting, as follows:

1. HbV was constructed from multiple components, including Hb, lipids, and iron from Hb. These components have potential risks for inducing harmful effects, when they accumulate at excessive levels in the body.
2. HbV is classified as a liposome preparation. It was previously reported that the pharmacokinetics of liposome-encapsulated amphotericin B differ between normal individuals and patients (Walsh et al., 1998; Bekersky et al., 2001).
3. The surface of HbV was modified by PEG to enhance the half-life in circulation and storage. It was recently reported that repeated injection of PEGylated liposomes influenced the pharmacokinetics of the second injected liposome (Dams et al., 2000; Ishida et al., 2003a).

Table 1. Pharmacokinetic properties of some artificial oxygen carriers.

	Perfluorocarbon-based oxygen carriers	Acellular-type HBOCs	Cellular-type HBOCs
Distribution	Liver, spleen	Liver	Liver, spleen
Metabolism	MPS	MPS	MPS
Excretion	Air	—	Internal Hb; urine outer membrane; feces
Half-life	~10 hours (rat)	~24 hours (rat)	30~40 hours (rat)
Existence in tissues	~1 year	—	~14 days

HBOCs, hemoglobin-based oxygen carriers; MPS, mononuclear phagocyte system.

Table 2. Physicochemical characteristics of HbV.

Parameter	
Particle diameter	ca. 250 nm
P_{50}	25–28 torr
Hb concentration	10 g/dL
MetHb	<3%
Colloid osmotic pressure	0 Torr
Intracellular Hb concentration	ca. 35 g/dL
Lipid composition[a]	DPPC/cholesterol/DHSG/DSPE-PEG_{5000}
Stability for storage at room temperature	Over 2 years, purged with N_2

[a]DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DHSG, 1,5-bis-*O*-hexadecyl-*N*-succinyl-L-glutamate; DSPE-PEG_{5000}, 1,2-distearoyl-*sn*-glycero-3-phosphatidyl-ethanolamine-*N*-PEG.

For these reasons, it becomes necessary to clarify the pharmacokinetic properties of HbV in various animal models and under conditions of repeated injection, if RBCs are to be used as a substitute in the future. For this purpose, 1) the disposition of HbVs was examined using isotope tracer techniques. In these experiments, ^{125}I-HbV, enclosed in HbVs, was radiolabeled with ^{125}I, and the lipid component vesicles of HbVs was radiolabeled with ^{3}H; 2) a pharmacokinetic study of HbVs in a rat model of hemorrhagic shock and hepatic chronic cirrhosis; 3) the repeated injection in normal and the hemorrhagic shock rat model; and 4) animal scale-up using an allometric equation, were conducted.

Some highlights of recent developments of our research related to the pharmacokinetic properties of HbV are discussed below.

The prior pharmacokinetic characteristics of HbV to stroma-free Hb

Two requirements need to be satisfied if HbV is to be accepted for use as an artificial oxygen carrier. For clinical applications, HbVs must have not only an acceptable physicochemical activity, but also must be safe for use in the clinic. In the latter case, the supply of oxygen tissues is one of the most important factors in sustaining the clinical effect of HbVs (Takaori, 2005). To fulfill these requirements, a prolonged half-life is a required property for HbVs.

We recently demonstrated that the half-life of HbV in mice was 30 times higher than that of stroma-free Hb at a dose rate of 1 mg Hb/kg (Table 3). Moreover, a dose-dependent study clearly showed that the plasma concentration curve and half-life of HbV in mice and rats increased with increasing doses of HbV (Figure 2, half-life; rats: 8.8±0.7, 11.5±0.3, and 30.6±4.0 hours at doses of 10, 200, and 1,400 mg Hb/kg, respectively; mice: 3.1±3.1, 3.6±1.3, 7.2±3.1, and 18.8±1.3 hours at doses of 1, 10, 200, and 1,400 mg Hb/kg, respectively) (Taguchi et al., 2009b).

These superior pharmacokinetic characteristic of HbV, compared to stroma-free Hb, could reflect their physicochemical differences, such as particle diameter, the absence or presence of a membrane structure, and PEG modification. In physiological conditions, free Hb that is released from ruptured RBC is rapidly bound to

Table 3. Pharmacokinetic parameters for HbV after the administration of ^{125}I-Hb and ^{125}I-HbV in mice at a dose of 1 mg Hb/kg.

	^{125}I-Hb	^{125}I-HbV	*p*
$t_{1/2}$ (hr)	0.1±0.1	3.1±1.0	<0.01
AUC (hr*% of dose/mL)	7.9±3.9	29.4±9.2	<0.001
CL (mL/hr)	12.7±2.1	3.4±0.1	<0.001
V (mL)	2.6±0.3	2.3±0.1	N.S.

$t_{1/2}$, half-life; AUC, are under the plasma-concentration versus time curve; CL, clearance; V, distributed volume; N.S., not significant.

haptoglobin (Hp), which promotes CD163 recognition in the liver (Kristiansen et al., 2001). When the Hb concentration exceeds the Hp-binding capacity, unbound Hb is removed by filtration through the kidney. Therefore, the reduction in HbV distribution in the liver and kidney could be due to the encapsulation of Hb by liposomes because this might not only suppress the binding of internal Hb to Hp, but also inhibit renal glomerular filtration. In fact, it was observed that the distribution of HbV in the liver and kidney was suppressed, compared with that of stroma-free Hb (Taguchi et al., 2009b). Moreover, the membrane surface modification by PEG also contributed to the increased half-life of HbV. In general, it is well-known that liposomes are scavenged and degraded by the MPS, such as Kupffer cells or macrophages in the spleen (Kiwada et al., 1998). PEGylation is a useful method for suppressing the capture of MPS, and the majority of the recently developed liposome formulations are modified with PEG (Noble et al., 2006; Sou et al., 2007; Okamura et al., 2009). Therefore, the modification of HbV with PEGylation is important to not only stabilize for a long-time storage, but also to maintain the good retention in the circulation. These balanced physicochemical activities result in a longer retention in the circulation, compared to stroma-free Hb and acellular-type HBOCs (Goins et al., 1995; Chang et al., 2003; Lee et al., 2006).

The disposition of HbV components

In clinical situations as a substitute of RBCs, massive amounts of HbV are typically given to patients. As a result, its associated components, including Hb, lipids from Hb, could result in undesirable consequences in the systemic circulation and organs during its metabolism and disposition. Such an extraordinary load of HbV components could result in the accumulation of components in the blood or organs, and has the potential to cause a variety of adverse effects, as follows: 1) high levels of lipid components, especially cholesterol, in the bloodstream, which are risk factors for kidney disease, arterial sclerosis, and hyperlipidemia (Grone and Grone, 2008); 2) Hb induces renal toxicity by dissociation of the tetramic Hb subunits into two dimers (Parry, 1988); and 3) free iron can trigger tissue damage induced by the Fenton reaction, which is mediated by heme (iron) (Balla et al., 2005). Therefore, it becomes necessary to clarify whether HbV and its components have favorable metabolic and excretion profiles. In order to investigate the disposition of each HbV component, Hb, enclosed in HbV, was radiolabeled with ^{125}I (^{125}I-HbV) or cholesterol, in the lipid component vesicles of HbV, was radiolabeled with ^{3}H (^{3}H-HbV).

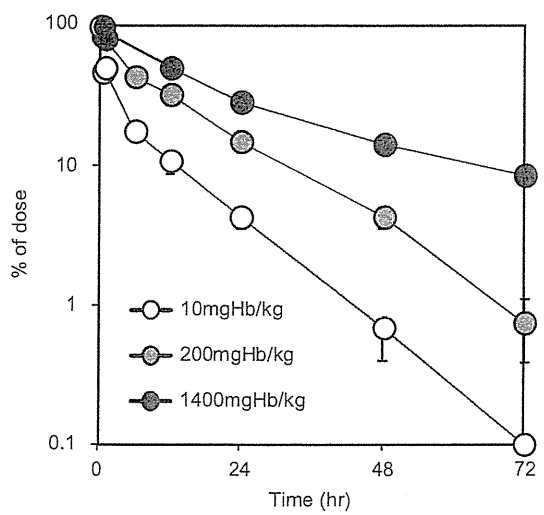

Figure 2. Dose-dependent plasma concentration curve of ^{125}I-HbV after administration of ^{125}I-HbV in rats. All rats received a single injection of ^{125}I-HbV at a dose of 10 (open squares), 200 (gray circles), and 1,400 mg Hb/kg (closed circles) containing 5% rHSA. At each time point (0.05, 0.5, 1, 6, 12, 24, 48, and 72 hours) after the ^{125}I-HbV injection, blood samples were collected from the tail vein, and a plasma sample was obtained. Each point represents the mean ± SD (n=3–5).

In the blood circulation, HbV typically maintains an intact structure for periods of up to 72 hours after injection, because similar plasma concentration curves for ^{125}I-HbV were observed for ^{3}H-HbV in rats (Figure 3A), and the pharmacokinetic parameters were also consistent between them (half-life: 30.6 ± 4.0, 30.9 ± 4.7 hours; clearance in plasma: 0.46 ± 0.04, 0.41 ± 0.02 mL/h, for ^{125}I- and ^{3}H-HbV, respectively). Moreover, ^{125}I-HbV and ^{3}H-HbV were mainly distributed in the liver and spleen (Figure 3B). Because HbV possesses a liposome structure, it would be predicted that it would be captured by the MPS in the liver and spleen (Kiwada et al., 1998). In fact, a previous *in vitro* study clearly demonstrated that HbV was specifically taken up and degraded in RAW 264.7 cells, which has been used as an alternative to Kupffer cells, but this was not the case for parenchymal and endothelial cells (Taguchi et al., 2009b). In addition, the uptake clearance (CL_{uptake}) in the liver and spleen were also similar between the two labeled preparations (liver: 1,141 ± 142, 1,098 ± 123; spleen: 619 ± 40, 518 ± 89 μL/h, for ^{125}I-HbV and ^{3}H-HbV, respectively). However, ^{125}I was more rapidly eliminated from each organ, and the activity essentially disappeared within 7 days. On the other hand, the elimination of radioactive ^{3}H was delayed, compared to that of ^{125}I, but nearly disappeared after 14 days. These data indicate that HbV is mainly distributed to the liver and spleen in the form of intact HbV, and that it was degraded by the MPS. In order to identify the excretion pathway of HbV, the levels of radioactivity of ^{125}I and ^{3}H in the urine and feces were measured. The radioactive ^{125}I was excreted mainly in the urine, whereas the majority of the ^{3}H was excreted in the feces. Based on the above findings, the disposition of HbV and its components, after circulating in the form of stable HbV, are distributed to the liver and spleen, where they are degraded by the MPS. Finally, the enclosed Hb and outer lipid components were mainly eliminated to the urine and feces, respectively, in the same manner as endogeneous substances (Figure 4). Similar results were also reported in mice and rabbits (Sou et al.,

2005; Taguchi et al., 2009b); these results indicate that HbV and its components have favorable metabolic and excretion profiles in mammalian species. In addition, the plasma concentration curve for heme (iron) derived from HbV was similar to that for ^{125}I-HbV and ^{3}H-HbV in mice (Taguchi et al., 2009b). Moreover, no significant differences in the ratio of the mercapt- (i.e., nonoxidized form) to the nonmercapt-form (i.e., oxidized form) of rat serum albumin, which serves as a marker of oxidative stress in the circulation system (Kadowaki et al., 2007; Shimoishi et al., 2007), were found between HbV and the saline administration groups for periods of up to 7 days after administration. These results suggest that excess free heme (iron) derived from HbV is not released in the plasma. However, the issue of the disposition of several HbV components, including PEG and phospholipid, was not clarified. It is also possible that these components in HbV are also metabolized and excreted in the same manner as endogenous substances, but further study will be needed to demonstrate this fact.

Pharmacokinetic properties of HbV under conditions of hemorrhagic shock

It is well known that clinical conditions can have an effect on the pharmacokinetics of numerous drugs (Abernethy et al., 1981; Turck et al., 1996). For example, it has demonstrated that the pharmacokinetics of liposome-encapsulated amphotericin B differ between normal individuals and patients in a clinical trial stage (Walsh et al., 1998; Bekersky et al., 2001). Consequently, it is possible that the pharmacokinetics of HbV would be also altered in the situation of a massive hemorrhage caused by injury, accidental blood loss, or a major surgery. To clarify this, we investigated the changes in HbV pharmacokinetics using a rat model of hemorrhagic shock induced by massive hemorrhage.

As shown in Figure 5, the retention of HbV in plasma under this condition was shorter, and the half-life of HbV was reduced significantly—by 0.66-fold—compared with the half-life of HbV in normal rats (30.6 ± 4.0, 18.1 ± 3.7 hours, for normal and hemorrhagic shock, respectively). At a glance, this appears to not be a desirable situation for the therapeutic use of HbV, because an important determinant of HbV efficacy is a long retention in the blood circulation. However, the distribution volume of the central compartment of HbV (V_1) was identical between normal and hemorrhagic shock rats, whereas the distribution volume of the peripheral compartment (V_2) in hemorrhagic shock rats was nearly 2-fold greater than that of normal rats (Figure 5, insert). Moreover, the time-course tissue distribution of HbV in the hemorrhagic shock rats was greater than normal rats. These findings indicate that the shorter half-life in hemorrhagic shock rats appears to be the result in an apparent reduction in HbV in the arteriovenous circulation. If this enhanced tissue distribution of HbV might be derived by an increased scavenging of HbV by the MPS, such as by Kupffer cells, red pulp zone splenocytes, and mesangial cells (Sakai et al., 2004a), it would not be expected to show significant pharmacological efficacy as an oxygen carrier, because HbV must maintain an intact structure to maintain its oxygen-carrying capacity. However, the pharmacological effect in the hemorrhagic shock model animal was significantly increased by the HbV treatment, similar to that for an RBC treatment (Sakai et al., 2004b; Terajima et al., 2006; Sakai et al., 2009). In addition, the amount of excretion into the urine, which is the major elimination pathway, did not differ between normal and hemorrhagic shock rats in our pharmacokinetic study. Therefore, HbV appears to be transferred from the arteriovenous blood to organ capillary beds as an intact structure, and is not excessively captured and metabolized by the MPS. These findings support the conclusion

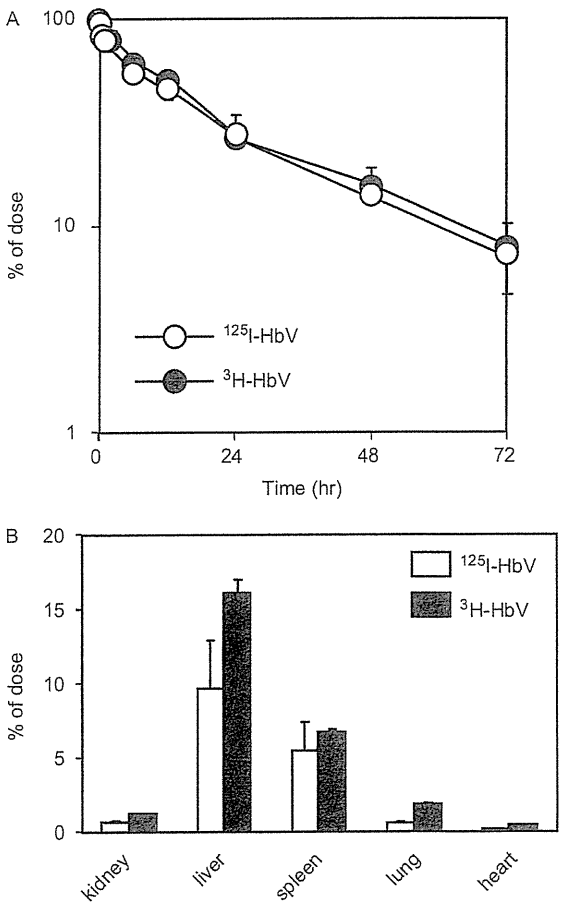

Figure 3. (**A**) Time course for the plasma level of ^{125}I-HbV (open circles) and ^{3}H-HbV (filled circles) after administration to rats. SD rats received a single injection of ^{125}I-HbV or ^{3}H-HbV to the tail vain at a dose of 1,400 mg Hb /kg. Blood was collected from the tail vein under ether anesthesia, and a plasma sample was obtained. Each point represents the mean ± SD (n=5). (**B**) Tissue distributions of ^{125}I-HbV (open bars) and ^{3}H-HbV (filled bars) at 24 hours after administration to mice. SD rats received a single injection of ^{125}I-HbV or ^{3}H-HbV from the tail vain at a dose of 1,400 mg Hb/kg. At 24 hours after injection, each organ was collected. Each bar represents the mean ± SD (n=5).

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

Pharmacokinetic properties of HbV in the condition with chronic liver failure

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

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

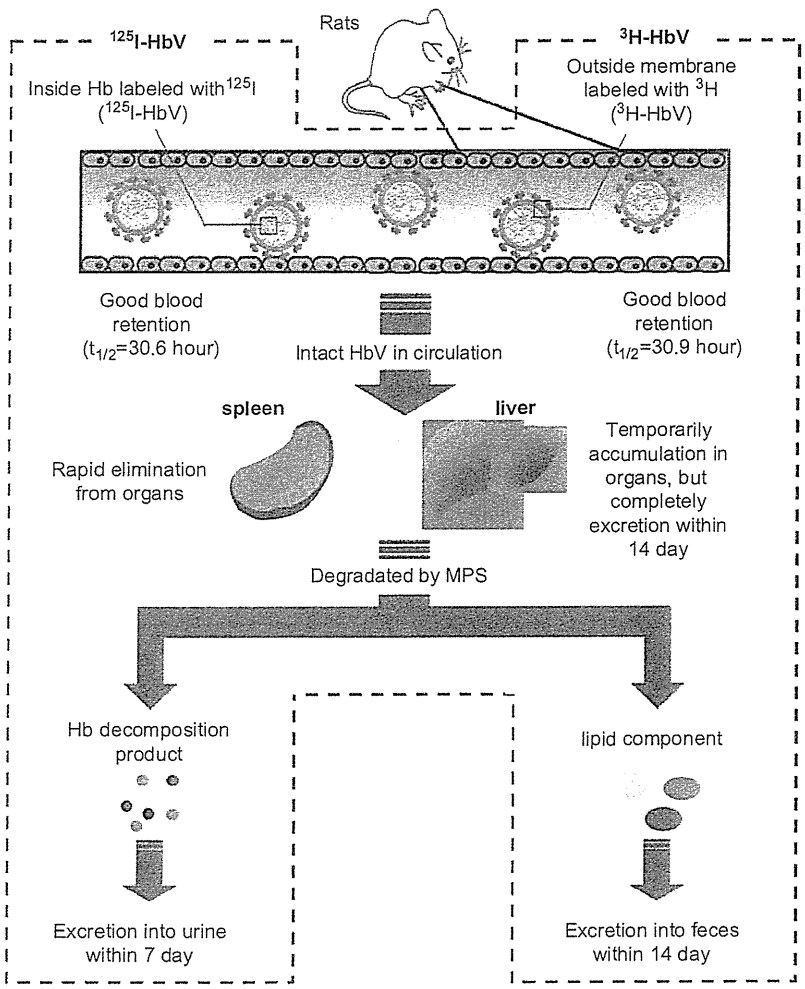

Figure 4. Representation of a sequence of HbV disposition, metabolism, and excretion from pharmacokinetic examinations, using ^{125}I-HbV and ^{3}H-HbV. After circulating in the form of stable HbV, it is distributed to the liver and spleen, where it is degraded by MPS. Finally, the enclosed Hb and outer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

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

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

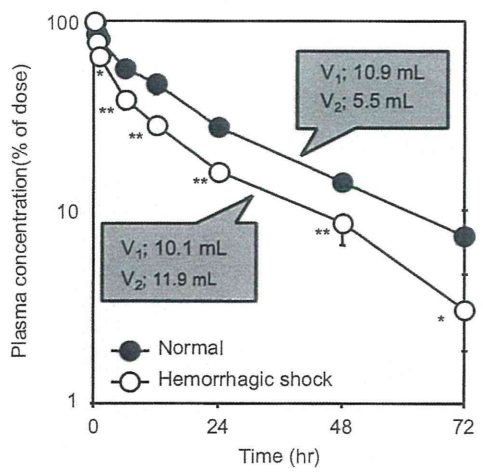

Figure 5. Relative plasma concentration of ^{125}I-HbV after administration of 1,400 mg Hb/kg via injection of normal (filled circles) or hemorrhagic shock rats (open circles). After inserting polyethylene catheters into the left femoral artery, SD rats received a single injection of ^{125}I-HbV to the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

Pharmacokinetic properties of HbV after repeated administration in mice

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

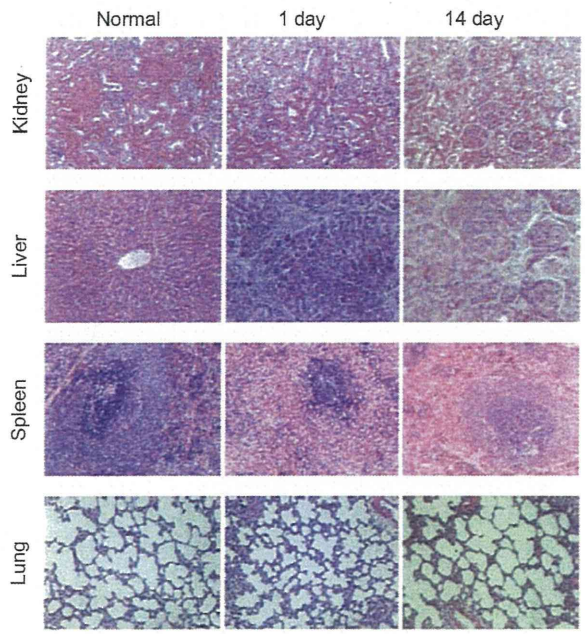

Figure 6. Light micrographs of kidney, liver, spleen, lung, and heart in CCl_4-treated rats after an HbV injection stained with hematoxylin and eosin (X100). Chronic cirrhosis model rats received a single injection of HbV at a dose of 1,400 mg Hb/kg. No noticeable changes were observed in all organs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

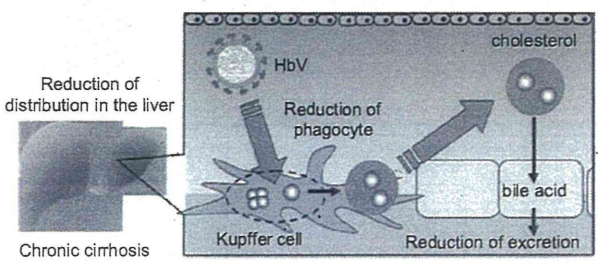

Figure 7. Representation of the pharmacokinetic properties of HbV in a rat model of chronic cirrhosis. Hepatic impairment altered the pharmacokinetic properties of HbV, such as blood retention, hepatic distribution, and fecal excretion, by a reduction in Kupffer cell phagocyte activity and damage to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

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

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

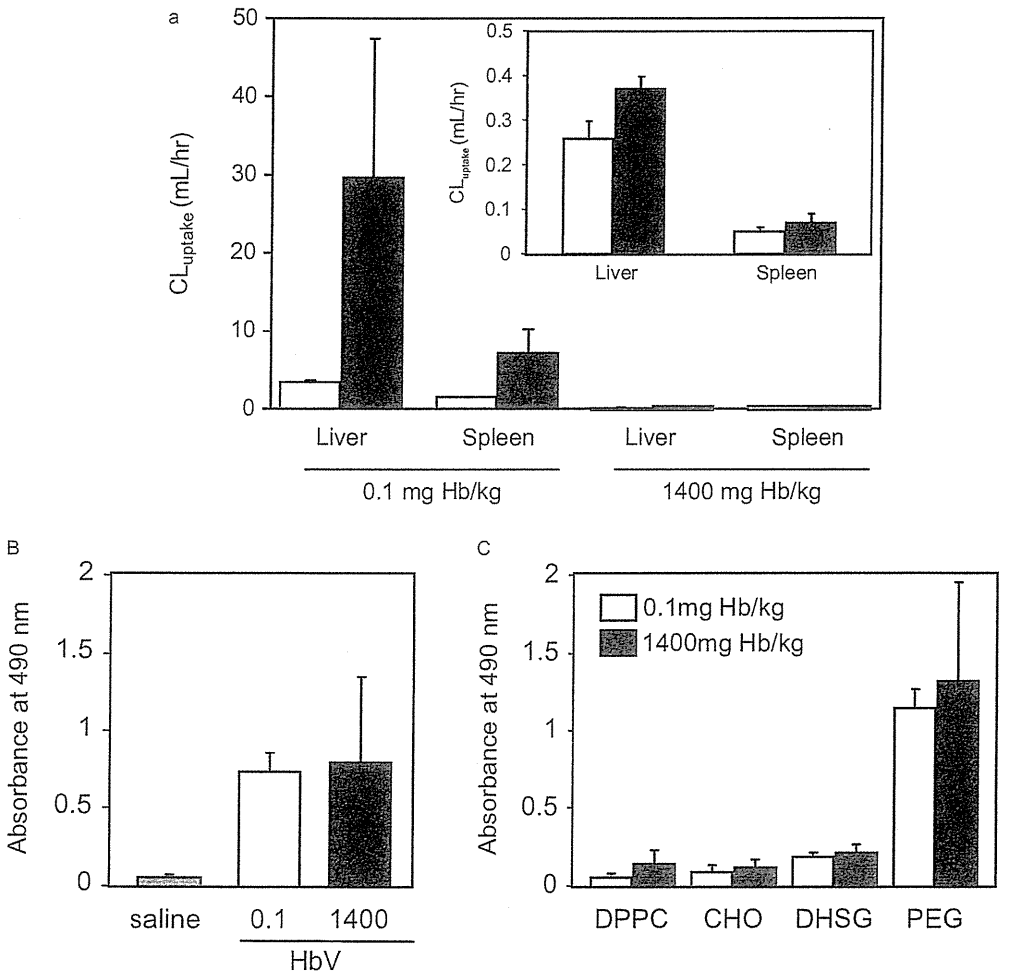

Figure 8. (A) Uptake clearance of HbV in the liver and spleen after 1 or 2 injections of ^{125}I-HbV. Uptake clearance for each organ was calculated by integration plot analysis at designated times from 1 to 30 minutes after injection. Each bar represents the mean ± SD ($n=4$). (B) Determination of IgM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

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

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

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

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

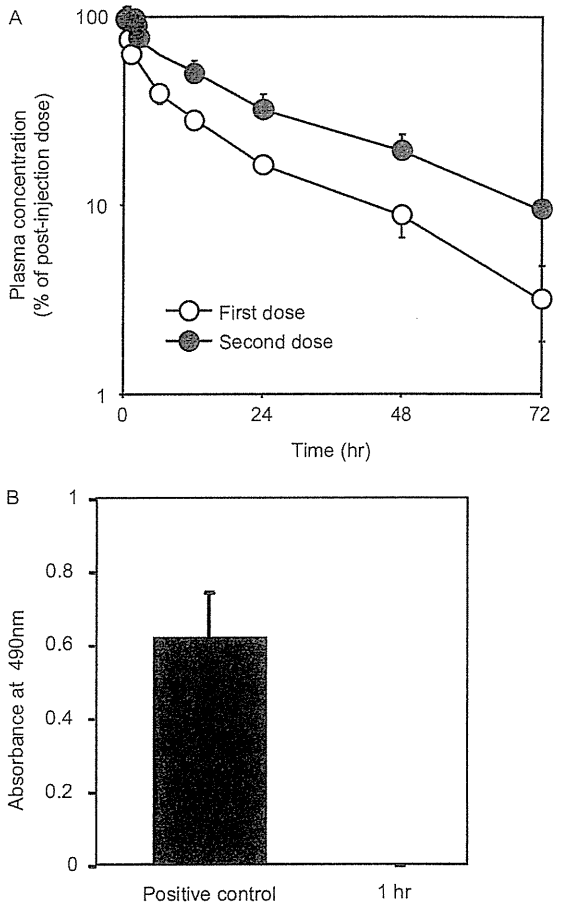

Figure 9. (A), Plasma concentration of ^{125}I-HbV as the percent of postinjection dose after the first (open symbol) or second dose (filled symbol) of ^{125}I-HbV to hemorrhagic shock rats at a dose of 1,400 mg Hb/kg for each injection. Each point represents the mean ± SD (n=5). Plasma concentration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