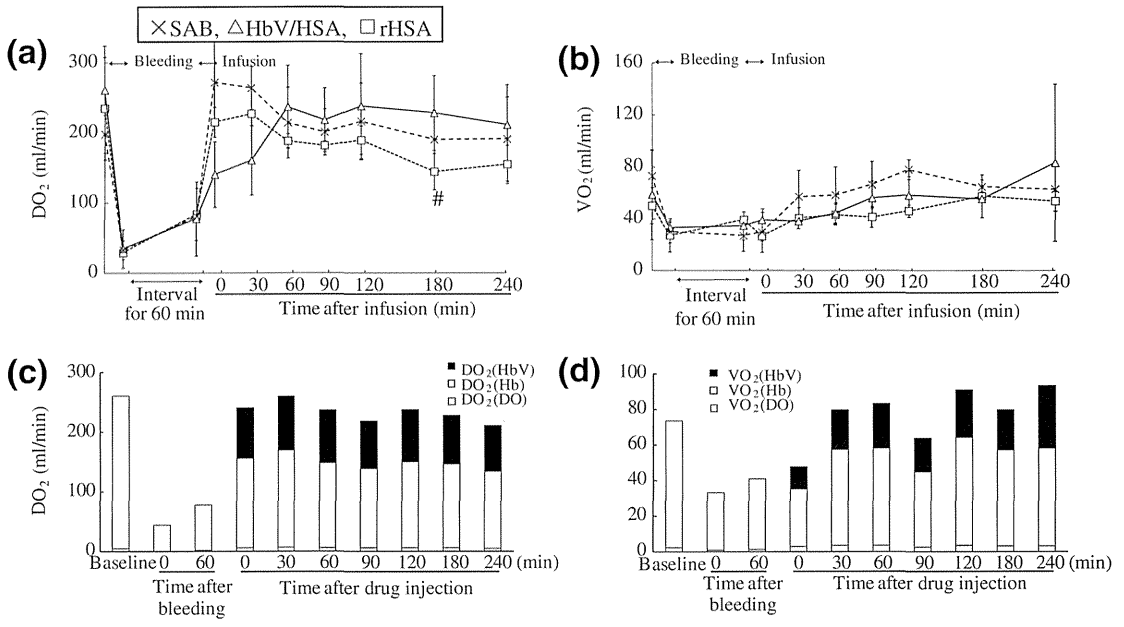

Fig. 28.4 Changes in oxygen delivery (DO_2), and oxygen consumption (VO_2) during hemorrhagic shock and resuscitation with infusion of rHSA alone, sSAB and HbV/rHSA (*Top*). The rates of oxygen delivery derived from dissolved oxygen in plasma (DO_2(Plasma)), hemoglobin of RBCs (DO_2(RBC)), and HbV (DO_2(HbV)) in total oxygen delivery (DO_2) of the HbV/rHSA group and oxygen consumption derived from dissolved oxygen in plasma (VO_2(Plasma)), hemoglobin of RBCs (VO_2(RBC)), and HbV (VO_2(HbV)) in total oxygen consumption (VO_2) of the HbV/rHSA group (*Bottom*). The values are mean ± SD. # significantly different versus the HbV/rHSA group ($p < 0.05$)

was approximately 35 %. It decreased to about 29 % for the HbV/rHSA group after recovery from acute phase study. However, it showed monotonic increase; at 7 days, the Hct showed a complete recovery to the baseline level (about 35 %). Although Ht recovered consistently in HbV/rHSA group, we could see distinguished significances until 2 months. White blood cell and platelet counts showed non-significant changes between the HbV/rHSA and SAB groups, and then maintained rather steady values.

Regarding the plasma biochemical tests, AST and ALT showed increases on Day 1 in both HbV/rHSA and SAB groups, but it reverted to the original level on Day 3 (Fig. 28.5). LDH showed decreases on Day 1 in both groups, but both groups showed gradual increases until Day 7. The HbV/rHSA group tended to show lower ALP than the SAB group, and showed significantly lower values than baseline after 168 and 365 days. γ-GTP, ChE, TP, and ALB showed stable values for 1 year. CPK showed increases on Day1 in both groups, but it reverted to the original level on Day 3. Amylase showed non-significant change between HbV/rHSA and SAB group for 1 year. Lipase showed significant decrease in HbV/rHSA group on Day 7, but it tended to show gradual recovery. LAP, BUN, Cre, and UA showed non-significant changes between HbV/rHSA and SAB group. Regarding plasma lipid components in the HbV/rHSA group, Total-cholesterol level and Free-cholesterol level showed significant increases on Day 7. However,

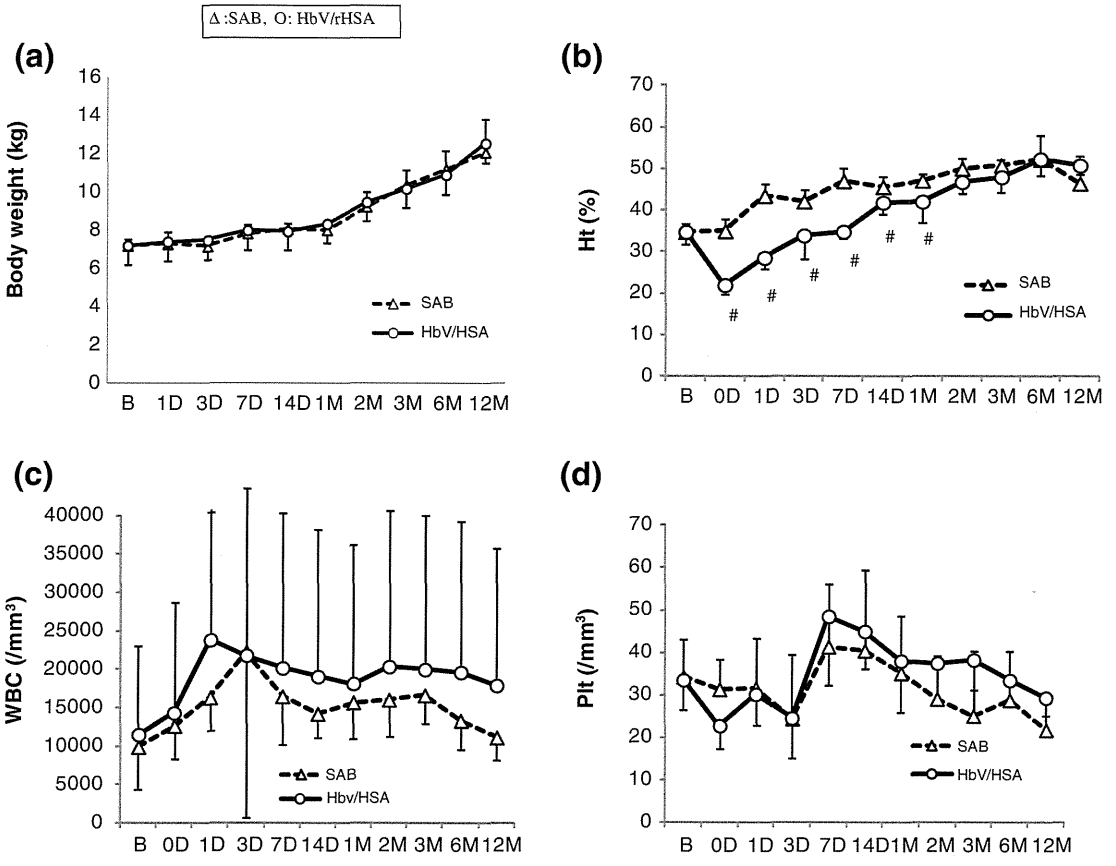

Fig. 28.5 One-year observation of changes in body weight, hematocrit (Hct), white blood cells count (WBC), and platelet count (PLT) after resuscitation with infusion of SAB and HbV/rHSA. The values are mean ± SD. # significantly different versus the autologous shed blood group ($p < 0.01$)

they returned to their original levels at Day 14 (Fig. 28.6). HDL-Chol (High Density Lipoprotein-cholesterol) showed significant decrease for HbV/rHSA group on Day 1, but it reverted to the original level on Day 3. Trigriseride (TG) and Free Fatty Acid (FFA) showed non-significant change between the HbV/rHSA and SAB groups. Phospholipids and Total Lipid showed significant increases in the HbV/rHSA group on Day 7, but they showed non-significant change after Day 3. Total bilirubin (T-Bil) and Fe maintained steady values. Copper ion showed significant increase in the SAB group on Day 1, but it showed similar tendency after Day 3. K, Ca, IP, and Mg showed stable values for 1 year.

28.3.3 Histopathological Study

In acute phase study, sinusoid of the liver showed the eosinophilic fine granular material in the HbV/rHSA group (Fig. 28.7). The red pulp zone of the spleen showed eosinophilic fine granular material in the HbV/rHSA group. These findings

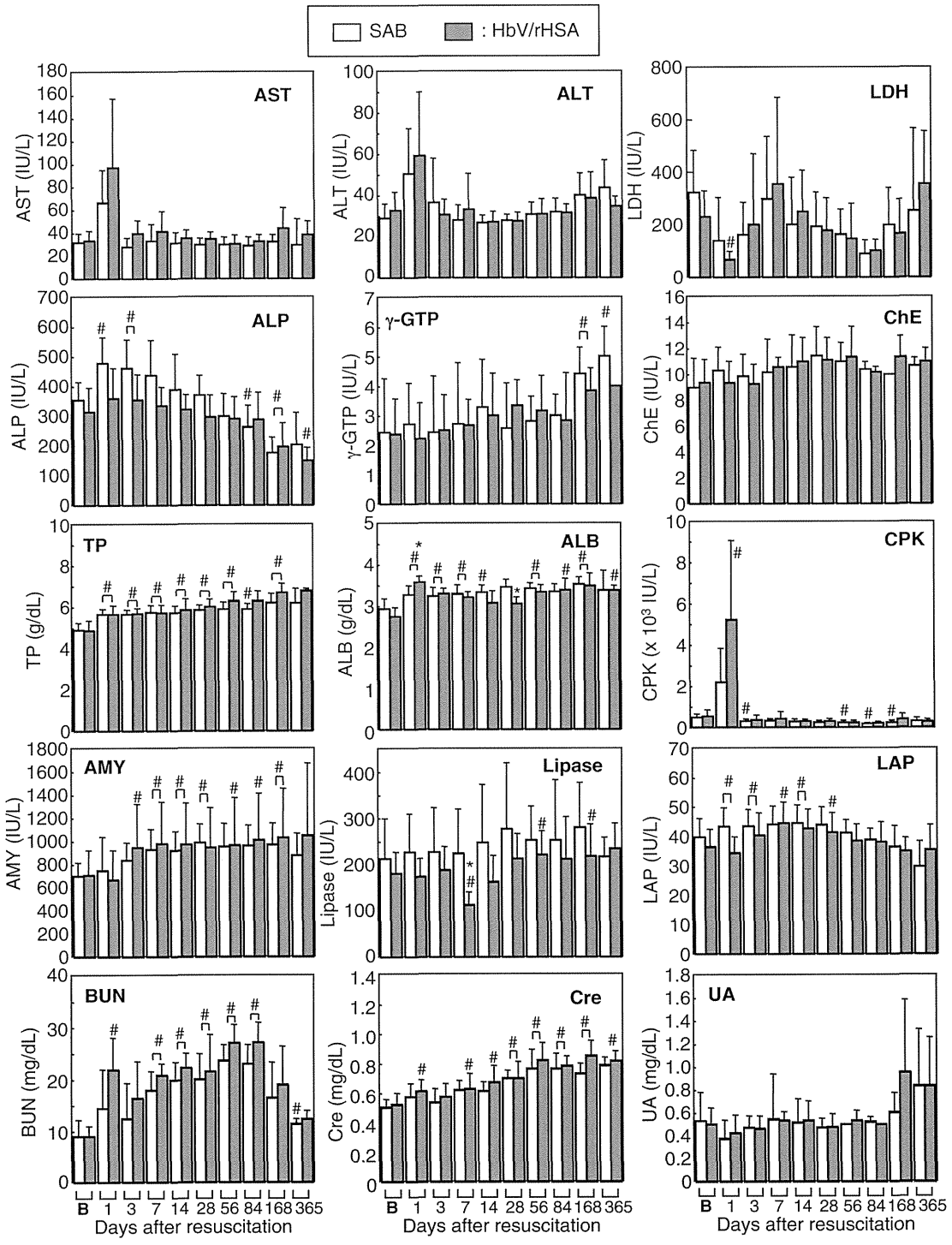

Fig. 28.6 Plasma biochemical tests reflecting the organ functions such as liver, pancreas, and kidneys during one year after 40 percent hemorrhagic shock and resuscitation with infusion of SAB and HbV/rHSA. The values are mean ± SD. # Significantly different from baseline ($p < 0.01$); * significantly different versus the autologous shed blood group ($p < 0.01$). *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *ALP* alkaline phosphatase, *GGTP* γ-glutamyltransferase, *ChE* cholinesterase, *TP* total protein, *ALB* albumin, *CPK* creatine phosphokinase, *AMY* amylase, *LAP* leucin amino peptidase, *BUN* blood urea nitrogen, *Cre* creatinine, *UA* uric acid

were considered significant amounts of HbV phagocytized by macrophages in the spleen and Kupffer cells in the liver were observed. In chronic phase study, the HbV/rHSA group on Day 28 showed brown pigment deposition in the spleen and the Kuppfer cells of the liver (Fig. 28.8). These findings were not observed at other time points of the HbV/rHSA groups and SAB groups. No significant changes were seen in the pancreas, lung, heart, and kidney (Fig. 28.9).

28.4 Discussion

Our primary finding in this study is that HbV suspended in an albumin solution showed a similar resuscitative ability to that of SAB. Cardiovascular function such as MAP, PAP, CVP, PCWP recovered after resuscitation, and there was not significant differences between all groups (HbV/rHSA group, rHSA group, and SAB group). We have reported the efficacy as a resuscitative fluid in hemorrhagic shock in canine model (Yamamoto et al. 2012). In the previous report, up-regulation of PAP in HbV group after resuscitation was significantly higher than the other groups (SAB, rHSA, and Lactate Ringer solution groups). We thought HbV has the constrictive potential to the pulmonary circulation. In this study, PAP recovered after resuscitation in all groups and there were no significant differences between groups. From these findings, we considered that spleen plays a primary role to mitigate the influence of HbV. Since spleen is a large RES organ, it could play a role as a filter of the particle that might influence on endothelium. Furthermore, it was the first time to clarify the long term safety of HbV using canine model as evidenced by the result that HbV did not disturb the cardiopulmonary circulation, and all the dogs survived for 1 year without any remarkable side effect for each organs.

In the acute phase study, the rHSA group tended to delay the recovery of decreased MAP, however, HbV/rHSA group showed the prompt response that was similar to SAB group. The change of DO_2 showed the similar characteristics to the change of MAP, and HbV contributed 26–29 % of the total DO_2 values. These findings showed that the resuscitative ability of HbV was better than that of rHSA and was equivalent to SAB, because of the enough ability of oxygen carrying capacity. Regarding the rHSA group, MAP tended to show lower values than the other groups, and SVR showed significantly lower value than the HbV/rHSA group. The remarkable increase of cardiac output (CO) was caused by the reduced blood viscosity and the increased HR to compensate for the inadequate oxygen carrying capacity. Due to the sufficient compensatory mechanism to this level of hemorrhagic shock and resuscitation, the blood gas parameters showed non-significant changes between rHSA and the other groups. By contrast, lactic acid showed lower value in the rHSA group than in other groups. Because all the dogs in the rHSA groups survived for 4 h, the rHSA fluid alone possesses a resuscitative ability to restore blood volume in spite of the lack of oxygen carrying capacity. Unlike a human spleen, a canine spleen is an important blood reservoir capable of

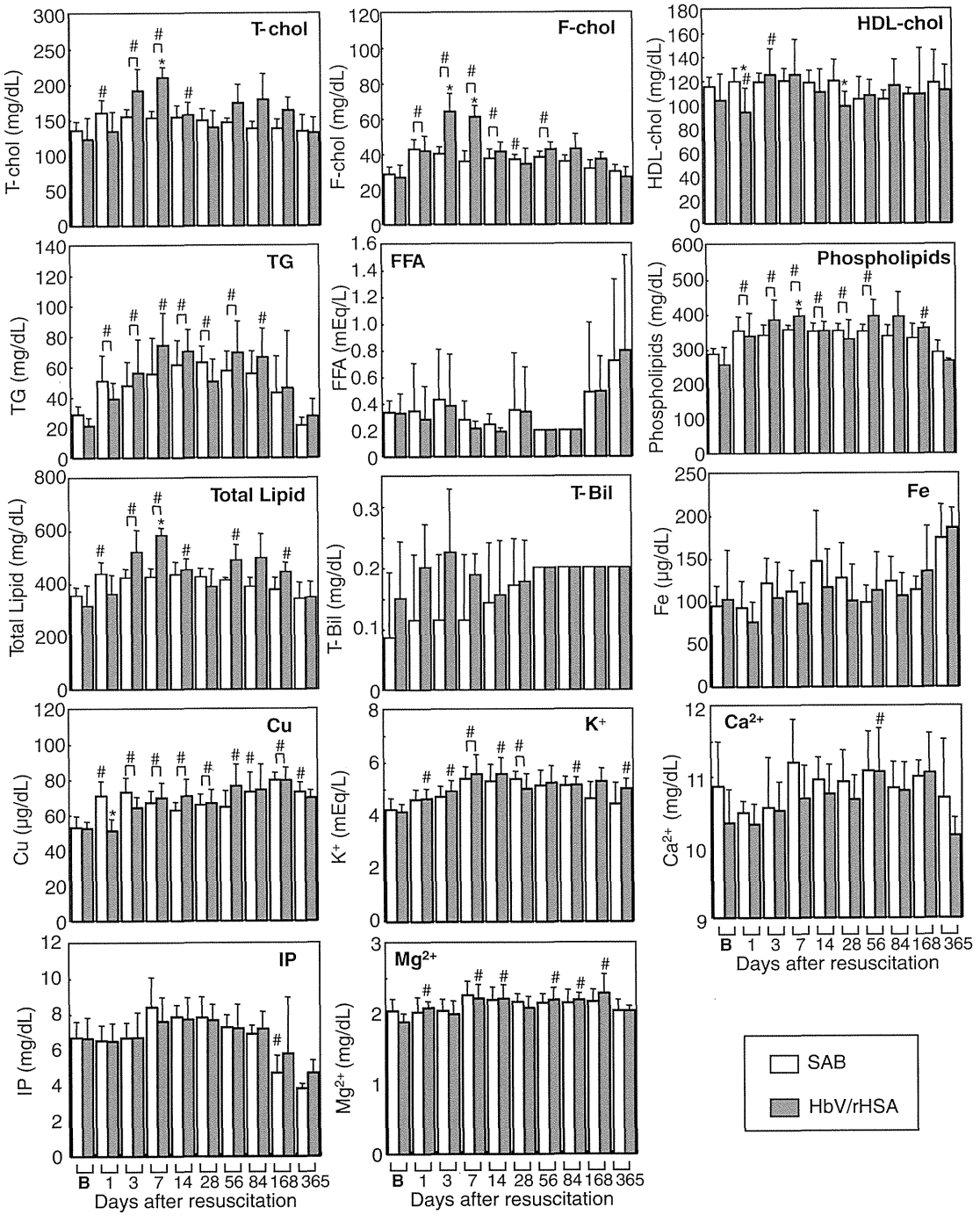

Fig. 28.7 Plasma biochemical tests relating the metabolism of the components of HbV (lipids and Hb), microelements, and electrolytes during one year after 40 percent hemorrhagic shock and resuscitation with infusion of SAB and HbV/rHSA. The values are mean ± SD. # Significantly different from baseline ($p < 0.01$); * significantly different versus the autologous shed blood group ($p < 0.01$). *T-chol* total cholesterol, *F-chol* free cholesterol, *HDL-chol* high density lipoprotein cholesterol, *TG* triglyceride, *FFA* free fatty acid, *T-Bil* total bilirubin, *IP* inorganic phosphate

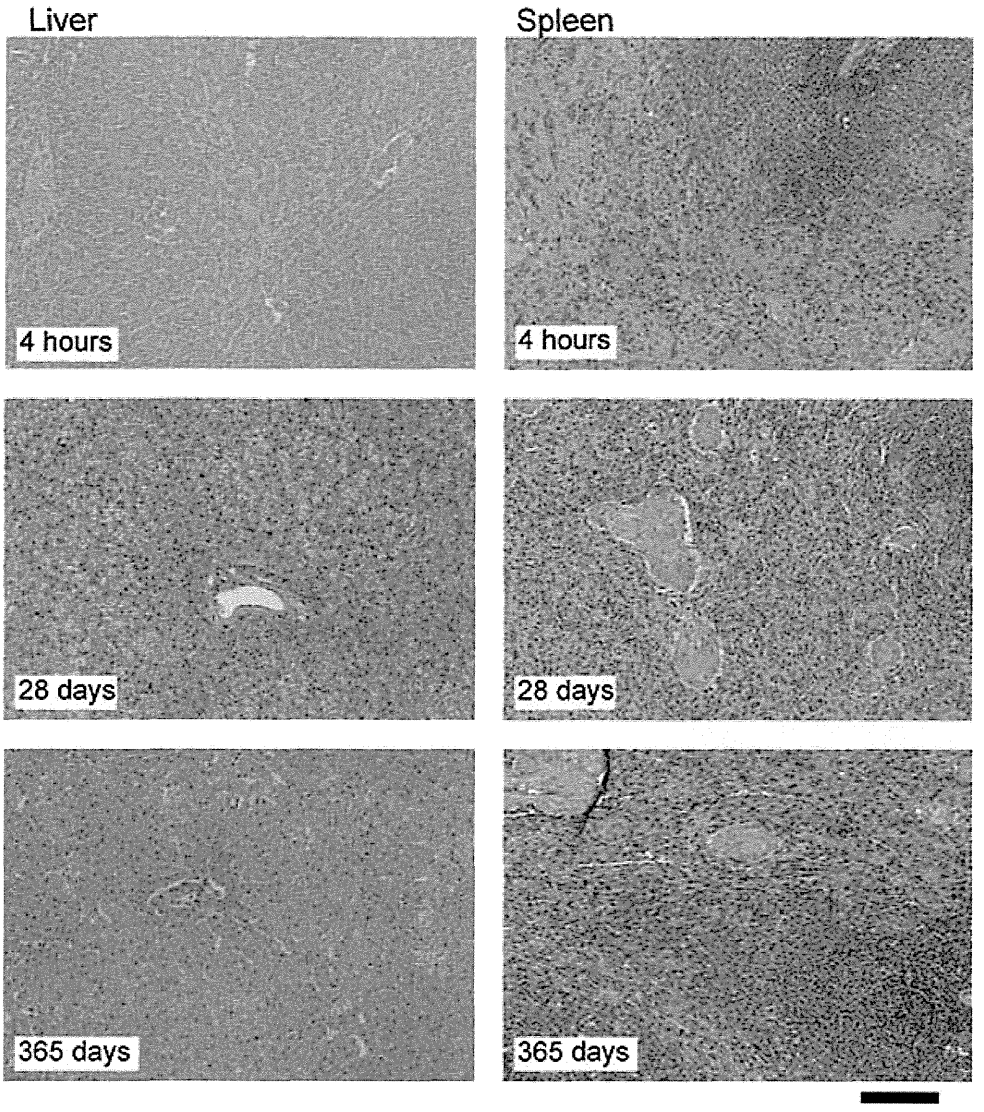

Fig. 28.8 Histology of spleen and liver of HbV/rHSA group at 4 h, and 28 and 365 days after resuscitation. The presence of spleen macrophages and liver Kupffer cells phagocytizing HbV particles was shown at 4 h. The liver and spleen at 28 days contained slight brown pigment deposition. No significant change is noted at 365 days. Scale bar, 100 μm. Hematoxylin and eosin stains

maintaining Hct at a stable level by "autotransfusion" in response to a blood loss such as hemorrhagic shock (Hoit et al. 1991). This might have caused the unexpectedly moderate resuscitative ability of the rHSA solution and without causing severe shock state. In previous report, while animals undertook splenectomy and 50 % hemorrhagic shock, in rHSA group, animals could maintain proper lactate level and showed good recovery of pH during resuscitation and observation phase (Yamamoto et al. 2012). In this respect, resuscitation potential of albumin solution was high, given the patient had enough cardiac reserve to restore organ perfusion, preventing hypoxia. In HbV/rHSA group, cardiac output, heart rate, and lactate level showed almost identical change compared with SAB group.

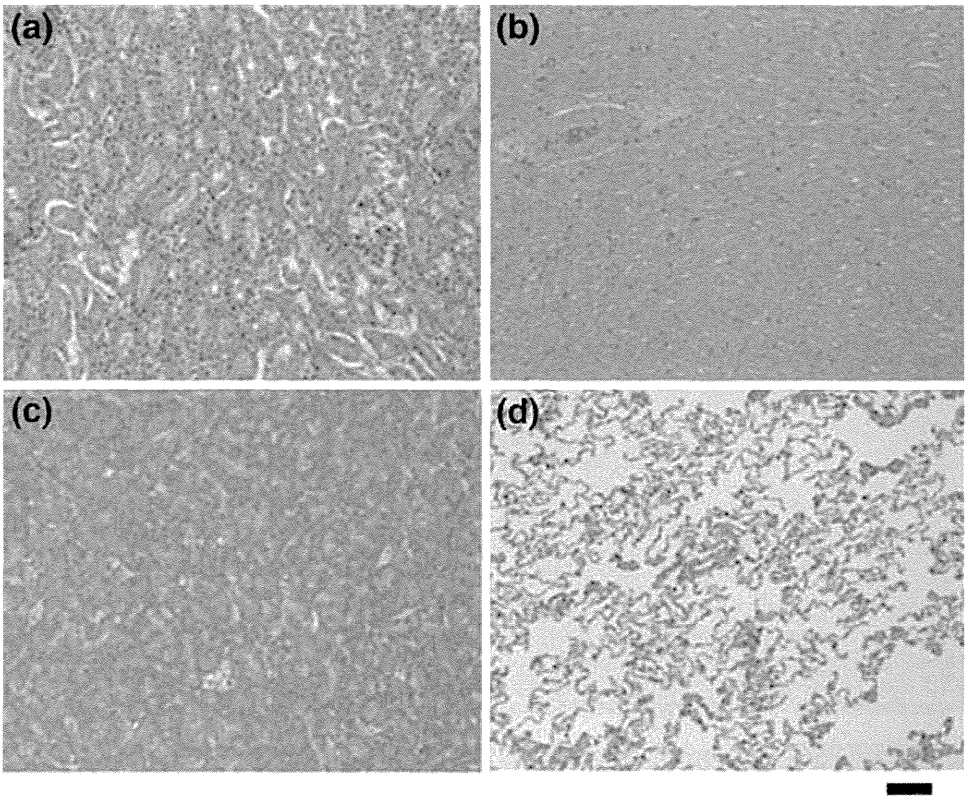

Fig. 28.9 Histology of kidneys (**a**), heart (**b**), pancreas (**c**), and lungs (**d**) of HbV/rHSA group at 365 days after resuscitation. No significant change is noted in these organs. Scale bar, 100 μm. Hematoxylin and eosin stains

It has to be emphasized that acute and chronic safety of HbV was shown in this study using the canine model in which a large amount of HbV was transfused.

It has been reported that resuscitation from hemorrhagic shock with acellular type HBOCs such as polymerized or intramolecular cross-linked Hb causes the elevation of MAP beyond the baseline values. The hypertension may be presumably due to the high affinity for nitric oxide of acellular type HBOCs and their smaller size that enables nitric oxide trapping in the proximity of the endothelium (Sakai et al. 2000a; Yu et al. 2008; Nakai et al. 1998; Driessen et al. 2001; Kasper et al. 1998; Natanson et al. 2008). In this study, the abnormal increase of MAP after infusion of resuscitative fluid was not seen. MPAP showed increase immediately after the infusion, and gradual decrease after 30 min from resuscitation. PVR showed the stable values after the infusion. As HbV/rHSA group did not show the specific elevation of MAP, MPAP, SVR and PVR, we considered that the cellular HbV presumably trap nitric oxide slowly as erythrocyte does. (Sakai et al. 2011; Arazov et al. 2011). On the other hand, it has been reported that the infusion of the other cellular type HBOCs cause the elevation of PVR in a beagle dog model and the elevation of SVR in a goat model (Pape et al. 2008; Kansaku et al. 2008). These circulatory abnormalities are not related the high affinity for nitric oxide because Hb is encapsulated, but may be presumably due to the activation of complement and platelet caused by the lipid component of the membrane

encapsulating the Hb (Abe et al. 2006; Sou and Tsuchida 2008). In the previous report, 50 % hemorrhagic shock model in beagle dog that underwent splenectomy showed transient increase of PAP after resuscitation while in the present study we could not find this change. The difference between previous study and acute phase of the present study is whether splenectomy was conducted. Spleen might have the ability to compromise the transient increase of PAP during resuscitation. Further study is required.

Pathological examination of the liver and spleen of 4 h after resuscitation showed accumulation of HbV (Sakai et al. 2001). Because the circulation half-life of HbV is about 35 h (Sou et al. 2005), the spleen had already started to show accumulation of HbV 4 h after resuscitation. The lung and kidney did not show any abnormalities such as embolism in the capillaries derived from the aggregation of vesicles (Rudolph et al. 1995). In the chronic phase study, Hct showed complete recovery to the baseline 7 days after resuscitation, although difference between SAB group remained significant until two months after experiment. While HbV have disappeared from circulating blood by 7 days after infusion because the circulation half-life of HbV is about 35 h, phagocytized HbV might made effect on the delay of erythropoiesis recovery. In contrast, WBC and Platelet didn't show any significant differences between SAB and HbV/rHSA groups. This fact showed that HbV didn't make an influence on the kinetics of WBC and platelet.

Hemorrhagic shock and resuscitation induce ischemia, hypoxia, and reperfusion injury, all of which influence organ functions. Many precedent papers have described the elevation of plasma enzyme levels, such as AST, ALT, and LDH after resuscitation with HBOCs or transfusion (Sakai et al. 2004c; Marks et al. 1987; Lehnert et al. 2003; Bosman et al. 1992; Mota-Filipe et al. 1999; McDonald et al. 2002; Young et al. 2007). In the present study, the elevations of these plasma enzyme levels were also seen for the SAB group, and these are the common reaction for this kind of shock study.

Lipase activity, but not amylase, significantly decreased in the HbV group, whereas no histopathological abnormality was seen in the pancreas. In our previous tests of daily repeated infusion for 14 days or bolus injection using rats, a transient increase in lipase activity was observed. This was thought to be due to the up regulation of lipase in response to the infusion of a large amount of lipids from the liposomes (Stuecklin-Utsch et al. 2002). However, in this study, the result was in conflict with the past results. The reason is not clear, but the difference of species might be one possible reason.

Liposome-encapsulated Hb without PEG-modification aggregated in the plasma and showed a slight accumulation in the kidneys (Rudolph et al. 1995). However, our PEG-modified HbV does not induce intervesicular aggregation, and does not have any deteriorating influence on the kidneys. In this study, no abnormal value was noted for BUN, Cre, and UA and there was no histopathological abnormality in the kidneys in the HbV/rHSA group.

The plasma lipid components; T-Chol, F-Chol, and Total lipid significantly increased after the infusion in HbV/rHSA group. They should be derived from HbV because it contains a large amount of cholesterol, and they would be liberated

after the HbV particles are captured and degraded in the reticuloendothelial system (RES) (Sakai et al. 2001, 2004a). Extensive studies of circulation kinetics and organ distribution of isotope-labeled HbV clarified that HbV accumulates preferentially in the RES (Awasthi et al. 2004; Sou et al. 2005). It is reported that once liposome is captured in the Kupffer cells, the diacylphosphatidylcholine is metabolized and is reused as a cell membrane component or excreted in the bile (Dijkstra et al. 1985). Cholesterol is finally catabolized as bile acids in the parenchymal hepatocytes. There should be no direct contact of HbV and the hepatocyte because HbV (diameter, 250 nm) cannot diffuse across the fenestrated endothelium into the space of Disse (Goda et al. 1998). Cholesterol of the vesicles should reappear in the blood mainly as lipoprotein cholesterol after entrapment in the Kupffer cells and should then be excreted in the bile after entrapment of the lipoprotein cholesterol by the hepatocytes (Kuipers et al. 1986). Actually, it was confirmed that ^{3}H labeled cholesterol was excreted in feces by the experiment of rat (Taguchi et al. 2009). In terms of PEG-lipid, we reported previously that PEG chain disappeared within 14 days in the liver and spleen by the experiment of rat (Sakai et al. 2009). In the present study, the plasma lipid components increased until 7 days, and recovered at 14 days, and so, it is supposed that the lipid components of HbV would be completely metabolized within 14 days.

During the metabolism of Hb, we would expect a release of bilirubin and iron. But they did not increase in the plasma. The released heme from Hb in HbV could be metabolized by the inducible form of heme oxygenase-1 in the Kupffer cells of the liver and the spleen macrophages (Sakai et al. 2004a; Finch and Huebers 1982). Bilirubin would normally be excreted in the bile as a normal pathway, and no obstruction or stasis of the bile should occur in the biliary tree. Normally, iron from a heme is stored in the ferritin molecule (Grady et al. 1989). Both ferritin and hemosiderin release iron. They are anticipated to induce hydroxyl radical production followed by lipid peroxidation (O'Connell et al. 1989). The iron release rate from hemosiderin, however, is substantially less than that from ferritin (Bennett and Kay 1981). Consequently, the excess amount of iron would then normally be stored in an insoluble and less toxic form as hemosiderin. We found iron deposit in the spleen and liver in long term study. The finding was the same with hemosiderosis often observed in patients who have received repeated blood transfusions.

The liver and the spleen are important organs for degradation of HbV in RES. Pathological examination of the liver showed evidence of Kupffer cells phagocytizing HbV; it disappeared within 7 days in the liver. In the spleen, substantial accumulation of HbV was confirmed in macrophages in the red pulp zone in the same manner as that in previous studies of bolus injection, daily repeated injections, and exchange transfusion (Sakai et al. 2001, 2004b, c). In the present study, hemosiderin deposition was detected in the liver of the HbV/rHSA group at 28 days. These results indicate that heme was metabolized in Kupffer cells of the liver and does not indicate a disability of liver function, as supported by the normal plasma enzyme levels.

We investigated lung, heart, liver, spleen, pancreas, kidney, adrenal glands, testis, trachea, esophagus, small intestine, and colon. Throughout the pathologic survey, we found no fibrotic change in organs. Perfluolochamical artificial oxygen carrier remained in the organs for over two years and induced fibrotic change in the liver (Kitazawa et al. 1982). Perfluorochamicals were said biological inert but it didn't have metabolic pathways. Therefore the material accumulate in the RES system and lymphnode. HbV were made of substantially biodegradable materials. Newly developed materials were minus charged lipid (DHSG) and PEG. Judging from the chemical structure, DHSG could be hydrolysed by non specific dehydrogenase, and the same pathways might be applied to PEG. We could not find any accumulation of these materials pathologically but further study was required.

In conclusion, resuscitation with HbV suspended in rHSA showed rapid recovery of hemodynamic parameters. There was no obvious side effect in hematological tests, plasma biochemical parameters, and histopathological examination within 1 year in comparison to the SAB group. Although transient but substantial accumulation of HbV in phagocytic cells raises concerns of the impact on the defensive function of the body, the present results using beagle dogs reassure that HbV suspended in rHSA shows a similar resuscitative ability and safety to that of SAB.

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Chapter 39
International Consortium for Development of Hemoglobin-Based Oxygen Carriers, Oxygen Therapeutics and Multifunctional Resuscitation Fluids–A White Paper

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39.1 Background

Today, allogeneic donor blood transfusion has evolved as a life-saving treatment for many acute anemic conditions. In developed countries, safe donor blood supply is generally adequate for routine clinical demands. However, in situations where demand greatly exceeds supply (e.g., natural or man-made massive disasters), matched donor blood is not immediately available (e.g., remote locations, battlefield, a rare blood type) or blood transfusion is not an option (e.g., certain religious group or patients with an unusual antibody status), currently there is no alternative treatment.

Based on the post-WWII experience, it is estimated that approximately 20 % of battlefield casualties are potentially salvageable (IOM 1999). The single most cause of death in battlefield casualties is hemorrhage. Therefore, there is greatest opportunity for reducing morbidity and mortality in this group if a safe and battlefield usable 'blood substitute' is available. In recognition of the potential life

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saving benefit, the US Department of Defense, Office of Naval Research, is supporting development of a multifunctional resuscitation fluid (MRF) that may contain a low volume electrolyte solution, an oxygen carrier and coagulation factor(s) in a ready to use battlefield friendly package (ONR 2010).

Of note, blood transfusion carries a risk of disease transmission (e.g., AIDS, hepatitis, malaria, STD, etc.) and certain non-infectious risks (e.g., clerical errors, transfusion reactions, TRALI, immuno-modulation). In sub-Saharan Africa, supply of safe donor blood is scarce because of high prevalence of HIV/AIDS (as much as 30 % in some countries or approx 25 million people) and other transmittable diseases among the donor population (e.g. malaria, trypanosomiasis, leishmaniasis) and inadequate donor blood screening due to limited resources (WHO 2008). Moreover, more than half a million women die each year of severe post-partum hemorrhage representing up 50 % of maternal death in some countries in Africa and Asia due to shortage of safe blood (WHO 2007). Even in the developed countries, safe donor blood is in greater demand as elderly population increases (who more likely to have surgery requiring transfusion) while eligible donor pools decrease due to stagnation in population growth and emergence of newly identified transfusion transmittable pathogens (e.g., vCJD, H1N1 and West Nile viruses, etc.) (Alter and Klein 2008).

Further, allogeneic donor blood can only be used in patient with compatible antibody status requiring typing and crossmatching before use. Donor blood is limited in supply and can only be stored for 5 weeks under refrigerated conditions. In addition, there is ongoing debate that blood transfusion (especially with older blood) may be harmful in certain situations (Alter 2008; Stowell 2010).

Considering these facts, there is a great need to develop universally compatible and readily available alternatives to allogeneic donor blood (red blood cells) for use especially when transfuable blood is not available or an option.(Weiskopf and Silverman 2013). A 'red blood cell substitute' that is safe and effective in saving lives by adequate oxygen delivery and tissue oxygenation preserving vital organ functions during the severe hypovolemia and other acute anemic conditions would be highly desirable.

39.2 Development Status of HBOC, Oxygen Therapeutics (OT) and Multi-Functional Resuscitation Fluids (MRFS)

Over the last 30 years, hemoglobin-based oxygen carriers (HBOCs) have been in development as safe and clinically effective therapeutics ('red cell substitutes') for treatment of hemorrhagic shock, acute anemia, ischemia and other conditions. For several HBOC candidates, preclinical studies were generally positive and some leading products have been tested in Phase III clinical trials, a final stage of development process (reviews by Kim 2004; Jahr 2011). However, observations of some serious adverse events (SAEs) including severe hypertension, MI, stroke and

Table 39.1 FDA summary of adverse events reported in HBOC clinical trials (modified from Silverman et al. 2008)

	Baxter		Biopure		Hemosol		Northfield		Sangart		Somatogen	
Cohort	T	C	T	C	T	C	T	C	T	C	T	C
Number of subjects	504	505	708	618	209	192	623	457	85	45	64	26
1. Death	78	61	25	14	1	4	73	39	2	0	*	*
2. Hypertention	76	38	166	59	113	75	*	*	7	1	8	0
3. Pulmonary Hypertension	1	0	3	0	*	*	*	*	*	*	*	*
4. Chest pain/chest tightness	*	*	21	16	*	*	*	*	*	*	6	0
5. Congestive heart failure	0	1	54	22	0	2	17	20	*	*	*	*
6. Cardiac arrest	*	*	17	6	1	1	14	9	*	*	*	*
7. Myocardial infarction	6	1	14	4	14	7	29	4	2	0	*	*
8. Cardiac arrhythmias/ conduction abnormalities	23	17	153	100	1	1	*	*	15	5	1	1
9. Cerebrovascular accident, cerebrovascular ischemia, TIA	*	*	16	3	2	1	3	1	*	*	*	*
10. Pneumonia	*	*	35	22	*	*	27	21	*	*	*	*
11. Respiratory distress/failure	*	*	22	12	*	*	21	17	*	*	*	*
12. Acute renal failure	1	3	10	4	2	2	*	*	*	*	*	*
13. Hypoxia, cyanosis, decreased oxygen saturation	*	*	76	35	1	1	*	*	*	*	3	1
14. Hypovolemia	*	*	19	4			*	*	*	*	*	*
15. Gastrointestinal	51	31	645	195	23	1	*	*			36	6
16. Liver, LFTs abnormal	27	8	20	5	8	0	*	*	57	20	6	3
17. Pancreatitis	11	0	5	3	1	0	*	*	*	*	*	*
18. Coagulation defect, thrombocytopenia, thrombosis	*	*	45	17	1	0	13	4	*	*	*	*
19. Hemorrhage/bleeding/ anemia	33	22	108	55	1	1	20	17	*	*	*	*
20. Sepsis, septic shock, MOF	2	2	15	6	0	1	26	20	*	*	*	*
21. Pancreatic enzyme inc	13	4	3	0	*	*	*	*	*	*	*	*
22. Lipase increase	29	9	48	12	19	2	*	*	8	4	7	1
23. Amylase increase	48	45	*	*	35	20	*	*	7	2	4	1

T HBOC treated group
C Control solution treated group
* No information available
Note Apex and Enzon also conducted clinical trials but data were not reported

death in recent HBOC clinical trials (Silverman 2009, Table 39.1) and a highly controversial Meta analysis that HBOCs are associated with increased risk of MI and death (Natanson 2008) are hampering further development of HBOCs as viable therapeutics. Recent workshops organized by NIH and FDA (Estep et al. 2008; Silverman 2009; NIH 2011) discussed current issues and provided recommendations on directions of future HBOC research and development.

The causality of HBOCs for the observed SAEs has not definitively been established. To elucidate the pathophysiologic mechanisms of AEs observed with HBOCs, it is essential to understand how HBOCs affect key organ systems and their physiologic functions not simply in normal subjects but in patients. Studies conducted in models of healthy young animals have failed to predict the pathophysiologic responses observed in actual patients who often are older and present with multiple co-morbid conditions (e.g., diabetes, hypertension, cardiovascular diseases, etc.). It is essential that preclinical safety studies be conducted in animal models that closely simulate target patient conditions. To further the development, investigations are required to establish causality of the observed serious adverse events (SAEs) and to test HBOC used and determine the pathophysiologic mechanism involved. Only when armed with accurate knowledge of pathophysiologic mechanisms of adverse events (AEs), may appropriate modifications be made to the current HBOC products or develop a new generation of safer products.

More recently, Mozzarelli (2011) presented a more open view on the strategies for designing a new generation of safer and effective products. Certain HBOCs are also being developed as oxygen therapeutics (OTs) targeted for treatments of ischemic tissues and organs (e.g., ischemic heart/limb, ischemic stroke). In addition, because many civilian and military hemorrhagic trauma victims are presented with coagulopathy, MRFs that contain procoagulation agents are also in development.

39.3 Current Issues and Barriers

The current impediment in the progress of HBOC development is in large part due to insufficient scientific understanding of some critical mechanisms. How an individual HBOC formulation, when administered intravenously, interacts with the host mechanisms in heightened or compromised state by disease, surgery or traumatic injury especially presented with concurrent underlying co-morbid conditions (e.g., hypertension, diabetes, cardiovascular diseases). It is an extremely complex dynamic process involving multiple cellular, tissue and organ systems which are ultimately integrated into the whole systemic response and its fate. To help facilitate HBOC development, a NIH-NHLBI organized working group workshops in 2006 (Estep et al. 2008) and 2011 (NIH 2011) and identified some of the key issues holding up the progress of the field (Table 39.2) and made a series of recommendations (Table 39.3). In addition, there are some inherent limitations in a traditional industry-centered collaboration model (Kim 2011).

Some of the key issues are:

- Animal models did not predict adverse effects observed in clinical trials Results of most preclinical animal studies conducted with various candidate HBOC products were generally positive. However, preclinical animal models did not predict the AEs observed in clinical studies. Therefore, there is a need to identify/develop animal models that are relevant to target patient conditions and

Table 39.2 Important issues to be addressed in HBOC development (Estep et al. 2008; NIH 2011)

- Development of animal models that more closely simulate human clinical conditions with co-morbidities (e.g., diabetes, hypertension, cardiovascular diseases)
- Development of new improved HBOCs (e.g., HBOCs with reduced vasoactivity and oxidative reactions, enhanced circulation time and shelf life)
- Investigation of mechanisms of cardiovascular and cerebrovascular events observed after blood substitutes infusion
- Significance of cardiac lesions observed after HBOC infusion
- Role of reactive oxygen species (ROS) in the etiology of human clinical side effects
- Exploration of interactive effects between blood substitutes infusion and concurrent fluid, drug and anesthetic therapies
- Effect of concurrent stress, particularly local or systemic inflammation, in response to blood substitutes infusion
- Investigation of the cause of bradycardia associated with HBOC administration
- Further study of mechanism and clinical significance of vasoactive effects of HBOCs
- Evaluation of the mechanism and clinical significance of gastrointestinal distress, pancreatic toxicity and liver and pancreatic enzyme elevation
- Comparative assessment of the antigenic and immunomodulatory properties of different blood substitutes
- Effects of formulation excipients
- Further study of the distribution and metabolism of HBOCs

Table 39.3 2006 NIH workshop recommendations (Estep et al. 2008)

- Exploration of the mechanism(s) of adverse side effects that have been observed during the clinical testing of HBOC formulations. Particular priority should be given to the investigation of cardiovascular and cerebrovascular events. The use of animal models with compromised cardiovascular systems and/or altered physiology in the evaluation of HBOC solutions is highly encouraged
- Further evaluation of the distribution and metabolism of different Hb derivatives, especially with reference to the role that these factors play in the etiology of adverse events and the determination of functional intravascular persistence
- Continued research into the physiology of oxygen delivery by acellular formulations ranging from subcellular to global levels of response, with emphasis on the microcirculation in different tissue beds
- Assessment of whether enhanced generation of ROS after HBOC infusion is responsible for clinically observed adverse events in humans
- Evaluation of the impact of HBOC formulation excipients on product toxicity and stability
- Development of new Hb active entities with improved adverse event profiles and enhanced intravascular functional persistence
- Identification and use of improved models for the comparative assessment of HBOC formulation safety and efficacy. Such models should be predictive of response in humans, incorporate stress conditions and be used to systematically evaluate the effect of variation in Hb structure, biochemistry and physical chemical properties
- Production and distribution of highly purified HBOC solution(s) in sufficient quantity to support the research and testing advocated elsewhere in this report
- Comprehensive assessment and reporting of the adverse events and physiologic response of HBOC solutions evaluated in clinical trials, recognizing that such an assessment would require the permission of commercial manufacturers and collaboration with the US FDA
- Development and validation of a noninvasive method for the routine clinical assessment of critical organ oxygenation to better inform decisions to transfuse HBOCs and blood

to employ more sensitive tests that could detect molecular and cellular dysfunction as well as organ-specific toxicities and systemic abnormalities.

- Limited availability of test materials (HBOCs/OTs)
  One well recognized issue in HBOC development is limited availability of test HBOC solutions for conducting independent investigations. Because most HBOC products are not yet marketed, independent investigators have difficulty obtaining test HBOC products to conduct evaluations. To allow more independent evaluations of candidate HBOC products, 2006 NIH-NHLBI Workshop (Estep et al. 2008) recommended that supply of sufficient amount of well characterized standardized HBOC formulations be made available to the general research community. To help accomplish that recommendation, NIH awarded a SBIR contract to a company that now make its products available for investigators albeit at a cost. However, because AEs and toxicities have been observed with different HBOC products, it is important that more than one product be made available to investigators. In addition, as deemed necessary certain experimental (as well as marketed) procoagulation products (e.g., platelets, fresh frozen plasma, cryoprecipitate, rFVIIa anti-fibrinolytics) should also be made available for development of MRFs. Therefore, this consortium will invite producers of HBOC/OT/MRF products to participate knowing that this will be a NIH/FDA guided pathway for possible regulatory approval. As such, the FDA will be invited in these deliberations and expected to provide advice/guidance to create a validated pathway for potential approval of products.
- Causality of HBOC in observed SAEs
  Cause(s) of the SAEs observed in HBOC clinical trials have not been thoroughly investigated. This is in part due to lack of detailed and objective information/data regarding the nature/circumstances of observed AEs. Currently, major HBOC developmental efforts are led by few companies that adopt a traditional industry-centered research model. This approach is a largely a 'closed' system in which most data (especially negative data) are kept confidential among the close collaborators only. The exact nature of negative results is not generally made available to a wider group of independent unbiased investigators. Therefore, outside researcher are often deprived of the opportunity to the timely investigation of SAEs and other side effects/toxicities. This 'closed' approach significantly hampers expeditious development of possible resolutions.

39.4 The HBOC/OT/MRF Research Consortium, a Way Forward

To facilitate progress of HBOC development, we propose a HBOC research consortium of key leading academic investigators and select HBOC producers from US, Europe and Asia. The consortium will serve as a think tank and a

coordinating body for collaborative efforts in investigation of the key unresolved scientific issues that are hampering further progress in HBOC development. The HBOC research consortium will facilitate development of viable HBOC products through concerted efforts of the some of the world's leading experts in the field. To encourage constructive discussions/solutions for key unresolved issues, the consortium will adopt an open communication policy and objective and transparent processes in the conduct of research. The goal of the consortium is to foster orchestrated collaborations and constructive discourse and to breakdown barriers that impede development of viable HBOC products.

Some key goals of the consortium are:

- Foster collaboration for expeditious resolutions of key unresolved issues in HBOC development.
- Coordinate collaboration to minimize unnecessary duplications/redundancies for maximum efficiency and conserve resources.
- More objective and transparent evaluation of candidate products by independent investigators exploiting state of the art methods to investigate physiological and biochemical mechanisms.
- Facilitate information/data exchange and prompt and timely dissemination of research findings through open presentations, publications and other media.
- Serve as a central 'library' for HBOC research and other relevant information obtained from public sources or voluntarily provided by authors, study sponsors and publishers. (copy right issue will be openly discussed and negotiated).
- Identify and secure funding for HBOC research/development (e.g., national and international, public and private funding agencies).
- Foster young investigators to enter into the field.
- Identify an optimal HBOC formulation and/or develop a new viable product in the next 10 years, including basic science, translational and FDA approved Phase 1 clinical trials.
- FDA will be invited to participate from the ground level to provide advice in design of experiments, protocol development and formulating guidelines to ensure that required preclinical and clinical studies are performed according to Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) and other regulatory guidelines. This FDA guided product development approach will serve as a 'validated' pathway to eventual product approval.

To achieve the stated goals, the consortium will utilize a multi-disciplinary approach. The core groups of the consortium will be US-based but to maximize 'brain power' for more expeditious development, a select group of leading international experts will also be included (see Table 39.4). In addition, to maximize the probability of successful outcome (discovery/identification of a successful product), the consortium will evaluate multiple candidate products with distinct characteristics. The candidate HBOC products will be studied (for selected projects) by member investigators with no conflict-of-interest issues with the products being tested. All investigators agree to participate in the consortium will be asked to disclose any conflict-of-interest issues. If found a clear conflict, he/she