刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
Biochim. Biophys. Acta **1668**, 145-155 (2005). "Antioxidant effect of bovine serum albumin on membrane lipid peroxidation induced by iron chelate and superoxide."	2005 年 2 月	Elsevier B.V.	K. Fukuzawa Y. Saitoh K. Akai K. Kogure S. Ueno A. Tokumura M. Otagiri A. Shibata
Biochim. Biophys. Acta **1747**, 81-88 (2005). "Effect of genetic variation on the thermal stability of human serum albumin."	2005 年 2 月	Elsevier B.V.	U. Kragh-Hansen S. Saito K. Nishi M. Anraku M. Otagiri
人工血液. **13**, 1-7 (2005) "血清アルブミンの構造特性と医薬への応用"	2005 年	日本血液代替物学会	安楽 誠 丸山 徹 小田切優樹
Brit. J..Haemato. **128**, 533-539 (2005) "Platelet glycoprotein Ib alpha polymorphisms affect the interaction with von Willebrand factor under flow consitions."	2005 年 2 月	Blackwell Publishing Ltd.	Y. Matsubara M. Murata T. Hayashi K. Suzuki Y. Okamura M. Handa H. Ishihara T. Shibano Y. Ikeda
Hepatology. **39**, 1099-1109, (2004) "Kupffer cells alter organic anion transport through multidrug resistance protein 2 in the post-cold ischemic rat liver."	2004 年 4 月	American Association for the Study of Liver Diseases	A. Kudo S. Kashiwagi M. Kajimura Y. Yoshimura K. Uchida S. Arii M. Suematsu
(2004) "代用血漿剤と臨床"	2004 年 6 月	克誠堂出版株式会社	小堀正雄 高折益彦

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
Artif. Cell. Blood Substit. Biotechnol. **33**, 65-73 (2005) "Approach to clinical trial considering medical ethics and efficacy for HbV , liposome encapsulated hemoglobin vesicle. "	2005 年	Taylor & Francis	M. Takaori
In: Artificial Oxygen Carrier: Its Front Line, Keio University International Symposia for Life Sciences and Medicine Vol.12, pp. 1-21, (2005). "Safety and Efficacy of Hemoglobin-Vesicles and Albumin-Hemes" .	2005 年	Springer-Verlag	K. Kobayashi H. Horinouchi M. Watanabe Y. Izumi Y. Teramura A. Nakagawa Y. Huang K. Sou H. Sakai T. Komatsu S. Takeoka E. Tsuchida
In: Artificial Oxygen Carrier: Its Front Line, Keio University International Symposia for Life Sciences and Medicine Vol.12, pp. 135-168, (2005). " Hemoglobin-vesicles (HbV) as artificial oxygen carriers" .	2005 年	Springer-Verlag	H. Sakai K. Sou S. Takeoka K. Kobayashi E. Tsuchida
Bioconjugate Chem. **15**, 1037-1045 (2004). "Reduction of Methemoglobin via Electron Transfer from Photoreduced Flavin: Restoration of O2-Binding of Concentrated Hemoglobin Solution Coencapsulated in Phospholipid Vesicles."	2004 年 9 月	American Chemical Society	H. Sakai Y. Masada H. Onuma S. Takeoka E. Tsuchida
Bioconjugate Chem. **15**, 1037-1045 (2004). "Reduction of Methemoglobin via Electron Transfer from Photoreduced Flavin: Restoration of O_2-Binding of Concentrated Hemoglobin Solution Coencapsulated in Phospholipid Vesicles. "	2004 年 9 月	American Chemical Society	H. Sakai Y. Masada H. Onuma S. Takeoka E. Tsuchida

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
J. Pharmacol. Exp. Ther. **311**, 874-884 (2004) "Physiologic capacity of reticuloendothelial system for degradation of hemoglobin-vesicles (artificial oxygen carriers) after massive intravenous doses by daily repeated infusions for 14 days."	2004 年 12 月	The American Society for Pharmaclogy and Experimental Therapeutics	H. Sakai Y. Masada H. Horinouchi E. Ikeda K. Sou S. Takeoka M. Suematsu K. Kobayashi E. Tsuchida
J. Pharmacol. Exp. Ther. **312**, 702-709 (2005). "Circulation kinetics and organ distribution of hb-vesicles developed as a red blood cell substitute."	2005 年 2 月	The American Society for Pharmaclogy and Experimental Therapeutics	K. Sou R. Klipper B. Goins E. Tsuchida W. T Phillips
Am. J. Physiol. Heart Circ. Physiol. (2005) "Oxygen transport by low and normal P50 Hb-vesicles in extreme hemodilution."	印刷中	The American Physiological Society	P. Cabrales H. Sakai A. G. Tsai S. Takeoka E. Tsuchida M. Intaglietta
J. Biomed. Mater. Res. **71A**, 63–69 (2004) "Exchange transfusion with entirely synthetic red-cell substitute albumin-heme into rats: Physiological responses and blood biochemical rests."	2004 年	Wiley Periodicals,Inc.	Y. Hunag T. Komatsu H. Yamamoto H. Horinouchi K. Kobayashi E. Tsuchida
Org. Biomol. Chem. **2**, 3108–3112 (2004). "Synthesis of protoheme derivatives with a covalently linked proximal-base and their human serum albumin hybrids as artificial hemoprotein."	2004 年 11 月	The Poyal Society of Chemistry	A. Nakagawa N. Ohmichi T. Komatsu E. Tsuchida
J. Am. Chem. Soc. **126**, 14304–14305 (2004) "Dioxygenation of human serum albumin having a prosthetic heme group in a tailor-made heme pocket. "	2004 年 11 月	American Chemical Society	T. Komatsu N. Ohmichi P. A. Zunszain S. Curry E. Tsuchida

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
J. Biomed. Mater. Res. **71A**, 644–651 (2004). "Exchange transfusion with synthetic oxygen-carrying plasma protein "Albumin-Heme" into an acute anemic rat model after seventy-percent hemodilution."	2004 年 12 月	Wiley Periodicals,Inc.	T. Komatsu H. Yamamoto Y. Huang H. Horinouchi K. Kobayashi E. Tsuchida
Bioconjugate Chem. **16**, 23–26 (2005). "Human serum albumin bearing covalently attached iron (II) porphyrins as O2-binding sites. "	2005 年 1 月	American Chemical Society	R.M. Wang T. Komatsu A. Nakagawa E. Tsuchida
「新訂版・表面科学の基礎と応用」第 5 章、第 12 節 pp. 1333-1336、"人工赤血球"	2004 年 6 月	エヌ・ティー・エス社	土田英俊 武岡真司 小松晃之 酒井宏水
体液・代謝管理 20, 3-6 (2004). "酸素輸送合成ヘム蛋白質 "アルブミン－ヘム" の創製と酸素輸液の展開"	2004 年	体液・代謝管理研究会	小松晃之 土田英俊
日本医師会雑誌 131, 907-910 (2004) "人工血液(人工赤血球)の開発動向"	2004 年 4 月	日本医師会	武岡真司
「周術期輸液の最前線」pp.162-168、"酸素輸液の展望"	2004 年 7 月	真興交易	武岡真司 宮尾秀樹
In: Organ Micocirculation: A Gateway to Diagnostic and Therapeutic Investigation. pp. 35-41, (2005) "Design and Modification of Nanoparticles for Blood Substitutes"	2005 年	Springer-Verlag	S. Takeoka
In: Artificial Oxygen Carrier: Its Front Line, Keio University International Symposia for Life Sciences and Medicine Vol.12, pp. 193-204 (2005). "Oxygen-carrying plasma hemoprotein including synthetic heme"	2005 年	Springer-Verlag	T. Komatsu E. Tsuchida
In: Artificial Oxygen Carrier: Its Front Line, Keio University International Symposia for Life Sciences and Medicine Vol.12, pp. 267-278 (2005). "Studies on Red cell Substitutes in Japan and Future Perspectives."	2005 年	Springer-Verlag	M. Takaori

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
人工血液 13, 34-41（2005）“完全合成型人工酸素輸液の開発”	2005 年 3 月	日本血液代替物学会	甲斐俊哉 木田善規 福富一平 帆足洋平 片山直久 山本尚志 大川裕紀 弘津一郎 佐藤 誠
人工血液、2005 “ヒト赤血球由来ヘモグロビンによるヘモグロビン小胞体の開発と酸素輸液としての医療応用、人工血液”	2005 年	日本血液代替物学会	高木智史 大村光代 太田勝次 須賀裕子 松浦昭宏
Eur. J. Cardio-thoracic Surg. **28,** 39-42 (2005). “The sealing effect of fibrin glue against alveolar air leakage evaluated up to 48h ;comparison between different methods of application”	2005 年	Elsevier B.V.	M. Kawamura M. Gika Y. Izumi H. Horinouchi N. Shinya M. Mukai K. Kobayashi
Am. J. Physiol. Lung Cellular Mol. Physiol. **289**, 956-953 (2005). “Role of Rho-kinase in reexpansion pulmonary edema in rabbits”	2005 年	American Physiological Society	M. Sawahuji A. Ishizaka M. Kohno H. Koh S. Tasaka Y. Ishii K. Kobayashi
Transfusion **46**, 339-347 (2006) “Acute 40% exchange transfusion with Hb-vesicles (HbV) suspended in recombinant HSA solution: Degradation of HbV and erythropoiesis in rat spleen observed for 2 Weeks.”	2006 年 3 月	American Association Of Blood Banks	H. Sakai H. Horinouchi M. Yamamoto E. Ikeda S. Takeoka M. Takaori E. Tsuchida K. Kobayashi.
外科, **68**, 82-87 (2006) “外科医のための輸血医学講座 人工血液の現在と将来.”	2006 年	泰山堂書店	小林紘一

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
ハートナーシング, **18**, 965-969 (2005). “人工赤血球(人工酸素運搬体)の臨床応用”	2005 年	メディカ出版	小林紘一 山崎真敬 饗庭 了 四津良平 土田英俊
検査と技術, **33**, 879-881(2005). “人工酸素運搬体の開発 現状と将来展望.”	2005 年	医学書院	堀之内宏久 泉 陽太郎 小林紘一 土田英俊
日本外科学会雑誌 **106**, 31-37 (2005). “外科領域における輸血と血液製剤の現状と展望. 7. 人工血液.”	2005 年	日本外科学会	小林紘一
Annual Review 呼吸器 2005. 152-155 (2005). Ⅲ.診断の進歩 “3. 細径気管支鏡.”	2005 年	中外医薬社	堀之内宏久 藤本博之 小林紘一
「医療用マテリアルと機能膜」, 第 5 章、pp 107-118, “人工赤血球”	2005 年 5 月	㈱シーエムシー出版	酒井宏水 宗 慶太郎 武岡真司 小林紘一 土田英俊
先端医療シリーズ３７「人工臓器・再生医療の最先端」 第 16 章-4. pp. 279-286, “酸素輸液(人工赤血球)の臨床応用”	2005 年 11 月	寺田国際事務所 / 先端医療技術研究所	土田英俊 酒井宏水 小松晃之 小林紘一
「麻酔科診療プラクティス 18. 周術期の輸液・輸血療法」 pp. 270-273, “(トピックス)輸血製剤への新しい取り組み:人工赤血球”	2005 年	文光堂	土田英俊 小林紘一
“Blood Substitutes” Ed. by Robert M. Winslow, Chapt. 44, pp. 514-522, “Hemoglobin-vesicles as a molecular assembly: Characteristics of preparation process and performances as artificial oxygen carriers.”	2006 年	Elsevier	H. Sakai K. Sou S. Takeoka K. Kobayashi E. Tsuchida

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
"Blood Substitutes" Ed. by Robert M. Winslow, Chapt. 46, pp. 532–539, "Albumin-heme: a synthetic heme-based oxygen carrier".	2006 年	Elsevier	T. Komatsu Y. Huang H. Yamamoto H. Horinouchi K. Kobayashi E. Tsuchida
Artif. Cells Blood Substitutes Biotechnol. **33**, 101-111 (2005). "Effects of hemoglobin vesicles on resting and agonist-stimulated human platelet in vitro."	2005 年	Taylor & Francis, Inc.	S. Wakamoto M. Fujihara H. Abe M. Yamaguchi S. Takeoka E. Tsuchida H. Azuma H. Ikeda
Artif Cells Blood Substitutes Biotechnol. **34**, 1-10 (2006). "Interaction of hemoglobin vesicles, a cellular-type artificial oxygen carrier, with human plasma: Effects on coagulation, kallikrein-kinin, and complement."	2006 年	Taylor & Francis, Inc.	H. Abe M. Fujihara K. Ikebuchi S. Takeoka E. Tsuchida H. Harashima H. Azuma H. Ikeda
Drug Metab Pharmacokinet. **20**, 309-323 (2005). "A molecular functional study on the interactions of drugs with plasma proteins."	2005 年	日本薬物動態学会	M. Otagiri
Drug Metab. Dispos. **33**, 1911-1919 (2005). "Hydrolysis of angiotensin II receptor blocker prodrug olmesartan medoxomil by human serum albumin and identification of its catalytic active sites."	2005 年	The American Society for Pharmaclogy and Exprimental Therapeutics	S. F. Ma M. Anraku Y. Iwao K. Yamasaki U. Kragh-Hansen N. Yamaotsu S. Hirono T. Ikeda M. Otagiri

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
J. Mol. Biol. **353**, 38-52 (2005). "Structural basis of the drug-binding specificity of human serum albumin."	2005 年	ELSEVIER	J. Ghuman P. A. Zunszain I. Petitpas A. A. Bhattacharya M. Otagiri S. Curry
Biochemistry **44**, 11122-11129 (2005). "Effect of nitric oxide in amyloid fibril formation on transthyretin - related amyloidosis."	2005 年	American Chemical Society	S. Saito Y. Ando M. Nakamura M. Ueda J. Kim Y. Ishima T. Akaike
Biochem. Biophys. Res. Commun. **334**, 1322-1328 (2005). "The structure and function of oxidized albumin in hemodialysis patients: Its role in elevated oxidative stress via neutrophil burst."	2005 年	ELSEVIER	K. Mera M. Anraku K. Kitamura K. Nakajou T. Maruyama M. Otagiri M. Otagiri
Biochim. Biophys. Acta. **1725**, 385-393 (2005). "Characterization of benzodiazepine binding site on human alpha1-acid glycoprotein using flunitrazepam as a photolabeling agent."	2005 年	ELSEVIER	V. T. Chuang M. Hijioka M. Katsuki K. Nishi T. Hara K. Kaneko M. Ueno A. Kuniyasu H. Nakayama M. Otagiri
FEBS Lett. **579**, 3596-3600 (2005). "Cooperative effect of hydrophobic and electrostatic forces on alcohol-induced alpha-helix formation of alpha1-acid glycoprotein."	2005 年	Elsevier BV	K. Nishi Y. Komine N. Sakai T. Maruyama M. Otagiri

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
J. Biochem. (Tokyo). **137**, 607-616 (2005). "CD36 is not involved in scavenger receptor-mediated endocytic uptake of glycolaldehyde- and methylglyoxal-modified proteins by liver endothelial cells."	2005 年	The Japanese Biochemical Society	K. Nakajou S. Horiuchi M. Sakai K. Hirata M. Tanaka M. Takeya T. Kai M. Otagiri
J. Neurochem. **96**, 1051-1059 (2006). "Involvement of organic anion transporters in the efflux of uremic toxins across the blood-brain barrier."	2006 年	Blackwell Science,	T. Deguchi K. Isozaki K. Yousuke T. Terasaki M. Otagiri
Biochim. Biophys. Acta **1747**, 81-88, (2005) "Effect of genetic variation on the thermal stability of human serum albumin."	2005 年	Elsevier B.L	U. Kragh-Hansen S.saito K.Nishi M.Anraku M.Otagiri
Hypertens. Res. **28**, 973-980 (2005). "Oxidation and Carboxy Methy Lysine-Modification of Albumin : Possible Invement in the Progression of Oxidative Stress in Hemodialysis Patients."	2005 年	日本高血圧学会	K.Mera M.Anraku K.Kitamura K.Nakajou T.Maruyama M.Otagiri
Thromb. Res. **115**, 191-197, (2005). "Factor XII Shizuoka, a novel mutation (Ala392Thr) identified and characterized in a patient with congenital coagulation factor XII deficiency."	2005 年	Pergamon Press	S. Oguchi K. Ishii T. Moriki, E. Takeshita M. Murata Y. Ikeda K. Watanabe
Neurosci. Lett. **374**, 132-135 (2005). "T280M and V249I polymorphisms of fractalkine receptor CX3CR1 and ischemic cerebrovascular disease."	2005 年	Elsevier Scientific Publishers Ireland	H. Hattori D. Ito N. Tanahashi M. Murata I. Saito K. Watanabe N. Suzuki

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
Blood Coagulation Fibrinolysis **16**, 9-16 (2005) "Genetic analysis of hereditary factor X deficiency in a French patient of Sri Lankan ancestry: *in vitro* expression study identified Gly366Ser substitution as the molecular basis of the dysfunctional factor X."	2005 年	Lippincott Williams And Wilkins	I. Isshiki R. Favier T. Moriki T. Uchida H. Ishihara P. Van Dreden M. Murata Y. Ikeda
J. Epidemiol. **5**, 203-10 (2005). "Increased risk of obesity resulting from the interaction between high energy intake and the Trp64Arg polymorphism of the beta3-adrenergic receptor gene in healthy Japanese men."	2005 年	Japan Epidemiological Association	K. Miyaki, S. Sutani H. Kikuchi I. Takei M. Murata K. Watanabe K. Omae
J. Hum. Genet. **50**, 241-8 (2005). "Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype."	2005 年	Springer Tokyo	K. Miyaki M. Murata H. Kikuchi I. Takei T. Nakayama K. Watanabe K. Omae
Thromb. Circ. **13**, 90-94 (2005). "PFA-100 による血小板凝集能測定."	2005 年	メディカルレビュー社	高橋信一 村田　満
Thromb. Circ. **13**, 20-24 (2005). "血小板機能と遺伝子多型（分子疫学的立場）"	2005 年	メディカルレビュー社	村田　満
日本医師会雑誌. **134**(特別号)S316-S319, (2005) "血栓性血小板減少性紫斑病　わかりやすい免疫疾患."	2005 年	日本医師会	鈴木美佐 村田　満
Circ. Res., **97**, e104-14 (2005). "Carbon monoxide from heme oxygenase-2 is a tonic regulator against NO-dependent vasodilatation in the adult rat cerebral microcirculation."	2005 年	American Heart Association	M. Ishikawa M. kajimura T. Adachi K. Maruyama N. Makino N. Goda T. Yamaguchi E. Sekizuka M. Suematsu

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
Antioxid Redox Signal., **7**, 788-794 (2005) "Hydrogen sulfide as an endogenous modulator of biliary bicarbonate excretion in the rat liver."	2005 年	Mary Ann Liebert, Inc.,	K. Fujii T. Sakuragawa M. Kashiba Y. Sugiura M. Kondo K. Maruyama N. Goda Y. Nimura M. Suematsu
Am. J. Physiol. Heart Circ. Physiol. **288**, H1885-H1892 (2005). "Oxygen transport by low and normal P_{50} Hb-vesicles in extreme hemodilution."	2005 年	American Physiological Society	P. Cabrales H. Sakai A. G. Tsai S. Takeoka E. Tsuchida M. Intaglietta
Crit. Care Med. **33**, 806-812 (2005). "New generation of hemoglobin-based oxygen carriers evaluated for oxygenation of critically ischemic hamster flap tissue."	2005 年	American Thoracic Society,	C. Contaldo J. Plock H. Sakai S. Takeoka E. Tsuchida M. Leuing A. Banic D. Erni
Am. J. Physiol. Heart Circ. Physiol. **288**, H2897-H2903 (2005). "Oxygen Release from low and normal P_{50} Hb-vesicles from Transiently Occluded Arteriole in the Hamster Window Model."	2005 年	American Physiological Society	H. Sakai P. Cabrales A. G. Tsai M. Intaglietta E. Tsuchida
Am. J. Physiol. Heart Circ. Physiol. **289**, H2624-H2631 (2005). "Is the Hb in Hb-vesicles infused for isovolemic hemodilution necessary to improve oxygenation in critically ischemic hamster skin?"	2005 年	American Physiological Society	A. Plock C. Contaldo H. Sakai E. Tsuchida M. Leunig A. Banic M. D. Menger D. Erni

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
Artif. Cells Blood Substitutes Biotechnol. **33**, 101-11 (2005). "Effects of hemoglobin vesicles on resting and agonist-stimulated human platelets in vitro."	2005 年	Taylor & Francis, Inc.	S. Wakamoto M. Fujihara H. Abe M. Yamaguchi H. Azuma H. Ikeda S. Takeoka E. Tsuchida
Bioconjugate Chem. **16**, 23–26 (2005). "Human serum albumin bearing covalently attached iron(II) porphyrins as O_2-binding sites"	2005 年	American Chemical Society	W. M. Wang T. Komatsu A. Nakagawa E. Tsuchida
J. Am. Chem. Soc. **127**, 15933–15942 (2005). "O_2 and CO binding properties of artificial hemoproteins formed by complexing iron protoporphyrin IX with human serum albumin mutants".	2005 年	American Chemical Society	T. Komatsu N. Ohmichi A. Nakagawa P. A. Zunszain S. Curry E. Tsuchida
Biomacromolecules **6**, 3397–3403 "Albumin clusters: structurally defined protein tetramer and oxygen carrier including thirty-two iron(II) porphyrins".	2005 年	American Chemical Society	T. Komatsu Y. Oguro A. Nakagawa E. Tsuchida
Bioconjugate Chem. **17**, 146–151 (2005) "Human serum albumin hybrid incorporating tailed porphyrinatoiron(II) in the $\alpha,\alpha,\alpha,\beta$ -conformer as an O_2-binding site".	2005 年	American Chemical Society	A. Nakagawa T. Komatsu M. Iizuka E. Tsuchida
Bioconjugate Chem. (2006) in press. "Poly(ethylene glycol) Conjugated Human Serum Albumin Including Iron Porphyrins: Surface Modification Improves the O_2-Transporting Ability."	2006 年	American Chemical Society	Y. Huang T. Komatsu R. M. Wang A. Nakagawa E. Tsuchida
バイオサイエンスとインダストリー **63**, 571-572, (2005) "目でみるバイオ「微小血管内を均一に流れる人工赤血球と その運命」"	2005 年	バイオインダストリー協会	酒井宏水 土田英俊
臨床麻酔 **29**, 721-726 (2005). "人工赤血球・人工血小板の開発の現状"	2005 年 4 月	真興交易(株)	武岡真司

刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行年月日	刊行書店名	執筆者名
In: "Microvascular Biology and Pathology. Vol. 2." Ed. by David Shepro. pp. 1095-1103. "Blood Substitutes' Efficacy: Microvascular and Theological Determinants."	2006 年	Elsevier	A.G. Tsai P. Cabrales H. Sakai M. Intaglietta
Clin. Hemorheol. Microcirc. **34**, 335-340 (2006). "Performances of PEG- modified hemoglobin-vesicles as artificial oxygen carriers in microcirculation."	2006 年	IOS Press	H. Sakai E. Tsuchida
人工血液 **13**, 12-18 (2005) "人工血液運搬体政策に関する基本的留意事項 (案) を解説する."	2005 年	日本血液代替物学会	高折益彦

その他の刊行物

1) 読売新聞朝刊「感染不安ない人工血液：早･慶大など開発、2 年後の実用化目指す」(H.16.1.25)
2) 毎日新聞朝刊「感染血液すり抜けなぜ？：－研究進む人工血液－まずは赤血球　実用化の期待」(H16.3.13)
3) 日本経済新聞　「人工赤血球を量産　二プロなど、安定供給に道」(H16.3.19)
4) 日本経済新聞「先端技術 21 世紀の気鋭、人工赤血球臨床へ、一つ一つ積み上げ成果」(H16.4.22)
5) Medical Tribune「人工血液がもたらす未来の救急医療像を模索」(H16.8.19)
6) 日本経済新聞 朝刊「血液型不適合で副作用、輸血の安全道半ば、普及する自己血、人工血液の開発も」(H16.11.28)
7) *Chemistry World*, 12, 18 (2005), Royal Society of Chemistry, "Albumin complex engineered for artificial blood"
8) TIMES 「Fake blood may save thousands of lives」(H16.3.15)
9) ニュートン誌「驚異の未来テクノロジー、人工赤血球で慢性的な輸血用血液の不足を補う」(H17.2.)
10) 日経産業新聞「酸素運搬体、実用化へ」(H17.2.3)
11) 日刊工業新聞「血液型照合なしで大量使用　酸素輸液の用途に」(H17.09.26)
12) 日刊工業新聞「人工酸素運搬体　実証プラント建設　ニプロが 20ℓ規模」(H17.10.25)
13) 日刊工業新聞「大学発ベンチャーの挑戦 75　献血血液で酸素輸液　早慶のシーズ事業化」(H17.12.27)

研究成果の刊行物・別冊

(2003. 4. ～ 2006. 3.)

Hemoglobin-vesicles suspended in recombinant human serum albumin for resuscitation from hemorrhagic shock in anesthetized rats*

Hiromi Sakai, PhD; Yohei Masada, MEng; Hirohisa Horinouchi, MD, PhD; Manabu Yamamoto, MD; Eiji Ikeda, MD, PhD; Shinji Takeoka, PhD; Koichi Kobayashi, MD, PhD; Eishun Tsuchida, PhD

Objective: **Hemoglobin-vesicle (HbV) has been developed to provide oxygen-carrying ability to plasma expanders. Its ability to restore the systemic condition after hemorrhagic shock was evaluated in anesthetized Wistar rats for 6 hrs after resuscitation. The HbV was suspended in 5 g/dL recombinant human serum albumin (HbV/rHSA) at an Hb concentration of 8.6 g/dL.**

Design: **Prospective, randomized, controlled trial.**

Setting: **Department of Surgery, School of Medicine, Keio University.**

Subjects: **Forty male Wistar rats.**

Interventions: **The rats were anesthetized with 1.5% sevoflurane inhalation throughout the experiment. Polyethylene catheters were introduced through the right jugular vein into the right atrium for infusion and into the right common carotid artery for blood withdrawal and mean arterial pressure monitoring.**

Measurements and Main Results: **Shock was induced by 50% blood withdrawal. The rats showed hypotension (mean arterial pressure = 32 ± 10 mm Hg) and significant metabolic acidosis and hyperventilation. After 15 mins, they received HbV/rHSA, shed autologous blood (SAB), washed homologous red blood cells (wRBC) suspended in rHSA (wRBC/rHSA, [Hb] = 8.6 g/dL), or rHSA alone. The HbV/rHSA group restored mean arterial pressure to 93 ± 8 mm Hg at 1 hr, similar to the SAB group (92 ± 9 mm Hg), which was significantly higher compared with the rHSA (74 ± 9 mm Hg) and wRBC/rHSA (79 ± 8 mm Hg) groups. There was no remarkable difference in the blood gas variables between the resuscitated groups; however, two of eight rats in the rHSA group died before 6 hrs. After 6 hrs, the rHSA group showed significant ischemic changes in the right cerebral hemisphere relating to the ligation of the right carotid artery followed by cannulation, whereas the HbV/rHSA, SAB, and wRBC/rHSA groups showed less changes.**

Conclusions: **HbV suspended in recombinant human serum albumin provides restoration from hemorrhagic shock that is comparable with that using shed autologous blood. (Crit Care Med 2004; 32:539–545)**

KEY WORDS: **blood substitutes; artificial red cells; liposome; resuscitation; transfusion**

A phospholipid vesicle encapsulating concentrated human hemoglobin (Hb) (Hb-vesicle, HbV) can serve as an oxygen carrier whose oxygen-carrying capacity can be formulated to be comparable to that of blood (1–4). HbV are void of blood-type antigens and infectious viruses and are stable and suitable for long-term storage (5). The cellular structure of HbV (particle diameter, ca. 280 nm) has characteristics similar to those of natural red blood cells (RBCs), because both have lipid bilayer membranes that prevent direct contact of Hb with the components of blood and the endothelial lining. Furthermore, Hb encapsulation in the vesicle suppresses hypertension induced by vasoconstriction, a mechanism presumably due to the effect of free Hb that scavenges the endogenous vasorelaxation factors nitric oxide and carbon monoxide (6, 7) consequent to their high affinity with Hb. Once in the circulation, HbV particles are captured by the phagocytes in the reticuloendothelial system (mainly the liver and spleen), and they are metabolized completely within 14 days, with no deposition of iron or lipid (8).

Oxygen-carrying fluids for blood replacement using molecular or encapsulated Hbs have been proposed for volume restoration in hemorrhagic shock (9, 10). We tested the efficacy of HbV suspended in plasma-derived human serum albumin (HSA) in extreme normovolemic hemodilution and found that they are comparable with RBCs (11, 12). In this report, we tested the HbV as a resuscitative fluid for hemorrhagic shock in anesthetized rats. HbV was suspended in recombinant HSA (rHSA), and the efficacy of the resulting HbV/rHSA was compared with that of shed autologous blood and of washed RBCs suspended in rHSA at the same Hb concentration. It has been extensively confirmed that the characteristics of the rHSA are identical with those of conventional plasma-derived HSA

*See also p. 612.

From the Advanced Research Institute for Science and Engineering (HS, YM, ST, ET), Waseda University, Tokyo, Japan; and the Departments of Surgery (HH, MY, KK) and Pathology (EI), School of Medicine, Keio University, Tokyo, Japan.

Supported, in part, by Health Sciences Research Grants (Research on Pharmaceutical and Medical Safety, Artificial Blood Project); by the Ministry of Health, Labor and Welfare, Japan; by Grants in Aid for Scientific Research from the Japan Society for the Promotion of Science (B12480268); by 21 COE "Practical Nano-Chemistry" from MEXT, Japan; and by Nipro, Osaka, Japan, which provided rHSA.

Address requests for reprints to: Eishun Tsuchida, PhD, Advanced Research Institute for Science and Engineering, Waseda University, Tokyo 169-8555, Japan. E-mail: eishun@waseda.jp; and Koichi Kobayashi, MD, PhD, Department of Surgery, School of Medicine, Keio University, Tokyo 160-8582, Japan. E-mail: kobayash@sc.itc.keio.ac.jp

DOI: 10.1097/01.CCM.0000109774.99665.22

(13, 14) and that rHSA will soon be approved as a promising plasma expander free from any pathogen from humans.

MATERIALS AND METHODS

Preparation of HbV and Washed RBCs Suspended in rHSA. HbV was prepared under sterile conditions as previously reported (7, 11). Hb was purified from outdated donated blood provided by the Hokkaido Red Cross Blood Center (Sapporo, Japan) and the Japanese Red Cross Society (Tokyo, Japan). The encapsulated purified Hb (38 g/dL) contained 14.7 mM of pyridoxal 5'-phosphate (Sigma Chemical, St. Louis, MO) as an allosteric effector at a molar ratio of pyridoxal 5'-phosphate/Hb = 2.5. The lipid bilayer was composed of a mixture of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine, cholesterol, and 1,5-bis-*O*-hexadecyl-*N*-succinyl-L-glutamate at a molar ratio of 5/5/1 (Nippon Fine Chemical, Osaka, Japan), and 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-poly(ethylene glycol) (NOF, Tokyo, Japan, 0.3 mol% of the total lipid) (15). HbVs were suspended in a physiologic salt solution at [Hb] = 10 g/dL, sterilized with filters (Dismic, Toyo Roshi, Tokyo, Japan, pore size, 0.45 μm), and deoxygenated with N_2 bubbling for storage (5). The content of lipopolysaccharide was <0.1 EU/mL.

Before use, the HbV suspension ([Hb] = 10 g/dL, 8.6 mL) was mixed with a solution of rHSA (25%, 1.4 mL, Nipro, Osaka, Japan) to regulate the rHSA concentration in the suspending medium of the vesicles to 5 g/dL. Under this condition, the colloid osmotic pressure of the suspension is about 20 mm Hg (Wescor 4420 Colloid Osmometer, Wescor, Logan, UT) (11). As a result, the Hb concentration of the suspension was 8.6 g/dL. The viscosities of the suspensions were measured with a capillary rheometer (Oscillatory Capillary Rheometer, OCR-D, Anton Paar GmbH, Graz, Austria). Physicochemical variables of the resulting HbV suspension in comparison with those of the other resuscitative fluids are listed in Table 1.

To prepare washed RBC suspended in rHSA (wRBC/rHSA), blood samples from donor Wistar rats were withdrawn into heparinized syringes and centrifuged to obtain an RBC concentrate. This was washed twice to remove plasma components and buffy coat by resuspension in 5% rHSA and centrifugation (3000 × *g*, 10 mins). The Hb concentration, measured with a cyanometHb method, of the resulting wRBC/rHSA was adjusted to 8.6 g/dL, equivalent to that of HbV/rHSA. The Hb concentration of the shed autologous blood was 13.4 ± 2 g/dL.

Animal Model and Preparation. The experimental protocol was fully approved by the Laboratory Animal Care and Use Committee of Keio University School of Medicine. It also complied with the Guide for the Care and Use of Laboratory Animals (16).

Experiments were carried out with 40 male Wistar rats (280 ± 27 g body weight; Saitama Experimental Animals Supply, Kawagoe, Japan). All animals were housed in cages and provided with food and water *ad libitum* in a temperature-controlled room with a 12-hr dark/light cycle. The rats were anesthetized with 1.5%-sevoflurane-mixed air inhalation (Maruishi Pharm., Osaka) with a vaporizer (TK-4 Biomachinery, Kimura Med., Tokyo) throughout the experiment (F_{IO_2} = 21%). Polyethylene catheters (SP-31 tubing, outer diameter 0.8 mm, inner diameter 0.5 mm, Natsume, Tokyo) filled with saline containing 40 IU/mL heparin were introduced through the right jugular vein into the right atrium for infusion and into the right common carotid artery for blood withdrawal. The catheter in the common carotid artery was connected to a Polygraph system (Nippon Koden, Polygraph LEG-1000). The body temperature of the rats was maintained between 37 and 38°C by an isothermal pad (Braintree Scientific, Braintree, MA) during the experiments.

Resuscitation From Hemorrhagic Shock. Hemorrhagic shock was induced by withdrawing 50% of the blood (28 mL/kg, 1 mL/min) from the carotid artery. Systemic blood volume was estimated to be 56 mL/kg body weight (3). Blood was withdrawn into a heparinized syringe and stored for 15 mins at room temperature for the resuscitation with shed autologous blood (SAB). Rats were resuscitated by the infusion of a volume of HbV/rHSA (n = 8), wRBC/rHSA (n = 8), rHSA alone (n = 8), or initially shed autologous blood (n = 8) in 5 min. The volume of the infused resuscitative fluid was identical to the shed volume (i.e., 50% of the blood volume at baseline). To monitor the severity of the shock, eight hemorrhaged rats were not resuscitated with any fluid (nonresuscitated group).

Measurements of Systemic Responses. Systemic variables and blood gases were evaluated before hemorrhage (baseline), after 50% hemorrhage, just after resuscitation, and 1.0, 3.0, and 6.0 hrs after resuscitation. Blood samples were collected in 70 IU/mL heparinized microtubes (125 μL, Clinitubes, Radiometer, Copenhagen) for blood gas analyses and in glass capillaries (Terumo, Tokyo) for hematocrit measurements. A pH/blood gas analyzer (ABL 555, Radiometer, Copenhagen) was used for analysis of Pao_2, $Paco_2$, pH, base excess (BE), and lactate. A recording system (Polygraph System 1000, Nippon Koden, Tokyo) was used for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR). Body temperature was monitored with a thermometer inserted into the anus.

Histopathological Examination and Serum Clinical Laboratory Tests. Six hours after resuscitation, about 5 mL of arterial blood was rapidly withdrawn into heparinized syringes, and the animals were laparotomized and killed by acute bleeding from the abdominal aorta. The liver, spleen, kidney, and then the lung, heart, and brain were resected for a histopathological study. The percentage of the area of ischemic changes (a pyknotic change of nuclei and an edematous change) in the cerebral hemisphere was measured with computer software (IPLab, Fairfax, VA). The blood samples were centrifuged at 3000 × *g* for 5 mins to obtain plasma. The HbV-containing plasma required further ultracentrifugation (50,000 × *g*, 20 mins) to obtain clear plasma avoiding the interference effect of the HbV particles (17). The samples of serum were stored at −80°C before the clinical laboratory tests (BML, Kawagoe). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum activities were measured. The organs were fixed in a 10% formalin neutral buffer solution (Wako Chemical, Tokyo) immediately after the resection, and the paraffin sections were stained with hematoxylin/eosin.

Data Analysis. Data are given as the mean ± SD for the indicated number of animals. Data were analyzed using analysis of variance followed by Fisher's protected least significant difference test between the groups. The Student's *t*-test was used for the comparisons with baseline values within each group. The level of confidence was placed at 95% for all the experiments.

Table 1. Physicochemical properties of four resuscitative fluids infused into hemorrhagic-shocked rats; hemoglobin-vesicles suspended in recombinant human serum albumin (HbV/rHSA) compared with shed autologous blood(SAB), washed red blood cells suspended in recombinant human serum albumin(wRBC/rHSA), and recombinant human serum albumin (rHSA)

Variables	HbV/rHSA	SAB	wRBC/rHSA	rHSA
Hemoglobin concentration, g/dL	8.6	13.4 ± 2	8.6	0
Particle diameter, nm	281 ± 11	ca. 7000	ca. 7000	—
P_{50}, torr	32	39[a]	39[a]	—
Colloid osmotic pressure, mm Hg	20	22	20	20
Viscosity, cP at 230/sec	2.8	5.2	2.1	1.1

[a]From Reference 12.

RESULTS

Survival Rate. All the rats in the HbV/rHSA, wRBC/rHSA, and SAB groups survived for 6 hrs after resuscitation until the kill. In the rHSA group, two of the eight rats died between 1 and 6 hrs (Fig. 1). Accordingly, hemodynamic and blood-

gas variables (Figs. 2 and 3) of the rHSA group were divided into the survivor group and the nonsurvivor groups. Therefore, the numbers of rats (n) for the rHSA (survivor) and rHSA (nonsurvivor) groups were six and two, respectively. All the rats in the nonresuscitated group died within 3 hrs.

Systemic Responses to the Hemorrhagic Shock and Resuscitation. MAP of the Wistar rats before hemorrhage was 99 ± 8 mm Hg on the average and declined to 32 ± 6 mm Hg after hemorrhage (Fig. 2*a*). Immediately after resuscitation, the MAP of the SAB group recovered to 110 ± 7 mm Hg, above the baseline value. The value was slightly reduced to 92 ± 9 mm Hg at 1 hr, and the level was maintained for 6 hrs. The MAP of the HbV/rHSA recovered upon retransfusion to 98 ± 8 mm Hg, the baseline level, which was significantly lower than that of the SAB group ($p = .027$). After 1 hr, there was no significant difference between the HbV/rHSA and SAB groups. The HbV/rHSA group showed significantly higher MAP than the rHSA (survivor) ($p = 0.0005$) and wRBC/rHSA ($p = .0032$) groups, whose MAPs at 1 hr were 74 ± 9 and 79 ± 8 mm Hg and remained at this higher level for 6 hrs. The MAP of the nonresuscitated group did not recover and remained at the lowest values. The average HR before hemorrhage was 405 ± 38 beats/min, and there was no significant change after hemorrhage. At 0 hr, the HbV/rHSA ($p = .0215$), SAB ($p = .0085$), and nonresuscitated ($p = .0076$) groups showed slightly lower HR than the basal values; however, there was no noticeable change after that (Fig. 2*b*).

The hematocrit before hemorrhage was 43 ± 2% and was reduced to 36 ± 2% after bleeding due to autotransfusion (Fig. 2*c*). After resuscitation, the hematocrit in the SAB group increased to 42 ± 4%. The hematocrit values in the rHSA (survivor), rHSA (nonsurvivor), and HbV/rHSA groups were significantly reduced to 19 ± 1, 18 ± 1, and 20 ± 2%, respectively ($p < .0001$ vs. baseline), due to the dilution of the blood with the different solutions. The HbV particles remained dispersed in the plasma phase in the glass capillaries for hematocrit measurements. The hematocrit of the wRBC/rHSA group (35 ± 3%) was significantly lower than that of the SAB group ($p < .0001$) corresponding to the lower Hb concentration in the fluid of the wRBC/rHSA groups (8.6 g/dL) than in that of the SAB groups (13.4 ± 2.0 g/dL). The hematocrit of the nonresuscitated group did not change after autotransfusion. The total Hb concentrations in blood after resuscitation with rHSA, HbV/rHSA, wRBC/rHSA, and SAB were estimated to be 6.3, 11, 11, and 13 g/dL, respectively.

Hemorrhagic shock induced metabolic acidosis shown by a decrease in pH from 7.48 ± 0.04 to 7.40 ± 0.09 on the average, a decrease in the BE from 4.5 ± 1.4 to −6.9 ± 3.4 mM, and an increase in lactate from 1.4 ± 0.5 to 6.2 ± 1.4 mM (Fig. 3). As a result, significant compensatory hyperventilation was observed as an increase in Pao_2 of 81 ± 8 torr to 103 ± 6 torr and a decrease in $Paco_2$ of 38 ± 5 torr to 26 ± 5 torr. All the resuscitated groups tended to recover immediately from the hyperventilation after infusion. The pH, BE, and lactate values did not show immediate recoveries after resuscitation but tended to recover at 1 hr. However, they did not return to the baseline level even after 6 hrs ($p < .05$ vs. baseline). There was no significant difference between the HbV/rHSA and SAB group. The nonresuscitated group remained with significant hyperventilation, acidosis, and reduction of BE at 0 hr ($p < .01$ vs. baseline). After that, the Pao_2 decrease and the $Paco_2$ increase were significant in the rats, leading to death. All the variables of the nonresuscitated group were significantly different from those of the HbV/rHSA group at 3 hrs. There was no clear difference between the rHSA (survi-

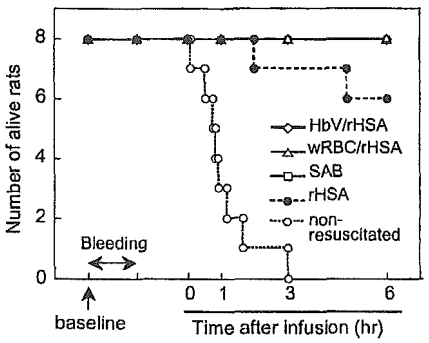

Figure 1. Survival rate of Wistar rats after resuscitation from hemorrhagic shock with infusion of hemoglobin-vesicles suspended in recombinant human serum albumin (*HbV/rHSA*), shed autologous blood (*SAB*), washed red blood cells suspended in recombinant human serum albumin (*wRBC/rHSA*), and recombinant human serum albumin (*rHSA*) alone. The nonresuscitated group did not receive a resuscitative fluid after the hemorrhage.

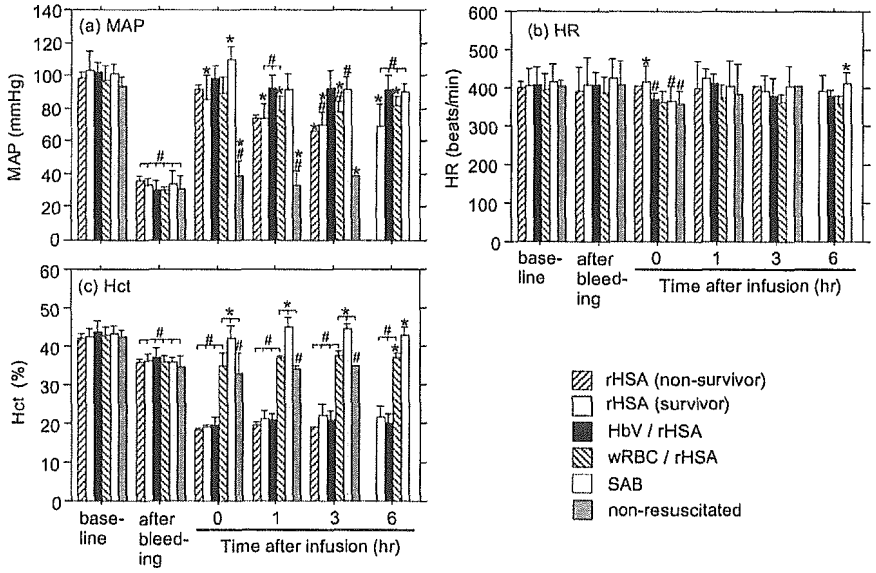

Figure 2. Changes in heart rate (*HR*), mean arterial pressure (*MAP*), and hematocrit (*Hct*) during hemorrhagic shock and resuscitation with infusion of hemoglobin-vesicles suspended in recombinant human serum albumin (*HbV/rHSA*), shed autologous blood (*SAB*), washed red blood cells suspended in recombinant human serum albumin (*wRBC/rHSA*), and recombinant human serum albumin (*rHSA*) alone. The nonresuscitated group did not receive a resuscitative fluid after the hemorrhage and died within 3 hrs (Fig. 1). The number of surviving rats was three at 1 hr. In the rHSA group, two of the eight rats died between 1 and 6 hrs. Accordingly, the rHSA group was divided into the rHSA (survivor) group and the rHSA (nonsurvivor) group until they died. Therefore, the numbers of rats (n) for the rHSA (survivor) and rHSA (nonsurvivor) groups were 6 and 2, respectively. #Significantly different from baseline ($p < .05$); *significantly different vs. the HbV/rHSA group ($p < .05$).

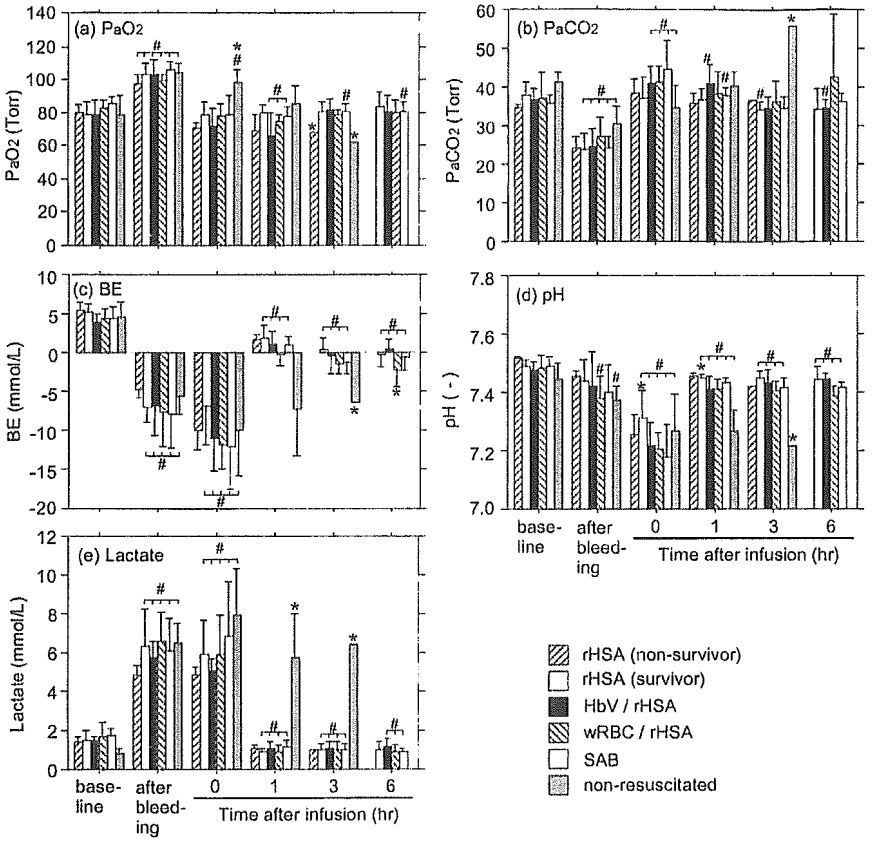

Figure 3. Changes in blood gas variables during hemorrhagic shock and resuscitation with infusion of hemoglobin-vesicles suspended in recombinant human serum albumin (*HbV/rHSA*), shed autologous blood (*SAB*), washed red blood cells suspended in recombinant human serum albumin (*wRBC/rHSA*), and recombinant human serum albumin (*rHSA*) alone. The nonresuscitated group did not receive a resuscitative fluid after the hemorrhage and died within 3 hrs (Fig. 1). The number of surviving rats was three at 1 hr. In the rHSA group, two of the eight rats died between 1 and 6 hrs. Accordingly, the rHSA group was divided into the rHSA (survivor) group and the rHSA (nonsurvivor) group until they died. Therefore, the numbers of rats (n) for the rHSA (survivor) and rHSA (nonsurvivor) groups were 6 and 2, respectively. #Significantly different from baseline ($p < .05$); *significantly different vs. the HbV/rHSA group ($p < .05$). *BE*, base excess.

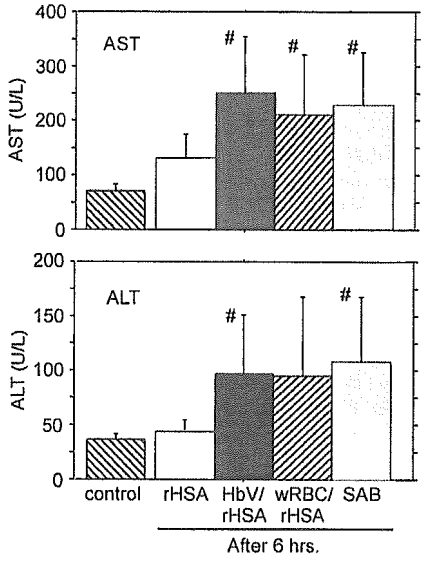

Figure 4. Aspartate aminotransferase (*AST*) and alanine aminotransferase (*ALT*) concentrations 6 hrs after resuscitation with infusion of hemoglobin-vesicles suspended in recombinant human serum albumin (*HbV/rHSA*), shed autologous blood (*SAB*), washed red blood cells suspended in recombinant human serum albumin (*wRBC/rHSA*), and recombinant human serum albumin (*rHSA*) alone. #Significantly different from the control group ($p < .05$).

vor) and rHSA (nonsurvivor) groups in MAP and HR in Figure 2. However, the rHSA (nonsurvivor) group tended to show a slightly lower Pao_2 than the rHSA (survivor) group at 0 and 1 hr and significantly at 3 hrs ($p = .0374$) in Figure 3.

Clinical Laboratory Tests of Blood Serum. Normal Wistar rats showed AST and ALT of 70 ± 13 and 37 ± 5 units/L, respectively (Fig. 4). The HbV/rHSA, wRBC/rHSA, and SAB groups showed significant or nonsignificant increases in AST ($p = .003$, .016, and .005, respectively) and ALT values ($p = .031$, .110, and .025, respectively) compared with the baseline values. On the other hand, the rHSA (survivor) group showed the smallest changes.

Histopathological Examination 6 Hrs After Resuscitation With HbV/rHSA. The hematoxylin/eosin staining of the rat organs demonstrated no significant morphologic abnormalities in the lung, kidney, and liver (data not shown). The red pulp zone of the spleen showed the accumulation of HbV particles as pink-colored dots (8). The myocardium showed focal minimal ischemic changes without apparent necrosis, probably due to the hemorrhagic shock. This histologic finding also was observed in other experimental groups including the rHSA (survivor) group. The cerebral hemisphere on the right side of the rHSA group showed significant ischemic changes, a pyknotic change of the nuclei, and an edematous change (34 ± 3% of the total section area), relating to the ligation of the right carotid artery. However, the other groups that were resuscitated with oxygen-carrying fluids showed minimal changes ($p < .001$ vs. rHSA; HbV/rHSA, 13 ± 5%; SAB, 11 ± 6%; wRBC/rHSA, 11 ± 3%). The nonresuscitated rats that died spontaneously did not show such ischemic changes.

DISCUSSION

One particle of HbV (diameter, ca. 250 nm) contains about 30,000 Hb molecules. HbV acts as a particle in the blood and not as a solute; therefore, the colloid osmotic pressure of the HbV suspension is nearly zero. It requires an addition of a plasma expander for a large substitution of blood such as normovolemic hemodilution to maintain blood volume (18). The candidates of plasma expanders are HSA, hydroxyethyl starch, dextran, or gelatin depending on the clinical setting, cost, countries, and clinicians (19). In this report we tested for the first time the addition of rHSA. The absence of any infectious disease from humans is the greatest advantage of rHSA, which will be soon approved for clinical use in Japan. Moreover, there should be no immunologic and hematologic abnormalities that are often seen with the use of dextran and hydroxyethyl starch (19). The virus inactivation and removal from a human-derived Hb solution can be aggressively

performed in our preparation process of HbV (20, 21). However, to completely avoid unknown infectious diseases, the combination of recombinant Hb-vesicles and recombinant HSA would be the most ideal "artificial red blood cells" in the future.

In our hemorrhagic shock model, all the rats in the nonresuscitated group, which did not receive any fluid, died within 3 hrs, indicating the severity of the shock state. The infusion of the resuscitative fluids resulted in the improvement of all the variables and survival, indicating the importance of the recovery in blood volume. Especially, our principal findings are that the infusion of HbVs suspended in rHSA restores the MAP and blood gas variables including BE and lactate after hemorrhagic shock and that all the rats survived 6 hrs after resuscitation despite the fact that in the rHSA group two rats among eight died within 6 hrs. This clearly shows that the ability of HbV/rHSA as an effective oxygen-carrying resuscitative fluid is comparable with shed autologous blood. After the resuscitation, there were minor differences in blood gas variables between the groups in Figure 3. It would seem that all the animals were hypervolemic in the initial phase of resuscitation, because they experienced autotransfusion during the shock period, although it lasted only 15 mins. This could be one of the reasons there were no significant changes in BE and pH between the groups. Moreover, after the infusion of rHSA, the Hb concentration should not be significantly lower than the transfusion trigger. However, the rHSA group dissociated into the survivor and nonsurvivor groups. There was no remarkable difference between the two, and it was difficult to determine the cause of death. If anything, the rHSA (nonsurvivor) group tended to show the lower Pao_2 values compared with the rHSA (survivor) group ($p = .037$ at 3 hrs), indicating that the respiratory function was not adequate to sustain metabolism under the condition of sevoflurane anesthesia and spontaneous breathing after resuscitation with nonoxygen-carrying fluid. In this case, the combination of the significant hypotension that was seen in all the rats in the rHSA group and the respiratory problem may be one of the causes of incidental death (22). Immediately after resuscitation, the HbV/rHSA group showed a recovery of MAP, Pao_2, and $Paco_2$ that was similar to that of the SAB group. However, the BE, pH, and lactate levels did not show immediate recovery due to the "washing out" of accumulated metabolites including nonvolatile lactate in the peripheral tissues (23). These values recovered 1 hr after resuscitation.

In our previous report (10), we tested HbV suspended in plasma-derived HSA for resuscitation from hemorrhagic shock of conscious small hamsters (ca. 60–70 g body weight) with much lower remaining hematocrit values, maintaining the MAP at 40 mm Hg for 1 hr. Even though the species was different and the observation period after resuscitation was only 1 hr, it seemed that the conscious hamsters showed sufficient compensation of hyperventilation even after resuscitation with HSA alone. In the present study, we could demonstrate the effectiveness of HbV/rHSA in anesthetized rats (280 ± 27 g body weight) as long as 6 hrs after resuscitation, where respiratory function was depressed and compensatory function was not sufficient. This indicates the effectiveness of HbV suspended in a plasma expander for resuscitation from hemorrhagic shock.

It has been reported that resuscitation from hemorrhagic shock with acellular Hb modifications such as polymerized or intramolecularly cross-linked Hb causes the elevation of MAP beyond the baseline values (9, 24, 25), whereas a refined polymerized human Hb that does not contain molecular Hb (<1%) shows no hypertension (26). The hypertension may be presumably due to the high affinity for nitric oxide of molecular Hbs and their smaller size that enables nitric oxide trapping in the proximity of the endothelium (7, 27). However, MAP did not exceed the baseline values after resuscitation with HbV. This is one advantage of cellular HbV in comparison with acellular molecular Hb modifications that may cause vasoconstriction and therefore hypoperfusion of peripheral tissues.

Interestingly, the HbV/rHSA group showed a significantly higher MAP than the wRBC/rHSA group and one that was comparable with that of the SAB group except immediately after resuscitation. It has been extensively confirmed that HbV is not vasoactive and does not induce hypertension (6, 7, 10). Because the total Hb concentrations are identical between the two resuscitative fluids (8.6 g/dL), one of the possible explanations could be related to the more effective oxygen transport by HbV than RBC to the myocardium where the oxygen consumption is significantly large and the oxygen tension gradient is steep. This is speculated from the facts that HbV distributes closer to the endothelial cell layer in the arteriolar blood flow whereas RBCs flow near the axial line (28). Another explanation should be related to the viscosity difference. The viscosity of HbV/rHSA (2.8 cP) is slightly higher than that of wRBC/rHSA (2.1 cP), and this may contribute to the higher vascular resistance and the resulting higher MAP. The slightly higher MAP for the SAB group immediately after infusion may be due to hypervolemia, the trace hemolysis that induces nitric oxide trapping and vasoconstriction, higher viscosity, or clotting during the preservation despite the heparinization.

Histopathological examination of the spleen showed accumulation of HbV in the red pulp zone as previously reported in the study of bolus infusion of HbV in normal rats (8). It was confirmed that HbVs, as foreign particles, were finally captured by the reticuloendothelial system mainly in the spleen and liver, and they were smoothly metabolized within 2 wks. Because the circulation half-life of HbV is about 35 hrs, the spleen had already started to show accumulation of HbV 6 hrs after resuscitation. The lung and kidney did not show any abnormalities such as embolism in the capillaries derived from the aggregation of vesicles (29). In our case, poly(ethylene glycol) modification of the surface of HbV guarantees the homogeneous dispersion and prompt blood flow in microcirculation (11, 30). The complete recovery of the blood gas variables and lactate concentration also supports the normal gas exchanging function of the lung and the excretion and decomposition of metabolites through the kidney and liver, respectively. The myocardium showed a slight influence of ischemic damage for all the groups. The significant difference was observed in the cerebral tissue between the groups receiving oxygen-carrying and noncarrying fluids. The rHSA group showed a significantly larger area with ischemic changes, a pyknotic change of the nuclei, and an edematous change, on the right side; however, other groups receiving oxygen-carrying fluids showed a significantly lower level of changes. We considered that ligation of the right carotid artery and the influence of hypoperfusion induced by the hemorrhagic shock caused ischemic environment in the right cerebral hemisphere, leading to pathologic and irreversible changes of cerebral tissues. The brain tissue was not

Hemoglobin-vesicle suspended in recombinant human serum albumin provides restoration from hemorrhagic shock that is comparable with that using shed autologous blood.

examined in our previous shock study using hamsters with ligation of a carotid artery in the same manner. In the present study, the significantly higher level of ischemic change only in the rHSA group may be caused by the prolonged hypotension and lower oxygen content in blood after resuscitation. Therefore, the cause of death in the rHSA group could be due, in part, to aggravated cerebral damage.

Even though histopathological examination of the liver did not show any abnormalities, the plasma clinical laboratory tests demonstrated elevation of AST and ALT for the HbV/rHSA, wRBC/rHSA, and SAB groups but not for the rHSA (survivor) group. Chemically modified Hbs also were reported to elevate AST and ALT after resuscitation (31, 32). This indicated that the resuscitation with oxygen-carrying fluids might induce ischemia/reperfusion injury that influences liver function (33, 34). However, because AST and ALT values represent the concentration in plasma, the difference in plasma volume between the groups should be considered. The plasma volume ratio should be calculated by subtracting the volumes of RBC and HbV from whole blood. Under the assumption that whole blood volume is equal between the groups, the rHSA group has a 1.35 and 1.20 times larger volume of plasma compared with the SAB group and the HbV/rHSA group, respectively, due to the reduced hematocrit for the rHSA group. Therefore, enzyme concentrations in the rHSA group may possibly be slightly underestimated. Including some antioxidative reagents such as active oxygen scavengers in the resuscitative fluid should be considered to obtain better resuscitation (35, 36).

CONCLUSION

HbV suspended in recombinant HSA at a concentration of only 8.6 g/dL of Hb showed effectiveness for resuscitation from hemorrhagic shock that was comparable to that using shed autologous blood. This acute study encourages us to continue further studies to optimize the physicochemical variables of the HbV suspension such as Hb concentration and oxygen affinity and to look at a longer term survival beyond 6 hrs to weeks using a larger animal model. Some of the polymerized Hbs are now in the final stages of clinical trials (37), and our HbV have to be compared with these materials in terms of safety and efficacy to demonstrate the advantage of cellular structure of HbV.

ACKNOWLEDGMENTS

We gratefully acknowledge K. Sou and N. Naito (Waseda University) for experimental assistance and M. Takaori (East Takarazuka Satoh Hospital) and Y. Izumi (Keio University) for meaningful suggestions on the experimental protocol.

REFERENCES

1. Chang TMS: Blood Substitutes: Principles, Methods, Products, and Clinical Trials. Basel, Switzerland, Karger, 1997
2. Djordjevich L, Mayoral J, Miller IF, et al: Cardiorespiratory effects of exchange transfusions with synthetic erythrocytes in rats. *Crit Care Med* 1987; 15:318–323
3. Izumi Y, Sakai H, Hamada K, et al: Physiologic responses to exchange transfusion with hemoglobin vesicles as an artificial oxygen carrier in anesthetized rats: Changes in mean arterial pressure and renal cortical tissue oxygen tension. *Crit Care Med* 1996; 24: 1869–1873
4. Rudolph AS, Klipper RW, Goins B, et al: *In vivo* biodistribution of a radiolabeled blood substitute: ^{99m}Tc-labeled liposome-encapsulated hemoglobin in an anesthetized rabbit. *Proc Natl Acad Sci U S A* 1991; 88: 10976–10980
5. Sakai H, Tomiyama K, Sou K, et al: Polyethyleneglycol-conjugation and deoxygenation enable long-term preservation of hemoglobin-vesicles as oxygen carriers in a liquid state. *Bioconjugate Chem* 2000; 11:425–432
6. Goda N, Suzuki K, Naito S, et al: Distribution of heme oxygenase isoform in rat liver: Topographic basis for carbon monoxide-mediated microvascular relaxation. *J Clin Invest* 1998; 101:604–612
7. Sakai H, Hara H, Yuasa M, et al: Molecular dimensions of Hb-based O_2 carriers determine constriction of resistance arteries and hypertension in conscious hamster model. *Am J Physiol Heart Circ Physiol* 2000; 279: H908–H915
8. Sakai H, Horinouchi H, Tomiyama K, et al: Hemoglobin-vesicles as oxygen carriers: Influence on phagocytic activity and histopathological changes in reticuloendothelial systems. *Am J Pathol* 2001; 159: 1079–1088
9. Loeb AL, McIntosh LJ, Raj NR, et al: Resuscitation after hemorrhage using recombinant human hemoglobin (rHb1.1) in rats: Effects on nitric oxide and prostanoid systems. *Crit Care Med* 1998; 26:1071–1080
10. Sakai H, Takeoka S, Wettstein R, et al: Systemic and microvascular responses to hemorrhagic shock and resuscitation with Hb-vesicles as oxygen carriers. *Am J Physiol Heart Circ Physiol* 2002; 263:H1191–H1199
11. Sakai H, Takeoka S, Park SI, et al: Surface-modification of hemoglobin vesicles with polyethyleneglycol and effects on aggregation, viscosity, and blood flow during 90%-exchange transfusion in anesthetized rats. *Bioconjugate Chem* 1997; 8:15–22
12. Izumi Y, Sakai H, Kose T, et al: Evaluation of the capabilities of a hemoglobin vesicle as an artificial oxygen carrier in a rat exchange transfusion model. *ASAIO J* 1997; 43: 289–297
13. Kobayashi K, Nakamura N, Sumi A, et al: The development of recombinant human serum albumin. *Ther Apher* 1998; 2:257–262
14. Nakatani T, Sakamoto Y, Ando H, et al: Effects of fluid resuscitation with recombinant human serum albumin solution on maintaining hepatic energy metabolism in hemorrhagic shock rabbits. *Res Exp Med* 1996; 195:317–325
15. Sou K, Endo T, Takeoka S, et al: Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly(ethylene glycol)-lipid into the vesicles. *Bioconjugate Chem* 2000; 11: 372–379
16. Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council–National Academy of Sciences: Guide for the Care and Use of Laboratory Animals. Washington, DC, National Academy Press, 1996
17. Sakai H, Tomiyama K, Masada Y, et al: Pretreatment of blood serum containing Hb-vesicles (oxygen carriers) for accurate clinical laboratory tests. *Clin Chem Lab Med* 2003; 41:222–231
18. Kobayashi K, Izumi Y, Yoshizu A, et al: The oxygen carrying capability of hemoglobin vesicles evaluated in rat exchange transfusion models. *Artif Cells Blood Substit Immobil Biotechnol* 1997; 25:357–366
19. de Jonge E, Levi M: Effects of different plasma substitutes on blood coagulation: A comparative review. *Crit Care Med* 2001; 29: 1261–1267
20. Abe H, Ikebuchi K, Hirayama J, et al: Virus inactivation in hemoglobin solution by heat treatment. *Artif Cells Blood Substit Immobil Biotechnol* 2001; 29:381–388