別添 4

表　研究成果の刊行に関する一覧表

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
平成 18 年度			
Use of hemoglobin vesicles during cardiopulmonary bypass priming prevents neurocognitive decline in rats. *Circulation.* 114(1 Suppl):I220-5 (2006).	2006 年 6 月	American Heart Association	Yamazaki M, Aeba R, Yozu R, Kobayashi K.
One-year observation of Wistar rats after infusion of Hb-vesicles (Artificial oxygen carriers). *Artif. Cells Blood Substitutes Biotechnol.* 35, 81-91 (2007).	2007 年 3 月	Informa Healthcare	H. Sakai, H. Horinouchi, E. Tsuchida, K. Kobayashi.
Hemoglobin-vesicles as a transfusion alternative. *Artif. Cells Blood Substitutes Biotechnol.* 34, 581-588 (2006)	2006 年 12 月	Informa Healthcare	E. Tsuchida, H. Sakai, H. Horinouchi, K. Kobayashi.
ヘモグロビン小胞体(HbV)-リコンビナントアルブミン分散溶液による 40%交換輸血:ラット脾臓内 HbV 代謝と造血に関する 2 週間の観察 (論文記事、Scondary Publication). *日本輸血細胞治療学会誌* 53, 47-55 (2007)	2007 年 3 月	日本輸血細胞治療学会	酒井宏水、堀之内宏久、山本学、池田 栄二、武岡真司、高折益彦、土田英俊、小林紘一
ヘモグロビン小胞体を用いた人工心肺充填液による高次脳機能保護効果 -ラット人工心肺モデルによる検討- *人工血液* 14, 62-65 (2006)	2006 年 9 月	日本血液代替物学会	山崎真敬、饗庭了、四津良平、小林紘一
Interaction of hemoglobin vesicles, a cellular-type artificial oxygen carrier, with human plasma: Effects on coagulation, kallikrein-kinin, and complement systems. *Artif. Cells Blood Substitutes Biotechnol.* 34, 1-10 (2006).	2006 年 4 月	Informa Healthcare	H. Abe, M. Fujihara, K. Ikebuchi, S. Takeoka, E. Tsuchida, H. Harashima, H. Azuma, H. Ikeda.
Effects of hemoglobin vesicles, a liposomal artificial oxygen carrier, on hematological responses, complement and anaphylactic reactions in rats. *Artif. Cells Blood Substitutes Biotechnol.* 35, 157-172 (2007).	2007 年 3 月	Informa Healthcare	H. Abe, H. Azuma, M. Yamaguchi, M. Fujihara, H. Sakai, S. Takeoka, E. Tsuchida, H. Ikeda.
Oxidation of Arg-410 promotes the elimination of human serum albumin. *Biochim. Biophys. Acta.* 1764: 743-749 (2006).	2006 年 4 月	Elsevier	Iwao Y, Anraku M, Yamasaki K, Kragh-Hansen U, Kawai K, Maruyama T, Otagiri M.

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
The structural and pharmacokinetic properties of oxidized human serum albumin, advanced oxidation protein products (AOPP). *Drug Metab. Pharmacokinet.* 21: 140-146 (2006).	2006 年 4 月	Japanese Society for the Study of Xenobiotics	Iwao Y, Anraku M, Hiraike M, Kawai K, Nakajou K, Kai T, Suenaga A, Otagiri M.
Recombinant human serum albumin dimer has high blood circulation activity and low vascular permeability in comparison with native human serum albumin. *Pharm. Res.* 23: 882-891 (2006).	2006 年 5 月	Springer	Matsushita S, Chuang VT, Kanazawa M, Tanase S, Kawai K, Maruyama T, Suenaga A, Otagiri M.
Chain length-dependent binding of fatty acid anions to human serum albumin studied by site-directed mutagenesis. *J. Mol. Biol.* 363: 702-712 (2006).	2006 年 10 月	Elsevier	Kragh-Hansen U, Watanabe H, Nakajou K, Iwao Y, Otagiri M.
S-Nitrosylation of Human variant albumin Liprizzi (R410C) confers potent Antibacterial and Cytoprotective properties. *J. Pharmacol. Exp. Ther.* 320: 969-77 (2007).	2007 年 3 月	American Society for Pharmacology & Experimental Therapeutics	Ishima Y, Sawa T, Kragh-Hansen U, Miyamoto Y, Matsushita S, Akaike T, Otagiri M.
The role of N-acetyl-methioninate as a new stabilizer for albumin products. *Int. J. Pharm.* 329: 19-24 (2007).	2007 年 2 月	Elsevier	Anraku M, Kouno Y, Kai T, Tsurusaki Y, Yamasaki K, Otagiri M.
新自己血液　改訂第3版	2006 年 3 月	克誠堂	高折 益彦
Hemoglobin vesicles containing methemoglobin and L-tyrosine to suppress methemoglobin formation *in vitro* and *in vivo*. *Bioconjugate Chem.* 17, 1241-1245 (2006).	2006 年 10 月	American Chemical Society	T. Atoji, M. Aihara, H. Sakai, E. Tsuchida, S. Takeoka.
Fluid resuscitation with hemoglobin-vesicle solution does not increase hypoxia or inflammatory responses in moderate hemorrhagic shock. *Biomed. Res.* 27, 283-288 (2006).	2006 年 12 月	Biomedical Research Press	Y. Goto, K. Terajima, T. Tsueshita, M. Miyashita, H. Horinouchi, H. Sakai, E. Tsuchida, A. Sakamoto.
Poly(ethylene glycol)-conjugated human serum albumin including iron porphyrins: Surface modification improves the O_2-transporting ability. *Bioconjugate Chem.* 16, 393−398 (2006).	2006 年 4 月	American Chemical Society	Y. Huang, T. Komatsu, R.-M. Wang, A. Nakagawa, E. Tsuchida.
PEGylated albumin-heme as an oxygen-carrying plasma expander: exchange transfusion into acute anemia rat model. *Biomaterials* 27, 4477−4483 (2006).	2006 年 6 月	Elservier	Y. Huang, T. Komatsu, H. Yamamoto, H. Horinouchi, K. Kobayashi, E. Tsuchida.
Poly(ethylene glycol)-conjugated phospholipids in aqueous micellar solutions: Hydration, static structure, and interparticle interactions. *J. Phys. Chem. B.* 11, 1393-1401 (2007).	2007 年 2 月	American Chemical Society	T. Sato, H. Sakai, K. Sou, E. Tsuchida.

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Hemoglobin vesicles reduce hypoxia-related inflammation in critically ischemic hamster flap tissue. *Crit Care Med.* 35, 899-905 (2007).	2007 年 3 月	Lippincott Williams & Wilkins	J. Plock, A. E. Tromp, C. Contaldo, T. Spanholtz, D. Sinovcic, H. Sakai, E. Tsuchida, M. Leunig, A. Banic, D. Erni
"Blood Substitutes", In: Wiley Encyclopedia of Biomedical Engineering. M. Akay (Ed.), pp. 613-621, 2006,	2006 年 4 月	John Wiley & Sons, Inc.	H. Sakai, E. Tsuchida.
Performances of PEG- modified hemoglobin-vesicles as artificial oxygen carriers in microcirculation. *Clin. Hemorheol. Microcirc.* **34**, 335-340 (2006).	2006 年 4 月	IOS Press	H. Sakai, E. Tsuchida.
ポリ（エチレングリコール）修飾アルブミン - ヘム : 酸素輸送血漿増量剤としての溶液物性と機能. *人工血液* 14, 47–54 (2006).	2006 年 9 月	日本血液代替物学会	小松晃之，黄　宇彬，　王栄民，中川晶人，山本尚志，堀之内宏久，小林紘一，土田英俊.
平成 19 年度			
救急医療の現場での輸血医療の実態と人工酸素運搬体への期待. *救急医学* 31, 981-986 (2007)	2007 年 8 月	へるす出版	高折益彦、堀之内宏久、小林紘一
Effect of hemoglobin vesicle, a cellular-type artificial oxygen carrier, on middle cerebral artery occlusion- and arachidonic acid-induced stroke models in rats. *Neurosci. Lett.* 421, 121-125 (2007).	2007 年 6 月	Elsevier	H. Komatsu, T. Furuya, N. Sato, K. Ohta, A. Matsuura, T. Ohmura, S. Takagi, M. Matsuura, M. Yamashita, M. Itoda, J. Itoh, H. Horinouchi, K. Kobayashi.
Hemoglobin-vesicles as artificial oxygen carriers: Present situation and future vision. *J. Intern. Med.* 263, 4-15 (2008).	2008 年 1 月	Blackwell Publishing Co.	H. Sakai, K. Sou, H. Horinouchi, K. Kobayashi, E. Tsuchida.
Hemostatic efficacy of a recombinant thrombin-coated polyglycolic acid sheet coupled with liquid fibrinogen, evaluated in a canine model of pulmonary arterial hemorrhage. *J. Trauma.* 63, 783-7, (2007)	2007 年 10 月	Lippincott Williams & Wilkins	Izumi Y, Gika M, Shinya N, Miyabashira S, Imamura T, Nozaki C, Kawamura M, Kobayashi K.

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
Effects of endogenous ligands on the biological role of human serum albumin in S-nitrosylation. *Biochem. Biophys. Res. Commun.* 364, 790-795, (2007)	2007 年 10 月	Elsevier	Ishima, Y., Akaike, T., Kragh-Hansen, U., Hiroyama, S., Sawa, T., Maruyama, T., Kai, T., and Otagiri, M.
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Effect of olmesartan on oxidative stress in hemodialysis patients. *Hypertens. Res.* 30, 395-402, (2007)	2007 年 5 月	The Japanese Society of Hypertension	Kadowaki, D., Anraku, M., Tasaki, Y., Kitamura, K., Wakamatsu, S., Tomita, K., Gebicki, J. M., Maruyama, T., and Otagiri, M.
Subdomain IIIA of dog albumin contains a binding site similar to site II of human albumin. *Drug Metab. Disposition* 36, 81-86 (2008).	2008 年 1 月	The American Society for Pharmacology and Experimental Therapeutics	Kaneko, K., Fukuda, H., Chuang, V.T.G., Yamasaki, K., Kawahara, K., Nakayama, H., Suenaga, A., Maruyama, T., Otagiri, M.
Effects of endogenous ligands on the biological role of human serum albumin in S-nitrosylation. *Biochem Biophys Res Commun.* 364, 790-5 (2007).	2007 年 12 月	Elsevier	Ishima Y, Akaike T, Kragh-Hansen U, Hiroyama S, Sawa T, Maruyama T, Kai T, Otagiri M.
Design and evaluation of S-nitrosylated human serum albumin as a novel anticancer drug. *J Pharmacol Exp Ther.* 325, 69-76 (2008).	2008 年 4 月	The American Society for Pharmacology and Experimental Therapeutics	Katayama N, Nakajou K, Komori H, Uchida K, Yokoe J, Yasui N, Yamamoto H, Kai T, Sato M, Nakagawa T, Takeya M, Maruyama T, Otagiri M.
周術期輸血	2007 年 6 月	克誠堂 東京	高折 益彦
Selective uptake of surface-modified phospholipid vesicles by bone marrow macrophages in vivo. *Biomaterials* 28, 2655-66 (2007).	2007 年 6 月	Elsevier	K. Sou, B. Goins, S. Takeoka, E. Tsuchida, W.T. Phillips
Rheological properties of hemoglobin vesicles (artificial oxygen carriers) suspended in a series of plasma substitute solutions. *Langmuir* 23, 8121-8128 (2007).	2007 年 6 月	American Chemical Society	H. Sakai, A. Sato, S. Takeoka, E. Tsuchida.
各種代用血漿剤に分散されたヘモグロビン小胞体(人工赤血球)とその血液混合系の	2007 年 12 月	日本ヘモレオロジー学会	佐藤敦、酒井宏水、武岡真司、土田英俊

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
レオロジー特性. 日本ヘモレオロジー学会誌. 10, 3-11 (2007)			
Encapsulation of concentrated hemoglobin solution in phospholipid vesicles retards the reaction with NO, but not CO, by intracellular diffusion barrier. *J. Biol. Chem.* 283, 1508-1517 (2008)	2008 年 1 月	The American Society for Biochemistry and Molecular Biology, Inc.	H. Sakai, A. Sato, K. Masuda, S. Takeoka, E. Tsuchida.
meso-Tetrakis(α , α , α , α -o-amidophenyl) porphinatoiron(II) bearing a proximal histidyl group at the β -pyrrolic position via an acyl bond: synthesis and O_2 coordination in aqueous media. *Chem. Lett.* 36. 640–641 (2007).	2007 年 5 月	日本化学会	A. Nakagawa, T. Komatsu, E. Tsuchida.
Influence of O_2-carrying plasma hemoprotein "albumin-heme" on complement system and platelet activation in vitro and physiological responses to exchange transfusion. *J. Biomed. Mater. Res. 81A*, 821–826 (2007)	2007 年 6 月	Wiley	T. Komatsu, Y. Huang, S. Wakamoto, H. Abe, M. Fujihara, H. Azuma, H. Ikeda, H. Yamamoto, H. Horinouchi, K. Kobayashi, E. Tsuchida.
Induced long-range attractive potentials of human serum albumin by ligand binding. *Phys. Rev. Lett. 98*, 208101-1–4 (2007).	2007 年 5 月	The American Physical Society	T. Sato, T. Komatsu, A. Nakagawa, E. Tsuchida.
O_2-binding albumin thin films: solid membranes of poly(ethylene glycol)-conjugated human serum albumin incorporating iron porphyrin. *Bioconjugate Chem. 18*, 1673–1677 (2007).	2007 年 9 月	American Chemical Society	A. Nakagawa, T. Komatsu, Y. Huang, G. Lu, E. Tsuchida.
Genetic engineering of the heme pocket in human serum albumin: modulation of O_2 binding of iron protoporphyrin IX by variation of distal amino acids. *J. Am. Chem. Soc. 129*, 11286–11295 (2007).	2007 年 9 月	American Chemical Society	T. Komatsu, A. Nakagawa, P. A. Zunszain, S. Curry, E. Tsuchida.
Hemoglobin vesicles to treat hypoxia in critically ischemic tissue. *Artif. Blood* 15, 58-64 (2007).	2007 年 12 月	日本血液代替物学会	D. Erni, R. Wettstein, C. Contaldo, J. Plock, N. Rafatmehr, H. Sakai, E. Tsuchida.
輸血の代替が可能な酸素輸液の実現と組織再生技術. *環境と健康* 20, 464-472 (2007)	2007 年 12 月	共和書院	酒井宏水、土田英俊.
Hemoglobin-vesicles for a Transfusion Alternative and Targeted Oxygen Delivery. *J. Liposome Res.* 17, 227- 235 (2007).	2007 年 7 月	Informa Healthcare USA, Inc.	H. Sakai, 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
Solution to the problems of acellular Hbs by encapsulation, and the intrinsic issues of Hb-vesicles as a molecular assembly. *Transfusion Alternatives in Transfusion Medicine* 9, 226-236 (2007).	2007 年 12 月	Blackwell Publising	H. Sakai, K. Sou, E. Tsuchida.
平成20年度			
Fluid resuscitation with artificial oxygen carriers in hemorrhaged rats: profiles of hemoglobin-vesicle degradation and hematopoiesis for 14 days. *Shock.* 31, 192-200 (2009).	2009 年 2 月	Lippincott Williams & Wilkins	Sakai H, Seishi Y, Obata Y, Takeoka S, Horinouchi H, Tsuchida E, Kobayashi K.
Enhanced radiation response of a solid tumor with the artificial oxygen carrier 'albumin-heme'. *Cancer Sci.* 99, 1274-1278 (2008).	2008 年 4 月	Wiley	Horinouchi H, Yamamoto H, Komatsu T, Huang Y, Tsuchida E, Kobayashi K.
Systemic administration of hemoglobin vesicle elevates tumor tissue oxygen tension and modifies tumor response to irradiation. *J. Surg. Res.* 151, 48-54 (2009).	2009 年 1 月	Elsevier	Yamamoto M, Izumi Y, Horinouchi H, Teramura Y, Sakai H, Kohno M, Watanabe M, Kawamura M, Adachi T, Ikeda E, Takeoka S, Tsuchida E, Kobayashi K.
Review of hemoglobin-vesicles as artificial oxygen carriers. *Artif. Organs.* 33, 139-145 (2009).	2009 年 2 月	Wiley	Sakai H, Sou K, Horinouchi H, Kobayashi K, Tsuchida E.
Histopathological changes of rat brain after direct injection of Hb-vesicles (artificial oxygen carriers) and neurological impact in an intracerebral hemorrhage model. *J. Biomed. Mater. Res. A.* (in press)	2009 年 (印刷中)	Wiley	Sakai H, Okamoto M, Ikeda E, Horinouchi H, Kobayashi K, Tsuchida E.
Hemoglobin-vesicles and red blood cells as carriers of carbon monoxide prior to oxygen for resuscitation after hemorrhagic shock in a rat model. *Shock* (in press)	2009 年 (印刷中)	Lippincott Williams & Wilkins	Sakai H, Horinouchi H, Tsuchida E, Kobayashi K.
FDA Workshop on Hemoglobin Based Oxygen Carriersに参加して. *人工血液* 16, 23-29 (2008).	2008 年 7 月	日本血液代替物学会	酒井宏水、堀之内宏久、小林紘一
Syndrome of inappropriate secretion of antidiuretic hormone after chemotherapy with vinorelbine. *Cancer Chemother Pharmacol.* 62, 331-333 (2008)	2008 年 7 月	Springer	Kuroda H, Kawamura M, Hato T, Kamiya K, Kawakubo M, Izumi Y, Watanabe M, Horinouchi H, Kobayashi K, Nakayama M.
Surgical outcomes for pulmonary metastases from hepatocellular carcinoma. *Eur J Cardiothorac Surg.* 34, 196-199 (2008).	2008 年 7 月	Elsevier	Kawamura M, Nakajima J, Matsuguma H, Horio H, Miyoshi S, Nakagawa K, Fujisawa T, Kobayashi K; Metastatic Lung Tumor Study Group of Japan.

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
Histopathological features and prognostic significance of the micropapillary pattern in lung adenocarcinoma. *Mod Pathol.* 21, 992-1001 (2008).	2008 年 8 月	Nature Publishing Group	Kamiya K, Hayashi Y, Douguchi J, Hashiguchi A, Yamada T, Izumi Y, Watanabe M, Kawamura M, Horinouchi H, Shimada N, Kobayashi K, Sakamoto M.
Disease-free interval length correlates to prognosis of patients who underwent metastasectomy for esophageal lung metastases. *J Thorac Oncol.* 3, 1046-1049 (2008).	2008 年 9 月	Lippincott Williams & Wilkins	Shiono S, Kawamura M, Sato T, Nakagawa K, Nakajima J, Yoshino I, Ikeda N, Horio H, Akiyama H, Kobayashi K; Metastatic Lung Tumor Study Group of Japan.
An alternative method for screening EGFR mutation using RFLP in non-small cell lung cancer patients. *J Thorac Oncol.* 3, 1096-103 (2008)	2008 年 10 月	Lippincott Williams & Wilkins	Kawada I, Soejima K, Watanabe H, Nakachi I, Yasuda H, Naoki K, Kawamura M, Eguchi K, Kobayashi K, Ishizaka A.
A case report of surgical correction for congenital mitral regurgitation with subvalvular apparatus abnormality. *Gen Thorac Cardiovasc Surg.* 56, 36-8 (2008).	2008 年 1 月	Springer	Kudo M, Yozu R, Aeba R, Kokaji K, Kimura N, Iwanaga S.
心臓手術の実際　許俊鋭　　編著　心内膜床欠損症に対する手術と体外循環　-慶應義塾大学-　p218-20. 2008	2008 年 10 月	秀潤社	饗庭　了
心臓血管外科テクニック　弁膜症編弁膜症の病態と治療戦略 肺動脈弁狭窄症・肺動脈弁閉鎖不全症　p49-59. 2009	2009 年 2 月	メディカ出版	饗庭　了
Influence of hemoglobin vesicles, cellular-type artificial oxygen carriers, on human umbilical cord blood hematopoietic progenitor cells in vitro. *J. Biomed. Mater. Res. A.* 88, 34-42 (2009).	2009 年 1 月	Wiley	Yamaguchi M, Fujihara M, Wakamoto S, Sakai H, Takeoka S, Tsuchida E, Azuma H, Ikeda H.
Biocompatibility study of hemoglobin vesicles, cellular-type artificial oxygen carriers, with human umbilical cord hematopoietic stem/progenitor cells using an in vitro expansion system. *ASAIO J.* (in press)	2009 年 (印刷中)	Lippincott Williams & Wilkins	M. Yamaguchi, M. Fujihara, S. Wakamoto, H. Sakai, E. Tsuchida, H. Azuma, H. Ikeda.
ヘモグロビン小胞体のin vitroにおける血液細胞および血漿タンパクへの適合性. *人工血液* 16, 212-220 (2008)	2009 年 2 月	日本血液代替物学会	藤原 満博、東　寛、池田 久實
Pharmacokinetics of single and repeated injection of hemoglobin-vesicles in hemorrhagic shock rat model. *J. Control. Release.* (in press)	2009 年 (印刷中)	Elsevier	Taguchi K, Maruyama T, Iwao Y, Sakai H, Kobayashi K, Horinouchi H, Tsuchida E, Kai T, Otagiri M.

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
Design and evaluation of S-nitrosylated human serum albumin as a novel anticancer drug. *J Pharmacol Exp Ther.* (2008) 325(1):69-76.	2008 年 4 月	American Society for Pharmacology and Experimental Therapeutics	Katayama N, Nakajou K, Komori H, Uchida K, Yokoe J, Yasui N, Yamamoto H, Kai T, Sato M, Nakagawa T, Takeya M, Maruyama T, Otagiri M.
S-nitrosylated human serum albumin-mediated cytoprotective activity is enhanced by fatty acid binding. *J Biol Chem.* (2008) 283(50):34966-75.	2008 年 12 月	American Society for Biochemistry and Molecualr Biology	Ishima Y, Akaike T, Kragh-Hansen U, Hiroyama S, Sawa T, Suenaga A, Maruyama T, Kai T, Otagiri M.
Altered chain-length and glycosylation modify the pharmacokinetics of human serum albumin.. *Biochim Biophys Acta.* (2008) in press.	2009 年 (印刷中)	Elsevier	Iwao Y, Hiraike M, Kragh-Hansen U, Kawai K, Suenaga A, Maruyama T, Otagiri M.
NO and CO binding profiles of hemoglobin vesicles as artificial oxygen carriers. *Biochim. Biophys. Acta.* 1784, 1441-1447 (2008).	2008 年 8 月	Elsevier	Sakai H, Sato A, Sobolewski P, Takeoka S, Frangos JA, Kobayashi K, Intaglietta M, Tsuchida E.
The role of luminal factors in the recovery of gastric function and behavioral changes after chronic Helicobacter pylori infection. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295, G664-G670 (2008).	2008 年 8 月	American Physiological Society	Verdu EF, Bercik P, Huang XX, Lu J, Al-Mutawaly N, Sakai H, Tompkins TA, Croitoru K, Tsuchida E, Perdue M, Collins SM.
Electrostatic interactions and complement activation on the surface of phospholipid vesicle containing acidic lipids: effect of the structure of acidic groups. *Biochim. Biophys. Acta.* 1778, 1035-1041 (2008)	2008 年 8 月	Elsevier	Sou K, Tsuchida E.
Structure, photophysical property, and cytotoxicity of human serum albumin complexed with tris(dicarboxy methylene)[60]fullerene. *Bioconjug Chem.* 19, 1556-1560 (2008).	2008 年 8 月	American Chemical Society	Qu X, Komatsu T, Sato T, Glatter O, Horinouchi H, Kobayashi K, Tsuchida E.
O_2 binding to human serum albumin incorporating iron porphyrin with a covalently linked methyl-L-histidine Isomer. *Bioconjugate Chem.* 19, 581–584 (2008).	2008 年 月	American Chemical Society	A. Nakagawa, T. Komatsu, M. Iizuka, E. Tsuchida,
Heme pocket architecture in human serum albumin: Regulation of O_2 binding affinity of a prosthetic heme group by site-directed mutagenesis. *Macromol. Symp.* 270, 187-192 (2008)	2008 年 10 月	Wiley	Komatu T, Nakagawa A, Tsuchida E.
多波長パルス分光法を用いたHb小胞体用パルスオキシメータに関する研究. *人工血液* 16, 198-204 (2008).	2009 年 2 月	日本血液代替物学会	須崎裕典、酒井宏水、小林直樹、池田達彦、堀之内宏久、小林 紘一、武田 朴、戸川 達男、土田英俊.
人工赤血球の過去、現在、未来. *ファルマシア* 45, 23-28 (2009).	2009 年 1 月	日本薬学会	酒井宏水、土田英俊.

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
Artificial Oxygen Carriers, Hemoglobin Vesicles and Albumin-Hemes, Based on Bioconjugate Chemistry. *Bioconjugate Chem.* (in press)	2009 年 (印刷中)	American Chemical Society	Tsuchida E, Sou K, Nakagawa A, Sakai H, Komatsu T, Kobayashi K.
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Hemoglobin-vesicle, a cellular artificial oxygen carrier, that fulfils the physiological roles of the red blood cells structure. *Adv. Exp. Med. Biol.* (in press).	2009 年 (印刷中)	Springer	H. Sakai, K. Sou, H. Horinouchi, K. Kobayashi, E. Tsuchida.
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Use of Hemoglobin Vesicles During Cardiopulmonary Bypass Priming Prevents Neurocognitive Decline in Rats

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Background—Homologous blood use is considered to be the gold standard for cardiopulmonary bypass (CPB) priming in infants despite exposure of the patient to potential cellular and humoral antigens. However, the use of hemoglobin vesicles (HbVs), artificial oxygen carriers that encapsulate a concentrated hemoglobin solution within phospholipid bilayer membranes, for CPB priming may prevent neurocognitive decline in infants. The goal of this study was to determine whether HbV use offsets hemodilution caused by patient/priming volume-mismatched CPB and thereby prevents the development of postoperative neurocognitive deficits.

Methods and Results—CPB was established in 28 male Sprague-Dawley rats (age, 14 to 16 weeks; weight, 450 grams) after cannulation of the tail artery and right atrium. The animals were randomly assigned to 1 of 3 groups: sham surgery (n=9), HbV (−) prime (n=10), or HbV (+) prime (n=9). CPB was conducted for 90 minutes at 200 mL/kg per minute. The hematocrit during CPB was 10.0±1.2% in the HbV (+) prime group and 9.9±1.3% in the HbV (−) prime group (P=not significant). Learning and memory function were evaluated using 2 different maze tests (Maze-1 and Maze-2, in which the arrival times to the target were measured on the first, third, fifth, and seventh postoperative days). Learning and memory function were significantly better in the HbV (+) prime group than in the HbV (−) prime group (Maze-1, P=0.012; Maze-2, P=0.042); there was no difference between the HbV (+) prime and the sham surgery group.

Conclusions—The use of HbV for CPB priming may serve as a substitute for homologous blood to prevent the unacceptable hemodilution and contribute to maintenance of intact neurocognitive function. (***Circulation***. **2006; 114[suppl I]:I-220–I-225.)**

Key Words: cardiopulmonary bypass ■ hemoglobin ■ nervous system ■ pediatrics

Homologous blood use continues to be the gold standard for cardiopulmonary bypass (CPB) priming in infants and neonates despite exposure of the patient to potential cellular and humoral antigens. Neurologic morbidity after CPB has become an increasing concern ever since surgical mortality has decreased in infants undergoing repairs of simple and complex congenital heart diseases. CPB itself can cause neurologic morbidity because CPB gives rise to a systemic inflammatory response that is responsible for decreased cerebral blood flow and cerebral dysfunction. Although hemodilution during CPB increases both early neurologic complications and late neurocognitive performance, the use of homologous blood potentially exaggerates the CPB-derived inflammatory response and may contribute to post-CPB neurologic morbidity. Though miniaturization of the CPB circuit has reduced priming volume,[1–3] at the present time, however, it is still not low enough to achieve an acceptable level of hemodilution in very small patients.

Hemoglobin vesicles (HbVs) have been developed for use as artificial oxygen carriers. HbV is a solution of purified Hb that is encapsulated within a phospholipid bilayer membrane. The oxygen-transporting ability of HbVs is comparable to that of blood.[4] Hb-based oxygen carriers have many potential advantages over homologous blood. First, HbV has no cellular and humoral antigens, which eliminates the risks of blood-type mismatch reaction and blood-transmittable infectious disease. Second, HbVs, which have a particle diameter of only 250 nm, are small enough to circulate through blood microvessels that can become constricted during and after CPB and through which red blood cells cannot pass. Third, HbV is very stable and can be stored as a powder for a long time.[5] Fourth, HbVs are captured by phagocytes in the reticuloendothelial system and are metabolized within ≈7 days, without iron or lipid deposition.[6] The only concern posed by HbV for clinical use is its short half-life of only 35 hours in the circulating blood. However, its quick disappearance from the circulation could be an advantage rather than a disadvantage when using HbV as a CPB priming solution in pediatric open heart surgery, because hemodilution occurs only during and soon after CPB, which is usually <2 hours in most cases.

Thus, using HbVs as the CPB priming solution instead of a crystalloid solution or homologous red blood cells could

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improve the neurologic and neurocognitive outcomes in very small patients undergoing open heart surgery. The purpose of this investigation was to determine the effects of HbVs on neurologic and neurocognitive function after CPB using a rat model.

Materials and Methods

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Preparation of HbV CPB Prime

HbVs were manufactured and provided by the Department of Polymer Chemistry, Advanced Research Institute for Science and Engineering, Waseda University (Tokyo, Japan). HbVs were prepared under sterile conditions as previously reported.[7] 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). HbVs were suspended in a physiological 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] Before use, the HbV suspension ([Hb]=10 g/dL, 8.6 mL) was mixed with a solution of human serum albumin (1.4 mL; Nipro, Osaka, Japan) to adjust the albumin concentration in the vesicle suspending medium to 5 g/dL. Under these conditions, the colloid osmotic pressure of the suspension is ≈20 mm Hg (Wescor 4420 Colloid Osmometer; Wescor, Logan, Utah).[7] As a result, the Hb concentration of the suspension was 8.6 g/dL.

Animal Model and Preparation

SD rats were puchased from Sankyo labo service Corp (Tokyo, Japan) The experimental protocol was 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*.[8]

Twenty-eight male SD rats, aged 14 to16 weeks and weighing 450 grams, were housed in cages and provided with food and water ad libitum in a temperature-controlled room with a 12-hour dark/light cycle. The animals were anesthetized with 3.0% sevoflurane-mixed air inhalation with a vaporizer. The rats were intubated (16-gauge intravenous catheter) and mechanically ventilated. The ventilator setting included FiO_2 of 0.21 and ventilatory rate of 70 cycles per minute. Anesthesia was maintained with 1.5 to 2.0% sevoflurane. Surgery was performed using aseptic technique.

CPB in the rat was performed using the surgical techniques described by Grocott et al.[9] Heart rate and rectal temperature were continuously monitored, and the rectal temperature was servo-regulated at 37.5°C. After systemic heparinization using 300 IU, the tail artery was cannulated with a 22-gauge angiocatheter. A 16-gauge multi-pore angiocatheter was introduced into the right internal jugular vein and advanced into the right atrium. A roller pump and custom-made CPB oxygenator/circuit were used for all the experiments.

The animals were randomly divided into the 3 experimental groups (Figure 1): (1) the sham surgery group (n=9); (2) the HbV (−) prime group (n=10); and (3) the HbV (+) prime group (n=9). In the sham surgery group, the animals were cannulated but CPB was not induced. In the other groups, the CPB circuit was primed with 60 mL of human serum albumin solution either with or without HbV (the HbV (+) prime group and the HbV (−) prime group).

Normothermic CPB with a flow of 200 mL/kg per minute was performed for 90 minutes. During CPB, 100% oxygen gas was delivered to the oxygenator at 1.0 L/min. The animals were separated from CPB without the use of any vasoactive agents. After removal of the cannula, the animals were ventilated for another 30 minutes, at which point all the blood that was left in the CPB circuit was collected and centrifuged at 2000 rpm for 5 minutes, and then the precipitates were returned intravenously. Arterial blood samples were collected after placement of the CPB cannulae (T-1), 45 minutes after CPB initiation (T-2), as well as after CPB and CPB blood return (T-3). A pH/blood gas analyzer (I-STAT; Fuso, Osaka, Japan) was used to determine arterial Po_2, Pco_2, and pH.

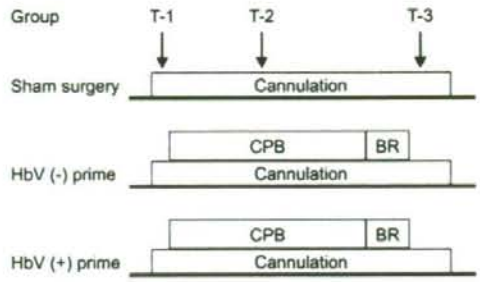

Figure 1. Experimental protocol. CPB indicates cardiopulmonary bypass; BR, blood return; T-1, T2, and T3, time for arterial blood sampling.

After the animals recovered from the effects of the general anesthetic, they were extubated and returned to their cages. The animals were observed for 7 days, during which time they had free access to water and food.

Neurologic and Neurocognitive Evaluation

Neurologic and neurocognitive outcomes were assessed by video-recording all behaviors of the animals, which a physician blinded to the groups reviewed collectively later.

Neurologic outcome was assessed on the days 1, 3, 5, and 7 after the operation using neurologic performance and functional disability scores.[10] The neurologic performance scale[11] consisted of a physical examination with points given for deficits. A normal examination score was 0, and the worst score was 95. The functional disability score was ranked from 1 to 5: *score 1* (no disability), able to run, explore the environment, and feed from the trough; *score 2* (mild disability), gait disturbances but able to ambulate, explore the environment, and feed from the trough; *score 3* (moderate disability), unable to walk and required bottle-feeding, but was alert and able to crawl; *score 4* (severe disability), not able to feed even with assistance and unable to crawl; and *score 5*, death.

To evaluate neurocognitive outcome, 2 different kinds of behavioral testing using maze tests (Maze-1 and Maze-2) were performed on days 1, 3, 5, and 7 after the operation.[12] The Maze-1 test is generally referred to as the Morris water maze test. Briefly, the Morris water maze consisted of a 1.5-m–diameter, 50-cm–deep water pool (27°C) with a submerged (3 cm below surface) hidden platform in 1 quadrant. The time to locate the submerged platform (defined as the latency) is measured to test for impairment in visual–spatial learning and memory. The animals underwent testing in the water maze with 4 trials per testing period. Each of the trials began from a separate quadrant. Testing was performed on days 1, 3, 5, and 7 after the operation. The Maze-2 test is of the type that actually has a maze-shaped pool of water with 5.5 m of total pathway length and 11 junction points, where the animals have to swim without rest until arriving at the sole exit. The time from the departure point to the goal point was measured in a similar way to that of Maze-1.

Histopathological Examination

After completion of the neurologic testing on day 7, the animals were euthanized with 3.0% sevoflurane inhalation. The brains were harvested and stored in 4% formalin. Paraffin-embedded brain sections were then serially cut (5-μm–thick sections) and stained with hematoxylin and eosin. A neuropathologist who was blinded to group assignment counted the total number of necrotic cells in the hippocampus (CA1–2) area.

Physiologic Data in Each Group

	T-1			T-2			T-3		
	Sham Surgery	HbV(−) Prime	HbV(+) Prime	Sham Surgery	HbV(−) Prime	HbV(+) Prime	Sham Surgery	HbV(−) Prime	HbV(+) Prime
Arterial pH	7.59±0.06	7.60±0.04	7.61±0.05	7.58±0.05	7.44±0.10*	7.50±0.10	7.55±0.03	7.36±0.07*	7.45±0.14
Arterial PCO_2, mm Hg	21.9±1.6	23.1±2.4	22.4±2.9	21.2±1.9	24.0±4.4	22.9±4.5	19.2±1.7	28.7±7.2†	27.2±7.9†
Arterial PO_2, mm Hg	92.9±10.5	85.2±8.9	88.4±10.7	96.8±8.9	460.3±37.3*	452.9±38.5*	95.4±7.6	79.1±13.7	79.8±19.7
Hematocrit, %	40.7±2.4	40.4±3.1	42.0±2.5	37.4±2.3	9.9±1.3*	10.0±1.2*	37.1±1.5	28.2±2.3	27.7±4.3

Values are mean ±SD. n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime.
*$P<0.01$ vs sham surgery; †$P<0.05$ vs sham surgery.
PCO_2 indicates partial pressure of carbon dioxide; PO_2, partial pressure of oxygen.

Statistical Analysis

All continuous numerical data were presented as means ± SD. Intergroup comparisons were made with 1-way analysis of variance. When a significant F ratio was obtained, further analysis was performed with Scheffe F post-hoc test. Nonparametric data were analyzed using the Kruskal-Wallis test. Statistical significance was assumed when $P<0.05$.

Results

All rats survived the entire period of time needed to complete the experimental protocol. The Table shows the baseline data for the 3 groups at T-1, T-2, and T-3.

The hematocrit was lower in the HbV (−) and HbV (+) prime groups than in the sham surgery group at T-2 ($P<0.01$). The hematocrit at T-2 was 9.9±1.3% in the HbV (−) prime group and 10.0±1.2% in the HbV (+) prime group (P=not significant). The arterial pH was lower in the HbV (−) prime group than the sham surgery group at T-2 and T-3 ($P<0.01$). The arterial PCO_2 was higher in the HbV (−) and HbV (+) prime group than in the sham surgery group at T-3 ($P<0.05$). At T-2, the arterial PO_2 was >400 mm Hg in the HbV (−) prime and HbV (+) prime groups, whereas that in the sham surgery group was 96.8±8.9 mm Hg at T-2 ($P<0.01$).

The neurologic examination showed no significant differences among the 3 groups with respect to performance and disability scores (Figures 2 and 3), and none of the groups showed any distinctly abnormal neurologic behaviors. All animals were able to feed by themselves, ambulate, and freely explore their surroundings.

Neurocognitive outcome is shown in Figures 4 and 5. The HbV (−) prime group had longer maze latencies for both maze tests compared with the other groups (Maze-1, $P=0.012$; Maze-2, $P=0.042$), indicating significant neurocognitive dysfunction after hemodilution. The maze latency curves were similar in the HbV (+) prime and the sham surgery groups. On day 1, the arrival times were similar among the 3 groups. However, subsequently, the HbV (+) prime and sham surgery groups had shorter arrival times than the HbV (−) group (Maze-1, $P<0.01$; Maze-2, $P<0.01$); the differences between the HbV(+) prime and the sham surgery groups were similar for all intervals.

Figure 6 represents the swimming speed of the rats. The swimming speed was similar among the 3 groups and did not show any chronological change from days 1 through 7 after the operation (P=not significant), indicating that exercise capacities were intact even in animals subjected to CPB.

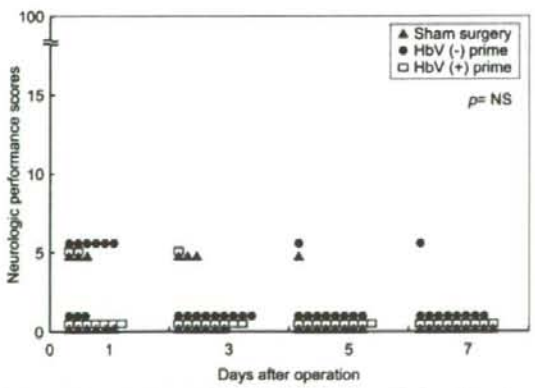

Figure 2. Neurologic performance scores after CPB on days 1, 3, 5, and 7 after the operation. Score 0 represents no neurologic deficits and 95 represents brain death. n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Neurologic performance scores were not different among the 3 groups. NS indicate not significant.

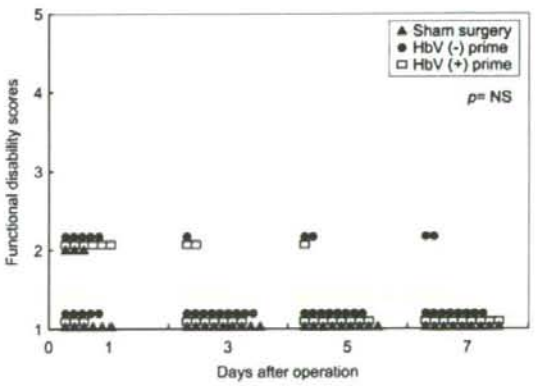

Figure 3. Functional disability scores after CPB on days 1, 3, 5, and 7 after the operation. *Score 1* indicates no disability; *score 2*, mild disability; *score 3*, moderate disability; *score 4*, severe disability; *score 5*, death. n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Functional disability scores were not different among the 3 groups.

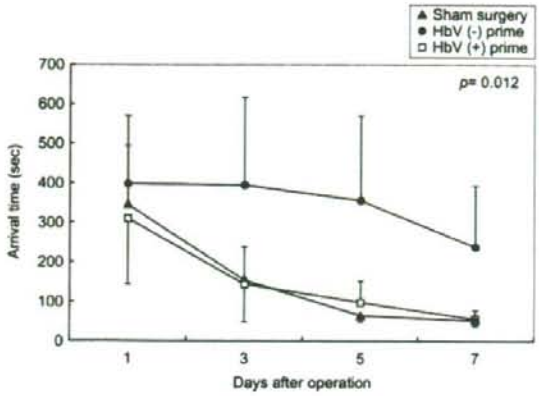

Figure 4. Neurocognitive outcome was assessed on days 1, 3, 5, and 7 after CPB by visual–spatial learning with the maze test (Maze-1). n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Analyzing the group mean latency in repeated measures analysis of variance, the HbV (−) prime group had longer latencies compared with the HbV (+) prime and the sham surgery group (P=0.012), indicating significant neurocognitive dysfunction. HbV (+) prime group was similar to the sham surgery group (P=NS).

On histology there was no difference among groups with respect to the total number of necrotic hippocampal neuron cells.

Discussion

For infants and neonates undergoing reparative and palliative surgery for simple and complex congenital heart disease, CPB techniques and treatment strategies have been rapidly evolving in the last decade. This has undoubtedly contributed to improved surgical outcomes. However,

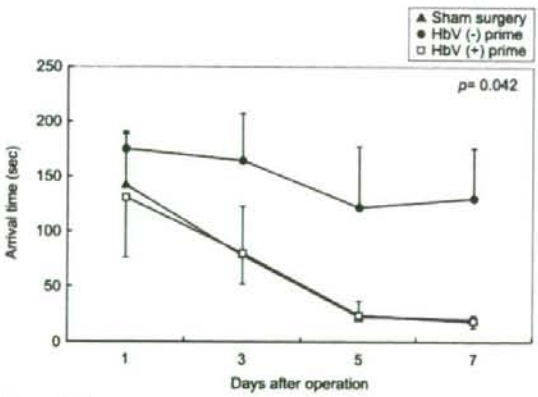

Figure 5. Neurocognitive outcome was assessed on days 1, 3, 5, and 7 after CPB by testing visual–spatial learning with the maze tests (Maze-2). n=8, sham surgery; n=7, HbV (−) prime; n=7, HbV (+) prime. Analyzing the group mean latency in repeated measures analysis of variance, the HbV (−) prime group had longer latencies compared with the HbV (+) prime and the sham surgery group (P=0.042), indicating significant neurocognitive dysfunction. HbV (+) prime group was similar to the sham surgery group (P=NS).

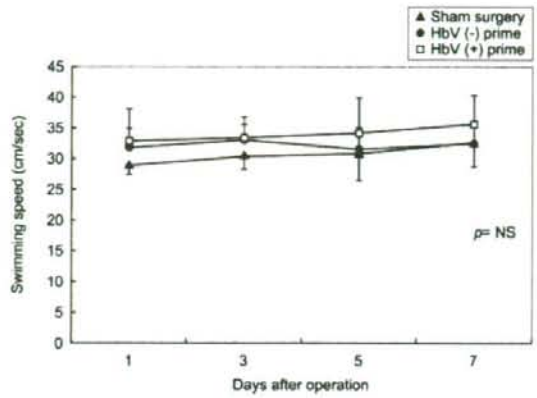

Figure 6. Water maze swimming speed was assessed in the rats. (n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime.) The swimming speed was similar among the 3 groups, and did not show any chronological change from days 1 through 7 after the operation.

CPB still results in an inflammatory systemic reaction, which can, in turn, cause dysfunction in many end organs. In the brain, CPB decreases cerebral endothelial function[13,14] and blood flow, a phenomenon known as "no reflow," which appears to be highly linked to postoperative neurologic morbidity.[15,16]

Thus, there is a dilemma in the use of homologous blood for CPB priming in infants. The priming volume for infants and neonates has been significantly decreased by the miniaturization of commercially available CPB circuits, which include tubing, bubble filter, and oxygenator.[5] This progress has decreased the ratio of CPB priming volume to circulating blood volume. However, this has helped only rather large infants who could have bloodless priming without unacceptable hemodilution and/or cases that require only a very short CPB duration. Otherwise, homologous blood is mandatory to prevent unacceptable hemodilution that leads to suboptimal oxygen supply, even though there is exposure to potential cellular and humoral antigens. In common with CPB, blood transfusion by itself stimulates systemic inflammatory cytokine production.[17,18] Thus, homologous blood use for CPB priming may also be a risk to cerebral blood flow and function.[19]

Artificial oxygen carriers could be a breakthrough that helps solve this dilemma. In the past, Fluosol was used in a pig CPB model to investigate its capability to augment myocardial perfusion.[20,21] However, a critical adverse effect developed; there was an increased level of ionized calcium that was associated with increased myocardial contractility and anaerobic metabolism. Therefore, the clinical use of Fluosol was aborted. Izumi, who is associated with our institute, has previously found that HbV has an equivalent oxygen transporting capability to red blood cells during CPB in a dog model.[22] HbV has no side effect of microvascular vasoconstriction, as commonly noted in many other Hb-based oxygen carriers. This background compelled us to perform the current study.

The clinical use of HbV in CPB priming of infants and neonate could ostensibly give rise to a variety of adverse effects during the several days before the reticuloendothelial system deals with the molecule. However, previous studies using rats found that a bolus large-dose HbV infusion was associated with minor and transient deterioration in major organ function.[6,23] Furthermore, these potential adverse effects could be minimized by using modified ultrafiltration after CPB,[24] which would eventually eliminate most of the HbV from the serum.

In our rat CPB model, the hematocrit during CPB was ≈10%, which in clinical practice should have been treated by blood transfusion. The animals had a lower pH when HbV was not added to the CPB priming solution. However, given the hypocarbia policy with ventilation and CPB during the entire period of anesthesia, the pH was maintained at ≥7.4 even in the HbV (−) prime group, and the rats all survived during CPB and for 7 days after CPB without any neurologic morbidity. On neurohistology 7 days after surgery, the findings were similar among all 3 groups. These results indicate that the rats in the HbV (−) prime group were over-hemodiluted in terms of oxygen supply, but this hemodilution was "subclinical" when surgical outcome was evaluated by gross observation and by neurohistology. Our model may be highly sensitive in detecting subtle injury of cerebral function. Learning and memory function is one of the highest levels of cerebral function and was evaluated by 2 maze tests. It may not be surprising that mild deficits in oxygen supply during CPB were only detected by impairment of neurocognitive function without any other findings.

Our model has several limitations with respect to extrapolating to the human clinical setting. The age of the animals was not matched to that of human neonates and infants. CPB was established using peripheral access with internal jugular venous and tail arterial return without opening of the chest, induced cardiac arrest, hypothermia, and circulatory arrest. All of these less invasive CPB cannulation techniques may have contributed to the 100% animal survival rate without neurologic morbidity and allowed successful neurocognitive evaluation after CPB. However, such an approach may not necessarily mimic the procedures used for human infants and neonates undergoing open heart surgery. Another concern is species difference. To prioritize the survival of the small animals we abandoned serial measurement of intracerebral oxygen tension and cerebral blood flow, as well as repeated blood sampling for lactate extraction, inflammatory cytokine concentrations, and serologic markers of brain injury, which might have provided important information to support our hypothesis. Finally, the current study lacked a control group with homologous blood priming because of technical issues involving blood-banking in rats.

Nevertheless, the results of the current study clearly indicate that HbV can serve as a substitute for homologous blood in CPB priming to prevent the unacceptable hemodilution caused by a large difference between circulating blood and CPB priming volume. The use of HbV for CPB priming may even be potentially superior to homologous blood priming with respect to the maintenance of intact neurocognitive function. These data also provide a rationale for further studies investigating the effect of HbV on cerebral oxygen metabolism and the inflammatory response in a larger animal CPB model.

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Disclosures

None.

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One-Year Observation of Wistar Rats after Intravenous Infusion of Hemoglobin-Vesicles (Artificial Oxygen Carriers)

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Abstract: Hemoglobin-vesicles (HbV) or liposome-encapsulated Hb are artificial oxygen carriers. Our previous studies of the bolus infusion of HbV into Wistar rats showed that HbV was captured by the reticuloendothelial system from the blood stream and degraded completely with no deteriorative effect for 2 weeks. However, one authority on artificial organs research suggested conducting a one-year observation because he experienced, with one lipid-emulsified perfluorocarbon (PFC), that rats died within one year from a pulmonary abnormality after receiving the PFC emulsion due to the unstable dispersion state (personal communication). We thought this would never happen for HbV because the dispersion state of HbV is stable with PEG-modification. To confirm this, we made one-year observations after HbV infusion as suggested. Five male

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Wistar rats intravenously received 20 ml/kg HbV suspended in saline ([Hb] = 10 g/dL). They were housed in separated cages and provided with food and water *ad libitum*. All rats survived one year, and were apparently healthy. Their body weights (821 ± 75 g) reflected obesity from their confinement in small cages. No histopathological abnormality was found in the lung. Plasma biochemical analyses showed overall normal organ functions. In our previous report, plasma lipid levels increased transiently at 1 or 2 days; then they reverted to the control level at 7 days. One year later, the rats showed much higher plasma lipid levels, a symptom of hyperlipidemia that is attributable to obesity and aging. It seemed the transient increases at the early days had no impact compared with the levels of hyperlipemia of the old rats.

Keywords: Blood substitute; Liposome; Metabolism; Reticuloendothelial system; Safety study

INTRODUCTION

Phospholipid vesicles or liposomes encapsulating hemoglobin (Hb-vesicles, HbV) can serve as an O_2 carrier with comparable ability to that of red blood cells (RBC) [1–5]. Advantages of the Hb-based O_2 carriers (HBOCs) are the absence of blood-type antigens and transmission of known and unknown blood-borne diseases, the possibility of improving rheological properties of blood flow according to patients' needs, and stable long-term storage [6]. These characteristics will enable the use of HBOCs both in elective and emergency situations. In this sense, the infusion of HBOCs becomes superior to the conventional blood transfusion, which still entails the potential for mismatching, viral infection by HIV and hepatitis virus, and problems inherent in the short 2–3 week preservation period. According to clinical conditions in which HbVs are expected to be applied, an organism is faced with the metabolism of large amounts of both Hb and lipids because the HbV dose rate is considerably large. The HbV particles, as well as phospholipid vesicles, which are infused in the blood stream are ultimately captured by phagocytes in the reticuloendothelial system (RES, or mononuclear phagocytic system, MPS) [7–9].

Through histopathological studies of rats receiving 20 ml/kg of HbV infusion, our previous reports clarified that the HbV particles were captured and metabolized within 7 days in RES, mainly in the spleen and liver [7,8]. Transmission electron microscopy provided a clear image of the HbV particles in the phagosomes 1 day after infusion, but they disappeared within 7 days. Staining with the anti-human Hb antibody, Berlin blue, and hematoxylin/eosin showed prompt metabolism of Hb

molecules with no morphological changes in the liver and spleen. Phagocytic activity decreased and then increased transiently, but tended to revert to the original level.

Plasma biochemical analyses for one week showed normal values overall, except that amylase and lipase activities showed reversible changes. However, no morphological changes were apparent in the pancreas. Plasma bilirubin and iron did not increase in spite of the fact that a large amount of Hb was metabolized in the macrophages. Lipid components increased transiently showing the maximum at 1 or 2 days, and returned to the control level at 7 days. They should be derived from membrane components of HbV that are liberated from macrophages that entrap HbV. Considering that result along with the previous report of prompt metabolism of HbV in the RES by histopathological examination, we conclude that HbV infusion transiently modified the values of the analytes at the bolus infusion rate of 20 ml/kg without any irreversible damage to the corresponding organs [7,8].

However, one authority in the research field of artificial organs strongly suggested that we conduct a one-year-observation because he experienced, unexpectedly, that rats died of pneumonitis within one year after receiving one lipid-emulsified perfluorocarbon (PFC) solution (personal communication) due to the insufficient dispersion stability [10]. In the case of HbV, the dispersion stability is ensured by the surface modification with polyethylene glycol (PEG) [6,10], and we thought this would never happen for our HbV. However, according to his suggestion, we conducted a simple test of topload bolus infusion of HbV at the dose rate of 20 ml/kg into Wistar rats. We confirmed the rats' survival, body weight increase, and conducted hematological, histological and plasma biochemical analyses, thereby verifying the safety of HbV.

MATERIALS AND METHODS

Preparation of Polyethylene Glycol (PEG)-Modified Hb-Vesicles (HbV)

The PEG-modified HbV was prepared in a sterile condition as reported previously in the literature [6,11,12]. Hb was purified from outdated donated blood provided by the Hokkaido Red Cross Blood Center (Sapporo, Japan) and the Society of Red Cross, Japan (Tokyo, Japan). The encapsulated hemoglobin (38 g/dl) contained 14.7 mM of pyridoxal 5′-phosphate (PLP; Sigma Chemical Co., St. Louis, MO) as an allosteric effector at a molar ratio of Hb/PLP = 2.5. The lipid bilayer was composed of Presome PPG-I [a mixture of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine, cholesterol, 1,5-dipalmitoyl-L-glutamate-*N*-succinic