概略を記載する。

生後9－12週齢のWistar系雄性ラット(Charles River Japan Inc.)を用いた。ヘパリン(ノボ・ヘパリン注1000、持田製薬) 1000 Uを腹腔内投与し、10分後に、xylazine hydrochloride 10 mg/kg (Sigma-Aldrich CO.)と ketamine hydrochloride (動物用ケタラール50、三共ライフテック) 90 mg/kgを腹腔内投与して麻酔した。開腹・開胸して心臓を取り出し、直ちに氷冷したKrebs-Henseleit buffer (NaCl 116 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, KH_2PO_4 1.2 mM, glucose 11.1 mM)(以下KH-bufferと省略する)に投入して心臓の拍動を停止させた。大動脈にカニューレを挿入し、KH-bufferを用いて静水圧100 cmH_2O、37℃でランゲンドルフ灌流した。KH-bufferは、実験開始前から終了するまで95% O_2 + 5% CO_2の混合ガスを通気し、pHを7.4に調整した。

左心室に生理食塩水を満たしたラテックス・バルーンを挿入し、圧トランスデューサー(P-50, Gould Inc.)を介して多チャンネル記録計(WS-641G, Nihon Kohden)に接続し、左室発生圧(LVDP)、左室拡張末期圧(LVEDP)、心拍数(HR)などを実験開始から終了まで連続的に記録した。バルーンの容積は、通常灌流時の左室拡張末期圧(LVEDP)が0-5 mmHgになるようにした。

3. Hb小胞体と空球小胞体のKH-bufferへの懸濁・希釈法：

この項目も昨年の報告書(1)で詳しく述べたので、実験に用いた懸濁・希釈液の種類とそのHb濃度のみを記載する。

(1) HbV 30倍希釈懸濁液：Hb濃度0.33 g/dL

(2) HbV 100倍希釈懸濁液：Hb濃度0.10 g/dL

(3)空球小胞体 30倍希釈懸濁液：Hb濃度0.33 g/dLに相当する

(4) 空球小胞体100倍希釈懸濁液：Hb濃度0.10 g/dLに相当する

こうして作製したHb小胞体と空球小胞体のKH-buffer懸濁液は37℃に加温し、95% O_2 + 5% CO_2の混合ガスを1時間以上通気した後実験に用いた。

4. 実験のプロトコール：

この項目も昨年の報告(1)で詳しく述べたので、以下に概略する。

(1) control群 (n = 6)：

control灌流を約30分間行った後に、灌流を停止させて虚血(ischemia)を惹起し、虚血を30分間継続した後再灌流を30分間行った。

(2) 空球小胞体0.10 g/dL群 (n = 7)：以下EV-a群

(3) 空球小胞体0.33 g/dL群 (n = 7)：以下EV-b群

(4) HbV 0.10 g/dL群 (n = 7)：以下HbV-a群

(5) HbV 0.33 g/dL群 (n = 6)：以下HbV-b群

20分間のcontrol灌流直後に灌流液を空球小胞体希釈懸濁液あるいはHbV希釈懸濁液に切り換え、同じ灌流圧で10分間灌流した。その後直ちに虚血を惹起し、対照群と同様に虚血30分-再灌流30分の処置を行った。

5. 灌流実験終了後の心臓の処理：

上述のプロトコールに従って灌流実験を行った心臓は、再灌流終了後に、液体窒素で冷却したWollenberger clampを用いて圧迫凍結した。液体窒素中で、この凍結心臓組織から、付着した灌流液などを取り除いた後、金属製の乳鉢と乳棒を用いて細かく粉砕し、以下の実験を行うまで－80℃に保管した。

6. 心臓組織からのglucoseとglycogenの抽出と測定：

心臓組織からのglucoseとglycogenの抽出と測定は、Keppler and Deckerの方法(2)に従って以下のように行った。

粉砕した心臓組織約50 mgを秤量し、5倍量(v/w)の0.6 M perchloric acid solutionを加えて氷冷下にhomogenizeした。このhomogenateの一部を採ってglycogenの測定に用いた。残ったhomogenateは約

2000×g、4℃で15分間遠心分離し、その上清をとって$KHCO_3$溶液と混合して中和し、$KClO_4$が沈殿した後の上清をglucoseの測定に用いた。遠心分離した後の沈査に0.1 M NaOH溶液を加えて溶解させ、BCA法で蛋白測定を行った。Glucoseの測定は、ATP/NADPを基質として、glucose-6-phosphate-dehydrogenaseとhexokinaseを用いて蛍光法で行った。Glycogenはamyloglycosidaseでglycogenをglucoseに分解した後、glucose測定と同様に行った。Glucose量、glycogen量のどちらも抽出された単位蛋白量当たりの量(μg/mg-protein)として表した。

7. 心臓組織からのsubcellular fractionの作製：

心臓組織からのsubcellular fractionの作製は、Camps et al.の方法(3)に従って以下のように行った。粉砕した心臓組織約50 mgを秤量し、10倍量(v/w)の抽出buffer (25 mM Hepes, 250 mM sucrose, 4 mM EDTA, pH 7.4)を加えて氷冷下にhomogenizeした。Homogenateを600×g、4℃で10分間遠心分離した(上清1と沈査1)。上清1はさらに10,000×g、4℃で10分間遠心分離した(上清2と沈査のcrude mitochondria分画)。Crude mitochondria分画はmitochondria懸濁用buffer (210 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM Hepes, pH 7.5, 1% Triton X-100)で懸濁し、酵素活性を測定するまで−80℃に保管した。

上清2は、さらに150,000×g、4℃で2時間遠心分離し、上清はcytosol分画として、酵素活性を測定するまで−80℃に保管した。また、沈査は少量の抽出buffer に懸濁しmicrosome分画として酵素活性を測定するまで−80℃に保管した。

沈査1は少量の抽出bufferに懸濁させた後、核分画遠心用buffer (0.9 M sucrose, 50 mM Tris-HCl,1 mM EDTA, 25 mM KCl, pH 7.5)の上に静かに上置し、1,800×g、4℃で20分間遠心分離した。こうして沈殿したものは、核分画として40% glycerol, 50 mM Tris-HCl, 5 mM $MgCl_2$, 0.1 mM EDTA, pH 8.0のbufferに懸濁させ−80℃に保管した。各分画の蛋白濃度の測定は、BCA法で行った。

8. Subcellular fractionの解糖系酵素活性の測定：

Cytosol分画、(crude) mitochondrial分画、microsome分画のHexokinase活性はEasterby and Saleheen Qadriの方法(4)に従って測定した。Cytosol分画のPyruvate kinase、Lactic dehydrogenaseの活性は、それぞれGardenasの方法(5)、Lee et al.の方法(6)に従って測定した。Hexokinase活性は、単位時間、単位蛋白量あたりのNADPHの生成量(NADPH ng/min/μg-protein)として、Pyruvate kinase活性とLactate dehydrogenase活性は、単位時間、単位蛋白量あたりのNADHの消失量(μg/min/μg-protein)として表した。

9. データの計算と統計処理：

全ての測定項目について、各実験群で測定した平均値(mean)と標準偏差(SD)を計算した。統計処理は、各測定項目について、各実験群のすべてのデータを用いて、対照群(control)の平均値に対するその他

Table 1. Langendorff 灌流した心筋組織中の glucose と glycogen 含有量

測定項目と単位	実験群名と例数				
	control 群 n = 6	EV-a 群 n = 7	EV-b 群 n = 7	HbV-a 群 n = 7	HbV-b 群 n = 6
glucose (μg/mg-protein)	14.5 ± 2.3	17.1 ± 1.6	16.7 ± 2.4	18.9 ± 4.6 *	18.7 ± 1.1 *
glycogen (μg/mg-protein)	7.5 ± 4.6	8.0 ± 6.2	12.2 ± 8.2	12.0 ± 6.3	12.6 ± 10.6

* $p < 0.05$, vs control 群 by Dunnett multiple comparison test

Table 2. Langendorff灌流した心臓の Subcellular fraction 中の解糖系酵素活性

測定項目と単位	実験群名と例数				
	control 群 n = 6	EV-a 群 n = 7	EV-b 群 n = 7	HbV-a 群 n = 7	HbV-b 群 n = 6
Hexokinase (cytosol分画) (NADPH ng/min/μg-protein)	27.4 ± 4.9	22.1± 5.1	23.5 ± 8.0	19.5 ± 3.7	20.5 ± 8.6
Hexokinase (mitochondria分画) (NADPH ng/min/μg-protein)	174 ± 48	176 ± 51	161 ± 37	178 ± 26	173 ± 44
Hexokinase (microsome分画) (NADPH ng/min/μg-protein)	82.0 ± 16.6	113.7 ± 38.5	93.0 ± 22.7	110.8 ± 30.3	91.0 ± 14.2
Pyruvate kinase (cytosol分画) (NADH μg/min/μg-protein)	1.32 ± 0.33	1.03 ± 0.41	1.05 ± 0.40	0.93 ± 0.28	0.94 ± 0.23
Lactate Dehydrogenase (cytosol分画) (NADH μg/min/μg-protein)	3.64 ± 1.64	2.90 ± 1.00	2.60 ± 1.17	2.43 ± 0.91	2.73 ± 1.53

の実験群の平均値の有意差をDannett多重比較法で検定し、$p<0.05$を有意とした。

C．研究結果

1. 心筋組織中のglucoseとglycogen含有量

各実験群の心筋組織中のglucose量とglycogen量(μg/mg-protein)を**Table 1**に示した。HVa群(Hb濃度0.10 g/dL)およびHVb群(Hb濃度0.33 g/dL)では、それぞれの懸濁液を虚血直前に10分間灌流し、虚血30分-再灌流30分処置を施した後のglucose量は、わずかではあるが、control群の値と比較して有意の高値となった。glycogen量については、測定値がばらついたため有意差は見られなかったが、HbV-b群で高くなる傾向が見られた。

2. Subcellular fraction中の解糖系酵素活性

各実験群の心筋組織のsubcellular fractionにおける Hexokinase 、 Pyruvate kinase と Lactate dehydrogenase活性の測定結果を**Table 2**に示した。測定したどの酵素活性においても、HbVの効果は見られなかった。

D．考察

以上に示した実験で、HbV原液をKH-bufferで希釈した懸濁液を、虚血を開始する前に10分間灌流すると、再灌流後に、心筋組織中のglucose濃度がcontrol群と比較して高値になることが判明した。しかし、glycogen濃度と測定した解糖系酵素活性については、HbVの効果は観察されなかった。また、昨年度に報告(1)したように灌流液中の乳酸濃度は、HbV懸濁液を灌流し始めた最初の5分と、再灌流直後1分間で、Hbの濃度に依存した低下が観察された。比較対照として用いた空球小胞体(EV)懸濁液ではほとんどこれらの効果が見られなかったことから、これらの結果は、HbVによって運ばれる余分の酸素が、ミトコンドリアでの酸化を促進すると同時に解糖系の活性を抑制する可能性を示唆した。

E．結論

ラット摘出心臓のランゲンドルフ灌流で、HbVを虚血の直前に10分間灌流し、その後虚血(30分)-再灌流(30分)処置を施した心臓の、組織中glucose、

glycogen濃度と心筋細胞のsubcellular fractionの解糖系酵素活性を測定した。HbV原液(Hbとして10 g/dL相当)をKrebs-Henseleit bufferで希釈した懸濁液(Hbとして0.33 g/dL相当および0.10 g/dL相当)を灌流した実験群では、心筋組織中のglucose濃度がcontrol群に比較して有意の高値となった。その他の項目では、HbVの効果は見られなかった。これらの結果は、昨年報告した結果と合わせて、HbVが心筋細胞の解糖系に抑制的な作用を示す可能性があることを示唆した。

(参考文献)

1. 大鈴文孝、楠原正俊、柳田茂樹、山岸正、加藤隆一、別所基明、浜御幸. Hb小胞体の心筋虚血-再灌流障害に対する保護効果.「救急・災害医療に利用可能な人工赤血球の開発に関する研究」平成17年度 総括・分担研究報告書、pp.29-34 (2006)

2. Keppler D and Decker K, 1.2 Glycogen, In: *Methods of Enzymatic Analysis*, 3rd Ed., Vol. VI, metabolites 1 carbohydrate, 11-18, Bergmeyer HU ed., (1984).

3. Camps M et al., Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose- transporter expression in insulin sensitive tissues. *Biochem. J.* 282, 765-772 (1992).

4. Easterby JS and Saleheen Qadri S, Hexokinase type II from rat skeletal muscle. *Methods Enzymol.* 90, 11-12 (1982).

5. Gardenas JM, Pyruvate kinase from bovine muscle and liver. *Methods Enzymol.* 90, 140-141 (1982).

6. Lee et al., Lactate dehydrogenase isozymes from mouse. *Methods Enzymol.* 89, 351-358 (1982).

F. 健康危険情報

該当なし

G. 研究業績

1. 論文発表

1. T. Yamagishi, M. Bessho, M. Hama, R. Katoh, S. Yanagida, M. Kusuhara, F. Ohsuzu. Hemoglobin Vesicle Aids Recovery of Cardiac Function during Ischemia-Reperfusion in Langendorff Perfused Rat Hearts. *人工血液* 14, 92-97 (2006).

2. M. Ayaori, S. Sawada, A. Yonemura, N. Iwamoto, M. Ogura, N. Tanaka, K. Nakaya, M. Kusuhara, H. Nakamura, F. Ohsuzu. Glucocorticoid Receptor Regulates ATP- Binding Cassette Transporter-A1 Expression and Apolipoprotein-Mediated Cholesterol Efflux From Macrophages. *Arterioscler Thomb Vasc Biol.* 26, 163-168 (2006).

3. R. Kato, Y. Momiyama, R. Ohmori, N. Tanaka, H. Taniguchi, K. Arakawa, M. Kusuhara, H. Nakamura, F. Ohsuzu. High Plasma Levels of Osteopontin in Patients With Restenosis After Percutaneous coronary Intervention. *Arterioscler. Thomb. Vasc. Biol.* 26, 1-2 (2006).

4. K. Isoda, F. Ohsuzu. The Effect of Interleukin-1 Receptor Antagonist on Arteries and Cholesterol Cholesterol Metabolism. *J. Atheroscler. Thromb.* 13, 21-30 (2006).

5. M. Kusuhara, K. Isoda, F. Ohsuzu. Interleukin-1 and Occlusive Arterial Diseases. *Cardiovascular & Hematological Agents in Medicinal Chemistry* 4, 229-235 (2006).

2. 総説・著書

1. 荒川 宏、大鈴文孝. 循環器疾患最新の治療 2006-2007. 山口 徹、堀 正二 編集 「心膜炎」pp.183-187. 2006 南江堂

2. 田中伸享、楠原正俊　アンチエイジング医学－その理論と実践.　吉川敏一　編集「エイジングによる疾患　2　動脈硬化（血管疾患）」pp.72-79. 2006 診断と治療社

3. 小倉正恒、楠原正俊.　アンチエイジング医学－その理論と実践.　吉川敏一　編集「エイジングの予防と治療　2　動脈硬化（血管疾患）」pp.209-212. 2006 診断と治療社

4. 石神徳郎、楠原正俊. 血管年齢の評価方法. *Moderan Physician* 26, 513-514 (2006).

3. 学会発表

1. 山岸正、別所基明、浜御幸、加藤隆一、柳田茂樹、楠原正俊、大鈴文孝／ラット摘出心臓での虚血-再灌流障害に対するヘモグロビン小胞体の保護効果－第2報／第13回日本血液代替物学会年次大会／2006.8.24-25／慶大医

2. T. Yamagishi, M. Bessho, M. Hama, R. Katoh, S. Yanagida, M. Kusuhara, F. Ohsuzu / Hemoglobin vesicle aids recovery of cardiac function during ischemia-perfusion / The 23rd Annual Meeting of ISHR Japanese Section / 2006.12.1-2.

H. 知的財産権の出願・登録状況(予定を含む)

2005年4月1日に、2005年度の報告書の内容で米国に仮出願(US60/667872)し、2006年4月3日に、2006年度の報告書の内容でPCT出願(PCT/JP2006/307509)を完了した。

別添 5

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
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.
心臓手術の実際. *Clinical Engineering* 17, 1116-11213 (2006).	2006 年 10 月	秀潤社	饗庭　了, 森田雅教
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
Orthostatic decrease in cardiac chaos during the head-up tilt test in patients with vasovagal syncope. *Circ. J.* 2006. 70, 902-8 (2006).	2006 年 7 月	Japanese Circulation Society	Suzuki M, Hori S, Tomita Y, Aikawa N.
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.
周術期／救急領域の赤血球輸血と人工酸素運搬体の展望：Hemoglobin Vesicle の可能性. *人工血液* 14, 71-78 (2006).	2006 年 12 月	日本血液代替物学会	寺嶋克幸、杖下隆哉、坂本篤裕
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.
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	Elsevier	Y. Huang, T. Komatsu, H. Yamamoto, H. Horinouchi, 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
Poly(ethylene glycol)-conjuated 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. Satoh, H. Sakai, K. Sou, E. Tsuchida.
Hemoglobin vesicles reduce hypoxia-related inflammation in critically ischemic hamster flap tissue. *Crit Care Med.* 35, 899-905 (2007).	2007 年 3 月	Society of Critical Care Medicine (Lippincott Williams & Wilkins)	J. Plock, A. E. Tromp, C. Contaldo, T. Spanholtz, D. Sinovcic, H. Sakai, E. Tsuchida, M. Leunig, A. Banic, D. Erni
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.
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. A* 80A (2007), in press	2007 年 (印刷中)	Wiley	T. Komatsu, Y. Huang, S. Wakamoto, H. Abe, M. Fujihara, H. Azuma, H. Ikeda, H. Yamamoto, H. Horinouchi, K. Kobayashi, E. Tsuchida.
Effects of hemoglobin vesicles, a liposomal artificial oxygen carrier, on hematological responses, complement and anaphylactic reactions in rats. *Artif. Cells Blood Substitutes Biotechnol.* (2007) in press.	2007 年 (印刷中)	Informa Healthcare	H. Abe, H. Azuma, M. Yamaguchi, M. Fujihara, H. Sakai, S. Takeoka, E. Tsuchida, H. Ikeda.
"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.
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.
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.

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
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
Hemoglobin Vesicle Aids Recovery of Cardiac Function during Ischemia-Reperfusion in Langendorff Perfused Rat Hearts. *人工血液* 14, 92-97 (2006).	2006年12月	日本血液代替物学会	T. Yamagishi, M. Bessho, M. Hama, R. Katoh, S. Yanagida, M. Kusuhara, F. Ohsuzu.
Glucocorticoid Receptor Regulates ATP-Binding Cassette Transporter-A1 Expression and Apolipoprotein-Mediated Cholesterol Efflux From Macrophages. *Arterioscler Thomb Vasc Biol.* 26, 163-168 (2006).	2006年1月	American Heart Association	M. Ayaori, S. Sawada, A. Yonemura, N. Iwamoto, M. Ogura, N. Tanaka, K. Nakaya, M. Kusuhara, H. Nakamura, F. Ohsuzu.
High Plasma Levels of Osteopontin in Patients With Restenosis After Percutaneous coronary Intervention. *Arterioscler. Thomb. Vasc. Biol.* 26, 1-2 (2006).	2006年1月	American Heart Association	R. Kato, Y. Momiyama, R. Ohmori, N. Tanaka, H. Taniguchi, K. Arakawa, M. Kusuhara, H. Nakamura, F. Ohsuzu.
The Effect of Interleukin-1 Receptor Antagonist on Arteries and Cholesterol Cholesterol Metabolism. *J. Atheroscler. Thromb.* 13, 21-30 (2006).	2006年2月	日本動脈硬化学会	K. Isoda, F. Ohsuzu.
Interleukin-1 and Occlusive Arterial Diseases. *Cardiovasc. Hematol. Agents in Med. Chem.* 4, 229-235 (2006).	2006年7月	Bentham Science Publishers	M. Kusuhara, K. Isoda, F. Ohsuzu.
循環器疾患最新の治療 2006-2007. 山口徹、堀 正二 編集 「心膜炎」pp.183-187. 2006	2006年2月	南江堂	荒川 宏、大鈴文孝
アンチエイジング医学－その理論と実践. 吉川敏一 編集「エイジングによる疾患 2 動脈硬化（血管疾患）」pp.72-79. 2006	2006年6月	診断と治療社	田中伸享、楠原正俊

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血管年齢の評価方法. *Modern Physician* 26, 513-514 (2006).	2006 年 4 月	新興医学出版社	石神徳郎、楠原正俊

その他刊行物

1. 週刊ダイアモンド「5年以内に花咲く凄い技術　夢の製品 人工血液 異例の長期保存可能に」2006.4.3
2. 日経産業新聞「人工赤血球 京都に製造設備 オキシジェニクス」2006.4.17
3. 日本経済新聞「ニプロ、人工血液事業参入、バイオVBと業務提携」2006.5.22
4. 日経産業新聞「人工血液事業化　ニプロと提携　オキシジェニクス」2006.5.24
5. 日刊薬業「オキシジェニクス　酸素運搬体製造でニプロと提携」2006.5.24
6. ガスレビュー No.601「輸血不足を解決するか　人工酸素運搬体(人工赤血球)」pp. 21-22, 2006.5.25
7. スーパーテクノロジー・ビジュアル報告、明日を一新する「値千金」の技術32.「人工赤血球が体の全細胞の呼吸に必要な充分量の酸素を運搬する」p.23, 2006.5.　(株)ニュートンプレス
8. THE MEDEICAL & TEST JOURNAL「オキシジェニクスがニプロと人工酸素運搬体の製造で契約」2006.6.11
9. 日本経済新聞「人工血液 研究盛んに　企業・大学、赤血球や血小板開発」2006.07.07
10. リポソーム封入人工赤血球OXY-0301, *New Current* 17, 6-7, 2006 (2006.9.1)
11. アルブミンを利用した完全合成赤血球 アルブミンヘム, *New Current* 17, 8-10, 2006 (2006.9.1)

研究成果の刊行物・別冊

(2006. 4. ～ 2007. 3.)

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.

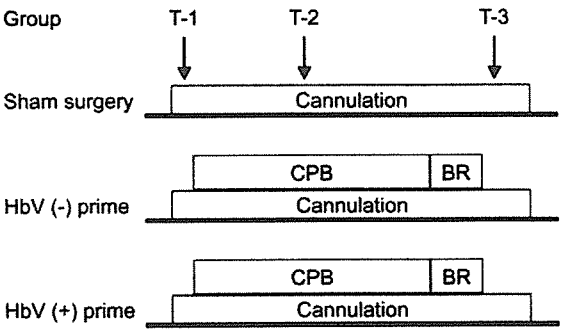

Figure 1. Experimental protocol. CPB indicates cardiopulmonary bypass; BR, blood return; T-1, T2, and T3, time for arterial blood sampling.

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.

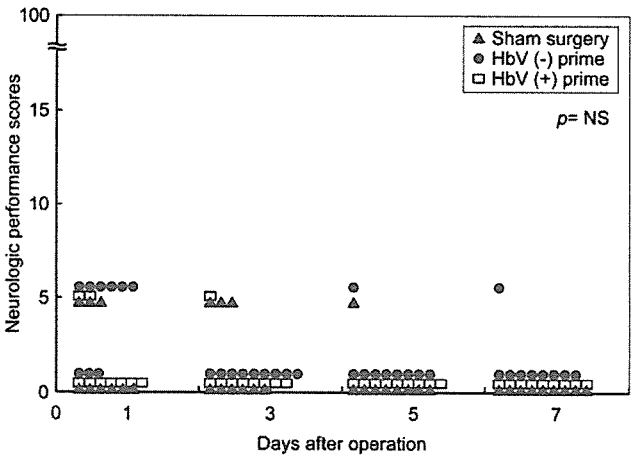

Figure 2. Neurologic performance scores after CPB on days 1, 3, 5, and 7 after the operation. Score 0 represents no neurologic deficits and 95 represents brain death. n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Neurologic performance scores were not different among the 3 groups. NS indicate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

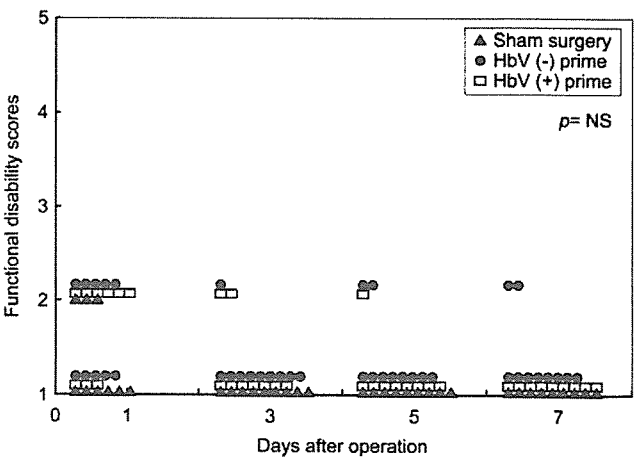

Figure 3. Functional disability scores after CPB on days 1, 3, 5, and 7 after the operation. *Score 1* indicates no disability; *score 2,* mild disability; *score 3,* moderate disability; *score 4,* severe disability; *score 5,* death. n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Functional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

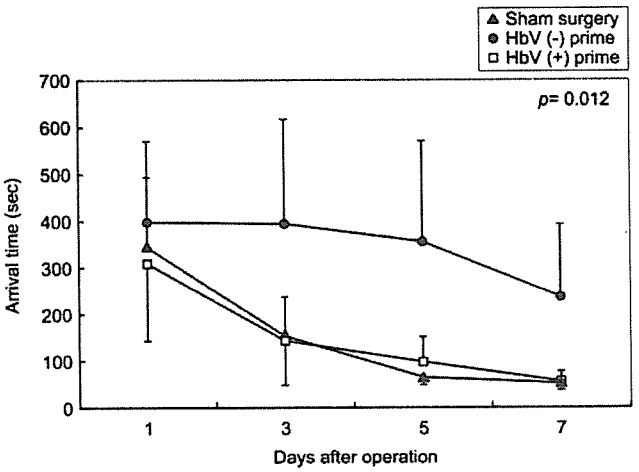

Figure 4. Neurocognitive outcome was assessed on days 1, 3, 5, and 7 after CPB by visual–spatial learning with the maze test (Maze-1). n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime. Analyzing the group mean latency in repeated measures analysis of variance, the HbV (−) prime group had longer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

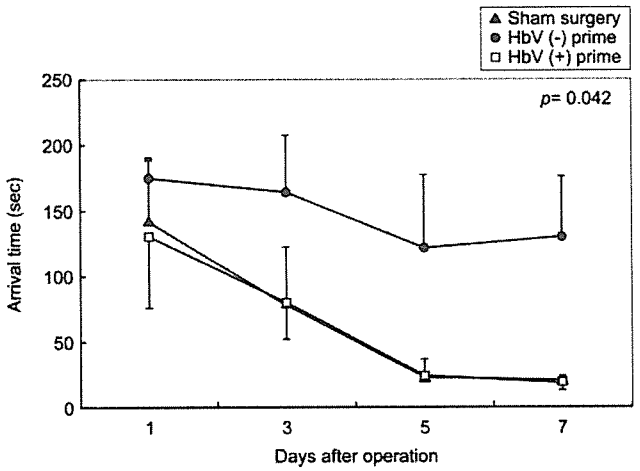

Figure 5. Neurocognitive outcome was assessed on days 1, 3, 5, and 7 after CPB by testing visual–spatial learning with the maze tests (Maze-2). n=8, sham surgery; n=7, HbV (−) prime; n=7, HbV (+) prime. Analyzing the group mean latency in repeated measures analysis of variance, the HbV (−) prime group 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).

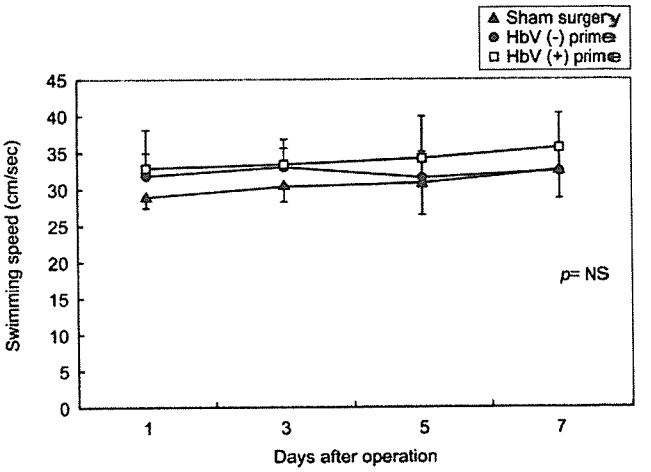

Figure 6. Water maze swimming speed was assessed in the rats. (n=9, sham surgery; n=10, HbV (−) prime; n=9, HbV (+) prime.) The swimming speed was similar among the 3 groups, and did not show any chronological change from days 1 through 7 after the operation.

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.[3] 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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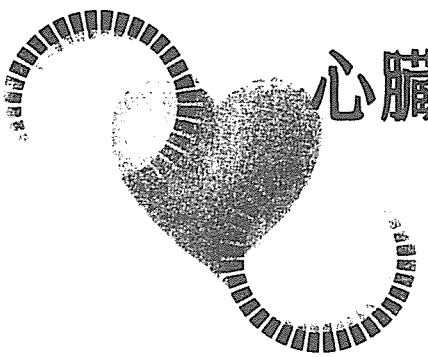
Disclosures

None.

References

1. Shapira OM, Aldea GS, Treanor PR, Chartrand RM, DeAndrade KM, Lazar HL, Shemin RJ. Reduction of allogeneic blood transfusions after open heart operations by lowering cardiopulmonary bypass prime volume. *Ann Thorac Surg.* 1998;65:724–730.
2. Merkle F, Boettcher W, Schulz F, Koster A, Huebler M, Hetzer R. Perfusion technique for nonhaemic cardiopulmonary bypass prime in neonates and infants under 6 kg body weight. *Perfusion.* 2004;19:229–237.
3. Karamlou T, Hickey E, Silliman CC, Shen I, Ungerleider RM. Reducing risk in infant cardiopulmonary bypass: the use of a miniaturized circuit and a crystalloid prime improves cardiopulmonary function and increases cerebral blood flow. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Ann.* 2005;8:3–11.
4. Izumi Y, Sakai H, Hamada K, Takeoka S, Yamahata T, Kato R, Nishide H, Tsuchida E, Kobayashi K. 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.
5. Sakai H, Tomiyama KI, Sou K, Takeoka S, Tsuchida E. Polyethyleneglycol-conjugation and deoxygenation enable long-term preservation of hemoglobin-vesicles as oxygen carriers in a liquid state. *Bioconjug Chem.* 2000;11:425–432.
6. Sakai H, Horinouchi H, Tomiyama K, Ikeda E, Takeoka S, Kobayashi K, Tsuchida E. Hemoglobin-vesicles as oxygen carriers: Influence on phagocytic activity and histopathological changes in reticuloendothelial systems. *Am J Pathol.* 2001;159:1079–1088.
7. Sakai H, Takeoka S, Park SI, Kose T, Nishide H, Izumi Y, Yoshizu A, Kobayashi K, Tsuchida E. 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.
8. 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.
9. Grocott HP, Mackensen GB, Newman MF, Warner DS. Neurological injury during cardiopulmonary bypass in the rat. *Perfusion.* 2001;16:75–81.
10. Priestley MA, Golden JA, O'Hara IB, McCann J, Kurth CD. Comparison of neurologic outcome after deep hypothermic circulatory arrest with alpha-stat and pH-stat cardiopulmonary bypass in newborn pigs. *J Thorac Cardiovasc Surg.* 2001;121:336–343.
11. Baker AJ, Zornow MH, Grafe MR, Scheller MS, Skilling SR, Smullin DH, Larson AA. Hypothermia prevents ischemia-induced increases in hippocampal glycine concentrations in rabbits. *Stroke.* 1991;22:666–673.
12. Mackensen GB, Sato Y, Nellgard B, Pineda J, Newman MF, Warner DS, Grocott HP. Cardiopulmonary bypass induces neurologic and neurocognitive dysfunction in the rat. *Anesthesiology.* 2001;95:1485–1491.
13. Stump DA. Embolic factors associated with cardiac surgery. *Semin Cardiothorac Vasc Anesth.* 2005;9:151–152.

14. Wagerle LC, Russo P, Dahdah NS, Kapadia N, Davis DA. Endothelial dysfunction in cerebral microcirculation during hypothermic cardiopulmonary bypass in newborn lambs. *J Thorac Cardiovasc Surg.* 1998;115: 1047–1054.
15. Langley SM, Chai PJ, Jaggers JJ, Ungerleider RM. Preoperative high dose methylprednisolone attenuates the cerebral response to deep hypothermic circulatory arrest. *Eur J Cardiothorac Surg.* 2000;17: 279–286.
16. Mezrow CK, Sadeghi AM, Gandsas A, Dapunt OE, Shiang HH, Zappulla RA, Griepp RB. Cerebral effects of low-flow cardiopulmonary bypass and hypothermic circulatory arrest. *Ann Thorac Surg.* 1994;57:532–539.
17. Darbonne WC, Rice GC, Mohler MA, Apple T, Hebert CA, Valente AJ, Baker JB. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J Clin Invest.* 1991;88:1362–1369.
18. Chai PJ, Williamson JA, Lodge AJ, Daggett CW, Scarborough JE, Meliones JN, Cheifetz IM, Jaggers JJ, Ungerleider RM. Effects of ischemia on pulmonary dysfunction after cardiopulmonary bypass. *Ann Thorac Surg.* 1999;67:731–735.
19. Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation.* 2003;10:319–327.
20. Engelman RM, Rousou JH, Dobbs WA. Fluosol-DA: an artificial blood for total cardiopulmonary bypass. *Ann Thorac Surg.* 1981;32:528–535.
21. Rousou JA, Engelman RM, Anisimowicz L, Dobbs WA. A comparison of blood and Fluosol-DA for cardiopulmonary bypass. *J Cardiovasc Surg.* 1985;26:447–453.
22. Izumi Y, Yamahata T, Yozu R, Kobayashi K, Mukai M. The oxygen transporting capability of neo red cells (NRC) evaluated under total cardiopulmonary bypass. *Jpn J Thorac Cardiovasc Surg.* 1998;46: 30–37.
23. Sakai H, Horinouchi H, Masada Y, Takeoka S, Ikeda E, Takaori M, Kobayashi K, Tsuchida E. Metabolism of hemoglobin-vesicles (artificial oxygen carriers) and their influence on organ functions in a rat model. *Biomaterials.* 2004;25:4317–4325.
24. Naik SK, Knight A, Elliot MJ. A successful modification of ultrafiltration for cardiopulmonary bypass in children. *Perfusion.* 1991;6:41–50.



心臓手術の実際
—外科医が語る術式，臨床工学技士が語る体外循環法—

監修—許 俊鋭 埼玉医科大学心臓血管外科

第12回

心内膜床欠損症に対する手術と体外循環法
—慶應義塾大学病院—

心内膜床欠損症(ECD)は，近年ではatrioventricular canal defectまたはatrioventricular septal defect（AVSD，房室中隔欠損症）と表記されることが多い．本稿では，ファロー四徴症(TOF)などの合併心疾患がなく，かつ二心室修復が可能なものを対象とする．また本疾患の半数以上はダウン症候群を合併している．今回，慶應義塾大学病院における心内膜床欠損症手術の術式について医師が，その術式に対応した体外循環法について臨床工学技士が解説する．

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心内膜床欠損の解剖学，病態生理

心内膜床欠損症(ECD)は，心室間交通量の度合により部分型，移行型，完全型に分類され，完全型はさらに共通前尖の形態によりRastelli A，B，C型に分類される．C型は共通前尖が分葉せずに心室中隔と腱索の連続がないものをいう．A型は共通前尖が分葉している部分において多数の細かな腱索によって支持されている．B型はまれである．しかし，心内膜床は発生学上，心房中隔と心室中隔とともに，僧帽弁や三尖弁に相当する左右房室弁を形成する基になる(図1)ので，ECDは形態上も病態生理上も症例ごとで異なり，分類することよりも1つのスペクトラムとしてとらえるほうが理解しやすい．

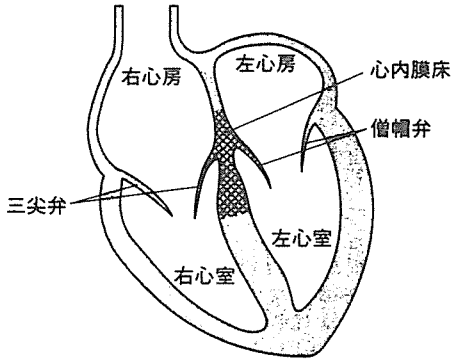

図1 心内膜床の位置

部分型は，心房位での左右短絡のみで心室間交通のないものをいい，一次孔欠損(ostium primum)と表記されることもある．左側房室弁(僧帽弁に相当)の前尖にはcleftといわれる裂け目があり，これは腱索付着による左室自由壁からの支持がないという点で，正常僧帽弁交連

の弁接合と大きく異なっている．左右房室弁は心室中隔の尾根と接合することにより連続しているが，それらの弁口は互いに独立している．修復術の適応が心房間交通のみである場合は，二次孔型心房中隔欠損と同様，3歳以上が手術時期となる．しかし，左側房室弁閉鎖不全による左心不全を呈する場合には早期に手術する．

移行型は，心室間交通が少量のみ存在するもので，一般的に部分型よりも心房位での左右短絡・僧帽弁閉鎖不全ともに高度で，しばしば，より早期に修復術が必要になる．また部分型と異なり共通前尖と共通後尖は連続していないが，ごく短い腱索により心室中隔の尾根に固定されている．

完全型は，心房位での左右短絡に加えて心室間交通が大きく，unrestrictive（血行力学上無制限な交通）になっているものを指す．乳児期に多量の心室間左右短絡による左心不全症状，または肺血管閉塞性病変の進行をきたすことが多いため，乳児期早期が修復術の適切な時期である．特にダウン症候群の場合には肺血管閉塞性病変の進行が速いので，特に修復術時期を遅らせないことが重要である．左右房室弁は共通となっていて，その形態も症例ごとで異なる．

外科的に最も重要な解剖学的ポイントは左側房室弁の形態であり，一般に左側弁尖の形成が良好なほど，左側方尖が付着している弁輪周囲に占める角度が大きいほど，また左側共通前後弁尖を支持する腱索が豊富なほど，修復が容易である．

心内膜床欠損症に対する手術

修復術の至適時期については前述の通りである．なお，肺動脈banding（絞扼）術の適応は，現在では人工心肺の絶対的禁忌（脳出血やRSウイルス感染など）に限定されている．手術術式は完全型を中心に記述する．

まず，胸骨正中切開し，胸腺部分切除の後，心房中隔パッチ用に心膜を採取し，動脈管は体外循環前に閉鎖しておく．人工心肺のカニュレーションは，上行大動脈に送血用を，上下大静脈に脱血用を挿入し，中等度低体温高流量体外循環とする．左室ベントを右上肺静脈より挿入する．上行大動脈を遮断し，順行性心筋保護液注入により心停止とする．房室間溝に平行に大きく右房切開をする．房室弁の形態を歪ませないように配慮して吊り糸をかけて視野を展開する．この手術では，十分な時間をかけて心内解剖を評価し，修復の全体的計画を決定してから修復の針糸をかけ始めることが特に肝要である．

左室内を晶液で充満させて共通房室弁を閉鎖位とし，心室中隔尾根上で共通前後弁尖のkissing point（接合点）を見付けて，これを中心として分割線を決める．分割線の約80%の長さを長径とした半弧形Gore-Tex®パッチを作成して心室中隔欠損閉鎖用とする．まずこのパッチの弧状下縁を心室中隔右側面に連続縫合で縫着する．腱索付着を可及的に温存する．次に，Gore-Tex®パッチの上縁，共通前後弁尖の分割線，心房中隔パッチ下縁の順でU字縫合をかけ固定する（図2）．心房中隔パッチの後側縫合線をとるときの房室結節の避け方は右房側と左房側の2法があり，我々は後者を採用している（図3）．なお，房室結節の位置の指標はあくまでも心室中隔と後側弁輪の接点であり，しばしば左房側変位がみられる冠静脈洞開口部ではない．

次に，左側房室弁形成のために再び左室を充満させる．弁形成の内容はcleft閉鎖と必要に応じてKay-Reed弁輪縫縮である．原則としてcleftは全長にわたり閉鎖する．理由は，この部分の残存/再発閉鎖不全が再手術の原因のほとんどであるからである．最後に，心房中隔パッチを連続縫合で縫着して心内操作を終了する．心拍動再開を待って右房切開を閉鎖し，体外循環から離脱する．心房と心室にペーシングリード，肺動脈カテーテルを挿入して閉胸する．

移行型，部分型においては，基本的に心室中隔欠損を除いた後半部分と同等と考えてよい．

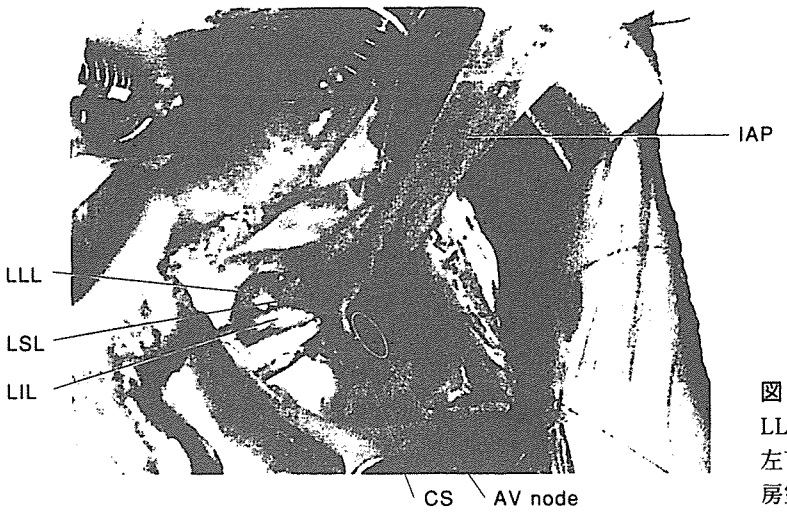

図2　術中写真
LLL：左側方尖，LSL：左上方尖，LIL：左下方尖，CS：冠静脈洞，AV node：房室結節，IAP：心房中隔パッチ．

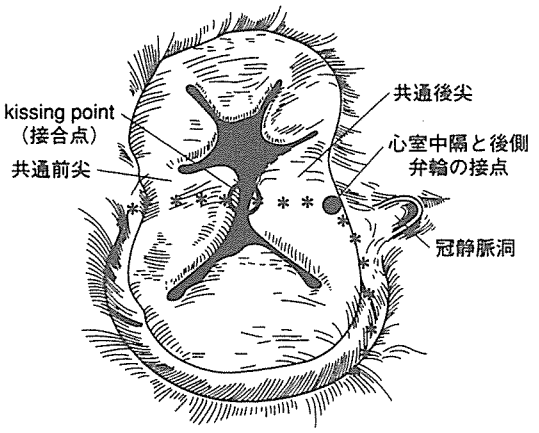

図3　共通房室弁と慶應義塾大学病院における心房中隔パッチ縫着線(＊)

術式に関連した周辺知識

体外循環離脱後，血行動態が不安定なときには，エコー検査で特に左側房室弁機能を評価することが肝要である．ECDは術後数日までの間に肺高血圧crisis（急激な発症）を引き起こすリスクが高いので，肺動脈圧を連続的に監視しながら，高濃度酸素，高換気量，筋弛緩による呼吸管理を必要な時間行うことにより，そのリスクを最小限とする．もし肺高血圧crisisを引き起こした場合には，一酸化窒素ガス吸入療法を行う．

慶應義塾大学病院における標準的小児体外循環システムと心内膜床欠損症手術に対する体外循環

標準的小児体外循環システム

1-1　人工心肺システム構成

STÖCKERT社製人工心肺装置「SIIIインファントモデル」（ポンプコンソール3基ベース）にて小児から成人の体外循環まで対応可能なシステムを構成している．ローラポンプはすべてダブルヘッドポンプ（直径85 mm）を使用する．送血ポンプは症例によりポンプヘッド部のチューブ径1/4インチ（流量≦1.5 *l*/min）と5/16インチ（流量≦2.0 *l*/min）を使用し，流量＞2.0 *l*/minの症例より遠心ポンプを使用する．脱血方法は全症例，陰圧吸引補助脱血とし，充填量の削減，操作性・視認性の向上を図っている．表1に体外循環の回路サイズと充填量を示す．また，このほか

表1　慶應義塾大学病院における体外循環の回路サイズと充填量

患者区分(体重)	≦10 kg	≦15 kg	≦20 kg	≦30 kg	≦40 kg	＞40 kg
回路区分	SSS	SS		S	M	L
ポンプ種類	ローラポンプ			遠心ポンプ		
ポンプチューブ［インチ］	1/4	5/16				
送血回路［mm］	4.5	6	6	6	8	10
脱血回路［mm］	6	6	6	8	10	10
充填量［m*l*］	230	265	375	450	650	850
充填薬剤組成	血液充填(体重＜7 kg) * 白血球除去洗浄赤血球 130 m*l* 新鮮凍結血漿(FFP) 80 m*l* 20%アルブミン溶液 25 m*l* 20%D-マンニトール溶液 4 m*l*/kg ヘパリン 2 m*l*			無輸血・無血液製剤充填(10 kg＜体重) 重炭酸リンゲル 適量 ヘスパンダー 5～10 m*l*/kg (最大 500 m*l*) 20%D-マンニトール溶液 4 m*l*/kg (最大300 m*l*)		
	アルブミン充填(7 kg＜体重＜10 kg) 5%アルブミン溶液 150 m*l* 重炭酸リンゲル 50 m*l* 20%D-マンニトール溶液 4 m*l*/kg ヘパリン 2 m*l*					

＊　重炭酸リンゲル液500 m*l*で洗浄限外濾過処理を行う.

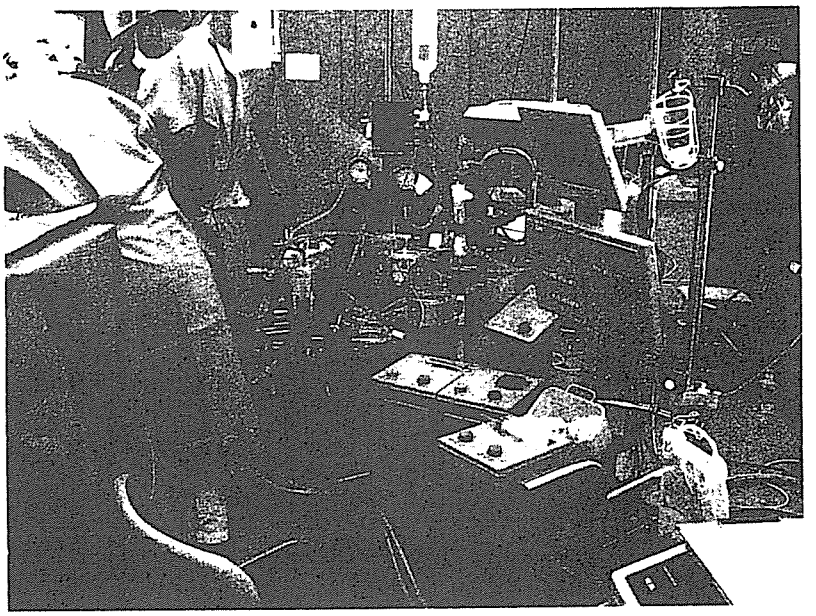

図4　慶應義塾大学病院における体外循環システム

にV-V MUF用にJMS社製血液ポンプ「MF-01」を2基搭載する(図4).

1-2　特徴

新生児・乳幼児は，成人に比べて毛細血管の透過性が高く，体外循環において水分・血漿成分が間質へ漏出しやすい状況にある．よって，体外循環中あるいは体外循環後の浮腫を軽減するためには，①希釈率を低くすること，②異物接触面積を少なくし，凝固線溶系，補体系，キニン・カリクレイン系，血小板，白血球，サイ