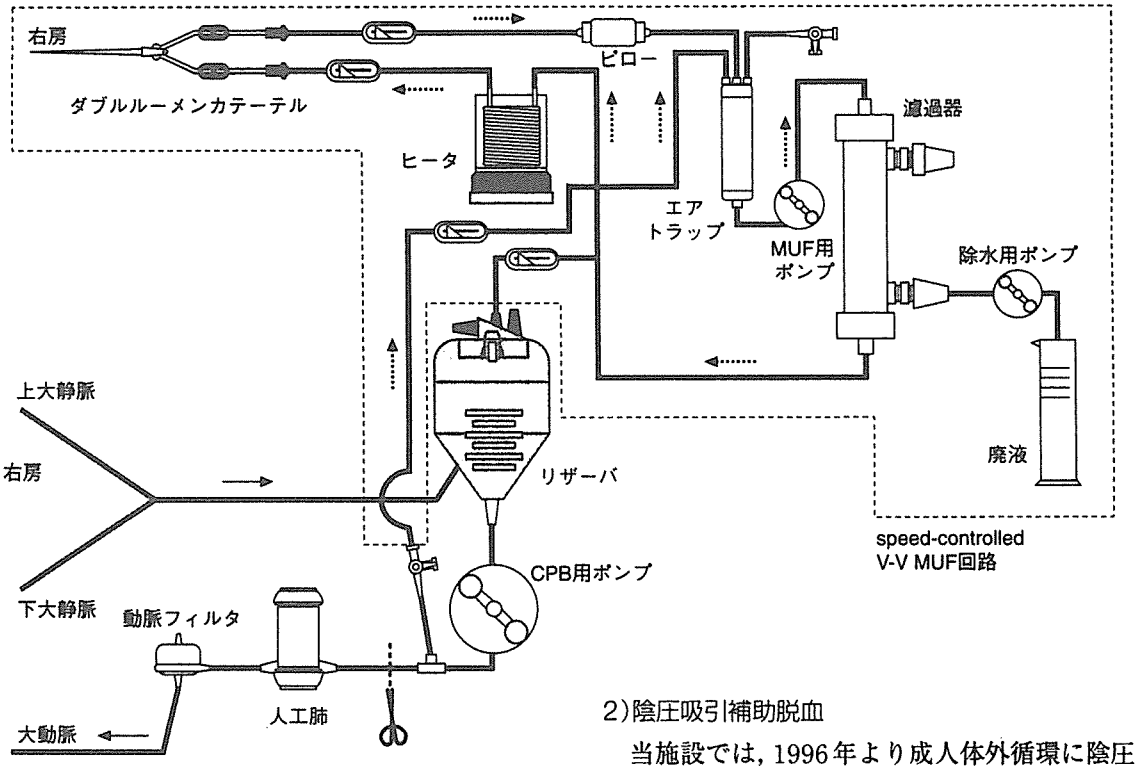

図5　MUF回路図

トカインなどの活性化あるいは亢進を抑えるために体外循環回路を小型化すること，③充填液中あるいは血中の有害物質を除去することが必要である[1]．当施設では，分離型ローラポンプの使用や陰圧吸引補助脱血を用いて他の脱血方法に比べ回路を短縮することで体外循環回路を小型化し，充填液の洗浄限外濾過処理，DUF，MUFを行うことで充填液中や血中の有害物質の除去を行っている．

1)充填血洗浄・血液充填組成

白血球除去洗浄赤血球(緊急手術の場合は赤血球MAP血)1単位(130 m*l*)，FFP 1単位(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*，重炭酸リンゲル液500 m*l*で体外循環回路の充填を行う．充填液が十分に撹拌されたことを確認した後，ヘマトクリット(Ht) 25～30%になるように500 m*l* + αの限外濾過を行い，カリウムをはじめとする電解質の補正および有害物質の除去を行う[2]．

2)陰圧吸引補助脱血

当施設では，1996年より成人体外循環に陰圧吸引補助脱血を導入し[3]，1999年以降，小児を含むすべての体外循環で陰圧吸引補助脱血を行っている．小児体外循環においては，落差脱血に比べ1～2サイズ細身のカニューレを使用し，体外循環開始時の陰圧を－30 mmHgとし，脱血量に合わせて適宜増減させる．最大陰圧を－80 mmHgとしているが，ほとんどの症例では－40～－50 mmHgの陰圧で必要脱血量を得ることができる．このとき，脱血回路への気泡の混入とコラップス*1に留意する．また，血液に掛かる陰圧を正確に測定するために，脱血回路において陰圧の測定を行っている．

3)speed-controlled V-V MUF

当施設では，心房中隔欠損症(ASD)を除く20 kg未満の体外循環症例にV-V MUFを施行している．MUFの血液ポンプのほか，濾過にもローラポンプを使用しているため，濾液のspeed-controlが容易[4]である(図5)．V-V MUF用のカ

＊1　コラップス
脱血カニューレが，過陰圧などにより血管壁に吸い付いてしまう現象．

テーテルは透析用ダブルルーメンカテーテル(12 Fr.)を右房より挿入し，血流量(Q_B)，濾過流量(Q_F)は体重に関係なく，Q_B 120 ml/min，Q_F 40 ml/min とし15分間行う．人工心肺(CPB) 離脱と同時にMUFを開始し，置換液は人工心肺回路内残血を使用，必要に応じて濾過型人工腎臓用補液，アルブミン製剤，重炭酸リンゲル液などを追加する．

2 心内膜床欠損症根治手術に対する体外循環

心内膜床欠損症(ECD)手術時の体外循環は次のように行う．

2-1 体外循環開始前

①充填血液の洗浄限外濾過処理の確認(Ht，カリウム濃度，pHなど)．

②中心静脈圧(CVP) のラインよりヘパリンを3 ml/kg 投与する．2分後，活性化凝固時間(ACT)の測定を行う．

③ACTが200秒を超えたら，サクションポンプを回し始める．送血のカニュレーションを開始する．

④送血回路接続後，拍動チェック，送りテストを行う．以降，血圧に留意しながら，脱血カニュレーション中の出血時など，必要に応じ適宜送血を行う．

2-2 体外循環開始時

①急激かつ異常な送血圧の上昇に注意しながら体外循環を開始する．

②陰圧を－30 mmHg から適宜増加させながら，予定灌流量まで血流量を上げていく(表2)．予定流量が得られなければ術者に報告し，脱血カニューレの位置を調整する．

③呼吸(換気)の停止．

表2 体外循環血流量

患者体重 [kg]	血流量 [ml/kg/min]
＜5	200 以上
5～8	180
8～10	160
10～12	150
12～15	130
15～20	120
20～30	100
30～40	80～100
40～50	70～90
50＜	60～80

2-3 完全体外循環(図6)

①上大静脈をスネアし，脱血量に変化がないか確認する．脱血量が減少するようであれば，上大静脈のカニューレの位置を調整する．

②下大静脈でも同様のテストを行う．上下大静脈とも問題なければ完全体外循環に移行する．

2-4 大動脈遮断

①大動脈遮断時の送血圧の上昇に注意する．

②心筋保護液を注入(10 ml/kg) し，心停止を得る．20 kg 未満の症例では，術野にてシリンジで注入する．

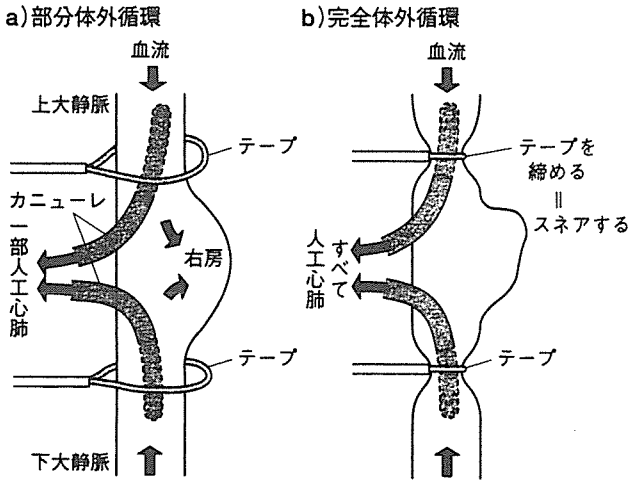

図6 部分体外循環と完全体外循環
人工心肺の開始時および離脱時には，生体での循環と人工心肺による循環の両方が行われる．このように，循環の一部分を人工心肺で担っている状態を部分体外循環(a) という．完全体外循環(b) は，開心操作を行うために上大静脈・下大静脈にそれぞれ通したテープで上下大静脈を締め(スネアするという)，上大静脈・下大静脈の血流を人工心肺に導き，体循環を人工心肺で維持する状態．

③局所心冷却法を施行する．以降10分ごとに局所心冷却を行う．
④必要な薬液の投与，輸液，輸血などを行う．
⑤大動脈遮断解除直前に，心筋浮腫軽減の目的で20%D-マンニトール2 ml/kgを投与する．
⑥体外循環離脱時，直腸温で35.5℃以上となるように，ゆっくりと復温・保温を開始する．

2-5　大動脈遮断解除～部分体外循環

①キシロカイン(4 mg/kg)を投与する．
②拍動の再開を確認・観察し，必要であれば除細動を行う．
③徐脈であればペーシング，あるいはイソプレナリン0.01 mgを投与し，心拍数を観察する．
④経食道エコーガイド下でルートおよびベントよりエア抜きを行う．
⑤必要であればカテコラミン・血管拡張剤などの投与を開始する．

2-6　体外循環離脱

①呼吸(換気)再開．
②塩化カルシウムを投与し，体血管側に容量負荷を行う．
③経食道エコーによる壁運動，房室弁の評価を行う．
④血圧，CVPを観察しながら徐々に血流量を下げる．血流量が半分になったら下大静脈のカニューレを抜去する．
⑤MUFを準備する．

2-7　体外循環離脱後

①上大静脈のカニューレを抜去し，そこからMUF用のダブルルーメンカテーテルを挿入し，速やかにMUFを開始する．
②MUFの効果を観察しながら，徐々にCVPを下げる．MUF施行中は体温の低下に注意する．
③MUF終了後，投与ヘパリンと等量の硫酸プロタミンを投与する．

心内膜床欠損症手術の体外循環のポイント

ECDにおける体外循環のポイントは次の通りである．

①ECDは完全型，不完全型に大別されるが，その中間型や移行型も存在し，ECDのタイプ・症状により，手術時期・患者体重に幅がある．
②当施設においてはASD，心室中隔欠損症(VSD)，ECD，ファロー四徴症(TOF)などの軽症例では，体外循環中の最低Ht値を20%以上としているため，7 kgを無輸血充填の限界としている．
③ECDの手術では，完全型，不完全型にかかわらず，僧帽弁の逆流テストを行うことが多い[5]．逆流テストの水分により体外循環中の血液の希釈が進むため，患者の体重によっては積極的に除水を行う必要がある．
④体外循環中の灌流圧は30～40 mmHgとし，クロルプロマジンを1～6 mg/kg分割投与するが，30 mmHgを下回る場合は血流量を上げて対処する．昇圧剤は使用しない．このことは尿量の確保においても重要である．
⑤小児開心術においては，腎臓の未熟性ゆえに術後腎不全の危険性が高い．術後腎不全の予防・術後の浮腫の予防のため尿量の確保は重要である．体外循環開始時にフロセミド5～10 mgを投与し，10～15分後，尿量が10 ml/kg/hrを下回るようであれば追加投与を行う．
⑥ECDの体外循環は，軽度低体温～常温で行う．

略語一覧

ACT:	activated coagulation time	**CS**:	coronary sinus
ASD:	atrial septal defect	**CVP**:	central venous pressure
AV:	atrio-ventricular	**DUF**:	dilution ultrafiltration
AVSD:	atrioventricular septal defect	**ECD**:	endocardial cushion defect
CPB:	cardiopulmonary bypass	**FFP**:	fresh frozen plasma

Ht:	hematocrit	**MAP:**	mannitol-adenine-phosphate
IAP:	inter-atrial patch	**RS:**	respiratory syncytial
LIL:	left inferior leaflet	**TOF:**	tetralogy of Fallot
LLL:	left lateral leaflet	**VSD:**	ventricular septal defect
LSL:	left superior leaflet	**V-V MUF:**	venovenous modified ultrafiltration

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トピックス

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－

Use of Hemoglobin Vesicles during Cardiopulmonary Bypass Priming Prevents Neurocognitive Decline in Rats

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研究要旨

新生児や乳児の開心術の成績は近年飛躍的に向上しているが，一般的には人工心肺回路の充填液として輸血が必須である．これは低体重の患者において無輸血充填を行った場合，高度の血液希釈が生じ，特に酸素需要の大きな脳の不可逆的障害を来たす可能性が高いためである．一方で，輸血には感染症，移植片対宿主反応，免疫抑制，炎症性生体物質活性化による臓器障害といった合併症の危険を伴う．こうした臨床上のジレンマの一解決手段として，我々は早稲田大学理工学総合研究センター及び慶應義塾大学呼吸器外科にて共同研究が進められているヘモグロビン小胞体（Hemoglobin vesicle, HbV）に着目した．本研究は，ラット人工心肺モデルを確立した後，人工心肺回路をHbVで充填した群において高次脳機能が維持されることを証明した．これはHbVの充填により末梢組織への酸素運搬が保持されたためと考えられ，HbVの有用性が明らかとなった．

A　緒言

先天性心疾患を伴う体重10kg以下の乳児の開心術においては，同種血輸血による人工心肺回路充填が一般に行われている．その理由は，人工心肺回路の充填液は300mlから400ml必要であり，これを晶質液で満たした場合，体重10kg以下の患者（循環血液量は約800ml）では，高度な血液希釈が生じ，酸素運搬を担う赤血球の相対的な減少により組織障害，特に酸素需要の大きな脳の障害を来たすためである．

このために，現在体重10kg以下の患者に体外循環を行う場合，赤血球輸血は避けられない状況にある．しかし一方で，輸血による感染症，移植片対宿主反応，免疫抑制といった合併症のリスクを伴い，社会的にも大きな問題となっている．また，炎症性生体物質の遊離を促進することによる脳障害の発生も指摘されており，できるだけ輸血を避けるように努めるべきである．

この臨床上のジレンマの解決手段として，我々は，人工酸素運搬体であるHbVに着目した[1-3]．現時点のHbVは半減期が約20から30時間と短い．しかしながら人工心肺運転中に生じる血液希釈状態という特殊な環境はおよそ数時間であり，その数時間だけ血液の役割を果たし，その後速やかに代謝される現在のHbVは小児心臓外科の立場から考えると臨床上のジレンマを解決に導く大きな利点となる．すなわち，HbVの短い半減期を活用するという逆転的発想により本研究は成り立っている．

本研究は，ラット人工心肺モデルにおいてHbVを用いた人工心肺充填液による高次脳機能保護効果の検討を目的に行われた．

B．方法

人工酸素運搬体は早稲田大学理工学総合研究センター及び慶應義塾大学呼吸器外科にて共同研究が進められているHbVを使用した[4]．ローラーポンプと特製膜型肺を用いて人工心肺回路を作成し（Fig. 1.）[5]，回路内に5%アルブミンを充填した群（HbV (-) prime群　n=7），HbVを充填した群（HbV (+) prime群 n=7），偽手術群（sham surgery群 n=7）の3群に分けて実験を行った．体重450g前後のSDラットをセボフルレンにて全身麻酔し，14G静脈留置針にて気管内挿管した．挿管後人工呼吸器管理とした．人工心肺の確立に際して，脱血管は右内頚静脈を介して右房へ，送血管は尾動脈に挿入した．胸骨正中切開は行わなかった．右内頚静脈にアプローチする際，ラットの右頚部に切開を加えるが，皮切線はわずかで動物への負担を最小にするように配慮した．人工心肺の運転は常温下，無拍動送血法で200ml/kg/minの流量で90分間行った．回路内充填量は60mlとした．HbV充填液のヘモグロビン濃度は8.6g/mLとした（Fig. 2.）．

人工心肺運転終了後は人工心肺回路内の残存血液を遠心分離し，沈殿した自己血を20分間かけて血管内に戻した．その後，

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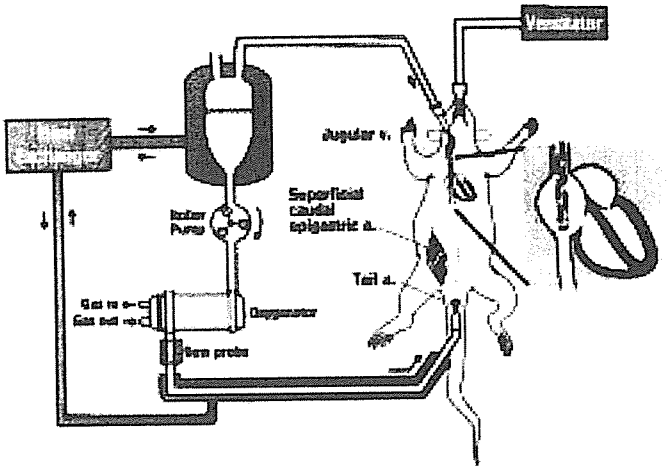

Fig. 1. Schema of cardiopulmonary bypass in a rat.

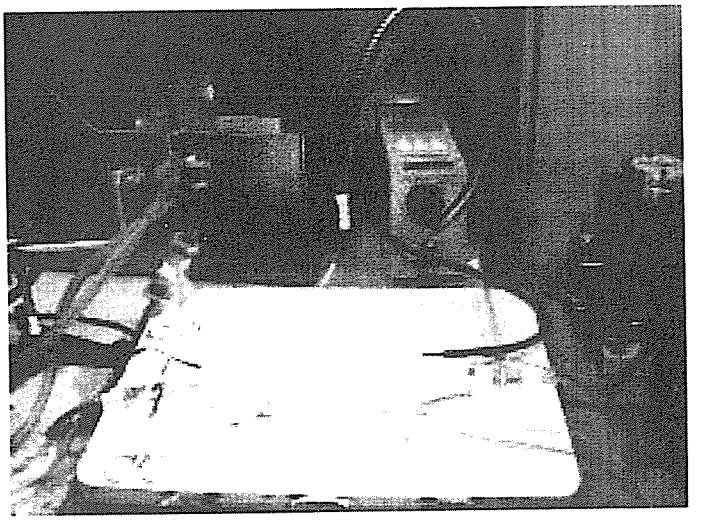

Fig. 2. Establishment of cardiopulmonary bypass in a rat.

脱血管及び送血管を抜去し，麻酔から覚醒させた後，ゲージにて飼育した．術後１，３，５，７日目に標準化神経学的機能試験及び２種の迷路試験（Morris water maze test）を行った[6,7]．標準化神経学的機能試験はneurologic performance scale, Functional disability scoresを用いて数値化した（Neurologic performance scale : 0-95, 0 = no deficit and 95 = brain death, Functional disability scores ：score 1 = no disability; score 2 = mild disability; score 3 = moderate disability; score 4 = severe disability; and score 5 = death.）[8]．

以上の試験はビデオ録画して，後に盲検となっている神経学者が一括して評価した．術後７日目に動物は犠牲死させ，脳の組織を採取し，海馬部の病理学検査を行った．

C. 結果

１．人工心肺運転中の検査所見

HbV (-) prime, HbV (+) prime, sham surgeryの３群における人工心肺運転中の採血データを検討した結果，Arterial pHに関してはHbV (-) prime群でアシドーシスの傾向があった．これは血液希釈によるものと考えられた．またHbVの充填に関わらず，人工心肺を運転した群に関しては人工心肺運転後においてArterial PCO_2の上昇を認めた．ただし，それ以外の大きな所見は得られなかった．３群ともいずれの症例も生存した．

２．標準化神経学的機能試験および迷路試験

術後１，３，５，７日目に標準化神経学的機能試験及び２種の迷路試験（Morris water maze test）を行った．各群のswimming speedには有意差がなかった．標準化神経学的機能試験はNeurologic performance scaleおよび，Functional disability scoreを用いておこなったが，３群ともに有意差を認めなかった．

迷路試験は認知記憶の評価で行われるもので，高次脳機能に関わる海馬の評価に用いられる．water maze test-1はFig. 3.に示すとおり，円形のプールに浮島を設けたもので一般的にMorris water maze testと呼ばれるものである．この結果からHbV (-) prime群は，HbV (+) prime群及びsham surgery群と比較して有意に到着時間が延長したことが分かった (p=0.005)．またHbV (+) prime群とsham surgery群には有意差がなかった．water maze test-2はFig. 4.に示すとおり，実際に迷路を正方形の枠の中に作成したものである．11箇所のjunction pointを設けた．この結果からもHbV (-) prime群は，HbV (+) prime群及びsham surgery群と比較して有意に到着時間が延長したことが分かった (p=0.05)．またHbV (+) prime群とsham surgery群には有意差がなかった．

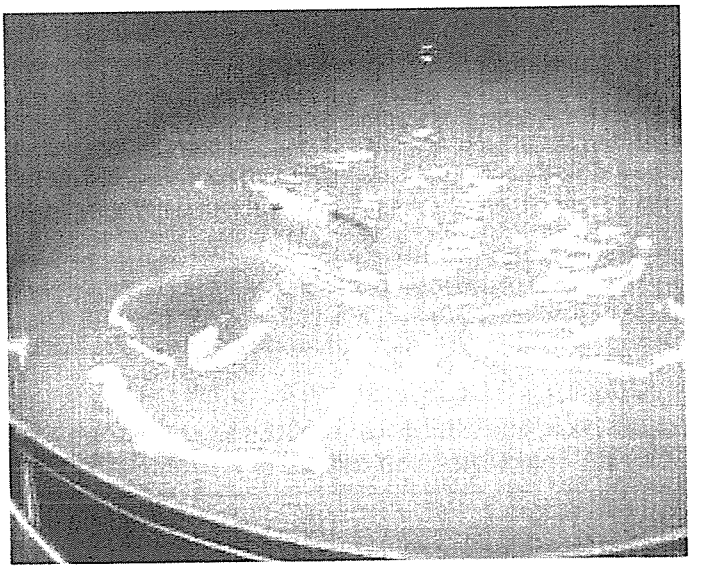

Fig. 3. Water maze test - 1
Neurocognitive outcome was assessed on the 1st, 3rd, 5th, and 7th days after CPB by visual-spatial learning with the maze test (Water maze test - 1).

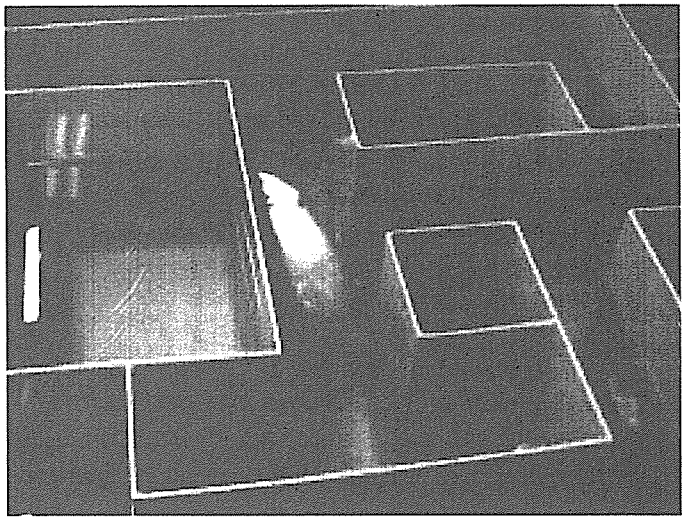

Fig. 4. Water maze test - 2
Neurocognitive outcome was assessed on the 1st, 3rd, 5th, and 7th day after CPB by testing visual-spatial learning with the maze tests (Water maze test - 2).

3．病理学的評価（海馬）

各群の海馬部においてヘマトキシリン・エオジン染色による病理学的評価を行ったが、異常所見を認めず、神経細胞数の比較においても有意差を認めなかった。

D. 考察

新生児及び乳児の先天性心奇形に対しての人工心肺技術と治療戦略は，この10年の間に急速に進化し外科的な治療成績を改善させてきた．しかしながら，全身的な炎症反応を起こすことなく人工心肺を用いることは未だ不可能であり，多くの終末臓器で多かれ少なかれ機能障害を引き起こす可能性がある．脳において，人工心肺の使用は脳血管内皮細胞機能と血流量を減少させる[9,10]．これはno-reflow現象としてよく知られており，術後の脳神経障害との関連も報告されている[11,12]．

血液希釈を可能な限り少なくするために，人工心肺充填量は回路の小型化によってかなり減少した[13-15]．しかしながら，施設間の差はあるにせよ現状ではかなり大きな乳児に対して無輸血充填が可能になっているにすぎず，それよりも小さい乳児及び新生児は種々の合併症のリスクがあるにもかかわらず同種血輸血の使用を余儀なくされている．人工心肺と同様に，同種血輸血も単独で全身の炎症性サイトカインを刺激し[16,17]，脳血流とその機能に対してリスクになりうるという報告も近年増加している[18]．

人工酸素運搬体は，このジレンマを解決するひとつの手段である．かつてFluosolが心筋の局所灌流を増やす能力を調査する為に，ブタの人工心肺モデルが用いられた[19,20]．しかしながら重篤な副作用によりFluosolの臨床使用は中止された．一方でIzumiは，イヌの急性期人工心肺モデルを用いて，同様の人工酸素運搬体が赤血球と同等の酸素運搬能を有していることを証明した[21]．HbVは他の多くのヘモグロビンに基づく酸素運搬体にみられるような微小血管の血管収縮の副作用もない．

HbVを新生児及び乳児の人工心肺の充填液に用いるためには，使用後数日の間に生じる細かな副作用を如何に少なくできるかが鍵となる．HbVをラットに使用した今までの研究で，大量のHbVを短時間で注入することが主要器官の機能を軽度ではあるが一過性に変動させることがわかっている[3,22]．したがって人工心肺運転後にmodified ultrafiltrationに準じた方法で[23]，充填液から大部分のHbVを除去することができれば，これらの潜在的な副作用は極めて少なくできると考えられた．

我々のラット人工心肺モデルにおいて，運転中のヘマトクリットはおよそ10%で，一般臨床上は輸血によって対処されるレベルのものであった．いずれの群のラットも７日間全て生存した．標準化神経学的機能試験においても有意差は認めなかった．手術７日後に犠牲死させた後に行った病理学的評価でも，３群間の所見は全て類似していた．HbV (-) prime群は，極度の血液希釈が生じており酸素供給に関して非常に不利ではあるが，標準化神経学的機能試験においては所見がなく，結果としてこの血液希釈は無症状だった．この結果は，我々のモデルが脳機能の微妙な損傷を見つける際に，非常に感度が高いことを示唆している．

学習と記憶は高次脳機能のうちの１つであり，これらを２つの迷路試験によって評価した．これらの結果，血液希釈を伴う体外循環が記憶学習機能に障害をもたらすと考えられた．人工心肺運転中における酸素供給のわずかな破綻が，他の標準化神経学的所見なしに，高次の認知機能障害のみによって発見されたことは，決して驚くべきことではない．

本研究の結果は，血液希釈を予防するためにHbVが同種血輸血の代わりに用いることができることを明らかに示している．認知機能評価の結果をふまえるとHbVの人工心肺使用は，潜在的に同種血輸血を充填液に使用するより優れていさえする可能性があった．

これらのデータの正当性を，今後より大きい動物の人工心肺モデルにて証明すると共に，脳酸素代謝と炎症反応に関してHbVの効果を評価していく必要がある．

E. 結論

ラット人工心肺モデルでのHbV使用は技術上問題なかった．このモデルにおいて，短時間の人工心肺運転であっても血液希釈を伴う体外循環は記憶学習機能に障害をもたらすと考えられた．一方HbVによる人工心肺回路充填で得られる酸素運搬量の増大が，高次脳機能の障害を軽減させる可能性が示唆された．

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Orthostatic Decrease in Cardiac Chaos During the Head-up Tilt Test in Patients With Vasovagal Syncope

Masaru Suzuki, MD; Shingo Hori, MD; Yutaka Tomita, MD*; Naoki Aikawa, MD

Background Autonomic dysfunction contributes to orthostatic intolerance in vasovagal syncope (VVS), but as it has not been identified by spectral analysis of heart rate variability (HRV) in previous studies, the present hypothesis was that nonlinear analysis of HRV would identify the orthostatic intolerance in VVS.
Methods and Results Twenty-six patients with VVS and 14 matched controls were subjected to 80-degree head-up tilt test (positive: 13 patients; negative: 13 patients and 14 controls). There were no differences in the orthostatic changes in the indices of spectral analyses of HRV among the 3 groups. The Lyapunov exponent (LE) was calculated from 200 consecutive RR-intervals to investigate chaotic behavior, and cardiac chaos was defined as the incidence of the presence of a positive finite LE. Orthostatic decreases in cardiac chaos were observed in the VVS patients (both the positive and negative groups), although there was no orthostatic decrease in the control group (ANOVA: $p=0.008$). The receiver-operator characteristic curve indicated that cardiac chaos during the tilt identified VVS regardless of the results of the tilt ($p<0.001$, sensitivity: 85.7%, specificity: 96.2%).
Conclusions The decrease in cardiac chaos during the tilt test was specific to patients with VVS, even if their response to the test was negative. (*Circ J* 2006; **70:** 902–908)

Key Words: Autonomic nervous system; Heart rate variability; Syncope

Vasovagal syncope (VVS) is a common manifestation of orthostatic intolerance in humans[1–7] and its diagnosis is made on the basis of the history, absence of any other proven etiology of the syncope, and a positive result for the head-up tilt test (HUT)[1,4,5,8] Autonomic behavior during the HUT has been considered indispensable to understanding the elusive pathophysiology of VVS[2,5,9] Previous studies have applied and assessed spectral analyses of heart rate variability (HRV) during the HUT in VVS patients[10–15] and most have shown a correlation between the spectral analyses of HRV and the results of the HUT. However, the analyses failed to identify VVS patients when the results of the HUT were negative[15]

Traditional analyses of HRV have used noninvasive methods for assessing changes in autonomic activity, but they are incapable of analyzing the nonstationary fluctuations[2,16] Techniques derived from nonlinear dynamics (eg, deterministic chaos) have complementary value in identifying patterns and mechanisms that cannot be detected by traditional statistic methods based on linear models[16–18] Because recent studies have indicated that some pathological conditions are accompanied by loss of chaos in the RR intervals (RRI)[17–21] application of chaos theory to analysis of RRIs during the HUT may provide a new indicator of orthostatic intolerance. We hypothesized that the chaotic behavior of RRIs would identify the orthostatic intolerance in VVS.

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Methods

Subjects

Twenty-six patients who presented to the emergency department with syncope and a history compatible with VVS participated in the present study. The diagnosis of VVS was confirmed by the history and/or tilt table testing according to the published guidelines on management of syncope[4] Of the 26 patients, 17 had a positive result for the HUT without any pharmacologic provocation, 4 had a positive result with pharmacologic provocation of isoproterenol infusion, and the remaining 5 each had a history compatible with VVS. None of the 26 patients had carotid sinus hypersensitivity or a history indicating situational syncope. They did not have hypertension, diabetes, or heart disease. The control group consisted of 14 healthy age- and gender-matched volunteers with no history of syncope (Table 1). All patients and volunteers gave their consent to participate before enrolment in the study. The study confirmed with the principles outlined in the World Medical Association's Declaration of Helsinki. The protocol and ethics were approved by the research committee of the Department of Emergency Medicine of Keio University Hospital.

HUT

The HUT was performed between 09.00–11.00 h in a quiet room at a controlled temperature (23–25°C). Patients with VVS underwent the HUT 0–47 days (median: 3 days) after spontaneous syncopal episodes. The tilting table was manually driven and equipped with a footplate support. Following a control period of 20 min in the supine position, each subject was tilted to 80° for a maximum of 30 min without the use of any provocative agents[8] If symptoms of impending syncope were elicited during the tilt, the patient was immediately returned to the supine position, and the test was stopped. A positive test was defined as the occur-

Table 1 Characteristics of the Study Group

	VVS patients		Controls (n=14)	p value
	HUT positive group (n=13)	HUT negative group† (n=13)		
Males, number (%)	7 (53.8)	5 (38.5)	8 (57.1)	0.59
Age (years), mean (SD)	23.5 (3.7)	25.5 (4.1)	23.4 (3.1)	0.28
Syncopal episodes*, median (range)	1 (1–3)	1 (1–3)	None	–

VVS, vasovagal syncope; HUT, head-up tilt test.
**Presyncopal episodes are not included; †13 patients had a negative result for HUT. In the HUT used to make a diagnosis of VVS, 4 patients had a positive result without provocation, 4 patients had a positive result using isoproterenol infusion; 5 patients had histories compatible with VVS.*

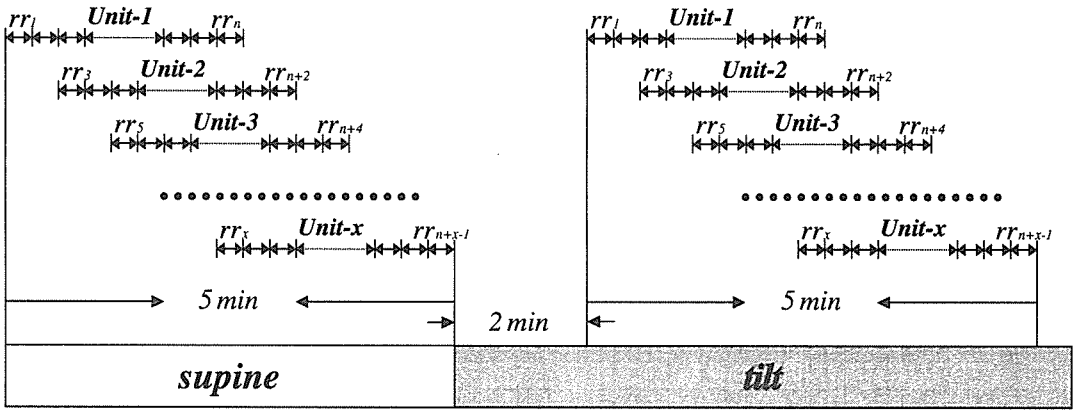

Fig 1. Extraction of the RR intervals (RRI) for calculating the Lyapunov exponent, which was calculated for units of consecutive RRI to investigate chaotic behavior. Consecutive units were extracted, shifting every 2 RRI, from 5-min RRI tracings recorded while supine and during tilting.

Table 2 AUC of the ROC for Diagnosing Vasovagal Syncope (Preliminary Study)

	Data points RR intervals	AUC (SE)	p value
HUT	25	0.50 (0.10)	1.00
	50	0.87 (0.08)	<0.001
	100	0.93 (0.05)	<0.001
	150	0.95 (0.04)	<0.001
	200	0.97 (0.02)	<0.001
	250	0.94 (0.04)	<0.001
	300	0.94 (0.03)	<0.001

AUC, area under the curve; ROC, receiver-operating characteristic; SE, standard error; HUT, head-up tilt test.

Table 3 Orthostatic Changes in Arterial Pressure and RR Intervals

	Supine Mean±SD	HUT Mean±SD	Repeated ANOVA
SAP (mmHg)			
VVS patients			
HUT positive group	116±19	107±34	p=0.27
HUT negative group	116±14	117±13	
Normal control	120±20	120±14	
DAP (mmHg)			
VVS patients			
HUT positive group	67±11	71±11	p=0.26
HUT negative group	66±12	73±13	
Normal control	68±11	73±10	
RRI (ms)			
VVS patients			
HUT positive group	1,048±169	807±104	p=0.39
HUT negative group	928±111	669±198	
Normal control	1,000±162	813±98	

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; RRI, RR-interval. Other abbreviations see in Table 1.

rence of syncope or presyncope associated with an increase in the RRI of more than 3 s and/or a decrease in systolic arterial pressure of more than 30 mmHg[2].

HRV

During the HUT, the ECG was monitored with 2-lead chest electrodes (Lifescope 8™, Nihon Kohden, Tokyo, Japan), and finger arterial pressure was monitored with a plethysmographic device (Finapres™, Ohmeda, Englewood, CO, USA). Data were stored on a DAT tape (DAT recorder, RD-130TE™, TEAC, Tokyo, Japan), and the RRIs were obtained with Fluclet™ software (Dai-nippon Pharmaceutical, Osaka, Japan) running on a personal computer. This software computes the spectral analyses of HRV based on wavelet transform and provides a description of the spectral parameters every second. The high-frequency component (HF) of the RRIs, defined as 0.15–2.00 Hz (ms^2/Hz), and the low-frequency component (LF), defined as 0.04–0.15 Hz (ms^2/Hz), were computed and measured[2]. The standard deviation of RRIs (SDNN) was also calculated.

Cardiac Chaos

Whether the behavior of RRIs is chaotic can be determined by calculating the Lyapunov exponent (LE). The presence of a positive LE is strong evidence that the system is chaotic and not just quasi-periodic or periodic[22–24] In the present study, LE was calculated thus:

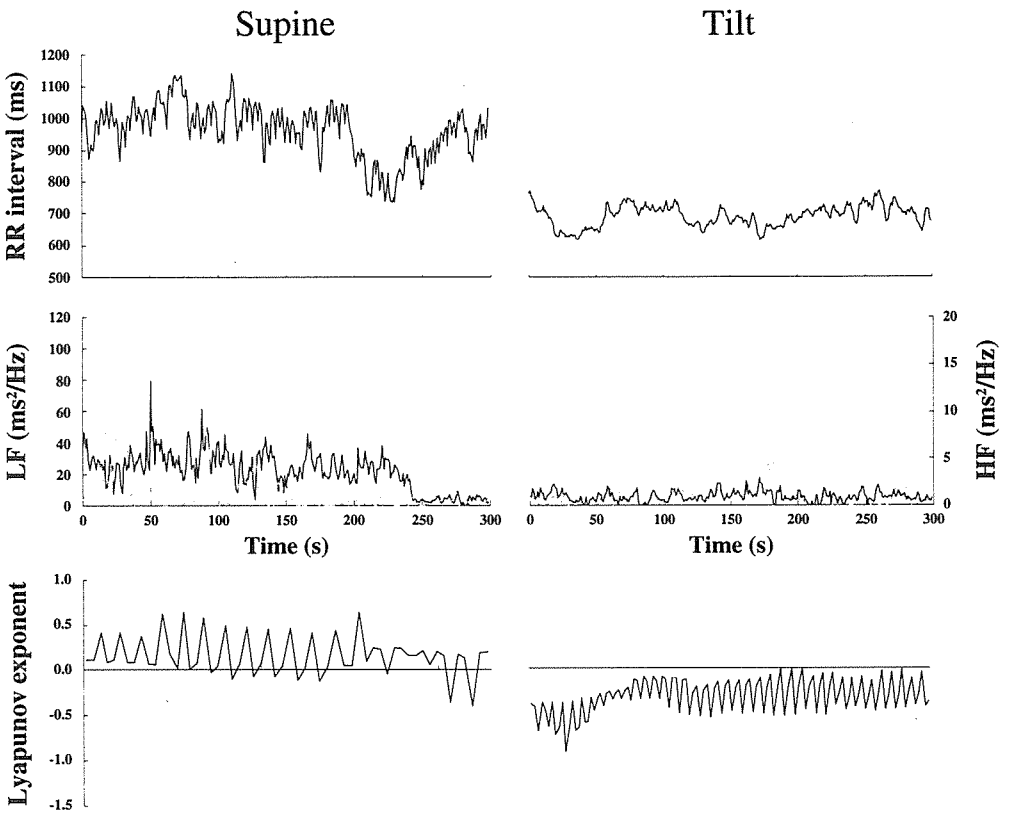

Fig 2. Time courses of the RR intervals (RRI) and heart rate variability (low-frequency component (LF), high-frequency component (HF), and Lyapunov exponent) in a vasovagal syncope patient with tilt-induced syncope. RRI, LF (gray lines), HF (black lines), and Lyapunov exponent are shown while supine (Left) and in the tilted position (Right). Positive Lyapunov exponents, which are strong evidence of chaos, were not observed in the tilted position.

$$LE=\sum_{i=2}^{n-1}\log\frac{|rr_i-rr_{i+1}|}{|rr_{i-1}-rr_{i+1}|}$$

The LE was calculated for units of consecutive RRIs (Fig 1). To quantify the chaotic behavior of the RRIs, the number of positive finite LEs during a 5-min tracing while supine and during the HUT was counted, and cardiac chaos was defined as the presence of a positive finite LE.

To choose the most suitable number of RRIs for identifying cardiac chaos, 25–300 RRIs were used to calculate LE in our preliminary study. Because the area under the receiver-operator characteristic (ROC) curve of cardiac chaos based on 200 RRIs had the largest area (Table 2), 200 RRIs were used to calculate LE in this study.

Data Analyses

To study the orthostatic changes in hemodynamics and HRV parameters, including LF, HF, LF/HF, SDNN and cardiac chaos, 5-min RRI tracings while supine and during the HUT were recorded: (1) with the subject supine before the start of HUT, and (2) 2 min after the start of HUT. The duration of the RRI recording was set at 5 min in this study, because 5-min RRI recordings have been preferred in previous investigations of short-term HRV[16]

Statistical Analysis

The means of paired samples were analyzed by repeated-measures ANOVA, and the means of unpaired samples were analyzed by one-way ANOVA. The chi-square test was used for comparisons of categorical data. The assessment of the diagnostic accuracy of the HRV parameters and cardiac chaos was expressed by the ROC curve, being the area under the curve equal to the probability to discriminate from having or not having VVS. The α level in the present study was 0.05. All statistical analyses were performed using SPSS™ 12.0J software (SPSS Inc, Tokyo, Japan).

Results

Results of the HUT

The arterial pressure and RRI responses to the HUT are summarized in Table 3. Of the 26 VVS patients, 13 experienced syncope and/or presyncope during the HUT (positive). There were no differences in the baseline characteristics of the patients with positive HUT results and those with negative results (Table 1). The positive responses were obtained 19.4±6.7 min (range: 8–29 min) after the start of the HUT.

Orthostatic Changes in HRV Parameters and LE

Representative changes in the RRI, HF, LF, and LE are shown in Figs 2–4; the LE decreased during the HUT in the VVS patients, both those who had positive and negative HUT results (Figs 2, 3), but did not decrease in the healthy volunteers (Fig 4).

There were no differences among the 3 groups in cardiac chaos while supine (Table 4). A significant orthostatic decrease in cardiac chaos was observed in the VVS patients ($p=0.008$), but no orthostatic decrease was observed in the controls. There were no differences in the other parameters

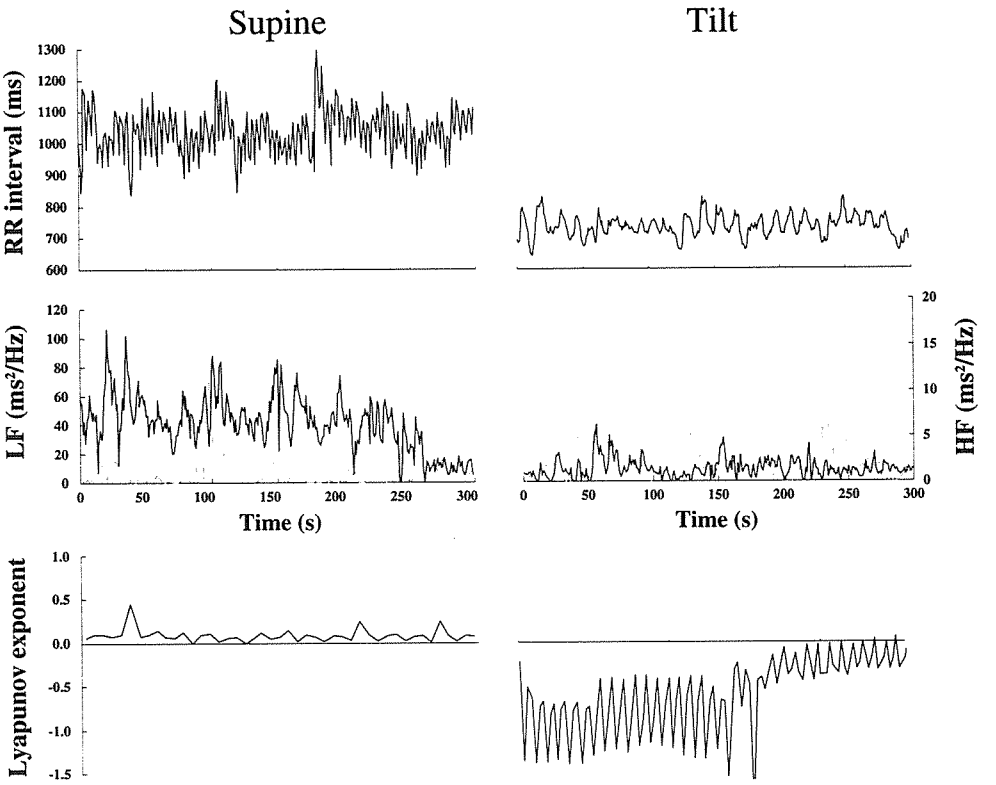

Fig 3. Time courses of the RR intervals (RRI) and heart rate variability (low-frequency component (LF), high-frequency component (HF), and Lyapunov exponent) in a vasovagal syncope patient without tilt-induced syncope. RRI, LF (gray lines), HF (black lines), and Lyapunov exponent are shown while supine (Left) and in the tilted position (Right). Positive Lyapunov exponents, which are strong evidence of chaos, were observed in the tilted position.

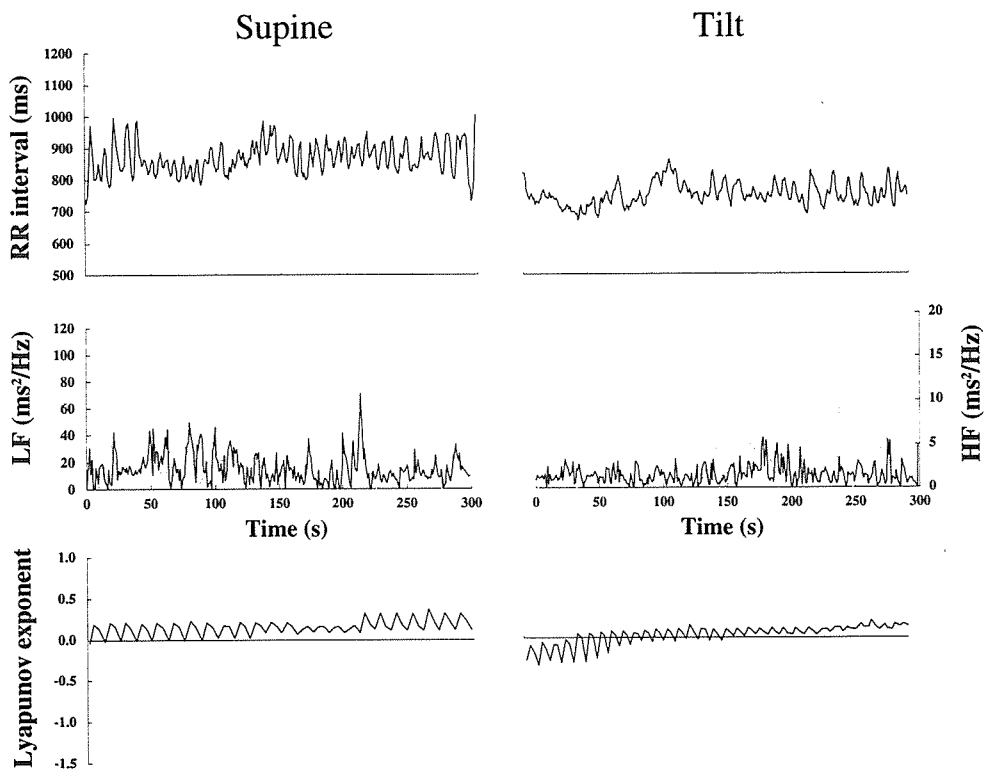

Fig 4. Time courses of RR intervals (RRI) and heart rate variability (low-frequency component (LF), high-frequency component (HF), and Lyapunov exponent) in a healthy control subject. RRI, LF (gray lines), HF (black lines), and Lyapunov exponent are shown while supine (Left) and in the tilted position (Right). Positive Lyapunov exponents, which are strong evidence of chaos, were observed in both positions.

Table 4 Orthostatic Changes in Heart Rate Variability

	Supine Mean±SD	HUT Mean±SD	Repeated ANOVA
Cardiac chaos (chaos/unit)			
VVS patients			
HUT positive group	*0.54±0.33*	*0.15*±0.17*	*0.008*
HUT negative group	*0.53±0.35*	*0.13†±0.10*	
Normal control	*0.64±0.24*	*0.57±0.25*	
LF (ms^2/Hz)			
VVS patients			
HUT positive group	*10.84±4.96*	*7.52±3.03*	*0.17*
HUT negative group	*9.31±3.95*	*8.96±4.30*	
Normal control	*11.95±7.12*	*14.05±10.02*	
HF (ms^2/Hz)			
VVS patients			
HUT positive group	*7.06±5.18*	*2.57±1.65*	*0.42*
HUT negative group	*5.56±2.75*	*2.31±1.72*	
Normal control	*9.43±7.14*	*4.12±2.72*	
LF/HF			
VVS patients			
HUT positive group	*7.15±9.98*	*19.67±20.67*	*0.32*
HUT negative group	*4.62±4.39*	*33.74±30.62*	
Normal control	*9.24±22.40*	*46.11±62.77*	
SDNN			
VVS patients			
HUT positive group	*77.51±26.69*	*63.92±22.61*	*0.66*
HUT negative group	*55.13±12.11*	*47.63±19.19*	
Normal control	*74.75±34.83*	*57.58±17.54*	

LF, low-frequency component; HF, high-frequency component; SDNN, standard deviation of RR-intervals. Other abbreviations see in Table 1.
**p=0.008 vs normal control. †p=0.004 vs normal control.*

Table 5 AUC of the ROC for Diagnosing Vasovagal Syncope

Position and HRV	AUC (SE)	p value
Supine		
Cardiac chaos	*0.59 (0.09)*	*0.34*
LF	*0.56 (0.11)*	*0.57*
HF	*0.60 (0.10)*	*0.29*
LF/HF	*0.44 (0.10)*	*0.55*
SDNN	*0.55 (0.10)*	*0.61*
HUT		
Cardiac chaos	*0.93 (0.05)*	*<0.001*
LF	*0.72 (0.09)*	*0.02*
HF	*0.71 (0.09)*	*0.03*
LF/HF	*0.51 (0.11)*	*0.91*
SDNN	*0.55 (0.09)*	*0.64*

Abbreviations see in Tables 1,2,4.

of HRV (Table 4).

Diagnosing VVS

Area under the ROC curves were drawn to elucidate the diagnostic accuracy of the parameters, and they showed that cardiac chaos, LF, and HF during the HUT had significantly larger areas (p<0.001, p=0.02, p=0.03, respectively). The parameters while supine were not predictive of VVS (Table 5).

Cardiac chaos during HUT had the largest area under the ROC curve (Fig 5). The optimal cutoff point for balancing sensitivity and specificity in cardiac chaos during the HUT was 0.36 (Fig 6), and at that cutoff value sensitivity was 92.3% and specificity was 96.3% (Table 6).

Discussion

In this study a decrease in cardiac chaos during the HUT

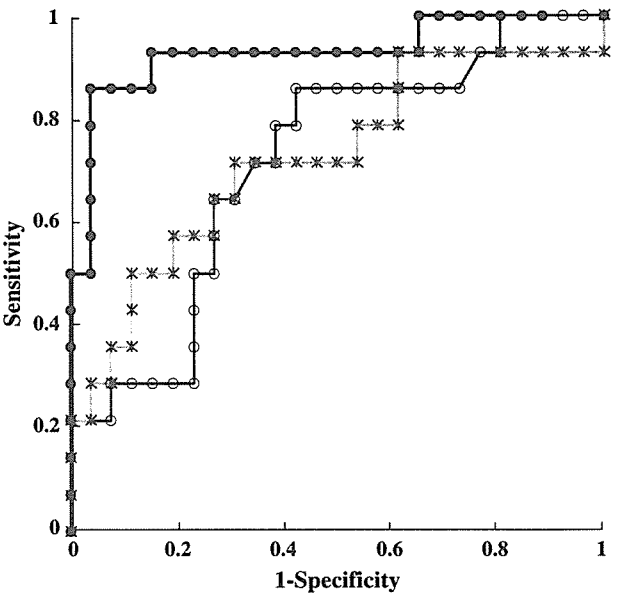

Fig 5. Receiver-operating characteristic curves for different strata of the indices of heart rate variability shown in Table 4. (●) Cardiac chaos, (×) low-frequency component, (○) high-frequency component. The largest area under the curve is observed in cardiac chaos.

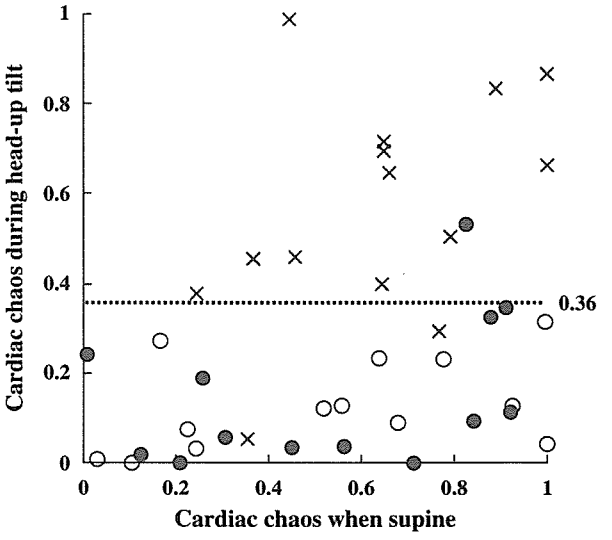

Fig 6. Relationship between cardiac chaos and the results of the head-up tilt test and position. Cardiac chaos in vasovagal syncope patients with (○) a negative head-up tilt test and (●) a positive head-up tilt test. (×) Cardiac chaos in healthy subjects. There were no differences among the 3 groups in cardiac chaos while supine, but cardiac chaos decreased in the patients when tilted regardless of the results of the head-up tilt test. A cutoff value of 0.36 is used in Fig 5 and Table 5.

Table 6 Cutoff Values for the Diagnosis of VS

Heart rate variability	Cutoff value	Sensitivity	Specificity
Cardiac chaos during HUT	*0.36*	*92.3%*	*96.3%*
LF during HUT (ms^2/Hz)	*8.66*	*76.9%*	*70.4%*
HF during HUT (ms^2/Hz)	*1.81*	*84.6%*	*55.6%*

Abbreviations see in Table 1,4.

was observed in the VVS patients regardless of the outcome of the HUT, suggesting that the chaotic behavior of the RRIs in reaction to the gravitational load differs between healthy subjects and VVS patients.

Loss of heart rate complexity, as measured by the cardiac chaos, was a more sensitive indicator of orthostatic intolerance than conventional analysis measures, and this observation is consistent with the recent widely accepted hypothesis that chaotic behavior is characteristic of normal biological systems and that diminished complexity indicates pathology.[16,25–28] A positive effect of chaos in hemodynamic regulation may serve as a response to altered external influences. According to several studies, the transition from a physiological contribution of hemodynamic regulation to a pathological state is accompanied by a reduction in LE and loss of the degree of chaos, and this has been demonstrated in studies of multiple sclerosis[24] and heart rate dynamics prior to sudden death.[20,21]

Although the mechanism responsible for the cardiac chaos was not clarified in the present study, cardiovascular baroreceptor function may play a role. Intact cardiovascular regulation involves a long feedback loop,[29] and the major feedback loop is controlled by arterial and cardiopulmonary baroreceptors that regulate autonomic nervous tone.[21] This feedback has nonlinear response characteristics. After baroreceptor denervation, hemodynamic control is less complex and less sensitive on initial conditions.[30]

Chaotic regulation may be advantageous in maintaining cardiovascular function in relation to gravitational load. Physiological systems normally operate to reduce variability and to maintain constancy of internal function, and the heart rate should return to its normal steady state after it has been altered. Chaotic systems operate under a wide range of conditions and are adaptable and flexible. This plasticity allows systems to cope with the exigencies of an unpredictable and changing environment.[25,26]

Previous studies have used different nonlinear measures during HUT.[19,31] One has reported that the LE values are not significantly different during tilt compared with supine posture in normal controls and patients with essential hypertension;[31] however, the subjects in that study were not syncopal patients. The other study indicated that there is a reduction of fractal dimension associated with presyncope during orthostatic stress using HUT and lower body negative pressure.[19] The reduction implies a loss of complexity in the underlying control of the heart rate response to orthostatic stress. These findings are compatible with the findings in the present study and represent the underlying complexity against which the cardiovascular system is able to recognize and respond to orthostatic stress.

A decrease in the chaotic behavior of the RRIs during HUT was observed in the VVS patients regardless of the outcome of the HUT, suggesting that the diagnostic yield of cardiac chaos is higher than that of the conventional HUT. The sensitivity and specificity of cardiac chaos for the diagnosis of VVS were greater than 85%, whereas the sensitivity of the conventional HUT is approximately 40–60%.[16,32,33] Recording the RRIs to analyze for cardiac chaos takes less than 10 min, whereas the conventional HUT takes at least 30–60 min.[16,32] Thus, analysis of cardiac chaos is an easier and more accurate test than the conventional HUT for diagnosing VVS.

Analysis of linear statistics, such as time domain and frequency domain analyses, does not directly address the complexity of the RRIs, and thus may miss potentially helpful information. Because the underlying mechanisms involved in the control of the RRIs are mainly nonlinear, application of nonlinear techniques seems appropriate. The results of this study support the clinical utility of the analysis of chaos based on nonlinear dynamics.

Study Limitations

Some questions remain concerning the significance of the cardiac chaos in the present study.

First, the mechanism that generates cardiac chaos was not clarified in this study. Analysis of RRI dynamics by methods based on chaos theory and nonlinear system theory has recently attracted interest and is being steadily developed.[34] However, it is impossible to record all the variables that affect it, and the exact total number of degrees of freedom is unknown. These are fundamental problems in selecting a valid mathematical model for analysis of the RRIs, and thus their applicability to specific conditions should be tested for diagnostic and clinical purposes.

Second, a previous study reported that LE decreases with age,[35] indicating that the RRIs become less chaotic as healthy subjects grow older. All of the subjects in the present study were young and healthy, and it is unknown whether our observations are valid for other age groups.

Third, the reproducibility and chronobiologic factors are crucial factors in determining the usefulness of this analysis as a diagnostic tool.[16,36] In fact, although the 8 of the 13 patients who were classified into the negative HUT group were diagnosed as VVS by positive HUT results with or without pharmacologic provocation (Table 1), orthostatic decreases of the cardiac chaos were observed during negative HUT response. This suggests that the analysis is reproducible; however, the reproducibility and chronobiology were not thoroughly evaluated in the present study. Further study is needed to elucidate the issue regarding reproducibility and chronobiology.

Fourth, 15 of the 26 VVS patients underwent the HUT within 5 days of spontaneous syncopal episodes. Their autonomic function may have been disturbed by the spontaneous syncopal attack. Our limited preliminary data suggested that a short period between spontaneous syncope and the HUT did not affect on the cardiac chaos, but this study was not designed to elucidate the impact of a spontaneous syncopal attack on cardiac chaos.

Finally, because there were no data for chaotic analysis of the RRIs in other types of orthostatic intolerance, including orthostatic hypotension, it is unknown whether our observations are applicable to the pathophysiology of other diseases.

Conclusions

A decrease in cardiac chaos during the HUT was specific to VVS patients, even when their response to the HUT was negative. Cardiac chaos may indicate adaptation to orthostatic stress, and the orthostatic decrease may play a role in the pathophysiology of VVS.

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Fluid resuscitation with hemoglobin-vesicle solution does not increase hypoxia or inflammatory responses in moderate hemorrhagic shock

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ABSTRACT

The aim of the present study was to compare the hypoxic and inflammatory effects of transfusing hemoglobin-vesicles (HbV) or lactated Ringer's (LR) solution on several organs in a hemorrhagic shock model. Hemorrhagic shock was induced in 48 anesthetized rats by withdrawing 28 mL/kg blood. The animals were resuscitated by replacing the blood with an equal volume of HbV solution or three times the volume of LR solution. The heart, lung, liver, kidney and spleen were extracted at different time points following resuscitation, and mRNA expression levels of hypoxia-induced factor 1-alpha (HIF-1α) and tumor necrosis factor-alpha (TNF-α) were determined. Blood lactate concentrations in the HbV group rapidly returned to baseline levels, whereas elevated lactate concentrations in the LR group were prolonged. There were no significant differences between the two resuscitation groups in terms of HIF-1α and TNF-α expression in the organs examined. HIF-1α and TNF-α expression in the lungs was significantly greater than in other organs. Our results suggest that resuscitation from hemorrhagic shock with HbV did not increase hypoxic or inflammatory effects in major organs, compared with resuscitation using LR solution, despite prolonged elevation of blood lactate.

Hemorrhagic shock is caused by hypovolemia and a loss of blood components, and it is usually corrected by infusion of crystalloids and colloids. Decreased blood flow and/or reduction in hemoglobin (Hb) during hemorrhagic shock can, however, lead to tissue hypoxia and critical anemia, which requires red blood cell (RBC) transfusion. During emergency care or perioperative periods in which RBCs are not available, RBC substitutes, such as those derived from Hb, are used (5, 8, 18, 28).

Hb-based oxygen carriers (HBOCs) are a valuable resource in prehospital care, large-scale disasters, and remote hospitals, in which stored blood is either not available or is rapidly depleted. The safety and efficacy of HBOCs can be evaluated in terms of hemodynamics (28), systemic and regional vasoconstriction (10, 11), tissue oxygenation, immunomodulation (13), and post-injury multiple organ failure (MOF) (20).

The standard approach to restoring oxygen delivery in hemorrhagic shock has been crystalloid administration to expand intravascular volume, followed by stored RBCs for critical anemia. However, the initial transfusion therapy after hemorrhagic shock may have adverse immunoinflammatory effects that increase the risk of MOF (20, 27, 32). Hb vesicles (HbV) are artificial oxygen carriers (24, 25, 28, 31). They consist of phospholipid vesicles (liposomes) that encapsulate purified human Hb with polyethylene glycol chains at the surface. The aim

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of the present study was to evaluate the use of HbV during resuscitation following hemorrhagic shock and to determine its oxygenation and proinflammation effects on multiple organs.

MATERIALS AND METHODS

Animal preparation. This study was approved by the Ethics Committee for Animal Experiments at Nippon Medical School, Japan. A total of 48 male Sprague-Dawley rats, aged 10 to 13 weeks weighing 308 ± 43 g (mean ± standard deviation (SD)), were anesthetized with 2–4% sevoflurane. Heated blankets were used to maintain core body temperature at 37°C. Lactated Ringer's solution (LR) was infused at a rate of 1 mL/kg/h via the tail vein, until baseline blood pressure measurements were obtained.

Following laparotomy, a 24G Teflon catheter was inserted into the inferior vena cava, and the common iliac artery was catheterized to allow mean arterial blood pressure (MAP) measurement and blood withdrawal for inducing hemorrhagic shock. Arterial pressure and central venous pressure (CVP) were measured with a pressure transducer (TP-300T; Nihon Koden, Tokyo, Japan) for 2 h following fluid resuscitation. The transducer was connected to a computer and electronic signals were configured to represent pressure changes by analysis software (MacLab/s; ADInstruments Japan, Nagoya, Japan).

Experimental procedure. Fifteen minutes after the preparation was complete, hemorrhagic shock was induced by withdrawing 28 mL/kg blood over 20 min, and maintaining the state for 15 min without fluid resuscitation. Animals were then randomly assigned to one of eight groups (n = 6 per group) based on treatment and time of sacrifice. Animals were resuscitated by infusing HbV solution (Oxygenix Co. Ltd., Tokyo, Japan, [Hb] = 10 g/dL) at the same volume as LR or by infusing three times the volume of LR. Each group was described according to the method of fluid resuscitation and the time of intentional sacrifice from the fluid resuscitation (*e.g.*, the group, which includes the animals resuscitated using HbV solution and sacrificed 2 h after the resuscitation, was described as HbV-2H). Arterial blood (0.2 mL) was sampled before hemorrhagic shock (baseline), after hemorrhagic shock (T1), and 1 h (T2) and 2 h (T3) after fluid resuscitation. An ABL 700 (Radiometer A/S, Copenhagen, Denmark) was used to measure Hb concentration, hematocrit, blood lactate concentration and pO2. MAP and CVP were recorded before and after blood withdrawal and 1 h and 2 h after fluid resuscitation.

RNA extraction and RT-PCR. Following a 2 h observation period, the heart, lung, liver, kidney, and spleen of animals in the 3XLR-2H and HbV-2H groups were removed. The same organs were removed from the remaining rats 24, 72, and 168 h after resuscitation under sevoflurane anesthesia. Organs were placed in liquid nitrogen and stored at −80°C pending RNA extraction. RNA isolation, quantification, and RT-PCR were performed according to established methods (6, 26).

Briefly, total RNA was extracted from each tissue sample using the chaotrophic Trizol method followed by Isogen-chloroform extraction and isopropanol precipitation. Residual genomic DNA was eliminated with DNase I (Takara Shuzo, Otsu, Japan). One microgram of each total RNA sample was reverse transcribed at 37°C for 1 h in a 20 μL solution with mouse Moloney leukemia virus reverse transcriptase and hexanucleotide random primers (Takara Shuzo). RNA was quantified by measuring absorbance at 260 nm, and each sample was diluted to 0.4 μg/μL.

PCR primers and TaqMan fluorogenic probes were designed using the Primer Express software program (Applied Biosystems, Foster City, CA) and had the following sequences: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward 5'-GAAGGTGAAGGTCGGAGTC-3', reverse 5'-GAAGATGGTGATGGGATTTC-3', and probe FAM-CAAGCTTCCCGTTCTCAGCC-Tamra. TNF-α: forward 5'-GCCTCAGCCTCTTCTCATTCCT-3', reverse 5'-GATGAGAGGGAGCCCATTTG-3', and probe FAM-ACCACGCTCTTCTGTCT-Tamra. HIF-1α: forward 5'-CACCTTCTACCCAAGTACCTCAAGA-3', reverse 5'-TGTCCGACTGTGAGTACCACTGT-3', probe FAM-ACCACTGCTAAGGCAT-Tamra. GAPDH was used as the housekeeping gene.

Quantitative PCR was carried out in a 50 μL solution containing 20 ng cDNA, 25 μL TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 900 nM forward and reverse primers, 200 nM TaqMan probe and deionized water. PCR conditions were 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The 6-FAM-labeled TaqMan probe was cleaved during amplification to generate a fluorescent signal that was measured using an ABI PRISM 5700 Sequence Detector (Applied Biosystems). Samples and calibration curve samples were run in triplicate. Values were interpolated automatically from the standard curve. A similar system utilizing a separate

GAPDH probe and primer set (TaqMan GAPDH control reagent kit; Applied Biosystems) was designed and run for GAPDH along with each sample to correct for total nucleic acid content. Relative amounts of mRNA were calculated by the comparative critical threshold (CT) method (Applied Biosystems).

Statistical analysis. Data are expressed as mean ± SD. Statistical analyses were performed with Statview® version 5.0 for Macintosh software (Abacus Concepts Inc., Berkley, CA). Differences in MAP, CVP, Hb concentration, blood lactate concentration and gene expression between resuscitation groups and time after resuscitation were analyzed with two-factor factorial ANOVA and the Tukey-Kramer test at the 95% confidence level. Within group differences were analyzed with one-factor ANOVA and the Tukey-Kramer test for comparison with each baseline value. p-values < 0.05 were considered statistically significant.

RESULTS

All rats tolerated hemorrhagic shock, and received fluid resuscitation period and survived until the time of sacrifice. MAP and CVP at baseline were similar in the 3XLR and HbV groups (Table 1). MAP was significantly reduced by hemorrhagic shock and returned to baseline values by fluid resuscitation in both groups. Hemorrhagic shock reduced CVP and fluid resuscitation increased CVP, but not significant. MAP was decreased in 3XLR group 2 h after resuscitation. Arterial blood lactate concentrations at baseline did not differ significantly between the 3XLR and HbV groups. Hemorrhagic shock increased arterial blood lactate concentration. Fluid resuscitation using HbV solution reduced the lactate level, but lactate concentrations in the 3XLR group remained elevated compared to baseline. Hb concentration and hematocrit at baseline were similar in both groups. After the fluid resuscitation, the Hb concentration and hematocrit in the HbV group were significantly higher than in the 3XLR group.

Expression of HIF-1α and TNF-α mRNA at various time points after fluid resuscitation is shown in Figs. 1 and 2. There were no significant differences in gene expression in any organ between the 3XLR and HbV groups. However, HIF-1α expression in the lung was significantly higher than in the heart, liver, and kidney in both 3XLR and HbV groups (Fig. 1). HIF-1α mRNA expressions in the heart were significantly lower than in the lung and spleen. HIF-1α mRNA expression peaked 24 h after fluid resuscitation and then decreased in the most organs. In contrast, TNF-α mRNA gradually increased during the 168 h following resuscitation (Fig. 2). TNF-α expression in the lung was significantly higher than in the other organs examined.

Table 1 *Hemodynamics and arterial blood values*

Measurement	Time point			
	Baseline	T1	T2	T3
Mean arterial blood pressure (mmHg)				
3XLR	83.3 ± 9.2	32.5 ± 4.0§	80.0 ± 13.7	56.0 ± 20.4§
HbV	80.5 ± 13.7	30.2 ± 3.1§	87.8 ± 14.8	68.7 ± 5.3
Central venous pressure (mmHg)				
3XLR	4.0 ± 0.6	3.3 ± 1.9	4.5 ± 1.4	3.7 ± 0.5
HbV	4.2 ± 1.2	4.0 ± 0.9	5.2 ± 2.1	4.7 ± 2.0
Hemoglobin concentration (g/dL)				
3XLR	11.1 ± 2.2	8.5 ± 2.4	7.4 ± 0.7§	8.0 ± 1.3§
HbV*	13.2 ± 1.5	9.5 ± 3.0§	13.5 ± 0.6	13.0 ± 2.1
Hematocrit (%)				
3XLR	34.2 ± 6.7	26.4 ± 7.1	23.0 ± 2.2§	24.9 ± 3.7§
HbV*	40.4 ± 4.6	29.4 ± 9.0§	41.6 ± 2.0	40.1 ± 6.3
Blood Lactate concentration (mmol/L)				
3XLR	2.1 ± 0.9	6.7 ± 2.6§	6.3 ± 2.6§	4.6 ± 2.7
HbV	3.2 ± 1.1	5.8 ± 1.0§	2.6 ± 0.5	2.6 ± 0.3

T1, immediately after fluid resuscitation; T2, 1 h after resuscitation; T3, 2 h after resuscitation
§ significantly different than baseline ($p < 0.05$)
*significantly different than LR group ($p < 0.05$)

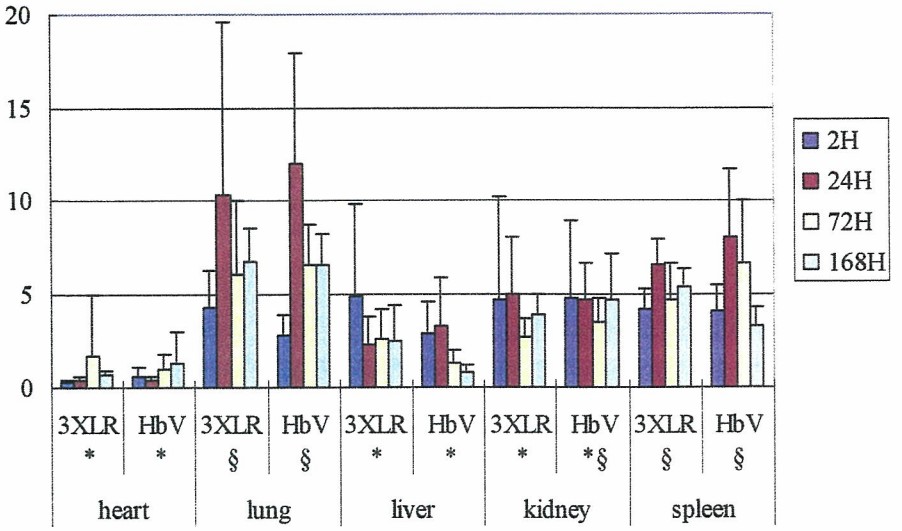

Fig. 1 HIF-1 α mRNA expression. *significantly different from lung ($p < 0.05$), § significantly different from heart ($p < 0.05$)

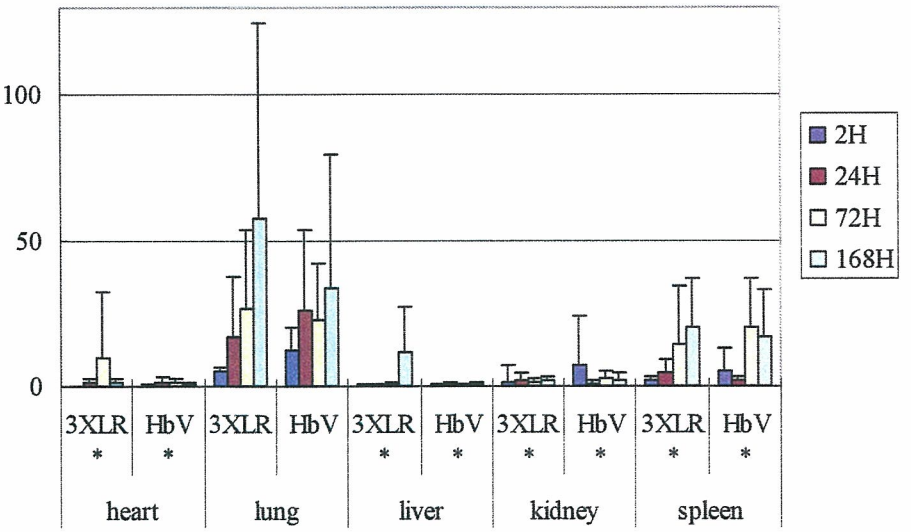

Fig. 2 TNF-α mRNA expression. *significantly different from lung ($p < 0.05$)

DISCUSSION

Critical acute anemia during emergency care and the perioperative period is usually treated with RBC transfusion. However, this requires time-consuming blood typing and cross matching tests, and the preservation time of blood products is limited. Stored RBCs cannot be supplied during prehospital care, and costs are incurred for their transport to remote hospitals. Moreover, there is increasing evidence that RBC transfusions are associated with adverse effects on the immune response to injury and illness (27). Transfusion of more than six units of RBCs within the first 12 h after injury is an independent risk factor for MOF (20), and aged packed RBC transfusion further increases the risk of postinjury MOF (32). HBOCs may attenuate adverse immunoinflammatory effects induced by allogenic RBC transfusion and ultimately reduce the incidence of postinjury acute respiratory distress syndrome (ARDS) and MOF (19).

In the present study, organ hypoxia and proinflammatory reactions were revealed by measuring HIF-1α and TNF-α mRNA expression after fluid resuscitation with crystalloid LR or HbV solutions. Despite a rapid recovery from elevated blood lactate

concentrations in the HbV group, no significant group differences were found in the expression of HIF-1α or TNF-α in intrathoracic and splanchnic organs. A reduction in hemorrhagic volume is sufficient to depress MAP and increase blood lactate concentrations, leading to a 40–50% loss of circulating volume (24, 25, 31). Infection and bacterial translocation in the gut, which commonly occur after injury, and coincidental hemorrhagic shock, can also prolong the increased HIF-1α response (14). Although fluid resuscitation for hemorrhagic shock using HbV solutions does not always modulate HIF-1α expression in the liver and kidney, an unmodulated HIF-1α response is beneficial in cases of anemia, as erythropoietin production remains unchanged, while vascular tone is adjusted (9). Differences in HIF-1α expression between organs can provide tolerance against hemorrhagic shock. Centralization of circulating blood and tolerance of acute isovolemic anemia (16) provide protection against moderate hemorrhage.

Tissue hypoperfusion and vasoconstriction followed by acute hemorrhagic shock can lead to tissue hypoxia. Acute hypoxia stimulates the expression of HIF-1α and p38 mitogen-activated protein kinase (MAPK), particularly in the lung, which are linked to the proliferation of pulmonary artery fibroblasts and remodeling (30). Pulmonary and systemic vasoconstriction and low peripheral perfusion are associated with use of a modified Hb tetrametric solution (11, 22), which causes scavenging of nitric oxide (NO) and enhanced endothelin release (10). NO scavenging enhances hypoxic pulmonary vasoconstriction (1), worsens pulmonary hypertension and reduces cardiac output after hemorrhagic shock. In the present study, HIF-1α expression, a marker of hypoxia, did not increase following HbV resuscitation compared to LR resuscitation. This suggests that HbV does not increase NO scavenging or impede microcirculation based on an endothelial cell disorder (23). A definitive difference is seen with the use of HBOCs that induce vasoconstriction (11). For instance, blood lactate concentration decreased following resuscitation with HbV.

Injury, hemorrhagic shock and fluid resuscitation can produce an inflammatory response such as postinjury ARDS, an acute inflammation of the lungs (17). Our results suggest that inflammation of the lungs, as measured by TNF-α and HIF-1α expression, far exceeded that of other organs. LR resuscitation was shown to produce a smaller hemorrhagic shock effect in the lung than the equivalent volume of normal saline (29). It is, therefore, of clinical interest that in the present study there were no significant differences between HbV and LR resuscitation groups in the extent of inflammation in the lung or other organs.

The key cellular mediators in the pathogenesis of proinflammatory effects are neutrophil polymorphonuclear leucocytes (PMN) (3, 4) and endothelial cells (15). PMNs are primed by plasma from stored RBCs; and the older the RBCs, the greater the priming effect (21). To reduce the induction of cytokines following RBC transfusion, PMNs can undergo prestorage leukoreduction treatment (2, 7, 12). However this does not eliminate all inflammatory reactions. The use of RBC substitutes may overcome the problems of inflammatory reactions. However, in future studies it will be important to demonstrate that, in critical situations, fewer inflammatory reactions are induced by artificial oxygen carriers than by RBCs.

In conclusion, we demonstrated that fluid resuscitation with HbV solution for moderate hemorrhagic shock did not influence the expression of HIF-1α and TNF-α mRNA in the heart, lung, kidney, liver and spleen compared with fluid resuscitation using LR solution, despite blood lactate concentrations changing after fluid resuscitation. Inflammation of the lung was significantly greater than that of other organs after hemorrhagic shock and fluid resuscitation, but the extent of inflammation did not differ according to the type of fluid resuscitation.

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