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第14回日本血液代替物学会年次大会

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大会長：半田　誠（慶應義塾大学　輸血・細胞療法部）
テーマ：人工血液の未来を語ろう

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総　説

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 Vesiclesの可能性-

Transfusion Overview in Perioperative/Emergent Field and Prospect of Artificial Oxygen Carriers -A Potential of Hemoglobin Vesicles-

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和文抄録

医療の飛躍的な発展にも関わらず，周術期や救急領域では多くの赤血球輸血の機会がある．近年の輸血療法に関する多くの報告を基に，日本赤十字社が輸血療法の実施に関する指針を発表し，改訂を重ねてきた．輸血を行うには，急性貧血時の代償機構と輸血の副作用を知らねばならない．輸血に対する安全性への努力はなされてきたが，根本的な問題の解決には至っていない．そこで，いくつもの人工酸素運搬体が開発された．しかし，安全性や効果の面で，一部のヘモグロビンを利用した人工酸素運搬体のみが本邦において前臨床応用段階で有望視されている．幸いにも，我々は最も臨床応用可能と思われるHemoglobin Vesiclesの安全性及び効果を研究する機会を得た．本稿では，麻酔や外傷治療における貧血への耐性と輸液・輸血後の予後，およびヘモグロビン利用酸素運搬体の可能性について，我々の調査結果を含め再考した．

Abstract

Despite the remarkable medical advances were made over recent years, erythrocyte transfusions are often still required during emergency care and the perioperative period. Several recent reports on blood transfusion have prompted the Japanese Red Cross Society to publish revised guidelines for implementing transfusion therapy. In order to perform this procedures, one must understand both the compensatory mechanism that occurs during acute anemia and the potential adverse reactions to transfusion. Although great efforts have been made to ensure the safety of blood transfusions, some fundamental issues have yet to be resolved. Consequently, several artificial oxygen carriers have been developed; however, in terms of their safety and effectiveness, only those that utilize hemoglobin have shown potential at the pre-clinical stage in Japan. We are privileged to have had the opportunity to study the safety and effectiveness of hemoglobin vesicles, which appear to have the greatest potential for clinical application. The present paper reviews tolerance to anemia under anesthesia and during trauma treatment, post-transfusion prognosis, and the potential application of hemoglobin-based oxygen carriers, with reference to the results of our own survey of these topics.

Keywords

Hemoglobin-based oxygen carrier, multiple organ failure, hemorrhagic shock, transfusion, red blood cell, fluid resuscitation

はじめに

麻酔中や救命救急領域では，出血や急性貧血に対する輸血の機会が非常に多い．昨年９月，「輸血療法の実施に関する指針」及び「血液製剤の使用指針」の改訂が行われた[1]．輸血方法を見直す報告が近年増加し，従来の輸血方法に対する批判も含まれている．特に，集中治療領域における輸血方法は患者予後に強く影響を及ぼす可能性がある．一方，血液代替物が開発されて75年が経過した．最近10年の進歩はめざましく，人工酸素運

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搬体は一部の国で臨床応用や臨床治験されるまでになった．現在，我が国でも数種類の人工酸素運搬体が動物試験されている．この論文では，酸素運搬体としての赤血球輸血とヘモグロビン利用の人工酸素運搬体に焦点を当て，麻酔／救急領域における赤血球輸血療法の適応と問題点，人工酸素運搬体利用による利点について我々の報告を含め概説する．

手術中の急性貧血に対する耐性

最も重要な赤血球の機能は，呼吸器（肺）から末梢組織への酸素運搬である．手術中のような院内出血は，晶質液や膠質液による循環血液量の補正後に必要な場合に対してのみ輸血が行われる．この時の輸血実施に対する判断は，生体の代償機能を超えるか否かの判断に基づく．この代償機能は，主に血行動態の変化とヘモグロビン酸素解離曲線の変化による[2]．循環血液量の保たれている急性貧血は，一回拍出量と心拍数の増加により心拍出量は増加する[3]．血液粘稠度の減少は，静脈還流増加による前負荷の増加と後負荷を軽減する[4]．そして，交感神経刺激は陽性変時・変力作用を来すことによる．従って，麻酔下の患者は，主に一回拍出量によって心拍出量を増加させる．全身的には，ヘモグロビン濃度が7 g/dLまでは酸素供給量は変化しない[3]．脳は酸素抽出率を増加することで急性貧血に対応するのに対し，心臓は酸素抽出率よりも酸素供給量の増加により代償する．微小循環に対しては血管床と血流の増大により酸素放出増加を可能にする[2]．また，貧血による2,3-DPGの増加は，酸素解離曲線を右方変位させ，末梢での酸素放出を容易にさせる．

麻酔中の冠動脈疾患患者での循環血液量の保たれている中等度急性貧血は許容されるようである．冠動脈バイパス術を予定されている患者のヘモグロビン濃度13.9 ± 1.3 g/dLから9.3 ± 1.0 g/dLへの血液希釈では，心筋虚血を示唆する心電図変化や局所壁運動異常，血行動態の変化は見られなかった[5]．このような臨床研究での対象患者は，予後を改善するために周術期もβ遮断薬による治療を継続している[6]．β遮断薬の使用の有無は，急性血液希釈による心拍出量の増加を阻害しない[7]．また，麻酔下の状況では陽性変時作用が見られないことも手術中のイベントの発生率を下げるかもしれない．さらに，オンポンプ冠動脈再建術患者のヘマトクリット値28%への急性血液希釈は，術後の血中トロポニンIやCK-MBレベルをコントロール群に比べ抑制し，カテコラミン必要量を低下させ術後不整脈発生率を低下した[8]．急性冠動脈症候群患者では，30日死亡の予測因子としてヘマトクリット値25%以上での輸血開始が挙げられた[9]．ただし，この研究は３つの急性冠動脈症候群に対する大規模研究データに基づくレトロスペクティブな研究であることを考慮しなければならない．

血液希釈によるヘモグロビン濃度6 g/dL以下の低下は，認知機能を低下させる[10-12]．ヘモグロビン濃度5 g/dLまでの低下による認知機能の低下は可逆的であり，新鮮血や保存血の違いに関わらずヘモグロビン濃度7 g/dLまで輸血することで回復する．また，吸入酸素濃度の増加によっても回復する．その障害時には，大脳誘発電位の一種である事象関連電位に見られる，刺激からの陽性波の発生までの時間，すなわちP300潜時の延長として表される可能性がある．

軽度から中等度の血液希釈は肺の換気血流不均等を改善させ[13]，肺高血圧を伴う慢性閉塞性肺疾患患者に対して肺動脈圧を減少させる[14] ことが示唆されている．血液粘度の低下や心拍出量の増加に加え，NOの増加による肺血管抵抗の減少が生じるためと考えられる．これにより，動脈血酸素分圧/吸入酸素濃度比は増加する．

腎血流量と血液分布は中等度血液希釈に影響を受けず，尿量と尿中ナトリウム排泄率は増加する可能性がある[15]．同様に，ヘマトクリット20 ± 1%までの血液希釈は肝臓や小腸，膵臓の血流を増加させる[16]．肝臓は，肝動脈の血流が86%増加し，門脈血流は28%増加した．小腸粘膜血流も増加した．ICG血管内半減期は短縮し，GPTは減少した．すなわち，肝機能を障害しないようである．

血液希釈による酸素供給量の重篤な低下は，主に乳酸値，重炭酸イオン，過剰塩基（BE），酸素抽出率，呼気二酸化炭素濃度，脈圧，心係数，収縮期血圧に影響を及ぼす[17]．臨床的に輸血を行う根拠の一つとして利用出来る可能性がある．

プレホスピタル出血性ショック

我々の関わるもう一つの分野に，救命救急や集中治療がある．救命救急の患者の多くは院外発症である．すなわち，院外発症の急性出血性ショック患者は通報により駆けつけた救急救命士により初期治療が施され，直ちに近くの救急対応病院に搬送される．このプレホスピタルの出血性ショックの特徴は，全ての血液成分が循環血液量とともに同程度に失われることである．残念なことに，我が国での救急救命士によるfluid resuscitation（輸液蘇生）は行われていない．欧米諸国では，患者搬送時間の問題もあるが，この救急救命士による初期輸液蘇生は急性出血性ショック患者の救命の一役をになっている．初期輸液蘇生の方法は，未だ発展途中である[18]．必要な止血処置とともに行われるプレホスピタルでの古典的輸液蘇生法は，大量の晶質液投与による循環維持をすることである．その後のインホスピタルで必要があれば，輸血が行われる．現在臨床治験中や開発中の人工酸素運搬体の役割の一つには，このプレホスピタルへの治療限界への挑戦が挙げられる．

人工酸素運搬体の過去の治験から学ぶ

修飾Hb４量体のヘムアシストは，第３相臨床試験で予期せず失敗に終わった[19]．このヘモグロビン利用酸素運搬体は，臨床治験前に少量投与で循環動態に強く影響することが分かっていた[20,21]．生来のHbと同じ様に，少量投与で，肺血管抵抗や体血管抵抗を増加し心拍出量を低下させた．これは，NOのスカベンジ[22-24] とエンドセリン放出増加，アドレナリン受容体の感受性増大[25-27]，二次的な動脈壁ずり応力の減少によるものと考えられている．出血性ショック患者へのプレホスピタルでの初期蘇生として，強力な血管収縮薬の投与は死亡率を増加させる結果となった．

Hemoglobin-Vesicles（HbV）の循環動態と臓器酸素化へ及ぼす影響

HbVは我が国で開発されたHBOCである．現在，開発した早稲田大学理工学部や慶応大学医学部を中心に精力的に研究活動を行っている．昨年，我々の教室でも，動物モデルでのHbVを用いた輸液蘇生での全身循環及び臓器酸素化への影響を調査した[28]．

対象動物は，生後12 - 14週齢の雄New Zealand white rabbitとした．早稲田大学理工学部の協力を得て，ウサギ赤血球とHbV（委託製造先，㈱オキシジェニクス）の酸素解離曲線を得た（Fig. 1.）．麻酔下に小開頭と開腹，大腿部切開し，脳／肝臓／腎臓／骨格筋に酸素電極を挿入した．ベースライン測定後，平均動脈圧が30-35 mmHgとなる様に脱血を行った．その後，脱血量と等量の５％リコンビナントアルブミン溶液＋HbV溶液（HbV＋alb群），５％リコンビナントアルブミン溶液（ニプロ㈱提供）（alb群），乳酸リンゲル液（LR群），さらに脱血の３倍量の乳酸リンゲル液（3XLR群）で急速輸液蘇生を行った．測定項目は，脱血量，動脈圧，中心静脈圧，心拍出量，組織酸素分圧とした．脱血量は群間に差がなかった．血行動態の変化をTable 1.に示す．LR群は蘇生終了15分後より，3XLR群は1時間後よりベースラインに比べ血圧が低下した．心拍出量はLR群と3XLR群ともに２時間後に低下した．それに対し，HbV＋alb群とalb群の心拍出量は蘇生後に回復し，実験期間中維持された．蘇生後15分でのHbV＋alb群の計算された全身血管抵抗は，他の群と差がなかった．HbV投与による蘇生後の組織酸素分圧は，他の蘇生群と比べ低下しなかった．脳や腎臓では，蘇生後15分以降ではLR群に比べ高く維持された．BEや血中乳酸値も他群に比べより早く回復した．これらの結果は，急性出血性ショックに対するHbV溶液の投与が，生体内で血管収縮による全身や局所の血管抵抗の増大を来さず，心拍出量と組織酸素分圧を回復させることを示唆する．さらに，全身の循環と酸素化改善により乳酸値とBEを早期に回復させることが示唆された．

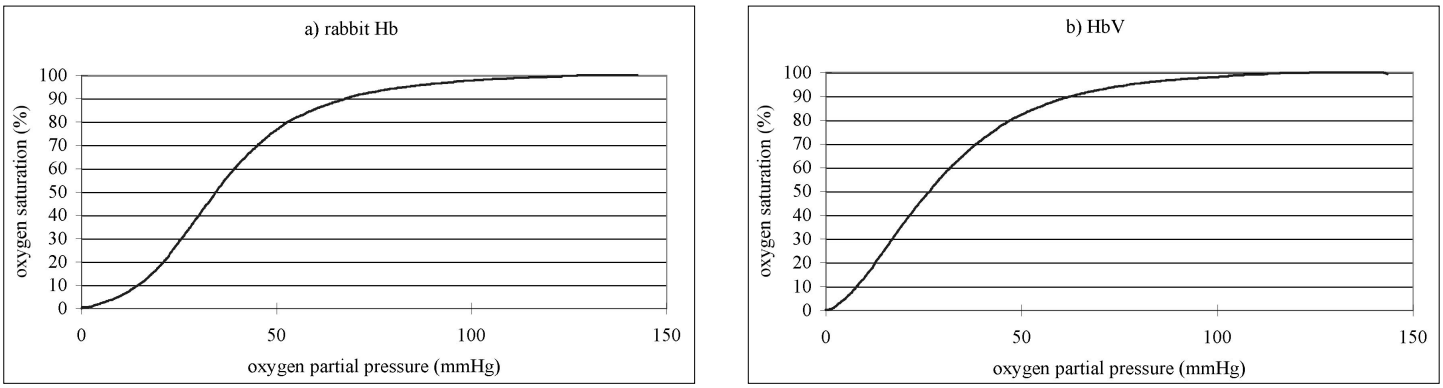

Fig. 1. The oxygen equilibrium curves for a) rabbit Hb and b) HbV.

Table 1. Hemodynamic variables in New Zealand white rabbits after inducing hemorrhagic shock (HS) by withdrawing blood and stabilization for 30 min. Animals were resuscitated using the same volume of HbV/rHSA, rHSA or RL, or using 3ɪRL, ove 15 min (RES). The hemodynamic variables were measured again after 15 min, 1 h and 2 h. *Significant difference from baseline ($p<0.05$). †Significant difference from the RL group ($p<0.05$). All values are presented as the mean ± SD (n = 6).

	Baseline	HS	30 min	RES	15 min	1 h	2 h
Mean arterial pressure (mmHg)							
HbV/rHSA	85 ± 10	33 ± 1*	43 ± 3*	88 ± 10†	90 ± 16†	90 ± 17†	90 ± 7†
rHSA	89 ± 11	32 ± 3*	36 ± 2*	68 ± 14	76 ± 9†	77 ± 9†	80 ± 9†
RL	78 ± 10	34 ± 1*	41 ± 9*	69 ± 9	56 ± 13*	51 ± 10*	54 ± 20*
3XRL	80 ± 17	31 ± 3*	38 ± 7*	67 ± 12	62 ± 17	48 ± 20*	46 ± 16*
Central venous pressure (mmHg)							
HbV/rHSA	4.5 ± 1.2	3.0 ± 0.9*	2.8 ± 1.5*	5.8 ± 1.5	5.8 ± 1.5	4.2 ± 1.2	4.7 ± 1.2
rHSA	5.2 ± 1.7	3.2 ± 1.2*	3.0 ± 1.3*	5.0 ± 1.7	4.8 ± 1.5	4.7 ± 0.5	4.2 ± 0.8
RL	6.2 ± 1.3	2.7 ± 0.8*	2.8 ± 0.4*	4.7 ± 1.4	4.0 ± 1.7*	3.8 ± 0.8*	3.5 ± 1.0*
3XRL	4.3 ± 1.0	2.8 ± 1.0*	2.8 ± 1.2*	5.5 ± 0.8	4.8 ± 1.2	3.7 ± 0.5	2.8 ± 1.0*
Cardiac Index (l·minute^{-1}·m^{-2})							
HbV/rHSA	2.9 ± 0.7		1.2 ± 0.4*		2.9 ± 0.2†	3.2 ± 0.8†	2.8 ± 0.4†
rHSA	2.7 ± 0.8		1.0 ± 0.2*		2.7 ± 0.7	2.3 ± 0.5†	2.4 ± 0.6†
RL	2.6 ± 0.9		1.1 ± 0.4*		1.8 ± 0.4	1.3 ± 0.3	1.1 ± 0.3*
3XRL	2.9 ± 0.5		1.1 ± 0.2*		2.6 ± 0.9	1.9 ± 0.6	1.4 ± 0.4*
Systemic vascular resistance (dyne·second·cm-5)							
HbV/rHSA	1579 ± 738		1839 ± 420		1516 ± 294	1498 ± 440	1516 ± 294
rHSA	1834 ± 494		1956 ± 394		1600 ± 569	1820 ± 549	1600 ± 569
RL	1559 ± 564		2012 ± 695		1578 ± 509	1993 ± 587	1578 ± 509
3XRL	1575 ± 465		1868 ± 370		1374 ± 334	1420 ± 717	1374 ± 334

Fluid resuscitationの歴史[29]

プレホスピタルでの輸液蘇生の進歩は，残念なことに大きな戦争ごとに見られるようである．戦場では医療に対して障害がある．初期の蘇生処置から根治的治療までの時間，治療薬の保存や運送面において効果的な初期治療のための方法と薬剤が求められる．

第一次世界大戦の時は，まだ術前のfluid resuscitationが導入させておらず，多くの兵を失った．第二次世界大戦や朝鮮戦争の時は，膠質液の投与と保存血の投与による蘇生が始まった．初期生存率の改善をもたらしたが，急性腎不全のために多くの死傷者が出た．ベトナム戦争の時には，以前の経験と大量等張晶質液による初期輸液蘇生が見直され，それまでのコロイド輸液から変わった．その結果，死亡率と急性腎不全の発生率は減少したが，adult respiratory distress syndrome（ARDS）の発生率とそれによる死亡率が増加した．1970年代より当時の先端医療技術とモニタリングなどの特徴を持つ集中治療室が発達し，ARDSでの死亡率は減少した．しかし，“多臓器不全”という現在でも外傷患者の死亡原因として最も重大な問題が明白となった．

後に，初期蘇生輸液の晶質液の種類についての研究が報告された[30]．中等度出血性ショックモデルに対する輸液／輸血蘇生では，生理食塩水を用いた群は乳酸リンゲル液での蘇生群に比べ，アシドーシス（7.14 ± 0.06対7.39 ± 0.04）と死亡率の増加（50%対0%）が観察された．

Small volume fluid resuscitationと permissive hypotension

急性ショック患者の主な目的の一つには，十分な酸素供給量に回復することである．酸素供給量に関わる要素は，ヘモグロビン濃度，酸素飽和度，心拍出量を含む．そのため，心拍出量を改善するための充分な輸液量と酸素運搬体の補充が必要となる．院内蘇生の最初の24時間の酸素供給量インデックスの目標は，現在のところ500 mL/minute/m2と報告された[31]．それ以上に酸素供給量を増加させても酸素消費量を増加させず，血中乳酸値は増加したままとなることがある．これは，末梢のミトコンドリア機能障害を示し，多臓器不全に導く[32]．また，過剰な輸液や輸血は，血圧上昇や凝固能への影響による出血量の増加や，低体温，アシドーシス，脳浮腫やARDS発生率の増加のような組織浮腫の可能性がある[33]．鈍的外傷による出血源のコントロールされていない患者に対する等容量輸液蘇生は，このような危険を含んでいる．

このような患者の輸液蘇生に対し，輸液量を制限する戦略や低血圧を維持させる戦略が始まった[34]．少量高張食塩水は大量晶質液と同等の心拍出量と血漿増量効果，血管内皮の膨化を防ぐことでの末梢循環改善効果，生存率改善が示された[35]．これは，心原性ショックに対しても末梢循環改善効果をもたらす[36]．しかし，この循環改善効果は出血量の増加をもたらした．高張食塩水の研究のメタアナリシスでの結果は，標準的な晶質液での輸液蘇生後の生存率を改善しなかった[37]．この生存率改善効果は，デキストランを添加することで可能性があることが示唆された[37,38]．さらに，多施設試験での蘇生後の臓器保護効果[39]やpermissive hypotensionによる蘇生後の予後を改善する効果[40]が示された．特に，ショックを伴う閉塞性頭部外傷患者では脳浮腫を軽減し頭蓋内圧を減少し，脳還流圧を上昇することが示された[41]．さらに，この高張食塩水の蘇生は，出血性ショックや虚血再還流において炎症反応を減少させることが示唆された[42-44]．

HbVの凝固機能への影響

出血性ショックに対する輸液蘇生時には，大量出血による凝固因子の流失，凝固機能亢進による消費，輸液蘇生に用いられる溶液の影響による出血量の増大が生じる可能性がある．さらに，前述の様に，末梢循環の改善効果のある溶液は，出血量を増加させる．出血性ショックモデルに対するHbV溶液を用いた輸液蘇生は，全身末梢血管抵抗に影響を及ぼさないようである[28]．そこで我々は，HbV溶液による血液希釈が凝固機能に与える影響をin vitroで調査し，報告した[45]．

健康成人男性のボランティア７人を対象とした．採血された血液サンプルを，直ちに乳酸リンゲル液，または，生理食塩水に分散した10%HbV溶液で，それぞれ，乳酸リンゲル液，または，生理食塩水が血液サンプルに対して０%，20%，40%，60%，80%になる様に希釈した．乳酸リンゲル液で希釈した血液は，フィブリノーゲン濃度，プロトロンビン時間，活性化部分トロンボプラスチン時間を測定した．また，全ての血液サンプルまたは希釈された血液はSONOCLOT（tm）（Sienco Company, Morrison, CO）で凝固機能を測定した．SONOCLOT（tm）は，血小板機能や血漿成分凝固因子，細胞成分凝固機能を，37℃における血液の粘度抵抗の経時的な変化に基づいてクロット形成と線溶現象を測定することで評価するものである[46,47]．希釈の程度に比例して，フィブリノーゲン濃度は低下した（Fig. 2a.）．プロトロンビン時間と活性化部分トロンボプラスチン時間は，希釈の程度以上に延長する傾向を認めた（Fig. 2b, 2c.）．SONOCLOT（tm）による測定値を示す（Fig. 3.）．60%希釈まではHbV溶液は乳酸リンゲル液に比べて差はなかった．しかし，80%希釈ではHbV溶液での希釈はSon-ACTを延長させた．HbV溶液による高度の血液希釈は，乳酸リンゲル液に比べ抗凝固作用を示す可能性が示唆された．ただし，60%希釈までの場合，ヒドロオキシエチルスターチに比べ影響が少ない可能性がある[46,47]．我々の研究では，60%までの希釈では，乳酸リンゲル液とHbV溶液のSONOCLOT（tm）による測定値は差がなかった．すなわち，HbV溶液で高度希釈を行うような特殊な場合を除き，臨床応用される状況下では凝固異常が問題となる可能性は低いことが示唆された．

赤血球輸血による外傷後多臓器不全と肺障害

輸血を必要とする外傷患者の蘇生後のMOFの発生率を低下させることは，現在の出血性ショック治療の主な関心の一つである[48]．Injury Severity Score（ISS）15以上の513人の外傷患者でのコホート研究では，外傷後12時間以内の6単位以上の輸血はMOF発生の独立危険因子であった[49]．また，最初の外傷後

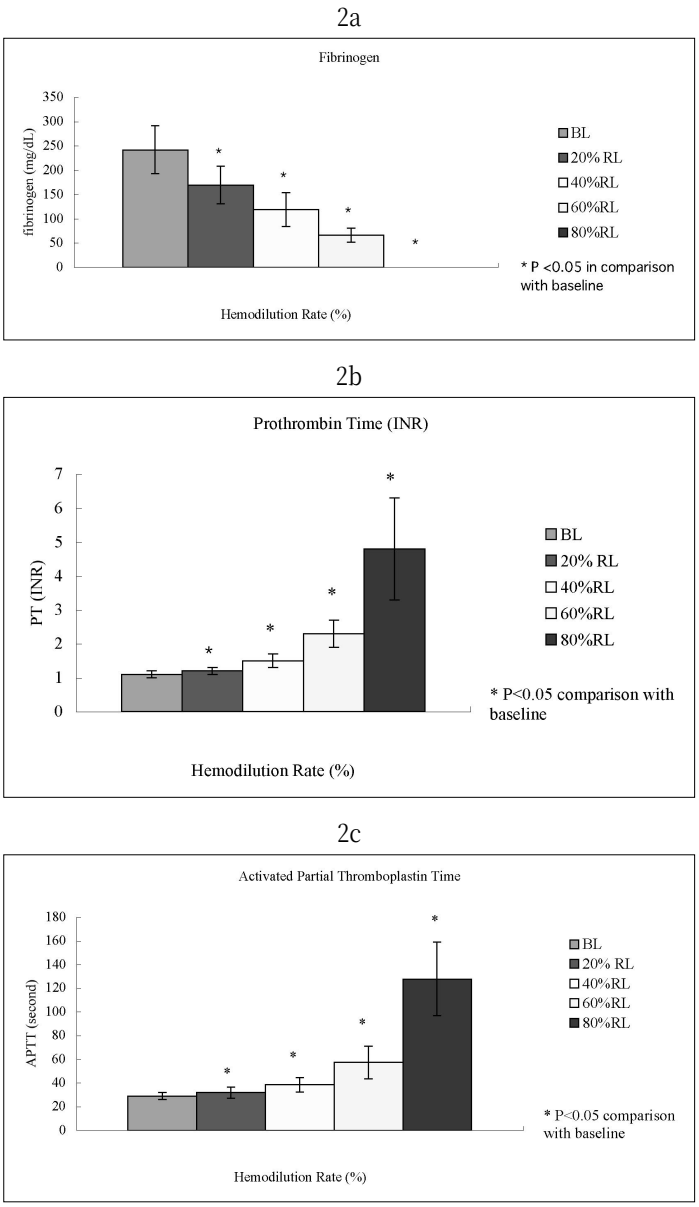

Fig. 2. Fibrinogen concentrations, prothrombin time and the activated partial thromboplastin time of diluted samples with isotonic Ringer's lactated solution (RL) or 10 g/dL Hemoglobin-Vesicles solution (HbV). Fibrinogen concentrations were decreased in inverse proportion to the dilution rate (2a). The prothrombin time and the activated partial thromboplastin time were in proportion to the square or cube of the dilution rate (2b, 2c).

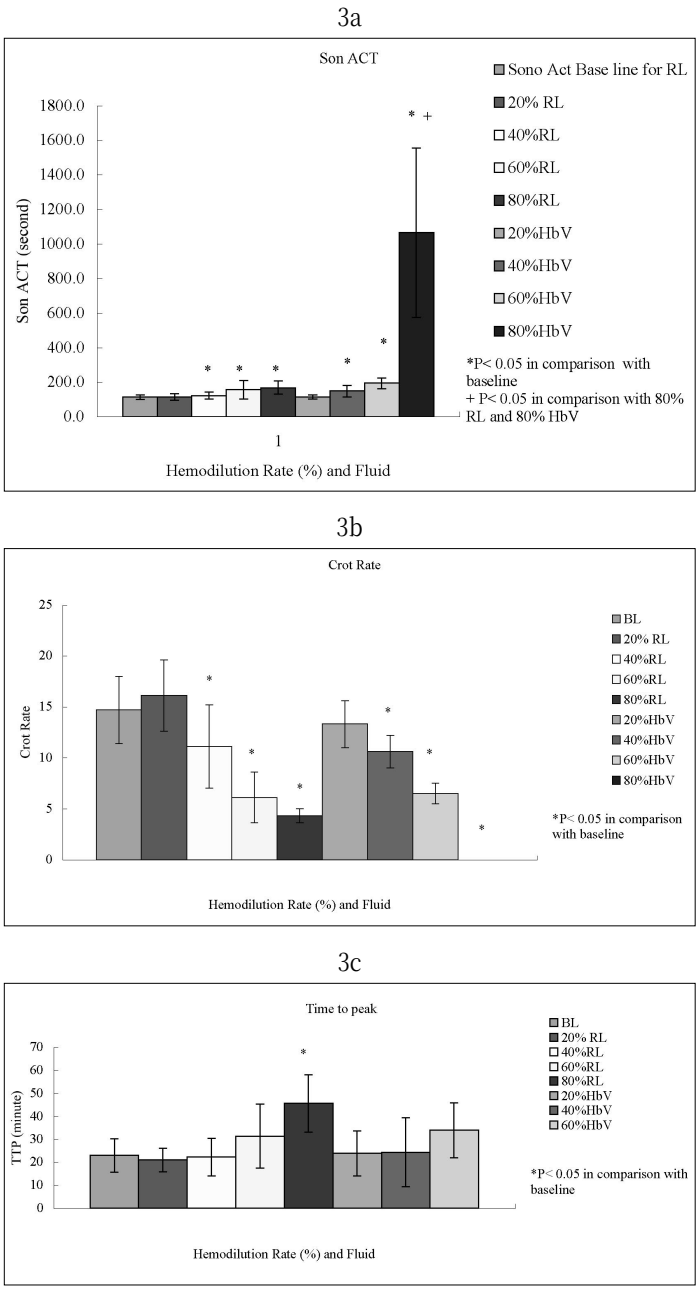

Fig. 3. SONOCLOT values of diluted samples with isotonic Ringer's lactated solution (RL) or 10 g/dL Hemoglobin-Vesicles solution (HbV). Activated coagulation time (ACT) and clot rate (CR) was prolonged in the 40% and 60% dilution with LR and HbV compared with baseline values, but there was no significance between the two groups (3a, 3b). In the 80% dilution by both solutions, ACT and time to peak was affected in comparison with baseline (3a, 3c).

６時間以内に投与された赤血球の保存期間の長さ（古さ）が外傷後MOFの発生に対する独立危険因子であった[50]．外傷後MOF発生のハイリスク患者では，循環中の好中球はプライミングされていてアポトーシスに抵抗性を示し[51]，血管内皮細胞のIntercellular adhesion molecule-1（ICAM-1）の発現が増加する．この原因の一つには，貯蔵赤血球バッグに混入する白血球があげられる[52]．そして，保存血の保存期間が長くなればなるほど，白血球や血小板由来の活性化物質は増加することが報告された[53]．さらに，貯蔵赤血球中の血漿と脂質は実験的に急性肺障害を生じることが示された[54]．

HbVの肺への影響

我々は，出血性ショックモデルに対するHbV投与後の肺への影響を調査し報告した[55]．週齢10-13週の雄Sprague-Dawleyラット72匹を対象とした．１）（単純投与）まず，36匹を自発呼

吸でセボフルレン麻酔下に，11.2 mL/kgの生理食塩水，または，生理食塩水に分散した10%HbV溶液を30分で経静脈投与した．投与後２時間，24時間，72時間に犠牲死させ，肺を摘出した．２）（出血性ショック後輸液蘇生）次に，36匹を自発呼吸でセボフルレン麻酔下に，28 mL/kgの血液を20分以上かけて脱血し，安定させた．その後，脱血量と等量の生理食塩水に分散した10%HbV溶液（HbV群），または，脱血量の３倍量の乳酸リンゲル液（RL群）で輸液蘇生を行った．ベースラインと脱血直後，蘇生後１時間，２時間後の血行動態とヘマトクリット値，BE，血中乳酸値を測定した．輸液蘇生後2時間，24時間，72時間に犠牲死させ，肺を摘出した．肺のhypoxic inducible factor 1 alpha subunit（HIF-1α），tumor necrosis factor alpha（TNF-α），interleukin-6（IL-6），heme oxygenase- 1（HO-1），inducible nitric oxide synthase（iNOS），intercellular adhesion molecule 1（ICAM-1）のmRNAの発現をRT-PCR法で測定した．脱血直後の平均動脈圧は，両群間で差はなかった（RL群33 ± 4 mmHg，HbV群30±3 mmHg）．蘇生後のヘマトクリット値は，1時間後，2時間後共に，HbV群の方が有意に高かった．蘇生後1時間後の血中乳酸値はRL群の方が有意に高かった．単純投与後は，いずれの測定時点でも，我々の測定したmRNAの発現には影響しなかった．HbV群は，RL群に比べ，投与２時間後のHIF-1αとIL-6が抑制されており，HO-1とTNF-αがより発現していた．また，72時間後のHO-1の発現が増加しており，IL-6の発現が抑制されていた．ICAM-1は単純投与でも出血性ショック後輸液蘇生でもHbV投与に影響を受けず，肺への血管内皮細胞への影響はないことが示唆された．また，Hb自体の影響として輸液蘇生直後のHIF-1αの発現抑制とHO-1発現増加が見られたと思われる．

海外でのHBOCsの研究報告と今後の展望

現在，南アフリカでは重合HbであるHemopure（Biopure Corp., Cambridge, MA）が急性出血に対して臨床利用されている．PolyHeme（Northfield Laboratory, Evanston, IL）は，大規模な第３相臨床研究が行われている[56]．基礎研究でも，外傷後MOFモデルとして２イベントモデルを作成し精力的な研究が行われている[57]．最終的な目的は，従来の赤血球輸血に比べてMOFやARDSの発生率を低下させ生存率を改善することだろう．しかし，臨床研究では対象患者の均一化や遺伝子多型性による反応の違い，フィジシャンの違い，目的の難しさなど様々な問題があり長い時間を費やす．HbVを含む人工酸素運搬体の臨床応用は，重大な副作用を来さず，緊急時の酸素運搬体としての簡便な適応に加えて，外傷直後の赤血球輸血を減らすことによるその後の急性肺障害や多臓器不全への発展を予防する可能性がある．血液成分を考えれば，酸素運搬体だけの投与は研究のプロトコルに制限があり，臨床適応するのが難しいと考えられる．このような困難にも関わらず，利用価値のある製品が十分な改良と適応を熟考された上で，臨床応用可能となることはきわめて合理的であると考えられる．我が国で開発されたHbVや海外で臨床治験中や臨床応用されているHBOCsの緊急時や手術中の患者に対する影響を理解することは，新たな種類の特徴ある人工酸素運搬体の有効な臨床応用を可能にするだろう．

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Newer Concepts of Oxygen Transport and Regulation: Relation to Vascular Physiology

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Introduction

Oxygen transport and blood flow regulation are linked by a variety of mechanisms but the linkage is complex and based on the interactions of a number of seemingly unrelated factors. The purpose of this presentation is to provide an overview of this area. As will become apparent in this presentation, the arteriole, which is the primary regulator of blood flow, is acted upon by a variety of stimuli that determine its degree of contraction and relaxation under different conditions.

Chemosensitive Mediation of Blood Flow

The metabolic hypothesis

The simplest relationship between the rate of blood flow and the rate of oxygen transport, is the metabolic hypothesis[1] as shown in Figure 1. This is based on the concept that arterioles possess chemosensitive properties.

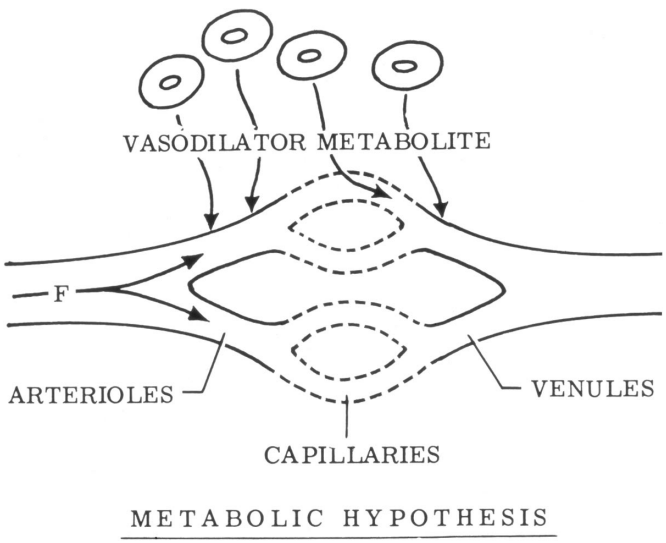

Fig. 1. Schematic diagram of a model of metabolic regulation of blood flow. Details are presented in the text. From (1) by permission.

According to this hypothesis the tissues are constantly producing metabolites that cause vasodilation of the arterioles. If oxygen delivery is adequate, a vasodilator product of aerobic metabolism, CO_2, will be produced as a function of the rate of aerobic metabolism. In turn, as carbonic acid is formed there will be an increase in H^+, which also has potent vasodilator effects/ In this case the rate of blood flow will be determined by the concentration of CO_2 acting on the arterioles. That concentration will be directly dependent on the ratio of oxygen consumption to blood flow. The attractive feature of this hypothesis is its simplicity and its ability to provide a direct linkage between the rate of oxidative metabolism and blood flow. And in fact, certain vascular beds such as myocardium[2] and brain[3] are sensitive to CO_2 and this hypothesis is a plausible possibility to explain, at least in part, blood flow regulation in those organs. However, in other vascular beds such as skeletal muscle there is a also direct relationship between blood flow and oxygen consumption but detailed studies have failed to reveal a significant role for CO_2 in flow regulation[4]. These findings have led researchers to examine other possible mediators which might be produced when the oxygen levels are sufficient overall but in localized areas are insufficient, leading to a shift to anaerobic metabolism in the parenchymal cells.

According to this hypothesis it is proposed that under normal conditions when oxygen levels are sufficient for most regions of the tissue there are certain tissue areas in which the oxygen supply is inadequate[5] causing these areas to rely on mediators such as adenosine and H^+ produced by anaerobic metabolism. Secondarily, depolarization of the parenchymal cells will cause release of K^+ from these cells. (A small increase in extracellular K^+ has been shown to increase K^+ conductance of the plasma membrane, leading to hyperpolarization and relaxation of vascular smooth muscle.) A logical location for a tissue area of anaerobic metabolism is the venous end of the capillary network where the oxygen

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concentration in the blood flowing through the capillary network reaches its lowest level. In addition, it is well known that flow rate of blood through the microcirculatory vessels and hematocrit in these vessels are both highly variable, lending credence to the possibility that such areas might normally be present. If they are present, the release of vasodilator products of anaerobic metabolism would provide a feedback system in which these vasodilator agents would diffuse to nearby arterioles and adjust the state of contraction of the vascular smooth muscle according to the degree of anaerobiosis present.

This hypothesis has been tested in our laboratory by monitoring the level of NADH fluorescence in microregions of the tissues 15 to 25 μm diameter in the vicinity of the capillary network of skeletal muscle as blood flow is reduced or stopped completely for a period of time. A rise in NADH level is taken as an indication of a shift from aerobic to anaerobic metabolism. We found that with complete occlusion of blood flow there was no change in NADH level for an average of 45 s and there was no difference in this latent period between the arteriolar and venous ends of the capillary network[6]. In these studies NADH increases at the earliest in 10 to 15 s after occlusion. When, instead of complete stoppage, blood flow was reduced by 50%, a minimum of 30 s passed before a detectable increase in NADH occurred[7]. Both of these findings suggest that under normal conditions there are no microregions of the tissue in the vicinity of the capillaries that are hypoxic or on the verge of hypoxia.

While these findings do not support the concept that the metabolic state of the parenchyma contributes to blood flow regulation under resting conditions, there is evidence that it is important when oxygen levels become inadequate. We have found that the magnitude of reactive hyperemia (the period of increased flow following a period of blood flow stoppage) is related to the duration of stoppage (unpublished findings). Interestingly, as the period of stoppage is increased from 5 s to 45 s, the amplitude of the reactive hyperemia following restoration of flow increases up to 4 times the control flow but the duration remains relatively constant at about 70s. However, when the flow stoppage exceeds 45 s NADH fluorescence of the tissue begins to increase and the duration of hyperemia increases as a function of the period of stoppage. This finding provides clear evidence that a shift to anaerobic metabolism in skeletal muscle can produce vasodilator metabolites, possibly including H^+, lactate and adenosine, and increase the duration of reactive hyperemia. Whatever the specific mediators may be, it appears that they remain in the tissues until they are reincorporated into the energy supply or washed out by the blood stream. We have some evidence in these studies that washout is more important than reincorporation.

Oxygen-dependent mediators at the arteriolar level

To this point we have focused on factors that may produce vasodilation in relation to the metabolic requirements of the parenchymal cells in the tissue. There are, however, a variety of oxygen-dependent mechanisms at the level of the arterioles that may play key roles in blood flow regulation. These mechanisms adjust the contractile state of the smooth muscle in accordance with the local PO_2.

Microcirculatory studies have revealed that elevation of the oxygen level in suffusing fluid over a surgically exposed tissue reduces blood flow due to constriction of the arterioles[8]. Several mechanisms may be involved as recently reviewed by us[9]. It appears that this response may be due to in part to release of a vasoconstrictor cytochrome P450 metabolite of arachidonic acid, 20-HETE, from the arteriole when arteriolar PO_2 is elevated. There is also evidence that release of vasodilator prostaglandins from the endothelium increases when PO_2 is reduced. Additionally, it has been shown that nitric oxide release from the endothelium increases when oxygen falls below normal levels. Finally, it appears that the endothelium also releases the vasodilator adenosine during hypoxia.

An additional mechanism that may be important is based on the finding that deoxygenation of the red cell may cause relaxation of the arterioles due to release of ATP which acts on purinergic receptors. It has been proposed also that as the red cell is deoxygenated in passage through the capillary bed ATP is released and acts on the venular endothelium to release vasodilator prostaglandins which would in turn diffuse to nearby arterioles[10]. This would provide a feedback arrangement in which greater oxygen extraction from the blood at the capillary level would automatically lead to arteriolar vasodilation and return of blood flow toward normal levels. The overall effect of the oxygen-dependent chemical mediators elicited at various levels as described above is summarized in Figure 2. In this figure the roles of the red cell, endothelium and the parenchymal cells and specific mediators in flow regulation are shown.

Mechanosensitive mechanisms

In addition to the chemosensitive mechanisms described above there is a separate class of important, mechanosensitive mechanisms that respond to physical stimuli. These are the myogenic response which is due to circumferential tension in the vascular smooth muscle

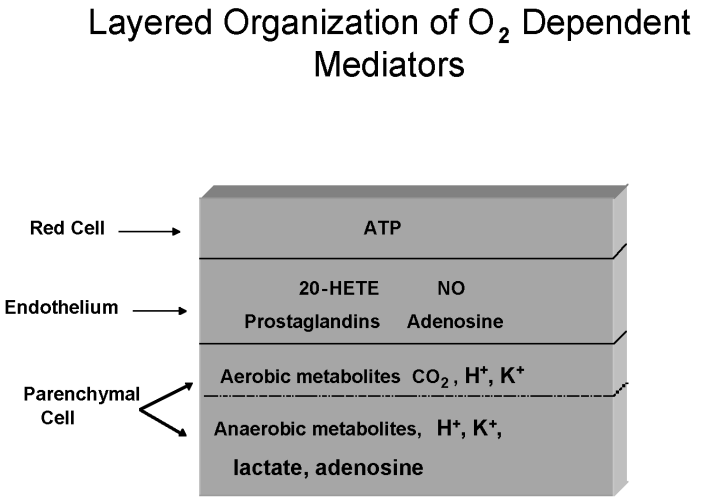

Fig. 2. Overview of chemosensitive factors that influence blood flow, Detail are presented in the text.

created by the intravascular pressure and the flow-induced vasodilation due to the wall shear stress exerted by the flowing blood on the endothelium.

The myogenic response

The myogenic response acts in such as way as to tend to maintain a constant circumferential tension in the arterioles despite a change in intravascular pressure[11]. A model description of this mechanism is shown in Figure 3[12]. Conceptually this mechanism considers the vascular smooth muscle cell to consist of a contractile element in series with a passively distensible sensor element. It is proposed that as the sensor element is stretched it causes depolarization of the smooth muscle cell and excitation of the contractile element. The length of the sensor element and its degree of deformation is dependent upon the circumferential tension (T). Tension is in turn dependent on both the intravascular pressure (P) and the vessel radius (R), according to the Law of Laplace $T = P \cdot R$. Consider now how the arteriole responds when pressure is elevated. As shown in Figure 3, an increase in pressure leads to an increase in vessel radius and deformation of the hypothetical sensor, which in turn causes shortening of the contractile machinery. Note that when the vessel radius returns to its initial level, the wall tension and sensor length are still elevated due to the increased pressure. This causes the smooth muscle cell to shorten further until an equilibrium point is reached at which the sensor is still somewhat deformed at an elevated pressure and as a consequence, the contractile element remains in a shortened state. While the final details are not clear, there is evidence that integrins, which connect the smooth muscle cell to its surroundings, may act as tension sensors to mediate the myogenic response, in part by causing depolarization in the fashion described above.

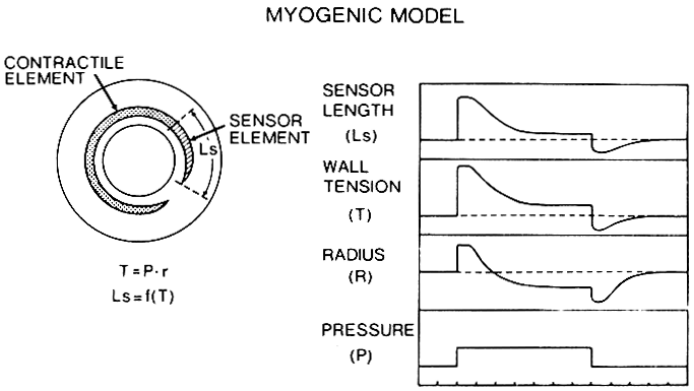

Fig. 3. Schematic diagram of a model of myogenic regulation of arteriolar diameter. Details are presented in the text. From (12) by permission.

The myogenic response is found in virtually all the arterioles in different organs and is one of the primary mechanisms that provides a basal level of vascular tone, that is a partial contraction of the arterioles. Of course the constant discharge of the sympathetic nervous system fibers acting on the arteries and arterioles also is important in providing a continuous state of partial contraction of the smooth muscle[13]. The functional importance of the myogenic response relates to the property shown in Figure 3, namely that it causes vascular tone to be inversely related to intravascular pressure. Experimental studies have shown that this mechanism tends to maintain capillary hydrostatic pressure constant as arterial pressure changes. It also tends to maintain blood flow to an organ constant as arterial pressure changes (autoregulation of blood flow).

Flow-induced dilation

The second mechanosensitive mechanism, flow-induced dilation, is a consequence of the wall shear stress dependent release of nitric oxide (NO) or, in certain vascular beds, prostaglandins, causing vasodilation[14]. This mechanism has been extensively studied and described elsewhere and will not be considered in detail here. Flow-induced dilation is found throughout the arterial and arteriolar networks. It is functionally important in a number of circumstances. For example, at the onset of muscular exercise the release of vasodilator metabolites from parenchymal cells causes relaxation of the arterioles in the muscle and increases flow through the arteries upstream that are outside the muscle

and thus not exposed to the vasodilator metabolites. The increased flow in these arteries increases wall shear stress on the endothelium that then leads to NO or prostaglandin release and dilation.

Influence of the Rheological Properties of Blood

The *chemosensitive* and *mechanosensitive* mechanisms regulating blood flow are influenced by the physical properties of the blood, most importantly the viscosity at the interface between the flow stream and the endothelium since shear stress in the flow stream is maximal at this interface. As blood travels through the vessels the red cells tend to migrate toward the center of the vessel, leaving a cell-free layer near the vessel wall[15]. The cell-free layer will influence the resistance to blood flow directly through its viscosity and also indirectly by influencing the geometric component of the flow resistance through the flow-induced dilation mechanism described above. We have found that the width of the layer increases from about 1 μm in arterioles of 10 μm i.d. to about 3 μm in 50 μm i.d. vessels (unpublished findings).

As mean width of the cell-free layer increases, the flow resistance would be reduced since viscosity in the cell-free layer is lower than in erythrocyte core. On the other hand, the irregular surface of the interface between the cell-free layer and erythrocyte core has been shown theoretically to increase the flow resistance[16]. Secondly, it has been shown in theoretical studies that an increase of the cell-free layer width would reduce NO scavenging by the red cell core[17], leading to a higher concentration of NO in the vicinity of the vascular smooth muscle, which would cause vasodilation. This observation suggests that the width and form of the cell-free layer near the vascular wall plays a significant role in blood flow regulation.

Effects of Blood Substitutes on Flow Regulation

The contribution of the cell-free layer to flow regulation may have significant implications for blood substitutes which generally consist of a solution containing hemoglobin in some form that increase the volume of the cell-free layer relative to the red cell component and reduce hematocrit. Since the width of the cell-free layer is inversely related to hematocrit, the increased width would reduce the scavenging of NO by the red cells. However, this effect may be partially offset by increased scavenging of NO if the blood substitute is hemoglobin-based.

At the systems level it is well known that reducing the oxygen level in the blood by reducing the PO_2 of the inspired gas has profound effects on the cardiovascular system, causing a reduction in arterial pressure and blood flow,[18]. These changes accentuate the local effects of the hypoxemia itself. It seems likely that blood substitutes would also introduce systemic as well as local effects.

From the considerations presented above, it is evident that the physiological effects of blood substitutes go well beyond those due to simply restoring the oxygen carrying capability. As a consequence, in designing a blood substitute its effects on the local regulatory mechanisms and the stimuli to which they are sensitive must be carefully considered.

Summary

In summary, blood flow to individual organs is determined largely by local regulatory mechanisms that respond to a variety of influences at the level of individual arterioles. These mechanisms fall generally into two main categories, chemosensitive and mechanosensitive. Chemosensitive mechanisms respond to changes in the environment of the arteriole and of the parenchymal cells as determined by oxygen levels in these regions. Mechanosensitive mechanisms respond to wall shear stress produced by the cell-free layer of the flow stream acting on the endothelium and circumferential stress acting on the vascular smooth muscle. Blood flow through each arteriole is determined by the summated effect of the stimuli presented to the arteriole by these operative mechanisms. In turn the organ flow represents the net effect of these independent processes occurring in individual arterioles.

Cardiac output then represents the summation of literally millions of the individual processes within each organ. In other words, cardiac output in the steady state situation is determined by the demand of the individual organs and not by the command of central mechanisms. When blood substitutes are introduced into the circulation local control mechanisms are significantly affected through changes in oxygen delivery, intravascular pressure, blood viscosity, wall shear stress and NO scavenging by the blood substitute and the red blood cells. A better understanding of the effects of blood substitutes on the chemo- and mechanosensitive factors that control blood flow through their effects on the arteriole may aid in developing more effective blood replacements.

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Review

Polyethylene Glycol Conjugated Albumin: A New Generation Plasma Expander

Nanae Hangai-Hoger and Marcos Intaglietta

Abstract

Polyethylene glycol conjugated albumin (PEG-Alb) in either normal saline or Ringer's lactate at concentrations in the ranging from 2.5 to 4.0 g/dl is a non-immunologic, non-antigenic plasma expander that has a long intravascular permanence and maintains central blood pressure, blood flow, and functional capillary density in extreme hemodilution, hemorrhagic shock and endotoxemia. It has longer lasting effects (half life of the order of 24 hours) and is more efficacious than colloids such as starch, albumin and dextrans and has the specific of restoring and improving microvascular function. These properties suggest that its use in critical illness and resuscitation can delay the use of blood transfusions, thus extending the transfusion trigger to lower hemoglobin levels.

Keywords PEG-Albumin, plasma expanders, shock, extreme hemodilution, endotoxemia, functional capillary density

INTRODUCTION

The need for an optimal plasma expander is as essential as that for a blood substitute. Factors that define an optimal plasma expander are the ability to sustain volume expansion for a prolonged period, maintenance of central blood pressure, lack of red blood cell (RBC) aggregation, effectiveness at low concentration and maintenance of tissue perfusion. How to achieve these properties has been a subject of controversy particularly regarding the viscosity, colloid osmotic pressure (COP) and type of material needed to obtain properties that insure adequate organ perfusion. Maintenance of tissue perfusion is probably the most important parameter in classifying the effectiveness of a plasma expander. Tissue blood flow is determined by perfusion pressure, vascular resistance and blood viscosity, factors that in rigid tubes are related by Poiseuille's equation. However, the introduction of plasma expander may lead to in vivo changes in oncotic and osmotic pressure and blood viscosity that interact with regulatory processes of peripheral vascular resistance arising from changes in the production of vasoactive mediators by the endothelium and their transport in the circulation.

Introduction of a plasma expander invariably reduces hematocrit (Hct), and unless its fluid viscosity is similar to that of blood it reduces blood viscosity. The reduction of blood viscosity (hemodilution) has been regarded as beneficial since antiquity. Currently the limit of hemodilution is the condition when perfusion and oxygen delivery are no longer able to maintain tissue metabolism, a point termed the transfusion trigger, where restoration of blood oxygen carrying capacity is considered necessary. Studies by Tsai et al. show that as blood viscosity is reduced by hemodilution, microvascular function is progressively impaired, jeopardizing tissue survival due to the local microscopic maldistribution of blood flow[1]. These effects take place at Hcts that are greater than those defining the oxygen supply limitation, a finding that leads to the hypothesis that the limit of hemodilution could be significantly decreased if the process of plasma expansion maintains microvascular function.

Recent studies show that microvascular function can be maintained in extreme hemodilution by increasing either blood or plasma viscosity[1-3]. Restoration of blood viscosity during hemodilution and hemorrhage is desirable, because it maintains functional capillary density (FCD), defined as the number of capillaries with passage of RBCs per unit surface of the field of view of a microscopically observed tissue. This microvascular parameter was found to be critical in defining tissue survival by Kerger et al.[4], who showed the direct correlation between maintenance of FCD above a specific

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threshold and survival in extended hemorrhagic shock[4]. FCD is also determined by the maintenance of capillary pressure, which in extreme hemodilution is obtained by using high viscosity plasma expanders[1].

The blood viscosity threshold that causes the decrease in FCD appears to coincide with the decision of transfusing blood. Therefore, the transfusion trigger may also be a "viscosity" trigger, and some of the results obtained with a blood transfusion may also be achieved by increasing plasma viscosity. Thus use of RBCs for the purpose of increasing blood viscosity is unnecessary if a material is introduced that increases plasma viscosity in the circulation. In this context it would seem that a desirable property for plasma expanders is that of increasing blood viscosity.

The viscosity of plasma expanders

The viscosity of plasma expander is determined principally by the size of the colloidal component and its concentration. Historical non-crystalloid plasma expanders were formulated to limit their viscosities. Gelatin, dextran and starch solutions were formulated in concentrations leading to viscosities in the range of 2 cp, prior to administration. The inherent dilution of the material upon their introduction into the circulation determine that final plasma viscosity was not very different from normal, i.e., about 1.0 to 1.2 cp. The viscosity of a colloidal solution is determined by both the number of particles per unit volume, and the molecular volume of the solute. Therefore augmenting concentration is not a mechanism for increasing viscosity of plasma expanders since this increases COP, bringing interstitial fluid into the circulation, diluting the material thus lowering viscosity, a self-limiting process.

A different approach for increasing solution viscosity is to increase molecular dimensions. Several molecular species have been proposed and tested as a basis for plasma expanders, including poly-vinyl pirrolidone (PVP), high molecular weight dextrans and starches, keratins, alginates and polyethylene glycol conjugated albumin (PEG-Alb).

With few exceptions high molecular weight material such as PVP, starches, dextrans and keratins tend to cause RBC aggregation when Hct is near normal. Alginates and PEG-Alb do not cause RBC aggregation and have similar properties since both materials trap a large amount of water in their molecular structure, which causes the increase of their effective dimensions. These materials have very different plasma expansion characteristics since alginates have COP of 2 - 3 mmHg when used at physiological concentrations (in the range of 0.7 - 0.8% by weight) while current formulations of PEG-Alb at about 4% concentration have COP of about 60 mmHg. At present alginates have not been extensively studied, while there is substantial information on the physiological characteristics on plasma expansion by PEG-Alb, also because it has the same biophysical properties as PEG-Hemoglobin, a material presently in clinical trials as an oxygen carrying blood substitute.

PEG-Albumin formulation and properties

PEG-Alb is formulated with either bovine or human albumin using similar procedures. Polyethylene glycol (PEG) has been used for modifying of proteins, peptides, enzymes and liposomes to extend plasma half-life, eliminate/lower toxicity, immunologic reactions and antigenicity, increase solubility in water and provide increased thermostability[5-9].

PEG-Alb is produced by conjugating the protein with PEG using a single step version of the thiolation mediated, maleimide chemistry based conservative PEGylation described by Acharya et. al.,[10]. Albumin (0.25 mM) is incubated with 5 mM 2-iminothiolane (BioAffinity Systems, Rockford, IL) with 7.5 mM maleimide phenyl PEG-5,000 in PBS overnight. The surface amino groups are thiolated and thiol groups generated on the protein in situ are derivatized by the maleimide PEG in the reaction mixture. The single step reaction used limits the oxidation of the thiols of the thiolated protein, and is a general approach for producing PEGylated proteins. Excess reagents are removed by tangential flow filtration after the overnight incubation and a 70 K membrane is used for diafiltartion and removal of unreacted PEG and excess immunothiolane. The material is prepared in concentration ranging from 2 to 4 g/dl (protein based). This chemical modification leads a colloidal osmotic pressure 40 mmHg, and a viscosity of 2 cp for a 2.5 g/dl concentration in saline.

The PEG molecule has the property of trapping a significant amount of water in its vicinity and a few PEG polymers attached to the surface of a protein trap a layer of water on it surface. This process causes the hydrodynamic dimensions of the protein to increase substantially augmenting its intrinsic viscosity and the COP of the solution. Biologically the water layer renders the molecule undetectable by receptors and the immune system which endows PEG treated molecules with "stealth" or "immunosilent" properties.

PEG-Alb is usually formulated with an average of 6-12 copies of PEG 5 kDa resulting in a molecular radius of the order of 8-9 nm vs. a radius of 2.5 nm for the natural albumin molecule. Such a large radius would cause a significant increase in viscosity, however the viscogenic properties are comparatively moderate, and changes in viscosity are not maintained when the material is introduced into the circulation because the high COP dilutes PEG-Alb to the extent that plasma concentrations are seldom beyond 1%, leading to plasma viscosities of 1.3 cp vs. the normal plasma viscosity of 1.2 cp.

At present there are no known biological or functional differences between PEG-Alb made with human, bovine or recombinant albumin, also because the PEG conjugation isolates the protein from the immune system.

PEG-albumin in extreme hemodilution

Extreme hemodilution is a condition that may be defined as the dilution of blood with plasma expanders beyond the transfusion trigger. It is seldom attained in clinical conditions unless continued blood losses are corrected with plasma expanders in the attempt to manage blood volume when blood is not available. Physiologically it is defined by the blood oxygen carrying capacity beyond which oxygen consumption becomes dependant on hemoglobin concentration. This critical condition provides an important experimental benchmark for studying the efficacy of blood substitutes, because changes in the circulating blood properties due to the presence of a test material clearly defines its effectiveness in either sustaining, improving or deteriorating systemic and microvascular parameters over a very narrow range of changes in blood composition. This acute anemic condition magnifies the effects of test compound since in this condition the organism is only marginally capable of compensating for changes in blood properties.

Cabrales et al., made extreme hemodilution experiments via a two step procedure with 6% dextran 70 (Dex70) to a Hct～18% and then hemodiluting to Hct 11% using 5% human serum albumin (HSA), 4% PEG-Alb (HSA)(MPA), Mal-PEG-hemoglobin, (MP4, P_{50} = 5.4 mmHg, Sangart Inc., San Diego, CA) and 6% Dex 70[11]. Systemic findings were that PEG-Alb provided a greater blood pressure than both HSA and Dex 70, and increased cardiac index 44% above baseline (no hemodilution). Notably systemic peripheral vascular resistance for PEG-Alb was 70% of that for the non PEG materials. Microvascular conditions were significantly improved for PEG-Alb since flow was normalized and FCD was above 60%, but not for Dex 70 and HSA whose flows were significantly below normal and FCD was below 50%.

These results should be in part due to the normalized oxygen delivery to the heart, since the microvascular conditions found in the window chamber tissue should be common to those of other tissues, as shown by a recent study of where organ flow distribution was measured[12] in extreme hemodilution. The results obtained appear to be primarily due to vasodilatation induced by PEG-Alb, while the opposite effect was seen for Dex 70 and HSA as shown in Figure 1. Vasodilatation and the significant decrease in peripheral vascular resistance may be also the reason why mean arterial blood pressure did not attain baseline values.

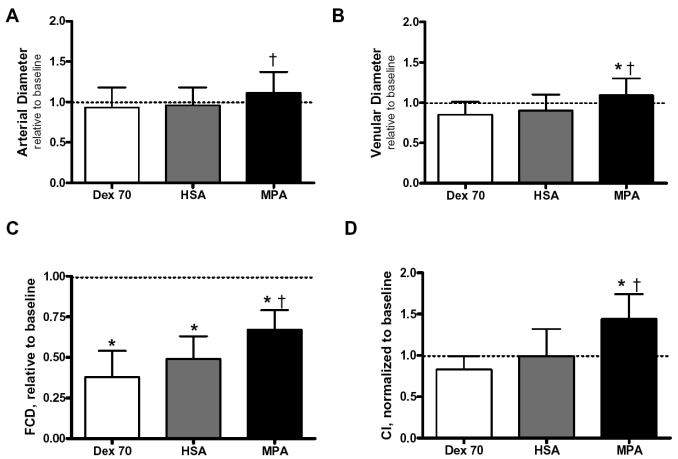

Fig. 1. Extreme hemodilution to Hct 11% with 4.2% PEG-Alb compared to 6% Dextran 70 kDa (Dex 70) and 5% human serum albumin HAS. Vessel diameter, functional capillary density and cardiac index after level 3 exchange are shown relative to baseline. There was no statically significant difference in plasma and blood viscosity between groups. Plasma COP was 21.4 ± 0.9 mmHg for PEG-Alb, 20.3 ± 1.1 mmHg for HAS, and 16.8 ± 0.8 for Dex 70. $P < 0.05$ vs. baseline (*), $P<0.05$ vs. Dextran 70 (†). Data from Cabrales et al., 2005.

PEG-Alb produces two vasodilatory effects, one radicated in the arteriolar and venular circulation and a second manifested by the increase in FCD. The former is clearly a consequence of a vasoactive phenomenon, since it is related to the reactivity of smooth muscle, which PEG-Alb causes to relax. The increase or maintenance of FCD is passive effect due to the transmission of blood pressure to the capillary network, which follows from the absence of vasoconstriction in the arteriolar circulation. A likely mechanism behind the vasodilatory effect of PEG-Alb should be NO management by PEG-Alb.

Nitric oxide mechanism and PEG-Alb

A mechanism that could in principle account for a vasodilatory effect is mechano transduction resulting from the maintenance or restoration of shear stress on the endothelium. In extreme hemodilution where PEG-Alb appears to be effective in maintaining flow and microvascular function shear stress is low due to the significant decrease in blood viscosity and the prevailing low flow conditions present with the use of low viscosity plasma expanders. Shear stress is a mediator of the production of NO by mechano transduction by the endothelium[13, 14], therefore increasing flow and viscosity should increase production of NO by the endothelium, an effect that was demonstrated by Tsai et al., using the high viscosity plasma expander Dextran 500 kDa. This effect has increased effectiveness in extreme hemodilution because the significant decrease in hematocrit lowers the NO scavenging properties due to the decrease of total hemoglobin in blood, near the source of production in the endothelial layer[15].

The mechano transduction hypothesis has been demonstrated for high viscosity plasma expanders such a dextran 500 kDa in extreme hemodilution[1], alginates in hemorrhagic shock[16], and the use viscogenic RBCs that do not carry oxygen (hemoglobin converted to CO-hemoglobin) in hemorrhagic shock, and therefore achieve resuscitation solely by virtue of their enhancement of blood viscosity[17]. However, the viscogenic properties of PEG-Alb are not sufficient to increase plasma viscosity significantly, also because the high COP dilutes the protein to the point that it is seldom possible to attain plasma concentrations greater than 2 g/dl. In summary, the mechano transduction hypothesis works when viscosity can be increased without increasing COP, which is not the case for PEG-Alb.

PEG-Alb appears to be unique in causing vasodilatation in conditions of anemia, low viscosity, and low oxygen tension. It is significant that the chemical process that leads to pegylation of albumin requires the thiolation of the surface amino groups leading to the production of extra thiol groups on the protein surface. Thiol groups have been implicated with the transport of NO from high concentration regions of the circulation, like the aortic wall, to the lower NO concentration regions of the microcirculation[18]. In this process PEG-Alb could provide a source of extra thiols if the chemical process of pegylation does not neutralize all active sites generated on the albumin by thiolation. These mechanisms have been proposed to occur in normal blood and to be a part of the regulatory control of ischemia via dilatation and increased perfusion[3]. According to this mechanism, the same process may be enhanced by the presence of unreacted thiols on the surface of PEG-Alb. Therefore the unusual vasodilatory capacity of PEG-proteins the enhancement of an NO transporting mechanism may be due to pegylation via thiolation.

PEG-albumin in hemorrhagic shock

Hemorrhagic shock results from the loss of circulatory volume and oxygen carrying capacity, causing increased heart rate, vasoconstriction, redistribution of blood flow away from nonvital organs and the decrease in capillary perfusions and FCD. Restoration of lost volume with plasma expanders is the initial therapy followed by blood transfusion.

Cabrales et al. compared the plasma expander HES 200 (Hydroxyl ethyl starch, Pentaspan, B. Braun, Medical , Irvine CA, 10% w/v COP 85 mmHg, viscosity 3.4 cp) with PEG-Alb (2.5 g/dl, COP 38 mmHg viscosity 3.4 cp) in a conventional shock protocol where 50% of blood volume is removed in 5 min, and 25% of blood volume is returned during resuscitation[19]. This study showed that PEG-Alb and HES provide initial identical systemic and microvascular recovery for about 15 min after resuscitation, when a trend started leading to statistically significant higher flow, mean arterial blood pressure, FCD and tissue pH for PEG-Alb in 30 min (Figure 2). Mechanistically the difference in recovery was related to the of lack of sustained recovery of arteriolar flow and FCD hindering the washout of metabolites from the tissue, preventing lactic acid from returning to normal levels causing the incomplete restoration of positive base excess and limiting the normalization of pH. The improved resuscitation found with PEG-Alb vs. HES should also be due to effects in the heart muscle and sustained improved cardiac function, as evidenced by the extended maintenance of blood pressure and improvement of arteriolar flow.

The difference in outcome could be due to the significant longer retention time of PEG-Alb a monodisperse material with molecular weight 130 kDa, vs. HES broad spectrum of molecular weights with a significant portion of material < 200 kDa. An alternative and/or complimentary explanation for these effects is that PEG-Alb caused a thiol mediated improved distribution of NO, leading to vasodilatation, improved flow and sustained cardiac function and blood pressure, as seen in extreme hemodilution, since there was no difference in blood viscosity, and plasma viscosity was 1.4 cp which is below the level needed to sustain FCD ranging from 1.8 cp[17] to 2.2 cp[1].

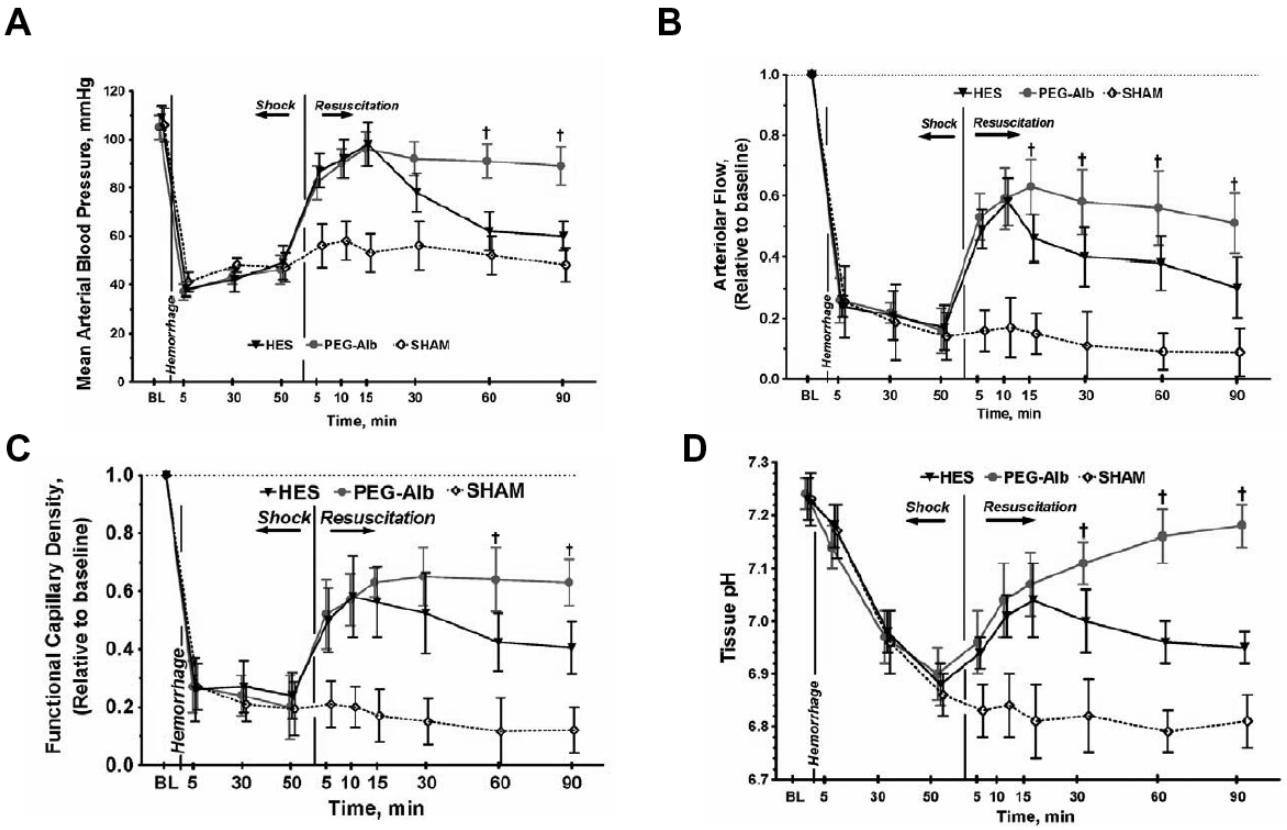

Fig. 2. Hemorrhagic shock resuscitation with 2.5% PEG-Alb vs. 10% hydroxyl ethyl starch, HES 200 (Pentaspan, B. Braun, Medical , Irvine CA). Systemic and microvascular parameters were maintained by PEG-Alb through the observation period. HES produced an initial recovery that was identical to PEG-Alb, but this was not sustained beyond the initial 15 min resuscitation period. Microvascular parameters diverged ahead to systemic parameters by 5 minutes after resuscitation. $P<0.05$ between HES and PEG-Alb (†). Data from Cabrales et al., 2005, reprinted by permission.

The significance of restoring oxygen carrying capacity in resuscitation from hemorrhagic shock was illustrated in the study of Wettstein et al. who used the conventional shock model resuscitated with PEG-Alb only and PEG-Alb and RBCs up to a concentration of 8 g/dl hemoglobin in the resuscitation fluid[20]. In this study, microvascular blood flow and FCD was significantly improved by avoiding the transfusion of additional RBCs while increasing the fraction of PEG-Alb solution in the resuscitation fluid. However, the highest oxygen delivery was obtained using PEG-Alb with 8 g/dl of hemoglobin in RBCs. As expected animals receiving RBC transfusion had higher oxygen tensions, however resuscitation without additional RBCs extracted a higher percentage of the available oxygen. A conclusion from this study is that microvascular recovery is a strong function of the biophysical properties of the plasma expander, and independent from the restoration of oxygen carrying capacity.

The relevance of oxygen carrying capacity in shock resuscitation was also tested by Wettstein et al., in the standard shock model using PEG-Alb and an identically configured PEG-Hemoglobin (PEG-Hb), where the latter is a molecule with a high affinity for oxygen, having a p50 of 5.4 mmHg[21]. These molecules, which are biophysically identical were used in the same concentration (4.2 g protein/dl), which was diluted to about 1 g/dl upon introduction into the circulation. This study showed that volume restitution in the hamster window chamber model of hemorrhagic shock did not show any microvascular or systemic functional differences between molecules, with identical recovery of systemic blood pressure, acid base balance and FCD. As expected oxygen delivery and consumption by PEG-Alb was significantly lower than that obtained with PEG-Hb, and tissue pO_2 was also significantly lower. Therefore this comparison further supports the concept that the characteristics of the plasma expander are critical for the recovery of hemodynamic parameters, which is not

necessarily related to the restoration of oxygen carrying capacity.

PEG-Albumin for the treatment of sepsis

Sepsis is an inflammatory response that produces proteolytic enzymes and oxygen metabolites that cause tissue damage. This damage is prevented by antioxidants, capable of blocking or inactivating the noxious products of inflammation. Sepsis can also be accompanied by hypotension, a condition called septic shock where, prior to the systemic circulatory collapsed there is wide spread microcirculatory impairment and tissue hypoxia leading to organ failure. The conventional therapeutic approach based on the VIP principle (ventilate, infuse, and pump)[22] is deployed in the early stage of sepsis.

We studied the effects infusing a PEG-Alb solution administered as 30 % of blood volume in a hypervolemic bolus in the hamster chamber window model of LPS induced endotoxemia[23]. This model showed a significantly decreased FCD (less than 10% from baseline) at 6 hrs after LPS injection, which did not recover during observation period (24hrs) without treatment. Fluid resuscitation was begun in early stage of endotoxemia as recommended using PEG-Alb infused with either 16 ml/kg (PEG-Alb-16) or 24 ml/kg (PEG-Alb-24) concentration intravenously during 1 hour, and compared this to Dex 70 (6% wt/vol molecular weight 70 kDa B. Braun Medical, Irvine, CA) infused at 24 ml/kg hr.

This study showed that treatment with PEG-Alb restored impaired microvascular function by increasing FCD and tissue pO_2 to near normal levels, while lowering perivascular NO concentration when compared to treatment using dextran 70 kDa. Endotoxemia significantly increased perivascular NO in arterioles and venules, which was also reduced by the increased perfusion due to PEG-Alb-24 treatment. There was also a significant decrease of perivascular tissue pO_2 at all time points of observation, which was in part corrected by the increased perfusion due to PEG-Alb-24, but not by dextran treatment. In a separate study recombinant human serum albumin (Nipro Medical Corp., Osaka, Japan) was used in the same protocol, which provided a similar recovery of FCD at 6 hrs after LPS as, PEG-Alb, however the latter had significantly better outcome after 12 hrs (Figure 3)[24].

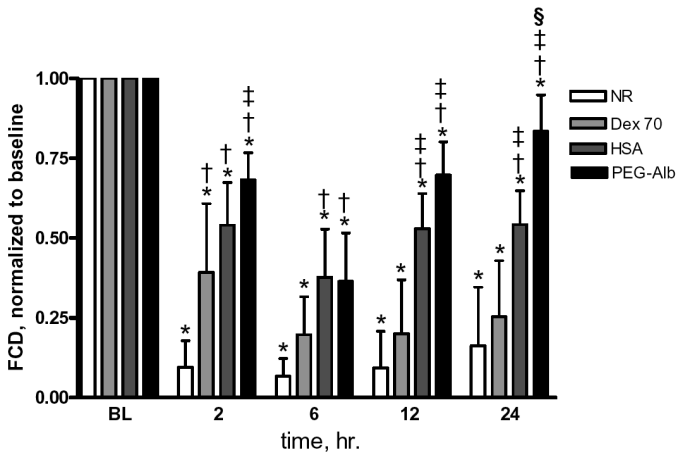

Fig. 3. Treatment of endotoxemia. No treatment (NR) reduced functional capillary density (FCD) to below 10% at 6 hr point, and remained below 20% of baseline for a 24 hr. period. Fluid resuscitation consisted in the infusion of 24 ml/kg/hr for one hour of 6% Dextran 70 kDa (Dex70) or 8.3% recombinant HAS (rHSA), and 2.5% PEG-Alb (PEG-Alb). Resuscitation with Dex 70 increased FCD, only at 2 hrs. rHSA increased FCD, which reached a maximum at 12 hrs and remained at this level until the end of observation period. PEG-Alb improved FCD gradually until the end of the experiment. $P<0.05$ vs. baseline (*); $P<0.05$ vs. NR (†); $P<0.05$ vs. Dex70 (‡); $p<0.05$ vs. rHSA (§). Data in part reported by Hangai-Hoger et al., 2006.

Microcirculatory impairment in sepsis is correlated with increased tissue NO concentration induced by iNOS[23, 25]. NO is known to have a dual personality[26] whereby NO induced by eNOS at physiological levels has beneficial circulatory effects, while NO induced by iNOS causes circulatory impairment, its level being 100-1000 times higher which is pathological. In the hamster model perivascular NO concentration was increased to mM level (nM being normal) and PEG-Alb resuscitation decreased NO to normal levels, an effect probably resulting from the combined action of PEG-Alb maintain microcirculatory flow and FCD, increasing the availability of RBC hemoglobin for scavenging NO. Likewise the mechanism of NO thiol transport may also have been operational, in a reverse mode, whereby NO is uploaded in the microcirculation and distributed to organs with lower iNOS activity.

CONCLUSIONS

Fluid therapy in critical care is still a matter of controversy. Colloidal solutions have advantages over crystalloids because they are effective in lower volumes. However, colloids are expensive, present the risk of allergic reaction and coagulopathic effects. Notably PEG conjugation of colloids should reduce this potential toxicity while also reducing the total amount of protein administered.

PEG conjugated colloids particularly albumin and hemoglobin have excellent plasma expansion properties, thorough effects that are not explained by their biophysical properties, also because these materials have high COPs causing their concentration in the circulation in most resuscitation scenario to be too small to affect blood viscosity, shear stress and mechano transduction. Analysis of the available data shows that both PEG-Alb and PEG-Hb cause vasodilatation in extreme hemodilution, which in the absence of mechano transduction would appear to be mediated via NO transport.

An important feature of PEG-colloids is that they have large hydrodynamic radii increasing their intravascular retention time, since they cannot be filtered into the tissues. Even though it would be in principle possible to simply provide albumin with extra thiols to obtain the NO mediated vasodilatation, this materials would extravasate according to the exchange transport dynamics of natural albumin which has a significantly lower intravascular residence time. In principle any molecular species of sufficient size including dextrans and starches could serve as a frame to support PEG molecules, however albumin (and hemoglobin) have the desirable feature of being uniform in dimensions with a predictable number of surface molecular structure for attaching PEG via thiolation.

It is not clear at this time what will be the economic scenario for PEG-Alb, however it is likely that it will be at least as costly as the basic colloid. This problem however should be compensated by its unique volume expansion and microvascular function restoration and enhancement properties, coupled with its effectiveness at weight concentrations that are significantly smaller than the unmodified colloid. An additional economy inherent to the use of this new type of plasma expander is that its ability of maintaining microvascular function allows extending the RBC transfusion trigger.

It is likely that PEG-HSA will be effective in extending the transfusion trigger; however an oxygen carrier is ultimately required when blood losses are extensive. Therefore it may be effective to consider the use of a plasma expender like PEG-Alb in combination with a material such as HbV (hemoglobin vesicles) which carries oxygen but like RBCs has no COP per se, and requires an appropriate fluid vehicle.

ACKNOWLEDGEMENTS

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Review

Hemoglobin Vesicle Aids Recovery of Cardiac Function during Ischemia-Reperfusion in Langendorff Perfused Rat Hearts

Tadashi Yamagishi, Motoaki Bessho, Miyuki Hama, Ryuichi Katoh,

Shigeki Yanagida, Masatoshi Kusuhara, Fumitaka Ohsuzu

Abstract

Background. - Formed from a lipid bilayer membrane, hemoglobin vesicle (HbV) is a small sphere (diameter ca. 250 nm) which contains hemoglobin, and is a candidate blood substitute. In this study, we examined whether HbV influences cardiac function during ischemia-reperfusion.

Methods. - Rat hearts were perfused according to the Langendorff method and subjected to 30 min of global ischemia and 30 min of reperfusion. HbV was made into a dispersion and diluted with Krebs-Henseleit buffer to achieve hemoglobin concentrations of 0.33 g/dL and 0.10 g/dL, and the hearts were perfused with this dispersion for 10 min immediately prior to ischemia. The same experiment was performed using the empty vesicle (EV; no hemoglobin). Cardiac functions were continuously monitored and coronary effluent collected every 5 min throughout the experiment.

Results. - In the HbV groups, between 10 and 30 min of reperfusion, there was a significant recovery in heart rate (to virtually pre-ischemia levels) as compared to the control group ($p<0.05$). A rise in endodiastolic pressure during reperfusion was significantly suppressed in the HbV group (30-60 mmHg) as compared to the control group (70-100 mmHg)($p<0.05$). In the HbV groups, there was a significant recovery in left ventricular developed pressure (LVDP) between 20 and 30 min of reperfusion, as compared to the control group ($p<0.05$). After 5 min of HbV perfusion and at 1 min of reperfusion, the lactate concentration in the coronary effluent was significantly lower in the HbV 0.33 g/dl group than in the control group ($p<0.05$).

Conclusion. - These results suggest that HbV changed cardiac metabolism before and during ischemia, and as a result, enhanced recovery of cardiac function during reperfusion.

Keywords hemoglobin-vesicle, cardiac functions, lactate, ischemia-reperfusion

Introduction

Artificial red blood cells have the following features that reduce problems in clinical blood transfusions: (1) no need for time consuming cross-matching or typing, (2) no need for refrigeration, and (3) no potential as infectious agents. Therefore, artificial red blood cell substances have been widely investigated as potentially useful blood substitutes since the 1960s[1]. They include perfluorochemicals, various types of chemically modified hemoglobin, and recombinant human hemoglobin[2-3], and are more simple oxygen-carriers than artificial red blood cells. In 1993, Takeoka et al.[4] developed a method for the production of hemoglobin vesicles covered with a lipid bilayer membrane that could serve as artificial red blood cells. Since then, many studies on the functions[5-6] of hemoglobin vesicles as a blood substitute and their biological safety[7] have been conducted using various animal models. The data obtained in these studies suggested that hemoglobin vesicles had no serious adverse effects on cardiac functions in the models in which they were used but their actual effects on cardiac functions were not clarified. Therefore, in the present study, we examined whether HbV influences cardiac functions during ischemia-reperfusion using isolated rat hearts and the Langendorff perfusion technique. We found that HbV aids recovery of cardiac functions during ischemia-reperfusion in this model.

Materials and Methods

Hemoglobin-vesicle and empty-vesicle

Hemoglobin-vesicle (HbV), a small sphere[8] (diameter ca. 250 nm) formed from a lipid bilayer membrane which

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contains hemoglobin, is supplied by Oxygenix Co., Ltd. as a saline-suspension containing 10 g/dL of hemoglobin. Also supplied as a suspension, empty-vesicle (EV) is identical to HbV except that it does not contain hemoglobin. The HbV suspension was diluted with modified Krebs-Henseleit buffer to final hemoglobin concentrations of 0.33 g/dL (30 times dilution) and 0.10 g/dL (100 times dilution). The EV suspension was diluted in the same manner as HbV with modified Krebs-Henseleit buffer.

Animals and experimental groups

Eight-week-old male Wistar rats were purchased from Charles River Japan Inc. The rats were maintained under specific pathogen-free conditions and a constant dark/light cycle (12 h each) in our animal facility at the National Defense Medical College throughout the experiment. They were given free access to a laboratory chow CE-7 (Clea Japan, Tokyo) and water for a few weeks after purchase. A total of 33 rats were included in the present study and the experiments were performed when they were 9 to 12 weeks old. They were divided into five experimental groups: control group (n = 6), hemoglobin-vesicle 0.33 g/dL group (HbV 0.33 g/dL group, n = 6), hemoglobin-vesicle 0.10 g/dL group (HbV 0.10 g/dL group, n = 7), empty-vesicle 0.33 g/dL group (EV 0.33 g/dL group, n = 7), and empty-vesicle 0.10 g/dL group (EV 0.10 g/dL group, n = 7). All experiments were performed in accordance with the *National Defense Medical College Institutional Animal Care and Use Committee Guidelines.*

Heart preparation and perfusion method

The rats were pre-medicated with heparin (1,000U, i.p.), and 10 min later anesthetized with ketamine hydrochloride (90 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.). The hearts were excised, put into ice-cold modified Krebs-Henseleit buffer (mKH buffer), quickly trimmed, weighed, and perfused according to the Langendorff mode (Fig. 1.). Perfusion was conducted at a constant perfusion pressure of 100 cmH_2O at 37 ℃ with modified Krebs-Henseleit (mKH) buffer solution, which comprised 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, and glucose 11 mM. The experimental buffer solutions used (mKH buffer solution, two HbV containing mKH buffer solutions, two EV containing mKH buffer solutions) were continuously aerated with 95% O_2 + 5% CO_2 and the pH was adjusted to 7.4. Cardiac function was monitored and recorded using a fluid-filled left ventricular balloon in line with a transducer (P-50, Gould Inc.) and a WS-641G multi-channel recorder (Nihon Kohden, Tokyo, Japan). The balloon volume was set to produce a left ventricular end-diastolic pressure (LVEDP) of 0-5 mmHg.

In the control group, each heart was perfused with mKH buffer for 20 min (control perfusion) and then subjected to 30 min of global ischemia by stopping the perfusion. This was followed by 30 min of reperfusion. In the other experimental groups, each heart was subjected to perfusion with mKH buffer for first 10 min of the control perfusion and then perfusion with the respective vesicle containing buffer solution for the remaining 10 min. They were then subjected to 30 min of global ischemia and 30 min of reperfusion in the same manner as for the control group. To measure the coronary flow rate (CFR) and lactate content, the coronary effluent was collected at 5-min intervals during the control perfusion. During reperfusion, it was collected at 1 min and 4 min, and then at 5-min intervals until the end of experiment. After measuring the volume of the effluent, part of it was centrifuged at 10,000 x g for 40 min, and the supernatant was frozen and stored at -80 ℃ until analysis for lactate content.

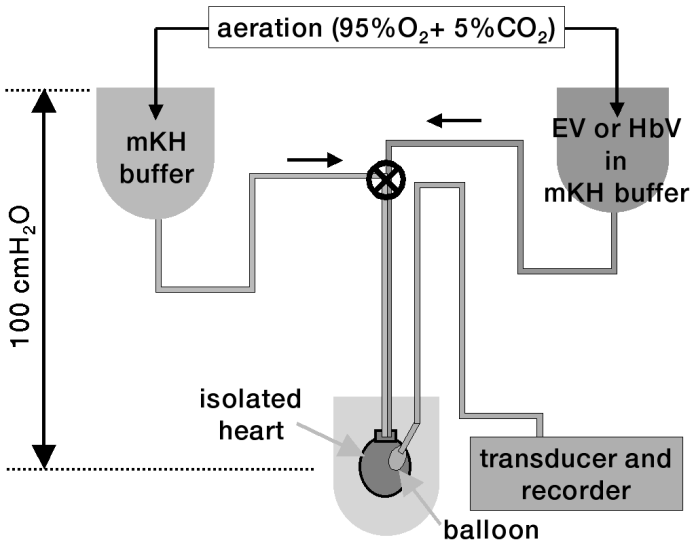

Fig. 1. Schematic presentation of Langendorff perfusion

Biochemical analysis and analysis of results

The coronary effluent was analyzed for lactate content enzymatically by the method of Lowry and Passonneau[9]. Cardiac function data [heart rate (HR), left ventricular endodiastolic pressure (LVEDP), and left ventricular developed pressure (LVDP)] were taken from the records made at 5-min intervals during the control perfusion and then at 10-min intervals until the end of experiment. In calculating the CFR for the 1st 5-min of reperfusion, the volume of the coronary effluents at 1-min and 4-min were added together and their sum taken as the volume for the 5-min interval. All values were calculated as mean ± SD but the SD has been

omitted to avoid confusion. All parameters were analyzed by means of time-series analysis of variance, and then the differences between the mean value in the control group and the corresponding values in the other experimental groups at each measuring time were analyzed using the Dunnett multiple comparison-test. A $p<0.05$ was considered as significant.

Results

Cardiac functions

Coronary flow rate (CFR)

The mean CFR in the control group gradually decreased from about 17 mL/min to 15 mL/min during the control perfusion. Just after the onset of reperfusion, CFR started to increase. Between 5 and 10 min, it recovered to a level near that in the control perfusion period, and then showed a slight decrease until the end of reperfusion. With the change to mKH buffer containing HbV or EV for the last 10 min of the control perfusion in the respective groups, though there was a larger decrease in the mean CFR in the HbV 0.33 g/dL group and EV 0.33 g/dL group than in the control group, this difference was not significant. Changes in CFR in the reperfusion period were similar in the control and experimental groups (Fig. 2.).

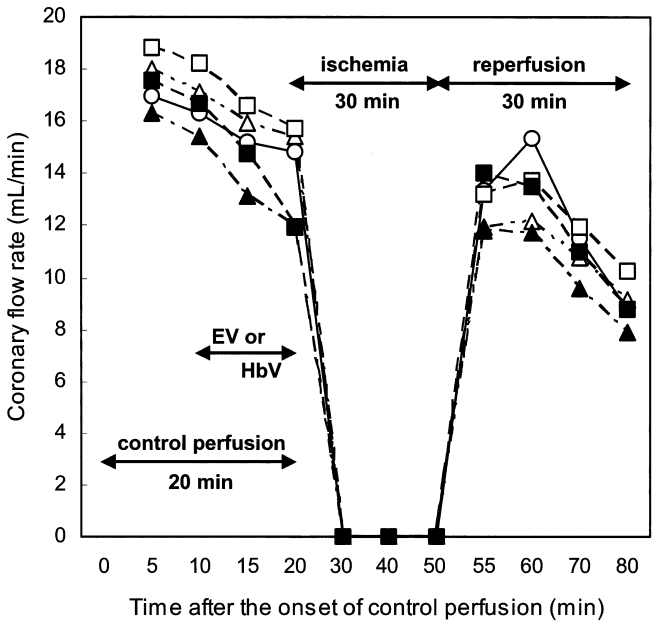

Fig. 2. Change in coronary flow rate (CFR) during experimental period
○: control group, □: HbV 0.10 g/dL group, ■: HbV 0.33 g/dL group, △: EV 0.10 g/dL group, ▲: EV 0.33 g/dL group

Heart rate (HR)

Mean HR values in the control group were maintained at about 260 beats/min during the control perfusion. HR was not affected by perfusing with mKH buffer solution containing HbV or EV for the last 10 min of the control perfusion. During the reperfusion period, there was no beating in any hearts of the control group apart from slight beating in one heart at 10 min. While the mean HR in the EV 0.10 g/dL group was similar to that in the control group, there was a slight recovery in HR in the EV 0.33 g/dL group, though this difference was not significant as compared to the mean HR in the control group. However, there was a significant recovery in HR in the two HbV groups (HbV 0.10 and 0.33 g/dL)($p<0.05$) compared with the control group at all measurement times during reperfusion. The mean HR in the HbV 0.33 g/dL group had recovered to the control perfusion level after 30 min of reperfusion (Fig. 3.).

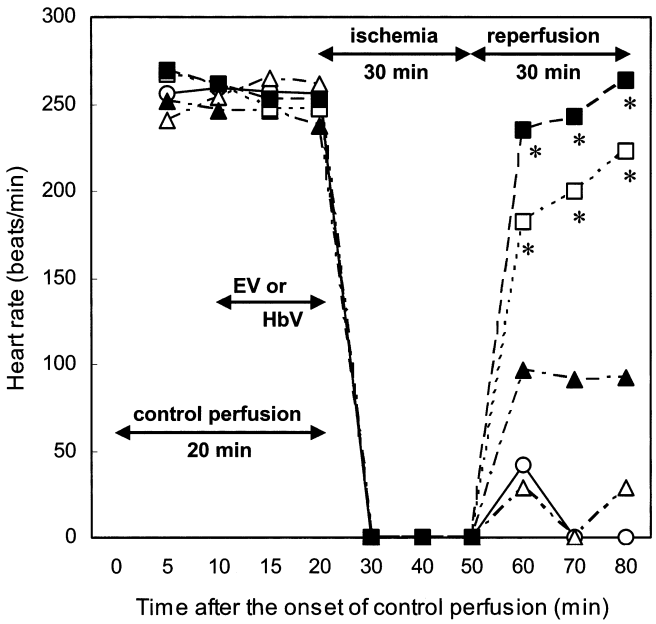

Fig. 3. Change in heart rate (HR) during experimental period
○: control group, □: HbV 0.10 g/dL group, ■: HbV 0.33 g/dL group, △: EV 0.10 g/dL group, ▲: EV 0.33 g/dL group
*$p<0.05$, vs. control group by Dunnett multiple comparison test

Left ventricular endodiastolic pressure (LVEDP)

During the control perfusion, the mean LVEDP in the control group was maintained at about 5 mmHg. This value was not altered by perfusing with the mKH buffer solutions containing HbV or EV for the last 10 min of the control perfusion period. After the onset of ischemia, the mean LVEDP in the control group started to rise gradually, and at 30 min of ischemia it had reached about 40 mmHg. The changes in mean LVEDP in the two EV groups were similar

to that in the control group during ischemia. The mean LVEDPs in the 2 HbV groups seemed to have risen to a lesser extent than that in the control group at 30 min of ischemia, though this difference was not significant when compared with the control group. During reperfusion, the mean LVEDPs rose further to 80-90 mmHg in the control and EV 0.10 g/dL groups, and this rise was maintained until the end of reperfusion. In the EV 0.33 g/dL group, the LVEDP had risen to about 80 mmHg at 10 min of reperfusion and then decreased to about 65 mmHg at the end of reperfusion, though the latter LVEDP was not significant when compared with the corresponding value in the control group. In the 2 HbV groups, rises in the mean LVEDP (30-60 mmHg) were significantly ($p<0.05$) suppressed as compared with the control group (70-100 mmHg) at 20 and 30 min of reperfusion (Fig. 4.).

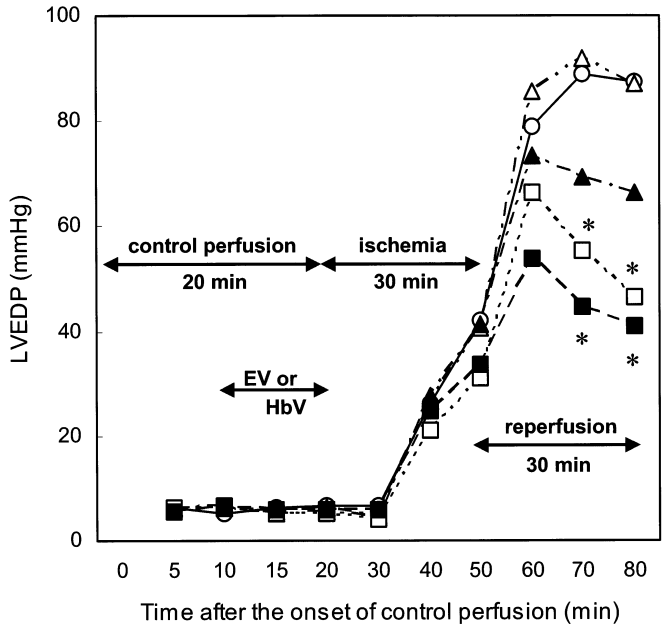

Fig. 4. Change in left ventricular endodiastolic pressure (LVEDP) during experimental period
○: control group, □: HbV 0.10 g/dL group, ■: HbV 0.33 g/dL group, △: EV 0.10 g/dL group, ▲: EV 0.33 g/dL group
*$p<0.05$, vs. control group by Dunnett multiple comparison test

Left ventricular developed pressure (LVDP)

During the control perfusion, the mean LVDP in the control group gradually decreased from about 175 mmHg to 155 mmHg, and a similar decrease was observed in the other experimental groups. During reperfusion, apart from the development of a small amount of pressure in 1 heart at 10 min, no recovery in LVDP was observed in any heart in the control group, and the mean LVDPs in the EV 0.10 g/dL group were nearly the same as those in the control group at all measurement times. While there appeared to be a recovery in LVDP in the EV 0.33 g/dL group, this was not significant when compared with the control group. In contrast, at 20 and 30 min of reperfusion, there was a significant ($p<0.05$) recovery in the mean LVDP in both HbV groups as compared with the control group (Fig. 5.).

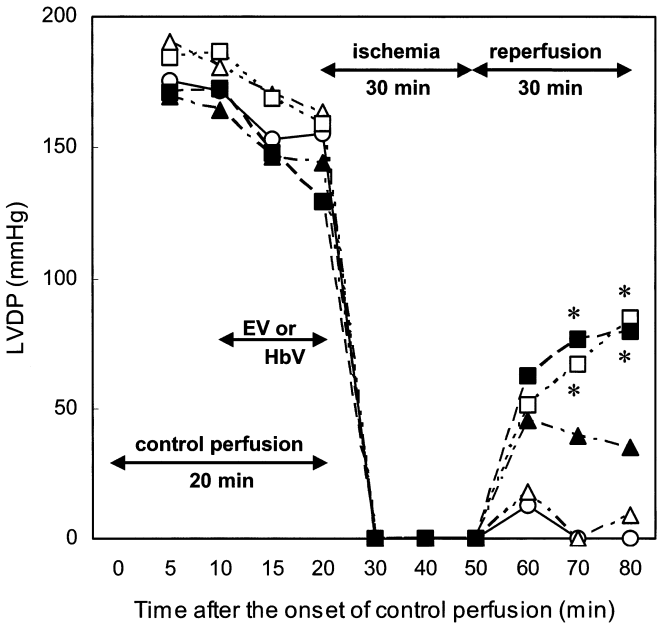

Fig. 5. Change in left ventricular developed pressure (LVDP) during experimental period
○: control group, □: HbV 0.10 g/dL group, ■: HbV 0.33 g/dL group, △: EV 0.10 g/dL group, ▲: EV 0.33 g/dL group
*$p<0.05$, vs. control group by Dunnett multiple comparison test

Lactate concentration of coronary effluent

During the control perfusion, there was a gradual increase in the mean lactate concentration of the coronary effluent in the control group, from about 10 μg/mL to 18 μg/mL. Though the increase in mean lactate concentration seemed to be slightly suppressed for perfusion with mKH buffer containing EV 0.10 g/dL or 0.33 g/dL, the difference from the control group was not significant. In the HbV 0.10 g/dL and 0.33 g/dL groups, the mean lactate concentrations for 5 min after the onset of HbV perfusion were significantly ($p<0.05$) lower than the corresponding control value (Fig. 6.A).

During reperfusion, there was a sharp increase in the lactate concentration of the coronary effluent in the control group in the first minute. In the next 4 min of reperfusion, it decreased rapidly to around the level in the control perfusion, and then continued at about this level until the end of reperfusion. Changes in the mean lactate concentration in the HbV 0.10 g/dL group were similar to those in the control group throughout the reperfusion period. The mean lactate

concentration in the HbV 0.33 g/dL group at 1 min of reperfusion was significantly ($p<0.05$) lower than that in the control group (Fig. 6.B). Though the mean lactate concentration in the HbV 0.33 g/dL group seemed to be slightly higher than that in the control group at 30 min of reperfusion, the difference was not significant.

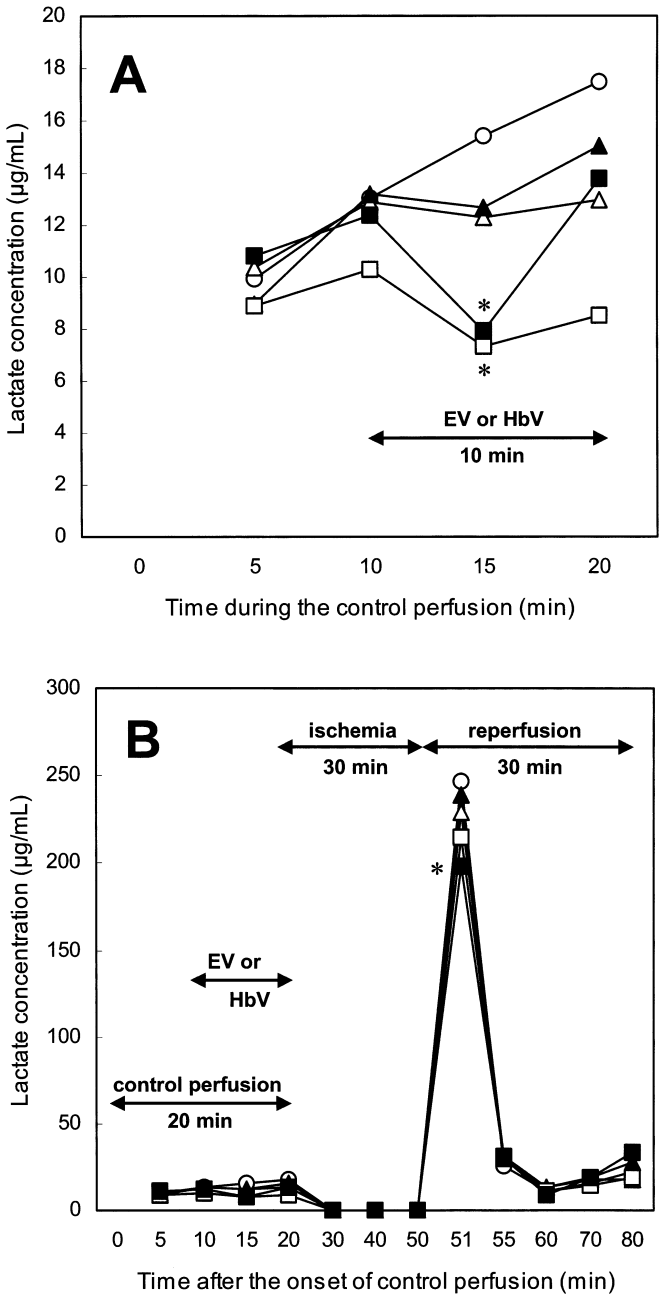

Fig. 6. Change in lactate concentration of coronary effluent during experimental period

A: Data obtained during control perfusion, B: Data obtained throughout the experiment. ○: control group, □: HbV 0.10 g/dL group, ■: HbV 0.33 g/dL group, △: EV 0.10 g/dL group, ▲: EV 0.33 g/dL group

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

Discussion

When an organ or a tissue is perfused with a buffer containing microcapsules like HbV and EV, we must be aware of the possibility of such microcapsules causing embolisms in the organ or tissue. Nakai et al.[10] reported that when isolated rat hearts were perfused with Krebs-Henseleit (KH) buffer containing hemoglobin-encapsulated liposomes (neo red cells, NRC), there was a sudden increase in perfusion pressure just after perfusion began, and their histological findings showed that embolisms were the likely cause of the increase in perfusion pressure. Further investigation revealed that inorganic crystals were formed after mixing NRC with the buffer, and these researchers suggested that the crystals were the cause of the embolisms. In this connection, Sakai et al.[11] showed that HbV caused no constriction of resistance arteries or hypertension in a conscious hamster model.

In the present study, we carefully observed cardiac functions while the hearts were perfused with mKH buffer containing HbV or EV for 10 min prior to ischemia. As mentioned in the results section, cardiac functions (CFR, HR, LVEDP, and LVDP) were not significantly affected by perfusing with mKH buffer containing HbV 0.33 g/dL or EV 0.33 g/dL. These results suggest that in contrast to the above research using NRC, embolisms did not occur in our study. Our findings with the higher concentrations of HbV and EV, however, require further clarification.

It is interesting that the lactate concentration of the coronary effluent was lower in the HbV 0.33 g/mL group than in the control group during the first 5 min of HbV perfusion and first minute of reperfusion. Pyruvate, the final substrate in the glycolytic pathway, is mainly oxidized in the mitochondria and partially converted to lactate by lactic dehydrogenase during control perfusion. Then, in ischemia, almost all pyruvate is converted to lactate since mitochondrial oxidation immediately stops, and this lactate is thought to be a factor in cardiac cell injury and delay in the recovery of cardiac functions during reperfusion. Therefore, we surmise that HbV suppressed lactate production in the cardiac cells during HbV perfusion and ischemia by stimulating mitochondrial oxidation, resulting in the significant recovery in cardiac functions in the HbV 0.33 g/dL group during reperfusion, though the mechanism by which HbV suppresses lactate production remains to be clarified.

In conclusion, our results suggest that HbV altered cardiac metabolism both before and during ischemia, and as a result, enhanced recovery of cardiac function during reperfusion.

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●編集後記●

私が編集委員になってから2年が経過した．武岡編集長はじめ編集委員各位，研恒社の担当者が本誌内容の充実のため献身的に作業されていることを目の当たりにした．私も微力乍らお役に立てるよう努力していきたい．さて，海外研究者に投稿を依頼することになり，私の留学先であったカリフォルニア大学サンディエゴ校（UCSD）の研究者二名に御願いし，本号の掲載にこぎつけた．Dr. Hangai（半谷先生）の総説は，第11回日本血液代替物学会年次大会（札幌）のシンポジウム講演の内容に基づいている．Dr. Hangaiは昨春UCSDを離れたが，PEG-albuminの新展開の礎を築き，UCSDでのmilestoneとなった．米国での今後のご活躍を期待したい．また，微小循環系の血流制御と酸素輸送に関する生理学の権威Prof. Johnsonが第12回大会（東京）で教育講演をされたことを受け，総説の執筆を御願いしたところ御快諾頂いた．Prof. Johnsonは昨夏UCSDを退官されたが，マイペースで研究を継続しておられるとのことである．永年に亘るProf. Johnsonの御業績に敬意を表したい．

編集委員：酒井宏水

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