行政院及所屬各機關出國報告書

心臟及心肺移植基礎與臨床之研究

服務機關：成功大學附設醫院
出 國 人 職稱：外科講師兼主治醫師
姓名：羅傳堯
出國地區：加拿大
出國日期：88.06.30~89.06.29
報告時間：91.03.10

封面格式

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（出國類別： 研究 ）

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報告名稱:

心臟及心肺移植基礎與臨床之研究

主辦機關:

國立成功大學醫學院附設醫院

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出國類別: 研究

出國地區: 加拿大

出國期間: 民國 88 年 06 月 30 日 -民國 89 年 06 月 29 日

報告日期: 民國 91 年 03 月 10 日

分類號/目: J2／西醫　J2／西醫

關鍵詞: 缺血性心臟病、血管型成術

內容摘要: 本人於民國 88 年 6 月接受教育部之補助，前往加拿大東部歷史最優久及最負盛名之麥吉爾大學(McGill University)心胸外科進修一年，進修之題目爲心臟及心肺移植基礎與臨床之研究，指導教授爲麥吉爾大學心胸外科主任邱智仁教授(Ray C-J Chiu)。並且經邱教授之推薦後註冊他們實驗外科碩士課程。Dr. Chiu 是心胸外科界國際知名的研究學者及教育家，他不僅在利用心肌型成術(dynamic cardiomyoplasty)治療心衰竭的領域上享譽國際，他所領導的研究小組更是連續二年獲得北美最佳研究首獎。在進修一年當中，除了修讀醫學碩士課程包括外科研究討論會(Seminars in Surgery Research)，外科研究統計學(Statistics for Surgical Research)，生物醫學研究之探討(Issues in Biomedical Research)，並發表包括在加拿大心血管協會之論文。最重要的是除了學習到所謂心臟移植及血管型成術(Angiogenesis)基本理論與概念外，並對於所謂外科研究領域有較深入之了解。

本文電子檔已上傳至出國報告資訊網

公務出國報告審核表

出國報告名稱：心臟及心肺移植基礎與臨床之研究	
出國計畫主辦機關名稱：國立成功大學附設醫院	
出國人姓名：羅傳堯 職稱：外科講師兼主治醫師 服務單位：心臟血管外科	
出國計畫主辦機關審核意見	☐1.依限繳交出國報告 ☐2.格式完整 ☐3.內容充實完備 ☐4.建議具參考價值 ☐5.送本機關參考或研辦 ☐6.送上級機關參考 ☐7.退回補正，原因：☐①不符原核定出國計畫 ☐②以外文撰寫或僅以所蒐集外文資料為內容 ☐③內容空洞簡略 ☐④未依行政院所屬各機關出國報告規格辦理 ☐⑤未於資訊網登錄提要資料及傳送出國報告電子檔 ☐8.其他處理意見：
層轉機關審核意見	☐同意主辦機關審核意見 ☐全部 ☐部分_____（填寫審核意見編號） ☐退回補正，原因：_____（填寫審核意見編號） ☐其他處理意見：

說明：

一、出國計畫主辦機關即層轉機關時，不需填寫「層轉機關審核意見」。

二、各機關可依需要自行增列審核項目內容，出國報告審核完畢本表請自行保存。

三、審核作業應於出國報告提出後二個月內完成。

目次

目的

1. 研究經心臟及心肺移植基礎與臨床之研究
2. 修讀外科研究碩士(Surgical Research M.Sc.)課程

過程

本人於 88 年 7 月 1 日到達加拿大蒙特婁綜合醫院並向 Dr. Chiu 報到。經過他熱心的介紹下與實驗室各個成員認識，包括動物實驗室二位技術員，Dianne，Marine 生物細胞室操作員 Minh 以及心胸外科實驗室二位工作伙伴，一位來自 Montreal Children Hospital 的 Dr. Shun-Tim 及心胸外科第三位住院醫師 Dr. Chedraway 。Dr. Chiu 隨即指派 Royal Victoria Hospital 另一位 Dr. Lachapelle 為我另一位 Supervisor 一起指導我作臨床心臟移植及經心肌血管型成術的研究工作。然因 McGill 大學心肺移植小組成員大都被紐約大學挖角，因此移植數目並不多，大部分的時間都是從事缺血性心臟病末期心衰竭病人之研究上。所使用的動物模式為利用鼠及豬引導成心肌缺血後再實行機械性刺激(needle)以誘導出新生血管進而改善心肌缺血及心室功能。

初期的實驗以豬為模式建立慢性缺血性心臟病，經機械性刺激後的確可以增加血管內皮細胞氧化氮合成酶(eNOS)之生成，此份結果並發表在第四屆 Terrence Donnelly Cardiac Residents Research(如附件一)及第五十三屆加拿大心血管年度大會上(如附件二)。隨後感覺血管之新生可能不僅是一氧化氮之生成刺激血管擴張而已，因此下一步驟是希

望藉由其他的血管刺激因子(Angiogenic factor)以刺激產生新生微血管及其他有功能性之血管。

進入九月份後，在邱教授的引薦下得以註冊成為 McGill University 外科研究學(Surgical Research)碩士班學生，所修讀之課程包括：外科研究討論會(Seminars in Surgical Research)，外科實驗統計學(Statistics for Surgical Research)，生物醫學研究之課題(Issues in Biomedical Research)(如附件三)，修讀時間為二學期，剩下的論文學分在回到成大後仍在進行中。

在修讀學位的過程中，整個實驗仍是繼續進行的。然而因為血管刺激因子來源的確造成很大的困擾(無法從其他實驗室中獲得)，因此我們修改研究的方向為經心肌機械性血管型成術之病理生理機轉。我們利用包括老鼠及豬之模式引發心臟缺血後，再經機械性刺激後，視心臟功能是否改善，心臟血流是否增加，帶有平滑肌細胞之小動脈是否有增加等。然因整個過程繁雜，而進修時間又極其有限，因此，所有的資料還在整理中。

在主要實驗進行的同時，另外一個有關 Brain protection during cardiopulmonary bypass 也在我們實驗室進行中。此大型實驗是由 Dr. Shum-Tim 主導，最主要是探討 Steroid 在 cardiopulmonary bypass 中對 brain protection 所扮演的角色，此次研究之成果也發表在 37 屆美國心胸外科大會上(附件四)及美國外科學院 Surgical Forum 上(附件五)。在離開 McGill 大學以前也在 Dr. Chiu 的指導下完成 **Optimal Vascular Delay in Cardiomyoplasty Following Latissimus Dorsi Muscle Isolation: A Canine Study** 並發表在台灣醫學會雜誌上(附件六)。

因時間上的因素，雖然有很多應做的工作，仍於 89 年 8 月回至成大醫院繼續臨床及研究工作。

心得

首先感謝邱教授能給我這個機會進入所謂真正外科實驗的領域。身為一位繁忙的心血管外科醫師，如何在有限的時間及体力從事所謂的外科研究實際上是一大難題。麥吉爾大學心胸外科從創立至今，這個問題的確是存在的，他們所有的心胸外科醫師都非常忙碌，其情況超乎我們想像之外，而且研究的財力及資源較之美國差距是非常大的，然而他們對研究的精神及其效率都比美國有名的學府有過之而無不及。為什麼在如此艱苦的環境下，他們還能夠有如此豐盛的成果呢？在我的觀察中，他們有著下列的特質：1. 對於學校及醫院有著強烈的使命感及企圖心，麥吉爾背負著加拿大歷史最優久以前是與 Harward 齊名的學校，學校的每一份子都為著 McGill 這個名字為榮。2. 有優良的傳統和完善的制度，比方說人員的訓練及選拔都有其辦法及標準。他們的心胸外科訓練是六年(還不包括 Fellowship)，其中有一年還必須完全到實驗室不能從事任何的臨床工作。另外，每年都有固定的客作教授講座及研究競賽。因此，對於所有從 McGill 出來的人對所謂外科研究都有著很深的体認，而且訓練完後大都有能力擔任其他醫院或大學心胸外科主管的角色。3. 他們有著願景(vision)，對於很多的辦法及制度都不是只顧著眼前的好處及利益，而是往前看，把握未來的方向。在有好的傳統及制度之下，縱使外界環境有變化，其結果不致有很大的偏頗。現在成大醫學中心雖然無法與他們的優良傳統匹比，然而現在與他們的情況有點類似；人力不足、經濟來源也不是很充足。在如此困難的環境中如何走出真正屬於成大的路是值得大家深慮的。

在我個人的研究的感想上，我似乎選擇了太多目標，要在一年中完成，似乎是犯了很大的忌諱。如二種動物模式一起進行，再加上碩士課程。因此至今似乎是沒有很具体的成果，這在以後的研究路上，似乎是要避免的事情。

建議

1. 在外科住院醫師的訓練中加入 1~2 年的研究工作，當然必須暫停臨床工作。雖然有點浪費人力，然對於整個專科醫師的養成有顯著的幫忙。現存之制度常希望魚與熊掌兼得，在未完全獨立成為一位合格專科醫師前，似乎會落得兩頭空。

2. 設立客座教授講學制度，各分科似乎有必要建立自己的講座，並有住院醫師研究評比。如此，除可達到相互交流之目的，並可以提升科內及住院醫師研究水準。

3. 有現在有臨床醫學研究所中，搭配碩士班課程配合住院醫師的需求而選修各種課程，當然如我在第一點所建議，在研讀過程中要暫停臨床工作一年。

4. 建議籌設第二動物中心，現有的動物中心都是以小型動物為中心，大型動物實驗手術室設備是不足的。然在進入人体實驗的過程中，以大型動物為介的實驗是必須的，因此，選擇適當的地點，建立以大型動物為主的第二動物中心是無可避免的趨勢。

Daniel Bonneau, MD
Stephanie Brister, MD
George T. Christakis, MD
John G. Coles, MD
Robert J. Cusimano, MD
Charles Cutrara, MD
Tirone E. David, MD
Lee E. Errett, MD
Christopher M. Feindel, MD
Stephen E. Fremes, MD
Bernard S. Goldman, MD
David A. Latter, MD
Yves Leclerc, MD
Lynda L. Mickleborough, MD
Charles M. Peniston, MD
Anthony Ralph-Edwards, MD
Gary C. Salasidis, MD
Hugh E. Scully, MD
Glen Van Arsdell, MD
Richard D. Weisel, MD
William G. Williams. MD
Terrence Yau, MD

Professors Emeriti
Ronald J. Baird, MD
Clare B. Baker, MD
Wilfred G. Bigelow, MD
George A. Trusler, MD
Donald R. Wilson, MD
James K.Y. Yao, MD

Scientists
Colin Bayliss, MD
David Courtman, PhD
Ren-Ke Li, MD
Carin Wittnich, DVM

THE FOURTH ANNUAL TERRENCE DONNELLY CARDIAC RESIDENTS' RESEARCH DAY

Friday, May 26, 2000
ROYAL YORK HOTEL
Toronto, Ontario

All sessions will be held in the Imperial Room

Terrence Donnelly Heart Centre

University of Toronto

ST. MICHAEL'S HOSPITAL

08:25 Welcome and Introduction
Dr. Richard Weisel, Chair
Division of Cardiac Surgery
University of Toronto

08:30 Morning Presentations

Moderator: Dr. Lee Errett

Marc Ruel, University of Ottawa Hea Institute
Is tranexamic acid safe in patients und coronary endarterectomy?

Bob B. Kiaii, University of Western C
A prospective, randomized multi-centre endoscopic versus conventional harves saphenous vein in coronary artery bypa

Philippe Demers, Montreal Heart Ins
Off-pump multi-vessel coronary artery revascularization in the elderly.

Camille L. Hancock-Friesen, Dalhou University
Does beating heart surgery compromis revascularization?

Subodh Verma, University of Calgar
Endothelin receptor blockade improve endothelial function in human internal arteries: Implications for CABG surge

Chwan-Yau Luo, McGill University
Increased endothelial Nitric Oxide synthase expression in transmyocardial mechanical revascularization (TMMR).

L. Ray Guo, University of Western Ontario
Effect of distal graft anastomosis site on retrograde perfusion and flow patterns of native coronary artery vasculature.

Gideon Cohen, University of Toronto
Superior hemodynamics with stentless versus stented valves: A prospective randomized trial.

Paul Fedak, University of Toronto
Open Hearts: The origins of direct-vision intracardiac surgery.

11:20 Visiting Professor

DR. DENTON COOLEY
TEXAS HEART INSTITUTE

"Current trends in Cardiovascular Surgery"

12:00-1:00 Lunch Break
Upper Canada Room

Eric de Broux, University of Montreal
Growth and pubertal development following pediatric heart transplantation, A 15-year experience.

Jean-Francois Legare, Dalhousie University
Allograft heart valve failure in rats is mediated by T Lymphocytes.

Hugues Jeanmart, University of Montreal
Cyclosporin causes a selective dysfunction of the coronary endothelium while Tacrolimus causes a generalized dysfunction in an in vitro porcine model.

Rakesh Arora, Dalhousie University
High thoracic spinal cord stimulation suppresses the excitatory effects of myocardial ischemia on the intrinsic cardiac nervous system.

Nicolas Noiseux, University of Montreal
PDGFR-β antisense suppresses intimal hyperplasia in injured rat carotids by preventing vascular smooth muscle cells migration.

2:30 Moderator: Dr. David Latter

Visiting Professor

DR. EDWARD BUSSE
UNIVERSITY OF SASKATCHEWAN

"Cardiac Surgery in the community setting. How can they manage without Residents?"

[illegible]
Hemodynamic unloading leads to [illegible] pulmonary vascular disease in rats.

Michael Moon, University of Manit
An organ culture model to study intin hyperplasia at the site of a coronary [illegible] anastomosis.

Richard Cook, University of Britisl
Potential deleterious effect of β-Andr stimulation during warm-blood cardi rabbit hearts.

Eddy Chedrawy, McGill Universit
Mesenchymal stem cell transplantati myocardial regeneration.

Pierre Voisine, University of Monti
Combination of internal thoracic art implantation and gene transfer of vas endothelial growth factor (VEGF) to perfusion in a canine model of chron ischemia

Meeting Adjourned

VET LAB AT ST. MICHAEL'S
Saturday, May 27, 2000
08:00 a.m.

CANADIAN CARDIOVASCULAR SOCIETY/SOCIÉTÉ CANADIENNE DE CARDIOLOGI

11:00-12:30 SIMULTANEOUS SESSIONS/*SÉANCES CONCOMITANTES*

CARDIAC SURGERY IV – FEATURED RESEARCH IN CARDIAC SURGERY
CHIRURGIE CARDIAQUE IV – RECHERCHE VEDETTE EN CHIRURGIE CARDIAQUE

Co-Chairs/*Co-Présidents* : **Richard Weisel, Arvin Koshal**

11:00 **Student Presentation Award competition**
Concours pour le Prix à un étudiant pour sa présentation

314. *Adjunctive gene therapy with VEGF$_{165}$: A preliminary Canadian experience.* AIM Campbell, DA Latter, LE Errett, NJ Camack, DW Courtman, DJ Stewart. Toronto

11:15 **Student Presentation Award competition**
Concours pour le Prix à un étudiant pour sa présentation

315. *Off-pump coronary artery surgery is associated with enhanced endothelial function in human internal mammary arteries.* S Verma, F Lovren, A Dumont, W Kidd, A Maitland, CR Triggle, TJ Anderson. Calgary, AB

11:30 **Featured research/*Projet vedette en recherche***

316. *Closed-chest coronary artery bypass grafting on the beating heart using a computer-enhanced surgical robotic system.* WD Boyd, R Rayman, AH Menkis, B Kiaii, S Ganapathy, W Dobkowski, G Jablonsky, FN McKenzie, RJ Novick. London Health Sciences Centre, London, Ontario

11:45 **Featured research/*Projet vedette en recherche***

317. *Enhanced myocardial angiogenesis by gene transfer using transplanted cells.* TM Yau, J Sarjeant, K Fung, RD Weisel, DA Mickle, R-K Li. Toronto

12:00 **Featured research/*Projet vedette en recherche***

318. *Increased endothelial nitric oxide synthase expression in transmyocardial revascularization.* C-Y Luo, K Lachapelle, VF Chu E Chedrawy, RCJ Chiu

12:15 **Featured research/*Projet vedette en recherche***

319. *Effects on coronary endothelial function of the Cohn stabilizer for minimally invasive coronary artery bypass surgery.* LP Perrault N Desjardins, M Carrier. Montreal

CLINICAL CARDIOLOGY (GENERAL) VII – CONGESTVE HEART FAILURE/TRANSPLANT
CARDIOLOGIE CLINIQUE (GÉNÉRALE) VII – INSUFFISANCE CARDIAQUE CONGESTIVE/ TRANSPLANTATION

Co-Chairs/*Co-Présidents* : **Ross Davies, TBA**

11:00

320. *Intrabreath diffusing capacity is a better predictor of lung function in heart failure patients before and after transplantation.* MA Quantz, SR Wilson, CA Smith, LW Stitt, D Ahmad. London, Ontario

11:15

321. *Outcome of heart transplantation for giant cell myocarditis.* RA Davies, J Veinot, S Smith, C Struthers, P Hendry, R Masters. University of Ottawa Heart Institute

11:30

322. *Safety and efficacy of long-term home intravenous milrinone therapy as a bridge to cardiac transplantation in patients with end-stage cardiomyopathy.* U Uppal, S Menon, S Jahania, K Yaqoob, T Mullett, J Sanchez, R Mentzer. Heart Failure and Cardiac Transplant Program, University of Kentucky, Lexington, KY, USA

11:45

323. *Beneficial clinical and neurohumoral effects of short-term inotropic support in decompensated congestive heart failure.* R Ibrahim, S Gagnon, L Whittom, A Ducharme, N Racine, M White. Montreal

12:00

324. *Atrial natriuretic peptide augments the variability of sympathetic nerve activity in human heart failure.* T Kubo, S Ando, P Picton, DJ Atchison, CF Notarius, MJ Pollard, BL Abramson, JS Floras. Toronto

12:15

325. *Skeletal muscle oxidative capacity has limited role in the exercise intolerance of patients with moderate heart failure.* LM Bussières, M Jadin, PF Gardiner, M White, M Juneau, JL Rouleau. Montreal

INTERVENTIONAL CARDIOLOGY II – NOVEL APPROACHES TO INTERVENTIONS IN THE CATH LAB/CARDIOLOGIE *INTERVENTIONNELLE II – NOUVELLES INTERVENTIONS AU LABORATOIRE DE CATHÉTÉRISME*

Co-Chairs/*Co-Présidents* : **Merrill Knudtson, Blair O'Neill**

11:00

326. *The impact of increased rate of radial angioplasty on the occurrence of vascular complication – 2027 coronary angioplasty review from 1997-1999.* D Hilton, X Wang, P Smith, M Williams, D Kinloch, R Mildenberger, E Fretz, P Klinke. Victoria Heart Institute, Victoria, BC

11:15 **Featured research/*Projet vedette en recherche***

327. *Predictors of failure of transradial approach: A multivariate analysis of a large series.* GR Barbeau, O Gleeton, L Roy, S Plante, G Proulx, JF Gobeil, S Simard, M-M Larivière. Institut de cardiologie de Québec, Ste-Foy, Canada

1 to 6 days) postoperatively. All patients are angina free and back to work at a mean follow-up of 128±54 days (range 13-198 days).
This report represents the first successful clinical use of a computer-controlled remote endoscopic surgical system in performing closed-chest, beating-heart coronary artery bypass. Further improvements in endoscopic stabilization and surgical instrumentation are required to facilitate broader clinical application.

317
ENHANCED MYOCARDIAL ANGIOGENESIS BY GENE TRANSFER USING TRANSPLANTED CELLS

TM Yau, J Sarjeant, K Fung, RD Weisel, DAG Mickle, R-K Li. Toronto

Purpose: The combination of myocardial cell transplantation and angiogenic gene transfer may improve postinfarction LV perfusion and function. We evaluated the effect of heart cells transfected with VEGF and transplanted into a myocardial scar on angiogenesis and regional blood flow.
Methods: Cultured rat heart cells were transfected with plasmids encoding $VEGF_{165}$ and green fluorescence protein. Syngeneic adult rats underwent LV cryoinjury to create a transmural scar. Three weeks later, 4×10^6 transfected heart cells (N=14), untransfected heart cells (N=13), or culture medium (N=16) were transplanted into the center of the scar. After 5 weeks, hearts were excised, fixed and sectioned for quantitative histologic analysis, or perfused with radiolabelled microspheres for evaluation of regional blood flow.
Results: Plates of heart cells transfected with $VEGF_{165}$ (at 25-30% efficiency) produced 6.1 times more intracellular VEGF and 3.8 times more secreted VEGF than nontransfected cells. Capillary density (mean ± standard error) per high power field in the center of the myocardial scar was 1.9±0.03 in controls, 6.4±0.10 in untransfected rats, and 8.7±0.16 in transfected rats (P=0.0002). Capillary density in the border zone around the scar was 1.1±0.02 in controls, 3.9±0.11 in untransfected rats, and 6.3±0.11 in transfected rats (P=0.004). Regional blood flow within the scar was 1.38% of normalized flow in untransfected hearts, but 2.94% (a ratio of 2.1) in transfected hearts.
Conclusions: Transplantation of heart cells transfected with VEGF induced greater angiogenesis than transplantation of unmodified cells. Combined gene transfer and cell transplantation strategies may improve postinfarction LV perfusion and function.

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INCREASED ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION IN TRANSMYOCARDIAL REVASCULARIZATION

C-Y Luo, K Lachapelle, VF Chu, E Chedrawy, RCJ Chiu

Objective: Nitric oxide (NO) is a potent vasodilator and is known to modulate the vasomotor tone of the coronary microcirculation. It is also important in vasculogenesis. We tested the hypothesis that transmyocardial revascularization (TMR) might stimulate the nitric oxide synthetase (NOS) enzymes and thus improve blood flow to ischemic myocardium by causing an increase in local NO production.
Methods: Chronic myocardial ischemia was created by placing ameroid constrictors around the circumflex artery in 15 pigs. Six weeks later, Group I (n=5) underwent a sham sternotomy with no TMR and served as ischemic controls. Group II (n=5) and group III (n=5) received TMR in the ischemic zone using 18 gauge needle punctures. Group I and II were sacrificed 1 week following the second procedure whereas group III was sacrificed at 4 weeks. The hearts were sectioned and specimens form the circumflex territory were studied for myocardial inducible nitric oxide synthase (iNOS) and endotheilial nitric oxide synthase (eNOS) expression using specific antibodies. The data was quantified using computer assisted morphometric analysis.
Results: Significantly increased expression of eNOS was noted at one week following TMR (Gr II=0.016±0.002 mm^2 vs Gr I=0.007±0.002 mm^2; P<0.001). Levels of eNOS decreased at four weeks after TMR and were similar to controls (Gr II=0.010±0.004 mm^2 vs Gr I=0.007±0.002 mm^2; P=0.3). The iNOS expression also increased at one week following TMR (Gr II=0.012±0.004 mm^2 vs Gr I=0.009±0.02 mm^2; P=0.15) but did not reach significance.
Conclusion: eNOS stimulation occurs soon after TMR and may be one of the immediate mechanisms involved in early anginal relief following TMR.

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EFFECTS ON CORONARY ENDOTHELIAL FUNCTION OF THE COHN STABILIZER FOR MINIMALLY INVASIVE CORONARY ARTERY BYPASS SURGERY

LP Perrault, N Desjardins, M Carrier. Montreal

Technical aids are required for optimal visualization of the operative field during the performance of coronary anastomoses and for stabilization during minimally invasive beating heart coronary artery bypass grafting (CABG). The Cohn stabilizer is an atraumatic technique of optimizing the operative field during the performance of beating heart CABG. We compared the effects on endothelial function of 2 commonly used stabilizing techniques: one using the Cohn stabilizer (used with proximal snaring) and the other by coronary shunting on an *in vivo* model of beating heart CABG. The 2 techniques were applied for 15 minutes on porcine epicardial coronary arteries after median sternotomy. Control rings were taken from the same coronary artery (left anterior descending and right coronary arteries at random). The endothelial function of control and instrumented arterial rings was studied in organ chamber experiments filled with modified Krebs-Ringer bicarbonate solution. After contraction to prostaglandin F2α, endothelium-dependent relaxation to serotonin (an agonist coupled to Gi-proteins) and bradykinin (a non-Gi-coupled agonist) and endothelium-independent relaxation to sodium nitroprusside (SNP), a nitric oxide donor, were compared in the Cohn retractor group, the shunting group, the snaring group, and in control segments. Evaluation of endothelial coverage at the site of application of the devices was performed with silver nitrate staining. Endothelium-dependent relaxations to serotonin and bradykinin were significantly decreased in the shunt group compared to control, Cohn stabilizer and snare groups. There were no significant differences in the relaxations to SNP. Greater endothelium-dependent relaxation in the Cohn stabilizer suggests a better preservation of the endothelial coverage at the site of application of the devices and lesser propensity to coronary spasm and later development of intimal hyperplasia.

CLINICAL CARDIOLOGY (GENERAL) VII – CONGESTIVE HEART FAILURE/ TRANSPLANT/CARDIOLOGIE CLINIQUE (GÉNÉRALE) VII – INSUFFISANCE CARDIAQUE CONGESTIVE/ TRANSPLANTATION

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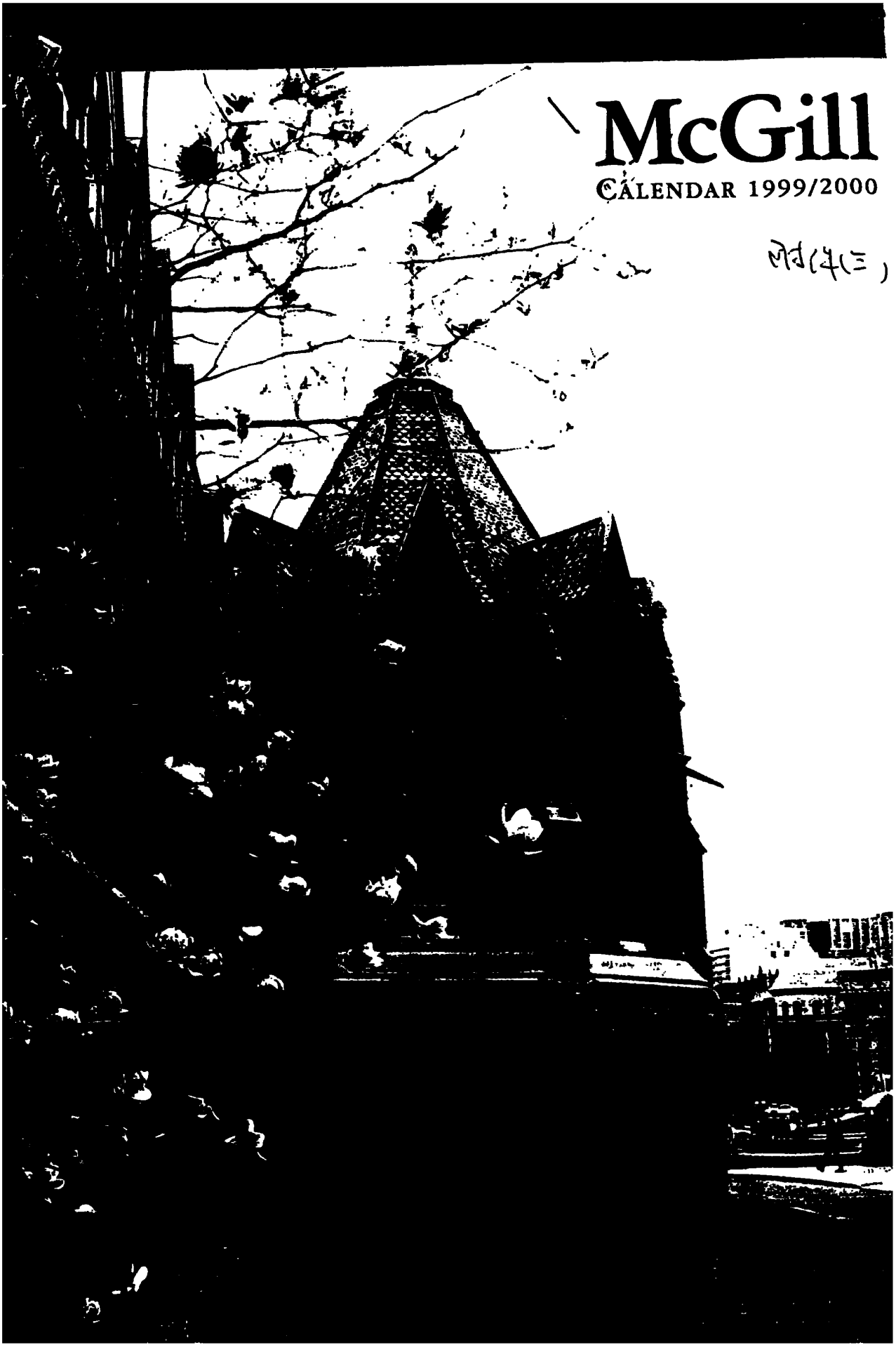
INTRABREATH DIFFUSING CAPACITY IS A BETTER PREDICTOR OF LUNG FUNCTION IN HEART FAILURE PATIENTS BEFORE AND AFTER TRANSPLANTATION

MA Quantz, SR Wilson, CA Smith, LW Stitt, D Ahmad. London, Ontario

Background and Methods: Pulmonary function testing using the intrabreath (IB) technique is a less stressful test for ill patients to perform and may be more accurate than the standard single breath (SB) technique. However, the accuracy of the IB technique has never been validated in patients with CHF or following heart transplantation. We compared IB and SB techniques in 75 patients with CHF prior to transplantation and 3 months postoperatively. Both techniques were performed sequentially on a Sensormedics 2200 according to ATS standards. Agreement between the alveolar volume (VA) and diffusing capacity (DLCO) as measured by IB were compared with the same parameters determined by SB using an analysis of intracorrelation coefficient (ICC). Results are expressed as ICC with 95% confidence intervals. Alveolar ventilation (VA) as determined by IB and SB was compared to the total lung capacity (TLC) using Pearson correlation coefficients.



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77 Surgical Research

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77.2 Programs Offered

The Department of Surgery offers graduate programs leading to M.Sc. and Ph.D. degrees.

The main research interests in the Department include projects in islet cell differentiation and islet transplantation, tissue engineering of cardiac muscle, immunopathogenesis of liver xenograft rejection, lung transplantation; tissue repair and engineering for plastic surgery applications; cartilage regeneration, osteoinduction and biomechanics; sepsis and multi-organ failure; biology of cancer; sexual dysfunction, prostate cancer and kidney stones; and surgical epidemiology.

A list of research directors and a description of their research topics, as well as application forms may be obtained from Mrs. Irene Sidorenko, Department of Surgery, Division of Surgical Research, email: irenes@med.mcgill.ca, to whom all enquiries are to be directed.

77.3 Admission Requirements

M.Sc. Program

Usually a B.Sc., M.D. or M.V.D. degree, with a minimum GPA of 3.2. Applications will be accepted from candidates sponsored by a research supervisor willing to provide laboratory space and direction for their research work.

Ph.D. Program

Admission is usually from the M.Sc. program either upon completion of the M.Sc. degree, or by transfer from the first year of M.Sc. to the second year of Ph.D. studies. Request for such transfer is to be made in writing by the thesis supervisor during the candidate's first year of M.Sc. studies, not later than March 30th for students enrolled in September, or November 1st for those registered in January. **Transfer is granted on the basis of an examination administered by the student's Research Advisory Committee.**

Students with an M.Sc. degree from other departments or from other recognized universities, whose M.Sc. topic is closely related to the subject of their Ph.D. research, may be given credit for one year of their M.Sc. and be admitted directly into the Ph.D. program, at the level of Ph.D.2, at the discretion of the Department. Exceptional students with a Master's degree unrelated to their proposed research may be admitted to Ph.D.1 directly.

77.4 Application Procedures

Applicants must submit a completed application form including a brief curriculum vitae, a short description of the proposed thesis research (prepared by the student and/or the prospective research director), a cheque for $60 payable to McGill University, as well as two copies of all academic transcripts and two letters of recommendation mailed directly to the Department.

Deadline for receipt of complete applications:
March 1st for the September term.
October 1st for the January term.
February 2nd for International students.

77.5 Program Requirements

M.Sc. Program

The M.Sc. program consists of research work in preparation of a thesis and completion of required courses for a total of 48 credits. The program is to be completed during three terms; an additional term is assigned for the preparation of the thesis.

The course requirements for a total of 15 credits are as follows:
519-601D Seminars in Surgical Research
519-606A Statistics for Surgical Research
519-605B Issues in Biomedical Research

A graduate level course in the student's specialty is also mandatory. Selection of the former and of additional courses, if required, will be in consultation with a Research Advisory Committee appointed for each student.

The laboratory research component of the program is given 33 credits.

Ph.D. Program

The minimum residence time in the program is three calendar years. In addition to the courses listed under the M.Sc. program, students are encouraged to select additional courses from allied

disciplines relevant to their research topic. To graduate, candidates will also have to pass a predoctoral examination.

Predoctoral comprehensive examination: All Ph.D. students (admitted directly into the Ph.D. program, or those allowed to transfer from M.Sc. 1 to Ph.D. 2 without writing an M.Sc. thesis) must take this examination.

The examination is to take place after 12 months of residence in the Ph.D. program, and will be administered by an expanded Research Advisory Committee under its Chair. The examination will have two components: an oral presentation of the candidate's research project, as well as preparation of a report in writing on an assigned research publication, and its oral presentation. The candidate must receive a pass mark in both components to continue in the Ph.D. program.

77.6 Courses

The course credit weight is given in parentheses (#) after the course title.

519-601D SEMINARS IN SURGICAL RESEARCH (6) (1½ hours/week) (Compulsory for graduate students in the Department of Surgery and available to others by permission of the coordinators.) Each session will consist of presentations by research directors and the graduate student. The fall term will feature invited speakers and the winter sessions will consist of presentations by research directors and the graduate student. The first will introduce the subject by highlighting the clinical/biological problem while the student will be expected to describe the project and methodology and integrate their findings with the overall approach presented. **Professors Alini, Lee, Mort and Philip**

519-606A STATISTICS FOR SURGICAL RESEARCH (3) (2 hours/week) (Compulsory for graduate students in the Department of Surgery and available to others by permission of the coordinators.) Introduction to basic statistical principles and methods as they could be applied to surgical research. The topics covered will include: descriptive statistics probability theory, statistical inference, bivariate techniques, analysis of variance, and introduction to multi-variate methods. **Professor Sampalis.**

519-605B ISSUES IN BIOMEDICAL RESEARCH (3) (2 hours/week) (Compulsory for graduate students in the Department of Surgery and available to others by permission of the coordinators.) Students will be introduced to current trends in important areas of surgical research such as inflammation, wound healing, immunity, tissue engineering, cancer and gene therapy. The impact of basic research on the practice of surgery and post-surgical patient care will be highlighted through lectures shared by clinical and basic scientists. In addition the course offers lectures on the art of grant and paper writing by experienced members of the staff.
Professors Brodt and Rosenberg.

519-604A SIGNAL TRANSDUCTION (3) (2 hours/week) (Open to graduate students with prerequisites and U3 undergraduates with special permission.) An in-depth course describing the cellular and molecular mechanisms involved in signal transduction by growth factors, cytokines and extra cellular matrix with emphasis on clinical relevance. The course will focus on how perturbation in signaling pathways may result in disease states and address the issues from a surgical research perspective. **Professors Philip and Chevalier**

519-690A M.Sc. RESEARCH I. (4)

519-691B M.Sc. RESEARCH II. (4)

519-692B M.Sc. RESEARCH III. (4)

519-693D M.Sc. THESIS. (21)

519-700D COMPREHENSIVE EXAMINATION.

78 Urban Planning

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Guest Lecturers
Cameron Charlebois, Luc Danielse, Marc Denhez, David Farley, Andrew Hoffmann, Peter Jacobs, Brenda Lee, Lloyd Sankey, Martin Wexler, Michel Frojmovic

78.2 Programs Offered

The objective of the School is to produce qualified professional urban planners for the public and the private sectors. Training is provided at the post-graduate level; the degree offered is the Master of Urban Planning (M.U.P.).

Upon completion of the two-year program of studies, graduates are expected to have acquired basic planning skills, a broad understanding of urban issues, and specialized knowledge in a field of their own choice. (The School also accepts a limited number of Ad-Hoc Ph.D. students.)

The program of study offered by the School is fully recognized by the Ordre des Urbanistes du Québec (O.U.Q.) and the Canadian Institute of Planners (C.I.P.). Graduates can become full members of these professional organizations after meeting their internship requirements.

Modern urban planning developed into a profession in the early decades of the twentieth century, largely as a response to the appalling sanitary, social and economic conditions of rapidly developing industrial cities. Initially the disciplines of architecture, civil engineering and public health provided the nucleus of concerned professionals; beautification schemes and infrastructure works marked the early stages of public intervention in the nineteenth century. Architects, engineers and public health specialists were joined by economists, sociologists, lawyers and geographers as the complexities of the city's problems came to be more fully understood and public pressure mounted for their solution. Contemporary urban and regional planning techniques for survey, analysis, design and implementation developed from an interdisciplinary synthesis of these various fields.

Today, urban planning can be described as the collective management of urban development. It is concerned with the welfare of communities, control of the use of land, design of the built environment, including transportation and communication networks, and protection and enhancement of the natural environment. It is at

Systemic Steroid Pretreatment Improves Cerebral Protection After Circulatory Arrest

Dominique Shum-Tim, MD, Christo I. Tchervenkov, MD, Al-Maleek Jamal, BS, Toni Nimeh, MD, Chwan-Yau Luo, MD, Edgar Chedrawy, MD, Eric Laliberte, CCP, Anie Philip, PhD, Colin P. Rose, MD, and Josee Lavoie, MD

Division of Cardiovascular Surgery, The Montreal Children's Hospital, and Divisions of Plastic Surgery, Cardiology, and Anesthesia, Montreal General Hospital, McGill University Health Center, Montreal, Quebec, Canada

Background. This study evaluates whether systemic steroid pretreatment enhances neuroprotection during deep hypothermic circulatory arrest (DHCA) compared with steroid in cardiopulmonary bypass (CPB) prime.

Methods. Four-week-old piglets randomly placed into two groups (n = 5 per group) were given methylprednisolone (30 mg/kg) into the pump prime (group PP), or pretreated intravenously 4 hours before CPB (group PT). All animals underwent 100 minutes of DHCA (15°C), were weaned off CPB, and were sacrificed 6 hours later. Postoperative changes in body weight, bioimpedance, and colloid oncotic pressure (COP) were measured. Cerebral trypan blue content, immunohistochemical evaluation of transforming growth factor-β_1 (TGF-β_1) expression, and caspase-3 activity were performed.

Results. Percentage weight gain (group PP 25.0% ± 10.4% versus group PT 12.5% ± 4.0%; $p = 0.036$), and percentage decrease in bioimpedance (PP 37.2% ± 14.5% versus PT 15.6% ± 7.9%; $p = 0.019$) were significantly lower, whereas postoperative COP was significantly higher in group PT versus group PP (PT 15.3 ± 1.8 mm Hg versus PP 11.6 ± 0.8 mm Hg; $p = 0.003$). Cerebral trypan blue (ng/g dry tissue) was significantly lower in group PT (PT $5.6 \times 10^{-3} \pm 1.1 \times 10^{-3}$ versus PP $9.1 \times 10^{-3} \pm 5.7 \times 10^{-4}$; $p = 0.001$). Increased TGF-β_1 expression and decreased caspase-3 activity were shown in group PT.

Conclusions. Systemic steroid pretreatment significantly reduced total body edema and cerebral vascular leak and was associated with better immunohistochemical indices of neuroprotection after DHCA.

(Ann Thorac Surg 2001;72:1465–72)

While cardiopulmonary bypass (CPB) makes open heart surgery possible, it also inflicts adverse effects that may be subtle in some respects and significant in other respects. After a complex surgical procedure for severely ill patients and for those of the extremes of age, the morbidity of CPB may adversely affect operative outcome [1]. Among the organ systems, postoperative neurodevelopmental impairment has become a more prominent complication that affects the quality of life of the surviving patients with congenital heart disease. Specifically, the brain may be injured by inadequate cerebral blood flow, embolism, and systemic inflammatory response. To provide a bloodless field for the precise repair of congenital cardiac malformations, a low-flow or deep hypothermic circulatory arrest (DHCA) is often necessary for pediatric cardiac surgery, creating ischemia-reperfusion injury to the brain. Embolic brain injury may occur as a result of air, thrombus, or debris. CPB injures blood elements causing protein denaturation and release of various proinflammatory cytokines [2]. These nonphysiologic reactions within the blood cause disturbances in capillary permeability, vascular tone, fluid distribution, and organ function collectively known as the "systemic inflammatory response," which may synergistically aggravate the other potential mechanisms of brain injury [3]. Steroids administration has been frequently used to counteract these inflammatory reactions of CPB. This study was designed to evaluate the systemic and neurologic effects of steroids in CPB prime versus systemic pretreatment in a piglet model of DHCA.

Material and Methods

Experimental Preparation

The surgical instrumentation in the piglet model was based on previously established methods detailed elsewhere [4]. Four-week-old Yorkshire piglets weighing 7.0 ± 0.8 kg were sedated with 45 mg/kg of intraperitoneal methohexital sodium. An intravenous line was immediately inserted into the ear vein followed by tracheal intubation with a 4.5-mm cuffed tube. Mechanical ventilation was supported by a pressure-controlled ventilator (ADS 1000; Engler Engineering Corporation, Hialeah, FL) set at a peak inspiratory pressure of 20 cmH_2O, inspired oxygen fraction (FiO_2) of 1.0, and a rate of 13 to 15 breaths per minute. After an intravenous bolus of fentanyl

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(25 μg/kg) and pancuronium (0.2 mg/kg), anesthesia was maintained with a continuous infusion of fentanyl (25 $\mu g \cdot kg^{-1} \cdot h^{-1}$), pancuronium (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) and midazolam (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) throughout the entire experiment except for the period of DHCA. Esophageal and rectal temperatures were maintained at 37°C to 38°C before and after CPB.

All surgical procedures were carried out under sterile conditions. A superficial branch of the left femoral artery was first cannulated with an indwelling catheter for continuous arterial blood pressure monitoring. Central venous pressure (CVP) in the right atrium (RA) was also recorded. After systemic anticoagulation with heparin (300 IU/kg), an 8-Fr arterial cannula (Medtronic; Bio-Medicus, Minneapolis, MN) was inserted into the right femoral artery followed by a 24-Fr venous cannulation (Stöckert Instrumente Gmbh, Lilienthalalle, Germany) of the RA appendage exposed through a right anterolateral thoracotomy. Once CPB was initiated, all animals were cooled to 15°C over 30 minutes, followed by 100 minutes of DHCA, then weaned off CPB after 40 minutes of reperfusion. Intravenous protamine (5 mg/kg) was given to reverse the heparin effect. Decannulation and closure of all incisions completed the operative procedure.

The experimental protocol was approved by McGill Animal Care Committee (No. 4064). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1985).

Extracorporeal Circuit Preparation

The CPB circuit consisted of a roller pump (Stöckert Instrumente, Munich, Germany), sterile tubing, and a membrane oxygenator (Lilliput1; Dideco, Mirandola, Italy). Fresh heparinized whole blood obtained from a donor animal harvested on the same day of experiment was used to prepare the pump prime solution. Crystalloid solution was titrated to achieve a priming hematocrit (Hct) of 25% in all groups. Cefazolin sodium (25 mg/kg), furosemide (0.25 mg/kg), and sodium bicarbonate (10 mEq) were standard additions to the prime. After baseline recording was obtained and instrumentation completed, full CPB support was established at a flow rate of 100 $mL \cdot kg^{-1} \cdot min^{-1}$ using the pH-stat strategy.

Before reperfusion, furosemide (0.2 mg/kg), mannitol (0.5 mg/kg), and sodium bicarbonate (10 mEq) were added to the pump. Each animal was rewarmed at similar flow rates for more than 40 minutes to achieve a rectal temperature of 35°C before weaning off CPB. The heart was defibrillated as necessary at 25°C. The Hct was maintained between 27% and 28% by blood transfusion or crystalloid infusion upon weaning off CPB. Mechanical ventilation with FiO2 of 1.0 was reestablished 10 minutes before CPB was discontinued. When hemodynamic stability was achieved, protamine was given, followed by decannulation and skin closure.

Experimental Groups

Ten piglets (n = 5 per group) were randomly assigned to two groups. In group PP, methylprednisolone sodium succinate (30 mg/kg; Upjohn Company of Canada, Don Mills, Ontario) was administered into the CPB prime. In group PT, a similar dose of intravenous steroid (30 mg/kg) was given 4 hours before CPB. No additional steroid was given in the prime otherwise.

Postoperative Management

All animals were monitored hemodynamically with an arterial line and CVP, with the core temperature maintained at normothermia using a warming blanket and a heating lamp, while they remained fully sedated, paralyzed, and mechanically ventilated. Crystalloid infusion was administrated to maintain CVP and Hct at about preoperative levels. Dopamine was titrated between 5 to 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ as required. Periodic blood gas and Hct levels were evaluated hourly for the first 3 hours and at 6 hours and corrected accordingly. Postoperative Hct was kept between 27% and 28% by crystalloid infusion or blood transfusion as indicated. Random blood sugar levels were assessed before and after surgery. Continuous monitoring and data collection were carried out until elective termination of the experiment at 6 hours after weaning off CPB.

Data Collection

Baseline body weight (BW) was recorded immediately after the induction of anesthesia and endotracheal intubation. Subsequent weight measurement was repeated at 6 hours postoperatively before termination of the experiment. Postoperative weight was expressed as percent increase from baseline and was calculated as follows: (postoperative BW − baseline BW) × 100% / baseline BW.

To obtain the bioelectrical impedance index (BEI), the baseline resistance and reactance were measured by a bioimpedance analyzer (BIA-101Q; RJL Systems, Inc, Clinton Township, MI) using two pairs of signal and detecting electrodes attached to the upper and lower extremities after intubation. Measurements were repeated upon weaning off CPB and at 6 hours postoperatively. The BEI was then derived from the following equation: $BEI = (resistance^2 + reactance^2)^{1/2}$. The total body water content (TBWC) has a reciprocal relationship with the BEI ($TBWC \times height^2/BEI$). Higher TBWC is associated with lower BEI because of the higher conductivity of water in the biologic system [4]. BEI expressed as percent decrease from baseline was calculated as follows: (baseline BEI − postoperative BEI) × 100% / baseline BEI.

To measure colloid oncotic pressure (COP), blood samples were obtained at baseline, immediately after weaning off CPB, and at 6 hours postoperatively. The blood COP was measured by a membrane colloid osmometer (Wescor 4420, Wescor, Utah) calibrated with 5% albumin (19.3 ± 1.4 mm Hg) with a molecular weight cut off of 30,000.

Trypan blue analysis was performed to assess the

cerebrovascular integrity and the extent of leakage at the blood brain barrier (BBB) after each experiment. Immediately upon elective termination of the experiment, all animals were euthanized. Each brain was perfused with 1 L saline, followed by 2% trypan blue infusion (approximately 1 mL/g of brain tissue). In situ brain fixation with 4 L of 4% paraformaldehyde was then carried out. After decapitation, the head was submerged in 10% formaldehyde solution for 7 days before the brain was removed from the skull and further preserved in formaldehyde. Three consistent areas of the frontal, temporal, and occipital cortices were sampled and submitted for spectrophotometric analysis [5]. The specimens were weighed and dried at 105°C for 24 hours, then reweighed and placed in Krebs-Ringer solution for another 24 hours. The samples were then homogenized in the Krebs-Ringer solution and mixed with 0.5 mL of 60% trichloroacetic acid to precipitate protein. The samples were then cooled for 30 minutes, centrifuged, and the supernatants measured at 610 nm for absorbance of trypan blue using a spectrophotometer (BIO-RAD Microplate Reader model 3550, Baltimore, MD). Cerebral trypan blue content is expressed as ng/g of dry weight calculated against a standard curve. The evaluation was performed in a blinded fashion. The mean values of the examined areas were used for statistical analysis.

Immunohistochemical analysis of cerebral transforming growth factor-β_1 (TGF-β_1) was performed to evaluate the expression of this neuroprotective agent after different steroid administration protocol set in this study. Brain specimens were fixed in formaldehyde, serially dehydrated in ethanol, and embedded in paraffin. The samples were sectioned at 6 μm, floated onto slides, deparaffinized, rehydrated and endogenous peroxidase activity blocked by incubating with 3% H_2O_2 in 99% methanol. The sections were then permeabilized using phosphate-buffered saline (PBS pH 7.5) containing 0.1% Triton X-100. Nonspecific binding was blocked with PBS containing 10% normal goat serum, 0.3% Triton X-100, and 0.5% bovine serum albumin. Anti–TGF-β_1 antibody was then applied to the sections for 1 hour at room temperature. The slides were incubated with biotinylated goat antirabbit secondary antibody and stained with 3-amion-9-ethyl-carbazole. The slides were washed, counterstained with Mayer's hematoxylin (Sigma, St. Louis, MO), and mounted. The anti–TGF-β_1 antibody has been shown to be specific by complete absorption of its immunoreactivity when incubated with TGF-β_1. Controls for the immunohistochemistry included experiments in which the primary antibody was omitted, and nonimmune IgG was substituted in place of primary antibody.

The apoptotic index of brain injury was assessed by immunohistochemical caspase-3 assay. Brain tissues from groups PP and PT were fixed in formaldehyde. The tissues were embedded in paraffin, cut into 6 μm thick slides, then processed for immunohistochemistry analysis. Caspase-3 fluorescent staining was carried out using a polyclonal antibody recognizing the active 17 kDa caspase-3 fragment (New England BioLabs, cat. #9661S). Digital images of the fluorescent staining were analyzed in a blinded fashion.

Statistical Analysis

All results are expressed as absolute mean ± SD with the exception of BW and BEI, which are expressed as percent change from baseline ± SD. Data were analyzed by unpaired *t* test for continuous data between groups. A p value less than 0.05 is considered statistically significant.

Results

Experimental Conditions

The experimental conditions were similar for each group throughout the experiment (Table 1). There were no significant differences regarding the baseline body weight, COP, BEI, blood gas, preoperative and postoperative Hct levels, systemic temperature, and hemodynamic variables between groups.

Operative Outcome

All animals survived the duration set for the experiment. One animal in group PP developed progressive hypotension and tachycardia with increasing Hct level (36%). A significantly distended abdomen was noted on examination, and in a near-arrest state at 90 minutes postoperatively, drainage of the abdominal ascites was necessary. The blood pressure improved from a mean of 40 mm Hg to 64 mm Hg after the drainage procedure and remained stable throughout the remaining course of the experiment. Approximately 370 mL clear ascites was drained immediately, and by 6 hours postoperatively a total of 1.1 L ascetic fluid had been drained. COP analysis of the ascetic fluid was performed and was at 13 mm Hg, while the blood taken simultaneously was at 12 mm Hg. The hemodynamic pattern of this particular animal resembled the clinical situation of severe capillary leak syndrome in an infant after CPB.

Changes in Body Weight

Baseline BW was comparable between the two groups (PP 6.9 ± 0.9 kg versus PT 7.1 ± 0.9 kg; p = not significant). Postoperative changes in body weight were expressed as percent increase from baseline ± SD. At 6 hours after discontinuation of CPB, the percent increase in BW was significantly higher in group PP (25.0% ± 10.4%) versus PT (12.5% ± 4.0%; $p = 0.036$).

Changes in BEI

There were no differences in BEI between groups before the operation (PP 164.5 ± 17.2 Ω versus PT 175.6 ± 14.9 Ω; p = NS). Subsequent changes in postoperative BEI are expressed as percent decrease from baseline ± SD. Immediately after coming off CPB, there was no significant difference in BEI between the two groups. However, at 6 hours postoperatively, the BEI in group PP was significantly decreased compared with group PT (PP 37.2% ± 14.5% versus PT 15.6% ± 7.9%; $p = 0.019$)

Table 1. Experimental Conditions

	Pump Prime Group	Pretreated Group	*p* Value
Baseline body weight (kg)	6.9 ± 0.9	7.1 ± 0.9	NS
Esophageal temperature (°C)			
Base line	37.9 ± 0.8	38.2 ± 0.5	NS
DHCA	14.4 ± 2.3	15.5 ± 2.1	NS
40 minute rewarming	37.2 ± 1.4	37.2 ± 0.9	NS
6 hour post-CPB	37.7 ± 0.4	37.5 ± 0.4	NS
Rectal temperature (°C)			
Base line	38.1 ± 0.6	38.2 ± 0.5	NS
DHCA	16.7 ± 2.0	17.2 ± 2.6	NS
6 hour post-CPB	37.7 ± 0.4	37.7 ± 0.3	NS
MAP (mmHg)			
Base line	74.8 ± 6.6	72.6 ± 7.6	NS
Cooling on CPB	66.0 ± 15.5	57.4 ± 11.4	NS
6 hour post-CPB	75.0 ± 12.5	73.6 ± 8.6	NS
Heart rate (beats/min)			
Base line	126.0 ± 8.5	125.0 ± 7.0	NS
6 hour post-CPB	137.0 ± 5.9	144.8 ± 17.0	NS
CVP (mmHg)			
Base line	7.8 ± 1.8	8.0 ± 1.6	NS
1 hour post-CPB	8.6 ± 2.3	9.0 ± 1.6	NS
6 hour post-CPB	11.8 ± 3.3	8.5 ± 1.3	NS
pH			
Base line	7.4 ± 0.1	7.5 ± 0.1	NS
1 hour post-CPB	7.4 ± 0.1	7.5 ± 0.1	NS
6 hour post-CPB	7.4 ± 0.1	7.5 ± 0.2	NS
pCO_2 (mmHg)			
Base line	41.8 ± 6.9	39.8 ± 7.7	NS
1 hour post-CPB	42.8 ± 9.2	35.4 ± 5.3	NS
6 hour post-CPB	39.0 ± 5.0	41.6 ± 13.7	NS
pO_2 (mmHg)			
Base line	515.8 ± 61.9	571.8 ± 66.5	NS
1 hour post-CPB	387.8 ± 213.8	469.6 ± 135.8	NS
6 hour post-CPB	293.6 ± 133.4	459.2 ± 109.2	NS
Hematocrit (%)			
Base line	26.4 ± 1.1	27.2 ± 1.6	NS
Cooling on CPB	26.0 ± 1.0	26.6 ± 0.9	NS
1 hour post-CPB	28.6 ± 4.4	28.6 ± 3.1	NS
6 hour post-CPB	26.4 ± 2.2	27.2 ± 2.4	NS

CPB = cardiopulmonary bypass; CVP = central venous pressure; DHCA = deep hypothermic circulatory arrest; kg = kilogram; MAP = mean arterial pressure; NS = not statistically significant.

suggesting a significant increase in TBWC in animals receiving steroids in pump prime.

Colloid Oncotic Pressure

The baseline COP values were similar between the two groups. Upon termination of CPB, the mean COP remained relatively unchanged with no significant difference between groups. At 6 hours postoperatively, the COP in group PP was significantly reduced compared with group PT (PP 11.6 ± 0.8 mm Hg versus PT 15.3 ± 1.8 mm Hg; $p = 0.003$; Fig 1).

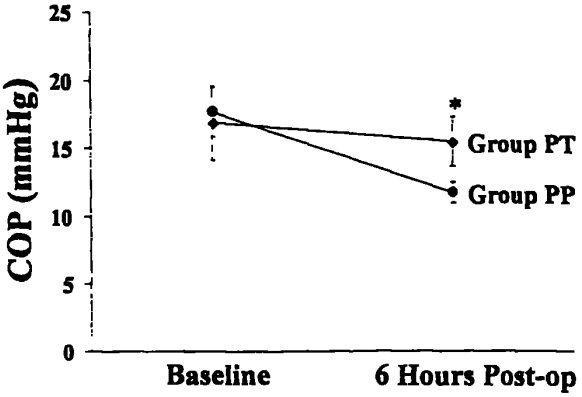

*Fig 1. Results of colloid oncotic pressure (COP, in mm Hg). The mean baseline COP was not different between groups but significantly decreased in the pump prime group (Group PP) versus the pretreated group (Group PT) at 6 hours postoperatively. (*p = 0.003.)*

Cerebral Trypan Blue Content

The values of trypan blue content are expressed as mean ± SD in ng/g of dry tissue. At 6 hours postoperatively, a significantly higher content of cerebral trypan blue was found in group PP ($9.1 \times 10^{-3} \pm 5.7 \times 10^{-4}$ ng/g dry tissue) than in group PT ($5.6 \times 10^{-3} \pm 1.1 \times 10^{-3}$ ng/g dry tissue; $p = 0.001$). This is suggestive of a better-preserved vascular integrity in the BBB among the steroid-pretreated animals undergoing prolonged DHCA (Fig 2).

Cerebral TGF-β_1 Expression

Immunohistochemical staining of TGF-β_1 in representative brain tissue is demonstrated in Figure 3. Two researchers blinded to the protocol performed independent assessment. Marked immunostaining for TGF-β_1 was observed in all the specimens analyzed in group PT, suggesting high expression of TGF-β_1 in this group. In

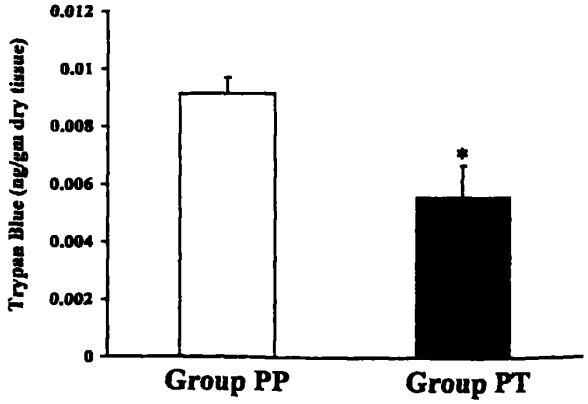

*Fig 2. Spectrophotometric analysis of cerebral trypan blue (ng/g dry tissue) showed significantly higher content in the animals receiving steroid in pump prime. (*p = 0.001.) (Group PP = pump prime group; Group PT = pretreated group.)*

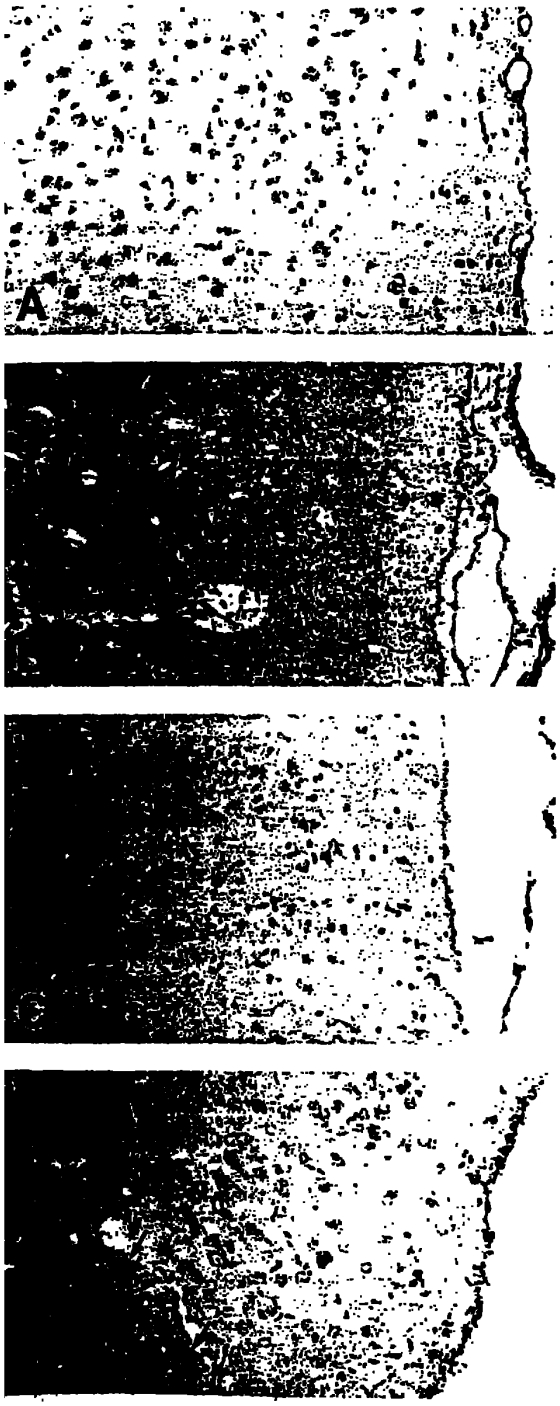

Fig 3. Representative immunohistochemical assays of cerebral TGF-β_1 (magnification ×40). (A) Negative control staining without primary antibody. (B) Positive TGF-β_1 expression (arrows) in sham brain without exposure to deep hypothermic circulatory arrest. (C) Absence of TGF-β_1 expression in the pump prime (group PP) animals. (D) TGF-β_1 expression in the pretreated (group PT) animals (arrows) resembled that in sham specimens.

contrast, the TGF-β_1 immunostaining was scarce in sections from group PP. Sham brain specimens prepared similarly to those of the experimental groups were obtained from a piglet not exposed to DHCA. The sham specimens exhibited intense TGF-β_1 immunostaining, similar to that observed in specimens from group PT.

Caspase-3 Activity

Positive fluorescent caspase-3 staining determined by immunohistochemical analysis showed extensive activation of the enzyme in the endothelial cells as well as perivascular parenchymal brain tissues in group PP. Remarkable caspase-3 activities were also found at areas distant from the vessels in group PP. In contrast, mainly vascular endothelial caspase-3 activities with minimal parenchymal involvement were identified in the group PT specimens (Fig 4).

Comment

Brain injury during cardiac surgery in infants has been attributed in part to the extreme manipulations of CPB flow rate or DHCA. Embolic brain injury represents another offending mechanism. The contact of the circulating blood with the synthetic surfaces of the extracorporeal circuits has been shown to activate a whole spectrum of inflammatory mediators and vasoactive compounds, collectively known as the "systemic inflammatory response" [2, 3]. In addition, total body water is relatively greater and capillary permeability is higher in newborns. Therefore, capillary leak syndrome is a more prominent morbidity of CPB in these pediatric patients [1]. These nonphysiologic reactions, while having the potential to directly inflict end-organ injury, can also further exacerbate the severity of cerebral ischemia-reperfusion injury associated with DHCA. In a group of patients undergoing CPB without circulatory arrest, Har-

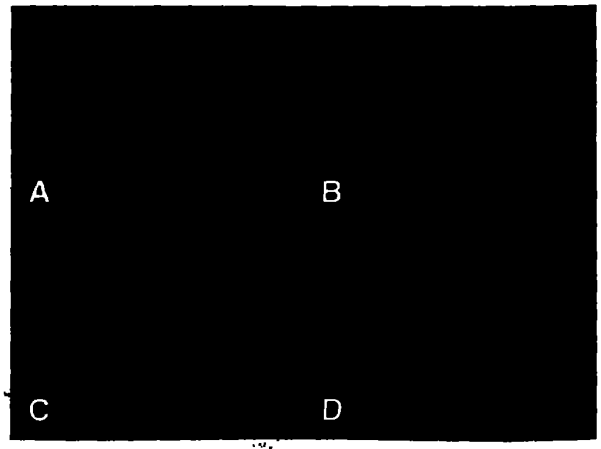

Fig 4. Immunohistochemical assays of caspase-3 activity in representative specimens. Note the extensive perivascular parenchymal involvement in addition to endothelial cells activation in the pump prime group (A and B) compared with the pretreated group (C and D; magnifications ×100).

ris and coworkers [6] have demonstrated remarkable swelling of the brain when examined by magnetic resonance imaging early after cardiac surgery. Although most of these early imaging changes in the brain subsided within days to weeks after surgery without major neurologic deficits, in the presence of hypoxic-ischemic brain injury caused by DHCA, their consequences may be further potentiated.

Steroids and Cardiopulmonary Bypass

The application of steroids in the early days of CPB was directed at reducing the negative hemodynamic effect of the extracorporeal circulation. Significant reduction of the vasoconstriction and low output state with subsequent improvement in peripheral perfusion has been reported using 30 mg/kg of methylprednisolone [7]. Although early studies have shown improvement in survival and outcome associated with the use of steroids, their mechanisms of action and biochemical benefits have only been recently documented [8]. It has been suggested that steroids may reduce the release of lipid mediators and the concentration of proinflammatory cytokines 1, 6, 8 and TNF-α while increasing the antiinflammatory interleukin 10 [8, 9].

This study has documented the beneficial effect of steroid pretreatment with respect to the systemic manifestation of capillary leak syndrome. In the steroid pretreatment group, there was a significantly better preservation of COP associated with less need for crystalloid infusion reflected by the changes in BW and BEI. In contrast, one extreme animal in group PP manifested a classic picture of severe capillary leak syndrome, with ascites, hypotension, increasing Hct, and low CVP despite fluid administration.

Steroids and Brain Injury

Steroid therapy has been applied in the setting of central nervous system injury. Its effectiveness has been a subject of controversy primarily because of undefined dosage, timing of administration, different mode of brain injury, and concern for side effects. However, Jane and coworkers [10] reported a significant improvement in severely injured patients who received an initial 15 to 30 mg/kg dose of methylprednisolone at the scene of the head-related accident. The mortality rate was strikingly reduced compared with that of a comparable group receiving no steroids.

In our setting of ischemia-reperfusion brain injury and inflammatory response to CPB, the trypan blue findings suggested significant disturbances in the neurovascular integrity of the BBB in the pump prime steroid group compared with steroid pretreatment. This increase in permeability of the BBB was translated into a higher index of apoptotic neuronal damage. All apoptotic caspases normally exist in cells as inactive enzymes. When cells undergo apoptosis, these caspases become activated through sequential proteolytic events that cleave the single peptide precursor into the active enzyme. Caspase-3 has been shown to play a central role in the regulation and execution of apoptosis [11]. In addition, there was a significantly better preservation of cerebral TGF-β_1 expression in steroid pretreated animals. Although TGF-β_1 is minimally expressed in intact adult brain, at a young age while organ development is very active, its expression is increased [12]. Increased TGF-β_1 expression has also been demonstrated after ischemic brain injury. This upregulation was thought to be a crucial response to injury since it possesses angiogenic and antiinflammatory properties that affect neurodegeneration, chemotaxis, and extracellular matrix remodeling. It also has a neuroprotective capacity to rescue cultured neurons from excitotoxic and hypoxic cell deaths, to reduce infarct size after cerebral ischemia [13]. The failure to activate otherwise normal amounts of TGF-β_1, on the other hand, is a negative prognostic factor.

Timing of Steroid Administration

Intuitively, it seems unlikely that a simple variation in the timing of steroids administration during CPB and DHCA can have different physiologic consequences. However, it is interesting to note that in the majority of reports demonstrating the benefit of steroids during extracorporeal circulation, the medication was administered directly into the patients before initiation of CPB [8, 14]. Although this detail has not been emphasized in their conclusions, more recent studies suggested that the timing of steroid administration played an important role. In a piglet model of DHCA, Lodge and associates [15] have shown that methylprednisolone pretreatment before surgery was associated with better pulmonary functions as compared with steroid in pump prime or no steroid groups. Similar findings were confirmed in clinical settings [8]. Our study incidentally revealed a strong tendency toward a decrease in arterial oxygen tension in group PP at 6 hours postoperatively. Steroid pretreatment has also been shown to have superior cerebral oxygen metabolism and recovery of cerebral blood flow after DHCA in a piglet model [16]. Our study results concurred with these previous reports and demonstrated that steroid pretreatment significantly decreased fluid accumulation, loss of COP, and trypan blue leakage across cerebral vasculature, with better indices of cerebral protection.

Several lines of evidence support the pharmacologic basis of these benefits. First, methylprednisolone is usually esterified with succinic acid to produce a water-soluble prodrug salt (methylprednisolone sodium succinate). Upon administration, this prodrug is hydrolyzed to the active moiety methylprednisolone by carboxylesterase enzymes. Among patients undergoing CPB, the peak concentration of methylprednisolone is reached approximately 1 to 2 hours after administration of methylprednisolone sodium succinate [17]. Second, steroids suppress inflammation by exerting their effects at various molecular levels. Through their receptors in the cytoplasm of target cells, they inhibit the adhesion molecule expression in the endothelial cells, which relates to the trafficking of leukocytes into the injured areas [18]. They also affect enzyme induction, protein synthesis, gene

transcription, and even chromatin structure [19]. In view of these pharmacokinetics and mechanisms of action of steroids, it is logical that the steroids should be prophylactically present at the target sites when activation of blood elements is triggered by the contact of the synthetic surfaces of the CPB circuits. Although it remains controversial whether blunting the activation and release of various proinflammatory cytokines alone may favorably improve clinical outcome, our study suggests that in the presence of cerebral ischemia-reperfusion injury related to DHCA, the seemingly benign inflammatory reaction might aggravate end-organ damage.

There are several limitations to the current study. This acute experiment reflects early indices of neurologic damage. The implications of these findings with regard to the neurologic outcome therefore remain undefined. Nevertheless, ischemic changes that occurred during the early postoperative period have been correlated with subsequent neurohistologic damage in surviving animals [20]. In addition, although we have not assessed the effect of no steroid treatment in this study, research in our laboratory is ongoing to address this issue. However, the data from Lodge and colleagues [15] suggested that in the presence of DHCA, pulmonary function was significantly worse for the groups not receiving steroids than for the groups receiving steroids either in the pump prime or by systemic pretreatment.

In conclusion, this study demonstrated that under the condition of prolonged DHCA, systemic steroid pretreatment resulted in significant reduced total body edema and cerebral vascular leak and was associated with decreased apoptosis, and therefore better indices of neuroprotection.

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DISCUSSION

DR ROSS M. UNGERLEIDER (Portland, OR): That is a nice study, and you and Dr Tchervenkov and your colleagues are certainly to be congratulated on trying to further our understanding of how to protect neonates during bypass. As you know, I'm a proponent of pretreatment with steroids, since Dr Lodge and Dr Jaggers in our group a few years ago described the effects of steroid pretreatment. I'm wondering if you could describe for us why you chose the model that you did. In your

two groups, you chose one group with 30 mg/kg of methylprednisolone in the pump prime, which is a fairly standard protocol used by several, and against that you compared a very high dose of 30 mg/kg of methylprednisolone given intravenously, about three times the dose that we've commonly pretreated patients with, and you've given it only 4 hours before exposure to your bypass model. There was no nonsteroid control. What I'm wondering is, could what you be seeing relate to just the dose effect of such a high dose of intravenous steroids given a very short time before exposure, since exposure to steroids in the pump prime would be diluted by the pump prime, and perhaps you're just seeing a dose-related response and nothing that relates to the pretreatment time.

The second thing I wonder about is why you chose just 4 hours of interval before exposure to bypass, as it may take steroids when used as a pretreatment mode much longer than 4 hours to have some kind of an effect. I think you have shown us in your study a change in the inflammatory response to steroids, but I wonder if you could describe a little bit for us why you chose the methodology that you used.

DR SHUM-TIM: Thank you for your kind comment. We are very aware of the literature originated from your lab and we must say that our current study is based on the previous findings from Lodge and Langley's studies who documented the beneficial effect of steroid pretreatment in regards of pulmonary function and cerebral oxygen metabolism. First of all, we chose this protocol for the evaluation of cerebral protection following deep hypothermic circulatory arrest because this is an established model that I have been made familiar with at the Boston Children's Hospital. Secondly, we chose to give the steroids 4 hours prior to surgery based on practicality. Obviously, as your previous papers suggested to give steroids 8 hours prior to surgery meant that if a patient was to have an operation at 8:00 in the morning, one had to start an intravenous and give a large dose of steroids at midnight. We found this was very impractical and inconvenient from a clinical point of view. Thirdly, there are certain pharmacologic studies that actually supported the benefits of using 4 hours pretreatment. There is a study looking at the pharmacokinetics of methylprednisolone in patients undergoing cardiopulmonary bypass, for instance. As you know, the active moiety of this steroid is the methylprednisolone. However, the preparation given clinically is the methylprednisolone sodium succinate. This preparation is to increase the water solubility of the medication so that it can be injected intravenously. This study found that in those patients undergoing cardiopulmonary bypass, the peak concentration of the active medication (ie, methylprednisolone) in the circulation is actually about 1 to 2 hours after the administration. Therefore, we think that the drug should be administered into the patient and allowed the active part of the medication to bind the proper receptors before activation of the blood element is initiated by contact with CPB. To answer your first question, the dose of 30 mg/kg of methylprednisolone is a very large pharmacological bolus given under normal circumstances. We do not think that giving it intravenously or giving it into the pump prime which, in our model, has approximately 300 cc of blood prime would create a difference in terms of the actual dose of steroids given. Therefore, to answer your first question, we do not think that the difference in the outcome measured in the study is based on a dose-related phenomenon. Rather, I think the bottom line is that the medication should be onboard at the time when all the inflammatory reactions are activated. It is also unlikely that the difference observed is due to a variation in the timing when the peak medication is present in the circulation. In other words, if we wait long enough, will the beneficial effects be similar? I don't have data to back up this statement but our clinical experience in which we give steroids directly to our patients intravenously, I can tell you that the hemodynamic effect and the remarkable difference in the extent of capillary leak syndrome are quite impressive. Therefore, I don't think that the beneficial effect of steroid pretreatment is solely based on the timing of the peak nor the difference in the concentration. I think the bottom line is the medication should be onboard before the injury is started.

DR JAKOB VINTEN-JOHANSEN (Atlanta, GA): Thank you for that presentation. How did your two interventional groups compare to a control untreated animal undergoing the same process? And secondly, what part of the inflammatory response do you think that the methylprednisolone is acting on? Is it at the endothelial level or at the neutrophil level? Is it inhibiting the generation of superoxide radicals through inhibition of neutrophils? Or at this point do you not know?

DR SHUM-TIM: To answer your last question, I think we all know that steroids have a very broad spectrum of activities and it works at several molecular levels. At this point, we don't have the data to show exactly at what level it works but I believe it works at the endothelial cells of the vessels as well as the steroid receptors on the plasma membrane of the target cells as suggested by the literature. It has also been shown that steroids affect the transcription of messenger RNAs and the protein synthesis. All in all, the systemic inflammatory reaction caused by cardiopulmonary bypass is so extensive and redundant. I believe that it takes something equally wide spectrum in order to effectively counteract these effects.

With respect to your first question, at the time when we submitted this abstract, we did not have the information regarding another group without steroid treatment at all. As a continuation of this study, which we have recently completed, to our surprise, the group without steroids versus the group with steroids in pump prime does not have significant differences.

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TIMING OF STEROID TREATMENT IS IMPORTANT FOR NEUROPROTECTION DURING CARDIOPULMONARY BYPASS AND CIRCULATORY ARREST

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IN PEDIATRIC CARDIAC SURGERY, the technique of cardiopulmonary bypass (CPB) management is unique. To facilitate the complex intracardiac repair in the presence of excessive noncoronary collaterals, complete cessation or reduction of blood flow has been widely used in infants. These CPB strategies conceivably may create severe neurologic insult compared with the standard technique in which extreme manipulations of temperature and flow rate are rarely used. Cardiopulmonary bypass is also known to activate the systemic inflammatory response within the blood vessels, which causes disturbances in vascular permeability, fluid distribution, and end-organ dysfunction. This nonphysiologic reaction to CPB can further aggravate cerebral ischemia-reperfusion injury during cardiac surgery. The administration of steroids has frequently been used to counteract these inflammatory reactions of CPB. This study was designed to evaluate the effects of CPB and deep hypothermic circulatory arrest (DHCA) on neuroprotection using 3 different steroid administration protocols in a piglet model.

MATERIALS AND METHODS

Eighteen 4-week-old piglets were divided into 3 groups. Methylprednisolone (30 mg/kg) was administered either intravenously 4 hours before CPB in group 1 (n = 6) or added in pump prime in group 2 (n = 6). Group 3 (n = 6) received no steroid. The hematocrit in the pump prime was 25% in all groups. Once the CPB was initiated, all animals were cooled to 15°C followed by 100 minutes of DHCA (15°C), then weaned off CPB after 40 minutes of rewarming. Animals were killed 6 hours after cessation of CPB support. Postoperative weight gain, bioelectrical impedance, and colloid oncotic pressure (COP) were evaluated. Neurovascular integrity was determined by trypan blue infusion. Cerebral transforming growth factor (TGF-β1) expression and caspase-3 activity as indicators of apoptosis were performed by immunohistochemical assays. Statistical analysis was performed with analysis of variance (ANOVA) and the Mann-Whitney rank sum test. A P value less than .05 was considered statistically significant.

RESULTS

Postoperative percentage of weight gain (13.0 ± 3.8 [group 1] vs 26.4 ± 9.9 [group 2] vs 22.6 ± 6.4 [group 3]; $P < .05$ by ANOVA); percentage bioimpedance change (14.3 ± 7.7 [group 1] vs 38.6 ± 13.4 [group 2] vs 30.3 ± 8.1 [group 3]; $P < .05$), and COP (mm Hg) (14.9 ± 1.8 [group 1] vs 10.9 ± 2.0 [group 2] vs 6.5 ± 1.8 [group 3]; $P < .05$) were significantly different between the groups. Spectrophotometric analysis of cerebral trypan blue (ng/g dry weight) was significantly different between the groups (0.0053 ± 0.0010 [group 1] vs 0.0096 ± 0.0026 [group

From the Division of Cardiovascular Surgery, Montreal Children's Hospital, McGill University Health Center, Montreal, PQ. *Supported by the Heart and Stroke Foundation of Quebec and Andy Collins for Kids Foundation. Technical assistance of Minh Duong and Dianne Murray is acknowledged.*

2] vs 0.0090 ± 0.0019 [group 3]; $P < .05$). Immunohistochemical scores for TGF-β1 expression were 3.3 ± 0.8 (group 1) vs 1.5 ± 0.8 (group 2) vs 1.5 ± 0.5 (group 3) ($P < .05$ group 1 vs 2 and group 1 vs 3 by Mann-Whitney test [1 = minimal expression, 4 = maximal expression as control brain not exposed to CPB]). Perivascular caspase-3 activity in groups 2 and 3 was remarkably higher than that of group 1.

DISCUSSION

Systemic steroid administration has been reported to improve outcome in head-related trauma and experimental acute hypoxic-ischemic brain injury.[1,2] These experimental studies and clinical observations raise optimism about the neuroprotective effect of high-dose steroids, which may significantly reduce brain edema and improve neurologic outcome if given early before or at the scene of injury. Significant decrease in the production of various proinflammatory cytokines and improved hemodynamic stability have also been reported in the setting of hypothermic CPB and DHCA.[3] While most of these studies administered systemic steroids before initiating CPB, it has been only recently that the literature has focused on the timing of administration. Lodge et al[4] have shown that methylprednisolone pretreatment before DHCA improved pulmonary function compared with steroids in pump prime or to groups without steroids. Yet, addition of pharmacologic agents in priming of extracorporeal circuits during CPB is still frequently used in many centers. In the present study, we suggested that the timing of steroid administration could critically influence its therapeutic effects. The experimental group that received steroid pretreatment before CPB and DHCA exhibited significantly reduced total body edema and increased cerebral protection. Since the activation of blood elements occurs when they contact the artificial surfaces of the bypass circuits, to have an optimal effect, the therapeutic agent should be administered before the initiation of CPB. In addition, steroids suppress inflammation by acting at various molecular levels.[5] On the basis of pharmacokinetics and mechanisms of action, it is logical that methylprednisolone should be prophylactically present at the target sites before activation of systemic inflammatory reaction is triggered by the contact of synthetic surfaces of the CPB circuit with the patient's blood. In conclusion, the administration of steroids in CPB prime does not offer additional benefits over no steroid treatment with respect to extracellular fluid accumulation and cerebral protection. In addition, our results strongly suggest that systemic pretreatment and, therefore, the timing of administration are essential for steroids to exert their neuroprotective effects.

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『Optimal Vascular Delay in Cardiomyoplasty Following Latissimus Dorsi Muscle Isolation』

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Optimal Vascular Delay in Cardiomyoplasty Following Latissimus Dorsi Muscle Isolation: A Canine Study

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Running Title: Vascular delay in latissimus dorsi dissection

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Abstract

Background and Purpose: Distal ischemia of the latissimus dorsi muscle (LDM) during cardiomyoplasty is a recognized complication that can reduce the muscle's function and mechanical effects. A 2-week vascular recovery period is recommended to allow revascularization and adhesion to the heart. It is not clear, however, that a 2-week vascular delay is optimal after LDM isolation. This study was designed to evaluate (i) the regional blood flow (RBF) of canine LDM flaps immediately after perforators were ligated, and (ii) the effect of 1-, 2-, and 3-week vascular delays on regional perfusion of LDM flaps without electrical stimulation.

Materials and methods: A catheter-access device connected to the left atrium was implanted in the left back subcutaneous layer in each of six adult mongrel dogs when the LDM was dissected. Five different colored microspheres were injected at five different time points, *viz.*, pre- and post-dissection, and after 1-, 2-, and 3-week vascular delays. At each time point, reference blood was withdrawn from the femoral artery at a fixed speed. The LDMs were removed post mortem for regional blood flow (RBF) determination. Both tissue and reference-blood samples were spectrophotometrically processed to quantify the amount of dye.

Results: Proximal RBF did not decrease immediately after dissection when compared to

the control (0.28 ± 0.10 vs. 0.26 ± 0.05 mL/g/min, $p > 0.05$), but it decreased after a 1-week vascular delay (0.11 ± 0.02 vs. 0.26 ± 0.05 mL/g/min, $p < 0.01$), and then returned to normal after a 3-week vascular delay (0.21 ± 0.06 vs. 0.26 ± 0.05 mL/g/min, $p > 0.05$). RBF decreased immediately after dissection in the middle and distal segments, and did not return to the control value even after a 3-week vascular delay.

Conclusion: The LDM's regional blood flow was depressed by surgical dissection in this canine model. Without electrical stimulation, the middle and distal portions of the LDM remained compromised even after a 3-week vascular delay. The clinical effects of progressive programmed electrical stimulation of the LDM at different periods of vascular delay remain to be studied.

Key words: cardiomyoplasty, vascular delay, regional blood flow, colored microsphere

Introduction

Dynamic cardiomyoplasty, in which a skeletal muscle is used to assist a failing ventricle, is a recently devised treatment option for end-stage heart failure [1-3]. The advantages of the treatment are that all patients have their own "power source", rejection is not a problem, and immunosuppression is not necessary. The latissimus dorsi muscle (LDM) is commonly used for this purpose because of its large mass and mobility [4].

Distal ischemia and necrosis of the LDM flap, however, are recognized complications of cardiomyoplasty [5]. The LDM is perfused principally by the thoracodorsal artery and perforators from the intercostal and lumbar arteries [6]. To mobilize the LDM flap for use in this cardiac-assist procedure, the perforators must be ligated. Because the ligations reduce the blood supply in the middle and distal regions of the muscle, distal ischemia and fibrosis will certainly influence muscle contractile function. Kratz et al [5] demonstrated that a 50% reduction in peak tension development was associated with muscle atrophy and fibrosis in the distal segment of swine LDMs 6 weeks after cardiomyoplasty. They therefore proposed a "vascular delay" period before stimulation to allow revascularization and adhesion to the heart, and to reduce the risk of muscle degeneration caused by ischemia. Two weeks---based on the "classic delay" period suggested by plastic surgeons---has been generally recommended. It is not clear,

however, that a 2-week vascular delay is optimal in dynamic cardiomyoplasty after LDM isolation.

This study was designed to evaluate (i) the regional blood flow (RBF) of canine LDM flaps immediately after perforators were ligated, and (ii) the effect of 1-, 2-, and 3-week vascular delays on regional perfusion of LDM flaps without electrical stimulation. We developed a procedure that allowed us to measure the segmental RBF of LDMs. These measurements were obtained at different time points before and after mobilization, vascular isolation, and reattachment of the LDMs.

Materials and Methods

All animals were cared for in a humane fashion and in accordance with guidelines published by the National Institutes of Health [7]. The Animal Care and Use Committee at the National Cheng Kung University Hospital and College of Medicine approved the protocol for the use of dogs in this study. Sterile techniques were used for all survival surgery.

Animal Preparation

Six adult mongrel dogs obtained from the county animal control authority were used in this study. The animals weighed between 15 and 20 kg. They were not fed on the night before the surgery, and they were all anesthetized in a similar fashion for all procedures, including the RBF measurements after the 1-, 2-, and 3-week vascular delays. The animals were premedicated with ketamine (12 mg/kg, intramuscular), atropine (0.02 mg/kg, intramuscular), and propionylpromazine (0.5 mg/kg, intramuscular) prior to anesthesia, then intubated and anesthetized with sodium pentobarbital (15 mg/kg, intravenous). Supportive fluids and anesthesia (sodium pentobarbital, 100 mg/hr) were administered intravenously. During surgery, the animals were ventilated at 300 ml tidal volume and at a rate of 18 cycles per minute. Each dog received one dose of intravenous

antibiotics (cefazolin sodium, 500 mg) at the induction of anesthesia. For 3 days, oral antibiotics (cephalexin, 30 mg/kg, every 12 hours) were given as a prophylaxis against post-surgery infection. Intramuscular analgesia (buprenorphine hydrochloride, 0.3 mg every 8 hours) was given as needed for one day postoperatively.

Operative Procedures

The operative field was prepared with an alcohol-iodine scrub. A transverse left axillary skin incision along the anterior border of the LDM was made and a skin flap was developed. After identifying the LDM border, a left thoracotomy was carefully performed through the 4th intercostal space without injuring the muscle. The pericardium was incised to expose the left atrium, and the tube of an implantable vascular device (Catheter Access, CAS-205P; Nissho Corporation, Osaka, Japan) was implanted into the left atrium with a purse-string suture. The body of the device was then embedded in the left back subcutaneous layer after the LDM was dissected. An 18-gauge intravenous catheter was inserted into the left or right femoral artery to measure the blood pressure and withdraw the reference blood simultaneously at a fixed speed, while concurrently injecting colored microspheres (CM) into the left atrium through the catheter-access device. Yellow CMs were injected into the LDM before the dissection. The anterior LDM muscle border was

then identified and dissection was extended into the submuscular plane. The vascular-delay operative procedure was accomplished by dividing all the perforating branches entering the costal surface from the underlying intercostal vessels, and the distal LDM was detached from its origin at the thoracolumbar fascia and the 10th rib. The thoracodorsal neurovascular pedicle, however, was left intact after dividing its insertion to the humerus. Immediately following vascular isolation, blue CMs were injected to measure regional RBF. After this mobilization procedure, the muscle was reestablished with sutures to restore the original anatomy and resting tension of the muscle. This procedure simulated cardiomyoplasty surgery in human patients, in which the LDM is mobilized and attached to the heart while maintaining the pedicle. Upon recovery, the animals were placed in a postoperative recovery room for overnight observation. The following day, all animals were examined by the team member and returned to the animal holding room, and then examined daily for the duration of the delay period.

RBF Measurement

RBF to the LDM was measured by the colored microsphere technique [8-10]. Five different CMs (0.2 ml/kg; yellow, blue, red, white, and purple, sequentially) (3×10^6/mL; 15 ì m diameter; Triton Technology, Inc., San Diego, CA, USA) were injected through

the vascular access device at 5 different time points: pre- and post-dissection, and after 1-, 2-, and 3-week vascular delays (Fig. 1). Each time, reference blood was withdrawn from the femoral artery at a fixed speed (7.64 mL/min, 90 seconds total). After each CM injection, the device was flushed with heparin (1000 units) to maintain the patency.

All dogs were anesthetized after 3 weeks with 20 ml of a saturated potassium chloride solution and a high dose of intravenous pentobarbital. Bilateral LDMs were removed for morphology analysis, but only left LDMs were taken for blood flow determination. The LDMs were equally divided into proximal, middle, and distal portions (Fig. 2). Five 2-g tissue samples were randomly collected from these three different regions of each LDM. Kidney samples were also taken to verify microsphere mixing.

For blood flow determination, each tissue sample and reference-blood sample was processed to spectrophotometrically quantify the amount of dye in it [7-9]. First, a 4-molar potassium hydroxide solution ([KOH] beads; Fisher Scientific, Springfield, NJ, USA) was added for digestion overnight. Then the samples were rinsed and filtered (nonelectrostatic vacuum filtration and polyester filter, pore size 8 ì m), and the CMs were suspended in 2% polysorbate 80 solution (Tween 80; Fisher Scientific). The CMs were then washed in 70% ethanol to reduce the amount of lipids and membranes attached to them. Dye was recovered from the CMs by adding dimethyl-foramide ([DMF]; Sigma

Co, St. Louis, MO, USA) as a solvent. The photometric absorption of each dye was determined with a spectrophotometer (DU 600; Beckman Coulter, CA, USA). The absorption spectrum of each dye was measured separately and served as a reference for matrix inversion, and was used to determine the absorption contribution of each color. The composite spectra measured in each color were as follows: 440 nm for yellow, 670 nm for blue, 525 nm for red, 360 nm for white, and 540 nm for purple. Finally, the RBF in each portion was calculated for each injection and then corrected for sample weight by the following equation:

$$[(\text{Calculated regional blood flow})_{tissue}] = [(\text{Measured blood flow})_{reference}] \times [(\text{Absorption})_{tissue} / (\text{Absorption})_{reference}]$$

Data Analysis

The mean RBF and standard error were calculated for each LDM proximal, middle, and distal portion at five different time points. Statistical significance for regional blood flow in proximal, middle, and distal portions for each time point was determined by a one-way ANOVA. When significant F ratios were obtained, statistical differences between different time points were determined by a Student's *t*-test, with a Bonferroni correction for multiple comparisons. Statistical significance was set at $p < 0.05$. All data are presented as mean ± SE.

Results

One dog did not survive after LDM isolation; it died of respiratory failure the day after surgery. The remaining five dogs underwent RBF measurement. Grossly, the studied muscles were atrophic and fibrotic compared to contralateral normal LDMs (Fig. 3). The length of the LDMs in this study were apparently shorter than normal; this may have been caused by edge fibrosis and shrinkage in the middle and distal portions.

Figure 4 shows the RBF responses in the proximal, middle, and distal portions at different time points. Apparently, the proximal RBF did not decrease immediately after vascular isolation when compared to the control (0.28 ± 0.10 vs. 0.26 ± 0.05 mL/g/min, $p > 0.05$); it decreased after one week (0.11 ± 0.02 vs. 0.26 ± 0.05 mL/g/min, $p < 0.01$), and then did not return to the baseline value until three weeks post-surgery (0.21 ± 0.06 vs. 0.26 ± 0.05 mL/g/min, $p > 0.05$). RBF in the middle and distal portions decreased promptly after surgical dissection (0.12 ± 0.02 vs. 0.19 ± 0.03 mL/g/min, $p < 0.01$; 0.10 ± 0.02 vs. 0.19 ± 0.04 mL/g/min, $p < 0.01$; respectively). More important, neither middle nor distal RBF returned to the baseline value even after a 3-week vascular delay (0.08 ± 0.01 vs. 0.19 ± 0.03 mL/g/min, $p < 0.01$; 0.07 ± 0.01 vs. 0.19 ± 0.04 mL/g/min, $p < 0.01$; respectively).

Discussion

Although dynamic cardiomyoplasty using the LDM is currently a treatment option for congestive heart failure, the objective hemodynamic results are inconsistent and correlate poorly with clinical improvement [11, 12]. Acker [12] summarized the results of dynamic cardiomyoplasty in more than 300 patients randomized for three different phases of a clinical study sponsored by Medtronic, Inc. (Minneapolis, MN, USA). Starting in 1994, a phase III, prospective, randomized clinical trial with an expected sample size of 400 cases comparing dynamic cardiomyoplasty with medical therapy was launched under FDA approval. Although it showed that operative mortality had decreased from over 20% in phase I to only 3% of 51 patients in phase III, it was a time- and money-consuming trial; it was also difficult in a short period of time to recruit nearly 400 cases to meet FDA requirements. In 1999, Medtronic announced the cessation of this clinical trial. In 2000, however, CCC del Uruguay, manufacturer of a new cardiomyostimulator, the LD-PACE II, initiated a clinical trial [13]. The LD-PACE II was designed for use in cardiomyoplasty, aortoplasty, skeletal muscle ventricles, and even as a pacemaker with a basic rate between 36 and 120 BPM. Because of this new piece of technology, we believe clinically oriented studies will resume in the future, and that it is important to continue and to publish the results of our ongoing cardiomyoplasty studies.

One established principle in dynamic cardiomyoplasty is based on evidence that electrical stimulation applied to a newly isolated LDM invariably causes muscle necrosis and fibrosis, presumably a result of the ischemic state of the LDM. Therefore, electrical stimulation training is not started until the LDM has completed a 2-week recovery period. The training begins with single impulses (bursts) of electricity and continues with progressively more complex electrical stimulation for 8 weeks before the LDM can contract strongly enough to assist the failing heart. This "classical" vascular delay was originally proposed by plastic surgeons to describe a procedure in which a pedicled flap is elevated in two stages separated by a delay of one to three weeks [14, 15]. It is now clear that in the first stage the tissue is made sub-lethally ischemic and that this ischemia stimulates a reorientation and revascularization of the tissue vasculature. The reorientation and revascularization play a major role in protecting the tissue when it is exposed to the second severe ischemic insult at the definite flap transfer. However, this is not the case in dynamic cardiomyoplasty. Instead of a second surgical procedure, the LDM must be electrically stimulated to transform it into a fatigue-resistant type-I muscle. It is recognized that chronic electrical stimulation improves vascularity in skeletal muscle, as evidenced by increases in capillary density, capillary-to-fiber ratio, and latissimus-derived collateral blood flow to the ischemic myocardium [16-18].

Nevertheless, Anderson et al [16] demonstrated that muscle degeneration and fibrosis are exacerbated when surgical dissection is combined with chronic electrical stimulation, even after a 2-week vascular-delay recovery period. They concluded that the ischemia caused by the surgical dissection is the major factor contributing to muscle degeneration.

The optimal vascular delay period before chronic electrical stimulation remains undetermined. If starting stimulation from two weeks post-dissection or earlier, the chronic electrical stimulation may increase vascularity, but it may also exacerbate necrosis and fibrosis in the ischemic muscle. This study of a canine model found that the RBF of the proximal portion resumed its perfusion 3 weeks after mobilization, and that the RBF of the middle and distal portions did not return to normal values even after a 3-week vascular delay. These data suggest that a vascular delay of longer than 2 weeks may be necessary before starting electrical stimulation in humans. Determination of the optimal delay will require experimental studies of programmed progressive electrical stimulation of the LDM with different periods of vascular delay.

There are also several options to provide cardiac assistance earlier after LDM isolation for patients with severe heart failure. The recent development of chronic electrical stimulation before grafting is one option for improving distal LDM blood flow. In their canine studies, Tang et al [19, 20] proposed prestimulation of the LDM *in situ*

Conclusion

Our data using a canine model show that dissection depresses the RBF of the LDM, and only after three weeks did RBF resume, and even then, only in the proximal portion but not in the middle or distal portions. Of course, the findings of our canine study cannot be applied directly to humans. They do, however, raise the possibility that the resumption of blood flow in the LDM in humans may not occur as quickly as currently believed. Without electrical stimulation, the middle and distal portions of the LDM in our canine model remained compromised even after a 3-week vascular delay. Determination of the effects of programmed progressive electrical stimulation of the LDM with different periods of vascular delay is needed. The effects of combining vascular delay with therapeutic angiogenesis also deserves further investigation.

before isolation. They demonstrated that electrical prestimulation could reduce the acute distal ischemia caused by surgical dissection and promote subsequent recovery of blood flow to base level within 5 days. This may not be feasible when applied to humans, who may need an extra operation before cardiomyoplasty to implant a generator in the back. These generators, however, produce a strong and uncomfortable "twitching" sensation, and it will undoubtedly require a few years to develop one that does not. A second approach is a cautious and variable electrical stimulation protocol that can prepare the LDM for cardiac assist; for example, a work-rest regimen at 15 contractions per minute [21]. A third possibility is that recently developed methods of therapeutic angiogenesis may also apply in such a case, involving either direct angiogenic factor injection or a gene transfer technique to restore blood perfusion in the ischemic portion after LDM mobilization. Each possibility---and therapeutic angiogenesis in particular---deserves further investigation.

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Legends for Figures

Figure 1. Schematic colored microsphere injection schedule at different time points.

Figure 2. Studied LDMs were divided equally into proximal, middle, and distal portions.

Figure 3. Representative left studied latissimus dorsi muscle (LDM) compared to right contralateral normal LDM. (A): LDM after 3-week vascular delay; (B): contralateral normal LDM

Figure 4. Regional blood flow in the proximal, middle, and distal portions of the latissimus dorsi muscle at different time stages. All compared to control group: $^{*}p > 0.05$, $^{**}p < 0.01$.