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Ten Hour Preservation of Guinea Pig Isolated Hearts Perfused at Low Flow with Air-Saturated Lior® Solution at Room Temperature: Comparison to ViaSpan®

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Brief title: Lior® solution to protect hearts

Ten Hour Preservation of Guinea Pig Isolated Hearts Perfused at Low Flow with Air-Saturated Lifor® Solution at Room Temperature: Comparison to ViaSpan®

Background -There is no suitable solution to preserve hearts for longer than 5 h between donor explant and recipient implant. Lifor® is a proprietary, fully artificial preservation medium containing both a non-protein oxygen and nutrient carrier (nanoparticles) and cellular nutrients, including amino acids and sugars. We proposed that recirculated Lifor solution would satisfactorily preserve guinea pig isolated hearts perfused at low flow with no added O₂ at room temperature for 10 h.

Methods and Results -Hearts were isolated from 21 guinea pigs and initially perfused at constant pressure with Krebs-Ringer's (KR) solution equilibrated with 97% O₂ and 3% CO₂ at 37°C. Heart rate, inflow and outflow O₂ tension, coronary flow and isovolumetric left ventricular pressure (LVP) were measured. After baseline values were obtained, hearts were perfused with 300 mL of recirculated Lifor or ViaSpan® equilibrated with room air at 15% of control flow at 26°C for 10 h. After the test period, hearts were perfused as during the baseline period for another 2 h. An untreated group perfused with KR solution for 15 h served as a time-control group. We found that Lifor arrested and protected the hearts against diastolic contracture and maintained a low level of O₂ extraction during the treatment period. Compared to the time control group, the Lifor group had a higher developed LVP than the time control group and coronary flow, %O₂ extraction and cardiac efficiency were similar between these two groups. Hearts similarly treated with ViaSpan solution exhibited diastolic contracture and lower %O₂ extraction during treatment and on warm reperfusion with KR exhibited continued diastolic contracture, no return of heart rate or contractility, low coronary flow and a high %O₂ extraction.

Conclusions -An effective and convenient preservation medium for hearts during a long transport would not require severe cooling and refrigeration and supplemental O₂ to restore viability during re-implantation. A suitable solution recirculated at low flow would also reduce the support apparatus required for transport. Lifor® preservation solution appears to be a step forward in meeting these requirements.

Key Words: heart preservation, ischemia reperfusion injury, nanoparticles

Introduction

In cardiac transplantation, the transport time between harvest and recipient is limited by the viability of the donor heart. Cold storage of human hearts for transplantation currently limits functional viability to 4-5 h despite the development and clinical availability of approximately ten different heart preservation solutions. There remains a lack of consensus on the ideal solution. Two major problems with current approaches are the need for severe hypothermia (3-6°C) and the lack of tissue perfusion during transport. Very low flow perfusion of hearts at room temperature without supplemental O₂ would facilitate a lengthening of the period of viability and reduce the need for complicated support equipment during transport. To do so requires a suitable preservation solution.

Our aim was to examine if Lifor® solution recirculated into hearts at room temperature and atmospheric conditions for at least 10 h would adequately preserve cardiac electrical, mechanical and metabolic function on warm reperfusion with a normal physiologic crystalloid solution. For comparison, another preservation medium, ViaSpan® was given as the treatment. A non-treated control group served to demonstrate changes in cardiac function over the same time period.

Methods

Langendorff heart preparation

The investigation conformed to the *Guide for the Care and Use of Laboratory Animal* (NIH publication 85-23, revised 1996). Prior approval was obtained from the Medical College of Wisconsin Biomedical Resource Committee. Our methods have been described in detail previously.¹⁻⁴ Ketamine (30 mg) and heparin (1000 units) were injected intraperitoneally into 21 guinea pigs (250-300 g), 15 min before the animals were decapitated when unresponsive to noxious stimulation. After thoracotomy, the aorta was cannulated distal to the aortic valve and the inferior and superior venae cavae were cut from the heart. Each heart was immediately perfused via the aortic root at 55 mmHg with a cold oxygenated modified Krebs-Ringer's (KR) solution (equilibrated with 97% O₂ - 3% CO₂) and rapidly excised. The KR perfusate (pH 7.39±0.1, pO₂ 562±11 mmHg) was in-line filtered (20 µm pore size) and had the calculated composition of (non-ionized, in mM) 137 Na⁺, 5 K⁺, 1.2 Mg²⁺, 2.5 Ca²⁺, 134 Cl⁻, 15.5 HCO₃⁻, 1.2 H₂PO₄⁻, 11.5 glucose, 2 pyruvate, 16 mannitol, and 0.05 EDTA, with 5

units/L of insulin. Perfusate and bath temperatures were maintained at $37.2\pm 0.1^{\circ}\text{C}$ before and after Lifor or ViaSpan treatments.

Lifor® (Lifeblood Medical Inc., Adelphia, NJ) is a proprietary solution containing sugars, amino acids, salts, buffers, colloids and lipid nanoparticles (295 ± 4 mOsm/L, pH 7.07 ± 0.01 , pCO_2 5.0 ± 0.2 mmHg, pO_2 169 ± 2 mmHg, Na^+ 98 ± 1 mM, K^+ 15.8 ± 0.4 mM, and Ca^{2+} 0.17 ± 0.02 mM equilibrated with room air at 26°C). Additives to Lifor® were adenosine ($10\ \mu\text{M}$) and butanedione monoxime (BDM, 10 mM). ViaSpan® (Barr Pharmaceuticals, Inc., Woodcliff Lake, NJ) is a proprietary, intracellular type solution containing energy precursors (phosphate, adenosine), impermeants (K^+ lactobionate, raffinose), antioxidants (allopurinol, glutathione), buffers, and colloids (pentafraction) (335 ± 4 mOsm/L, pH 7.33 ± 0.01 , pCO_2 6.7 ± 2.3 mmHg, pO_2 167 ± 8 mmHg, Na^+ 39 ± 2 mM, K^+ 94 ± 2 mM, Ca^{2+} 0.08 ± 0.01) equilibrated with room air at 26°C . Bartel's® antibiotic solution (3%, containing gentamycin, streptomycin, and amphotericin B) were added to both Lifor and ViaSpan solutions because the hearts were not harvested in a sterile manner and the solutions were exposed to room air. During the 10 h treatment period with either 300 mL of recirculated and filtered ($20\ \mu\text{m}$) Lifor or ViaSpan coronary inflow was set at 15% of the baseline flow, which was approximately 17 mL/min, so that the volume was recirculated through the vasculature approximately 5 times. Recirculation was achieved by a pump and tubing between the right ventricle (coronary sinus) and aortic inflow (coronary ostia) cannula. Solution pO_2 was 168 ± 2 mmHg (room air) and temperature was $26.2\pm 0.6^{\circ}\text{C}$ (room temperature).

Left ventricular pressure (LVP) was measured isovolumetrically with a transducer connected to a thin, saline-filled latex balloon inserted into the LV through the mitral valve from a cut in the left atrium. Balloon volume was initially adjusted to a diastolic LVP of zero mmHg so that any subsequent increase in diastolic LVP reflected an increase in LV wall stiffness or diastolic contracture. Bipolar electrodes were placed in the right atrial appendage and in the right ventricular free wall to monitor spontaneous heart rate. Coronary flow (aortic inflow) was measured by an ultrasonic flowmeter (T106X, Transonic System; Ithaca, NY) placed into the in-flow line. Coronary sinus effluent was collected from a small catheter placed into the right ventricle through the pulmonary artery after ligating both venae cavae. Coronary effluent Na^+ , K^+ , Ca^{2+} , pO_2 , pCO_2 , and pH were measured

intermittently. Coronary outflow (coronary sinus) O₂ tension was also measured in-line with a Clark-type O₂ electrode. Myocardial O₂ consumption (MVO₂) was calculated as (coronary flow/g heart weight) x (arterial pO₂-venous pO₂) x 24 μL O₂/mL (37°C) or 26.5 μL O₂/mL (26°C) at 760 mmHg; and cardiac work efficiency was calculated as systolic-diastolic LVP x heart rate/MVO₂.

If ventricular fibrillation occurred on reperfusion, a bolus of 250 μg lidocaine was given within 20 s. Lidocaine injection and washout had no lasting effect on LVP. All data were collected from hearts in sinus rhythm at baseline. After the 10 h treatment period and a 2 h reperfusion period with KR, hearts were removed and the ventricles were cut into 4-5 horizontal sections and stored overnight in 10% formaldehyde. Ventricular infarct size (% total ventricular weight) was determined using the 2,3,5-triphenyltetrazolium chloride (TTC) staining method. TTC stains the non-infarcted myocardium a brick red color, which indicates the presence of a formazan precipitate that results from the reduction of TTC by dehydrogenase enzymes present in viable tissue.

Protocol

Each experiment lasted 14 h beginning after 30 min of equilibration. Ten second recordings of atrial and ventricular electrograms, LVP, coronary flow, venous pO₂, perfusion pressure and temperature were recorded automatically every 30 min (PowerLab®, ADInstruments, info@adinstruments.com). Hearts were randomly divided into three groups: 1) non-treated hearts perfused continuously at 55 mmHg with non-recirculated, 97% oxygenated KR solution at 37.1±0.04°C for the 10 h treatment period (Time control, n = 6); 2) recirculated, room air saturated ViaSpan® solution (Viaspan, n = 7); and 3) recirculated, room air saturated Lifor® solution (Lifor, n = 8). Wet heart weight was not different among groups: Time control, 1.46±0.05 g; Viaspan, 1.45±0.09 g; Lifor, 1.43±0.07 g. Hearts were allowed to cool from 37°C to 26.2±0.05°C over 10 min at the initiation of treatment and were rewarmed from 26.2°C to 37°C over 10 min on reperfusion with KR as during baseline conditions. LVP, coronary flow, and coronary sinus pO₂ were measured continuously before, during and after treatment. Hearts arrested immediately during initiation of Lifor and ViaSpan treatments.

Statistical Analysis

All data are expressed as means \pm SEM. Analysis of variance for repeated measures (Super Anova 1.11 software for Macintosh from Abacus Concepts; Berkeley, CA) was used to assess within group differences over time. Within group data were analyzed by one-way analysis of variance for comparison of data collected at the selected times of 4 and 12 h (during treatment), and 13 and 15 h (after treatment) compared to that at 2 h (37°C pre-treatment baseline). Among group data were analyzed by two-way analysis of variance and compared with each other at the baseline (2 h) and at 4, 12, 13, and 15 h. If F values from the analyses of variance were significant, Student Newman Keuls's multiple-comparison post hoc tests were used to differentiate within or among group differences. Differences among means were considered significant when $P < 0.05$ (two-tailed).

Results

Heart rates at 2 h (baseline values) in the Time control, Lifor and ViaSpan groups were not different (223 ± 6 , 226 ± 6 , 227 ± 7 beats/min, $p > 0.05$). At 15 h (2 h after end of treatment) heart rates were similar in the Time control (217 ± 6 beats/min) and Lifor groups (212 ± 4 beats/min), whereas in the ViaSpan group hearts did not beat. Each heart in the ViaSpan group had no ventricular rhythm and occasional erratic atrial dysrhythmias throughout the post treatment period. One heart in the Lifor group exhibited ventricular fibrillation at 10 min of reperfusion but this reverted to sinus rhythm within 30 s without intervention. Percent ventricular infarct size after 2 h warm reperfusion was greater in the ViaSpan group, $41.6 \pm 2.6\%$ than in the Lifor group, $15.5 \pm 3.5\%$.

Systolic and diastolic LVP (Figure 1) fell slightly but significantly below the baseline levels within 2 h of perfusion in the Time control group; by 15 h of perfusion systolic LVP was approximately 30% below baseline. Diastolic LVP was unaltered throughout the study. In the ViaSpan group diastolic LVP rose within 30 min of treatment and remained elevated both during treatment and during warm reperfusion with KR solution; systolic LVP was equal to the diastolic LVP throughout treatment and reperfusion, i.e., there was no phasic contractile effort. In the Lifor group diastolic and systolic LVP were near zero mmHg during treatment; on warm reperfusion with KR solution diastolic LVP remained near zero and systolic LVP returned abruptly to the pretreatment baseline levels.

Percent O₂ extraction (Figure 2) was unchanged in the Time control group throughout perfusion with KR solution. During the low flow, room temperature treatments, % O₂ extraction was higher in the Lifor group than in the ViaSpan group. On warm reperfusion % O₂ extraction in both treatment groups was equivalent to that in the Time control group.

Coronary flow (Figure 3) remained unchanged in the Time control group throughout perfusion with KR solution. Coronary flow was set constant to 15% of the baseline flow for each heart during treatments with ViaSpan and Lifor at 26°C. Set coronary perfusion pressures before and after treatment, respectively, were 55±2 and 55±2 mmHg in the Lifor group and 53±2 and 55±4 mmHg in the ViaSpan group. Perfusion pressure (at constant flow) increased slightly from 22±1 to 25±2 mmHg from 1 to 10 h of Lifor treatment but from 5±3 to 33±1 mmHg from 1 to 10 h of ViaSpan treatment ($p<0.05$ ViaSpan vs Lifor). Coronary flow after Lifor treatment on warm reperfusion with KR solution was nearly twice that after ViaSpan treatment and nearly as high as in the Time control group.

Cardiac efficiency (Figure 4) slowly declined slowly with time in the Time control group and the decline was significant from 8 to 15 h of perfusion with warm KR solution. Cardiac efficiency was zero during treatments as hearts did not beat or generate pressure. On warm reperfusion after ViaSpan cardiac efficiency remained at zero, but after Lifor cardiac efficiency returned to the levels found for the Time control group.

Discussion

This is the first report on Lifor®, a nanoparticle solution containing amino acids, salts, sugars and other additives, as a heart preservation solution. In our model Lifor was a much better heart preservation solution than ViaSpan solution and could be a more suitable alternative to other common preservation solutions, especially when used at room temperature and air conditions. We found that re-circulated Lifor solution, supplemented with adenosine and BDM and given both as a cardioplegic agent and as a preservation medium, well protected hearts against damage for 10 h at 26°C. BDM and adenosine were added because we reported improved function with these additives in a severe cold storage heart model.^{1,5} Moreover, Lifor treated hearts exhibited a return of developed LVP that was higher than that found in the non-treated Time control group. Under the same experimental conditions

as for the Lifor solution, hearts treated with ViaSpan solution were completely non functional (no heart beat or contractile effort) during the 2 h warm reperfusion period with KR.

The low and then marked increase in coronary perfusion pressure at low constant flow during ViaSpan treatment, but during Lifor treatment, may indicate an initial enhanced osmotic or oncotic effect to siphon H₂O from the interstitial space and decreasing perfusate viscosity, which is later replaced by an increase in osmolarity in the interstitial space. In contrast, perfusion pressure was not significantly altered during the 10 h ViaSpan treatment. After stopping Lifor and perfusing with warm KR solution, coronary flow and %O₂ extraction returned to baseline levels, while cardiac efficiency returned to levels found in the Time control group. Infarct size after ViaSpan treatment was nearly 3 times that after Lifor treatment. The value for Lifor (15.5%) is within the detection error of the method⁶ because the TTC staining by weight method to determine the percentage of infarcted tissue is not reliable at lower degrees of infarction, as indicated by an apparent 11±3% infarct size measured after 3 h KR perfusion without ischemia.⁷

The quest for better and longer preservation of hearts for transplant can be attested to by over 600 articles published in the past 10 yr on cardiac preservation solutions and techniques. While there are suitable preservation techniques and solutions for the liver and kidney, there remains a need for better methods to protect the heart for periods longer than 4-5 h.⁸⁻¹² More popular clinically used solutions include ViaSpan®¹³⁻¹⁵ (also called UW solution), HTK (histidine-tryptophan-ketoglutarate based solution, or Bretschneider's),^{16, 17} Celsior® (antioxidant based solution with mannitol, reduced glutathione, plus high Mg²⁺, lactobionate, glutamate),¹⁸⁻²² and STH (St. Thomas' Hospital, a high K⁺, high Mg⁺, low Ca²⁺, lidocaine containing solution);²³⁻²⁶ others are Euro-Collins' and Stanford's solutions.

There is no clear consensus on which solution is better than another. All have limitations on the adequacy and length of protection²⁷ and their protective effects are dependent on the conditions of the study.^{23, 28, 29} Many of the heart studies compare one preservation solution with another,^{17, 20-24, 26, 30-35} and with or without additives, such as channel activators or blockers,^{1, 5, 36-40} exchange inhibitors,⁴¹⁻⁴³ anesthetics,^{2, 40, 44} or BDM.^{1, 21, 45} For example Celsior preserved function better than Viaspan in several studies,^{35, 46} whereas ViaSpan was better than Celsior in others.^{20, 21} HTK was found to be more

protective than ViaSpan;³⁴ in another study ViaSpan was reported to be better than HTK.¹⁷ A newer solution, LYPS (extracellular type with low Ca^{2+} and Mg^{2+} , added pyruvate, polyethylene glycol and chlorpromazine), was well designed and tested using a biopsy technique for tissue viability to evaluate the independent effects of 19 compounds found in other preservation solutions.⁴⁷ They found this solution better preserved pig hearts stored for 8 h at 4°C much better than did St. Thomas' solution.⁴⁷ In a rat model (8 h, 4°C) LYPS was better than ViaSpan (intracellular type UW, or extracellular type UW-1), but was equivalent to Celsior.³²

A potential problem with ViaSpan and other so-called intracellular solutions is endothelial damage due to its very high K^+ and high viscosity,^{29, 33, 48} although this may also occur with Celsior.³⁰ Our use of ViaSpan as a low flow perfusate clearly decreased coronary vascular compliance over time. Another problem with these viscous, high K^+ solutions as a storage rather than a perfusion medium is that a more typical extracellular type cardioplegic solution should be given first to flush the vasculature and arrest the heart before perfusing the storage solution; post storage the solution needs to be again flushed out.²⁰

Perfusion storage of hearts is not often used clinically compared with simple immersion into an ice-jacketed container because this is more complicated and costly to accomplish. Depending on the preservation conditions and time line, perfusion storage may require a mechanical pump, a cooling system, an O_2 supply tank, and a very large volume of non-recirculated solution to perfuse the coronary vasculature. Moreover, to be warranted as the best technique, perfusion preservation must lead to superior return of function after a long period compared to simple storage, particularly if severe cooling is to be avoided. A recent review suggests that a perfusion system is needed to effectively preserve hearts for increasing longer periods between explant and implant.⁹ Animal studies show the superiority of low flow perfusion techniques.^{1, 2, 49, 50}

Another concern for long-term protection of hearts is the need for severe hypothermia. The colder the hearts the longer they can be protected against no flow ischemia.^{41, 43, 51} Severe hypothermia reduces energy demand and so is useful to protect hearts metabolically against ischemic injury during cardiac storage prior to transplantation. Hypothermia preserves essential mechanisms during heart transport to rapidly regenerate ATP on reperfusion by decreasing energy utilization. Although

hypothermia is the most effective method to preserve hearts during ischemic storage, hypothermia itself has deleterious effects on contractile element and endothelial cell function the more severe is the cooling. Two of these effects are cytosolic⁵² and mitochondrial³ Ca^{2+} loading; another is excess release of O_2 species (ROS).⁵³ Either of these can result in mitochondrial and cellular damage proportional to the degree and duration of hypothermia. For example, we reported that cardiac perfusion at 17°C before ischemia itself caused a moderate and steady-state increase in mitochondrial Ca^{2+} , a more reduced mitochondrial redox state (increased NADH), and moderate production of ROS.³ Under different mitochondrial conditions, either low or high tissue O_2 levels can lead to ROS generation.^{4, 54}

Decreasing the degrees of cooling and oxygenation in cell free preservation solutions should be offset by methods to increase tissue O_2 and nutrient delivery, particularly if a solution is to be recirculated to reduce the volume of coronary perfusate required. Our goal in this experimental model was to apply this approach but to preserve hearts at room temperature rather than expose them to severe hypothermia and to do so with no added O_2 .

Heart transplant programs would benefit from a cardiac preservation technique with a single solution that did not require severe cooling of the heart or supplemental O_2 , and required only a small volume, recirculated, coronary perfusate for transport between centers. Lifer solution may lead to attainment of that goal. A prolonged preservation time, particularly at room temperature, would lead to an increase in the available donor pool of viable hearts and improve post-transplant outcomes. An increase in preservation times and improvements in banking and transport of hearts over greater distances should greatly increase the availability of viable hearts with good tissue matches to needy recipients.

Conclusions and Limitations

In this experimental model, which consisted of a very low flow coronary recirculation system at room temperature and room air, Lifer solution was superior compared to Viaspan for heart preservation up to 10 h. The experimental conditions of this study were set up to mimic the condition of transporting and preserving human hearts for transplant. An obvious limitation is that the use of a small animal, non-working heart model for a study of preservation solutions may not reflect the clinical conditions or use

of these solutions in the human heart. Additional studies are needed to determine the optimal conditions and maximal length of protection afforded by Lifor preservation solution and to compare Lifor with other available preservation solutions.

Disclosure

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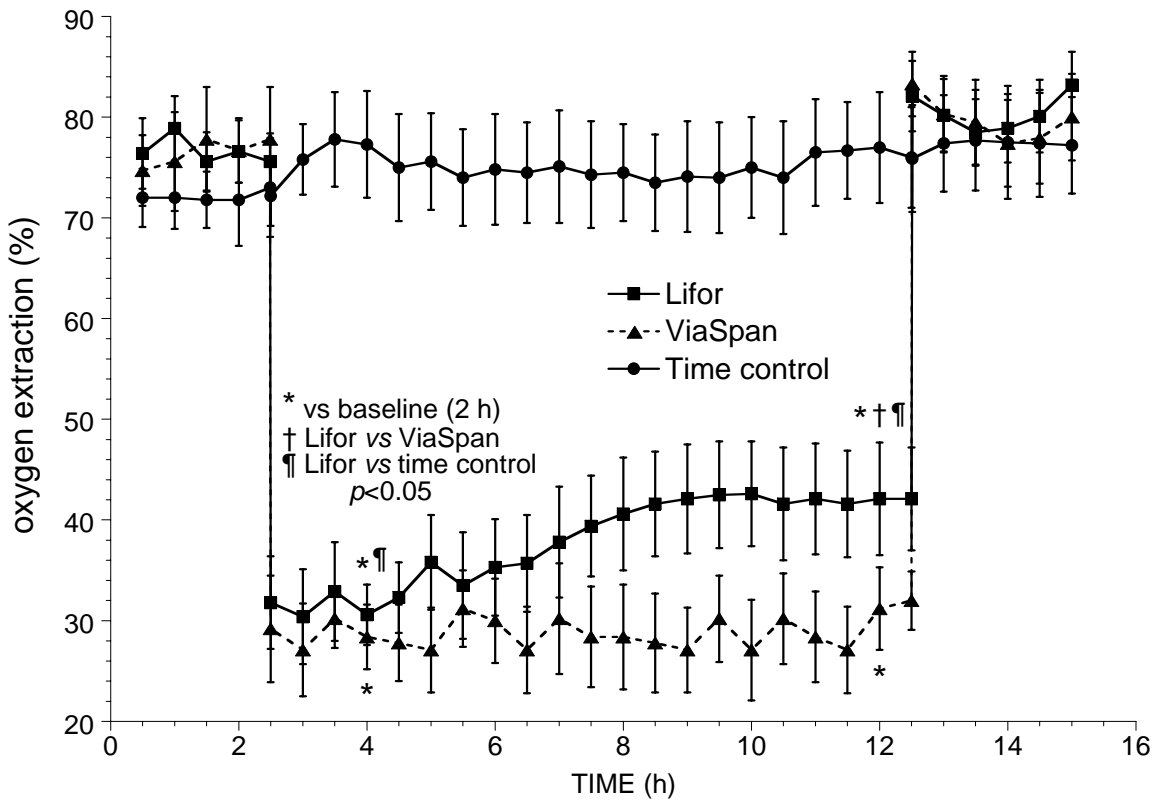
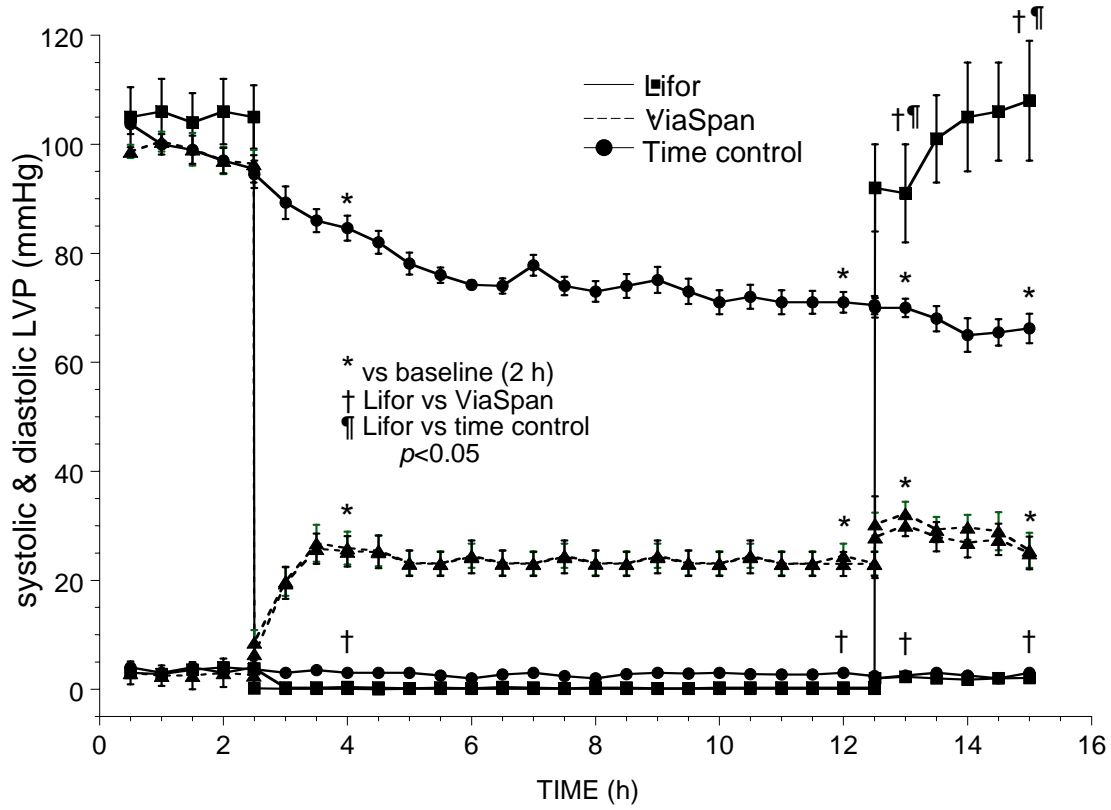
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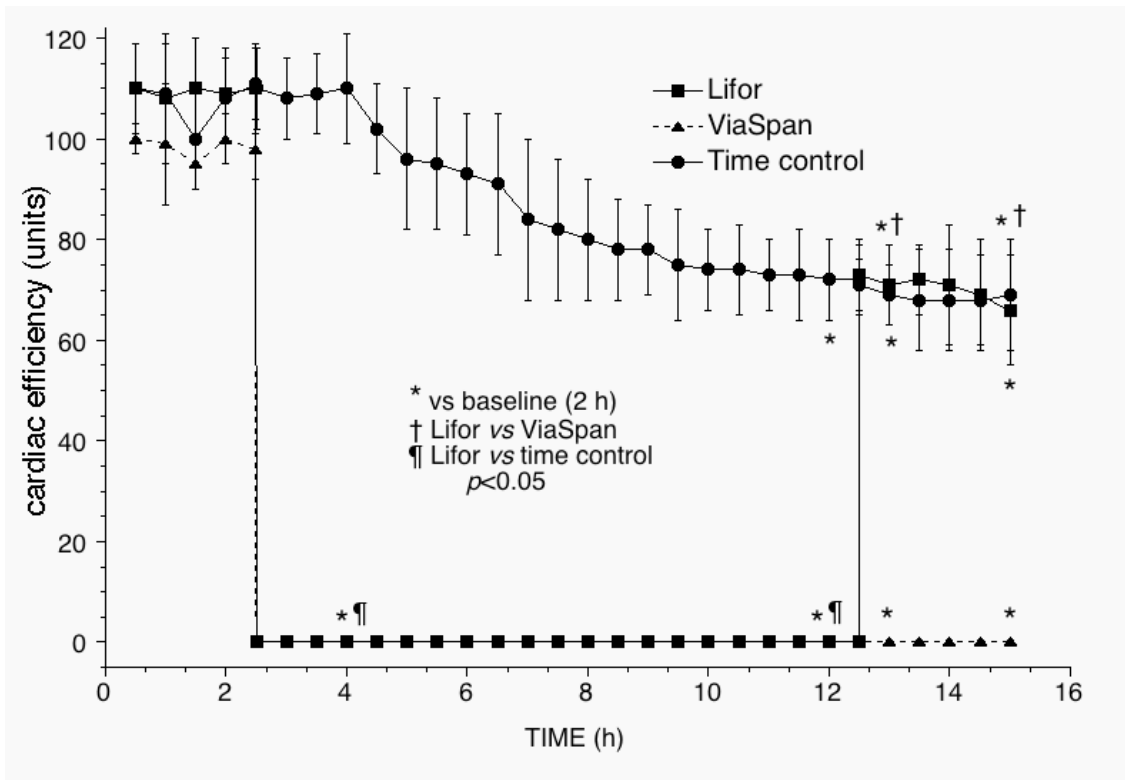
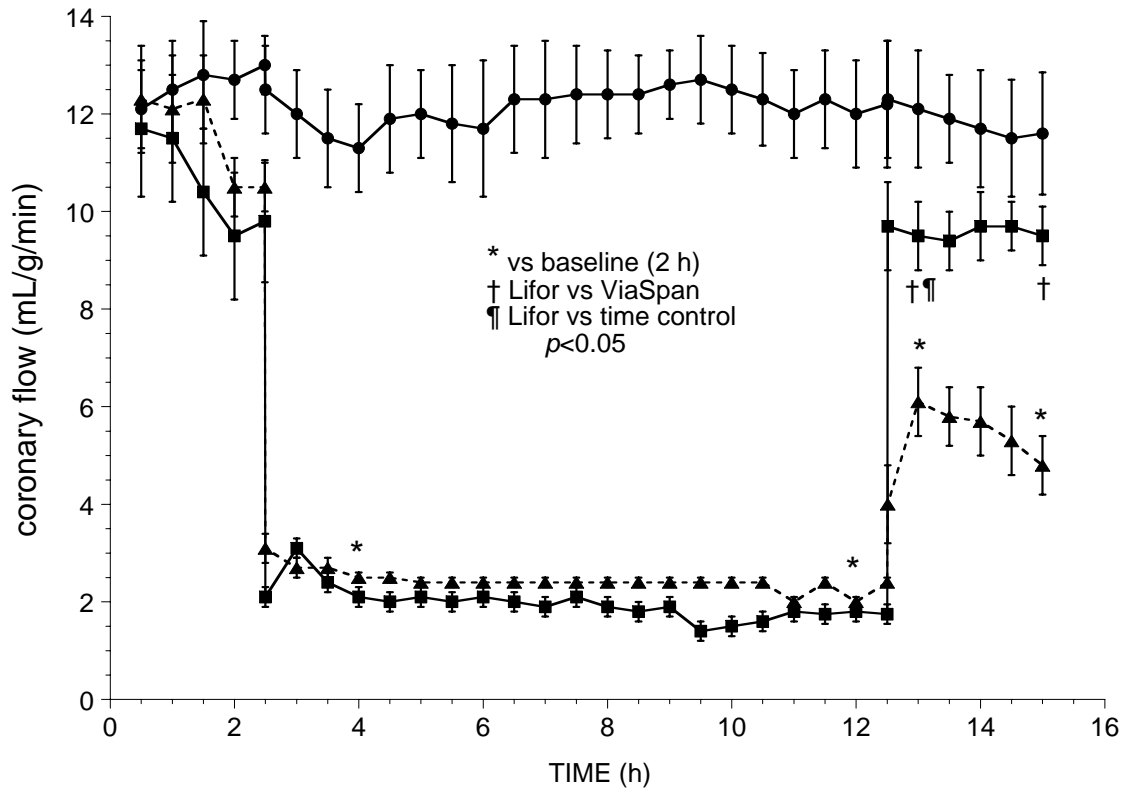
Figure 1. Systolic and diastolic LVP before, during and after treatment with air equilibrated, re-circulated Lifer or ViaSpan for 10 h at 26°C at 15% of baseline coronary flow, or with no treatment (Time control). Statistical notations are given for selected time points; baseline values (at 2 h) are not different among groups. Note the restoration of systolic LVP and the absence of diastolic contracture after Lifer treatment compared to the diastolic contracture and the absence of contractility after ViaSpan treatment.

Figure 2. Percent O₂ extraction before, during and after treatment with air equilibrated, re-circulated Lifer or ViaSpan for 10 h at 26°C at 15% of baseline coronary flow. Note that during Lifer treatment, hearts extracted O₂ better than during ViaSpan treatment and that %O₂ extraction in the ViaSpan group after treatment was accompanied by diastolic contracture and no phasic contractile effort.

Figure 3. Coronary flow before, during and after treatment with air equilibrated, re-circulated Lifer or ViaSpan for 10 h at 26°C at 15% of baseline coronary flow. Flow was natural at constant pressure before and after treatment, and pump perfused during treatment. Note that coronary flow was much lower after ViaSpan treatment than after Lifer treatment or in the Time control group.

Figure 4. Cardiac efficiency (heart rate • systolic-diastolic LVP/O₂ consumption rate) before, during and after treatment with air equilibrated, re-circulated Lifer or ViaSpan for 10 h at 26°C. Units for cardiac efficiency are: mmHg•beat•1 μL O₂/g. Note that on warm reperfusion after treatment cardiac efficiency after Lifer treatment was similar to that of the time controls, whereas after ViaSpan cardiac efficiency was zero.





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