Ten-hour preservation of guinea pig isolated hearts perfused at low flow with air-saturated Lifor solution at 26°C: comparison to ViaSpan solution

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Stowe DF, Camara AK, Heisner JS, Aldakkak M, Harder DR. Ten-hour preservation of guinea pig isolated hearts perfused at low flow with air-saturated Lifor solution at 26°C; comparison to ViaSpan solution. Am J Physiol Heart Circ Physiol 293: H895-H901, 2007. First published April 13, 2007; doi:10.1152/ajpheart.00149.2007.—There is no suitable solution to preserve hearts for longer than 5 h between donor explant and recipient implant. Lifor is a fully artificial preservation medium containing both a nonprotein 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. Hearts were isolated from 21 guinea pigs and perfused with Krebs-Ringer (KR) solution (97% O2 and 3% CO2) at 37°C. Heart rate, inflow and outflow O2 tension, coronary flow, left ventricular pressure (LVP), and maximal and minimal rate of change in LVP (dLVP/dt) were measured. After baseline measurements, hearts were perfused with recirculated Lifor or ViaSpan equilibrated with room air at 15% of control flow at 26°C for 10 h. Hearts were then perfused at 100% flow with KR for 2 h at 37°C. A time control (untreated) group was perfused only with KR solution for 15 h. Lifor arrested and protected hearts against diastolic contracture and maintained a low O2 extraction. Compared with time controls, Lifor led to a higher developed LVP and coronary flow; %O2 extraction and cardiac efficiency were similar between these two groups. Hearts similarly treated with ViaSpan exhibited diastolic contracture and lower %O2 extraction during treatment and, upon reperfusion with KR, exhibited continued diastolic contracture, no return of heart rate or contractility, low coronary flow, low %O2 extraction, and marked infarction. For long-term cardiac protection, a suitable preservation solution recirculated at low flow and room temperature without supplemental O2 would reduce the support apparatus required for transport. Lifor was far superior to ViaSpan in meeting these requirements.

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 ~10 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. Successful 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 would require a suitable preservation solution.

Our aim was to examine whether 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 physiological crystalloid solution. For comparison, another preservation medium, ViaSpan, was given as the treatment. A nontreated control group served to demonstrate changes in cardiac function over the same time period.

MATERIAL AND METHODS

Langendorff heart preparation. This investigation conformed to the “Guide for the Care and Use of Laboratory Animals” [DHHS Publication No. (NIH) 85-23, Revised 1996, Office of Science and Health Reports, DRR/NH, Bethesda, MD 20205]. Prior approval was obtained from the Medical College of Wisconsin Biomedical Resource Committee. Our methods have been described in detail previously (23, 37, 46, 47). Ketamine (30 mg) and heparin (1,000 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 (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 (nonionized, in mM) 137 Na+ 98, 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 U/l insulin. Perfusion and bath temperatures were maintained at 37.2 ± 0.1°C before and after Lifor or ViaSpan treatments.

Lifor (Lifeblood Medical, Freehold, NJ) is a proprietary solution containing sugars, amino acids, salts, buffers, colloids, and lipid nanoparticles (295 ± 4 mosmol/L, pH 7.07 ± 0.01, PCO2 5.0 ± 0.2 mmHg, Po2 169 ± 2 mmHg, Na+ 98 ± 1 mmHg, K+ 15.8 ± 0.4 mmHg, Ca2+ 0.17 ± 0.02 mmHg) equilibrated with air room at 26°C. Additives to Lifor were adenosine (10 μM) and butanedione monoxime (BDM; 10 mM). ViaSpan (Barr Pharmaceuticals, Woodcliff Lake, NJ) is a proprietary, intracellular-type solution containing energy precursors (phosphate, adenosine), impermeants (K+ lactobionate, raffinose), antioxidants (allopurinol, glutathione), buffers, and colloids (pentafracion) (335 ± 4 mosmol/L, pH 7.33 ± 0.01, PCO2 6.7 ± 2.3 mmHg, Po2 167 ± 8 mmHg, Na+ 39 ± 2 mmHg, K+ 94 ± 2 mmHg, and Ca2+ 0.08 ± 0.01) equilibrated with room air at 26°C. Bartel’s antibiotic solution (3%; containing gentamicin, streptomycin,
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Abacus Concepts, Berkeley, CA) was used to assess within-group differences over time. Within-group data were analyzed using 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 with that at 2 h (37°C pretreatment baseline). Among-group data were analyzed using 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 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). We had full access to the data and take responsibility for its integrity; we have all read and agree with the manuscript as written.

RESULTS

Heart rates at 2 h (baseline values) in the time control, Lifor, and ViaSpan groups were not different (223 ± 6, 226 ± 6, and 227 ± 7 beats/min, respectively; 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, heart rates did not beat. Each heart in the ViaSpan group had no ventricular rhythm and occasional erratic atrial dysrhythmias throughout the posttreatment 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.

Systolic LVP (Fig. 1A) fell slightly but significantly below the baseline levels within 3 h of perfusion in the time control group; at 15 h of perfusion, systolic LVP was ~30% below baseline. In the Lifor group, systolic LVP returned abruptly to the pretreatment baseline level during warm reperfusion with KR solution. The return of systolic LVP after Lifor treatment was higher than in the time control group. Diastolic LVP (Fig. 1B) was unaltered throughout the study in the Lifor and time control groups. In the ViaSpan group, diastolic LVP rose within 30 min of treatment and remained elevated 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 during or after ViaSpan treatment.

Maximal dLVP/dt, an index of contractility (Fig. 2A), fell significantly within 8 h of perfusion in the time control group; at 15 h of perfusion, this value was ~33% less than baseline. In the Lifor group, maximal dLVP/dt was not significantly different from baseline and was greater than in the time control group on reperfusion immediately after treatment, but it was less than baseline after 2 h of reperfusion. Similarly, minimal dLVP/dt, an index of relaxation (Fig. 2B), fell significantly within 10 h of perfusion in the time control group; at 15 h of perfusion, this value was ~38% below baseline. In the Lifor group, minimal dLVP/dt returned to the baseline level on initial reperfusion after treatment and was greater than in the time control group, but it was less than baseline at 2 h of reperfusion. Because hearts did not beat in the ViaSpan group on reperfusion after treatment, maximal and minimal dLVP/dt approached zero.

Percent O2 extraction (Fig. 3A) was unchanged in the time control group throughout perfusion with KR solution. During the low-flow treatments at room temperature, 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...
Fig. 3B 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 ViaSpan and Lifor treatments 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 on warm reperfusion with KR solution was nearly twice that after ViaSpan and nearly as high as in the time control group.

Cardiac efficiency (Fig. 4A) 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, since hearts did not beat or generate pressure. On warm reperfusion after ViaSpan treatment, cardiac efficiency remained at zero, but after Lifor treatment, cardiac efficiency returned to the levels found for the time control group. Percent bi-ventricular infarct size (Fig. 4B) after 2 h of warm reperfusion was very large only in the ViaSpan group, reflecting the lack of function in this group; there was no statistical difference between the time control and Lifor groups. Representative cross sections of one heart from each group (Fig. 5) show the marked light-colored (infarcted) areas of the ViaSpan-treated hearts compared with the dark-colored (noninfarcted) areas of the Lifor-treated and time control hearts.

**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 and could be a
more suitable alternative to other common preservation solutions, especially when used at room temperature and air conditions. We found that recirculated Lifor solution, supplemented with adenosine and BDM and given as both a cardioplegic agent and a preservation medium, well protected hearts against damage for 10 h at 26°C. BDM and adenosine were added because our group reported improved function with these additives in a severe cold storage heart model (44, 46). Moreover, Lifor-treated hearts exhibited a return of developed LVP and minimal dLVP/dt (relaxation), and initially, maximal dLVP/dt (contractility), that was higher than that found in the nontreated time control group. Under the same experimental conditions as for Lifor, hearts treated with ViaSpan were completely nonfunctional (no heart beat or contractile effort) during the 2-h period of warm reperfusion with KR.

The low and then marked increase in coronary perfusion pressure at low constant flow during ViaSpan, but not during Lifor, may indicate an initial enhanced osmotic or oncotic effect to siphon H2O from the interstitial space and a decrease in 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 Lifor treatment. After Lifor was stopped and hearts were perfused with warm KR solution, coronary flow and %O2 extraction returned to baseline levels, whereas cardiac efficiency returned to levels found in the time control group. Infarct size after ViaSpan was
72 ± 2% of the total ventricular weight. The apparent small infarct size in the Lifor and time control groups could represent true infarction, but it is rather likely a result of the inaccuracy in identifying and cutting out suspected small infarcted areas for weighing. The values for Lifor and time control groups are within the detection error of the method (36), because the method of TTC staining by weight 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 of KR perfusion without ischemia in another study (38).

The quest for better and longer preservation of hearts for transplant can be attested to by over 600 articles published in the past 10 years on cardiac preservation solutions and techniques. Although 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 (9, 10, 32, 39, 50). More popular clinically used solutions include ViaSpan (22, 28, 51) (also called UW solution), HTK (histidine-tryptophan-ketoglutarate based, or Bretschneider solution) (16, 41), Celsior (antioxidant-based solution with manitol, reduced glutathione, plus high Mg2+, lactobionate, and glutamate) (5, 17, 20, 33, 34), and St. Thomas Hospital solution (STH; a high-K+, high-Mg+, low-Ca2+, lidocaine-containing solution) (4, 8, 21, 25); others are Euro-Collins and Stanford solutions. There is no clear consensus on which solution is better than another. All have limitations on the adequacy and length of protection (43), and their protective effects are dependent on the conditions of the study (19, 21, 31). Many of the heart studies compare one preservation solution to another (5, 8, 16, 20, 21, 24, 25, 30, 33, 35, 52–54) and with or without additives, such as channel activators or blockers (11, 12, 18, 26, 40, 44, 46), ion exchange inhibitors (6, 42, 48), anesthetics (2, 40, 47), or BDM (20, 46, 49). For example, Celsior preserved function better than ViaSpan in several studies (29, 52), whereas ViaSpan was better than Celsior in others (5, 20). HTK was found to be more protective than ViaSpan (24); in another study ViaSpan was reported to be better than HTK (16). A newer solution, LYPS (extracellular type with low Ca2+ and Mg2+, 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 (14). This solution was found to preserve pig hearts stored for 8 h at 4°C much better than St. Thomas solution (14). 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 (30).

A potential problem with ViaSpan and other so-called intracellular solutions is endothelial damage due to its very high K+ level and high viscosity (27, 31, 53), although this may also occur with Celsior (35). 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 the storage solution is perfused; after storage, the solution needs to be again flushed out (5).

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 O2 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 with 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 increasingly longer periods between explant and implant (39). Animal studies show the superiority of low-flow perfusion techniques (13, 15, 46, 47).

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 (1, 6, 48). Severe hypothermia reduces energy demand and so is useful to protect hearts metabolically against ischemic injury during cardiac storage before 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 cytosolic (45) and mitochondrial (37) Ca2+ loading; another is excess release of O2 species (ROS) (7). Either of these can result in mitochondrial and cellular damage proportional to the

Fig. 5. Representative midventricular cross sections in 1 heart from each group. Note that the darker tissue (red) in the Lifor and time control hearts indicates viable tissue, whereas the lighter tissue in the ViaSpan group indicates infarcted tissue.
degree and duration of hypothermia. For example, our group
(37) reported that cardiac perfusion at 17°C before ischemia
itself caused a moderate and steady-state increase in mitochon-
drial Ca2+, a more reduced mitochondrial redox state (in-
creased NADH), and moderate production of ROS. Under
different mitochondrial conditions, either low or high tissue O2
levels can lead to ROS generation (3, 23).

Decreasing the degrees of cooling and oxygenation in cell-
free preservation solutions should be offset by methods to
increase tissue O2 and nutrient delivery, particularly if a solu-
tion 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 O2.

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 O2 and
required only a small-volume, recirculated coronary perfusate
for transport between centers. Lifor solution may lead to
attainment of that goal. A prolonged preservation time, partic-
ularly at room temperature, would lead to an increase in the
available donor pool of viable hearts and improve posttrans-
plant outcomes. An increase in preservation times and im-
provements in banking and transport of hearts over greater
distances should greatly increase the availability of viable
hearts with good tissue matches to needy recipients.

Limitations and conclusions. In this experimental model,
which consisted of a very low-flow coronary recirculation
system at room temperature and room air, Lifor solution was
superior compared with 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, nonworking heart model for a study of preser-
vation 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.
The mechanism of protection by nanoparticle-based solutions
is a focus of future studies.

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