



Room Temperature Pulsatile Perfusion of Renal Allografts With Lifor Compared With Hypothermic Machine Pump Solution

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ABSTRACT

This pilot study compared the use of the Lifor Organ Preservation Medium (RTLf) at room temperature with hypothermic Belzer machine preservation solution (CMPS) and room in vitro temperature Belzer machine preservation solution (RTMPS) in a porcine model of uncontrolled donation after cardiac death (DCD). In this study, 5 porcine kidneys for each perfusate group were recovered under a DCD protocol. The kidneys were recovered, flushed, and placed onto a renal preservation system following standard perfusion procedures. The average flow rate for CMPS was 36.2 ± 7.2549 mL/min, RTMPS was 90.2 ± 9.7159 mL/min, and RTLf was 103.1 ± 5.1108 mL/min. The average intrarenal resistance for CMPS was 1.33 ± 0.1709 mm Hg/mL per minute, RTMPS was 0.84 ± 0.3586 and RTLf was 0.39 ± 0.04 . All perfusion parameters were statistically significant ($P < .05$) at all time points for the CMPS when compared with both RTMPS and RTLf. All perfusion parameters for RTMPS and RTLf were equivalent for the first 12 hours; thereafter, RTLf became significantly better than RTMPS at 18 and 24 hours. It appears that both RTMPS and RTLf have equivalent perfusion characteristic for the initial 12 hours of perfusion, but LF continues to maintain a low resistance and high flow up to 24 hours. The results of this pilot study indicate that RTLf may represent a better alternative to pulsatile perfusion with CMPS and requires validation in an in vivo large animal transplant model.

DESPITE SIGNIFICANT changes in immunosuppressive regimens, limited progress has been made in the science of organ preservation.¹ Currently, the 2 most common solutions used for static cold preservation of renal allografts have been in use for >20 years, and neither has been shown to confer a significant advantage over the other.² We continue to have a practical limit of 24–48 hours of viable cold storage time. The alternative to cold renal storage, machine pulsatile perfusion (PP), has never been clearly shown to be superior to static preservation methods and uses similar preservation solutions.³ The accepted prognosticating standards for renal perfusion have relied on intrarenal resistance (IR) of pumped kidneys since the mid 1980s when Henry et al⁴ first reported their findings. Henry further reported that IR < 0.250 mm Hg/mL per minute predicted an immediate functioning renal allograft, an IR of 0.250–0.400 mm Hg/mL per minute predicted delayed renal allograft function (the need for dialysis within the first week posttransplantation), and IR > 0.400 mm Hg/mL per minute led to a primary nonfunctioning renal allograft.⁴

Although cold PP is the current standard, studies of warm machine perfusion of organ allografts have resulted in deteriorating perfusion characteristics as manifested by an increasing intrarenal resistance and decreasing flow.⁶ This effect is due to the failure to maintain the integrity of the vascular endothelium and an inability to deliver adequate amounts of nutrients and oxygen.⁶ An alternative to cold PP is the use of a new perfusion agent, Lifor OPM (Lifeblood Medical, Freehold, NJ), which has been developed to maintain organ integrity at room temperature. Lifor OPM is a novel agent that includes nutrients, amino

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Table 1. Temperature of Perfusate Within Perfusion Apparatus (°C)

Time (Hour)	C MPS (n = 5)			RT LF (n = 5)		RT MPS (n = 5)			RT LF (n = 5)	
	Mean	St Dev	P	Mean	St Dev	Mean	St Dev	P	Mean	St Dev
1	6.9	1.084	<.0001	19.1	1.6	23.0	1.5643	.0042	19.1	1.6
4	5.8	1.1524	<.0001	20.6	2.1	23.7	1.2478	.0250	20.6	2.1
6	5.7	0.9685	<.0001	21.1	2.4	24.2	0.753	.0224	21.1	2.1
12	5.3	0.886	<.0001	21.7	2.2	23.6	1.0977	.1244	21.7	2.2
18	5.3	0.911	<.0001	21.2	2.7	23.4	1.09	.1200	21.2	2.7
24	5.4	0.9072	<.0001	21.0	2.4	23.7	1.3517	.0716	21.0	2.4
Mean	5.7	0.109		20.8	0.4	23.6	0.2753		20.8	0.4

acids, growth factors, salts, and buffers. It is designed to prevent ischemia–reperfusion injury. Previous studies in rodent kidney transplants (unpublished data) and heart preservation in guinea pigs have demonstrated that Lifor is superior to University of Wisconsin solution in protecting against warm or cold ischemia–reperfusion injury.⁷

In this pilot study, we have compared Lifor OPM at room temperature (RTLF) with standard hypothermic (CMPS) and room temperature (RTMPS) perfusion with Belzer machine perfusate (Transmed, Elk River, Minn) in a porcine model of donation after cardiac death (DCD). We sought to determine if room temperature machine perfusion of porcine kidneys with LF OPM would result in similar or improved perfusion characteristics versus standard CMPS.

METHODS

Using an approved IACUC protocol (NIH # UOB 006) kidneys were recovered from an area slaughterhouse duplicating a Maastricht I donor with warm ischemia time between 25 and 35 minutes from exsanguination to initial flushing. The kidneys were flushed with cold lactated Ringer's solution, followed by flush and storage with cold MPS and transported back to the laboratory on ice within 2 hours. Kidneys were then placed onto a Waters RM3 renal preservation system for 24 hours primed with 1 of the 3 perfusate groups ($n = 5$ for each group; CMPS, RTMPS, and RTLF). The

Waters RM3 Preservation System (Waters Medical Systems, Rochester, Minn) is the only FDA-approved renal preservation system that has an oxygenator in the perfusion circuit.

The systolic perfusion pressure for all groups was maintained at 50 mm Hg with pulse rate of 70 bpm and room air blown across the oxygenator to oxygenate the perfusate; IR (mean pressure/flow) was allowed to fluctuate according to the kidney's perfusion flow output and these results were recorded from the RM3. Temperature was maintained between 5–7°C or at ambient room temperature (Table 1). Statistical analysis was performed using an unpaired Student *t*-test and a paired, 2-tailed $P < .05$ was considered significant.

During perfusion, 2-mL aliquots of perfusate were drawn and flash frozen in cryotubes for protein quantitation using SearchLight Porcine Cytokine Array 1 (Thermo Scientific, Inc, Woburn, Mass). Samples were diluted 2-fold before analysis. ELISA plate images were taken with a Fuji LAS 3000 CCD camera and analyzed using SearchLight Array Analyst Software. Calculated concentrations were baseline corrected to the minimum detectable standard value per cytokine per plate. To account for subtle effects on binding owing to differences in perfusate content, these corrected values were converted to a value proportionate to respective values at time of perfusion initiation ($T = 0$). To each time point, the Mann–Whitney U test was applied to compare each group with CMPS.

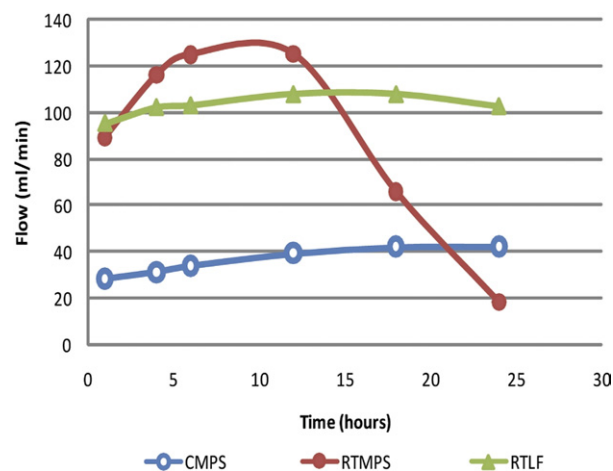


Figure 1. Relationship between the kidney's output flow in (ml/min) over the perfusion time for the 3 perfusates.

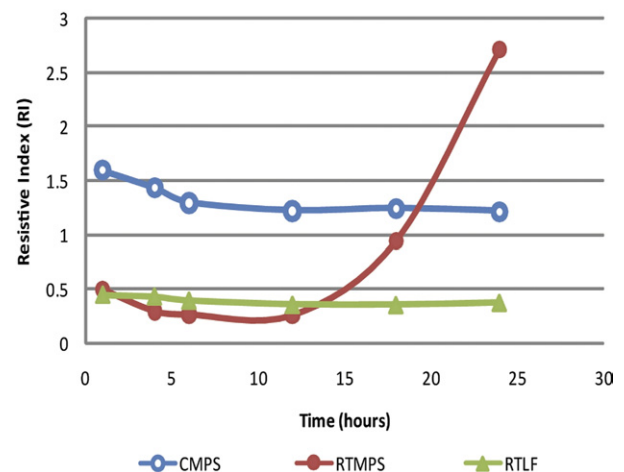


Figure 2. Relationship between intrarenal resistance of the kidneys' (mm Hg/mL per minute) over the perfusion time for the 3 perfusates.

RESULTS

The mean temperatures ($^{\circ}\text{C}$) for the 3 treatment arms were 5.7, 23.6, and 20.8 for CMPS, RTMPS, and RTLTF, respectively (Table 1). Overall, the mean flow (mL/min) for the 3 groups was 36.2, 90.2, and 103.1, the average flow rates for each treatment arm at specific time points are shown in Figure 1. The average IR (mm Hg/mL per minute) measured for the 3 groups was 1.33, 0.84, and 0.39, respectively; the average resistances for each treatment arm at specific time points are shown in Fig 2. Differences in flow and resistance between the treatment groups were found to be significant at all the CMPS, 18 and 24 hours RTMPS time points (all $P < .05$).

To initiate identification of mediators of reperfusion injury, cytokine-specific ELISAs were used herein. No difference in measurable interleukin (IL)-8 or tumor necrosis factor (TNF)- α concentration was observed between RTLTF and CMPS within the first 12 hours of perfusion. At 24 hours, the change in RTLTF (8.34-fold) and CMPS (1.37-fold) became significantly different ($P = .032$). Strikingly, RTMPS demonstrated significant increases in IL-8 as

opposed to those in CMPS at 6 hours (15.34-fold; $P = .017$), 12 hours (65.65-fold; $P = .016$), and 24 hours (39.02-fold; $P = .008$) after perfusion initiation. These changes in the pro-inflammatory chemokine IL-8 were followed by a dramatic decrease in flow rate (Figs 1 and 3). Additionally, the relative TNF- α concentration in RTMPS (4.65-fold) was significantly greater than in CMPS (1.00-fold; $P = .008$). Although not significant, marked increases in concentration were observed with RTMPS for IL-1 β and interferon (IFN)- γ (Fig 3). These changes seem to be blunted by the RTLTF, as demonstrated by little or delayed average increases in concentration.

DISCUSSION

It has been shown that hypothermic organ preservation is a serious barrier to the logistics of preserving transplant organs.⁸ These barriers are greater when cold ischemia is preceded by warm ischemia as is the case in DCD donors. Although some studies have shown that normal renal function may be possible after as much as 2 hours of warm ischemia, this period is typically limited to 45 minutes.⁹ The

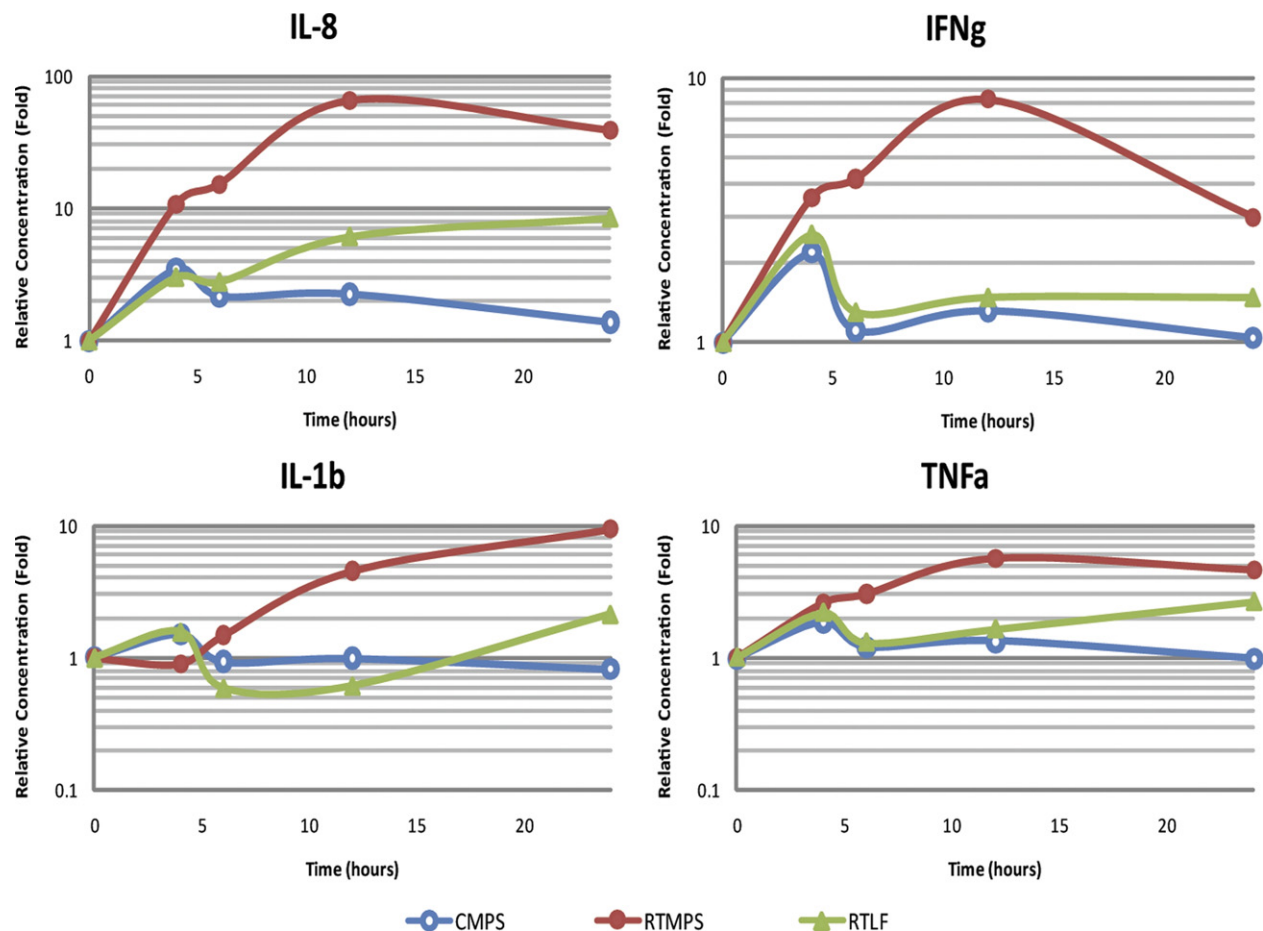


Figure 3. Relative concentration of perfusate (CMPS, RTMPS, and RTLTF) cytokines during the perfusion period. PP with both CMPS and RTLTF seems to blunt pro-inflammatory cytokine (IL-8, TNF- α , IL-1 β , and IFN- γ) production when compared with RTMPS.

theoretical basis for hypothermic preservation has been the decrease in metabolic rate achieved by lowering tissue temperature. However, hypothermia can lead to changes in membrane integrity that worsen reperfusion injury.¹⁰ These changes include a decrease in the adenine pool, accumulation of metabolic byproducts, dysfunction of ion pumps, edema, and deterioration of the vascular structures. In contrast, warm perfusion *ex vivo* may result in induction of factors that mitigate reperfusion injury.¹⁰

Results of this pilot study of Lifer OPM and Belzer Machine Perfusion Solution indicate that perfusion with RTLF and RTMPS results in significant improvement in resistance and flow rates during the first 12 hours when compared with CMPS. During the subsequent 12 hours of perfusion, RTMPS demonstrates considerable deterioration of perfusion characteristics while RTLF maintains favorable flow (>100 mL/min)⁵ and resistance (<0.40 mm Hg/mL per minute).⁵ Therefore, despite prolonged warm perfusion with LF OPM, porcine kidneys maintained excellent flow characteristics over 24 hours. Additionally, both RTLF and CMPS seemed to attenuate a pro-inflammatory response as measured by cytokine analysis. This differs from the results of warm perfusion reported by other investigators using acellular solutions.^{11,12} The results of previous studies of warm perfusion using University of Wisconsin and Dextran, respectively, demonstrated sharp deterioration of perfusion characteristics concurrent with tissue injury.^{11,12} Our results were similar to several other investigators who have studied warm perfusion using liquid media coupled with an oxygenator and tight control of pH, PCO₂ and temperature.^{8-10,12-15} In contrast to their studies, our results have achieved similar favorable perfusion characteristics, without the need for perfusates or perfusion circuits, which may result in cost savings.

In conclusion, this study demonstrated that room temp PP with LF OPM results in improved perfusion characteristics and a seemingly blunted inflammatory response. As a result of the pilot nature of this study, these observations need to be validated in a large animal transplant model before undertaking a clinical trial.

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I am a military service member (or employee of the US Government). This work was prepared as part of my official duties.

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The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Resource Council, National Academy Press, 1996.

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