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Research Article

Pro-Inflammatory Milieu in Human Kidney Perfusates Treated with Hypothermic Pulsatile Machine Perfusion

Emmett Ratigan[3], Sashi Kasimsetty[1*], Jeffrey Cohen[3], Alana Shigeoka[2], Lisa Stocks[3,5], Kristin Mekeel[3,4], Robert Steiner[3], Dianne B. McKay[1,2,3]

[1]Department of Immunology and Microbiology, Scripps Research Institute, La Jolla, CA, USA

[2]Scripps Clinic and Green Hospital, La Jolla, CA, USA

[3]Department of Medicine, University of California San Diego, La Jolla, CA, USA

[4]Department of Surgery, University of California San Diego, La Jolla, CA, USA

[5]Lifesharing, Camino del Rio South, San Diego, CA, USA

[*]**Corresponding author:** Dianne B. McKay, Department of Immunology and Microbiology, Scripps Research Institute, 10466 N Torrey Pines Rd, La Jolla, CA 92037, USA. Tel: +1 858-784-9716; Fax: +1-8582461191; Email: dmckay@scripps.edu

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Abstract

Hypothermic Machine Perfusion (HMP) has been shown to decrease Delayed Graft Function (DGF) and improve outcomes of deceased donor kidney transplants. Although the mechanisms of protection afforded by HMP have not yet been defined, this study tested the hypothesis that HMP improves transplant outcomes by clearing pro-inflammatory molecules from the allograft during pumping. Here we report on 26 allografts preserved via pulsatile HMP at our organ procurement organization that were prospectively evaluated for the time-dependent clearance of cytokines, chemokines and injury markers during their time on pump. Of the cytokines and chemokines measured, IL-1RA, IL-6, IL-18, INFγ, and MCP-1 were increased in the pump perfusate over time and were correlated with markers of kidney injury. Over time on pump, the 'Danger' molecule HMGB1 appeared in the perfusate, and perfusate samples activated T-cells *ex vivo*. The data from this prospective study show that HMP is associated with a time-dependent clearance of pro-inflammatory cytokines, chemokines and danger molecules from donor allografts during the process of pumping, which may in part explain the beneficial effects of HMP.

Keywords: Hypothermic Machine Perfusion; HMGB1; Inflammation; Kidney Transplant; Pro-inflammatory Molecules; Renal Tubular Epithelial Cells; T cells

Abbreviations

HMP : Hypothermic Machine Perfusion

DGF : Delayed Graft Function

Introduction

Pulsatile Hypothermic Machine Perfusion (HMP) has emerged as an important technique for preserving kidney allografts prior to transplantation. Since the early 1960's investigators have recognized that deceased donor kidneys treated with HMP before transplantation experienced reduced Delayed Graft Function (DGF) and improved graft survival [1-6]. The reason for the protective effect of pumping is not completely understood, but the positive effect has been associated with delivery of energy substrates, elimination of toxic metabolites, reduction of organ edema, and prevention of vasospasm [7-10]. Recent reports have shown that even a short period of HMP following prolonged cold storage is beneficial [9,10].

Several studies have demonstrated that the pump perfusate contains metabolic or injury markers released from the kidney [11-14], however the utility of these molecules as biomarkers has not been established. It is well known that the donor kidney produces inflammatory molecules during the processes of brain death and organ procurement that are immunogenic, and when the kidney is transplanted in the recipient these pro inflammatory molecules incite anti-donor immune responses that impact the outcomes of the transplanted kidney. The transcriptome profile of the donor kidney has in fact been suggested to predict the recipient's immune response and subsequent susceptibility to post-transplant Delayed Graft Function (DGF) [15-17]. To further investigate how HMP influences the inflammatory milieu of the donor organ we designed a prospective study to evaluate key inflammatory cytokines/

chemokines in perfusates of human kidneys treated over time with HMP. The study also characterized the ability of molecules in the perfusate to activate naïve T cells.

Methods

Study Protocol

Twenty-six sequential deceased donor kidneys procured by the San Diego-Imperial County OPO, Life sharing®, preserved utilizing the Life-port Kidney Transporter® Hypothermic Pulsatile Machine Perfusion (HMP) system, were analyzed in this study. Kidneys included in this study were those whose family had previously authorized use of their organs for research and who were placed on the HMP machine prior to transplantation. The study was reviewed by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the Declaration of Helsinki (as revised in Brazil 2013). Each of the kidneys underwent an intraoperative wedge biopsy at the time of procurement. CDC high-risk donors were identified by Life sharing® (defined as IV drug user, hemophiliac, prostitution history, high risk sexual activity, exposure to HIV and jail sentencing) and were excluded from this study. After procurement, all organs were flushed with SPS-1® static preservation solution (formally Belzer UW® cold storage solution), containing Hydroxyethyl starch (50 gm), Lacto bionic acid (35.83 gm), Potassium phosphate monobasic (3.4 gm), Magnesium sulfate heptahydrate (1.23 gm), Raffinose pentahydrate (17.83 gm), Adenosine (1.34 gm), Allopurinol 0.136 gm), Glutathione (0.922 gm), Potassium hydroxide (5.61 gm), Sodium hydroxide/Hydrochloric acid (adjusted to ph7.4) in each 1L. The kidneys were then placed on the Life-port Kidney Transporter® and perfused in 1L of KPS-1® machine perfusion solution (containing Calcium chloride 0.068 gm, Sodium hydroxide 0.70 gm, HEPES 2.38 gm, Potassium phosphate 3.4 gm, Mannitol 5.4 gm, Glucose 1.8 gm, Sodium gluconate 17.45 gm, Magnesium gluconate D gluconic acid/Hemi-magnesium salt 1.13 gm, Ribose d 0.75 gm, Hydroxyethyl starch 50 gm, Glutathione 0.92 gm, Adenine 0.68 gm). When the pump was turned on, a 10-ml vial of perfusate was aspirated from the sample port and labeled as 0 hour (n=21). A 10-ml vial of perfusate was then aspirated after 2 hours (n=25), 4 hours (n=25), and 8 hours (n=17) on the machine pump. The 0 hour and 2 hour samples were collected at these time points. Four-hour samples were collected between 4-6 hours of pumping, whereas 8-hour samples were collected between 7-9 hours of pumping. Perfusates were stored at 4˚C immediately after collection and then stored at -80˚C thereafter prior to analyses.

Analyte Quantification

Direct measurement of perfusate samples was performed using a Bioplex Magpix Multiplex Reader® via Biorad Bio-plex ProHuman Cytokine® (no dilution) and RBM Human Kidney Toxicity 1® (1:4 dilution) assays for IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-10, IFNγ, MCP-1, MIP-1α, TNFα, IL-18 Calbindin, Clusterin, GST-pi, and KIM-1. An ELISA for NGAL was performed according to protocol using R&D Systems DuoSet®, #DY1757 at a 1:25 dilution.

Western Blot

Equal volumes of perfusate (27 µl) derived from three pumped allografts procured from unique donors sampled at 0, 2, 4, and 8 hours on pump were loaded onto a 10% polyacrylamide gel and separated by SDS electrophoresis. Proteins were transferred to a polyvinylidene difluoride membrane, blocked with 5% milk in TBST and probed for 4 hours at room temperature with anti-HMGB1 (Abcam, ab18256) at 1:1000 in TBST. After incubation with primary antibody, the blots were washed and probed with anti-rabbit horseradish peroxidase-conjugated antibody (ECL, #NA934V) at 1:5000 for 45 minutes at room temperature and then visualized using Super Signal® West Femto Maximum Sensitive Substrate.

Protein Precipitation

Samples taken from allografts after 4-6 hours of pumping were precipitated with 1:1 ice-cold acetone overnight. The following day the samples were centrifuged to solidify the protein pellet prior to aspirating the supernatant. The pellet was then let air dry prior to the addition of fresh RPMI media (GIBCO) in equal volume to the original volume of perfusate precipitated. As a comparator, sterile perfusate was precipitated and re-suspended in the same fashion.

Migration Assay

Perfusate circulated on pump for 4-6 hours (n=25) was protein precipitated using ice-cold acetone and re-suspended in RPMI media. For comparison, sterile perfusate was precipitated and re-suspended in RPMI with (positive control, n=6) or without (negative control, n=6) the added chemokine CCL21 (2 µg/ml). The samples were added to the lower chamber of Costar 3421® 5.0 µm trans well permeable support chambers (600 µl). Human T cells (Kit225 cells) were cultured in 10K media containing 200U of IL2, transferred to RPMI media and then seeded in the upper chamber at a density of $5x10^5$ cells per 100µl. After 4 hours a cell count was performed using the Nexcelom Cello meter K2®.

T-cell Proliferation

Perfusate circulated on pump for 4-6 hours (n=25) was protein precipitated using ice-cold acetone and re-suspended in RPMI. For comparison, sterile perfusate was precipitated and re-suspended in RPMI (control, n=10). One hundred µl of HMP or sterile perfusate was added to 50K Kit225 cells suspended in 100µl of RPMI (1:1 dilution) and seeded in Costar 3799® 96 well cell round bottom culture plates. Following 1, 2, and 3 days of incubation, Kit225 cells were analyzed for proliferation using the

Pro-Mega Cell Titer 96® assay. Samples were run in duplicate. The mean of both observations was used for analysis.

Donor Demographics and Normalization of Size

Using United Organ Sharing (UNOS) identification numbers, allografts were paired with de-identified donor information provided by Life sharing® that included information regarding the donor's demographics, prior medical history, reason for admission, cause of death, medication administration, laboratory results, diagnostic imaging, procedures, and intraoperative flow sheets. This review was exempt from IRB oversight by the UCSD Human Research Protections Program (HRPP) as it involved de-identified data that was collected from deceased individuals.

To account for variability of allograft size, analytes were normalized to kidney volume in Cubic Centimeters (cm). At the time of procurement, the length, width, and depth of the allograft was measured, and the measured dimensions were used to estimate kidney volume. The analyte concentrations were reported per milliliter, and the concentration was multiplied by the total milliliters of perfusate in which it was diluted, and then divided by the estimated allograft volume in cubic cm, which yielded the normalized value.

Statistical Analysis

All statistical analyses were performed using IBM SPSS version 22® software. For continuous variables, normality was evaluated using the Shapiro-Wilk test with the null hypothesis being rejected at the 0.05 level. For normally distributed data, means and standard deviations are reported and compared using t-tests. Abnormally distributed data are reported by median and interquartile ranges or minimum-maximum and are compared using Mann-Whitney U tests. Correlation was performed with Spearman's rho. All p-values reported are asymptotic, 2-sided.

Results

This prospective study was designed to evaluate whether pro-inflammatory molecules were released from donor kidneys treated with HMP. The immunologic activity of the perfusates from kidneys on pump was also tested to determine whether inflammatory mediators present within the perfusates contributed to T cell activation.

Study Population

The demographic and clinical characteristics pertaining to the allografts examined in this study are shown in Table 1. The majority of allografts were procured from male donors (10/14 donors) who were of Caucasian race (13/14 donors). Overall, the mean Kidney Donor Profile Index (KDPI) was high (77%), which may be reflective of the type of donor selected by our study protocol, i.e., donors requiring intraoperative biopsies whose allografts are subsequently preserved with HMP. The cause of death was most commonly Cerebral Vascular Accident (CVA) (9/14 donors). Of the remaining allografts 2 were procured from one donor who died of head trauma, while seven allografts were procured from 4 donors listed as dying from "Anoxic" injuries. More specifically, "anoxic" death included 3 presumed cardiovascular events (5 allografts) and one drowning (2 allografts). Five of our 14 donors were procured by from Donation after Cardiac Death (DCD) donors (10/26 allografts) with warm ischemic periods ranging from 20-43 minutes. The time elapsed between procurement and pumping was similar between paired allografts and on average was 135 minutes. The total time spent on pump averaged approximately 12 hours. Samples were obtained from 21 allografts at 0 hours, 25 allografts at 2 hours, 25 allografts at 4-6 hours, and 17 allografts at 7-9 hours of HMP.

Parameter	Donors (n=14)[a]
Total allografts (n)	26
Age (years)	57 +/- 9
Male sex	10 (71%)
BMI (kg/m^2)	29 +/- 6
Race	
Caucasian	13 (93%)
Hispanic/Latino	1 (7%)
History of hypertension	8 (57%)
History of diabetes	2 (14%)
Length of hospitalization (days)	4.5 +/- 1.6
Terminal Injury	
CVA	9 (64%)
Head trauma	1 (7%)
Anoxia	4 (29%)
Terminal serum creatinine (mg/dl)	1.4 +/- 0.7
Kidney Donor Profile Index (KDPI)	77 +/- 18%
Donation after Cardiac Death (DCD)	5 (36%)
Time elapsed between procurement and pumping (min)	135 +/- 113
Duration of pumping (min)	711 +/- 395
[a]Results are presented as mean +/- SD.	

Table 1: Donor Demographic and Clinical Characteristics.

Analyte Evaluation

As shown in Figure 1, the cytokines IL-1RA, IL-6, IL-18, IFNγ and chemokine MCP-1 were detected in perfusates from all allografts, and their concentrations in the perfusates increased over time. The renal tubular injury marker NGAL was similarly detected and increased in a time-dependent fashion. The appearance rate of these inflammatory mediators over the HMP period is shown graphically in Figure 1 for all 26 allografts. In contrast, IL-1β, IL-2, IL-4, IL-10, MIP-1α, TNFα, calbindin, clusterin, GST and Kim-1 were either undetectable or present at very low-levels (data not shown). Over the duration of HMP, the appearance rate of all cytokines and chemokines in the perfusate slowed, and presuming active production is minimal while hypothermic, a steady state between graft and perfusate may have been achieved for the production of these analytes, as has been reported by others [13].

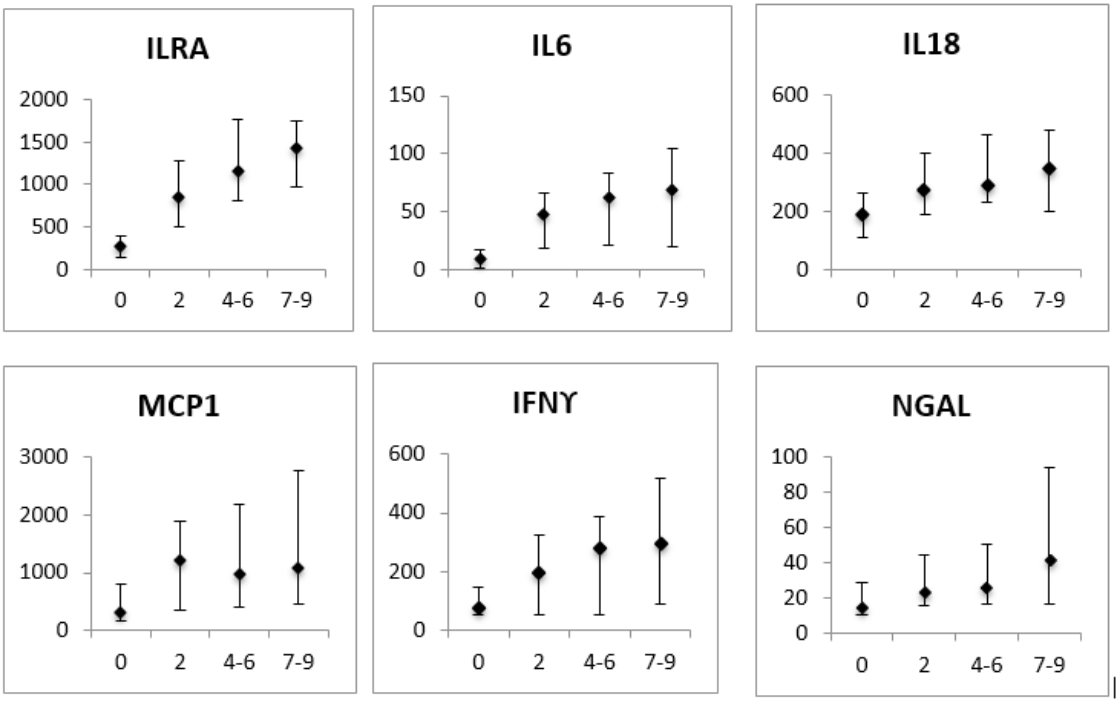

Figure 1: Inflammatory molecules detected in perfusates over time on HMP. Samples collected while on pump at 0 hour (n=21), 2 hours (n=25), 4-6 hours (n=25), and 7-9 hours (n=17). Data points represent median values (expressed as pg/cm^3 for all cytokines and ng/cm^3 for NGAL) and error bars represent interquartile range.

Amongst the detected analytes, there was a significant correlation between IL-1RA, IL-6, MCP-1, and the kidney injury marker NGAL ($P<0.01$) (shown in Table 2), suggesting that these molecules were associated with the kidney injury that occurred during procurement. To further evaluate the effect of HMP on innate immune activators, we also assayed the perfusates of three kidneys over time for the presence of the Damage Associated Molecular Pattern (DAMP) molecule HMGB1, and found that HMGB1 was detected over time in the sampled perfusates (Figure 2).

	IL1RA	IL6	IL18	INFγ	MCP1	NGAL	Median	IQR
IL1RA	1						1167	803-1762
IL6	0.78**	1					62	22-84
IL18	0.45*	0.41*	1				290	230-465
INFY	0.22	-0.01	-0.02	1			275	52-385
MCP1	0.62**	0.67**	0.49*	-0.37	1		965	408-2192
NGAL	0.64**	0.66**	0.37	-0.22	0.68**	1	26	17-51
Performed with Spearman's rho. *Correlation significant at the <0.05 level; **Correlation significant at the <0.01 level.								

Table 2: Correlation amongst perfusate inflammatory/injury markers measured at 4-6 hours normalized to volume.

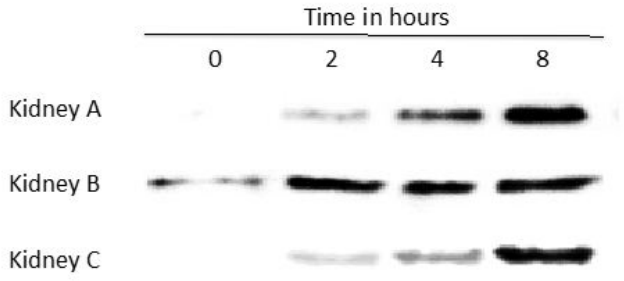

Figure 2: HMGB1 in perfusates of kidneys on HMP, over time. Each of the three Western Blots (WB) represents a different kidney perfusate in which the same kidney was sampled over time. Each WB depicts a time-dependent analyses of HMGB1 when equal volumes of perfusate from the same allograft sampled at 0, 2, 4, and 8 hours were compared. The three kidney allografts are derived from unique donors.

Association with Donor Exposures

We next analyzed the association between exposures in the donor and the measured analytes (Table 3). Amongst the 14 donors evaluated, MCP-1 appeared to be significantly increased in donors dying of "Anoxia" whereas IFNγ was significantly elevated in donors dying of CVA or head trauma. It also appeared that donors who had experienced AKI during the admission had significantly increased levels of MCP-1 and NGAL. There did not appear to be a significant association between terminal creatinine or procurement method with the studied inflammatory and injury markers. Furthermore, there was no association between the inflammation scores on pre-transplant allograft biopsy to these analytes, however there was very little inflammation on any of the pre-transplant allograft biopsies (data not shown).

Donor Characteristic (n)	**IL1RA** (pg/cm^3)	**IL6** (pg/cm^3)	**INFγ** (pg/cm^3)	**MCP1** (pg/cm^3)	**IL18** (pg/cm^3)	**NGAL** (ng/cm^3)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Terminal Injury						
CVA/Head Trauma (10)	1125 (732-1772)	46 (17-97)	307 (103-539)*	697 (391-1201)**	284 (234-443)	22 (16-51)
Anoxia (4)	1473 (685-1869)	82 (44-97)	34 (30-221)	2528 (2183-4796)	418 (198-505)	43 (42-125)
AKI[b]						
No (6)	951 (732-1263)	33 (17-107)	196 (60-376)	495 (334-1201)*	272 (228-385)	18 (14-28)*
Yes (8)	1645 (685-2124)	76 (39-97)	284 (33-554)	2150 (840-2621)	447 (231-513)	43 (29-90)
Terminal Creatinine						
≤1.0 mg/dl (6)	993 (732-1357)	55 (17-128)	91 (36-290)	776 (391-2101)	272 (219-385)	21 (16-43)
>1.0 mg/dl (8)	1645 (633-2124)	60 (28-91)	335 (97-586)	1529 (579-2298)	447 (231-513)	42 (22-90)
Procurement method						
BD (9)	1167 (500-1645)	51 (25-96)	283 (36-357)	811 (455-2236)	284 (234-447)	42 (20-60)
DCD (5)	1551 (951-2774)	70 (22-150)	115 (48-804)	1896 (641-3829)	410 (217-525)	25 (14-97)

[a]For kidney pairs from the same donor analytes were averaged for analysis. [b]AKI defined by AKIN criteria for serum creatinine. *Significant at <0.05, **Significant at <0.01

Table 3: Relation of donor characteristics to inflammatory markers at 4-6 hours normalized to kidney volume (cm^3)[a].

Perfusate Inflammatory Phenotype

The inflammatory phenotype of the perfusate was evaluated by transferring precipitated protein fraction of the 4-6 hour MP samples (n=25) into fresh media to evaluate its ability to activate T-cells. For T-cell assays, an immortalized line of T-lymphocytes (Kit225) was used. Table 4 shows the results of the T-cell migration assay in which the protein fraction of the HMP perfusate samples significantly increased migration across trans well membranes compared to sterile perfusate ($p<0.01$). Of the analytes evaluated, only the chemokine MCP-1 showed a significant correlation to T-cell migration ($p=0.02$). We then evaluated the HMP protein fraction's effect on T-cell proliferation and found that the HMP perfusate induced significantly more T cell proliferation than sterile perfusate not pumped through the kidneys, $p<0.01$ (Figure 3A). T cell proliferation was also noted by visually by inspecting cultures of Kit225 cells exposed to optimal stimulating media (10K media

+ 200U Il-2), sterile perfusate or HMP perfusate (Figure 3B).

Sample (n)	Median cell count	Range	p-value[b]
Pumped kidney protein fraction (24)	32	6-126	<0.01
Positive control (6)	77	6-550	<0.01
Negative control (6)	0	0-2	
[a]Migration occurred over 4 hours at 37°C. [b]The p-value represents comparison to the negative control.			

Table 4: The protein fraction of pumped kidney perfusate is capable of inducing T-cell migration *in vitro*[a].

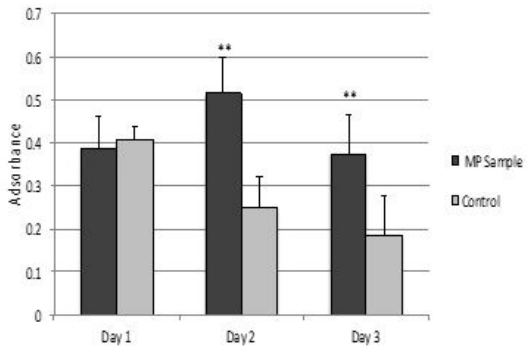

Figure 3A: The protein fraction of HMP kidney allografts is capable of activating T-cell proliferation *ex vivo*. The protein fraction from HMP allografts at 4-6 hours (n=25) was transferred to RPMI media and incubated with 50,000 Kit225 cells at a 1:1 dilution in fresh RPMI for 3 days (dark grey bars, MP sample). For comparison, sterile perfusate, which was acetone-precipitated and re-suspended in RPMI, was also incubated with Kit225 cells as above (n=10) (light grey bars, Control). T cell proliferation was evaluated each day using the Pro-Mega Cell Titer 96®, tetrazolium based assay. Results are presented as the mean; error bars denote SD. **p<0.01.

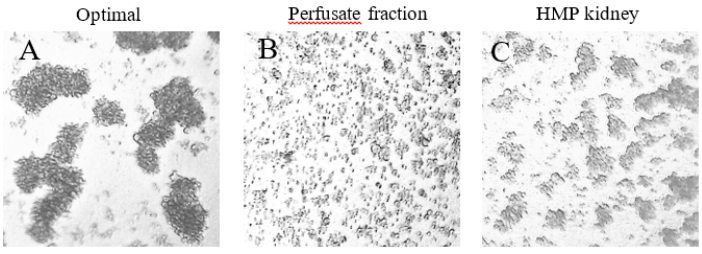

Figure 3B: Perfusate from kidneys on HMP induces T cell activation *ex vivo*. The results demonstrate that the protein fraction from HMP samples at 4-6 hours, acetone-precipitated and re-suspended in RPMI media (C) is capable of inducing T cell proliferation. Comparison is made to Kit225 cells grown in both optimal media (10K media+200U IL-2) (A) and the protein fraction of sterile perfusate (B). Images are taken at 40X magnification after 2 days of growth.

Discussion

This preliminary study asked whether pro-inflammatory molecules were released into perfusates from human kidneys treated with HMP. In the HMP treated kidneys there was a steady increase in pro-inflammatory cytokines, chemokines and renal tubular injury molecules released into the perfusates for up to 4-6 hours on pump, after which time the production of these molecules appeared to have ceased in cooled kidney. Many studies have shown that ischemic kidneys produce stress-induced cytokines and chemokines, and that production of these molecules in the kidney is reduced by HMP [18,19].

Of the inflammatory mediators detected in this study, IL-1RA, IL-6, IL-18, IFNγ, and MCP-1 appeared within the first two hours on pump and their concentrations increased over 4-6 hours, after which time they appeared to reach steady state. A significant correlation was noted between the kidney injury marker NGAL and IL-1RA, IL-6 and MCP-1. NGAL and MCP-1 concentrations were the highest in donors that experienced AKI prior to donation, which is consistent with reports of these molecules as biomarkers of AKI [20,21]. IFNγ levels were higher in the perfusates of kidneys procured from donors dying of a CVA. Several studies have reported that ischemic stroke activates the peripheral immune system and increases serum IFNγ in donors [22,23]. Hence IFNγ cleared from allografts of patients with CVA may be a consequence of systemic inflammation in the donor rather than de novo production within the donor kidney. Although human data is limited, ischemia-reperfusion experiments in mice have clearly shown rapid induction of NGAL and MCP-1 following ischemic kidney injury [24,25]. MCP-1 regulates the migration and infiltration of T cells, monocytes and NK cells [26,27], and since the perfusate samples caused activation of T cells *ex vivo*, it is possible that elimination of MCP-1 during HMP might ultimately dampen immunogenicity of the donor kidney.

Renal ischemia causes release of the Damage Associated Molecular Pattern (DAMP) HMGB1, which activates the innate immune system [28,29]. Over time HMGB1 was detectable in the perfusates of the pumped kidneys. While we do not know whether tissue production of this DAMP was altered by HMP, Gallinat, et al. demonstrated that HMP treatment significantly lowered HMGB1 levels in the kidneys and reduced innate immune activity in a porcine auto-transplant model [9]. Given that our data demonstrate nonspecific clearance of several proteins from the allograft, other DAMPs and injurious molecules are likely to be cleared similarly.

Of measured inflammatory mediators, IL-6, IL-18, IFNγ, and MCP-1 are pro-inflammatory and amplify the immune response [30]. Given that various other pro and anti-inflammatory mediators are surely present, we tested the effect of perfusate protein fractions on T cell activation *ex vivo* and found that they induced T-cell proliferation and migration. Therefore, it appears that substrates

responsible for T-cell activation appear in the perfusate and it is possible that eliminating these molecules from the donor kidney contributes to downregulation of the adaptive immune response upon transplant.

Taken together these findings support the possibility that the clearance of the inflammatory milieu from the donor organ in part underlies the efficacy of HMP in kidney allograft preservation. This concept is supported by data showing HMP was associated with fewer inflammatory transcripts than cold storage preservation in a transcriptome analysis of pre-implantation cadaveric kidney biopsies [31]. The results of this preliminary study warrant further analyses to determine the effect of HMP on clearance of injurious molecules from the donor kidney in large patient groups in which HMP is compared to other forms of donor kidney preservation (e.g. cold static storage, or forms of warm storage). Despite limitations of small sample size, data from this preliminary study show that inflammatory cytokines and chemokines are cleared from the allograft over time in kidneys treated with HMP. We propose that donor exposures determine the magnitude of pro-inflammatory molecule expression in the donor kidney and that treatment with HMP helps to dampen immunogenicity of the donor organ prior to transplant. Further studies are ongoing to determine whether the clearance of cytokines and chemokines during HMP are of benefit, particularly to allografts from donors with predictable hyper-inflammatory profiles.

Conclusion

This study demonstrates that amongst renal allografts preserved via pulsatile hypothermic machine perfusion, there is a time-dependent clearance of pro-inflammatory cytokines, chemokines and danger molecules from the allograft. Furthermore, while all studied allografts released an inflammatory milieu, characteristic profiles of inflammation may differ based on donor exposure. We propose that the clearance of these pro-inflammatory mediators serves to condition the allograft prior to transplantation and may in part account for the beneficial effect of HMP in kidney transplantation.

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Disclosures

None

Author's contributions

Emmett Ratigan; concept design, drafting article, data acquisition and analysis/interpretation; Kasimsetty, Cohen, Shigeoka; performed experiments critical to manuscript data; Stocks, Hemming, Mekeel; acquired and provided samples used in the experiment, data interpretation and critical review and editing of manuscript; Steiner; concept/design, critical manuscript review; McKay; concept/design, data analysis/interpretation, critical revision of article, funding.

References

1. Vetto RM, Belzer FO, Rogers RC, Park HY (1967) Rapid flow, isolated renal perfusion. The Journal of surgical research 7: 520-526.
2. Belzer FO, Ashby BS, Gulyassy PF, Powell M (1968) Successful seventeen-hour preservation and transplantation of human-cadaver kidney. The New England journal of medicine 278: 608-610.
3. Kaths JM, Paul A, Robinson LA, Selzner M (2017) *ex vivo* machine perfusion for renal graft preservation. Transplantation reviews 2017.
4. Moers C, Smits JM, Maathuis MH, Treckmann J, Gelder FV, et al. (2009) Machine perfusion or cold storage in deceased-donor kidney transplantation. The New England journal of medicine 360: 7-19.
5. Matsuoka L, Shah T, Aswad S, Bunnapradistb S, Choa Y, et al. (2006) Pulsatile perfusion reduces the incidence of delayed graft function in expanded criteria donor kidney transplantation. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 6: 1473-1478.
6. Schold JD, Kaplan B, Howard RJ, Reed AI, Foley DP, et al. (2005) Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 5: 1681-1688.
7. Fuller BJ, Lee CY (2007) Hypothermic perfusion preservation: the future of organ preservation revisited? Cryobiology 54: 129-145.
8. Yuan X, Theruvath AJ, Ge X, Floerchinger B, Jurisch A, et al. (2010) Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. Transplant international: official journal of the European Society for Organ Transplantation 23: 561-570.
9. Gallinat A, Paul A, Efferz P, Lüer B, Kaiser G, et al. (2012) Hypothermic reconditioning of porcine kidney grafts by short-term preimplantation machine perfusion. Transplantation 93: 787-793.
10. Gallinat A, Efferz P, Paul A, Minor T (2014) One or 4 hours of "In-house" reconditioning by machine perfusion after cold storage improve reperfusion parameters in porcine kidneys. Transplant international: official journal of the European Society for Organ Transplantation 27: 1214-1219.
11. Nath J, Smith TB, Patel K, Ebbs SR, Hollis A, et al. (2017) Metabolic differences between cold stored and machine perfused porcine kidneys: A 1H NMR based study. Cryobiology 74: 115-120.
12. Bhangoo RS, Hall IE, Reese PP, Parikh CR (2012) Deceased-donor kidney perfusate and urine biomarkers for kidney allograft outcomes: a systematic review. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 27: 3305-3314.

13. Moers C, Varnav OC, van Heurn E, Jochmans I, Kirste GR, et al. (2010) The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. Transplantation 90: 966-973.

14. Hoogland ER, de Vries EE, Christiaans MH, Winkens B, Snoeijs MG, et al. (2013) The value of machine perfusion biomarker concentration in DCD kidney transplantations. Transplantation 95: 603-610.

15. Mueller TF, Reeve J, Jhangri GS, Mengel M, Jacaj Z, et al. (2008) The transcriptome of the implant biopsy identifies donor kidneys at increased risk of delayed graft function. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 8: 78-85.

16. Avihingsanon Y, Ma N, Pavlakis M, Chon WJ, Uknis ME, et al. (2005) On the intraoperative molecular status of renal allografts after vascular reperfusion and clinical outcomes. Journal of the American Society of Nephrology: JASN 16: 1542-1548.

17. Hall IE, Yarlagadda SG, Coca SG, Wang Z, Doshi M, et al. (2010) IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. Journal of the American Society of Nephrology: JASN 21: 189-197.

18. Henry SD, Nachber E, Tulipan J, Stone J, Bae C, et al. (2012) Hypothermic machine preservation reduces molecular markers of ischemia/reperfusion injury in human liver transplantation. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 12: 2477-2486.

19. Tozzi M, Franchin M, Soldini G, Ietto G, Chiappa C, et al. (2013) Impact of static cold storage VS hypothermic machine preservation on ischemic kidney graft: inflammatory cytokines and adhesion molecules as markers of ischemia/reperfusion tissue damage. Our preliminary results. International journal of surgery 11: S110-S114.

20. Munshi R, Johnson A, Siew ED, Ikizler TA, Ware LB, et al. (2011) MCP-1 gene activation marks acute kidney injury. Journal of the American Society of Nephrology: JASN 22: 165-175.

21. Alge JL, Arthur JM (2015) Biomarkers of AKI: a review of mechanistic relevance and potential therapeutic implications. Clinical journal of the American Society of Nephrology: CJASN 10: 147-155.

22. Offner H, Subramanian S, Parker SM, Afentoulis ME, Vandenbark AA, et al. (2006) Experimental stroke induces massive, rapid activation of the peripheral immune system. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism 26: 654-665.

23. Seifert HA, Leonardo CC, Hall AA, Rowe DD, Collier LA, et al. (2012) The spleen contributes to stroke induced neurodegeneration through interferon gamma signaling. Metabolic brain disease 27: 131-141.

24. Safirstein R, Megyesi J, Saggi SJ, Price PM, Poon M, et al. (1991) Expression of cytokine-like genes JE and KC is increased during renal ischemia. The American journal of physiology 261: F1095-F1101.

25. Sung FL, Zhu TY, Au-Yeung KK, Siow YL, O K (2002) Enhanced MCP-1 expression during ischemia/reperfusion injury is mediated by oxidative stress and NF-κB. Kidney international 62: 1160-1170.

26. Rollins BJ, Yoshimura T, Leonard EJ, Pober JS (1990) Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. The American journal of pathology 136: 1229-1233.

27. Lloyd CM, Minto AW, Dorf ME, Proudfoot A, Wells TNC, et al. (1997) RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. The Journal of experimental medicine 185: 1371-1380.

28. Rosin DL, Okusa MD (2011) Dangers within: DAMP responses to damage and cell death in kidney disease. Journal of the American Society of Nephrology: JASN 22: 416-425.

29. Ratliff BB, Rabadi MM, Vasko R, Yasuda K, Goligorsky MS (2013) Messengers without borders: mediators of systemic inflammatory response in AKI. Journal of the American Society of Nephrology: JASN 24: 529-536.

30. Akcay A, Nguyen Q, Edelstein CL (2009) Mediators of inflammation in acute kidney injury. Mediators of inflammation: 137072.

31. Mas VR, Archer KJ, Dumur CI, Scian MJ, Suh JL, et al. (2012) Reduced expression of inflammatory genes in deceased donor kidneys undergoing pulsatile pump preservation. PloS one 7: e35526.