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Deletrious effect of prolonged cold ischemia on renal function

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Abstract The detrimental effect of prolonged cold ischemia (CI) on posttransplant renal function has long been recognized. However, the cellular consequences of CI have not been clearly defined. This study describes a model for the identification of CI-induced injury by evaluating ex-vivo renal metabolism and function prior to reperfusion. Small bovine kidneys were cold stored in Viaspan for 24-, 48-, 72-, and 96 h. Kidneys were then warm perfused (32 °C) using Exsangiunous Metabolic Support (EMS) technology and evaluated for oxidative metabolism, vascular dynamics and function. Oxygen consumption, vascular resistance, and diuresis were stable in kidneys with CI up to 48 h. After 72- and 96 h of CI, vascular resistance was increased while oxygen consumption and diuresis were reduced ($P < 0.05$). Glomerular filtration rate was diminished at CI greater than 24 h ($P < 0.05$). Results show that function was compromised with CI greater than 24 h and preceded the loss of cell viability following 48 h of CI.

Keywords Cold ischemia · Kidney · Preservation · Warm perfusion · Cold storage

Abbreviations ATP Adenosine triphosphate · CI Cold ischemia · DGF Delayed graft function · EMS Exsangiunous metabolic support

Introduction

Cold ischemia (CI) time, defined as the period between the initial cold perfusion of the kidney and reperfusion after reimplantation, is one of the best documented parameters in transplantation studies. The majority of all renal transplantation studies recognize the influence of CI time on posttransplant outcomes. Based upon these studies, there is general consensus that the longer the CI time, the greater is the extent of damage to a transplanted organ. In clinical renal transplantation, prolonged cold storage has been recognized to be strongly associated with delayed graft function (DGF) [4, 20, 21, 22, 23, 26, 31, 32]. Early effects of DGF include prolonged hospitalization, additional imaging studies, biopsies, dialysis, or inappropriate immunosuppression. Increases in costs and patient dissatisfaction are frequent consequences [12, 19]. In addition, an emerging body of evidence suggests that DGF may have detrimental effects on graft function and survival [25, 35]. But despite this large amount of information, the impact of CI as an independent marker on graft outcome is much less clear. It often has been difficult to separate injury produced by CI per se from that occurring during reperfusion. Reperfusion initiates a complex interplay between the endothelium, different types of blood cells, and resident leukocytes, adding to the injury obtained during CI.

The present study was designed to analyze CI-induced injury prior to actual reperfusion. We identified the effect of prolonged CI alone on renal metabolism and function using exsanguinous metabolic support (EMS) technology, an acellular ex-vivo warm perfusion technology, thereby eliminating the deleterious effects

of actual reperfusion. Following CI times varying from 24–96 h, oxidative metabolism, vascular dynamics and organ function were evaluated during EMS perfusion.

Materials and methods

Animal model

Bovine kidneys were harvested from healthy 7-day-old calves scheduled for slaughter. The kidneys, weighing 70–100 g, were excised immediately after the animals were killed via a captive bolt from a humane stunner to the cerebrum. The initial postmortem warm ischemic period was less than 15 min (mean). Following harvesting, a cannula was placed in the renal artery after which the kidneys were flushed (± 200 ml) and statically stored in UW preservation solution at 4 °C. CI times were 24-, 48-, 72-, and 96 h respectively ($n = 8$ in each group).

EMS technology

After the designated CI periods, the kidneys were re-flushed (± 200 ml) and transitioned to a pressure-controlled warm temperature perfusion (32 °C) system including an oxygenator and a pulsatile pump, retrofitted with controllers to maintain PaO_2, $PaCO_2$, pH, and temperature (Breonics, Schenectady, N. Y.). Both flush solution and perfusate consisted of a highly enriched tissue culture-like solution comprising more than 70 ingredients including amino acids, lipids, carbohydrates, ions, proteins, trophic factors, vasodilators, radical scavengers, and adenine compound substrates, adjusted to a pH of 7.4 (Breonics, Schenectady, N. Y.). The perfusate was supplemented with an oxygen carrier (perfluorocarbon emulsion) to provide adequate oxygen to support ongoing renal metabolism.

Evaluation of renal metabolism, function and vascular dynamics

Metabolism was assessed by measuring the renal oxygen consumption during EMS perfusion. PaO_2 analysis of pre-renal and post-renal samples were performed on a Radiometer ABL5 blood gas analyzer. Oxygen consumption (mm Hg/ml per min per 100 g) was calculated with the following formula:

Oxygen consumption = [(PaO_2 art-PaO_2ven) × flow rate]/ weight

Function was assessed by urine production (ml/min per 100 g) during EMS perfusion. Furthermore, creatinine was added to the recirculated perfusate as a tracer molecule at a starting concentration of 6 mg/dl. This allowed for urinalysis of tracer creatinine using an Ames Seralyzer III. GFR (ml/min per 100 g) was then calculated by the following method:

GFR = [(creatinine concentration urine) × (diuresis)] / (creatinine concentration perfusate)

Flow and pressure where monitored during warm perfusion and used to calculate vascular resistance:

vascular resistance = mean arterial pressure / mean flow rate

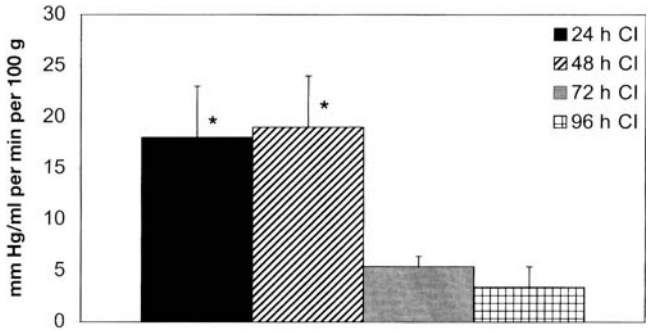

Fig. 1 Oxidative metabolism during EMS perfusion. * = $P < 0.05$ with respect to 72- and 96 h of CI

Histological evaluation

Following the 4 h perfusion period, wedge-shaped biopsies were taken and fixed in 4% neutral-buffered formalin, dehydrated, and paraffin-embedded. Four micron sections from each kidney were made and stained using haematoxylin and eosin for light macroscopic evaluation. The morphological characteristics of the kidneys (patchy lesions of swollen tubular cell cytoplasm, loss of tubular epithelium, necrosis of epithelial cells, and the presence of shed cells in the lumen) were determined by blinded histological evaluation

Data analysis

All reported values are the means along with the calculated standard error. For statistical analysis of differences in obtained data between the experimental groups the Student T-test was used. The level of significance was set at 5%.

Results

Oxidative metabolism (Fig. 1)

During the first 60 min of perfusion, the oxygen consumption of all kidneys stabilized and stayed constant during the remaining perfusion period. Following 4 h of EMS perfusion, oxygen consumption was found to be equal in kidneys cold stored for 24 h and 48 h (respectively 18 ± 5 and 19 ± 5 mm Hg/ml per min per 100 g). When the CI time was increased to 72 h, the oxygen consumption was markedly reduced (5.4 ± 1 mm Hg/ml per min per 100 g) and was further reduced at 96 h of CI (3.4 ± 2 mm Hg/ml per min per 100 g). The differences between kidneys cold stored up to 48 h and kidneys stored for a longer period reached statistical significance ($P < 0.05$).

Vascular dynamics (Fig. 2)

During EMS perfusion, the vascular resistance was equivalent in test groups exposed to CI up to 48 h (re-

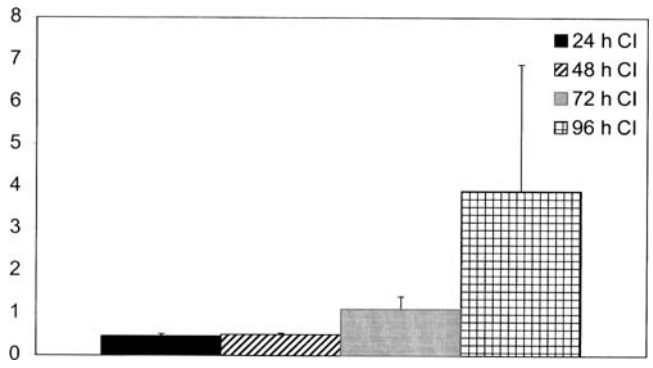

Fig. 2 Vascular resistance during EMS perfusion

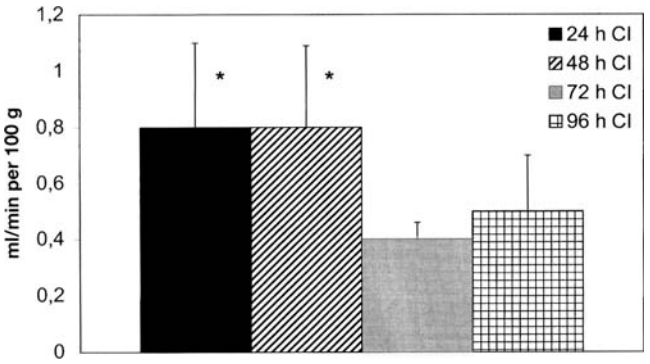

Fig. 3 Urine production during EMS perfusion. * = $P < 0.05$ with respect to 72- and 96 h of CI

spectively 0.46 ± 0.05 and 0.5 ± 0.03). With CI exposure of 72 h, the vascular resistance doubled, and again doubled when CI was increased to 96 h (1.1 ± 0.3 and 3.9 ± 3 respectively). However, none of these differences between groups reached statistical significance.

Organ function (Figs. 3 and 4)

The pattern of urine flow during EMS perfusion was very similar to that observed with oxygen consumption and vascular resistance. Again, urine flow was only compromised with CI times exceeding 48 h. The rate of urine production at 24 h and 48 h of CI measured both 0.8 ± 0.3 ml/min per 100 g. Much lower urine flow was seen at 72 h of CI (0.4 ± 0.06 ml/min per 100 g) and at 96 h of CI (0.5 ± 0.2 ml/min per 100 g). Urine flow after 72 h and 96 h of CI was significantly diminished, compared to kidney stored up to 48 h ($P < 0.05$). GFR was the only measured parameter by which it was possible to determine the effect of CI during the first 48 h of hypothermic preservation. CI exposure of 24 h provided a GFR of 4.2 ± 0.5 ml/min per 100 g. This dropped by 60% at 48 h of exposure to CI (1.6 ± 0.6 ml/min per 100 g) and by 85% at both 72- and 96 h of CI (0.5 ± 0.1 and 0.6 ± 0.4 respectively). Therefore, in contrast to the metabolic parameters, a significant reduction of GFR was seen after 24 h of CI ($P < 0.05$).

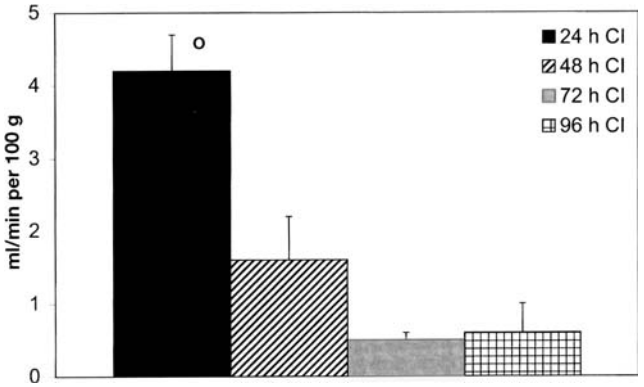

Fig. 4 Glomerular filtration rate during EMS perfusion. ° = $P < 0.05$ with respect to 48-, 72-, and 96 h of CI

Histological evaluation

Light microscopic analysis revealed no signs of vascular or glomerular damage in all kidneys. However the occurrence of extensive tubular necrosis (> 50% of tubules affected) was observed at time points of CI exceeding 48 h, the severity depending on the duration of the CI period.

Discussion

There are two areas in transplantation where improvements must be made. One is an understanding of the changes that take place during the period of CI; the other is an understanding of the injurious events that are the result of reperfusion injury. CI causes damage through a gradual process in which biochemical alterations lead to functional impairment, followed by structural changes and ultimately cell death [27]. Reperfusion injury is an additional hazard contributing to organ damage upon revascularazation after CI. When blood flow is restored, oxygen influx leads to the formation of active free radicals that, by modulating leukocyte-endothelial cells interactions, produce further cellular-, membrane- and microvascular injury [6, 7, 13].

The aim of this study was to determine the effects of CI on restored ex-vivo kidney metabolism and function prior to reperfusion. Restoration of renal metabolism and function was achieved by ex-vivo warm perfusion, using EMS technology. The perfusate used during ex-vivo warm perfusion was designed to support the nutritional and metabolic needs of the vascular endothelium

within a graft, thereby maintaining the integrity of the vasculature and subsequently the normal permeability of the organ. Inhibition of radical oxygen species formation by addition of scavengers, together with the absence of inflammatory cells in the EMS perfusate was thought to eliminate much of the compounding damage seen during actual reperfusion with blood.

Effect of CI on oxidative metabolism

Renal cell viability was compromised after CI times exceeding 48 h. The reduced capability to restore metabolism observed after prolonged CI has its origin in the negative effects of ischemia. During ischemia, cell metabolism continues, resulting in decreasing adenosine triphosphate (ATP) levels. The loss of ATP may be a critical event in initiating the cascade of events leading to cell death. Cells can certainly tolerate very low concentrations of ATP for a long period, however loss of ATP causes loss of control over metabolism with inhibition of ATP-dependent reactions while catabolic reactions continue. ATP is also necessary for the maintenance of the integrity of membranes. One of the most ATP sensitive cell functions is the sodium and water homeostasis via the sodium-potassium pump. As this pump fails, due to lack of ATP, cellular swelling and eventually rupture can occur [8, 16]. Therefore, although hypothermia reduces the kidney's metabolic demands for nutrients and oxygen, thereby extending the period during which anoxic tissue stays viable, the result is merely a change in the processes leading to cell death [27].

Effect of CI on organ function

Loss of renal function occurred sooner as compared to cell viability, with a marked deterioration of GFR by 48 h of CI ($P < 0.05$). An explanation for the more rapid loss of function might be the increased susceptibility of the proximal tubule cells to ischemic injury. In the literature, these cells have been described as the primary cause for the pathophysiological and clinical aspects of ischemic acute renal failure [29, 30]. Ischemia leads to a loss of surface membrane polarity and the integrity of the tight junction. Integins are redistributed towards the apical surface [5], and dead and live cells slough into the tubular lumen, thereby contributing to cast formation [24]. The cast causes increased intratubular pressure and a reduced GFR. Loss of the epithelial cell barrier and of the tight junctions between viable cells can result in back leakage of the GFR, further reducing effective GFR.

Based upon the results, static cold storage seems to be a method of limited utility for high quality, long term organ preservation. This seems to be due to an exhaustion of nutrients and an accumulation of waste products during the period of preservation [15]. Improved preservation could be obtained if catabolism were suppressed and anabolic metabolism stimulated. This has been accomplished by both hypothermic machine perfusion [1] and intermittent normothermic perfusion [14, 33, 34]. During machine perfusion, a cold perfusate is pumped through the organ at low pressure simulating metabolism by supplying oxygen and nutrients and removing metabolic end products. This method appears to give a good quality of long preservation for kidneys [11, 17, 18]. Kootstra's group demonstrated that intermittent perfusion with normothermic blood in the course of cold preservation extended the preservation times significantly. It was postulated that the beneficial effect of this intermittent perfusion could be attributed to a washout of metabolic waste products, and a restoration of exhausted enzyme systems and energy sources. Nevertheless, none of the two described preservation methods has been able to play a dominant role in clinical organ preservation.

Another approach to stimulate anabolic metabolism during preservation in order to prolong adequate preservation time, could be the perfusion of organs at warmer temperatures [28]. In an environment where renal metabolism is continuously supported instead of inhibited, and waste products are removed, the limitations of static storage may be avoided. In this study we used a limited period of warm perfusion with EMS technology as a tool to identify the deleterious effects of CI on renal metabolism and function. Our next goal is to apply EMS technology to successfully perfuse organs over a longer period of time, thereby eliminating cold preservation as a whole. Obviously obstacles will need to be overcome before warm perfusion preservation can become a reality. Contamination and long term metabolic support are just a few of the areas which will need to be addressed. Nevertheless there is growing evidence postulating the feasibility of warm temperature organ preservation [2, 3, 9, 10].

The present study focused on the impact of CI on the kidney prior to reperfusion. A better understanding of this injury may facilitate in the development of more effective preservation technologies designed to minimize the ischemic damage. Using EMS perfusion, we were able to observe a loss of function after 24 h of CI and a reduction of cell viability after 48 h. With the clinically available cold storage techniques, the currently held view in clinical transplantation of attempting to routinely limit CI to 24 h appears to be justified.

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