The Effect of Machine Perfusion on the Arteries of Porcine Kidneys

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Objectives: **Machine perfusion is an excellent method of assessing the viability of a kidney graft and can also potentially improve the quality of an equivocal kidney. Several authors have expressed concerns that machine perfusion can potentially damage the vessels of the kidney but until now, no studies have been performed to clarify this issue. We aimed to examine the effect of machine perfusion on the renal arteries of porcine kidneys.**

Materials and Methods: **Eight pairs of kidneys were removed from pigs in the abattoir. One kidney of each pair was preserved on ice for 24 hours. The other kidney from the same animal was initially stored on ice until arrival at the laboratory when it was perfused on the RM3 machine for 4 hours and then stored again on ice for the remainder of the 24 hours. After 24 hours, since the retrieval and initial storage on ice at the abattoir, tissue samples were obtained from all renal arteries at 3 different sites. These samples were sent for histologic evaluation.**

Results: **Machine perfusion caused more damage at a statistically significant level compared with simple cold storage only for the first sample site, which was the part of the renal artery closest to the perfusion cannula.**

Conclusions: **Our experiments suggest that machine perfusion, even when it is done lege artis, can damage the part of the renal artery closest to the adaptor, which can potentially result in a higher incidence of posttransplant arterial thrombosis. Therefore, excision of the first part of the renal artery should be considered prior to transplantation, and modifications of the perfusion technique must be developed to minimize damage to the renal arteries.**

Key words: *Renal artery damage, renal perfusion, renal preservation, renal transplantation, non–heart-beating donor*

During the last 10 years, there has been continual increase of the number of patients with end-stage renal failure on waiting lists for a kidney transplant and a continuous decrease of cadaveric donors [1, 2]. This trend has been seen in the United States and in Europe, has led to a dramatic shortage of organs for transplantation, and has initiated a search for other potential sources of organs. Several centers have renewed their interest in the use of non–heart-beating donors [2, 3] to increase the donor pool and face the crisis of organ shortage. The operation of kidney transplantation from non–heart-beating donors is technically very successful with the same low rate of technical complications as heart-beating donors; however, there is an increased rate of delayed graft function of up to 80% [4, 5] and most importantly, a higher incidence of primary nonfunction, which can be as high as 15% [5].

Clearly, it is paramount to develop a method of assessing the viability of a kidney to minimize the probability of implanting an organ that will never work. The viability of a kidney can be assessed in several ways [6, 7]. Donor history [8, 9], macroscopic evaluation of the graft, and microscopic evaluation of biopsies [7] are factors that have been used to assess kidney viability. For machine perfusion, the kidneys are connected via the renal artery to the machine and are perfused by means of a pulsatile pump using a preservation solution. This allows monitoring of flow, resistance, and pressure in the

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intraparenchymal vessels over time and therefore offers a more objective way of assessing the viability of a kidney graft [7]. In addition, during the perfusion, clots are cleared, and free radicals, which are released during the period of warm ischemia, can be washed away. The American Waters Medical RM3 renal perfusion machine (Waters Medical Systems, Rochester, MN, USA) is an improved version of Belzer's machine and is currently the only commercially available perfusion system; however, several centers use alternative home-made perfusion systems [10].

Although machine perfusion has been shown to improve the quality of non–heart-beating donor kidneys, reduce the delayed graft function rate, and be an important tool in testing the viability of a kidney [11-14], several authors have also warned that machine perfusion in itself can potentially be damaging to the kidney [15, 16]. One unanswered question so far is the potential damage that machine perfusion can cause to the renal arteries. The aim of this study was to investigate this issue.

Materials and Methods

The kidneys used for this study were obtained from pigs slaughtered in an abattoir. In a clinical setting, these kidneys would be category III non–heart-beating donors according to the Maastricht classification [3, 6, 7, 17-19] (ie, the patient is residing in the intensive care unit, the emergency room, or another ward; any cardio-respiratory support is withdrawn; cardiac arrest is anticipated; and when it occurs, the retrieval of the organs takes place). The category III donors are the vast majority of non–heart-beating donors.

Because of the method of processing the pigs in the abattoir, it was impossible to determine exactly the time between death by exsanguination and retrieval of the organs (warm ischemia time). We estimated that, on average, it took about 15 to 20 minutes to process a pig through the chain of events and receive the kidneys en block with the aorta and vena cava.

As soon as the kidneys had been retrieved, they were transferred to the bench and placed in a kidney dish filled with ice and Eurocollins solution (Soltran, Baxter, Compton, UK) (pairs 1-4) or University of Wisconsin solution (Belzer MPS) (pairs 5-8). Both perfusion solutions are commonly used in clinical practice. A 20-F Foley catheter was inserted in the aorta above the iliac arteries and was kept in position with a silk tie. The aorta was tied off proximally above the renal arteries with a silk tie, and the tributaries were ligated. Perfusate (500 mL) (Eurocollins for pairs 1-4 and UW for 5-8) was then allowed to pass through the Foley catheter and into the aorta and renal arteries with hydrostatic pressure. The bag of perfusate was fastened to the wall about 1 m above the bench. Perfusion was confirmed when blood-stained fluid was noticed draining from the vena cava. Perfusion via the aorta and not directly via the renal arteries was used to avoid any damage to the renal arteries, which would bias the study. After perfusion, the kidneys were separated from the aorta and vena cava. The arteries were preserved with a cuff of aortic tissue, but the veins were cut close to the renal pelvis. Both kidneys of each pair were placed together in a plastic bag filled with perfusion solution (Eurocollins for pairs 1-4 and UW for 5-8) and were preserved on ice for transport to the laboratory. Placement of the kidneys on ice at the abattoir was the beginning of the total of 24 hours of simple cold storage or cold storage plus machine perfusion prior to obtaining samples for histology studies from the renal arteries.

On arrival to the laboratory, one kidney of each pair was removed from the bag, while the other was kept on ice. The kidney that had been removed from ice was then attached to the RM3 perfusion machine (Waters Medical Systems, Rochester, MN, USA). A plastic adaptor (Henleys Medical Supplies Ltd, Hertfordshire, UK) was inserted in the renal artery, and the artery was tied to the adaptor as close as possible to the aortic patch to limit immediate damage to the renal artery. The machine was operated under standard settings. Priming was done with 600 mL of perfusion solution (Eurocollins for pairs 1-4 and University of Wisconsin solution for 5-8). The solution was allowed to pass through the kidney with a pressure of about 40 mm Hg. The temperature of the perfusate was maintained below 10°C, and the pulses per minute were kept around 60. The flow volume (mL/min) and resistance (mm Hg/mL/min) in the kidney were documented at 30-minute intervals. Perfusion was maintained for 4 hours, similar to clinical practice, after which the kidney was removed from the machine and stored on ice for the remainder of 24 hours since the initial placement on ice at the abattoir.

Twenty-four hours after the initial placement on ice, samples (full-thickness rings approximately 15

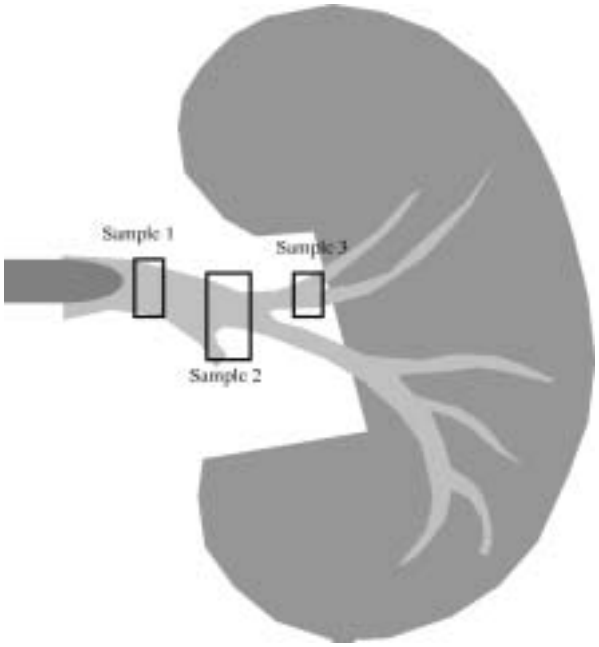

Figure 1. This figure is the graphic representation of the 3 sample sites. Sample 1 was taken just distally to the adaptor, sample 2 was taken at the first bifurcation of the renal artery, and sample 3 was taken at the next divergence of the renal artery near the renal pelvis.

mm long) were taken from the renal arteries of all kidneys at 3 sites (Figure 1). In the case of machine perfusion, sample 1 was taken away from the adaptor, nearer to the renal pelvis to avoid bias in the results owing to adaptor-related damage. Sample 2 was taken at the first bifurcation of the renal artery, and sample 3 was taken at the second divergence of the renal artery. The samples were preserved in 10% formaldehyde, and they were sent to researchers, blinded to the study's parameters, for histologic evaluation. The samples were evaluated microscopically after Hematoxylin and Eosin staining. The damage to the arteries was graded from 0 to 3.

0 = **no** disruption or edema of the endothelial cells
1 = **mild** disruption and edema of the endothelial cells
2 = **moderate** disruption and edema of the endothelial cells
3 = **severe** disruption and edema of the endothelial cells

Fisher's exact test was used to compare damaged and nondamaged arteries. The Wilcoxon signed rank test was used to compare test results for each group. Values for P less than .05 were considered statistically significant.

Results

All kidneys showed excellent perfusion characteristics while on the RM3 machine. Although flow patterns varied, the flow remained sufficient, and the resistance (mm Hg/mL/min) remained low in all perfused kidneys. Flow is usually measured in mL/min/100 g tissue. In our setting, we did not weigh the kidneys. A pig kidney weighs between 80 and 180 g. To calculate the flow, we used the maximum weight of 180 g for each kidney and the lowest flow measurement during perfusion. Talbot and coworkers use a flow rate of 25 mL per minute per 100 g kidney tissue as the cutoff rate during viability testing with machine perfusion [16]. To calculate the perfusion flow index, we used the maximum perfusion pressure recorded during perfusion for each kidney. In using these measurements for calculations, we obtain the worst values possible. A kidney with a perfusion flow index greater than 0.4 mL per minute per 100-g kidney / renal artery systolic pressure is considered to be viable and thus suitable for transplantation [16]. The flow in all of the kidneys remained above 25 mL per minute per 100-g kidney tissue, and the perfusion flow index remained above 0.4 mL per minute per 100-g kidney/renal artery systolic pressure (Table 1). According to these results, these kidneys would all be considered suitable for transplantation in a clinical setting.

Table 1. Flow and PFI for each machine perfused kidney. Kidney 2 did not produce any results owing to a faulty probe.

	Flow (mL/min/100 g)	Index (mL/min/100 g/mm Hg)
Kidney 1	61.666	1.312
Kidney 3	58.333	1.356
Kidney 4	33.888	0.788
Kidney 5	56.111	1.335
Kidney 6	75.555	1.798
Kidney 7	71.111	1.616
Kidney 8	60.555	1.376

PFI: Perfusion flow index

The histologic results are shown in Table 2. There was a significant difference between machine perfusion and cold storage for sample site 1, P = .023. There was no statistically significant difference between machine perfusion and cold storage in sample sites 2 and 3, P = .655 and P = .257 respectively. There also was no statistically significant difference (P = .194) when the sample means of

Table 2. Histologic results according to grade of damage (0 = none, 1 = mild, 2 = moderate, and 3 = severe endothelial disruption and edema) of each sample site and each kidney.
*The renal artery of kidney 5 in the cold storage arm was damaged during retrieval of the organs in the abattoir, and this kidney was therefore excluded.

	Kidney pair 1	Kidney pair 2	Kidney pair 3	Kidney pair 4	Kidney pair 5	Kidney pair 6	Kidney pair 7	Kidney pair 8
Machine Perfusion								
Sample 1	2	2	2	1	1	1	1	0
Sample 2	1	1	1	0	2	0	1	0
Sample 3	0	0	2	1	0	1	0	0
Cold Storage								
Sample 1	2	0	0	0	*	0	0	0
Sample 2	2	0	1	1	*	1	1	0
Sample 3	0	0	0	0	*	0	0	0

damage for kidney pairs 1-4 (perfused with Eurocollins) were compared with the sample means for kidneys 5-8 (perfused with UW). Microscopic views of the different grades of damage to the renal arteries can be seen in Figure 2.

Discussion

Donor and organ shortages are major problems in modern transplantation. The acceptance of non–heart-beating donors is one possible way to deal with the problem. It is estimated that fully developed non–heart-beating donor programs can increase the available kidneys for transplantation between 20% and 40% [20]. However, kidneys retrieved from non–heart-beating donors endure a period of warm ischemia, which can potentially damage them significantly. It is therefore of the greatest importance to assess the viability of the kidneys prior to transplantation, and machine perfusion seems to be the most objective way to predict which kidneys, if they get transplanted, might not function. The possibility that machine perfusion can harm the renal arteries, something that could potentially lead to technical failure, is one of the reasons for the reluctance of many centers to develop non–heart-beating donor programs using kidneys tested with machine perfusion.

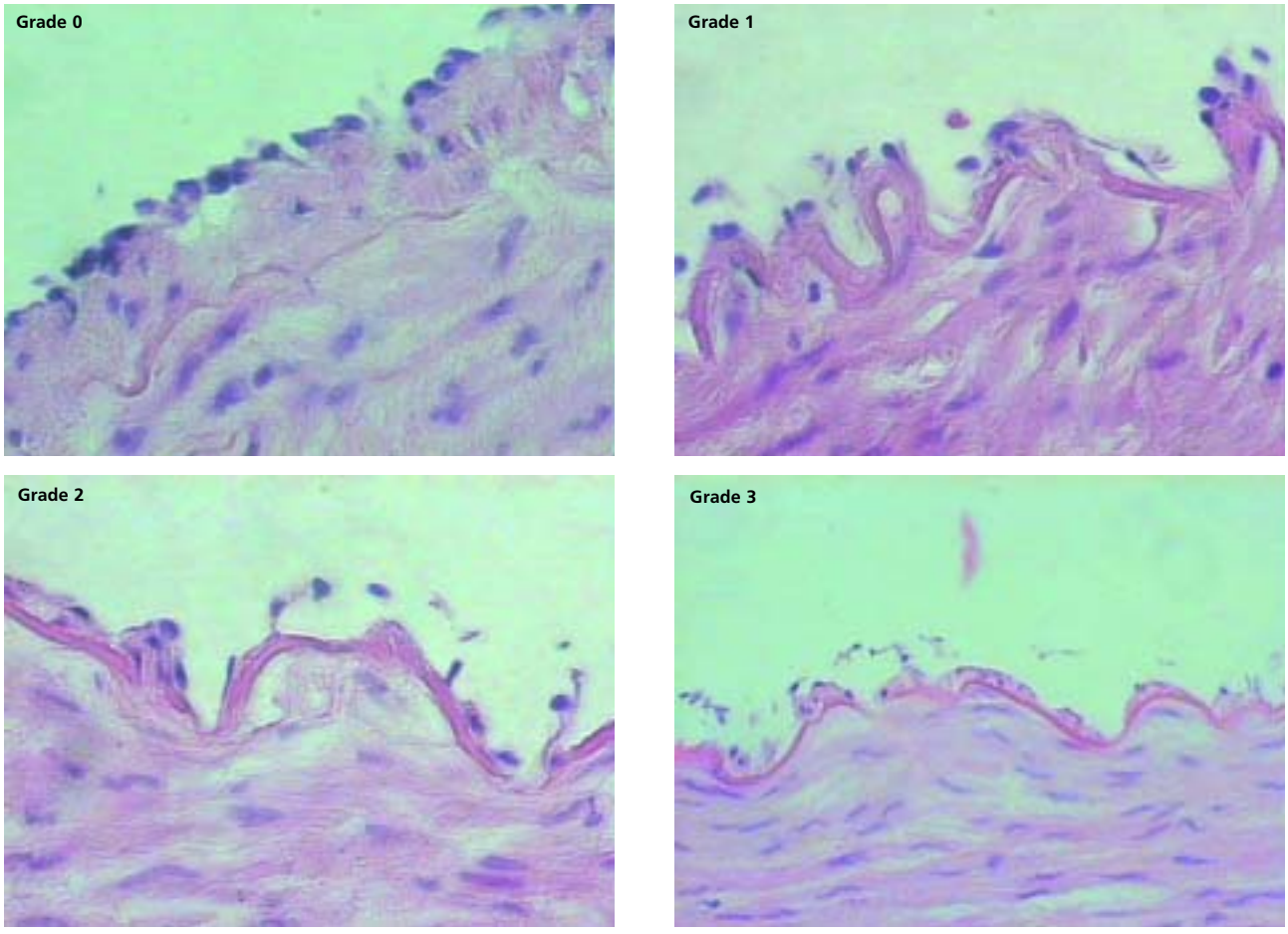

Figure 2. Microscopic views of each grade (0 = none, 1 = mild, 2 = moderate, 3 = severe) of damage. Samples were stained with Hematoxylin and Eosin.

The aim of this study was to investigate the effect of machine perfusion on the renal arteries using a model, which is as close as possible to the clinical setting. We selected pigs because they are large animals, and their kidneys have a similar size and anatomy compared with humans. This makes them ideal to use for perfusion on the RM3 perfusion machine, which is used in human organ transplantation. In addition, all the animals were young and healthy and therefore, the renal arteries did not have atheromatous and hypertensive changes, which could be a bias for the study. The way the pigs die in the abattoir is similar to the circumstances of category III non–heart-beating donors, which represents the vast majority of non–heart-beating donors. In this category, cardiac arrest is anticipated, the patient dies, and after an interval of 10 minutes, in situ cooling is initiated.

The retrieval, initial cooling, and storage on ice of the porcine kidneys was done in a way that was almost identical to the clinical practice, and this was also the case for machine perfusion in the laboratory using the RM3 machine [16]. The average cold ischemia time in kidney transplantation without machine perfusion is around 24 hours. In addition, centers that perform machine perfusion do not usually transplant the kidneys immediately after the completion of machine perfusion and this, most of the times, is due to the lack of availability of operating theatres. The kidneys are stored on ice after machine perfusion, and the total cold ischemia time is again around 24 hours [21]. Therefore, the fact that the kidneys in our experiments were stored (either simple cold storage or machine perfusion + cold storage) for 24 hours was also very similar to what happens in clinical practice. The majority of the kidneys that were perfused on the RM3 machine did well although some showed relatively reduced flow near the end of the machine perfusion, which in case of human kidneys, could affect the final decision for their clinical use. Nevertheless, all the values were above the cutoff standards and therefore, if in a clinical setting, all these kidneys would be viable for transplantation.

Histopathological study of the renal arteries showed that the difference between machine perfusion and cold storage was significant only for the first sample, the one closest to the adaptor. This indicates that machine perfusion perhaps causes endothelial injury in the first part of the renal artery. This finding in combination with the fact that non–heart-beating donors are often elderly donors with atherosclerosis and therefore, additional problems with their arteries might indicate that in a clinical setting, it would probably be advisable to cut the potentially damaged part of the artery prior to transplantation. This will decrease the chances of dissection, aneurism formation, or occlusion due to thrombosis, which invariably results in loss of the graft.

Although it is not clear why machine perfusion can damage the renal arteries, some possible explanations are as follows:

- Although the adaptor used was the same as the one used in clinical practice, and care was taken to obtain samples away from the adaptor, it is possible that the shape of the adaptor could be the source of the damage to the renal arteries. It is therefore important to test adaptors with another shape or a smoother surface.
- The way the artery rests in the machine during perfusion and the length of the artery allow for slight kinking in the area where the artery is attached to the machine. This might be responsible for narrowing, increased speed of the flow, and increased friction at that site and thus increased damage to the artery (Bernoulli's law). In future experiments, the artery could be shortened and the kidney positioned in such way that kinking of the artery during machine perfusion would be avoided.
- The pulsatile flow of the machine has a higher pressure at the site of entrance than it does further down the tributaries, which might contribute to proximal renal artery damage. A different machine might be able to provide a continuous flow as opposed to the pulsatile flow of the RM3 machine and potentially avoid the damage to the renal arteries.

Our study demonstrates that although machine perfusion is the best way to determine the viability of kidneys from non–heart-beating donors, its use could be associated with damage to the renal arteries. Since kidneys from non–heart-beating donors are a vital source of organs, it is important to investigate further other potential problems of machine perfusion and try to introduce possible improvements of its application in clinical practice.

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