

The Viability of Liver Graft for Transplantation After Prolonged Warm Ischaemia

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Abstract

Maximum duration of warm ischaemia within which the liver graft is viable for transplantation remains undefined. Published data on porcine allogeneic liver transplantation (LTx) using non heart beating donors (NHBDs) are conflicting because technical details like the hepatic artery status, systemic heparinisation of donor animals and duration of rewarming were not addressed. We described a novel porcine model which simulate conditions of transplantation from NHBDs. The pigs were divided into three groups of 6 each. Groups I, II and III were subjected to 60, 90 and 120 minutes of warm ischaemia, respectively. Liver viability was assessed using four parameters: serum liver function tests (serum bilirubin and transaminase), dynamic liver function test i.e. the monoethylglycinexylidide (MEGX) formation test, morphological assessment and animal survival. All animals in groups I and II (90 minutes of warm ischaemia or less) survived but 50% of animals in group III died of massive liver failure. Given that rewarming period required in actual allogeneic LTx is about 60 minutes, the safe period for intervention in NHBDs is determined to be about 30 minutes. Allogeneic porcine LTx using NHBDs with 30 minutes of cardiac arrest were performed in 5 animals. All of them survived.

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Key words: Allogeneic transplantation, Cold ischaemia, Non heart beating donor, Porcine model

Introduction

Liver transplantation (LTx) is the treatment of choice for many patients with end stage liver disease.¹ However liver allograft availability prevents its widespread application. Non heart beating donors (NHBDs) are a potential source of donors. The main problem with the utilisation of liver grafts from this subset of donors is the susceptibility of the liver to ischaemic injury. Maximum duration of warm ischaemia within which the liver graft is viable for transplantation remains undefined. Published data on porcine allogeneic LTx using NHBDs are conflicting. Hoshino et al^{2,3} and Kim et al⁴ reported no survivors in recipients of allografts from NHBDs subjected to 5 to 20 minutes of warm ischaemia. Takada et al⁵ found that liver allografts remain suitable for transplantation up to 60 minutes of warm ischaemia. LTx is a technically demanding procedure involving multiple anastomosis. The hepatic artery anastomosis is

especially crucial and results in failure of transplantation if thrombosed. The status of the hepatic artery was not described in these papers. In two reports,^{4,5} systemic heparinisation was given to the donor prior to induction of cardiac arrest. Rewarming time which is the time taken for completion of anastomosis, is a significant factor in graft injury.^{6,7} These factors which were not addressed accounted for the wide discrepancies observed in the previous studies.

We describe a novel porcine model which simulate conditions of transplantation from NHBDs. The duration for intervention in NHBDs was then determined and porcine allogeneic LTx were then performed to validate the findings.

Materials and Methods

The Model (Fig. 1)

A porcine animal model was used. Warm ischaemia

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was induced by total vascular exclusion of the liver i.e. portal vein clamping, ligation of hepatic artery and supra and infra hepatic inferior vena cava (IVC) clamping. Systemic heparin was not given until total vascular exclusion was secured and confirmed on colour Doppler duplex ultrasound. Portal and systemic decompression during total vascular exclusion was achieved via venovenous bypass into the internal jugular vein using a roller pump. The liver was subjected to warm ischaemia *in situ*. The core liver temperature was maintained at 35°C to 37°C with the use of warming blanket, moist warm abdominal packs and overhead warmer. At the end of warm ischaemia, the liver was packed and insulated from surrounding organs and cold perfused *in situ* with 1 L of 4°C Ringer's lactate. Cold ischaemia was maintained *in situ* by packing the liver with Ringer's lactate ice sludge for 30 minutes. With this method the core liver temperature was maintained at 4°C to 8°C. Prior to revascularisation the liver was rinsed with 500 ml of warm Ringer's lactate. Portal vein and infrahepatic IVC were repaired. Suprahepatic IVC was unclamped and hepatic artery ligatures were released, allowing for a rewarming period of 10 minutes. Liver viability was assessed using four parameters: serum liver function tests (serum bilirubin and transaminase), dynamic liver function test (monoethylglycinexylidide (MEGX) formation test), morphological assessment and animal survival.

Animals and Experimental Procedure

Eighteen Yorkshire pigs weighing 35 to 40 kg were used. The animals were handled according to the institution's guidelines for handling and care of laboratory animals.⁸ They were fasted for 48 hours prior to surgery but allowed to have water. Pigs were sedated with intramuscular ketamine (Ketapex, Apex Lab Pte Ltd, St Marys, NSW, Australia) 20 mg/kg. They were weighed, anaesthetised with intravenous pentobarbitone (Nembutal, Sanofi, Cedex, France) 10 to 12 mg/kg, intubated and maintained on spontaneous ventilation with supplemental oxygen. Anaesthesia was maintained with isoflurane 1% to 2%. Orogastric tube was inserted for gastric decompression. Warming blanket was used. Non invasive blood pressure via a tail cuff (Hewlett Packard neonatal cuff #1) and continuous electrocardiography were monitored. Central line inserted into the internal jugular vein for fluid therapy. Intravenous amoxicillin 250 mg and cloxacillin 250 mg were given.

Laparotomy was via midline incision under aseptic conditions. Bowels packed to the left. Infrahepatic IVC exposed, mobilised and controlled. Portal vein isolated and skeletonised of its overlying lymphatics and small vessels and mobilised proximally to the level of the splenic vein and distally to its bifurcation. All its other tributaries were ligated and divided. Hepatic artery was

traced proximally to its common hepatic branch and distally to its division into the left and right branches. Gastroduodenal artery and hepatic communications with the left gastric artery were ligated. Gastrohepatic ligament was completely divided to ensure that any accessory communications with the hepatic artery was ligated. Cystic artery was ligated and divided. Bile duct was skeletonised. The suprahepatic IVC was then isolated and controlled.

Total hepatic exclusion was then commenced by double ligating the hepatic artery, clamping the portal vein and the infrahepatic IVC above the renal veins. The suprahepatic IVC was then cross-clamped. Total vascular exclusion was confirmed by colour Doppler ultrasound (Acuson 128XP/10). Intravenous heparin 200 iu/kg was given only after total vascular exclusion of the liver. Porto-caval to internal jugular veno-veno bypass was then commenced at rate of 25 to 30 ml/kg/min using a roller pump (Sarns 8000, 3M Ann Arbor, Michigan, USA). During the anhepatic phase isoflurane was reduced to 0.5% and 50 ml of 8.4% NaHCO₃ given intravenously.

Core liver temperature was monitored (Hewlett Packard Temperature Probe 21075A). It was maintained at 35°C to 37°C with the use of a warming blanket, moist warm abdominal packs and an overhead warmer (Vickers Medical, Model 183, England). At the end of the warm ischaemic period the liver was cold perfused with 1 L of 4°C Ringer's lactate via the portal vein and vented through an opening made in the infrahepatic IVC (Fig. 1). The liver was subjected to 30 minutes of cold ischaemia *in situ*. Core liver temperature was kept at 4°C to 8°C by insulating the liver from surrounding organs and covering it with Ringer's lactate ice sludge. Prior to revascularisation the liver was flushed with 500 ml of warm (38°C) Ringer's lactate. Portal vein and infrahepatic IVC were repaired with polypropylene 5/0 sutures. Hepatic artery ligatures removed and pulsation checked. Rewarming period was kept to 10 minutes. Anticoagulation was reversed with protamine sulphate 2 mg/kg.

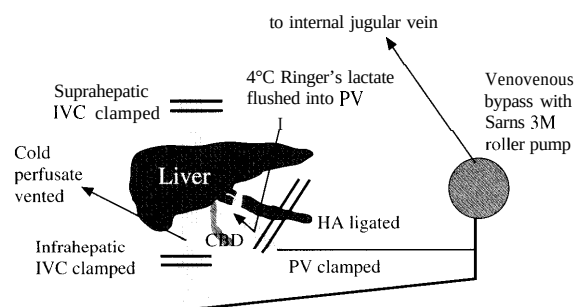


Fig. 1. The circuitry of the porcine model used to simulate conditions in NHBDS. CBD: common bile duct; HA: hepatic artery; IVC: inferior vena cava; PV: portal vein

Postoperatively they were given intravenously 40 ml dextrose 50% and maintained on intravenous dextrose-saline solution 1 L/day until they were able to take it orally.

Experimental Groups

The pigs were divided into three groups of 6 each. Groups I, II and III were subjected to 60, 90 and 120 minutes of warm ischaemia respectively. All the animals were subjected to 30 minutes of cold ischaemia and rewarming was kept to 10 minutes.

Parameters

Baseline blood specimens were taken for liver function tests. MEGX test was performed using intravenous 1 mg/kg lignocaine (Delta West Pty Ltd, Bentley, West Australia) with blood samples taken at 0 and 15 minutes. Assays were measured by fluorescence polarisation immunoassay (TDx, Abbott Diagnostics Division, Abbott Park, IL, USA). Liver biopsy was taken prior to ischaemia. Blood samples for aspartate transaminase (AST), alanine transaminase (ALT) and bilirubin were collected at the end of warm ischaemia, 1 hour post revascularisation, the first, second, fourth and eighth postoperative days. MEGX test was repeated on the first, second, fourth and eighth postoperative days.

Survival for 8 days after the operation was considered successful. On the eighth day, the animals were sacrificed and the liver examined grossly and histologically. Autopsy was performed immediately if the animal died prior to the eighth day. Gross specimens were examined for extent of necrosis. Multiple biopsies were taken from different lobes and grossly abnormal areas. These were fixed in 10% formalin, processed in paraffin wax, sectioned at 3 µm and stained with haematoxylin and eosin for light microscopy. The slides were then reviewed by a pathologist who was blinded to the different groups. Assessment of ischaemic injury was based on three parameters: cellular vacuolation, sinusoidal dilatation and necrosis. Cellular vacuolation and sinusoidal dilatation was categorised to none/mild, moderate or severe/extensive changes. Necrosis was categorised into none/minimal, patchy or frank necrosis.

Statistics

Values are expressed as median (range). Statistical analysis was performed with the SPSS 6.0 program. Differences among groups were analysed using Chi-square test for categorical data, Kruskal-Wallis and Mann-Whitney U test for continuous data.

Porcine Allogeneic LTx using NHBDs

Duration for intervention in NHBDs was calculated as follows:

Safe duration for intervention in NHBDs = Maximum tolerable warm ischaemia – Rewarming time

In our experience the rewarming time required was 60 minutes. To validate the findings in the model, allogeneic LTx was then performed on 5 pigs using NHBDs. Five pairs of Yorkshire pigs between 40 and 55 kg were used. Donors and recipients were litter mates. Donors were anaesthetised as described above. Midline laparotomy was performed. One unit of blood was collected from infrarenal aorta into acid citrate dextrose bags for later use in recipients. Warm ischaemia induced by cross clamping the supraceliac aorta and intracardiac injection of 10 ml of 7.45% KCl to induce cardiac arrest. After 30 minutes of warm ischaemia the liver was cold perfused using the standard flush technique² via the portal vein and aorta. One litre of Ringer's lactate were flushed through the portal vein and aortic cannula separately followed by 2 litres of University Wisconsin (UW) solution (Viaspan, Du Pont Pharmaceuticals, Delaware, USA) into the portal vein. The liver was procured. After 3 hours of cold preservation in UW solution, they were implanted into the recipients using standard orthotopic transplantation technique.⁹ Veno-veno bypass with a roller pump was used (Sarns 8000, 3M Ann Arbor, Michigan, USA) at rate of 25 to 30 ml/kg/min. Vascular anastomoses were hand sutured incorporating growth factor with polypropylene sutures. Biliary anastomosis was hand sutured over a stent. Survival more than 4 days were considered successful.

Results

One animal from group II was excluded from analysis because the hepatic artery was lacerated during the operation.

Survival

All animals in groups I and II survived (Table I). Postoperatively animals in groups I and II recovered well. They were able to drink on the first day and all were able to stand and feed by the second day. Group III fared significantly worse. Half of the animals in group III died. At post mortem there was evidence of extensive massive liver necrosis involving all lobes of the livers. All 3 animals died of liver failure.

TABLE I: SURVIVAL RATES OF ANIMALS IN THE MODEL SIMULATING CONDITIONS IN NHBDs

	Duration of warm ischaemia (min)	n	No. of animals alive on day 8	Survival*
Group I	60	6	6	100%
Group II	90	5	5	100%
Group III	120	6	3	50%

* $P=0.035$

Biochemistry

Serum bilirubin levels (Fig. 2) were raised in groups II and III on the first postoperative day but soon returned to baseline levels by the fourth day. Group I did not show any significant rise. Median level in group III on the first postoperative day was significantly raised compared to those in group I. The rise in group III from baseline median level of 7.0 $\mu\text{mol/l}$ (range 2 to 12) to 25.0 $\mu\text{mol/l}$ (range 7 to 46) was greater in magnitude compared to that in group II which increased from median level of 5.5 $\mu\text{mol/l}$ (range 3 to 9) to 11 $\mu\text{mol/l}$ (range 3 to 25) but this did not reach statistical significance.

Serum transaminases levels (Fig. 3) were markedly elevated reaching its peak on the first postoperative day in all groups. Animals that survived in group III and all animals in groups I and II showed recovery of transami-

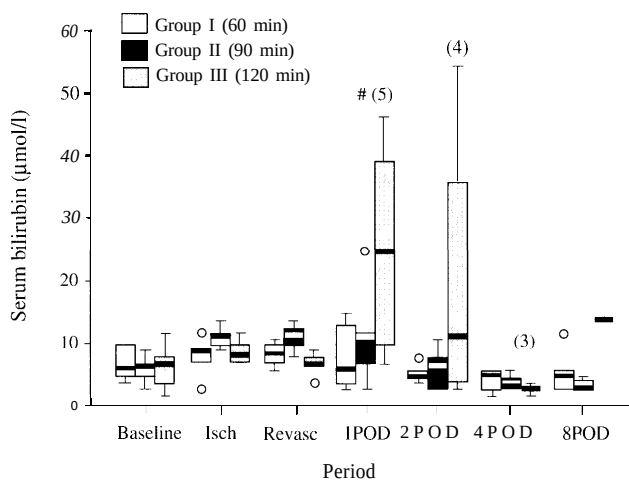


Fig. 2. Serum bilirubin levels.

Isch: post warm ischaemia; POD: postoperative day; Revasc: 1 hour post revascularisation.

$P = 0.0547$ (Mann-Whitney U) between group I and group III. Numbers of animals in group III which survived indicated in ().

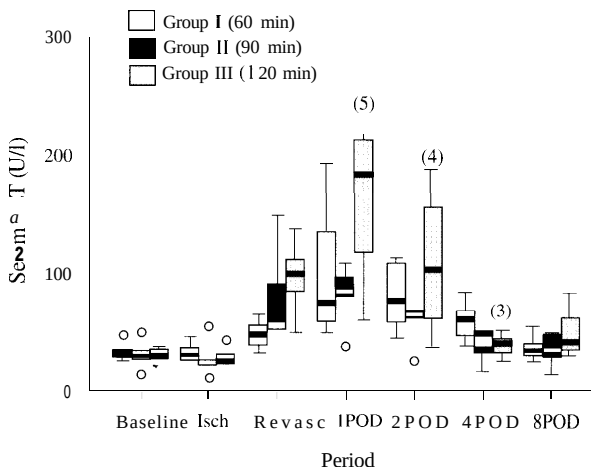


Fig. 3. Serum ALT levels.

Isch: post warm ischaemia; POD: postoperative day; Revasc: 1 hour post revascularisation. Numbers of animals in group III which survived indicated in ().

nase levels to normal values between fourth and eighth postoperative day.

MEGX Test

Baseline median levels for MEGX (Fig. 4) in groups I, II and III were 51 ng/ml (range 20 to 90), 27 ng/ml (range 17 to 73), and 33 ng/ml (range 17 to 39), respectively. This rapidly deteriorated on the first day and recovered to reach their respective baseline values by the eighth day among the animals that survived. This trend of improvement in liver metabolism of lignocaine correlates with the improvement in the clinical condition of the animal and the biochemical profile.

Morphological Studies

In both groups I and II, no necrosis was seen at sacrifice on the eighth day. There was no evidence of portal vein or hepatic artery thrombosis. Three of the animals that died in group III showed extensive necrosis involving all lobes. The other three that survived did not show gross necrosis.

Histological examination did not reveal any necrosis in groups I and II, but in group III, 2 had areas of frank necrosis and 1 with patchy necrosis (Fig. 5). The difference in median score among the three groups was statistically significant. All animals in group III had severe sinusoidal dilatation (Fig. 6). The difference in median score among the three groups for sinusoidal dilatation was statistically significant. Group III again had more severe cellular vacuolation although these did not reach significance (Fig. 7).

Orthotopic Allogeneic Transplantation

All 5 recipients survived more than 4 days. Two died of gastrointestinal haemorrhage on the sixth postoperative day, one had acute rejection on the eighth day, and two were still alive at 2 weeks and 5 months.

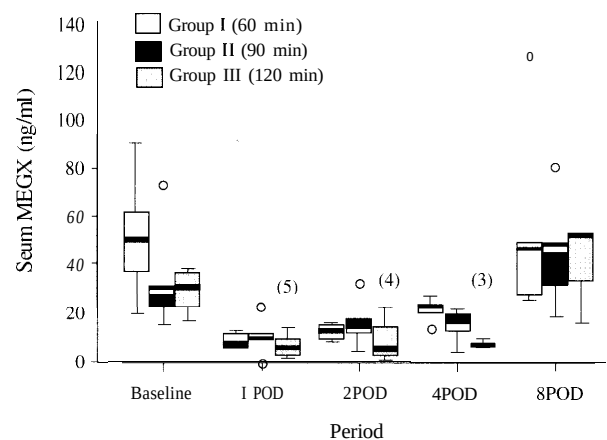


Fig. 4. Serum MEGX levels.

POD: postoperative day.

Numbers of animals in group III which survived indicated in ().

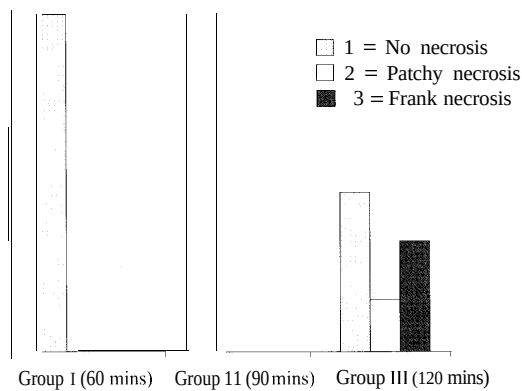


Fig. 5. Distribution of histological score for necrosis

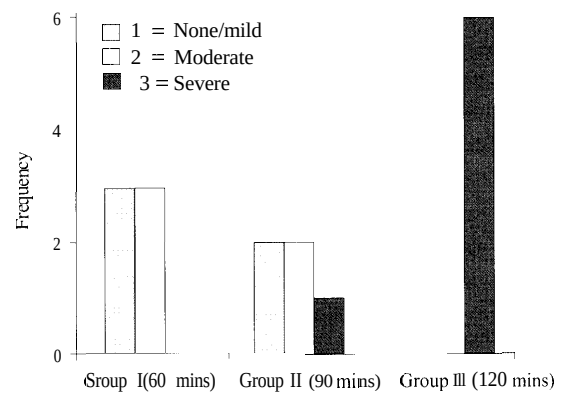


Fig. 6. Distribution of histological score for sinusoidal dilatation.

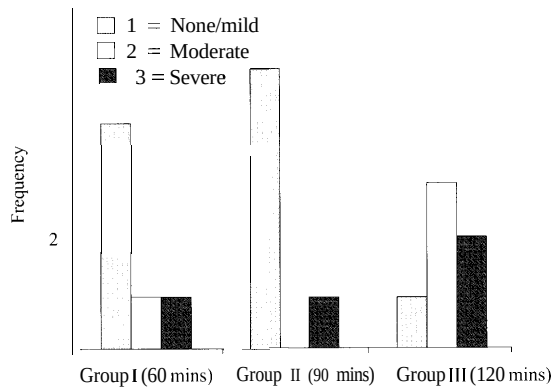


Fig. 7. Distribution of histological score for cellular vacuolation

Discussion

Clinical experience with liver ischaemia for hepatic resection suggests that the liver is able to tolerate ischaemia of 60 minutes at normothermia.^{10,11} Porcine experimental models showed that with prior heparinisation the liver tolerates inflow occlusion of 120 to 180 minutes.^{12,13} These models however do not mimic conditions in NHBs. Ischaemia was incomplete on account of the reflux of venous blood into the liver via the hepatic veins. In most NHBs there is no opportunity for heparinisation prior to cardiac arrest. In this model systemic heparinisation was given only after complete vascular exclusion, hence simulating conditions in NHBs. For the purpose of this experiment cold ischaemia was induced for 30 minutes. This suffices as it has been shown that only minimal cell damage occurs in livers stored for up to 4 hours. There is also no difference in amount of cellular damage when compared to those stored for 8 hours. Duration of cold ischaemia becomes an important determinant of graft failure only in livers stored longer than 10 hours.^{14,15} This whole animal model mimics conditions in NHBs and animal survival can be used as measure of liver viability.

Failures in LTx are often multifactorial. It is a technically exacting procedure involving 5 anastomosis.

Discrepancies in results of LTx using NHBs from studies alluded to earlier²⁻⁴ may be due to some of these factors. Rewarming time required for vascular anastomosis was not addressed in those studies. The rewarming period is the time taken for implantation—the time allograft is removed from its cold preservation until completion of the portal vein anastomosis. Studies have shown that the core liver temperature rises rapidly during this period to reach $17.2^{\circ}\text{C} \pm 3.1$ by the time of portal anastomosis.¹⁶ This period results in large reduction in glycogen content of the liver.¹⁷ Duration of rewarming affects the outcome in transplantation.¹⁸ This period contributes to the total duration of warm ischaemic injury. In contrast to Tanaka's experiment, in this study heparin was not given prior to induction of cardiac arrest in the donors. In the model used here, anastomosis was avoided. The venous system was excluded by cross clamping. The hepatic artery was ligated with silk suture during ischaemia and subsequently released during revascularisation. A technically demanding anastomosis was avoided and the risk of hepatic artery thrombosis which is usually fatal was reduced. Using this model the liver subjected to sequential warm ischaemia, cold ischaemia and rewarming and revascularisation was able to tolerate period of warm ischaemia up to 90 minutes without any fatality.

In animals that survived, the serum bilirubin, ALT and AST, which were elevated postoperatively, returned to its baseline values by the fourth day. MEGX tests normalised by the eighth day. MEGX is a metabolite formed from lidocaine via oxidative N-deethylation by the hepatocyte cytochrome P-450 system. Hence MEGX formation kinetics reflect hepatic function dynamically. This test has been used to assess graft viability prior to transplantation.¹⁹ It has also been reported to be more useful for evaluating liver function than indocyanine green test.²⁰ In our series the baseline levels were lower than that reported for human beings. We are uncertain if this is due to species differences. Nevertheless a definite trend can be seen in the animals that survived,

where the metabolic function of the livers recovered to baseline levels in a fashion that correlated with the improvement in serum transaminases. This functional improvement also bears out in a morphologically near normal appearance of the liver histologically and in the gross specimen.

Allogeneic porcine LTx using NHBDs with 30 minutes of cardiac arrest was carried out. This was to adjust for the approximately 60 minutes of rewarming required for vascular anastomosis during implantation of the allograft. The results of the allogeneic porcine LTx suggest that 30 minutes is a safe period to intervention in NHBDs. This concur with clinical experience of NHBDs in human LTx. Belzer et al²¹ reported 5 cases of human LTx using NHBDs. Warm ischaemic time was 15.4 min $\pm$ 10.7 (range 4 to 34). There was no mortality. Primary graft non function occurred in 1 patient, attributed to technical reasons. The patient was retransplanted. Starzl et al²² reported 12 cases. Allografts from 14 uncontrolled NHBDs (patients whose organs were harvested following a period of cardiopulmonary resuscitation) with warm ischaemia of 37 min $\pm$ 29 were harvested. Six liver allografts were transplanted. Primary graft non function occurred in 3 patients (50%). Retransplantation was required in 5 patients (83%). This is in contrast to the controlled NHBDs group (patients whose organs were procured following cardiopulmonary arrest in the operating room setting). There were 10 patients in this group with a mean warm ischaemia of 23.8 min $\pm$ 11. Six liver allografts were transplanted. There was no primary graft non function. Retransplantation was required in 2 patients (33%).

The relatively short duration for intervention limit the clinical application of NHBDs. Moreover in the clinical situation, haemodynamic instability prior to cardiac arrest may contribute a significant but unquantifiable amount of warm ischaemic injury. Hence the result of this study need to be cautiously interpreted.

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