Urol Res (1992) 20:415–417

Urological Research

Study of kidney and liver viability in the rat after exclusive aortic perfusion using intracellular ATP measurement

H. Bittard, L. Chiche, M. Moukarzel, D. Douguet, and G. Benoit

Laboratory of Experimental Surgery, University of Paris, Bicêtre Hospital, Bicêtre, France

Accepted: May 26, 1992

Summary. To find whether the liver can be procured after exclusive aortic perfusion, three organ perfusion models were used in three groups of donor rats. Group 1 underwent liver wash-out via the portal vein; in group 2, the kidneys alone were perfused via the aorta; and group 3 underwent simultaneous aortic perfusion of liver and kidneys. All perfusion flow rates in the three groups were adjusted to physiological values. Harvested organs were transplanted and recipient animals were killed 4 h after transplantation to study liver and kidney viability by using intracellular ATP measurement. Liver ATP was lower ($P < 0.005$) in the portal perfusion group (group 1: 1.396 ± 0.412) than in the aortic perfusion group (group 3: 2.181 ± 0.061). Kidney ATP was comparable in groups 2 and 3: 1.066 ± 0.09 vs 1.059 ± 0.273 (μmol/g) tissue). Liver cooling was quicker with portal perfusion than with the aortic flush (20° C in 20 s vs 15° C in 60 s). Aortic perfusion at a physiologic flow rate has no detrimental effect on renal viability studied by intracellular ATP measurement. We conclude that liver cooling via the aortic route only is a good alternative to portal perfusion and seems to give good preservation. Application of this observation to emergency procurement in humans is still the subject of controversy.

Key words: Aortic kidney perfusion – Aortic liver perfusion – Organ transplantation

Livers are procured after portal and aortic perfusion. An urgent and increasing need for organs has led to extension of the conditions for acceptance, so that donors with unstable hemodynamic conditions, such as collapse or cardiac arrest, are now considered acceptable: urgent intraaortic perfusion is begun via a double balloon catheter placed through the femoral artery in such circumstances [1, 2, 7, 8]. In these cases, kidneys are the only organs salvaged: the liver is not harvested because of lack of portal perfusion. The aim of this work was to study liver and kidney function after urgent exclusive aortic perfusion in the rat model, compared with livers after portal perfusion.

Materials and methods

BN syngeneic rats weighing 250 g were used as donors and recipients. Perfusion flow rates were adjusted to physiological values, using a small perfusion pump with a flow rate capacity ranging between 10 and 100 ml/mn (Watson, Marlow, Bucks., UK).

Organ temperature was measured using an electrical thermometer (51 kJ thermometer mesurix). Organs were perfused until complete wash-out. Quality of and time to complete wash-out were noted. Harvested organs were placed in cold normal saline with rapid cooling (2–4° C) in a few seconds. Cold ischemia time varied between 15 and 30 min. Three donor groups were considered:

Group 1: Liver wash-out via the portal vein at normal physiologic perfusion rate (20 ml/mn) ($n = 4$), followed by transplantation.

Group 2: Intra-aortic perfusion of kidneys only, still at normal flow rate of 10 ml/mm ($n = 5$). The aorta was ligated above and beneath the renal arteries. Transplantation followed immediately.

Group 3: Simultaneous aortic perfusion of liver and kidneys at the normal physiologic flow rate of 20 ml/mm ($n = 5$); this flow rate is the total of liver, mesenteric and renal flow rates [16]. This third type of perfusion is similar to that obtained with a double occlusive balloon catheter. After complete organ wash-out (liver, intestine, pancreas, kidneys), the liver and one kidney were harvested and transplanted to two different recipients. Recipients were kept for only 4 h after transplantation, and then organs were removed and immediately frozen for intracellular ATP measurement after perchloric extraction for enzymatic determination [10] (Boehringer Mannheim test). Reference liver and kidney ATP values were obtained and controlled in normal animals. SGPT and SGOT were measured just before liver recipients were killed. The Mann-Whitney test was used for statistical analysis.

Results

Normal reference values for intracellular ATP were
- Liver ATP ($n = 5$): 2.171 ± 0.225 μmol/g tissue
- Renal ATP ($n = 5$): 1.567 ± 0.174 μmol/g tissue

The major characteristics of the three groups are summarized in Table 1. Results for the livers are shown in Table 2 and those for the kidney in Table 3.

Table 1. Characteristics of the three groups

	Group 1 ($n=4$)	Group 2 ($n=5$)	Group 3 ($n=5$)
Perfusate temperature (°C)	2.9 ± 1.1	2.7 ± 0.9	3.1 ± 0.9
Recipient portal cross-clamping time (min)	18 ± 1	–	18 ± 2
Recipient caval cross-clamping time (min)	27 ± 1	–	25 ± 4
Rewarming time for kidney (min)	–	23 ± 2	24 ± 2

Table 2. Results recorded in livers in groups 1 and 3

	Group 1 ($n=4$)	Group 3 ($n=5$)
Complete wash-out time (min)	0.1	0.8 ± 0.2
Liver temperature (°C)	14 ± 0.1/15″	18.1 ± 1.4/1
ATP 4 h after transplantation (μmol/g tissue)	1.396 ± 0.412	2.281 ± 0.061
SGPT (IU/l)	42.5 ± 20.9	45.4 ± 18.1
SGOT (IU/l)	275.9 ± 113.1	227.3 ± 67.2

Table 3. Results recorded in kidneys in groups 2 and 3

	Group 2 ($n=5$)	Group 3 ($n=5$)
Complete wash-out time (min)	1.7 ± 0.8	3.6 ± 0.9
Kidney temperature at 1 min (°C)	19.2 ± 1.5	19.9 ± 0.4
Kidney temperature at 5 min (°C)	15.1 ± 0.1	15.5 ± 1.1
ATP 4 h after transplantation (μmol/g tissue)	1.066 ± 0.09	1.059 ± 0.273

Liver wash-out via the portal route was immediate, while it took 1 min when only aortic perfusion was done. Liver cooling was clearly quicker with portal perfusion (14°C in 15 s) than with aortic flush (18°C in 1 min) ($P<0.05$). Liver ATP concentration was lower in the portal perfusion group than the ATP values in the aortic perfusion group ($P<0.05$). SGPT and SGOT were comparable in recipients in these two groups.

When kidneys were the only abdominal organs perfused (group 2), complete wash-out was quicker than in group 3 (1.7 min vs 3.6 min). Renal temperature was almost the same in both groups (groups 2 and 3): 19.2°C vs 19.9°C at 1 min and 15.1°C vs 15.5°C at 5 min. ATP values were also comparable in these two groups (groups 2 and 3): 1.066 μmol/g vs 1.059 μmol/g (Table 3).

Discussion

ATP represents chemical energy indispensable to cellular functioning (synthesis of components, transmembranous ionic and molecular transport, cellular or intracellular movements [4]. ATP is synthesized (and stocked) from glucose degradation by mean of two successive biochemical steps: cytosolic anaerobic glycolysis followed by intramitochondrial oxidative phosphorylation, quantitatively the most important (respectively 2 and 32 molecules of ATP are synthesized from 1 glucose molecule). During ischemia, this second step is stopped by lack of oxygen. Since mitochondria are the cellular components that are most sensitive to ischemia [14], the capacity of a correct ATP level restoration after reperfusion was recently used as a helpful index of organ preservation and viability. Thus, Sumimoto et al. [16] showed in a rat liver transplantation model that the rate of ATP resynthesis 4 h after transplantation was correlated with bile flow recovery and survival rate of the animals. In an experimental model system for kidney transplantation, Tatsukawa [17] showed the same correlation between ATP resynthesis, survival of the animals, and the kidneys' viability. For these reasons, we chose to use cellular ATP content 4 h after transplantation for assessment of organ viability in our study. This parameter appeared to us to be simple, quantifiable, and easily comparable for our three groups of animals and to give precise information on mitochondrial postischemic activity and therefore on the preservation of kidney and liver cells. We do not think that other criteria of preservation reported in the literature, such as more complex nucleotides measurements (ADP + AMP/total adenine nucleotides) [11, 13, 18], NAD measurement [18], or ^{31}P nuclear magnetic resonance spectroscopy [3, 19], would yield more substantial information for our work. Obviously, the assay of ATP content on the removed organ does not allow measurement of animal survival.

Our group 3 model is almost similar to multi-organ perfusion in human organ procurement with a double balloon intra-aortic catheter or an Anaisse tube without portal perfusion, which is done via the mesenteric venous return. Our perfusion flow rate of 20 ml/min in the rat correlates with optimal flow values stated in the literature [18].

In the case of urgent procurement (unstable donors, cardiac arrest etc.) a double balloon aortic catheter [8] is quickly inserted, either in the operating room or in the intensive care unit, using the femoral or the iliac artery [6]. In these cases, portal perfusion is often difficult, and we believe that liver procurement can be done in cases where no warm ischemia is noted before the start of aortic perfusion.

Our animal study shows that multi-organ aortic perfusion through the renal arteries, the celiac trunk and the superior mesenteric artery has no detrimental effect on renal wash-out and renal cooling at 1 and 5 min. ATP levels 4 h after reperfusion show good energetic stock generation in both liver and kidney.

On the other hand, liver cooling was quicker in group 1, with portal perfusion, than in group 3 with aortic perfusion only (20°C in 20 s vs 15°C in 60 s); and ATP

values were significantly higher in group 3, which seems to confirm that rapid cooling could be deleterious, as already indicated by other authors [5, 9, 12, 20].

Obviously, the numbers of animals in the three groups are small and the standard deviations relatively large: these facts may perhaps militate slightly against our conclusions.

The comparison between rat and human procurement is a matter of discussion, expecially as in the rat we compared portal perfusion alone against exclusive aortic perfusion. However, we may conclude the following:
- Liver cooling by the aortic perfusion is possible, and it is smooth and progressive compared to portal wash-out.
- This seems to give good preservation (measured by the ATP value), and we therefore believe that livers can be safely procured with kidneys when donors are perfused by the aorta only, with almost no warm ischemia time.

Conclusion

Cellular ATP measurements 4 h after liver and kidney transplantation have shown that exclusive perfusion at a normal physiologic flow rate is an efficient technique for preparation of abdominal organs (kidneys and liver) for harvesting.

References

1. Anaise D, Yland MJ, Walteztr WC, Frischer Z, Rapaport FT (1987) A new perfusion tube for multiple organ procurement. Transplant Proc 19:4129
2. Benoit G, Bensadoun H, Borie I, Vavasseur G, Gillot C (1990) Sonde a double ballonet pour prélèvements d'organes abdominaux. Ann Urol 24:199
3. Bretan PN, Baldwin N, Novick AC, Majors A, Easley K, Thian NG, Stowe N, Rehm P, Streem SB, Steinmuller DR (1989) Pretransplant assessment of renal viability by phosphorus-31 magnetic resonance spectroscopy. Transplantation 48:48
4. Duval D (1979) Biochimie, vol 2 Biochimie métabolique. In: Presses Universitaires de France (ed), pp 7–44
5. Francavilla A, Brown TH, Fiore R, Cascardo S, Taylor P, Groth CG (1973) Preservation of organs for transplantation: evidence of detrimental effect of rapid cooling. Eur Surg Res 5:384
6. Fries D, Charpentier B, Benabdallah T, Neyrat D, Houssin D, Smadja C, Castaing D, Benarbia S, Hammouche M, Brocard JF, Alsabbagh A, Benoit G (1982) In situ cadaver kidney cold perfusion for transplantation. Transplant Clin Immunol Experta Clin 14:(whole issue)
7. Garcia Rinaldi R, Lefrak EA, Defore WU (1975) In situ preservation of cadaver kidneys for transplantation. Ann Surg 182:576
8. Gillot C (1961) La cavographie occlusive: note préliminaire présentation d'une sonde. Presse Med 65:193
9. Jacobsen IA, Chemnitz E, Kemp E, Buhl MR (1980) The effect of cooling rate during perfusion on function and morphology of rabbit kidney grafts. Scand J Urol Nephrol [Suppl] 54:2
10. Jaworek D (1974) Methods for enzymatic analysis, 3rd edn. In: Bergmeyer HU (ed) Verlag Chemie, Weinheim, p 2147
11. Kamike W, Burdelski M, Steinhoff G, Ringe B, Lauchart W, Pichlmayr R (1988) Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. Transplantation 45:138
12. Otto G, Wolff H, Uerlings I, Gellert K (1986) Preservation damage in liver transplantation. Influence of rapid cooling. Transplantation 42:122
13. Palombo JD, Pomposelli JJ, Hirschberg Y, Blackburn GL, Bistrian BR (1989) Glycolytic support of adenine nucleotides in rat liver flush-preserved with UW or Collins' II. Transplantation 48:901
14. Pegg DE (1986) Organ preservation. Surg Clin North Am 66:617
15. Sheldon W (1979) Morphophysiology. In: Baker HJ, Russel-Linsey J, Weisbroth SH (ed) The laboratory rat, vol 2. Academic Press, New York, p 87
16. Sumimoto K, Inagaki K, Yamada K, Kawasaki T, Dohi K (1988) Reliable indices for the determination of viability of grafted liver immediately after orthotopic transplantation. Bile flow rate and cellular adenosine triphosphate level. Transplantation 46:506
17. Tatsukawa Y (1979) The role of coenzyme Q10 for preservation of the rat kidney: a model experiment for kidney transplantation. Life Sci 24:1309
18. Tokunaga Y, Ozaki N, Wakashiro S, Ikai I, Kimoto M, Morimoto T, Shimahara Y, Kamiyama Y, Yamaoka Y, Ozawa K, Nakase Y (1988) Effects of perfusion pressure during flushing on the viability of the procured liver using noninvasive fluorometry. Transplantation 45:1031
19. Vine W, Gordon E, Alger J, Flye MW (1986) Hepatic preservation assessed by MRS. Transplant Proc 18:577
20. Winchell RJ, Halasz NA (1998) The effects of cooling rates and storage temperature on the function of 24 hour cold preserved kidneys. Transplantation 46:918

Dr. G. Benoit
Service d'Urologie
Hôpital de Bicêtre
78 rue du général Leclerc
F-94275 Bicêtre Cedex
France