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The value of dextran 12,000 in ischemically damaged canine kidneys during machine perfusion

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Abstract The use of non-heartbeating (NHB) donor kidneys has led to the search for new methods of viability-testing. We investigated, in a canine model, the relationship between the filtration of dextran 12,000 into urine and a certain period of warm ischemic time (WIT) during machine perfusion. Twenty-four canine kidneys were divided into three groups, sustaining 0 min, 30 min or 60 min of WIT. After cooling and flushing, the kidneys were perfused on a perfusion machine for 8 h. Three hundred milligrams of dextran 12,000 was added to the perfusate. In the perfusate, dextran and lactate dehydrogenase (LDH) concentrations were measured. Dextran concentrations were also analysed in urine. Intrarenal vascular resistance (IRR) was calculated from pressure and flow characteristics. The 30WIT group showed a higher dextran excretion rate than the other two groups. IRR and LDH measurements showed lower levels in the ischemic groups compared with the control group. Dextran 12,000 is not suitable as a viability test but does show interesting results regarding the low LDH and IRR levels in the ischemic groups.

Key words Non-heartbeating · Ischemic · Kidney · Dextran · Effect · Canine

Introduction

Due to the increasing demand for donor kidneys, extensive research is carried out in the quest for new sources. One approach is the use of non-heartbeating (NHB) donor kidneys. By further improvement of viability-testing techniques, NHB donor kidneys can alleviate the organ shortage by 20 to 40% [8, 24].

The main problem in the use of NHB donor kidneys is the inevitable period of warm ischemic time (WIT). WIT is defined as the period between cardiac arrest and the start of the cooling of the organs. Frequently, WIT is based on an estimation and therefore of unknown duration. Because organ viability decreases with prolonged WIT, it is important to be able to evaluate ischemic damage of the kidneys before transplantation. Research is being done to find a parameter that could estimate WIT and provide an idea of the viability of the kidney [6, 9, 14, 23].

Machine perfusion (MP) has proven to be superior to cold storage in preserving NHB donor kidneys [3, 4, 17]. Furthermore, MP offers the opportunity of viability assessment. Intrarenal vascular resistance (IRR) [12] and release of the enzymes lactate dehydrogenase (LDH) [15] and α-glutathione *S*-transferase (αGST) [9, 14] into the perfusate can be analysed for viability testing. Besides these tests, donor data and macroscopic and microscopic appearance of the kidney remain important criteria for accepting or discarding an organ for transplantation. Despite the use of this range of parameters, a certain percentage of kidneys fail to function [7, 14].

In a canine model with standard periods of WIT induced in the kidneys, a small type of the isoelectrical macromolecule dextran was added to the perfusate in order to assess nephron damage. Dextrans are known to be biologically and chemically inert, and they might therefore provide a suitable parameter for specific

Table 1 Number of kidneys, weight gain (grams) after eight hours of MP and total urine production (ml.) for each group. Results are presented as means ± SEM

	number of kidneys	weight gain (weight before MP)	total urine production
0 WIT	8	18.3 ± 2.3 (81.4)	37.8 ± 11.6
30 WIT	8	19.0 ± 3.3 (83.1)	13.4 ± 5.5
60 WIT	8	20.2 ± 1.6 (72.6)	13.1 ± 1.7

membrane characteristics such as leakage and other gradients.

Materials and methods

Twelve mongrel dogs weighing between 23 and 34 kg. were used. The harvested kidneys were divided into three groups: A, B and C. Each group consisted of eight kidneys (four right and four left kidneys), which sustained 0 min, 30 min and 60 min of warm ischemia (0WIT, 30WIT and 60WIT groups, respectively).

Twelve hours prior to surgery, the dogs were fasted, with free access to water. Premedication consisted of acepromazine 1 mg/ml, oxicodon 10 mg/ml and atropine 0.5 mg/ml; this mixture was given in a dose of 1 ml/5 kg i.m. Anaesthesia was introduced with sodium thiopental 15 mg/kg i.v. and maintained with 2% O_2, 4% N_2O and 1% halothane/min.

The kidneys were exposed through a midline incision and the renal vessels were identified. Heparin 100 IU/kg was given i.v. prior to clamping the arteries. After harvesting, the kidneys were flushed with 200 ml of cold (4 °C) histidine tryptophan ketoglutarate (HTK) solution.

The kidneys were weighed, the ureter was catheterised and the kidneys were connected to a Gambro PF-3B perfusion machine (Gambro, Lund, Sweden). For perfusate, 500 ml UW-gluconate (Belzer's) solution was used [19]. Before perfusion, 300 mg of dextran 12,000 (Fluka Chemie, Switzerland) was added to the perfusate. (The actual weight of dextran 12,000 is 11,600 Da.) The Einstein-Stokes radius of dextran 12,000 is 25.06 Å, using the formula [log r(Å) = 0.472 log mol wt–1.196] [10, 26]. During MP, the temperature was maintained between 4 and 6 °C. At the start of perfusion, the flow was set at a systolic pressure of 60 mmHg. Systolic pressure was allowed to range between 50 and 70 mmHg and was adjusted when above or below these values. In the organ chamber, 150 ml of oxygen/min was blown over the surface of the perfusate.

During the first 8 h, perfusion pressure (systolic, diastolic and mean), flow and urine production were recorded hourly. IRR was calculated by dividing mean pressure by recorded flow and was corrected for kidney weight and expressed per 100 g.

Perfusate samples for LDH and dextran were taken at the same time intervals after starting MP. Urine samples were collected at hourly intervals, provided there was a minimum of 1 ml urine produced, necessary to measure dextran concentrations. If less then 1 ml of urine was produced, the collected urine would be added to the urine production of the following hour.

Before dextran analysis, urine and perfusate samples were filtered through a myoglobin filter (30,000 Da) in order to extract the hydroxyethyl starch (HES) from the perfusate, which would otherwise disturb dextran analysis. The analysis of the dextran concentration was done as described earlier by van Kreel et al. [16]. In short, the free glucose molecules originally present in urine and perfusate were removed, after which the dextran molecules were hydrolysed in an acid environment to form glucose molecules. These newly formed glucose molecules are used as the final parameter for dextran analysis. A ratio was calculated by dividing glucose concentration in urine to the glucose concentration in perfusate (U/P). This U/P fraction was calculated to prevent any bias due to changes in dextran concentrations in the perfusate, caused by cellular oedema for example.

LDH levels were determined by the standard colorimetric assay (Boehringer Mannheim, Almere, The Netherlands). LDH levels were calculated per 100 g. of kidney weight.

The Kruskal-Wallis test was used to test for differences between the groups at each time point. Differences were regarded significant when $P < 0.05$.

Results

The number of kidneys, weight gain after 8 h of MP and total urine production in each group are presented in Table 1.

Dextran

The dextran concentration as a fraction of the concentration in urine and perfusate shows a significant difference at $T = 1$ between 0WIT and 30WIT groups and at $T = 3$ between 30WIT and 60WIT groups (Fig. 1). The 60WIT group showed almost similar results to the 0WIT group. Some data consist of less than eight measurements because of low urine production. Figure 2 shows dextran levels in the first sample of urine produced by each kidney in each group. Differences were significant between the 0WIT and 30WIT groups ($P = 0.036$) and between the 30WIT and 60WIT groups ($P = 0.009$).

Dextran concentrations in perfusate increased during MP by30–38%. The dextran concentrations at $T = 1$ and $T = 8$ and the mean concentration increase are presented in Table 2.

IRR and LDH

IRR was lower in the 60WIT group at $T = 4, 5, 6, 7$ and 8 compared with the 0WIT group ($P = 0.045, 0.021, 0.021, 0.027$ and 0.007, respectively). Results are shown in Fig. 3. LDH levels showed means of the ischemic groups to be lower than the control group, but were not statistically different (Fig. 4).

Discussion

It is known that ischemia induces damage to the glomerulus and tubuli [2, 13, 25]. It is therefore likely that the filtering function of the nephron alters due to a certain

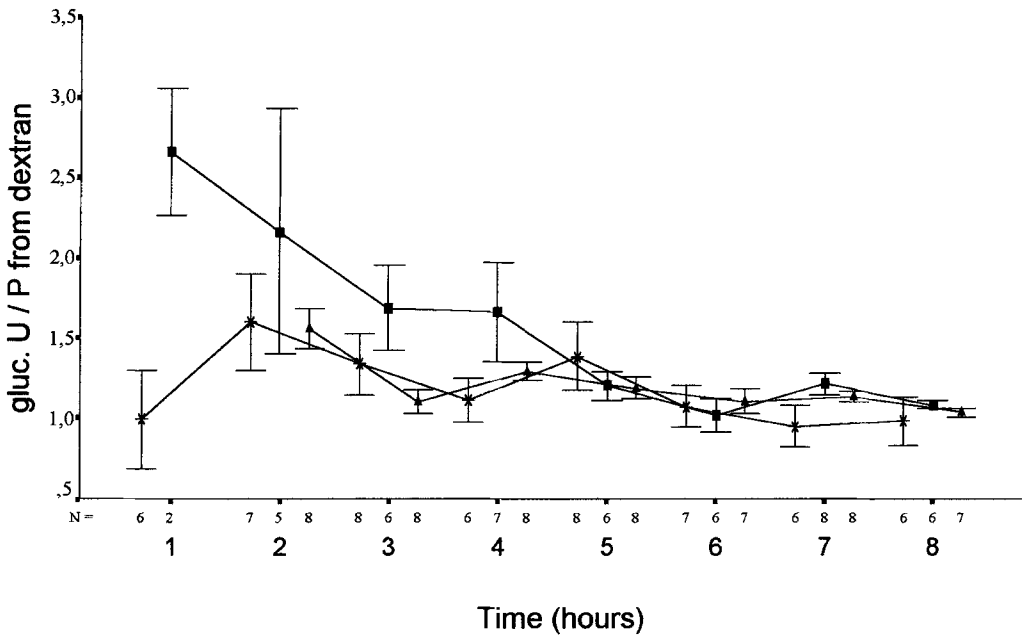

Fig. 1 Changes in dextran level as a fraction of the concentration in urine and perfusate between the time intervals for each individual group. Dextran levels are displayed as glucose concentration. *0 min warm ischemic time (WIT); *black squares*, 30 min WIT; *black triangles*, 60 min WIT. Means ± SEM

Table 2 Dextranconcentrations at T = 1 and T = 8 and dextranconcentration increase for each group. Results are presented as means ± SEM. Differences between groups are not significant

	Dextranconcentration in perfusate T = 1	Dextranconcentration in perfusate T = 8	Average dextran concentration increase
0 WIT	4.26 ± 0.31	5.89 ± 0.64	38 %
30 WIT	4.39 ± 0.36	5.81 ± 0.73	32 %
60 WIT	3.59 ± 0.25	4.68 ± 0.36	30 %

period of WIT. If the change in sieving capabilities could be correlated to the sustained WIT, this might be used as an estimate of the amount of ischemia suffered by a NHB donor kidney. Damage induced by ischemia include the formation of tubular casts [2, 13, 20], structural changes in glomerular endothelial fenestrae, epithelial cell foot processes in the glomeruli [2, 13, 22, 25] and back-leak of tubular fluids into the interstitium [20]. In this study, the changes in the dextran 12,000 sieving-capacity of the nephron were measured in correlation to the WIT.

Dextran

Before discussing the results, it is important to realise that the increase in dextran concentrations in perfusate are corrected for. Table 2 shows that the concentration of dextran in perfusate increases by 30–38 %. This can partly be explained by a relative loss of water, which is due to the weight gain by the kidneys (Table 1), probably caused by cellular oedema. The rest of the increase in concentration is probably caused by a gradual dissolving of dextran that was precipitated after adding it to the cold perfusate. Since the urinary dextran excretion by the kidney is dependent on the concentration present in the perfusate, correction for the concentration increase is important. Therefore, all the results concerning dextran concentrations are presented as U/P fractions.

As can be seen in Fig. 1, only the first 4 h of perfusion show marked differences in the excretion of dextran by the kidneys. The fact that the dextran excretion stabilises in the three groups during the last 4 h of MP points to the development of a halt in the processes described in the first paragraph of this Discussion. This might be the result of the perfusion machine, bringing the kidney to a steady state environment.

One of the problems encountered in this study was the low urine production by some of the kidneys, especially the ischemic ones (Table 1), which decreased the number of measurements in the first few hours of perfusion. In order to overcome this problem, the data of the first samples of urine produced were collected, irrespective of the time of production. Presented in Fig. 2, the results from the 60WIT group show a discrepancy with theoretical expectations. On theoretical grounds, one would expect the U/P fraction in the 60WIT group to be higher than the groups with less ischemia (the more

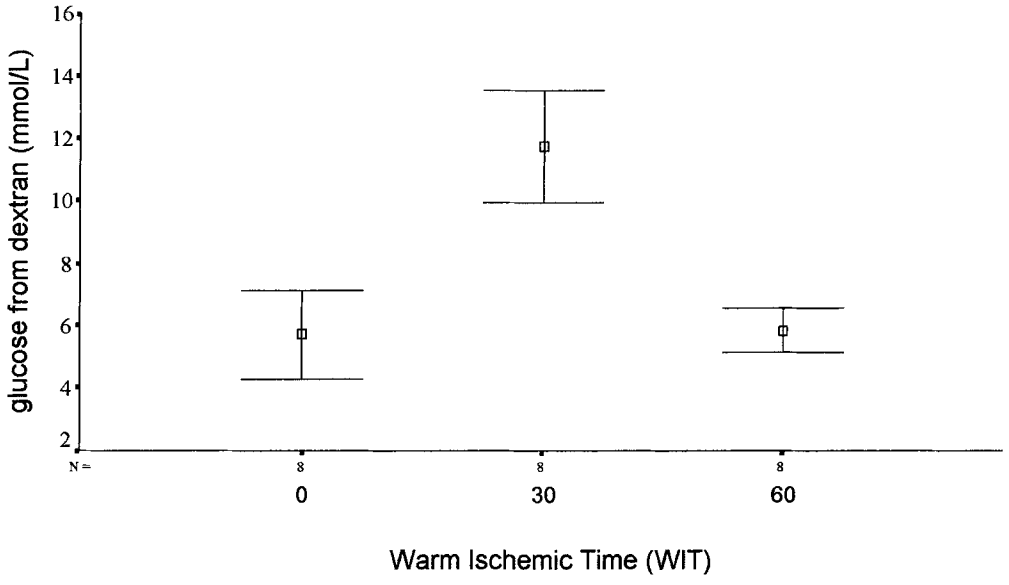

Fig. 2 Changes in dextran level in the first sample of urine produced by the kidneys between the time intervals for each individual group. Dextran levels are displayed as glucose concentration. Means ± SEM

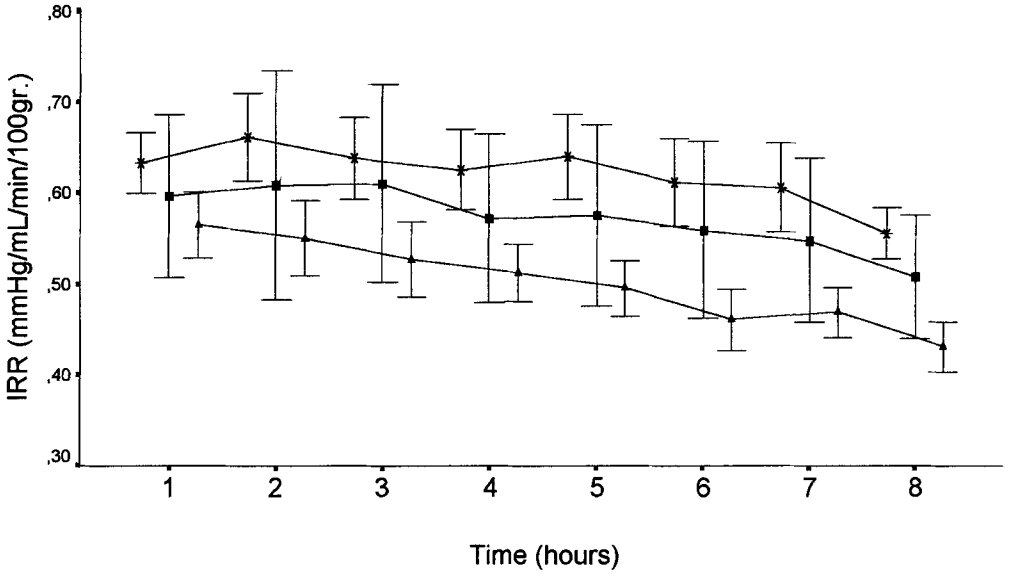

Fig. 3 Changes in intrarenal vascular resistance between the time intervals for each individual group. *0 min WIT, *black squares*, 30 min WIT; *black triangles*, 60 min. WIT. Means ± SEM

damage, the more filtration). Instead, the 30WIT group shows a significantly higher dextran concentration in the urine compared with the 0WIT and 60WIT groups.

This experiment does not explain the cause of this phenomenon. It is likely that the diameter of the endothelial fenestrae becomes smaller as a result of ischemia [2, 22], and thus the most probable cause of the increased dextran concentration in urine in the 30WIT group would be the process of back-leak. This is known to occur as a result of tubular endothelial damage and the formation of tubular casts [20]. These casts cause an increased intraluminal pressure, which in turn stimulates back-leak of tubular fluids into the interstitium. This is likely to happen to small molecules such as water. Larger molecules such as dextran 12,000 possibly stay in the tubular lumen. Eventually, this should result in an increased dextran concentration in the urine. As for the reason why the 60WIT group shows a reduced excretion of dextran compared with the 30WIT group, one can only speculate, because of the absence of similar experiments in the literature. Since there is no difference in urine production between the 30WIT and 60WIT groups and there is a difference in dextran concentration between the same groups, the most likely conclusion is that the urine in the 60WIT group is no longer concentrated by a process such as back-leak. Although this is interesting from a physiological point of view, this does lead to the conclusion that dextran

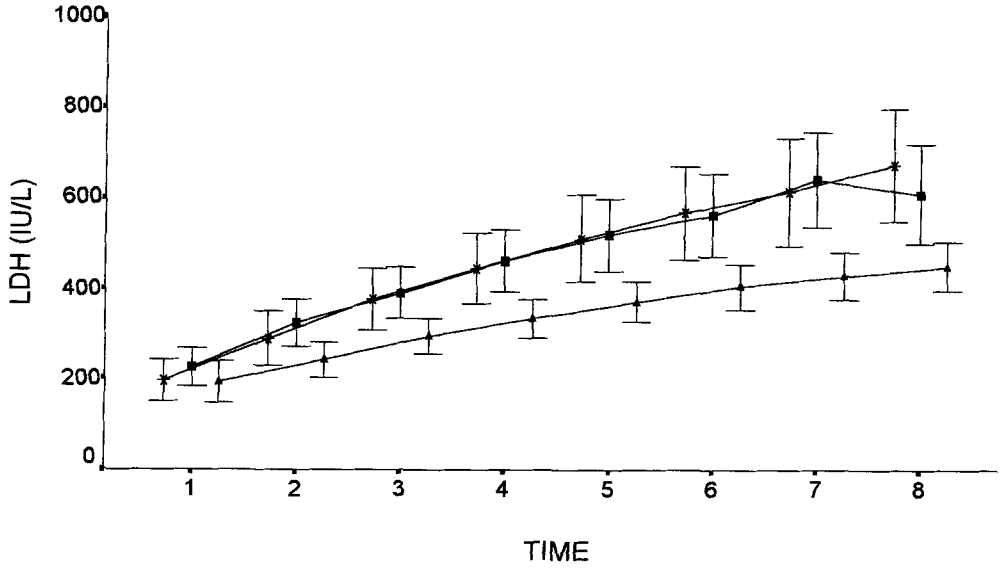

Fig. 4 Changes in lactate dehydrogenase release into the perfusate for each individual group. *0 min WIT; *black squares*, 30 min WIT; *black triangles*, 60 min WIT. Means ± SEM

12,000 in this study proves unsuitable as a viability test, since there is no difference between the control group and the 60WIT group.

IRR and LDH

That IRR is lower in the 60WIT group than the 0WIT group is remarkable. One would expect the vascular resistance to be higher in the ischemic groups [7, 12]. The same conclusion is applicable for the lower LDH in perfusate of the ischemic groups. Although the differences are not statistically significant, one would expect the mean LDH concentration to be higher in the ischemic groups, instead of being lower than the control group [1, 11]. Confounding of LDH analysis due to the presence of dextran was ruled out by testing known LDH levels in samples with and without dextran.

In comparison with the normal human NHB donor kidney preservation protocol, the only change made was the addition of dextran 12,000 to the perfusate. Dextrans are known to be biologically inert molecules [5], but one might suspect this to be untrue for the smaller types such as dextran 12,000, regarding the decreased IRR and LDH levels in the ischemic groups. In a literature review, no report about a biological effect of dextran 12,000 was found. Larger dextrans, such as dextran 70,000, are widely in use as plasma expanders with very few side effects. There are some reports of dextrans having anti-thrombotic properties in vivo, but the exact physiological mechanism remains unknown [18, 21].

In this study, no intent was made to prove a biological effect of dextran 12,000 on the kidney. Even if dextran had any interactive properties, one would expect these to be minimal at 4–6 °C, as metabolism is reduced to a minimum. However, IRR and LDH levels – being established viability parameters – are lower in the ischemic groups. Despite these results, it would be premature to deduce a protective effect of dextran 12,000 on the machine perfused NHB donor kidney. Further investigation will be needed to verify these observations.

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