Nifedipine improves recovery function of kidneys preserved in a high-sodium, low-potassium cold-storage solution: study with the isolated perfused rat kidney technique

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Abstract
Background. Extracellular types (high-Na) of cold-storage solution (CSS) have been shown to be more effective in preserving kidneys than intracellular CSS (high-K). On the other hand, calcium entry blockers (CEB) have been demonstrated to improve graft function when administered after and/or prior to transplantation. The ischaemia reperfusion syndrome involves, in part, an alteration in intracellular calcium metabolism that induces an increase in renal vascular resistances (RVR) and other cellular dysfunction, and high-K CSS per se are vasoconstrictive. Since CEB act via a modification in intracellular calcium metabolism on vascular smooth muscle, glomerular, and tubular cells, we evaluated the actual benefit on CEB on clinical investigations have been undertaken to study enhancement of hypothermic protection with the addition of calcium entry blockers (CEB). Several studies showed that the administration of CEB seems to reduce the incidence of delayed graft function, prevents acute cyclosporin toxicity and lessens the number of rejection episodes in the first few weeks after transplantation [3,4]. Recent studies [5,6] suggest that the short-term improvements noted in both graft function and rejection episodes with CEB are maintained for the first year post-transplantation and furthermore, the use of CEB results in improved 1 year graft survival. However, as usually in clinical studies, some drawbacks may raise uncertainty about results, such as cyclosporin-associated treatment and dosage that may vary during the follow-up, the possibility that the different CEB may act differently on the metabolism of cyclosporin, and the lack of standardization of CEB dosing that has to be adapted to blood pressure. In several experimental models of ischaemic acute renal failure, CEB have been reported to be of value in preserving organ function but have not been extensively studied with respect to renal preservation [7]. Despite some disadvantages and limitations the isolated perfused kidney model (IPK) offers many advantages. The experiments are relatively simple, rapid, and inexpensive. The methods of evaluation are reproducible and more precise than the results based on the...
Renal perfusion pressure and perfusion rate were related to changes in renal vascular resistances. Extensive work with this system studying adenosine nucleotide metabolism and relative preservative effects of various flushing solutions has been reported [9–11]. We have previously demonstrated that a high sodium version of UW cold-storage solution seemed to be less injurious to recovery function than the original high potassium UW Belzer’s solution [12]. In the present study we used the IPK model to elucidate the actual benefit of adding the CEB nifedipine to both cold-storage solutions.

Subjects and methods

The IPK technique

Sprague–Dawley rats (weighting 200–250 g) were used. Unless otherwise indicated in the method section, the technique for IPK has been previously described in detail [12]. Briefly, the kidneys were perfused with a Krebs–Henseleit bicarbonate solution containing bovine albumin 6%. Polyfructosan (Inutest®, Laevosan, Linz, Austria) was added to measure glomerular filtration rate (GFR). The solution was perfused at 37 °C in a closed thermostatically controlled circuit with a peristaltic pump. When perfusion pressure of 90–100 mmHg was reached, the pump flow rate was maintained constant, and subsequent changes in perfusion pressure were related to changes in renal vascular resistances (RVR). Renal perfusion pressure and perfusion rate were continuously monitored. Following an equilibrium period of about 30 min after the onset of perfusion medium, urine was collected for two consecutive 30 min periods. For each period, GFR, absolute (ARNa) and fractional (FRNa) sodium reabsorption, and RVR were measured. RVR was the ratio of perfusion pressure to perfusion rate.

Cold-storage solutions

Two cold-storage solutions, derived from University of Wisconsin (UW) Belzer’s solution and prepared in the Pharmaceutical department [12], were compared:

1. K-UW solution identical to the original Belzer’s high-potassium UW solution (125 mmol/l potassium and 30 mmol/l sodium).
2. Na-UW solution contained the same agents as the original UW solution but with a high-sodium and low-potassium concentrations (125 mmol/l sodium and 30 mmol/l potassium).

Experimental protocols

Effects of cold-storage solutions on renal vascular resistances of the IPK

To evaluate the effects of cold-storage solutions on renal vascular resistances, the following experiments were performed. After cannulation of mesenteric artery as described previously, the kidneys were placed in the isolated perfusion circuit and perfused with Krebs–Henseleit solution without recirculation of the effluent, at a rate of 10 ml/min until stabilization of renal perfusion pressure. Therefore the cold-storage solution was infused through the main perfusate line for 1 min every 5 min, four times, at increasing flow rates consecutively (3, 5, 7, and 10 ml/min). When the cold-storage solution infusion was started, the rate of Krebs–Henseleit perfusion was reduced so as the kidney was perfused at a constant rate of 10 ml/min, i.e. when the rate of infusion of the cold-storage solution was increased from 3 to 5, 7, and 10 ml/min, the perfusion rate of Krebs–Henseleit solution was reduced to 7, 5, 3, and 0 ml/min respectively. As a result, recorded changes in perfusion pressure are actually in relation to changes in RVR and not in the rate of perfusion. Final potassium concentration that reached the kidney was respectively 41, 68, 89, and 125 mmol/l when the rate of K-UW infusion was increased, and 12.5, 17.5, 22.5, and 30 mmol/l when Na-UW was infused. For each cold-storage solution, four rats were used.

Effects of nifedipine on the changes in renal vascular resistances induced by cold-storage solutions

After cannulation of the kidney and its perfusion with Krebs–Henseleit solution as described above, the cold-storage solution was infused during 1 min every 5 min for 10 times, at a rate of 10 ml/min. When the cold-storage solution infusion was started, the Krebs–Henseleit perfusion was stopped. After the cold-storage solution had been infused for consecutive times, to confirm the reproducibility of the induced changes in perfusion pressure, an infusion of nifedipine (10–6 M in NaCl 9‰) was added during 10 min and then withdrawn. For each cold-storage solution, 4 rats were used.

Influence of nifedipine on renal function of the IPK

Without nifedipine (N−). In the control group (n = 6), the kidneys were perfused normothermically with the Krebs–Henseleit albumin solution immediately after they were harvested. In the cold-stored group, kidneys were catheterized and isolated without interruption of blood flow and flushed with the cold-storage solution cooled at +4 °C for 10 min at 3 ml/min. The kidneys were then placed with their catheters in a small container with 30 ml of the cold-storage solution and stored at 0–4 °C for 24 h. Afterwards, the organs were reperfused on the isolated perfusion circuit with perfusion medium at 37 °C to measure renal function and haemodynamics. In these experiments 14 rats were used, seven for each cold-storage solution.

With nifedipine (N+). The same experiments were performed as indicated above, but nifedipine 10–6 M was added to the cold-storage solution and to the Krebs–Henseleit perfusion medium. In these latter experiments 18 rats were used, six controls and six for each cold-storage solution.

Statistical analysis

Results are expressed as the mean ± SEM. Renal function parameters were expressed as the mean ± SEM of the two consecutive 30-min urine collection periods. Data between groups were compared with one-way analysis of variance (ANOVA) followed by Fischer’s protected least-significant
Effects of nifedipine on recovery function of cold-stored kidney

Fig. 1. Effects of cold-storage solutions on perfusion pressure of the IPK. K+ concentration indicates final potassium concentration reaching the kidney and obtained by increasing the infusion rate of each cold-storage solution. *P<0.05 vs control perfusion pressure.

Results

Effects of cold-storage solutions on renal vascular resistances of the IPK

Figure 1 shows changes in perfusion pressure obtained after infusion of the cold-storage solutions. Increments of infusion rates of K-UW from 3 to 5, 7 and 10 ml/min which result in an increase in final concentrations of potassium of 41, 68, 89 and 125 mmol/l, induced significant increases in perfusion pressure of 44±8, 82±28, 86±16 and 90±20 mmHg respectively. Conversely, an identical increase in infusion rate of Na-UW providing final concentrations of potassium to the IPK of 12.5, 17.5, 22.5 and 30 mmol/l induced a slight but non-significant decrease in perfusion pressure below base line of −9.5±0.6, −7.5±2.9, −8.8±4.8 and 9.5±0.6 mmHg respectively.

Effects of nifedipine on the changes in renal vascular resistances induced by cold-storage solutions (Figures 2 and 3)

Before adding nifedipine, the infusion of K-UW for 3 consecutive times induced a reproducible increase in perfusion pressure of 54±13 mmHg above baseline. In similar conditions, the infusion of Na-UW induced a slight decrease in perfusion pressure of 8±3 mmHg below baseline. The addition of nifedipine to K-UW produced a significant reduction of the peak perfusion pressure to only 17±6 mmHg, whereas Na-UW infusion was not influenced by nifedipine (−3±1 mmHg). Figure 3 depicts a typical graph of changes in perfusion pressure obtained after the infusion of K-UW and the inhibiting effect of nifedipine.

Influence of nifedipine on renal function of the IPK (Table 1)

Control group. Table 1 depicts the results of renal function and haemodynamics of control IPK measured immediately after kidneys have been harvested. The addition of nifedipine did not cause any significant alteration in the functional parameters. Cold-stored group without nifedipine. After 24 h cold-storage, GFR was dramatically decreased by 90% compared to control for the two solutions. Na reabsorption was also considerably altered by cold-storage. However, both FR_{Na} and AR_{Na} of kidneys preserved in Na-UW were significantly higher than in K-UW. There was no difference in RPF or RVR between cold-stored kidneys and control.

Cold-stored group with nifedipine. Nifedipine did not significantly influence renal function of kidneys flushed and stored with K-UW. In contrast the addition of nifedipine to kidneys stored in Na-UW induced a significant increase in GFR, FR_{Na} and AR_{Na}. Table 1 shows that renal function of Na-UW-nifedipine became significantly higher than that of K-UW-nifedipine. There was no difference in RPF or RVR between K-UW, Na-UW and control.

Discussion

Cold-storage solutions commonly used for preservation of organs before transplantation, as EuroCollins (EC) and Belzer UW solutions, have a high potassium concentration [13]. These solutions are supposed to limit potassium leakage from stored cells and thereby maintain a more normal intracellular milieu. However, high-potassium solutions have several disadvantages.
Inhibitory effect of nifedipine on the peak-pressure induced by K-UW infusion. After the end of nifedipine infusion there was a progressive restoration of the K-UW-induced vasoconstriction.

In particular, the high potassium concentration causes depolarization of vascular smooth muscle and epithelial cell membrane, and can lead to blood vessel constriction and rapid cellular swelling.

In a recent study using the IPK model, we have shown that a high sodium version of Belzer’s cold-storage solution (Na-UW) was less deleterious to diffusion of the cold-storage solution and ameliorate preservation. Our results showed that the addition of nifedipine to K-UW produced a significant reduction of the peak perfusion pressure, while Na-UW infusion was not influenced by this CEB (Figures 2 and 3).

Previous studies using the IPK have clearly demonstrated that nifedipine inhibits the KCl induced vasoconstriction after 24 h cold-storage.

In the first part of the present study we tried to verify the vasoconstrictive effect of K-UW. Our results clearly demonstrate that K-UW, but not Na-UW, induced a reproducible and ‘dose-dependent’ (K-concentration-dependent) increase in vascular resistances of the IPK (Figure 1). To our knowledge these results are the first to clearly illustrate a vasoactive effect of high-potassium cold-storage solutions.

We tried in the second part of the study to inhibit vasoconstriction by adding nifedipine to the cold-storage solutions, since manoeuvres which would prevent vasoconstriction may favour the intraorgan diffusion of the cold-storage solution and ameliorate preservation. Our results showed that the addition of nifedipine to K-UW produced a significant reduction of the peak perfusion pressure, while Na-UW infusion was not influenced by this CEB (Figures 2 and 3).

In the third part of the study we evaluated the functional recovery of kidneys after 24 h cold-storage in K-UW and Na-UW with or without nifedipine.

In control IPK (without cold-storage) infusion of nifedipine did not modify haemodynamics or tubular...
function. Under conditions of in vitro perfusion with perfusion pressure of 80–100 mmHg, the IPK appears to possess little intrinsic vascular resistance, and in the absence of exogenous vasoconstrictors, CEB exert no effect on renal perfusate flow or glomerular filtration rate in this model [6,15].

As others and we demonstrated previously, cold-storage of kidneys for 24 h resulted in a considerable alteration in GFR and tubular function as assessed by FRNa and ARNa in the IPK, whatever the composition of the cold-storage solution could be [9–12], and we confirmed that Na-UW appears slightly more effective than the original high-potassium UW solution. These results have been recently validated in the isogenic graft model in the rat [16]. Other authors reported similar results by using other experimental animals or other low-potassium, high-sodium solutions [17,18].

Finally we added nifedipine to K-UW and Na-UW during flush and storage for 24 h, and then to the normothermic perfusate of the kidney. As shown in Table 1, nifedipine did not induce any significant change in GFR, or in tubular function or haemodynamics in IPK cold-stored in K-UW. In contrast the addition of nifedipine to Na-UW improved both GFR and tubular function of the cold-stored kidney, while Na-UW without nifedipine ameliorate only tubular function.

Shapiro et al. [19] were the first to demonstrate a significant protection of the CEB verapamil of ischaemic renal injury in the IPK model. In this study, verapamil improved GFR and ARNa after warm ischaemia [40 min at 37°C]. Nakamoto et al. [20] showed in another study that the addition of verapamil to the IPK preserved during 24 h improves renal function parameters significantly, but they used hypothermic continuous perfusion (4–7°C) instead of static cold-conservation. Mills et al. [21] demonstrated that the pharmacological active isomer of Emopamil, an analogue of verapamil, has salutary effects on IPK function following warm and cold ischaemia in EC solution, but storage was performed for only 4 h.

Alterations in cellular calcium homeostasis are commonly involved in the pathogenesis of ischaemia reperfusion syndrome, although it is not clear whether the increase in intracellular calcium is the cause or rather the consequence of the damage [2]. It is noteworthy that the addition of nifedipine to Na-UW ameliorated GFR without altering RPF. Thus it seems, as suggested by other studies as well, that there is a dissociation of the vasodilatory effects of CEB from their effects on organ function [22]. As a matter of fact increased intracellular calcium may affect not only vascular smooth muscle, but also glomerular mesangium and tubular epithelium [2]. Therefore the consequences may be not only an increase in renal vascular resistances, but also a decrease in glomerular capillary surface and permeability, and tubular cell injury. CEB may interact with the process controlling GFR at multiple sites. First, the increase in GFR without alteration of RPF may suggest a direct action on glomerular permeability, i.e. an increase in ultrafiltration coefficient without modification in capillary glomerular hydrostatic pressure [23]. On the other hand, previous studies have actually demonstrated a prominent effect of CEB on glomerular afferent artery, and a decrease in glomerular afferent arteriolar resistances without change in efferent resistances may account for an increase in GFR without change in RPF [15].

Furthermore, in our study, nifedipine clearly ameliorate tubular function in IPK preserved in Na-UW, but not in K-UW. The effects of CEB on renal tubular epithelium have been examined in experimental studies. In addition to morphological alterations, studies performed in suspension of proximal nephron segments have demonstrated a profound and significant increase in 45Ca uptake after 30 min of anoxia [23]. CEB, either verapamil or nifedipine, prevented the increase in calcium uptake and improved membrane integrity as evidenced by electron microscopy. In this connection, the improvement of tubular function in the Na-UW group added with nifedipine may account for the observed increase in GFR via the tubuloglomerular feedback mechanism. As a matter of fact the improvement of NaCl reabsorption may modulate NaCl concentration in tubular urine, and it is clearly demonstrated that a change in NaCl concentration at the contact with the macula densa induces opposite changes in GFR [24]. Interestingly, Figure 4 shows that there is a negative correlation between the amount of Na reabsorbed by the IPK tubules and final concentration of Na in urine, when all groups are plotted. On the other hand the urinary concentration of Na in urine is correlated with GFR. In the IPK model distal nephron function is compromised as can be seen by a defect in urinary concentration ability due to the lack of vasopressin and the supraphysiological RPF, and suppression of Na reabsorption due to the lack of aldosterone [8]. Thus sodium concentration in final urine may reflect that of the end of the thick ascending limb of Henle’s loop, and the lower sodium concentration, the higher GFR increases by an inhibition of the tubuloglomerular feedback mechanism. Changes in GFR by this mechanism may be mediated either by dilatation of afferent arteriole or by an increase in glomerular permeability [23] or both.

In conclusion, this study using the IPK model shows that a high-potassium UW but not high-sodium UW cold-storage solution induced an increase in RVR when flushed into the kidney. This vasoconstriction is prevented by nifedipine. However, addition of nifedipine to the flush, the cold-storage solution for 24 h and to the normothermic perfusate, improved the recovery function of the IPK cold-stored in high-sodium UW but not in high-potassium UW. Thus, nifedipine may be of potential effects in preventing or attenuating ischaemic injury by a mechanism which does not involve its vasodilatory properties. This drug may interfere on the hypoxic-induced disturbance in cellular calcium metabolism or act by other mechanisms, such as inhibition of ion channel activities or prevention of
cell swelling. Further studies are needed to clearly evaluate the potential clinical role of CEB in the prevention of ischaemic acute renal failure post transplantation.

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Abbreviations

AR\textsubscript{Na} Absolute Na reabsorption  
CEB Calcium entry blockers  
EC EuroCollins’ cold-storage solution  
FR\textsubscript{Na} Fractional Na reabsorption  
GFR Glomerular filtration rate  
IPK Isolated perfused rat kidney  
RPF Renal perfusate flow rate  
RVR Renal vascular resistances  
UW University of Wisconsin cold-storage solution

References


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