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# Na-K/2Cl transporter inhibition for reduction of postischemic kidney failure tested in autologous reperfusion

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**Abstract** Postischemic kidney function may be influenced by donor conditioning. The sulfamoylbenzoate "piretanide" (P) is a diuretic agent with an inhibitory effect on the luminal Na-K-2CL-transporter system in the ascending part of the loop of Henle. A clinical pilot study demonstrated a lower rate of organ dysfunction following transplantation in humans when the donor organs were pretreated with piretanide. In an experimental ex vivo model the effect of piretanide on immediate organ function following long or short cold ischemia was studied. Porcine kidneys ($n = 36$) were removed after in situ transaortal hypothermic flushing with 2 l Eurocollins solution. Following short storage (1 h, $n = 18$) or long storage (24 h, $n = 18$) the kidneys were reperfused with intraoperatively drawn heparinized autologous blood diluted with Ringer's lactate to a hematocrit of 25 %. Urine flow was higher in the piretanide-pretreated group (p), especially after long storage. The electrolyte loss was comparable in both groups. Postischemic endogenous creatinine clearance was significantly elevated in the treatment group (4.45 ± 0.6 ml/min per 100 mg in P vs 1.91 ± 0.4 ml/min per 100 mg, in control, $P < 0.05$ Mann-Whitney test). Renal hemodynamics were improved by piretanide, resulting in significantly lower resistance and allowing higher flow during pressure-controlled perfusion. $O_2$ consumption, representing general metabolic activity, was higher after long storage, indicating an earlier recovery from cold ischemia. In this ex vivo model, autologous reperfusion of porcine kidneys could be improved by piretanide pretreatment. Autoregulation of kidney vasculature was maintained as well as functional parameters such as creatinine clearance or gluconeogenesis. Therefore, piretanide may be used in larger clinical trials to further improve organ quality in times of donor shortage.

**Key words** Piretanide · Kidney transplantation · Donor conditioning · Ex vivo hemoperfusion

## Introduction

The concept of improving organ quality by donor pretreatment has gained importance in clinical transplantation. The incidence of early graft failure (ARF) following allogeneic cadaver kidney transplantation is an important clinical parameter [12] independent of immunological aspects such as HLA mismatch or antibody status of the recipient. Piretanide (P), a loop diuretic with a similar chemical structure to furosemide but different pharmacodynamic properties [3], inhibits the energy dependent luminal Na-K/2Cl ion transportation in renal

tubular cells and thus contributes to higher ATP concentrations during and after the cold ischemia period [8]. In addition, piretanide influences arachidonic acid metabolism resulting in vascular effects that attenuate postischemic vasoconstriction [9]. Both effects may be of advantage in kidney conservation if piretanide is given to the donor. In a clinical pilot study, a beneficial effect of this concept could be demonstrated through a reduction of the ARF rate in patients receiving piretanide-pretreated donor organs [1]. Because of these direct clinical implications, it was the aim of this experimental study to verify these preliminary results and to describe early functional characteristics of piretanide-pretreated kidneys after short and long cold ischemia time in a standardized autologous perfusion model.

## Material and methods

Thirty-six kidneys from German Landrace pigs (mean weight 22.7 ± 3 kg) were studied. The experiments were approved by the local board of animal protection, as required. Under deep anesthesia following arterial and venous catheterization, a laparotomy was performed and the bladder canulated. Mean arterial pressure, heart rate and $PO_2$ were continuously monitored. The treatment group ($n = 18$) received 1.6 mg/kg body weight i.v. piretanide for 30 min before organ removal, followed by systemic heparin administration (3000 I.E.). Transaortal in situ hypothermic perfusion with 3000 ml Eurocollins (EC) solution was used and warm ischemia was avoided.

Half the organs (mean weight 63 ± 5 g) were stored for 1 h on ice and the other half for 24 h, resulting in four groups: piretanide short storage, piretanide long storage, control short storage and control long storage (each $n = 9$) (P = piretanide, C = control, SS = short storage, LS = long storage).

Intraoperatively drawn autologous heparinized blood was diluted and kept at a constant hematocrit of 25 %, resulting in a volume of 450 ml per experiment. The pH value was maintained at physiological values using bicarbonate titration. $PO_2/PCO_2$ were adjusted by changing the gas flow to the system (resulting in $PO_2$ of > 200 mm Hg and $PCO_2$ of 37–40 mm Hg). The perfusion system (Fig. 1) consisted of two separate precision pumps, one to control oxygenator circulation into a reservoir and one to control pulsatile kidney perfusion at a defined pressure of 100 mm Hg. White blood cell counts did not significantly decrease during oxygenator passage as controlled by washout experiments. Perfusate flow was recorded on-line using a digital interface. The ratio of flow:pressure was calculated as whole organ resistance (R).

The kidney was positioned in the system following arterial canulation and ureteral canulation. The first fraction of 70 ml venous outflow was separately collected as effluate (high potassium, EC solution), the experiment was then continued in a closed circulation. Changes in hematocrit were substituted with 0.9 % NaCl solution according to the amount of collected urine and according to the measured hematocrit. The experimental period was limited to 1 h.

At 0, 5, 15, 30, and 60 min blood samples were taken and at the end the kidney was removed for weight determination and prepared for histological examination.

For statistical purposes, Student's *t*-test or the Mann-Whitney test was used for the comparison of the numerical data of two independent groups.

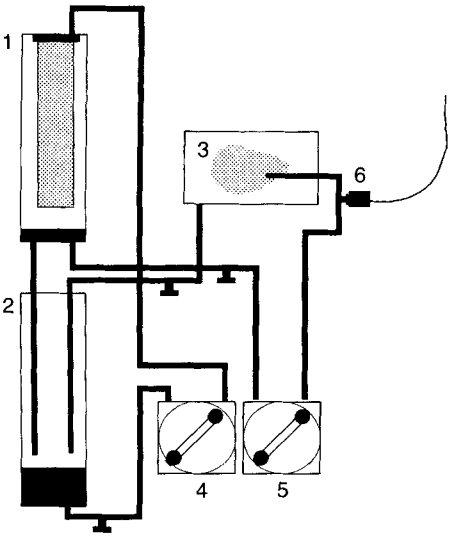


**Fig. 1** Depiction of perfusion system. (*1* oxygenator, *2* reservoir, *3* perfusion chamber, *4* oxygenation pump, *5* perfusion pump, *6* pressure monitoring)

## Results

### Weight

Significant differences in weight increase were found only following short storage (9.5 ± 2.9 g P vs 23.4 ± 7.1 g C; $P < 0.01$). Long storage resulted in comparable weight increases (24.8 ± 6.1 g C vs 25.1 ± 4.2 g P; n.s.).

### Urine flow

There were no statistical differences in urine flow or osmolarity with or without piretanide pretreatment. However, following SS, urine flow was about 50 % higher (3 ml/min) compared to LS (2 ml/min).

### Creatinine clearance

Creatinine clearance was improved by P in both SS and LS, reaching statistical significance. Clearance of control organs in situ before surgical manipulation of the donor animal was 40 ml/min per 100 g.

### Resistance

Piretanide lowered whole-organ resistance in both SS and LS (Fig. 2). After SS, P led to a decrease to 52 % of baseline value, whereas in C resistance values remained significantly higher after 20 min until the end of the experiments. Similar results were seen in the LS group,

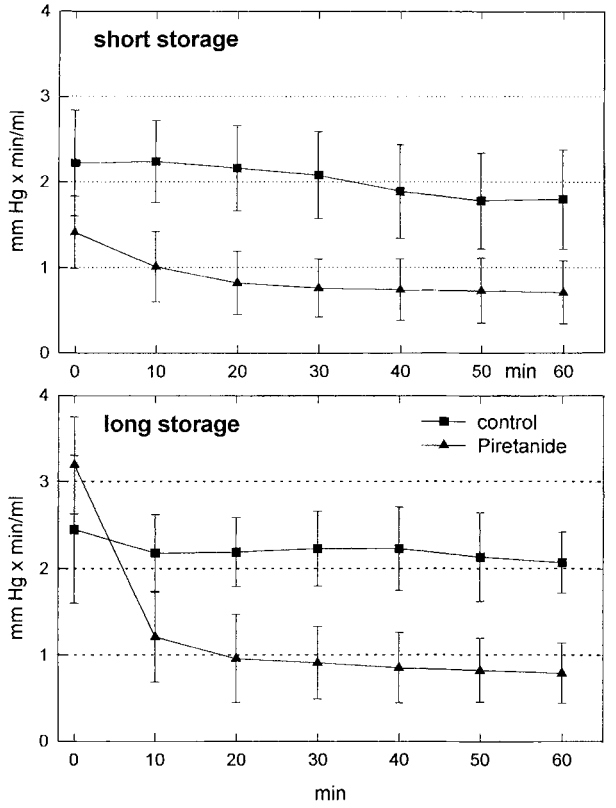


**Fig. 2** Whole-organ resistance of kidneys, a recorded during perfusion. $P < 0.05$, Mann-Whitney test P vs C)

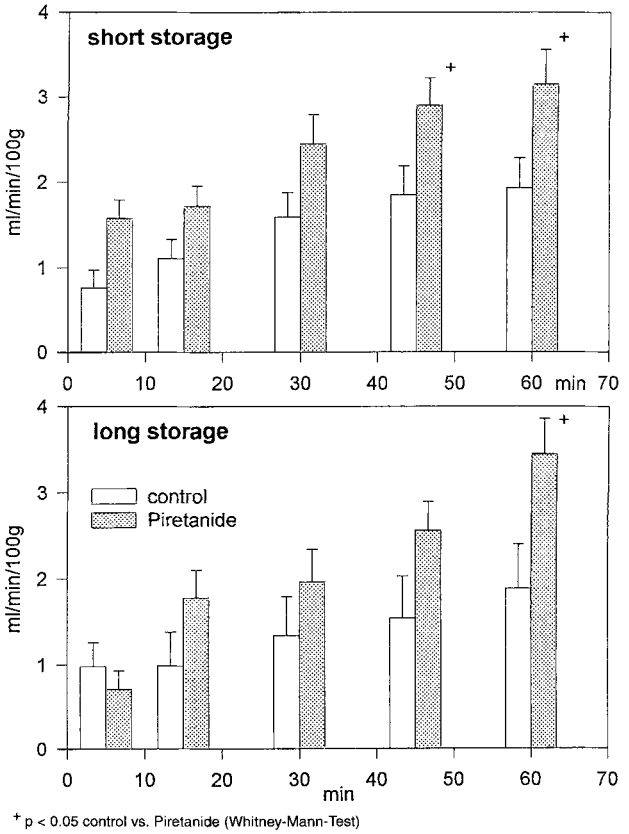


**Fig. 3** Oxygen consumption ($AVDO_2$) of reperfused kidneys

with the difference that P kidneys needed 10 min of low-flow perfusion before reaching physiological resistance values of $< 1.5$ mm Hg $\times$ min/ml. When calculating flow:GFR (vascular resistance), the differences were more obvious, but due to large SD values did not reach significance.

## Oxygen consumption

Oxygen consumption was quantified by the $AVDO_2$ measured in the arterial inflow line and in the venous outflow of the organ (Fig. 3). All kidneys of the treatment group exhibited a higher $AVDO_2$, reaching statistical significance in SS after 30 min of reperfusion and in LS after 45 min ($P < 0.05$, Mann-Whitney test).

## Serum blood glucose levels

No glucose was added to the blood during the experimental closed circulation. P kidneys led to significantly lower glucose levels in both SS and LS at all time points, thus effectively reducing gluconeogenesis.

## Cumulative electrolyte excretion

Special attention was paid to electrolyte excretion in the urine. Even though creatinine clearance was higher in P, the cumulative electrolyte loss was equal in P and C. Depending on the total urine flow, there were differences between SS and LS. All electrolytes showed a similar linear profile of excretion (potassium excretion was higher in LS compared to SS) thus P showed, despite an increased creatinine clearance, a relative sodium and potassium sparing effect.

## Histology

In paraffin-embedded sections, differences were observed between the two groups which became more pronounced following LS. In the treatment group, medullary edema and tubular damage, such as epithelial disruption and cell debris in the tubular lumen, was reduced. Glomerula of untreated kidneys showed retraction of capillaries, loose cytoplasm, and flat epithelium with vacuolisation. These changes were not seen in the piretanide group.

## Discussion

This study was undertaken to investigate kidney function following donor pretreatment with piretanide in a transplantation model. Autologous blood rather than allogeneic blood was used for reperfusion to exclude immunological, antibody-mediated reactions, e.g., isoagglutinin-associated blood group incompatibility. The pig is known to have 15 blood groups, with occasional isoagglutinins [5] that might interfere with mere reperfusion phenomena. Another advantage of ex vivo hemoperfusion, as developed by our group, is the possibility of pressure-controlled perfusion, which is not possible in other transplantation models. To judge kidney protection during ischemia, physiological parameters of reperfusion are accepted as being most adequate.

In our experiments, the plasma filtration of the reperfused kidneys was sufficient, as judged by the amount of urine produced. The observed creatinine clearance was lower than expected (1–5 ml/min), but no data exist for comparing how isolated porcine kidneys function in the first hour of hemoperfusion. However, our results are comparable with the results of Bretschneider obtained from canine kidneys after in situ ischemia with EC or HTK solution [6].

A positive effect of piretanide pretreatment could nevertheless be demonstrated, since in both groups (short and long storage) creatinine clearance was increased. Another very important parameter is the profile of renal hemodynamics, because kidney microvasculature is subject to autoregulation. The calculated whole-organ resistance reflects the quality of reperfusion; in our system pressure-controlled perfusion was used at 100 mm Hg mean arterial pressure, which resembles the physiological situation. Microcirculation of the renal cortex is difficult to monitor. Intravital microscopy can only visualize capsular capillaries which do not necessarily reflect the situation in vessels of the renal cortex [2]. A new possible way to assess cortical perfusion is the use of a laser doppler perfusion imaging system that allows continuous scanning of the organ surface [15].

In our study, whole-organ resistance was significantly reduced by piretanide in the reperfusion period, thus allowing a higher renal blood flow with higher glomerular filtration. This mechanism is probably due to altered arachidonic acid metabolism [9]. Postoperative vasoconstriction can also be reduced by direct cyclooxygenase inhibition, which leads to a decrease in thromboxane release in the venous effluate directly after declamping [16]. It can be speculated, that piretanide acts in a similar way.

Improved capacity to consume oxygen is a further important parameter reflecting early recovery after nephroplegia. During cold ischemia, anaerobic glucolysis maintains structural metabolism, and, at reperfusion, the organ switches again to aerobic metabolism. Considering their relative weight in the organism, kidneys are the organs with the highest potential for gluconeogenesis [13]. In our experiments, there was an improved blood glucose regulation in piretanide-pretreated kidneys, in the sense of reduced hyperglycemia in the perfusate. In transplantation, this effect is difficult to observe, since released glucose is immediately metabolized by other organs (liver, muscle and brain). To our knowledge, not much attention has been paid to the phenomenon of glucose release by reperfused kidneys. The mechanism, by which piretanide influences glucose release, can only be speculated upon. A link to improved ATP content in the distal tubular cells and the reduced need for anaerobic glucolysis is a possible hypothesis that should be the object of further investigation.

All the observed parameters of kidney function suggest an advantage in using piretanide over no treatment. A direct comparison with furosemide was not done, because furosemide pretreatment does not seem to influence subsequent renal allograft function [7, 14]. Piretanide has a higher potential for diuresis and electrolyte excretion than furosemide [10], as has been shown in patients with renal insufficiency [11]. Another advantage is the reduced ototoxicity of piretanide [4]. In the clinical setting, the transplanted patient is treated with high doses of furosemide and dopamine in the postoperative phase. These measures were not used in our experimental study, but, in the already mentioned clinical pilot study, there was an effect of piretanide on the ARF rate [1].

For further investigation of the multiple effects of piretanide pretreatment for postischemic kidney function independent of the postoperative treatment, transplantation studies must be carried out, since in an ex vivo model it is not possible to imitate the ARF situation. Different dosages and combinations of pre- and postischemic treatment should also result in improved organ protection in humans.

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