Kidney International, Vol. 37 (1990), pp. 1240–1247

Red cell trapping after ischemia and long-term kidney damage. Influence of hematocrit

P. OLOF A. HELLBERG, ALFRED BAYATI, ÖRJAN KÄLLSKOG, and MATS WOLGAST

Department of Physiology, University of Uppsala, Biomedical Center, Uppsala, Sweden

Red cell trapping after ischemia and long-term kidney damage. Influence of hematocrit. The influence of the hematocrit (Hct) on the trapping of red blood cells (RBC) in the renal microvasculature and its effect on the long-term outcome following unilateral ischemia were investigated in the rat. The results showed that an increase in the duration of ischemia increased the RBC trapping, as measured by ^{51}Cr-labeled erythrocytes, in a dose-dependent manner. At normal Hct (46%) the period of ischemia producing half-maximum RBC trapping was 45 minutes, whereas after hemodilution (Hct = 31%) or hemoconcentration (Hct = 60%) the corresponding periods were 80 and 25 minutes, respectively. Regarding the long-term outcome, 45 minutes of ischemia with a normal Hct was associated with a marked decrease in kidney weight, GFR and urine osmolarity after four weeks of recovery, which could be prevented to a large extent by hemodilution. Conversely, with hemoconcentration there was severe damage after only 25 minutes of ischemia. It is suggested that these long-term effects are attributable to RBC trapping in the microvasculature of the outer medulla, which may cause added ischemia in this area of the kidney. It is also suggested that cortical atrophy is secondary to the medullary injury, and is brought about to avoid extensive water and salt losses.

Most authors have focused attention on the renal cortex in attempts to explain the functional deficit in ischemic acute renal failure (ARF) [1]. However, the inability to produce hyperosmolar urine and to secrete potassium, both common features of ischemic ARF, suggests an impairment of the function of the renal medulla [2–6]. In ARF, caused by clamping of the renal artery, trapping of red blood cells (RBC) in the microvasculature of the renal outer medulla is a constant phenomenon, which has been suggested to cause a regional reperfusion deficit in this part of the kidney [4–11]. The pathophysiological significance of this phenomenon, however, is controversial. Mason and coworkers have even suggested that there is a direct correlation between the RBC trapping and the decrease in the glomerular filtration rate (GFR) in the acute stage of postischemic renal failure [4, 5]. On the other hand, administration of hyperosmolar mannitol or sucrose *after* the ischemic injury is able to improve renal clearance parameters even though it does not reduce the RBC trapping [12, 13]. Likewise, prevention of the RBC trapping by administration of free radical oxygen scavengers is associated with only little, if any, improvement in renal clearance parameters [12, 14]. A finding of no relationship between the RBC trapping and tubular necrosis after 24 hours of recovery has also been reported [15].

It seems possible, however, that in the acute stage of renal failure the functional impairment and morphological signs of injury resulting from the ischemia-reperfusion per se could obscure the damage caused by the RBC trapping in the outer medullary capillaries. Nevertheless, this trapping and consequently further medullary ischemia might influence the long-term outcome.

It has been proposed that RBC trapping is preceded by capillary leakage and subsequent hemoconcentration [10, 14, 16]. To investigate this possibility the hematocrit in renal venous blood during reperfusion was measured. The effect of hemodilution and hemoconcentration on RBC trapping was estimated from the intrarenal distribution of ^{51}Cr-labeled RBCs or visualized by scanning electron microscopy. Since the hematocrit was found to substantially affect RBC trapping in the acute stage of postischemic ARF, its influence on renal function and gross morphology, was investigated after four weeks of recovery from ischemia.

Method

Effects of the length of the ischemic period and the hematocrit on the RBC trapping

These studies were performed on 48 male Sprague-Dawley rats (Möllegaard, Denmark) weighing 200 to 270 g. They had free access to tap water and standard rat chow (1324, Altromine International, FRG). Anesthesia was induced by an intraperitoneal injection of Inactin® (Byk, Gulden, Konstanz, FRG) in a dose of 120 mg · kg^{-1} body weight. The animals were then placed on a servo-controlled heating pad, which kept the body temperature at 37.5°C, and tracheostomized. Catheters were inserted into the right femoral artery and vein, the former for monitoring of blood pressure and withdrawal of blood samples and the latter for continuous infusion of Ringer solution (containing 120 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl and 0.75 mM $CaCl_2$) at a rate of 5 ml · kg^{-1} body wt · hr^{-1}. The left kidney was exposed through a midline incision and dissected free. After completion of the surgical procedures the hematocrit was either left unaltered, elevated or reduced as follows:

A high hematocrit was evoked by transfusion of 5 ml of red cell concentrate prepared on the day of the experiment. For this purpose whole blood was collected from a donor animal into an

Received for publication February 21, 1989
and in revised form December 4, 1989
Accepted for publication December 18, 1989

acid-citrate-dextrose solution. After centrifugation the plasma fraction and the leukocyte-rich "buffy coat" were removed and the red blood cells were washed once in saline. The red cells were then resuspended in saline to a packed cell volume of about 80% and 5 ml of this suspension was slowly injected into the femoral vein.

A low hematocrit was produced by isovolemic hemodilution. For this purpose 5 ml of a 5% albumin-Ringer solution (Human albumin, Kabi, Sweden) was infused into the femoral vein while an equal volume of whole blood was withdrawn from the femoral artery simultaneously. After completion of the transfusion, the rats were allowed to recover for 30 minutes.

The intrarenal distribution of red blood cells was measured essentially as described by Karlberg et al [10]. Briefly, 5 ml of blood was withdrawn from a donor animal into an acid-dextrose solution. After centrifugation and removal of the plasma fraction and "buffy coat", the red cells were incubated with about 100 μCi of sodium-^{51}chromate (Amersham Int., Amersham, UK) for one hour at room temperature. The erythrocytes were then washed three times in saline and kept cold (4°C) until used. As a rule 0.2 ml (5 μCi) of the ^{51}Cr-labeled erythrocytes, injected intravenously before induction of ischemia, was used to determine the regional renal red cell volume.

Ischemia was evoked by occluding the left renal artery and ureter, the latter to avoid collateral circulation by this pathway, for either 10, 25, 45, 60 or 90 minutes, during which time the abdomen was kept closed. The occlusions of the renal artery and ureter were then released and after 15 minutes of reperfusion both the left postischemic and the right intact kidney were removed together with a reference blood sample. Using a razor blade, the kidney was dissected under a microscope into the cortex, outer stripe and inner stripes of the outer zone of the medulla and the inner zone of the medulla, as described elsewhere [10]. The kidney specimens were weighed and analyzed for their ^{51}Cr activity in a gamma-spectrophotometer. The fractional distribution volume of red blood cells could then be calculated as the activity in the individual kidney specimens divided by the activity in the systemic blood.

Scanning electron microscopy (SEM)

A total of 28 Sprague-Dawley rats (210 to 250 g body wt) were used. After anesthesia and surgical preparation as described above, the left kidney was subjected to 45 minutes of ischemia. Prior to the ischemia the hematocrit was either reduced, elevated or left unaltered as described above. The following groups, each containing four animals, were studied: unaltered hematocrit and 15 minutes, two hours and 24 hours of reperfusion; hemoconcentration with two hours and 24 hours of reperfusion; and hemodilution with two and 24 hours of reperfusion.

In animals subjected to 24 hours of reperfusion, anesthesia was performed as in the long-term studies described below.

For preparation of tissue sections for the SEM study, the kidneys were immersion-fixed for 24 hours at room temperature in 2.5% glutaraldehyde (Kebo Lab AB, Stockholm, Sweden) in a phosphate buffer solution (pH 7.4). With use of an Oxford vibratome 100 μm thick sections were cut and post-fixed at room temperature in 1% osmium tetroxide (Expectron Man. AB, Stockholm, Sweden) for 24 hours. After dehydration in graded series of acetone, the sections were dried in a critical point drying apparatus (Polaron, Agar, UK), mounted on Cambridge aluminum stubs and coated with a 400 Å gold layer in an ion sputter (Jeol JFC 1100, USA). A Philips 525 scanning electron microscope set at an acceleration voltage of 20 kV was used.

To exclude preparation artifacts, parallel studies were performed on the contralateral kidney in each animal.

Measurement of hematocrit in renal venous blood

Ten Sprague-Dawley rats (body wt 210 to 240 g) were used. After induction of anesthesia, as described above, a catheter was placed in the femoral artery for continuous measurement of blood pressure. The left kidney was exposed through a flank incision and supported in a lucite cup. In order to cut off collateral circulation the left ureter was divided and the distal end was supplied with a ligature. The left renal artery was then occluded for 5, 25, 45, 60 or 90 minutes (N = 2). During ischemia a 20 mm long silicone catheter with a diameter of 1 mm was inserted into the collapsed renal vein. On termination of the ischemic period the renal artery occlusion was removed. When blood appeared in the renal vein ten samples of venous blood were collected into 100 μl microcaps (Drummond Scientific Company, USA) at intervals of 3 seconds. The hematocrit was determined by sedimentation. Protein concentrations in plasma were measured by the method of Lowry et al [17]. Renal blood flow was calculated from the volume of the samples.

Long-term studies

This series comprised 48 male Sprague-Dawley rats weighing 190 to 225 g, which had free access to tap water and standard rat chow (1324, Altromine International, FRG).

Anesthesia was induced by Brietal® (Lilly International, USA) given as an intraperitoneal injection in a dose of 50 mg · kg^{-1} body wt, followed by repeated small doses when required. Otherwise the surgical procedure was identical to that described above. The animals were divided into six groups with eight in each.

Group 1. Hemoconcentration, as described above, and 45 minutes of ischemia.

Group 2. No transfusions and 45 minutes of ischemia.

Group 3. Hemodilution, as described above, and 45 minutes of ischemia.

Group 4. Hemoconcentration and 25 minutes of ischemia.

Group 5. This group was used to investigate whether the transfusion procedure per se, that is, irrespective of the hematocrit, contributed to the long-term kidney damage. For this purpose red blood cells were resuspended in saline to a final concentration of 45%. Five milliliters of this suspension was injected into the femoral vein, while at the same time an equal volume of blood was withdrawn from the femoral artery.

Group 6. These animals served as a sham group and were treated in the same way as the others except that there were no transfusions or ischemia.

After completion of the transfusion procedures the animals were allowed to recover for 30 minutes. Ischemia was then evoked by ligation of the left renal artery and ureter, during which time the abdomen was kept closed. After removal of the ligatures the abdomen was sutured, the catheters were removed, and the corresponding femoral vessels were ligated. When they woke the animals were returned to their cages.

Nephron function after four weeks of recovery

Four weeks after ischemia, the animals were anesthetized by an intraperitoneal injection of Inactin® (Byk, Gulden, Konstanz, FRG) in a dose of 120 mg · kg^{-1} body wt, placed on a servo-controlled heating pad and tracheostomized.

Catheters were inserted into both ureters, through a supravesical incision. The right femoral artery was catheterized for monitoring of blood pressure and withdrawal of blood samples and the femoral vein for infusion of Ringer-bicarbonate solution (5 ml kg^{-1} body wt · hr^{-1}); this solution also contained 5 μCi · ml^{-1} of ^{3}H-inulin. After a bolus injection of 1 ml of the above solution followed by continuous infusion, the animals were allowed to recover for one hour.

Urine was then collected from both kidneys for one hour. In the middle of this period a reference blood sample was withdrawn. The ^{3}H activity in the plasma and urine were measured by the liquid scintillation technique.

Urine osmolarity was determined by the freezing point depression method. The sodium and potassium concentrations in the plasma and urine were determined by flame photometry.

Histological procedure

After completion of the functional studies, 2 ml of 1% Alcian blue (Kebo AB, Stockholm, Sweden), a stain which because of its positive charge adheres to the glomerular basement membrane, was injected intravenously. After five to ten minutes both kidneys were removed, bled out, separated from the capsule, weighed, and deep-frozen.

By means of a cryomicrotome, a central 250 μm thick sagittal section was cut. The section was put under a microscope and the cortex was separated from the medulla with a tiny knife. Using 40 times magnification, the glomeruli, which were easily identified by their blue color, were counted. All kidney specimens were then dried at 100°C for 24 hours and weighed. The number of glomeruli per mg dry weight cortex tissue could then be calculated. The number of glomeruli in the kidney as a whole was calculated by multiplying this number by the dry weight of the entire cortex, the latter being assumed to be 63% of the total kidney dry weight, since in a normal rat the cortex accounts for about 63 volume % of the total renal parenchyma [18].

In one representative kidney in each group the diameter of at least 50 randomly selected glomeruli were determined, using a microscope with 400 times magnification. In these kidneys the cortex to medulla dry weight ratio was estimated by comparing the dry weights of the cortical and medullary specimens of the central sagittal slice.

Statistics

All values are given as mean ± 1 SE. Statistical evaluation was performed by one factor ANOVA and Sheffe's test was used to compare groups. A P value less than 0.05 was considered significant.

Results

Figure 1 shows the intrarenal RBC distribution after 25, 45, 60 and 90 minutes of ischemia in animals with a normal hematocrit. Values for nonischemic kidneys were obtained from the contralateral kidneys. It should be pointed out that these figures refer to a bled-out kidney, which explains the low values for the normal kidney.

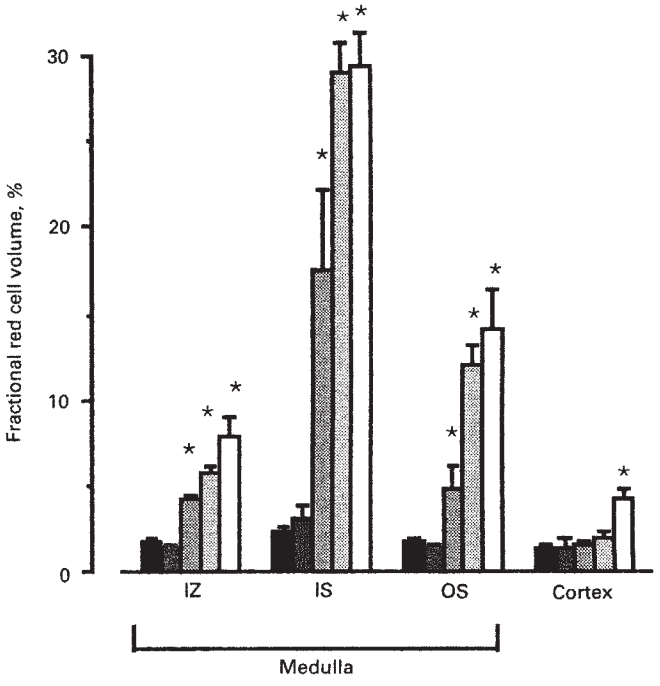

Fig. 1. *Fractional distribution volume of red blood cells in the kidney after 0 to 90 min of ischemia and 15 min of reperfusion.* The kidney was dissected into cortex, outer stripe (OS), inner stripe (IS) and inner zone (IZ) of the medulla. Values are means ± 1 SE. The value for a normal kidney refers to the contralateral kidney. (*) indicates a significant difference compared with the contralateral kidney ($P < 0.05$). Symbols are: (■) 0, (▩) 25 min, (▨) 45 min, (▧) 60 min, (□) 90 min ischemia.

Evidently the RBC trapping was concentrated to the renal medulla, and in particular to the inner stripe of the outer medulla. In contrast, the inner medulla was much less affected. Only after 90 minutes of ischemia was there any evidence of RBC trapping in the renal cortex.

After hemodilution, which reduced the hematocrit from 46 ± 1 to 31 ± 1%, the RBC trapping in the inner stripe of the outer medulla was considerably decreased (Fig. 2). Conversely, hemoconcentration, which increased the hematocrit to 60 ± 1%, was associated with substantial RBC trapping after only 25 minutes of ischemia.

Although the time of ischemia required to produce RBC trapping was longer in the hemodiluted and shorter in the hemoconcentrated animals, as compared with the normal hematocrit groups, the intrarenal RBC distribution was the same, that is, when it occurred, the RBC trapping was confined to the outer medulla (Table 1). It may also be noted that in the intact (right) kidney, the RBC distribution volume varied only in proportion to the hematocrit.

Scanning electron microscopy

After 45 minutes of ischemia and 15 minutes of reperfusion in animals with a normal hematocrit, most capillaries in the outer medulla were filled with densely packed erythrocytes (Fig. 3B). As a rule, however, the vasa recta in the center of the vascular bundles contained no RBC aggregates but only a few normally

Table 1. Fractional red cell volume after 15 minutes of reperfusion

Hematocrit	Ischemia *min*	Cortex	Outer stripe	Inner stripe	Inner zone
			%		
Normal	0	1.2 ± 0.1	1.7 ± 0.2	2.3 ± 0.2	1.0 ± 0.1
Normal	25	1.4 ± 0.5	1.4 ± 0.2	3.1 ± 0.5	1.4 ± 0.2
Normal	45	1.6 ± 0.2	4.9 ± 1.3	18 ± 4.5	4.5 ± 0.4
Normal	60	1.9 ± 0.4	12 ± 1.2	29 ± 1.7	5.9 ± 0.4
Normal	90	4.3 ± 0.6	15 ± 2.3	30 ± 1.8	8.1 ± 1.2
Hemodilution	0	0.6 ± 0.1	0.9 ± 0.1	1.7 ± 0.1	0.6 ± 0.1
Hemodilution	45	0.9 ± 0.2	1.3 ± 0.2	2.0 ± 0.4	0.5 ± 0.1
Hemodilution	60	1.6 ± 0.4	6.0 ± 2.2	12 ± 3.7	3.2 ± 0.6
Hemodilution	90	2.5 ± 0.4	9.1 ± 1.8	32 ± 1.6	6.7 ± 1.0
Hemoconcentration	0	1.3 ± 0.2	1.8 ± 0.2	3.3 ± 0.3	1.4 ± 0.4
Hemoconcentration	10	1.1 ± 0.2	1.6 ± 0.3	4.6 ± 1.0	1.9 ± 0.2
Hemoconcentration	25	1.8 ± 0.4	11 ± 2.0	23 ± 4.6	7.2 ± 2.4
Hemoconcentration	45	3.6 ± 1.2	18 ± 2.0	32 ± 1.6	9.3 ± 2.2

Values are means ± SE. Each group consisted of 4 animals. The values for 0 ischemia were obtained from the contralateral kidneys in each group.

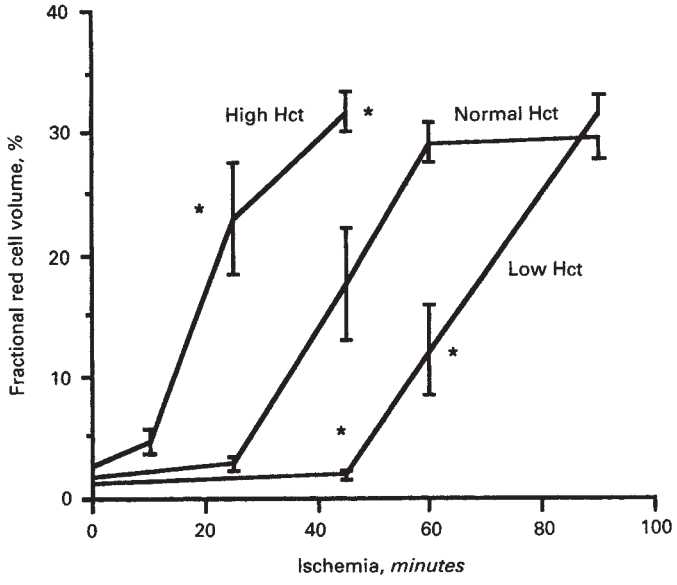

Fig. 2. *Fractional red cell volume in the inner stripe of the outer medulla after various periods of ischemia and 15 min of reperfusion in animals with a high (60 ± 1%), normal (46 ± 1%) and low (31 ± 1%) hematocrit.* Values are means ± 1 SE. Each group consisted of 4 rats. (*) indicates a significant difference compared with the normal hematocrit group.

shaped erythrocytes. The tubules appeared compressed by the distended capillaries. This should be compared to the findings in the normal contralateral kidney (Fig. 3A) where the tubules appeared with their normal round configuration and the capillaries with their normal narrow lumen containing only a few erythrocytes. In the inner medulla and the renal cortex RBC aggregates were found only occasionally. After two hours there was no notable difference as compared to 15 minutes of reperfusion, and most capillaries in the outer medulla remained filled with RBC aggregates (Fig. 3C). After 24 hours of reperfusion, however, RBC aggregates were found only occasionally and the majority of capillaries in the outer medulla were open.

In hemoconcentrated animals virtually all capillaries in the outer medulla, except for the vasa recta, were filled with RBC aggregates after two hours of reperfusion. In contrast to the finding in animals with a normal hematocrit, an extensive number of capillaries in the outer medulla remained occupied by RBC aggregates after 24 hours of reperfusion (Fig. 3D). It was also evident that clotting is not present since neither fibrin streaks nor thrombocytes were found (Fig. 3B–D).

In animals subjected to hemodilution, RBC aggregates were virtually absent both after two and 24 hours of reperfusion.

Hematocrit in renal venous blood during reperfusion

The arterial pressure remained at the control level during the first 30 seconds of reperfusion, except in animals subjected to only five minutes of ischemia, in which the blood pressure decreased during the last four sampling periods, probably as a result of hypovolemia.

The renal blood flow during the initial reperfusion period was markedly reduced as compared with that in a normal kidney. The values obtained after 5, 25, 45, 60 and 90 minutes of ischemia were 1.60, 0.78, 0.42, 0.23 and 0.20 ml · min^{-1} · 100 g^{-1} body wt, respectively.

As shown in Figure 4, transient increases in the hematocrit of equal magnitude were found after 5 and 25 minutes of ischemia. In both instances the protein concentration in renal venous plasma increased simultaneously to a maximum value of 9.9% and declined to 5.8% after 30 seconds of reperfusion. After 45 minutes of ischemia there was no further increase in the protein concentration in renal venous plasma, while the hematocrit reached as high as 80%. Further lengthening of the duration of ischemia caused no further increase either in hematocrit or in the protein concentration in renal venous blood.

Long-term outcome

After four weeks of recovery all animals appeared healthy and there was no difference in body weight or in increase in body weight between the groups. Table 2 summarizes the urine excretion data obtained in the different groups. When the hematocrit was normal, 25 minutes of ischemia was followed by complete recovery, but after hemoconcentration this duration of ischemia resulted in severe depression of nephron function, with GFR only 25% of the sham value and the ability to produce hyperosmolar urine and excrete potassium severely reduced.

With a normal hematocrit, 45 minutes of ischemia resulted in

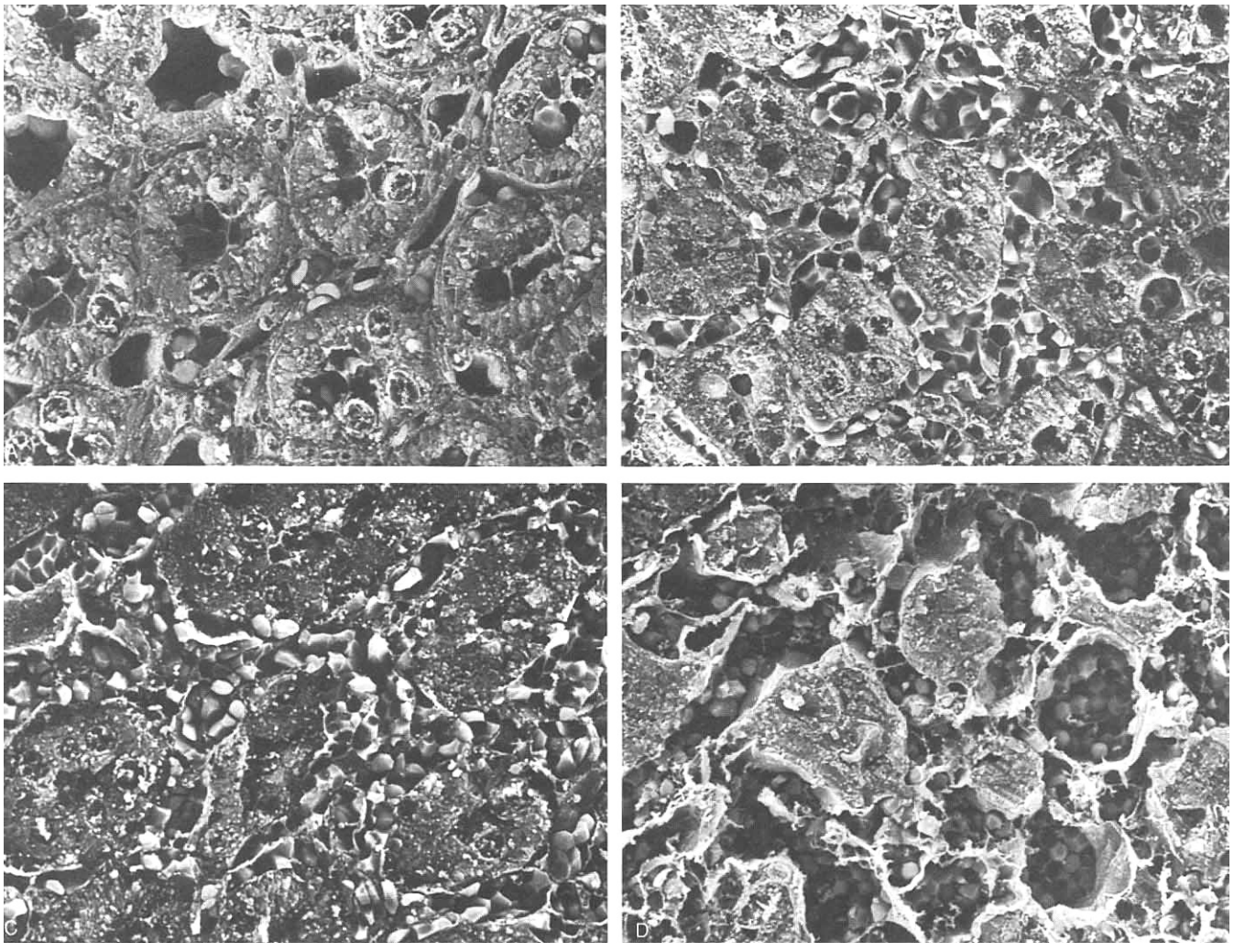

Fig. 3. *Scanning electron microscopy (× 1100) of the inner stripe of the outer medulla of a normal kidney* **(A)** *a kidney subjected to 45 min of ischemia and 15 min* **(B)** *and 2 hr* **(C)** *of reperfusion.* Fig **(D)** shows a kidney subjected to hemoconcentrated, 45 min of ischemia and 24 hr of reperfusion.

a decrease in GFR towards 15% of the sham value, urine osmolarity close to isosthenuria and potassium excretion less than half of the value in the sham group.

As expected, hemoconcentration resulted in even more severe impairment of these parameters, whereas hemodilution prevented the kidney damage considerably. Thus in the latter group GFR was about 60% of the sham value, and the potassium excretion and urine osmolarity were next to normal.

In the contralateral kidney, a compensatory increase in GFR was found. The impaired excretion of osmolar components and potassium in the damaged kidney was thus compensated by increased excretion in the contralateral kidney. Almost perfect balance between the postischemic and contralateral kidney regarding the sodium concentration in the urine was noted in all groups.

Postischemic atrophy

The kidney weights and the cortex/medulla dry weight ratios of the central sagittal slice of the kidneys are presented in Table 3. The size and number of glomeruli are also shown. Although there was a substantial reduction in kidney mass in the animals subjected to hemoconcentration and 45 minutes of ischemia, the cortex/medulla dry weight ratio did not differ from that in the sham group. In the most severely damaged kidneys there was a reduction in glomerular size, but not in the number of glomeruli.

It may be mentioned here that a glomerulus which is sectioned close to the equator will display a normal diameter and hence be recorded as a whole glomerulus. This will lead to an overestimation of the number of glomeruli. This error is simply R/L, where R is the radius of the glomerulus and L is the thickness of the section [for details see 19, 20]. Thus in the sham group the overestimation would be 20%. Since this error will vary with the size of the glomeruli, the corresponding errors in the small hypotrophic and the large contralateral hypertrophic kidneys would be 16% and 22%, respectively. This could explain the apparent decrease in the number of glomeruli in the small atrophic kidneys and the increase in the large hypertrophic ones.

Table 2. Nephron function after four weeks of recovery

Group	Body weight *g*	C_{In} *ml · min^{-1} 100 g^{-1} body wt*	U/P_{In}	U_{Osm} *mOsm · liter^{-1}*	U_K *mmol · liter^{-1}*	U_{Na} *mmol · liter^{-1}*	V_U *µl · min^{-1} 100 g^{-1} body wt*
High Hct 45 min ischemia	365 ± 10	0.03 ± 0.01[a]	60 ± 9[a]	462 ± 134[a]	55 ± 9[a]	16 ± 4[a]	0.41 ± 0.07
(Contralateral kidney)		(0.56 ± 0.03)[a]	(475 ± 44)	(2070 ± 96)	(315 ± 38)	(19 ± 3)[a]	(1.26 ± 0.16)[a]
Normal Hct 45 min ischemia	369 ± 7	0.06 ± 0.01[a]	145 ± 39[a]	575 ± 236[a]	92 ± 16[a]	31 ± 11[a]	0.51 ± 0.07
(Contralateral kidney)		(0.52 ± 0.04)[a]	(480 ± 78)	(1800 ± 187)	(280 ± 32)	(30 ± 6)[a]	(1.27 ± 0.18)[a]
Low Hct 45 min ischemia	379 ± 13	0.23 ± 0.03[a,b]	604 ± 133[b]	1479 ± 155[a,b]	248 ± 28[b]	35 ± 7	0.47 ± 0.06
(Contralateral kidney)		(0.39 ± 0.03)	(681 ± 95)	(2095 ± 161)	(291 ± 32)	(36 ± 6)	(0.68 ± 0.12)[b]
High Hct 25 min ischemia	373 ± 11	0.11 ± 0.02[a,b]	320 ± 47[a,b]	936 ± 88[a,b]	157 ± 21[a,b]	23 ± 4[a]	0.39 ± 0.06[b]
(Contralateral kidney)		(0.50 ± 0.03)[a,b]	(478 ± 62)	(2086 ± 114)	(390 ± 28)	(28 ± 4)[a]	(1.12 ± 0.01)[a,b]
Normal Hct 25 min ischemia	373 ± 12	0.41 ± 0.02	625 ± 79	1943 ± 123	278 ± 37	39 ± 9	0.75 ± 0.12
(Contralateral kidney)		(0.36 ± 0.03)	(704 ± 101)	(1956 ± 170)	(246 ± 46)	(36 ± 8)	(0.57 ± 0.08)
Sham	361 ± 12	0.40 ± 0.03	769 ± 111	2015 ± 140	251 ± 28	54 ± 9	0.61 ± 0.11
(Contralateral kidney)		(0.38 ± 0.04)	(759 ± 136)	(1970 ± 139)	(280 ± 29)	(54 ± 8)	(0.68 ± 0.16)

Values are means ± SE. All groups consisted of 8 rats

[a] significant difference compared with the sham group ($P < 0.05$)

[b] significant difference compared with animals with a normal hematocrit subjected to ischemia of equal duration ($P < 0.05$)

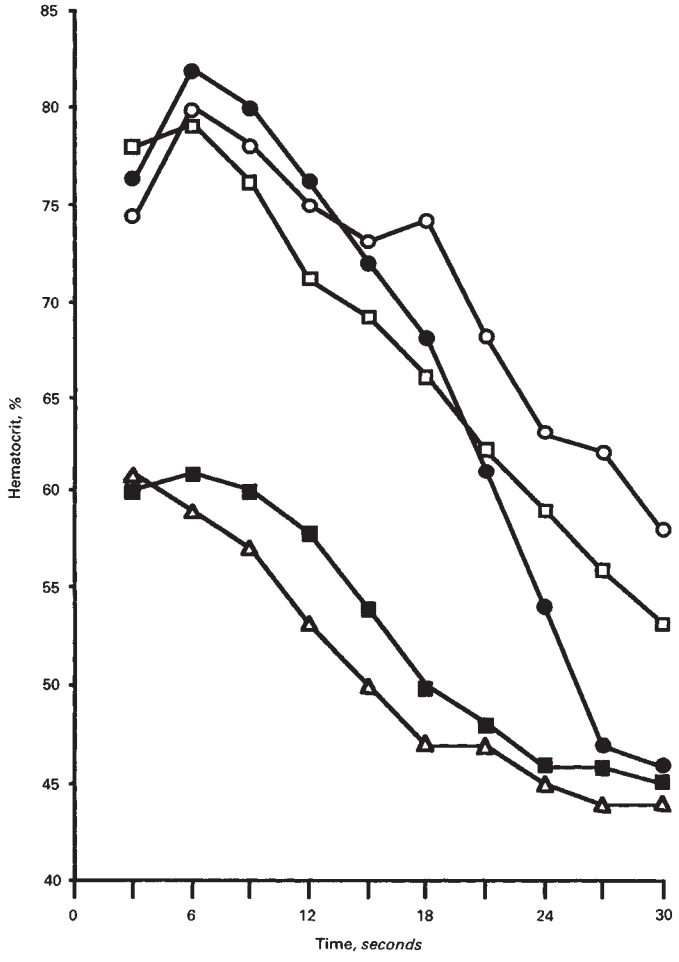

Fig. 4. *Hematocrit in renal venous blood during the first 30 seconds of reperfusion after 5 to 90 min of complete renal ischemia.* Symbols are: (△) 5 min, (■) 25 min, (●) 45 min, (□) 60 min, (○) 90 min ischemia.

Discussion

It is evident from the present study that the trapping of blood cells in the microvasculature of the renal outer medulla is related to the duration of ischemia in a dose-dependent manner. After hemodilution a longer ischemic period is required to produce the same extent of RBC trapping and after hemoconcentration a shorter ischemic period is required. Irrespective of the treatment, however, the same distribution of red cells and the same maximum red cell volume of about 30% in the inner stripe of the outer medulla was observed, probably representing the maximum vascular space in this area of the kidney [18, 21, 22]. Thus the trapping of blood cells in the vasculature of the outer medulla seems to depend both on the duration of the ischemia and on the systemic hematocrit.

It may be added here that RBC trapping is most likely not a consequence of blood clotting. Thus, neither heparin nor antithrombocyte aggregation drugs prevent the RBC trapping [5]. Furthermore, from the appearance of this trapping on scanning electron microscopy thrombocytes and fibrin streaks are clearly absent.

In our view, the RBC trapping in outer medullary capillaries occurs during the early reperfusion period [10] and is preceded by a local hemoconcentration, which if it reaches a critical level will lead to complete cessation of blood flow.

It is evident from the present study that even a period of ischemia as short as five minutes is associated with a transient increase in the hematocrit of renal venous blood during reperfusion to a value of about 60%, and with a simultaneous increase in the plasma protein concentration of the same magnitude. This is most likely a consequence of ultrafiltration in the glomerular capillaries and delayed reabsorption from the renal tubules. It is reasonable to assume that reabsorption cannot start before the renal tubules, which will collapse during ischemia, are refilled with glomerular filtrate.

When the length of the ischemic period exceeded 45 minutes there was no further increase in the protein concentration in renal venous blood, while the hematocrit rose to about 80%, indicating a loss of whole plasma during reperfusion. The latter is most likely a consequence of increased macromolecular permeability caused by ischemia-reperfusion injury [14, 16, 23–25]. Obviously if the systemic hematocrit is reduced, more severe capillary leakage, that is, a longer period of ischemia, will be required to produce critical local hemoconcentration.

Table 3. Renal atrophy after four weeks of recovery

Group	Wet weight *g*	Dry weight *mg*	Fractional dry weight ratio *cortex/medulla*	Glomerular diameter *μm*	Numer of glomeruli mg^{-1}*(d.w.) cortex*	Number of glomeruli *whole kidney*
High Hct 45 min ischemia	0.45 ± 0.05[a,b]	76.8 ± 8[a,b]	53/47	82 ± 0.5	771 ± 82[a,b]	35200 ± 3500
(Contralateral kidney)	(1.67 ± 0.06)[a]	(377 ± 8)[a]		(110 ± 0.5)	(197 ± 9)	(43100 ± 4500)[a]
Normal Hct 45 min ischemia	0.70 ± 0.07[a]	125 ± 12[a]	49/51	89 ± 1.0	467 ± 74[a]	33200 ± 2700
(Contralateral kidney)	(1.54 ± 0.07)[a]	(328 ± 17)[a]		(112 ± 1.1)	(190 ± 10)	(39000 ± 2100)
Low Hct 45 min ischemia	1.28 ± 0.06[b]	256 ± 16[b]	50/50	98 ± 0.9	231 ± 41[b]	38600 ± 2400
(Contralateral kidney)	(1.44 ± 0.04)	(343 ± 12)[a]		(104 ± 0.6)	(169 ± 8)	(36300 ± 1100)
High Hct 25 min ischemia	0.99 ± 0.10[a,b]	227 ± 19[a,b]	47/53	92 ± 0.9	260 ± 27[b]	34300 ± 1900
(Contralateral kidney)	(1.60 ± 0.06)[a,b]	(345 ± 8)[a,b]		(108 ± 0.7)	(165 ± 7)	(35700 ± 1100)
Normal Hct 25 min ischemia	1.37 ± 0.04	315 ± 8	54/46	100 ± 0.4	196 ± 11	39500 ± 2300
(Contralateral kidney)	(1.33 ± 0.05)	(314 ± 6)		(103 ± 0.8)	(181 ± 7)	(35700 ± 1300)
Sham	1.29 ± 0.05	292 ± 11	48/50	101 ± 0.8	203 ± 12	37000 ± 1600
(Contralateral kidney)	(1.35 ± 0.05)	(292 ± 12)		(102 ± 0.4)	(196 ± 11)	(35800 ± 1200)

Values are means ± 1 SE. All groups consisted of 8 rats. The glomerular diameter and fractional dry weight ratio of cortex/medulla were determined in one representative kidney from each group and refer to the central sagittal section of the kidney.

[a] significant difference compared with the sham group ($P < 0.05$)

[b] significant difference compared with animals with a normal hematoctrit subjected to ischemia of equal duration ($P < 0.05$)

Conversely, a short ischemic period may cause RBC trapping if the systemic hematocrit is high.

The RBC trapping is restricted to the medulla and in particular the outer zone, while the renal cortex is not affected. The latter may be explained by a higher blood flow velocity in the post-glomerular capillaries of the renal cortex as compared with the renal medulla [11, 26]. The longer transit time in the medullary capillaries will thus permit more plasma leakage and further hemoconcentration. The blood flow velocity will also influence the apparent viscosity of the blood [27], thus producing a higher resistance in the capillaries of the medulla and eventually complete cessation of blood flow with RBC trapping. It should be emphasized that this trapping occurs mainly in the outer medulla, while the inner zone is much less affected. The reason for this difference between the inner and outer medulla is not completely understood. However, the larger diameter and the more straight arrangement of the vasa recta, supplying the inner medulla, as compared with the capillaries in the outer medulla [28], may offer an explanation. Furthermore, RBC trapping in the outer medullary capillaries may cause shunting of blood through the outer to the inner medulla [11], which will facilitate the wash-out of highly viscous blood and thereby prevent RBC trapping in the inner medulla. Thus, the occurrence of RBC trapping in the outer medulla is not necessarily in conflict with the recent reports of an increase in blood flow in the inner medulla after ischemia [11, 29].

Regarding the long-term outcome, it was evident in the present study that just as hemodilution prevented the kidney damage so hemoconcentration enhanced it. It would seem most probable that this effect of the hematocrit is due to an influence on the RBC trapping in the outer medulla. This trapping will produce secondary hypoxia in the outer medulla, which means that the real ischemic period in this area of the kidney is not 25 or 45 minutes, but much longer. Regarding the duration of the RBC trapping, Mason, Thorust, and Welsch [4] found macroscopic evidence of such trapping after 18 hours of recovery following 45 minutes of ischemia, whereas Karlberg and coworkers [30] observed no persistent RBC trapping one day after the ischemic insult. In the present study extensive RBC trapping was evident on scanning electron microscopy after 45 minutes of ischemia and two hours of reperfusion in animals with a normal hematocrit, and after 24 hours of reperfusion in animals subjected to hemoconcentration. Thus, with high hematocrit the RBC trapping is not only more pronounced in the very acute stage of reperfusion, but also seems to last longer. The latter would seem most relevant for the long-term outcome.

In accordance with this reasoning, damage mainly to the outer medulla would be expected in the hemoconcentration animals. The present observation of a reduced potassium concentration and osmolarity in the urine in these kidneys as compared with those of the sham-operated animals indicates a functional deficit in the renal medulla.

Nevertheless, in these animals there was a proportionally equal reduction of cortical and medullary parenchyma and a substantial decrease in inulin clearance. However, none of these observation is necessarily indicative of a primary injury to the renal cortex. Thus, if a functional deficit of the renal outer medulla occurs, a reduction in GFR would seem essential for the body as a whole to avoid salt and water depletion.

Based on micropuncture experiments in which the single nephron filtration rate was compared with the whole kidney GFR, a reduction in the nephron population after ischemic injury has been suggested [30, 31]. This might seem contradictory to the present observation that the number of perfused glomeruli was equal in all groups. However, after ischemia there is a considerable difference between different nephrons [2, 3, 32, 33]. Thus, the number of perfused nephrons, as measured here, and the number of filtering ones are probably not equal, and a substantial number of perfused but not filtering glomeruli are likely to atrophy later on in the recovery phase.

It is concluded that RBC trapping in the outer medullary vasculature is related to the length of the ischemic period in a dose-dependent manner. This trapping may cause secondary hypoxia in the renal outer medulla, with long-term damage as a result. Prevention or enhancement of the RBC trapping by hemodilution or hemoconcentration, respectively, could influence the long-term outcome of renal ischemia.

Reprint requests to Dr. Olof Hellberg, Department of Physiology and Medical Biophysics, University of Uppsala, Biomedical Center, Box 572, S-751 23 Uppsala, Sweden.

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