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Failure of loop diuretics to improve the long term outcome of ischaemic damage in rat kidneys.

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ABSTRACT

The effects of furosemide administered at the onset of postischaemic renal failure were investigated in Sprague-Dawley rats one month after exposing the left kidney to 45 min of renal ischemia. In the experimental group, 13 mg furosemide was given intravenously both before and a few minutes after induction of the ischaemia and then, by an osmotic pump, in a daily dose of 2–3 mg for the following 7 days. The animals of the control group were treated similarly but with saline alone. After one month, the glomerular filtration rate (GFR) in the damaged left kidneys of the furosemide-treated rats was 0.5 ± 0.08 ml/min, which was not significantly different from that in the untreated control rats, of 0.8 ± 0.14 ml/min. As expected, the right intact kidneys responded with an increase in GFR to about 2 ml/min. Further effects that were similar in the damaged kidneys of the furosemide-treated and untreated animals were a decrease in potassium secretion and in the urine concentration ability; the urine osmolality in the diseased left kidneys was thus 1 000–1 500 mOsm/kg, as against over 2000 mOsm/kg in the right, intact kidneys. The function of the individual nephrons in terms of such variables as single nephron filtration rate, fractional fluid reabsorption and tubular and vascular hydrostatic pressures remained unaltered, however. Hence, the severe reduction in whole kidney GFR appeared to be due to a loss of nephrons rather than to an equal decrease in each individual nephron. It is also clear that furosemide did not improve the long-term outcome of acute post-ischaemic renal failure.

Key words
Acute renal failure, furosemide, ischemia, kidney, loop diuretic

INTRODUCTION

There are a number of reasons why treatment with loop diuretics may be anticipated to prevent acute renal failure. For instance, through their inhibition of the tubuloglomerular feedback mechanism, loop diuretics may be expected to increase the renal blood flow and glomerular filtration rate (GFR). The maintenance of a brisk tubular fluid flow may also be beneficial, since it will reduce the risk of trapping of cells and cell debris in the tubular lumen and hence prevent tubular obstruction, which, at least in the acute stage, is generally considered to be the main cause of the

severe reduction in GFR that occurs in renal failure. It has also been suggested (Epstein et al., l987, Schurek, 1988) that because of the counter current arrangement of the medullary capillaries, the ambient oxygen tension in the mitochondria rich cells of the thick ascending limb of the loop of Henle hovers close to the critical pO_2 and hence that inhibition of the transport processes in this particular segment by furosemide, for example, might lessen the risk for hypoxic damage.

Unfortunately, however, opinions diverge as to the efficacy of therapies based on loop diuretics. Although the controversy seems to derive partly from the use of different models of acute and chronic renal failure, even when the aetiology is clearly defined and the renal function can be precisely measured, the results remain conflicting. This also applies to the present model of post-ischaemic renal failure, on the basis of which some authors claim that early administration of loop diuretics is of major benefit (Mark et al., 1986, Mann et al, 1986, Prestel et al. 1996) or have even considered these diuretics to be the treatment of choice (Gienello, l990). In other studies no such beneficial effect has been observed either in acute or in chronic renal failure (Cronin, 1986, Middeke et al., 1985, Satta et al., 1985, Lewis et al.1984, Shilliday et al. 1997).

In our previous series of studies on the long-term outcome of post-ischaemic acute renal failure in rats (Bayati et al., l990a, Bayati et al., l990b), admini-stration of furosemide or piretanide during the first "critical" week was found to improve, though modestly, the GFR. Similarly, the loop diuretics also improved the urine concentration ability; after one month the urine osmolality reached 1300–1500 mOsm/kg, compared with about 1000 mOsm in untreated animals. The same pattern was also evident regarding the ability of the diseased kidney to excrete potassium. In contrast, however, the kidney dry weight remained unaffected by the treatment.

As proposed above, the improved recovery might also be due to the diuresis *per se*. In order to exclude this possibility, in our previous investigation relatively low, non-diuretic dosages of both furosemide and piretanide were used. Obviously these low dosages might also explain why the effects of the therapy were fairly moderate.

The present study aims at the control of the effects of furosemide on the long-term outcome of ischaemic damage to the kidney. In order to ensure a constant drug concentration in the body fluids during the first 7 days after the ischaemic insult, the drug was administered via an osmotic pump implanted subcutaneously between the two scapulae.

MATERIAL AND METHODS

The studies were performed on 17 male Sprague-Dawley rats (Möllegaard, Denmark) weighing 300 ± 8.0 g in the first session. They had free access to water and rat chow throughout the experimental period.

First session

In the first session the rats were anaesthetized with an intraperitoneal injection of 15 mg of pentobarbital (Mebumal, Vet, Nord Vacc, Sweden) and then placed on a servo-controlled heating pad which stabilized the body temperature at 37.5 °C.

The kidneys were exposed through a midline abdominal incision and the left renal artery and left ureter were dissected free. The abdomen was then closed and the rat was allowed to recover for approximately 30 min to ensure that the body temperature returned to 37.5°C.

Acute renal failure was induced by clamping the renal artery and ureter for 45 min. During this time the kidney was placed in its natural position and the abdomen was closed. In addition, cotton wool was placed over the incision, i.e. in order to maintain the temperature at 37.5 °C. After termination of the warm ischemia, the clamps were removed, the abdomen was sutured and the animals were returned to their cages.

In order to prevent coagulation, each rat received 100 IU of Heparin (Löven, Sweden).

The loop diuretic furosemide (Lasix, Hoechst AG, Frankfurt am Main, Germany), in a dose of 13 mg was administered via a catheter inserted into the femoral vein both before and a few minutes after the clamping of the kidney. This was followed by continuous administration for 7 days by an osmotic pump (Alzet, Alza Corporation, Palo Alto, CA, USA) implanted subcutaneously between the two scapulae. In the experimental group the pump was loaded with 20 mg furosemide dissolved in 2 ml of a sterile saline such that the animals received a daily dose of 2–3 mg furosemide. In order to secure immediate onset of function, the pump was first soaked in physiological saline at 37°C for 4 hours.

The animals in the control group were handled identically, except that they were infused with saline alone.

Removal of the osmotic pump

After 8 days, the osmotic pump in both the untreated and furosemide-treated groups was removed under Mebumal anaesthesia. In all the cases the osmotic pump was found to work properly. As a further control, the water intake of 4 of the animals receiving furosemide was found to be 32±4.7 ml/day as against 18± 5.6 ml/day in the control series.

Second session

The kidneys were examined one month after the primary ischaemic insult: The rats were anaesthetized with Inactin (Byk Gulden, Konstanz, Germany) given intraperitoneally in a dose of 120 mg/kg body weight, tracheotomized and placed on a servo-controlled heating pad which established the body temperature at 37.5°C.

The left femoral artery and vein were both catheterized – the artery for continuous monitoring of blood pressure and for withdrawal of blood samples and the vein for administration of test substances and infusion of isotonic Ringer-bicarbonate solution at a rate of 5 ml/h per kg body weight.

The left kidney was exposed through a subcostal flank incision, dissected free, suspended in a lucite cup and, in order to prevent drying, superfused with heated mineral oil. The ureter was cannulated close to the pelvis.

In the micropuncture studies the hydrostatic pressures of the tubular and vascular

structures was measured by the servo-nulling device described by Wiederhielm et al. (1964), using sharpened glass capillaries with a tip diameter of 2–5 μm.

The glomerular capillary pressure was estimated by the indirect "stop flow technique" (Gertz et al., l966). For this purpose an early proximal tubular segment was punctured with a 10 μm glass capillary followed by injection of castor oil until the filtration ceased. The glomerular capillary pressure was then calculated by adding this "stop flow" pressure to the colloid osmotic pressure of systemic plasma of 20 mm Hg, a technique which has previously been shown to permit accurate determination of the glomerular capillary pressure (Källskog and Wolgast, 1983). The single nephron glomerular filtration rate, SNGFR, was measured as the nephron clearance of tritiated inulin (NEN, Boston, MA, USA); the inulin was infused at a constant rate of 50 μCi/h, preceded by a bolus injection of 50 μCi. The plasma activity was measured repeatedly Five to eight samples were taken from each animal.

The collection of tubular fluid was initiated by injection of a stable oil block of about 10 tubular diameters in length, followed by sampling of tubular fluid for 3–4 min at a rate which kept the oil block in position. During this time the pressure proximal to the block was monitored, to ensure that the pressure was close to that found under free flow conditions. In the present series SNGFR was measured from punctures of both proximal and distal tubules.

It should be noted that the tubules investigated were selected at random. However, in the present series this did not cause any obstacle, beacuse the tubules either had a normal function or were grossly dilated with no signs of fluid turnover.

Whole kidney GFR was measured as the total clearance of tritiated inulin. For this purpose urine was sampled at 15-min intervals throughout the experiment. The plasma activity was estimated from arterial blood samples drawn in the middle of these periods. However, since it will take about 5 min for the filtrate to reach the urine, the blood sample has to be taken 5 min before the middle of the sampling period.

Standard formulae were used to calculate GFR and SNGFR.

RESULTS

At the time of induction of acute renal failure, the weights of the rats in the furosemide and control group were about the same, viz 299 ± 11 and 300 ± 12 g respectively, with virtually no change during the first week. During the following weeks the rats in both groups showed a normal weight gain, and after one month they weighed 413 ± 12 and 422 ± 13 g respectively.

On examination of the kidneys one month after the primary ischaemic insult, the left, diseased kidneys in both groups (see Table I) had their normal pale appearance. Microscopically, however, the left kidneys showed numerous scars and crypts. Grossly dilated, almost cyst-like tubules with no sign of fluid turnover were also observed.

Regarding the renal function (Tables I and II; Figs 1 and 2), the most striking finding was that the changes in the two groups were virtually identical. In other words, the treatment with furosemide seemed to have had no effect on any of the variables studied.

The ischaemic insult resulted in a reduction, though moderate, in the weight of the left, diseased kidneys, (Fig.1, upper panel) and compensatory growth of the right

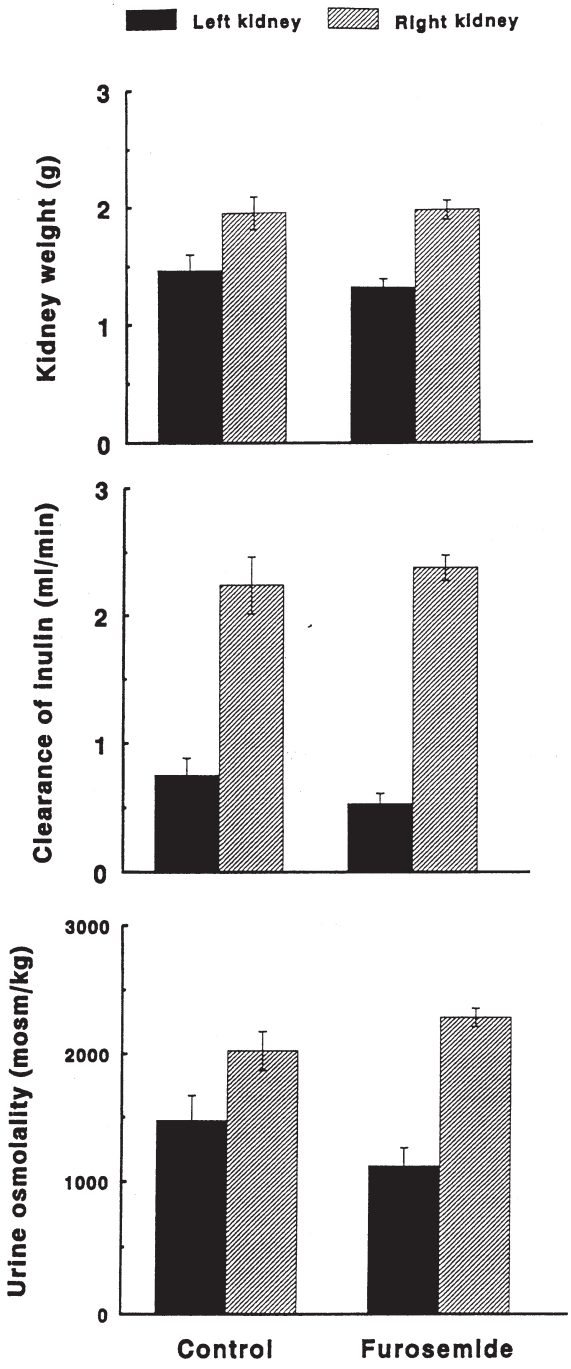

Fig 1. Weight (upper panel), whole kidney GFR (middle panel) and urine osmolality (lower panel) in the left and right kidneys one month after exposure of the left kidney to 45 min of warm ischaemia, mean ± S.E. (n=9). The bars on the left-hand side refer to furosemide-treated animals and the bars on the right-hand side to untreated animals.

The ischemic damage evidently resulted in a decrease in both kidney weight, whole kidney GFR and urine osmolality. It is also clear that the treatment with furosemide did not affect these parameters.

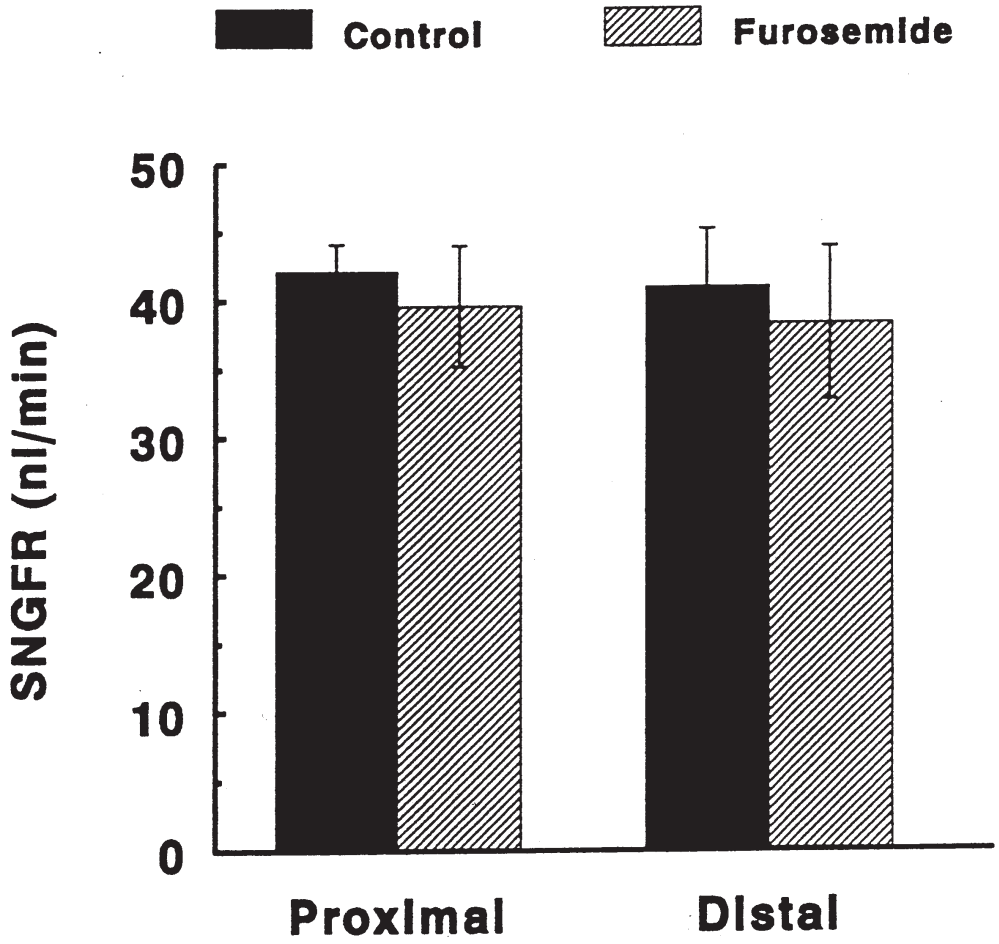

Fig 2. Single nephron filtration rate, (SNGFR) determined from proximal and distal tubules one month after exposure of the kidney to 45 min of warm ischaemia in furosemide-treated (n=9) and untreated animals (n=8), mean ± S.E.

The figure demonstrates that in spite of the ischaemic damage, SNGFR was normal in both groups of animals. Also the SNGFR data obtained from proximal and distal tubules were virtually identical.

intact kidneys, both of which changes were to be expected. As stated above, there was no difference in response between the furosemide-treated animals and the untreated controls.

It may be added here that the kidneys were drained off before weighing, which means that the weight of the extracellular fluid will not be incorporated in the total weight of the kindeys. However, since after one month, the nephrons undergoing degeneration still remain, the data will overestimate the weight of the nephrons in function.

The loss of kidney mass was accompanied by an even greater reduction in the clearance function of the kidneys (Fig. 1, middle panel). In the untreated animals GFR in the left, diseased kidney was estimated at 0.75 ± 0.14 ml/min and in the furosemide-treated animals it was about the same, 0.53 ± 0.08 ml/min ($P>0.1$). Thus

Table I.

	After furosemide		Untreated animals	
Variable	Left kidney	Right kidney	Left kidney	Right kidney
GFR ml/min	0.5±0.08* §	2.4±0.10	0.8±0.14*	2.2±0.23
Urine flow µl/min	1.9±0.3* §	6.2±0.7	1.7±0.2*	4.6±0.6
U/P_{inulin}	337±53‡ §	397±25	421±62*	515±65
Urine Na mM	25±4.4‡ §	33±5.5	32±6.5‡	32±5.7
Urine K mM	123±29* §	290±23	117±29‡	197±30
Urine Osm mOsm/kg	1 119±146* §	2 288±70	1 472±201*	2 026±152

Function of the right, intact and left, damaged kidney one month after exposure of the left kidney to 45 min of warm ischaemia in untreated animals and in animals treated with furosemide. Mean±S.E. *= P<0.05 left kidney versus right kidney. ‡= P>=.05 left kidney versus right kidney. §= P>0.05 left treated kidney versus left untreated kidney.

the GFR was some 30–50% of that in normal kidneys. As expected, the contra lateral, intact kidneys responded with a compensatory increase in GFR to 2.2 ± 0.23 ml/min and 2.1 ± 0.10 in the untreated and furosemide-treated animals, respectively. However, if the GFR is expressed in ml per gram tissue the figures were about the same as in normal kidneys.

It may also be stated that, taken together, the GFR of the two kidneys was thus essentially normal.

Another disturbance typical for ischaemic renal failure was a reduction of the urine osmolality (Fig. 1, lower panel), which was estimated to be 1 000–1 500 mOsm/kg in the left, diseased kidneys in the two groups as against more than 2 000 mOsm/kg in their right, intact counterparts. The same pattern was observed for the ability to secrete potassium (Table I).

Regarding the function of the individual nephrons, none of the variables studied (Table II) were different from those in normal kidneys. A slight increase in the proximal and distal fluid flow and concomitant decrease in the respective Tf/P-inulin was possibly perceptible. Again, the data obtained in the animals treated with furosemide did not differ from those in the untreated controls.

Table II

Variable	After furosemide	Untreated animals
Glomerular capillary pressure mmHg	59.1±0.8 NS	58.6±0.6
Proximal tubular pressure mmHg	15.2±0.5 NS	14.9±0.6
Welling point pressure mmHg	15.4±0.8 NS	15.4±0.9
Peritubular capillary pressure mmHg	10.3±0.3 NS	11.0±1.0
Proximal tubular fluid flow nl/min	28.7±3.3 NS	29.6±1.9
Distal tubular fluid flow nl/min	9.6±1.9 NS	8.5±1.2
TF/P_{inulin}, proximal tubules	1.45±0.04 NS	1.58±0.06
TF/P_{inulin}, distal tubules	5.50±0.85 NS	5.32±0.43
SNGFR from proximal tubules nl/min	39.6±4.4 NS	42.1±2.0
SNGFR from distal tubules nl/min	38.3±5.6 NS	41.0±4.3

Micropuncture data for the left diseased kidney one month after exposure to 45 min of ischaemia 1) under control conditions and 2) after treating the animals with furosemide during the first week following the ischaemic insult. Mean±S.E. NS= P>0.05 treated versus untreated animals.

It was concluded that since the glomerular filtration in the individual nephron remained unaltered (Fig. 2), the pronounced reduction in the whole kidney GFR must have been due to the loss of functioning nephrons. As the whole kidney GFR was reduced to 30–50% of the normal, then 30–50% of the nephrons would have regenerated, whereas the majority, 50–70%, would have undergone complete degeneration. The scars, crypts and cysts were probably remnants of this nephron degeneration.

DISCUSSION

In the pathogeneses of ischaemic renal failure, it seems likely that the severe impairment of the clearance function seen in the acute stage is due to a blockade of tubules with cells and cell debris rejected from the more proximal parts of the nephron (Thiel et al., l980, Karlberg et al., l982a, Flamen-baum, l984).

In addition, the damage to the tubular walls also results in a considerable leakage of inulin estimated at about 30% (Hellberg and Källskog, 1989).

In contrast, the possibility that the reduction in GFR is due to a decrease in renal blood flow is not very likely, since in most cases this blood flow will show a rapid recovery both after warm ischaemia (Conger and Schrier, 1980, Mason, l988, Karlberg et al., l983, Thiel et al., l980, Wolgast et al., l982) and after the cold ischaemia at 4^{o}C that precedes transplantation (Norlén et al., l978).

Where the recovery phase is concerned, Bayati et al., (1990d) have suggested that the depression of GFR is primarily due to the formation of "new", more homogeneous casts in the thick ascending limb of the loop of Henle (Patel et al., l964, Mc Kenzie and Mc Queen, 1969, Hoyer et al., l979) which, almost certainly, are composed of Tamm/Horsfall protein (Tamm and Horsfall, l950).

The formation of these cylinders seems to start very soon after the onset of circulation following the ischaemic insult. On slices of the outer medulla, the morphometric area occupied by Tamm/Horsfall cylinders amounted to 5–10% (Bayati l990) of the total area as early as 4 hours after the beginning of recirculation. It then increased to a maximum of about 20% after 2 days, decreasing again to some 15% one week after the primary ischaemic insult. The Tamm/Horsfall deposits persisted for periods varying up to one month, but then dispersed freely in the form of casts into a widened interstitium with no signs of an epithelial lining (Bayati 1990).

From these observations it was suggested that *if* the Tamm/Horsfall cylinders persist beyond the first week, the entire nephron will degenerate. However, *if* a tubule is able to clear itself of the entrapped material within the first week, the nephron will regenerate completely.

The rational of the treatment with loop diuretics, is that our previous study (Bayati et al.,1990d) suggested that they might interact with the formation of Tamm/Horsfall protein. It was not known, however, whether these diuretics would reduce or augment the formation/deposition of this protein. Greven (1983) has reported that furosemide may bind to the protein, whereas Brunisholz et al.,(l987) refutes this possibility.

In line with the assumption of a protective effect of furosemide, Bayati and

coworkers found that the total GFR was improved one month after the damage, being about 35% of the normal GFR after treatment with the loop diuretics as against 20–25% of the normal GFR in untreated controls. This difference was also statistically significant – although only on the 5% level.

The present findings are in accordance with our previous study in the sense that, although the total GFR was greatly reduced, both the glomerular capillary pressure and the filtration rate in single nephrons were normal; in other words, the reduction in the total GFR appeared to be due to loss of functioning nephrons rather than to a decrease of equal magnitude in the function of all nephrons. This proposed loss of nephrons was also suggested by the loss of weight, although, after one month, also scarred tissue will contribute to the renal mass.

However, as shown by Leyssac et al (1991), the glomerular ultrafiltration rate is also dependent on the flow resistance in the tubular system, a resistance which in fact is of the same order of magnitude as the glomerular net driving force *per se*. Among the factors governing this resistance also the tubular fluid reabsorption will contribute. In the present study the Tf/P-inulin in both proximal and distal tubules was thus depressed, i.e. indicative of a reduced tubular reabsorption. Consequently the proximal tubular pressure was increased with about 10%, a rise which thence will depress the glomerular filtration rate.

As expected, the contralateral kidny responded with a compensatory hypertrophy. However, the mechanism behind this change remains to be clarified.

We have thus failed to establish a beneficial action of furosemide, i.e. contradictory to our previous result, although we cannot exclude the possibility that a lower, nondiuretic dose may act differently from a diuretic dose of this drug.

However, this does not exclude the previous suggestion of a central role for the Tamm/Horsfall protein, although a diuretic dosage of furosemid does not seem beneficial.

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