Studies on the Mechanism of Non-Oliguric Experimental Acute Renal Failure

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Although acute renal failure, caused either by renal ischemia or nephrotoxic agents, is usually characterized by oliguria, a severe fall in glomerular filtration rate, and a fall in renal blood flow, some patients and experimental models display a non-oliguric pattern of renal injury. The present study was designed to evaluate the mechanism of preservation of high urinary flow rate under this condition.

Following the administration of the aminoglycoside gentamicin to rats for five days, a decrease in concentrating ability was demonstrated, caused by impaired vasopressin-mediated water transport. Further treatment resulted in a fall in C_in to 15 percent of control, although RBF was reduced to only 67 percent of control, and urine flow rate rose above control levels. Induction of acute renal failure with dichromate was associated with variable high or low urinary flow rates according to pre-injury intake of sodium. Urine volume correlated directly with cortical blood flow.

These data suggest that the non-oliguric pattern of acute renal injury is caused by preservation of cortical perfusion in the setting of severe tubular injury.

Acute renal failure (ARF), caused either by renal ischemia or nephrotoxic agents, is typically characterized by oliguria, a severe reduction in glomerular filtration rate (GFR), and a variable fall in renal blood flow (RBF). The urine is isotonic and tubular reabsorption of sodium is markedly impaired. Recent studies report, however, that an increasing proportion of patients with acute renal failure display a non-oliguric pattern of renal injury [1,2]. Nephrotoxins, and especially aminoglycoside antibiotics, are cited as important etiologic factors in many of these cases. Since the mechanism responsible for a high urinary flow rate in the presence of acute renal failure has not been elucidated, the present study was performed to analyze the characteristic features of this pattern of renal injury in an experimental model of gentamicin-induced non-oliguric renal failure. The aminoglycoside gentamicin has a well-documented nephrotoxic potential [3], and produces in both humans and experimental animals a dose-related progressive acute renal failure which is typically non-oliguric [4]. The possible role of hemodynamic factors in the mechanism of non-oliguric ARF was also investigated in another model of nephrotoxic ARF induced by dichromate. Since urinary flow rate can be modulated by dietary sodium intake in animals with dichromate-induced ARF [5], cortical bloodflow was correlated with urinary flow rate in this model of ARF as well as in gentamicin-treated animals.

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METHODS

Experiments were performed in male Sprague-Dawley rats weighing 180 to 250 g at the initiation of drug administration. All animals were fed standard Purina rat chow and allowed ad-lib access to tap water.

GENTAMICIN-INDUCED ACUTE RENAL FAILURE

Experimental animals were given subcutaneous injections of gentamicin sulfate (Garamycin, Schering Corp.) at a dose of 80 mg/kg body weight per day for up to 12 days. Control animals were treated with an approximately equal volume (0.5 ml) of 0.15 M NaCl during similar time intervals.

Determination of Urine Flow Rate, Concentrating Ability, and Free Water Clearance

Animals were placed in metabolic cages to collect total urine volume under oil without fecal contamination during consecutive 24-hour intervals. After an adaptation period of three days, treatment with either gentamicin or 0.15 M NaCl was begun. Daily urine volumes were determined in a graduated cylinder.

To determine urinary concentrating ability, conscious control and experimental animals were studied one day before, on the day of initiation of treatment, and on the fifth and tenth day after treatment with subcutaneous gentamicin. Animals were placed in metabolic cages, deprived of access to water, and administered 0.5 units of aqueous vasopressin (Pitressin, Parke-Davis) subcutaneously on study days. One hour following administration of vasopressin, animals were induced to void by light suprapubic pressure and urine was collected quantitatively for two subsequent hours, at which time voiding was again induced. The osmolarity of the urine was measured with a Wescor-Vapor pressure osmometer (Model 5100 B, Wescor, Inc., Logan, Utah).

To examine the mechanism(s) of the observed impairment in concentrating ability further, free water clearance was determined in additional conscious experimental animals after five days of treatment with gentamicin and in control animals. A water diuresis was produced by the oral load of 5.0 ml water/100 g body weight given by gavage, under light ether anesthesia. After induced voiding, animals were placed in metabolic cages and urine was collected during two consecutive 60-minute intervals. At the end of each interval, voiding was induced. At the termination of the final collection, a sample of aortic blood was obtained and animals were sacrificed. Urine and plasma was analyzed for osmolarity (Wescor-Vapor pressure osmometer).

Determination of GFR, RBF, and Electrolyte Excretion

Anesthesia was induced with Inactin (Promonta, Hamburg) at a dose of 40–120 mg/kg body weight to produce a similar level of anesthesia in all groups. Animals were placed on a heated board; a tracheostomy was performed and the bladder and one external jugular vein were cannulated with PE-50 tubing. A left carotid arterial catheter and femoral artery catheter were inserted.

After replacement of surgical fluid losses with 0.15 M NaCl at one percent of body weight, a priming dose of 10μCi of inulin-methoxy-3H (New England Nuclear Corp.) was given intravenously and was followed by a sustaining dose of 10μCi in a volume of 1.2 ml/hour of 0.15 M NaCl. After 45 minutes of equilibration, timed urine collections at ten-minute intervals were begun and tail blood samples obtained at the midpoint of each collection. At the termination of the final urine collection, a sample of blood was obtained from the left renal vein with a 25-gauge needle.
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After completion of the three clearance periods, total and regional blood flow were determined using radioactive microspheres as previously described [6]. Microspheres, 15 ± 2 μ in diameter and labeled with Sr85, were dissolved in 10 percent dextran containing three drops of Tween 80. Microspheres were mixed for one minute with an ultrasonic dismembrator (Artex Systems Corp., Farmingdale, N.Y.) prior to injection. During injection of 0.2 ml (approximately 120,000 microspheres) of the mixture into the carotid artery catheter, blood was simultaneously withdrawn for one minute from the femoral artery at a fixed rate of 1.03 ml/min. The animals were sacrificed, the kidneys removed, the capsule stripped, and a midsagittal section of each kidney was made. Each kidney was placed on a microtome and the outer 0.5 mm of cortex was excised, weighed, and labeled “OC.” The remainder of the kidney was weighed and the sum of the weights of the two outer cortical slices plus the remainder was calculated as total kidney weight (“TC”). The activity of the isotope in each outer cortical slice, as well as the remainder, was determined in a gamma counter (Beckman Instruments, NJ), and the sum of the activities for each kidney was determined.

The radioactivity of inulin-methoxy-3H in plasma and urine was determined in a liquid scintillation counter (Tri-carb). The concentration of sodium and potassium was measured with a flame photometer with an internal standard. Standard formulae were used to calculate C_in and the urinary excretion of electrolytes. Renal blood flow was determined by two methods for control and five-day experimental animals: (a) RBF = RPF / 1 − Hct, where RPF = C_in / E_in. The value E_in was determined from A_in − V_in/A_in, where A and V represent arterial and renal venous concentrations of inulin. (b) RBF = Sr85 CPM whole kidney/CPM femoral arterial blood × 1.03 ml/min (the femoral arterial flow rate). Proportional flow to the outer cortex (OT/TC ratio) was calculated as CPM/g outer cortex + CPM/g whole kidney. In the ten- and 12-day experimental groups, since E_in was markedly reduced and RBF could not be accurately estimated by this method, only the microsphere method was employed.

Morphologic Correlates of Functional Abnormalities

Additional groups of control animals and experimental animals treated with gentamicin for five days were anesthetized with Inactin. The chest was entered via a transverse incision below the sternum and in vivo perfusion and fixation was performed with a catheter positioned in the aorta. For renal cortical histologic sampling, a 350 mM NaCl in 3 percent dextran solution was first flushed through the catheter and followed by Karnovsky’s fixative [7] diluted 1:3 with 0.1M sodium cacodylate buffer and 175 mM NaCl in 3 percent dextran. For medullary sampling, the vasculature was flushed with 550 mM NaCl in 3 percent dextran followed by the same fixature and buffer in 350 mM NaCl plus 3 percent dextran. Samples of cortical and medullary tissue were placed in Karnovsky’s fixative, washed in cacodylate buffer, and post fixed in osmium tetroxide. After embedding in Epon, sectioning was performed on an LKB ultratome III and grids were scanned and photographed in Zeiss SM9S electron microscope.

DICROMATE-INDUCED ACUTE RENAL FAILURE

To compare the findings in aminoglycoside ARF to another model of non-oliguric nephrotoxic ARF, animals were offered either food containing no sodium and tap water (low-salt group) or regular Purina Chow and 0.15 m NaCl to drink (high salt group). After two weeks of dietary preparation, each group was divided into experimental animals, which were treated with potassium dichromate in a dose of 15
mg/kg (subcutaneously) and control animals which received 0.15 M NaCl in an equivalent volume of 0.1 cc.

Twenty-four hours after treatment, measurement of GFR, RBF, renal blood flow distribution, and urinary flow rate were made using the same methods described above.

Results are expressed as the mean ± SEM and the Student's test was used to compare groups of animals.

RESULTS

Gentamicin-Induced Acute Renal Failure

a. Urinary flow rate, concentrating ability, free water clearance:

The administration of gentamicin caused a progressive increase in urinary flow rate (Fig. 1). By day seven after initiation of treatment, urine volume was significantly increased in the gentamicin treated animals.

The upper panel of Fig. 2 displays the results of maximum concentrating ability following exogenous vasopressin administration. Mean urine osmolarity on day zero was 1,829 ± 100 mOsm/kg H$_2$O and was not significantly different following the administration of gentamicin for one day. Following five days of treatment, however, means osmolarity decreased by 35 percent to 1,183 ± 168 ($p < 0.005$) and by day ten decreased further to 600 ± 44 mOsm/kg H$_2$O ($p < 0.001$).

As shown in the lower portion of Fig. 2, C$_{H2O}$, at comparable levels of distal solute delivery (C$_{Na^+}$ + C$_{H2O}$), was similar in both control and experimental animals treated for five days. Since C$_{in}$ was similar in controls and the group treated with gentamicin for five days, these values were not factored by C$_{in}$. It is important to note that during water diuresis $\dot{V}$ (6.4 ± 0.4 ml/hr), $P_{Osm}$ (278.4 ± 2.6 mOsm/kg H$_2$O) and

![FIG. 1. Daily urine volume during gentamicin treatment (80 mg/kg/day). The arrow represents the first day of drug injection. Values represent mean ± SEM (n = 12-18 animals).](image-url)
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**FIG. 2. Upper panel.** Maximum urinary concentrating ability following vasopressin administration. Day 0 represents first day of drug injection. Values represent mean ± SEM (n = 10 animals). **Lower panel.** Free water clearance versus distal sodium delivery. Open circles represent control animals (n = 5 animals); closed circles represent animals treated with gentamicin for five days (n = 5 animals); C_H2O and C_Na + C_H2O in ml/hr.

U_Osm \((132.4 ± 25.4 \text{ mOsm/kg H}_2\text{O})\) in experimental animals were not statistically different from control.

b. GFR, RBF, and electrolyte excretion:

The results of determination of C_in, RBF, \(\dot{V}\), and sodium excretion are shown in Table 1. Control animals treated with subcutaneous injections of saline for five, ten, or 12 days showed no differences in regard to parameters of renal function and are listed collectively.

At five days of treatment with gentamicin, there was no significant change in C_in or \(\text{FE}_{\text{Na}}\) and, although \(\dot{V}\) was increased 50 percent the rise was statistically insignificant. However, after ten days of treatment, experimental showed a 48 percent reduction in C_in from the control value of 1,158 ± 60 \(\mu\text{l/min/100 g body weight}\) to 602 ± 130 \(\mu\text{l/min/100 g body weight}\) \((p < 0.01)\). C_in was further reduced to about 15 percent of control values (176 ± 47 \(\mu\text{l/min/100 g body weight}\)) in animals treated for 12 days. These reductions in C_in were associated with large and significant increases in urine flow rate to 4.95 ± 0.76 \(\mu\text{l/min/100 g body weight}\) at ten days \((p < 0.01)\) and 7.56 ± 1.77 \(\mu\text{l/min/100 g body weight}\) at 12 days \((p < 0.01)\). \(\text{FE}_{\text{Na}}\) was also markedly increased on days ten and 12 of treatment.

Gain in body weight was not significantly different from control (6.1 ± 0.4g/day) in gentamicin-treated animals through the fifth day of treatment. Beyond that time, however, body weight fell in association with severe renal failure.

Mean values of total renal blood flow (RBF) and cortical blood flow distribution (OC/TC) are also shown in Table 1. The values of total renal blood flow determined
by the microsphere method did not differ significantly from values estimated by the clearance and extraction of inulin; results from the microsphere method alone are shown in the table. Although RBF was not reduced by the fifth day of treatment compared to the control value 5,979 ± 514 μl/min/100 g BW, blood flow was reduced to 70 percent and 67 percent of control on the tenth and twelfth days of treatment, respectively. Proportional flow to the outer cortex (OC/TC) was not significantly different from control (1.55 ± 0.03) on days five and ten, but rose above control to 1.78 ± 0.06 on day 12 (p < 0.05).

c. Morphologic correlates of functional abnormalities:

After five days of treatment with gentamicin, proximal tubular cells displayed prominent alterations of lysosomes and large numbers of “myeloid bodies,” as previously reported [8]. Rare lysosomal myeloid bodies were noted in distal tubular cells, but never to the extent found in the proximal tubules. There were no other apparent differences between the control and experimental groups on morphologic examination.

### Dichromate-Induced Acute Renal Failure

Changes in GFR, RBF, and urinary flow rate observed in the animals treated with potassium dichromate after dietary sodium manipulation are shown in Table 2. In animals injected with 0.15 M NaCl, C_{in}, RBF, and V were similar in both high-salt and low-salt groups. Proportional blood flow, however, was reduced in animals on a sodium-free diet (1.47 ± 0.03) compared to the group receiving a high sodium load (1.63 ± 0.03, p < 0.01).

Twenty-four hours after administration of dichromate, C_{in} was markedly reduced in both groups of animals to 33 percent and six percent of control values in high and low sodium groups, respectively. In sodium-deprived animals, RBF fell 75 percent compared to saline-injected animals and proportional flow to the outer cortex decreased in association with a significant fall in V. In contrast, both total RBF and proportional flow to the outer cortex were unaltered in the high-salt intake group, which was also non-oliguric. In these animals with dichromate-induced ARF therefore, the preservation of RBF and OC/TC (high-salt group) was associated with a non-oliguric pattern of ARF whereas a marked reduction in RBF and OC/TC (low-salt group) was characterized by a more severe fall in C_{in} and an oliguric pattern of ARF.

|                | Control | 5 Days | 10 Days | 12 Days |
|----------------|---------|--------|---------|---------|
| N              | 12      | 7      | 11      | 10      |
| Final Body weight (g) | 294 ± 9 | 260 ± 16 | 220 ± 11* | 228 ± 11* |
| V (μl/min/100gBW) | 1.99 ± 0.25 | 3.29 ± 1.20 | 4.95 ± 0.76* | 7.56 ± 1.77* |
| C_{in} (μl/min/100gBW) | 1158 ± 60 | 1130 ± 130 | 602 ± 130* | 176 ± 47* |
| RBF (μl/min/100gBW) | 5979 ± 514 | 4638 ± 723 | 4160 ± 480* | 3998 ± 721* |
| OC/TC          | 1.55 ± 0.03 | 1.55 ± 0.06 | 1.59 ± 0.05 | 1.78 ± 0.06* |
| FE_{Na}        | 0.04 ± 0.01 | 0.13 ± 0.07 | 2.16 ± 1.00* | 2.75 ± 0.48* |

Values represent mean ± SEM; N indicated the number animals studied. The following symbols denote a statistical difference compared to the control group; *p<0.05; *p<0.01.
TABLE 2
Effect of Potassium Dichromate on Parameters of Renal Function

|                     | High Sodium | Low Sodium |
|---------------------|-------------|------------|
|                     | Saline-Injected |          | Dichromate-Injected |          |
| \( \dot{V} \), \( \mu l/min \) | 4.7 ± 0.5   | 4.5 ± 0.5  |
| \( C_{in} \), \( \mu l/min/100gBW \) | 1040 ± 50   | 977 ± 60   |
| \( RBF \), \( \mu l/min/100gBW \) | 5330 ± 310  | 5970 ± 530 |
| Proportional blood flow OC/TC | 1.63 ± 0.03 | 1.47 ± 0.03*  |
|                     | 3.8 ± 1.0   | 1.8 ± 0.3±  |
|                     | 370 ± 100±  | 60 ± 30±   |
|                     | 5260 ± 750  | 1480 ± 320± |
|                     | 1.69 ± 0.04 | 1.32 ± 0.03± |

Values are mean ± SEM
*\( p<0.01 \) High Sodium vs. Low Sodium Group
±\( p<0.01 \) Saline-Injected vs. Dichromate-Treated Group

DISCUSSION

Over the past decade, the pathophysiology of acute renal failure has received considerable attention. Primary attention has been focused on the mechanisms responsible for the reduction in glomerular filtration rate and diminished urine output [9]. Considerably less emphasis, however, has been given to the mechanisms involved in non-oliguric acute renal failure, although this type of acute renal failure has been recognized with increased frequency in clinical reports [1,2]. The present study was designed to investigate the potential mechanism(s) responsible for high urine flow rate in two experimental models of non-oliguric acute renal failure.

In animals treated with gentamicin, concentrating ability was impaired on day five of treatment in the absence of changes in glomerular filtration rate, total renal blood flow, or blood flow distribution. Consequently, the fall in concentrating ability could not be attributed to a decrease in solute delivery to the loop of Henle or wash out of the medullary concentrating gradient. Moreover, the concentrating defect was unlikely to be due to reduced solute reabsorption in the ascending limb, since \( C_{H2O} \) was not reduced when solute delivery to the distal nephron was comparable to control, or by diminished medullary urea concentration due to protein deprivation, since animals maintained normal weight gain through day five of treatment. In addition, primary polydipsia seems unlikely since urine flow rate was not significantly increased on day five. The present data therefore, suggest that the gentamicin-induced concentrating defect observed early in the course of treatment was due to impairment of vasopressin-mediated water movement in the collecting duct system. Since myeloid bodies were abundant in proximal tubules, but were not prominently discernable in distal nephron segments on day five, the present study indicates an apparent dissociation of structure-function relationships in the early phase of this type of nephrotoxicity.

Treatment with gentamicin for ten and 12 days led to the characteristic features of
non-oliguric acute renal failure. Despite a fall in $C_{in}$ to 15 percent of control and a severe impairment in sodium reabsorption, urine flow rate remained high and actually increased above control values. Despite the severe fall in $C_{in}$, total renal blood flow decreased to only 67 percent of control and proportional blood flow to the outer cortex rose to levels above control. These data suggest that the high urine flow rate may be related to the maintenance of relatively high rates of cortical blood flow at a time when $C_{in}$ was severely impaired.

Since gentamicin may have a unique action on tubular reabsorption of water, additional experiments were performed in another model of nephrotoxic ARF to determine whether high rates of urine flow would also correlate with preservation of cortical perfusion. Potassium dichromate was chosen as the nephrotoxin since manipulation of dietary sodium intake has been shown to influence urine flow rate after induction of injury [5]. Animals on a low sodium diet developed a severe oliguric ARF associated with a marked fall in total RBF and outer cortical perfusion. In contrast, in animals on a high sodium diet the fall in $C_{in}$ to 30 percent of control was associated with well-maintained urine flow rate and preservation of cortical perfusion at control levels. Thus, despite treatment with the same nephrotoxin, marked differences in urine flow rate in this model of acute renal failure were observed. Animals with non-oliguric dichromate-induced ARF exhibited hemodynamic changes similar to those observed in gentamicin-treated non-oliguric ARF. These data therefore suggest that the preservation of cortical perfusion in the presence of diminished $C_{in}$ may be a characteristic feature of non-oliguric ARF.

Recent studies have suggested that microspheres are subject to axial streaming and, thereby, overestimate absolute outer cortical perfusion in the rat [10,11]. In the present studies, microspheres were used only as an index of outer cortical perfusion, rather than as a determinant of absolute outer cortical perfusion. Moreover, since previous studies have demonstrated a preferential increase in vascular resistance in the outer cortex during acute renal failure [12], it seems likely that axial streaming, if it were to occur, would favor a redistribution of blood flow away from rather than toward the outer cortex as found in gentamicin-treated animals. Whether or not current methodology available for estimating renal blood flow distribution provides a useful index, it should be recalled that approximately 85 to 90 percent of blood flow to the kidney perfuses the cortex. In both models of non-oliguric acute renal failure studied in these experiments the maintenance of near normal levels of total blood flow indicates relatively high rates of cortical perfusion.

Previous studies from our laboratory have documented preferential reperfusion of the outer cortex during the diuretic phase of the recovery from an oliguric ischemic or toxic acute renal injury and after release of bilateral ureteral occlusion [13,6,14]. In each of these experimental conditions there was a reduction in $C_{in}$ which was associated with increased urine output and increased fractional sodium excretion. It is possible, therefore, the preservation or restoration of cortical perfusion in the setting of reduced glomerular function and acute tubular injury results in glomerular-tubular imbalance, which is expressed as a non-oliguric pattern of ARF due to increased fractional excretion of water and solute.

In conclusion, this study provides data which indicates: (1) gentamicin impairs vasopressin-induced water transport early in the course of treatment and suggests that (2) preservation of cortical blood perfusion following nephrotoxic injury is associated with a non-oliguric pattern of acute renal failure.
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