Renal Responses to Atrial Natriuretic Peptide (ANP) in Rats with Non-Oliguric Acute Renal Failure Induced by Cisplatin

Nobuo NAGANO, Mikio YAGI, Shinichiro KATO, Yoshihiro FURUYA, Sonoe MIYATA, and Noboru MANABE

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd. 3, Miyahara-cho, Takasaki, Gunma 370-12, and 1Department of Animal Science, Kyoto University, Kyoto 606-01, Japan

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ABSTRACT. This study was designed to compare the renal effects of atrial (A-type) natriuretic peptide (ANP) on control (saline-injected) rats and rats with non-oliguric acute renal failure induced by cisplatin. The results obtained here are summarized as follows: (1) In the metabolic cage study, cisplatin-treated rats showed increases in blood urea nitrogen and serum creatinine while creatinine clearance decreased to the lowest levels on day 4. A transient increase in urinary protein was observed at day 4. (2) ANP infusion significantly increased urine flow rate (UFR), creatinine clearance (CCr), fractional excretion rates of sodium (FENa) and chloride (FECl), and urinary phosphorus and magnesium (Mg) excretions in a dose-dependent manner without affecting renal plasma flow and fractional excretion rates of potassium and urea in cisplatin-treated rats. (3) Renal effects of ANP on UFR, CCr, FENa, FECl and excretion of Mg were more pronounced in cisplatin-treated rats compared to control rats although markedly blunted responses to ANP have been reported in nephrotic patients and nephroptic animals induced by adriamycin and aminonucleoside. (4) Histological examination showed extensive necrosis of the S2 segment of the proximal tubule located in the outer stripe of the outer medulla with minimal glomerular abnormalities in the kidney of cisplatin-treated rats. In conclusion, the main mechanism of the increased renal responses to ANP is considered to be due to an increased delivery of sodium, fluid and ANP itself to the inner medullary collecting duct which is the major renal site of action of ANP under the condition of acute proximal tubular necrosis by cisplatin. — KEY WORDS: atrial natriuretic peptide (ANP), cisplatin, non-oliguric acute renal failure, rat.


Atrial (A-type) natriuretic peptide (ANP) is found in the heart and has a number of renal actions, such as diuresis and natriuresis [13]. It has been reported that natriuretic and diuretic responses to ANP are markedly reduced in nephrotic patients [8] and in nephrotic animals induced by adriamycin (ADR) [1, 12, 14, 15] and aminonucleoside (PAN) [7, 11, 16]. These two experimental nephrotic models have the common properties of sodium (Na) and fluid retention associated with decreased urinary excretion of fluid and Na. It has been suggested that the blunted responses to ANP are due to retention of fluid and Na, elevated levels of endogenous ANP, decreased affinity or number of ANP receptors, changes of angiotensin II system and heightened renal sympathetic nerve activity. However, the mechanisms of the blunted responses to ANP and Na retention in the two kinds of nephrotic animal models have not been fully elucidated.

Cisplatin, an antineoplastic agent, causes nephrotoxicity characterized by decreased glomerular filtration rate and reduced reabsorption of fluid and Na in rats [5, 19]. Cisplatin-treated rats also show increases of urine flow rate (UFR) and fractional excretion of electrolytes. The purpose of the present study was to induce non-oliguric acute renal failure in rats treated with cisplatin, and then compare the renal effects of ANP in the control (saline-injected) and cisplatin-treated rats.

MATERIALS AND METHODS

Animals: A total of 26 seven-week-old, male Sprague-Dawley rats (Charles River, Japan) were used in this study. The animals were fed with a pellet diet (CE-2, CLEA, Japan) and water ad libitum.

Cisplatin-induced acute renal failure rats (metabolic cage study): Rats were injected with a single dose of 7.5 mg/kg of cisplatin (cis-platinum (II) diammine dichloride, Sigma) intravenously, and then housed in metabolic cages with food and water for 24 hr. The urine was collected at day 0, 1, 4, 7 and 12 and 20. A 300 μl sample of blood was collected from the tail artery of each of the animals on the same experimental days. Control animals were injected with the same volume of saline (3.0 ml/kg, i.v.).

Perfusion study: At day 4 after the administration of saline or cisplatin, rats were anesthetized with thiopental sodium (80–120 mg/kg, i.p., Tanabe, Japan) and placed on a thermoregulated blanket system (KN-474, Natume, Japan) designed to hold their body temperature at 37°C with a rectal probe monitor. A catheter (PE50) was placed in the right carotid artery to monitor mean arterial pressure (MAP) with a polygraph system (Nihon Kohden, Japan).

The left femoral vein was cannulated with a PE 50 catheter, and then lactated Ringer’s solution (Na+, 130 mEq/l; K+, 4 mEq/l; Ca2+, 3 mEq/l; Cl-, 109 mEq/l; lactate, 28 mEq/l; Lactac Injection, Otsuka, Japan) containing 0.5% p-aminohippuric acid (PAH, Wako, Japan) was constantly infused at a flow rate of 2.0 ml/hr with infusion pump system (STC-523, Terumo, Japan). Urine was collected via a short catheter (PE50) placed in the bladder through a suprapubic incision. The dome of the bladder was tied off with a ligature to keep the dead-space volume as small as
possible. After equilibration for 2.0–2.5 hr, baseline clearance period for 30 min was obtained (basal phase: 0–30 min). Then, human ANP (1–28) (M.W. 3080.5, Peptide Institute, Japan) at a dose of 10 pmol/kg/min was infused for 30 min (30–60 min). After that, the concentration of the peptide was increased to 30 pmol/kg/min (60–90 min) and then to 100 pmol/kg/min (90–120 min) with 30 min urine samples taken at each dose infusion. After perfusion of the peptide infusion, lactated Ringer’s solution was infused for 30 min (recovery phase: 120–150 min). A 500 μl sample of blood was collected with a catheter (PE50) inserted in the right jugular vein at the midpoint of every urine collection period, and the same volume of lactated Ringer’s solution without PAH was injected via a catheter (PE50) inserted in the right femoral vein just after every blood collection.

Analytical procedures: The concentrations of urea nitrogen, creatinine, protein, cholesterol, calcium (Ca), phosphorus (P) and magnesium (Mg) were determined with commercial test kits (Wako, Japan). PAH concentrations in urine and serum were determined by the Brun method [4]. The concentrations of electrolytes (Na+, K+, and Cl−) were measured by an automated electrolyte analyzer (PVA-tII, A & T). The clearance, fractional excretion rate and water reabsorption rate were calculated by using standard formulas.

Histological study: After the perfusion study, the left kidneys of the control and cisplatin-treated rats were rapidly removed and fixed in neutral phosphate buffered 10% formalin and embedded in paraffin. Three μm sections were stained with periodic acid–Schiff reagent (PAS) and post-stained with hematoxylin.

Statistical analysis: Data are expressed as the mean ± SEM. Statistical significance of the differences were tested with Student’s t test.

RESULTS

Cisplatin-induced acute renal failure rats: Cisplatin-treated rats showed symptoms of acute renal failure associated with decreased body weight (control: 247.6 ± 8.8 g; cisplatin group: 219.2 ± 5.9 g), polyuria (control: 21.2 ± 4.0 ml/day; cisplatin group: 26.0 ± 2.3 ml/day), proteinuria, azotemia and hypercholesterolemia (control: 63.2 ± 4.0 mg/dl; cisplatin group: 92.9 ± 5.8 mg/dl) at day 4 after the administration. Increases of urinary protein, BUN and serum creatinine were observed with peaks on day 4, while

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Fig. 1. The effects of single administration of cisplatin (7.5 mg/kg, i.v.) on the urinary protein (A), blood urea nitrogen (BUN) (B), serum creatinine (C) and creatinine clearance (CCR) (D). The open circle and the closed circle represent the results in control (saline-treated) rats and cisplatin-treated rats, respectively. Each point represents the mean ± SEM of values obtained from five rats. *: p<0.05, and ***: p<0.001, as compared with control rats.
creatinine clearance (CCr) was decreased and reached the lowest levels on day 4 (Fig.1).

**Perfusion study.** About 2-fold increase of UFR and marked decreases of CCr, renal plasma flow (RPF) were observed at day 4 after injection of cisplatin (Fig. 2). Water reabsorption rate was 99.5 ± 0.2 % in control and 94.1 ± 0.6 % in cisplatin group. Then, fractional excretion rates of sodium (FE\textsubscript{Na}), potassium (FE\textsubscript{K}) and chloride (FE\textsubscript{Cl}) (Fig. 3) were markedly increased, but urinary phosphorus (P) and magnesium (Mg) excretions (Table 1) were decreased in cisplatin-treated rats compared to control rats. In contrast, there was no differences in MAP (Fig. 2), fractional excretion rate of urea (FE\textsubscript{urea}) (Fig. 3) and urinary calcium excretion (Table 1) between control rats and cisplatin-treated rats.

The ANP infusion slightly increased UFR, RPF, CCr, FE\textsubscript{Na}, FE\textsubscript{K} and FE\textsubscript{urea} in control rats. In contrast, the ANP infusion significantly increased UFR, CCr, FE\textsubscript{Na}, FE\textsubscript{K} and excretions of P and Mg in a dose-dependent manner without affecting RPF, FE\textsubscript{urea} in cisplatin-treated rats (Figs. 2 and 3; Table 1). When ANP infusion was stopped (120-150 min), these values almost returned to basal phase levels (0-30 min) in both the control and cisplatin-treated rats. The peptide infusion decreased MAP in control rats to the same extent as in cisplatin-treated rats (Fig. 2). The effects of ANP on UFR, CCr, FE\textsubscript{Na}, FE\textsubscript{K} and excretion of Mg were more marked in cisplatin-treated rats compared to control rats. In addition, ANP decreased urinary P excretion in control rats but increased that in cisplatin-treated rats, while increased excretion of Ca was significantly observed in control rats (Table 1).

**Histological study.** Histological examination showed extensive necrosis of the S\textsubscript{3} segment of the proximal tubule located in the outer stripe of the outer medulla in the kidney of the cisplatin-treated rats (Fig. 4). There was no obvious differences in the part of S\textsubscript{1}, S\textsubscript{2} and the glomerulus between control and cisplatin-treated rats. In the distal nephron, PAS-positive cast formation was occasionally observed in cisplatin-treated rats. The high-magnification observation showed that the injured S\textsubscript{3} segment of tubular cells lost the brush border and sloughed into the lumen in cisplatin-treated rats. Regenerative cells at this part were not observed at day 4 after injection of cisplatin.

**DISCUSSION**

It is well known that natriuretic and diuretic responses to ANP are markedly reduced in nephrotic patients [8] and in nephrotic animals treated with ADR [11, 12, 14, 15] and PAN [7, 11, 16]. In contrast to these observations, the

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**Fig. 2.** The effects of ANP infusion on urine flow rate (UFR) (A), renal plasma flow (RPF) (B), creatinine clearance (CCr) (C) and mean arterial pressure (MAP) (D) in control rats (open circle) and cisplatin-treated rats (closed circle). Each point represents the mean ± SEM of values obtained from 6-8 rats. **:** p<0.001, as compared with control rats. #: p<0.05, and ##: p<0.01, as compared with basal phase (0-30 min period).
Fig. 3. The effects of ANP infusion on fractional excretion rates of sodium (FE\text{Na}) (A), potassium (FE\text{K}) (B), chloride (FE\text{Cl}) (C) and urea (FE\text{ure}) (D) in control rats (open circle) and cisplatin-treated rats (closed circle). Each point represents the mean ± SEM of values obtained from 6-8 rats. *: p<0.05, **: p<0.01, and ***: p<0.001, as compared with control rats. #: p<0.05, as compared with basal phase (0–30 min period).

Table 1. The effects of ANP infusion on urinary excretions of calcium (Ca), phosphorus (P) and magnesium (Mg) in control rats (control) and cisplatin-treated rats (cisplatin)

<table>
<thead>
<tr>
<th></th>
<th>Basal phase</th>
<th>Human ANP 30 pmol/kg (60–90 min)</th>
<th>Human ANP 100 pmol/kg (90–120 min)</th>
<th>Recovery phase (120–150 min)</th>
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<tr>
<td></td>
<td>(0–30 min)</td>
<td>(30–60 min)</td>
<td>(90–120 min)</td>
<td>(120–150 min)</td>
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<tr>
<td>Urinary Ca (µg/30 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.1 ± 1.5</td>
<td>16.0 ± 1.8</td>
<td>36.4 ± 6.0*</td>
<td>22.8 ± 3.7#</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>20.3 ± 6.1</td>
<td>22.2 ± 7.5</td>
<td>33.7 ± 12.5</td>
<td>17.8 ± 5.9</td>
</tr>
<tr>
<td>Urinary P (mg/30 min)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1.27 ± 0.14</td>
<td>1.05 ± 0.11</td>
<td>0.97 ± 0.09</td>
<td>0.56 ± 0.08*</td>
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<tr>
<td>Cisplatin</td>
<td>0.41 ± 0.05***</td>
<td>0.45 ± 0.07**</td>
<td>0.61 ± 0.08*</td>
<td>0.61 ± 0.04#</td>
</tr>
<tr>
<td>Urinary Mg (µg/30 min)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>175.4 ± 23.8</td>
<td>176.4 ± 23.7</td>
<td>193.7 ± 23.6</td>
<td>154.1 ± 19.5</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>66.6 ± 7.6**</td>
<td>78.9 ± 10.3*</td>
<td>116.3 ± 9.0***</td>
<td>86.7 ± 8.7*</td>
</tr>
</tbody>
</table>

Each point represents the mean ± SEM of values obtained from 6-8 rats. *: p<0.05, **: p<0.01, and ***: p<0.001, as compared with control rats. #: p<0.05, and ##: p<0.01, as compared with basal phase (0–30 min period).
The present study showed increased responses to ANP in cisplatin-treated rats as compared with control rats. Since ANP infusion decreased MAP in the control rats to the same extent as in the cisplatin-treated rats, the cause of the increased response to ANP in nephrotic rats is not considered to be mediated by the systemic effects of ANP. Several intrarenal mechanisms for the increased diuretic and natriuretic responses to ANP in cisplatin-treated rats may be hypothesized.

First, cisplatin-treated rats do not show fluid and Na retention while nephrotic animals treated with ADR and PAN show reduced basal levels of UFR and urinary Na excretion. Hildebrandt and Banks [7] have suggested that the altered responsiveness to ANP in nephrotic rats may contribute to the fluid and Na retention in this disorder, and there might be differences in the endogenous ANP levels and responsiveness to ANP between cisplatin-treated rats and nephrotic rats treated with ADR and PAN. In contrast, it has been reported that plasma ANP levels [1] and the density and affinity of ANP receptors in renal tissues [15] are not altered in ADR-treated rats. In addition, Radin and McCune [16] have reported that there is no significant differences in plasma ANP levels and in binding affinity of glomerular ANP receptors in vehicle-treated rats and PAN-treated rats. Thus, it may be considered that plasma ANP levels and receptors in the distal nephron is independent of occurrence of fluid and Na retention. Therefore, it is improbable that plasma ANP levels and receptors might be changed in cisplatin-treated rats.

Second, ADR and PAN damage to glomerular epithelium without injuring the distal nephron [2, 3], but cisplatin damages to tubular cells. In cisplatin-treated rats, fluid and Na reabsorption by the tubular cells is thought to be reduced by acute tubular necrosis [5, 19]. In the present study, increased UFR, FE_{Na} and decreased fluid reabsorption rate were observed in cisplatin-treated rats. Furthermore, cisplatin-treated rats showed extensive necrosis of the proximal tubule located in the outer stripe of the outer
medulla with minimal glomerular abnormalities. Thus, decreased fluid and Na reabsorption at the proximal nephron could result in the delivery of much fluid and Na to the inner medullary collecting duct which is the major renal site of action of ANP [20].

Third, it has been reported that neutral metalloendopeptidase EC3.4.24.11, an ANP-degrading enzyme, is located predominantly in the luminal membranes of proximal tubules [10, 18]. Therefore, a high concentration of ANP could be delivered to the inner medullary collecting duct since ANP could not be inactivated at the proximal tubules in cisciplatin-treated rats. This is supported by the report that marked responses to ANP are observed in rats with acute renal failure induced by gentamicin which also damages the proximal tubular cells [17].

Fourth, there may be differences in other aspects, such as renal sympathetic nerve activity, between cisciplatin-treated rats and nephrotic rats treated with ADR and PAN. Koeppke and DiBona [12] have shown that renal denervation partially reversed the blunted natriuretic and diuretic responses to ANP in ADR-treated rats.

It has been shown that ANP causes natriuresis and kaliuresis, together with an increase in the excretion of Ca, Mg and P [6, 9]. In the present study, ANP increased the excretion of Ca and Mg in a dose-dependent manner in the control and cisciplatin-treated rats. The effect of ANP on the excretion of Mg was more marked in cisciplatin-treated rats than in control rats. In contrast, the response to ANP in the excretion of Ca was more potent in control rats as compared with the cisciplatin-treated rats. It is known that the mechanism for the reabsorption of Mg at the proximal tubular cells is different from those of Na, K and Ca. In the present study, the basal levels of Ca excretion in cisciplatin-treated rats were higher than those in control rats. This observation may suggest that the reabsorption site of Ca in the proximal tubular cells might be damaged by cisciplatin. It is very interesting that ANP decreased urinary P excretion in control rats but increased that in cisciplatin-treated rats. The decreased excretion of P in control rats might be due to lack of P in the lactated Ringer's solution.

In conclusion, the main mechanism of the increased renal responses to ANP is considered to be due to an increased delivery of sodium, fluid and ANP itself to the inner medullary collecting duct which is the major renal site of action of ANP under the condition of acute proximal tubular necrosis by cisciplatin. Further studies will be necessary to characterize the renal actions of ANP in nephrotic syndrome.

REFERENCES


