

## Original Article

# The Effect of 72 Hours' Continuous Infusion of Long Acting Natriuretic Peptide on Acute Ischemic Renal Failure in the Rat

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**ABSTRACT**

**Objectives:** Atrial natriuretic peptide (ANP<sub>1-28</sub>) protects the kidneys against acute renal failure in animals; however, its use in humans has been disappointing. Long acting natriuretic peptide (LANP<sub>1-30</sub>) has natriuretic and diuretic actions similar to ANP<sub>1-28</sub>, but it has a longer half-life and a different receptor site of action. These differences comprise this study, specifically, to see if LANP<sub>1-30</sub> has better renal protection than ANP<sub>1-28</sub>, which may make it useful in the treatment of acute renal failure. **Subjects/Methods:** Three groups of male Sprague-Dawley rats were used, each with a body weight between 250-300 gm. Group 1 (ischemia only, n=6) had right nephrectomy followed by 30 minutes of left renal pedicle clamping. Group 2 (LANP Peptide treated, n=7) had renal ischemia similar to Group 1, followed by an intraperitoneal bolus of 10 mg of LANP1-30 and

placement of mini-osmotic pumps delivering LANP<sub>1-30</sub> at a rate of 1mg/hr for 72 hours. Group 3 (controls, n=6) was used to measure the baseline creatinine level and had no renal ischemia or surgery.

**Results:** 72 hours post renal ischemia, the weight loss in the ischemia group was similar to the peptide treated group (7.65 ± 1.14% and 10.03 ± 0.9% body weight loss, respectively, p=0.126). The ischemia group had significantly higher creatinine levels compared to the controls (66.3 ± 5.3 versus 30.1 ± 0.9 mmol/l, p=0.002). The peptide treated group had higher creatinine (174.1 ± 77.8 versus 66.3 ± 5.3 µmol/l, p=0.035) and LANP<sub>1-30</sub> levels (673.14 ± 69.64 versus 45.83 ± 8.45 pg/ml, p=0.001) than the ischemia group.

**Conclusion:** Prolonged use of LANP<sub>1-30</sub> has no renal protective effect.

KEYWORDS: acute renal failure, ischemia, long acting natriuretic peptide

**INTRODUCTION**

Since the discovery of atrial natriuretic peptide (ANP<sub>1-28</sub>) in 1981<sup>[1]</sup>, many experiments have been done on animals to evaluate its effect on acute renal failure<sup>[2-4]</sup>. Although ANP<sub>1-28</sub> has been shown to protect against acute renal failure in animals<sup>[5,6]</sup>, its use in humans with acute renal failure has been disappointing<sup>[7]</sup>.

ANP is a 28 amino acid peptide, derived from the carboxy end terminus of the 128 amino acid pro-ANP<sup>[8]</sup>. It has a 17 amino acid ring structure, formed by the joining of two cysteine residues<sup>[9]</sup>. ANP induces natriuresis and, in turn, diuresis, through its action on membrane guanylate cyclase (GC) receptors, located at the basolateral side of renal tubular cells, mainly in the collecting tubule<sup>[10]</sup>. Activation of guanylate cyclase leads to the formation of a second messenger cyclic GMP, which reduces the sodium channels' opening time at the luminal side of the plasma membrane, leading to the observed natriuresis<sup>[11]</sup>. ANP is

rapidly cleared from the circulation via C-type GC receptors present in many tissues, leading to its short duration of action<sup>[11]</sup>. It is also a vasodilator that can lead to hypotension, as seen in animal models and in humans<sup>[12,13]</sup>.

The use of ANP for a short period of time in animal models of acute renal failure, the short duration of action of ANP, and its hypotensive effects, are perhaps the negative factors that have hampered its usefulness in human subjects. Long acting natriuretic peptide (LANP<sub>1-30</sub>) is another natriuretic peptide that circulates freely in human plasma and is similar to ANP<sup>[14]</sup>. LANP<sub>1-30</sub> is a linear 30 amino acid peptide derived from the N-terminus end of ANP<sub>1-98</sub><sup>[15]</sup>. It has a longer duration of action than ANP and acts at different and distinct GC receptors<sup>[16]</sup>. These differences to ANP prompted the use of LANP chronically (72 hours intraperitoneal peptide infusion) in animal models with acute ischemic renal failure, to see whether it provides better renal protection.

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## MATERIALS AND METHODS

### Peptide and Mini-Pumps

LANP was purchased from TANA Laboratories (Houston, Texas, custom synthesized), peptide integrity was analyzed by reverse-phase high-pressure liquid chromatography (reverse-phase HPLC), and HPLC purified to 95% by the manufacturer (amino acid composition analysis report and HPLC-analysis graph were supplied by the manufacturer).

On the day of the experiment, LANP<sub>1-30</sub> was reconstituted with 0.1 Molar acetic acid at a concentration of 1 mg/ml. Mini-osmotic pumps (capacity 100 ml to deliver at a rate of 1 ml/hr for 72 hours, ALZA Corporation, Palo Alto, CA) were used to deliver LANP. Mini-osmotic pumps were filled with LANP, then primed in a normal saline bath at 37 °C for four hours, prior to being placed intraperitoneally in the treated animals.

### Animal surgery

Male Sprague-Dawley rats (Harlan, Indiana) weighing 250-300 grams were used. Six rats were used as ischemia only (Group 1) and seven as the peptide-treated group (Group 2). Each animal received an intraperitoneal injection of pentobarbital for anesthesia at a dose of 50 mg/kg. A midline incision was then made and the right kidneys were removed in both groups. Both groups of animals had the renal pedicle of the left kidney clamped for 30 minutes. Following unclamping of the left renal pedicle, the treated group received a 10 mg loading dose of LANP intraperitoneally, followed by placement of primed mini-pumps. The ischemia group received no treatment. All animals were returned to their cages, where they had free access to food and water.

Another group of six male Sprague-Dawley rats of similar weight (Group 3) served as controls for the measurement of plasma creatinine only. This group had no surgery or any renal ischemia.

### Creatinine and LANP measurements

Seventy-two hours after the induction of renal ischemia, all animals were weighed and decapitated. The blood was collected in tubes containing EDTA. The blood was centrifuged immediately at 4 °C for 20 minutes and the plasma collected for analysis. Creatinine was measured by Vitros CREA slides (Bay Pines Va Medical Center, Bay Pines, FL, USA).

LANP<sub>1-30</sub> measurements were done using a radioimmunoassay kit purchased from Peninsula Laboratories (Peninsula Laboratories, Belmont, CA, USA). One ml of plasma was added to 1 ml of 1% trifluoroacetic acid (TFA) and centrifuged for 20 minutes at 4 °C. Sep-columns containing 200 mg of

**Table 1**

Characteristics of the three groups of animals used in the study

Characteristic	Animal Group			p-Value
	Control group	Ischemia group	Peptide group	
IBW (gm) <sup>a</sup>	309.83 ± 6.48	294.66 ± .64	301.85 ± 4.79	0.277
EBW (gm) <sup>b</sup>	N/Ae	271.83 ± 5.51	271.43 ± 3.63	0.951
%BWD (gm) <sup>c</sup>	N/A	7.65 ± 1.14	10.03 ± 0.90	0.126
Creatinine (mmol/l)	30.1 ± 0.9	66.30 ± 5.3	174.1 ± 77.8	0.001
LANPLevel(pg/ml)d	ND <sup>f</sup>	45.83 ± 8.45	673.14 ± 69.64	0.001

Legends: a = Initial body weight (IBW), b = End Body Weight (EBW), c = Percent Body Weight Difference (% BWD), d = Long Acting Natriuretic Peptide (LANP), e=not applicable (N/A), f=not done (ND).

C18 were equilibrated by washing once with 1 ml of a buffer containing 60% acetonitrile in 1% TFA, followed by 3 ml of 1% TFA three times. The plasma was then loaded onto the pre-treated sep-columns. The columns were then washed with 3 ml 1% TFA twice, and the wash was discarded. The peptide was then eluted with 60% acetonitrile in 1% TFA, and the eluant was evaporated to dryness using nitrogen gas. Each residue was dissolved in an RIA buffer supplied with the RIAkit, using a volume of 250 ml for a two-tube assay, followed by vortexing and centrifuging. An aliquot of 100 ml was taken for each assay tube. A 100 µl of primary antibody (rabbit anti-peptide serum) was added to each tube and incubated at 4 °C for 16-24 hours. Upon completion of the incubation period, a 100 ml of <sup>125</sup>I-peptide tracer (10,000-15,000 cpm/100 ml) was added to each tube, followed by incubation for 16-24 hours at 4 °C.

At the end of the last incubation period, a 100 µl of goat anti-rabbit IgG serum was added to each tube, followed by a 100 ml of normal rabbit serum. The contents of each tube were vortexed and incubated at room temperature for 90 minutes, then 500 ml of RIA buffer and 500 ml of polyethylene glycol were added to each tube and the contents centrifuged at 3,000 rpm for 20 minutes at 4 °C. The supernatant was then decanted and the pellets were placed as duplicates in a gamma counter for peptide measurements.

### Body Weight Measurements

All animals had their body weight taken just before surgery (Initial Body Weight, IBW). The ischemia groups and the peptide treated group were weighed again at 72 hours post-ischemia (End Body Weight, EBW). Controls had only one body weight measurement taken at the time of blood collection. Body weight was measured in grams. The difference between the IBW value and EBW for Groups 1 and 2 was calculated and divided by their respective IBW, then multiplied by 100 to obtain a percentage body weight difference (% BWD).

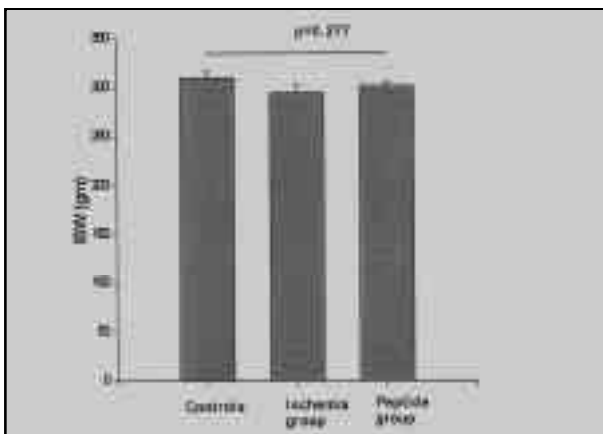


Fig. 1: Initial Body Weight (IBW) for all groups. Controls IBW 309.83 ± 6.48 gm, the ischemia group IBW was 294.66 ± 7.64 gm, and the peptide treated group had IBW 301.85 ± 4.79 gm. There was no statistically significant difference between all groups. ANOVA, p = 0.277.

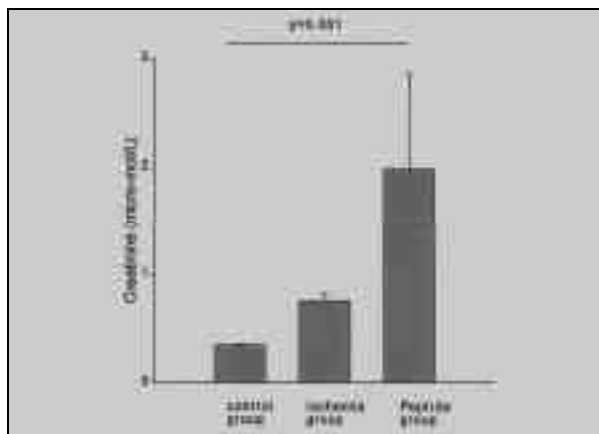


Fig. 3: Creatinine levels for the ischemia and the peptide treated groups 72 hours post-renal ischemia, and baseline creatinine for the control group. Control group 30.1 ± 0.9, ischemia group 66.3 ± 5.3, and peptide treated group 174.1 ± 77.8 μmol/l. The difference in mean creatinine levels between all groups was statistically significant (p = 0.001).

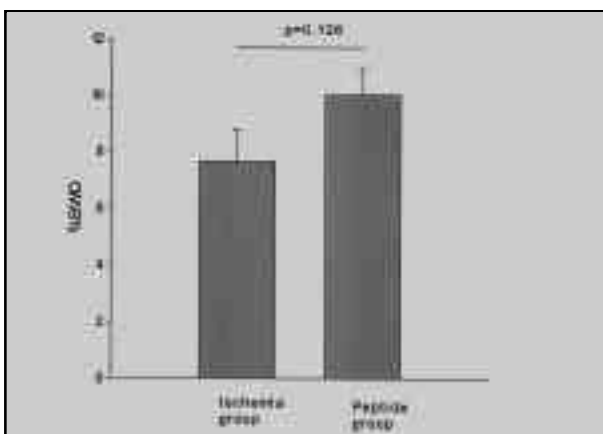


Fig. 2: Percent Body Weight Difference (% BWD) between the ischemia and the peptide treated group. The peptide treated group lost 10.03 ± 0.90 gm% of their initial body weight, compared to the ischemia group, which lost 7.65 ± 1.14 gm%. The difference between the two groups was not statistically significant (p = 0.126).

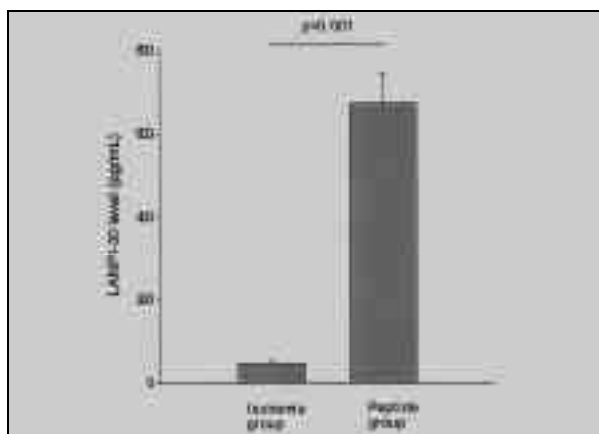


Fig. 4: Long Acting Natriuretic Peptide (LANP) levels for the ischemia and the peptide treated groups. The ischemia group had a level of 45.83 ± 8.45 pg/ml, while the peptide treated group had a level of 673.14 ± 69.64 pg/ml. The difference between the two groups was statistically significant (p = 0.001).

**Statistical Analysis**

LANP1-30 levels were taken as the average of two measurements of each plasma sample that was run in duplicates. The ANOVA test was used to compare the differences between the creatinine and IBW in the three groups. The Student t-test was used to compare differences between the ischemia group and the peptide-treated group in terms of creatinine and LANP levels, EBW, % BWD, and the intra-weight difference. Sigma Stat Jandel scientific statistical software was used for all statistical analysis. Values are expressed as means ±SE (Standard Error). P<0.05 was accepted as statistically significant.

**RESULTS**

**Body weight**

The ischemia group had an IBW mean value of 294.66 ± 7.64 gm, and the peptide treated group had a mean value of 301.85 ± 4.79 gm. The controls had a mean body weight of 309.83 ± 6.48 gm. There was no

statistically significant weight difference between the three groups (ANOVA p=0.277) (Fig. 1). The mean EBW for both the ischemia group and the peptide treated group was lower than at the beginning of the experiment. For the ischemia group, the mean EBW was 271.83 ± 5.51 gm, which was statistically significant compared to their IBW (p=0.036). For the peptide-treated group, EBW reached a mean of 271.43 ± 3.63 gm, which was also statistically significant compared to their IBW (p<0.001). For the ischemia group, the loss in body weight, calculated as a % BWD, was 7.65 ± 1.14 gm %, and for the peptide-treated group it was 10.03 ± 0.90 gm %. The % BWD between the ischemia group and the peptide-treated group, however, was not statistically significant (p=0.126) (Fig. 2), nor was the difference in EBW between the two groups (p= 0.951).

**Creatinine levels**

Mean creatinine levels for the ischemia group and controls were 66.3 ± 5.3 versus 30.1 ± 0.9 mmol/l.

For the peptide treated group, mean creatinine was  $174.1 \pm 77.8$  mmol/l. The difference between the three groups was statistically significant (ANOVA,  $p=0.001$ ) (Fig. 3). The difference in the mean creatinine level between the peptide treated group and the ischemia group was statistically significant ( $p=0.035$ ).

#### LANP<sub>1-30</sub> level

The ischemia group had a mean LANP<sub>1-30</sub> level of  $45.83 \pm 8.45$  pg/ml, and the peptide treated group had an LANP<sub>1-30</sub> level of  $673.14 \pm 69.64$  pg/ml. The mean LANP<sub>1-30</sub> level difference between the ischemia group and the peptide treated group was statistically significant ( $p=0.001$ ) (Fig. 4).

#### DISCUSSION

ANP<sub>1-28</sub> is a 28 amino-acid peptide of the carboxy terminal end of the 126 amino-acid pro-ANP<sup>[8]</sup>. It has been shown to impart renal protection when used in animals with acute renal failure<sup>[5,6]</sup>. Its use in humans, however, has been disappointing.

Long Acting Natriuretic Peptide (LANP<sub>1-30</sub>) is a linear 30 amino-acid natriuretic peptide derived from the N-terminus end of the prohormone pro-ANP<sub>1-98</sub><sup>[15]</sup>. LANP<sub>1-30</sub> has similar natriuretic and diuretic activities to ANP<sub>1-28</sub>, activating a particulate guanylate cyclase, resulting in an increase in intracellular cGMP<sup>[14]</sup>. Cyclic GMP, in turn, leads to a reduction in sodium entry via the amiloride-sensitive cation channel<sup>[15]</sup>. LANP<sub>1-30</sub> freely circulates in plasma, has a longer half-life than ANP<sub>1-28</sub>, and acts at distinct and separate GC receptors than ANP<sub>1-28</sub><sup>[16]</sup>. These differences prompted the use of LANP<sub>1-30</sub>, to see if it would lead to better renal protection in animal models with acute ischemic renal failure.

As seen in Table 1, all animals had a similar body weight at the start of the experiment ( $p=0.277$ ). Base line creatinine, as determined in Group 3, was  $30.1 \pm 0.9$   $\mu$ mol/l. After 72 hours post-acute ischemic renal failure, body weight loss was significant in the ischemia group ( $p=0.036$ ). The same group also had significantly higher creatinine than the baseline that was determined in controls ( $p=0.002$ ). This weight loss in the setting of acute renal failure could be due either to urinary fluid loss, poor intake secondary to uremia, or a combination of both; however, the exact mechanism was not determined in this study.

In the peptide treated group, body weight loss as absolute weight loss (IBW-EBW) or % BWD was not different from the ischemia group ( $p=0.951$  and  $p=0.126$ , respectively). The LANP<sub>1-30</sub> treated animals had significantly higher creatinine than the ischemia group 72 hours post-acute ischemic renal failure ( $p=0.035$ ). That the ischemia and the peptide treated

rats had similar weight loss, yet the peptide treated group had higher creatinine level, points against weight loss as the factor that led to this difference.

LANP<sub>1-30</sub> measurements by radio-immunoassay showed that the LANP<sub>1-30</sub> treated group had up to 15 times higher levels of LANP<sub>1-30</sub> in their plasma compared to the ischemia group ( $p=0.001$ ). The use of mini-osmotic pumps is, therefore, a simple and a viable way of delivering peptides intraperitoneally over prolonged periods of time.

Compared to the use of LANP<sub>1-30</sub>, the use of ANP in animals with acute renal failure has shown some protection as shown by Neumayer in the dog<sup>[17]</sup>. Other models of ischemic acute renal failure have shown similar results. In the rat, infusion of atrial natriuretic factor improved renal function<sup>[18]</sup>, and in the norepinephrine induced acute renal failure<sup>[19]</sup>.

In conclusion, Long Acting Natriuretic Peptide (LANP<sub>1-30</sub>, ANP<sub>1-30</sub>) has no protective effect in the setting of acute ischemic renal failure in the rat. Moreover, it leads to worsening of renal function when used continuously for a prolonged period of time.

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