

Effect of Etanercept on Atrial Natriuretic Peptide Induced Changes in Heart Rate and Biochemical Parameters

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ABSTRACT

The atrial natriuretic peptide (ANP), a member of the natriuretic peptide family, is a cardiovascular hormone which possesses well defined natriuretic, diuretic and vasodilating properties. Most of the biological effects of ANP are mediated through its guanylyl cyclase coupled receptor. ANP and its receptors have been shown to be expressed and differentially regulated in the immune system, and it has been suggested that ANP has an immuno-modulatory potency. A regulatory role for ANP has been suggested for the secretion of Tumor necrosis factor- α (TNF- α) in whole blood. In this study, we investigated the effect of pre-treatment with etanercept on ANP induced changes in heart rate, serum uric acid and creatinine levels. Our study suggests that etanercept modulated the ANP induced change in heart rate and serum creatinine but did not significantly affect the serum uric acid levels.

Key Words: Etanercept, ANP, Uric Acid, Heart Rate, Creatinine, TNF-Alpha

INTRODUCTION

Heart failure (HF) is a serious anomaly with high mortality rates. Levine *et al.*, (1998) demonstrated that circulating levels of TNF- α were elevated in patients with end stage heart failure. Short and mid-term pharmacological perspectives target hormonal systems and cytokines: endothelin-receptor antagonists, inhibition of natriuretic peptide degradation (via neutral endopeptidase), and newer drugs acting against TNF- α such as etanercept. It has recently been suggested (Murray *et al.*, 2002) that plasma natriuretic peptide concentrations might provide a simple biochemical means of tailoring therapy for the treatment of heart failure. Intensification of treatment, aimed at normalizing natriuretic peptide concentrations, might improve outcome.

On the other hand, the natriuretic peptides exert their biological actions through three receptors, two of which are membrane bound guanylyl cyclases (NPR-A and NPR-B). The guanylyl cyclases NPR-A and NPR-B contain an extracellular, ligand binding domain whereby NPR-A binds ANP and brain natriuretic peptide (BNP), and NPR-B binds CNP. The third receptor serves as clearance receptor (C-receptor). NPR-C has the potency to internalize and clear the natriuretic peptides. Moreover, an increasing number of reports show that several biological effects of ANP are mediated through this “clearance” receptor (NPR-C) (Vollmar *et al.*, 2005). These effects seem to be related to a G-protein coupled inhibition of adenylyl cyclase. The action of ANP in the cardiovascular system has been well studied and

investigations have concentrated mainly on the diuretic, natriuretic and vasodilating properties of ANP. Of further importance is that ANP was found to exert an inhibitory action on the production of TNF- α in activated rodent macrophages and in the whole human blood. Taken together, ANP is suggested to be a regulator of macrophage/leukocyte activation and therefore, of inflammation.

Uric acid is known to induce the nuclear transcription factor (NF- κ B) and monocyte chemoattractant protein-1 (MCP-1). Regarding TNF α it has been shown that serum uric acid levels significantly correlate with TNF α concentrations in congestive heart failure and as a result Olexa P *et al.* concluded that serum uric acid may reflect the severity of systolic dysfunction and the activation of an inflammatory reaction in patients with congestive heart failure (Olexa *et al.*, 2002). Serum uric acid represents the strongest predictor of elevated urinary albumin concentrations in HF. Additionally, uric acid also stimulates human mononuclear cells to produce interleukin-1 β , IL-6, and TNF α (Johnson *et al.*, 2003). The macrophage cytokine TNF- α , together with other inflammatory mediators (Weber *et al.*, 2003), plays a key role in many pathophysiologic conditions, such as rheumatoid arthritis (Neale *et al.*, 1989), atherosclerosis, or septic shock (Rhodes *et al.*, 1992). Atrial natriuretic peptide (ANP) has been shown to inhibit the TNF- α induced adhesion molecule expression in endothelial cells (Kierner *et al.*, 2002). ANP has also been shown to inhibit TNF- α production in

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interferon-gamma-activated macrophages (Kiemer *et al*, 2000). ANP was previously shown to be an autocrine regulator of iNOS (Vollmar *et al*, 1995), an enzyme strongly induced in inflammatory processes (Pedram *et al*, 2001) such as septic shock (Aiura *et al*, 1995).

This study was hitherto carried out to investigate the effect of etanercept pretreatment on ANP induced changes in heart rate and serum biochemical parameters like uric acid and creatinine in healthy albino Wistar rats.

MATERIALS AND METHODS

Animals and housing condition

Experiments were performed on Wistar albino rats of either sex weighing, between 180-220 g obtained from experimental animal center of Christian medical College, Vellore, India. Animals were housed in groups of 5-6 animals in polypropylene plastic cages under hygienic conditions, lined with paddy-husk bedding. Animals were housed in a colony room once the experiments completed under controlled temperature (25 $\pm$ degree C), relative humidity Of (60 $\pm$ 2%) and were exposed to a 12 hour light :12 hour dark cycle, with food and water available *ad libitum*. All experiments were conducted during the light phase, between 8.00- 13.00 hours. Experimental protocol was approved by Institution animal ethics committee (IAEC).

Drugs and chemicals used

The following drugs and chemicals were used for this study: Etanercept injection (Pfizer Limited), Atrial natriuretic peptide (Sigma chemical Co.,India). Other chemicals and reagents obtained from commercial sources were 0.9% Saline Solution, uric acid standard solution, Phosphotungstic acid, Sodium tungstate, standard creatinine, Picric acid, NaOH and Sulphuric acid.

Estimation of cardiac rate:

Wistar albino rats weighing between 180 to 220 g were utilized for this study. Animals were anesthetized with Ketamine and Diazepam. Electrocardiography was conducted using the limb lead II on a physiograph (INCO, India) using a speed of 10 mm/second. Drugs like etanercept and ANP were administered to the animals intravenously into the cannulated jugular vein. Heart rate was estimated from the ECG tracings by counting the number of 'R' waves per minute as per earlier described technique (Tyagi and Thomas *et al*, 1999).

Procedure for estimation of serum creatinine:

Estimation of serum creatinine was determined in Wistar rats (Nirmala *et al*, 2010). Blood was collected from the

cardiac puncture (by glass capillary method) or from the sub-mandibular region of the animals. 2.5-5ml of blood was collected according to standard procedures in glass tubes. The whole blood was fractioned by centrifuging at 1500-2000 X g for 10-15 min at room temperature. This would separate the blood into an upper serum layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs. A sterile plastic or glass pipette to transfer the serum into a clean and dry boiling tube was used. 4 ml of serum was pipette out into the boiling tube. Into the boiling tube, added 6 ml of water, which was followed by the addition of 2 ml of 10% sodium tungstate and 4 ml of 2/3 N H₂SO₄. The contents were mixed in the boiling tube thoroughly and let stand for 10 minutes. The contents of the tube filtered through a Whatman No.1 filter paper and preserved the filtrate for creatinine estimation by spectro-photometric technique.

Estimation of serum uric acid:

Estimation of serum creatinine was determined in Wistar rats. Blood was collected from the cardiac puncture (by glass capillary method) or from the sub-mandibular region of the animals. 2.5-5ml of blood was collected according to standard procedures in glass tubes. The whole blood was fractioned by centrifuging at 1500-2000 X g for 10-15 min at room temperature. This would separate the blood into an upper serum layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs. For the estimation of serum uric acid, serum was deproteinised. The filtrate containing uric acid reduces phosphotungstic acid and alkaline medium to form a blue complex, tungsten blue. The intensity of blue colour that develops was measured using a colorimeter.

RESULTS AND DISCUSSION

Effect of Etanercept and ANP on Heart Rate

The results of our study on the effect of drugs on the heart rate are shown in the Table 1. Three separate control groups (n=6) were used for this study. In the test groups the animals received intravenous injections of either etanercept (0.3 ml, 5mg/ml concentration) or ANP (25 μ g concentration, 0.2 ml) alone or in combination. Three separate recordings of the ECG were done and heart rate estimated. The statistical inference is shown in the Table 1.

Serum Creatinine Levels Estimation in Wistar Rats

The results of our study on the effect of etanercept and ANP on serum creatinine levels are shown in Figure 1. The animals were injected intra peritoneally with etanercept (5 mg/ml; dose 0.3 ml) alone for 40 minutes or before ANP (Dose 0.2 ml / rat, concentration: 25 μ g/ ml)

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Table 1. The percentage change in heart rate of rats (n=6) after Etanercept and ANP treatment. The results depict the standard deviation and statistical significance values.

S. No.	N	Pretreatment	Treatment	Heart rate (Beats/minute)	% change from control	SD	P-value
1.	6	Nil	Control ¹	365	0	34.05	----
2.	6	Nil	Etanercept	333	8.76	34.30	0.008
3.	6	Nil	Control ²	309	0	20.46	----
4.	6	Nil	ANP	321	3.73	22.22	0.015
5.	6	Nil	Control ³	360	0	35.07	----
6.	6	Etanercept	ANP	340	5.55	32.04	0.031

N= number of animals

Control¹= Control for etanercept

Control²= Control for ANP

Control³= Control for combination (Etanercept + ANP)

SD= Standard Deviation

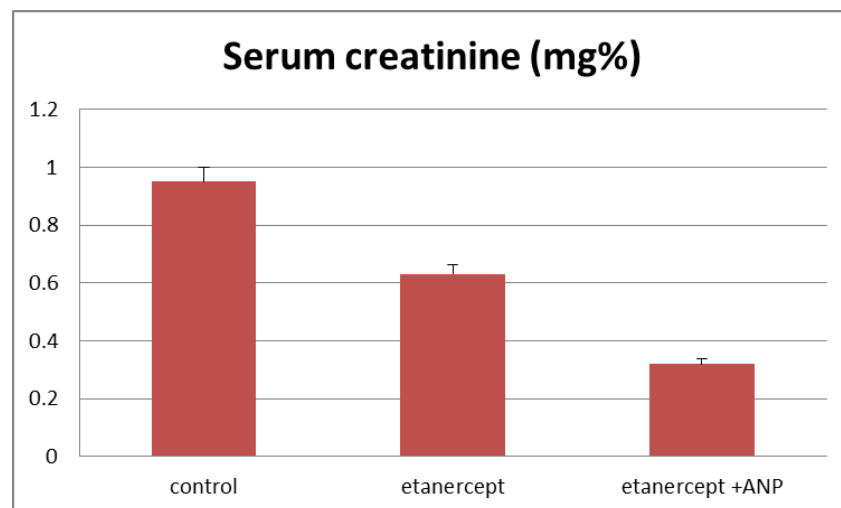


Figure 1. The effect of Etanercept and ANP on serum creatinine levels depicted as mg %.

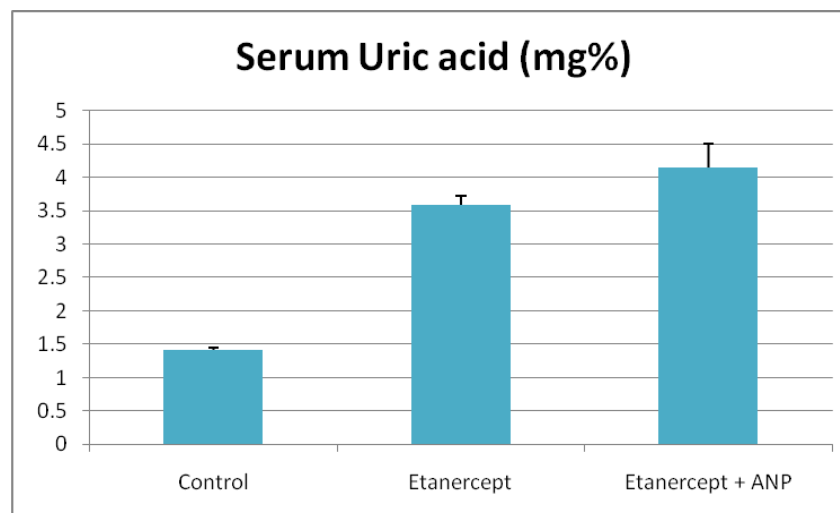


Figure 2. The effect of Etanercept and ANP on serum uric acid levels depicted as mg %.

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and the blood collected for serum creatinine estimation. The results with serum creatinine show that etanercept pretreatment attenuated the levels of serum creatinine post ANP treatment.

Serum Uric Acid Levels Estimation In Wistar Rats

The results of our study on the effect of etanercept and ANP on serum uric acid levels are shown in Figure 2. The animals were injected intra peritoneally with etanercept (5 mg/ml; dose 0.3 ml) alone for 40 minutes or before ANP (Dose 0.2 ml / rat, concentration: 25 µg/ ml) and the blood collected for serum uric acid estimation. The results with serum uric acid show that etanercept pretreatment increased the levels of serum uric acid post ANP treatment.

Although heart failure has traditionally been viewed as a hemodynamic disorder, the currently accepted paradigm has evolved to reflect the contribution of neurohormonal and proinflammatory cytokine activation to the progression of the disease (Werden, 1998, Beg *et al*, 1996). In this regard, TNF-alpha is a pro-inflammatory cytokine that has been implicated in the pathogenesis of cardiovascular diseases, including acute myocardial infarction, chronic heart failure, atherosclerosis, viral myocarditis, cardiac allograft rejection, and cardiac dysfunction associated with sepsis (Meldrum 1998). An effective blocker of the TNF-alpha is etanercept and this drug has been introduced in the market for the treatment of rheumatoid arthritis. Plasma ANP levels are also affected in congestive heart failure (CHF), hypertension, and septic shock (Levin *et al*, 1998) presumably as a result of the adaptive response, which indicates that ANP and its receptors, may play important roles in the pathophysiology of these disorders.

The atrial natriuretic peptide, ANP was the first described member of the natriuretic peptide family, a family of cardiovascular cyclic peptide hormones. Due to its natriuretic and diuretic properties, ANP exhibits strong cardiovascular effects, such as regulation of blood pressure and plasma volume expansion. ANP mediates most of its cardiovascular and renal effects through interaction with the guanylyl-cyclase-coupled natriuretic peptide receptor, NPR-A, via cGMP as second messenger (Levin *et al*, 1998). In our study, pretreatment with etanercept caused a reduction of heart rate after ANP treatment as depicted in Table 1. This decreasing effect of ANP can be attributed to the possible effects of ANP on guanylate cyclase enzyme in the heart after TNF alpha attenuation (Pandey *et al*, 1994). ANP also binds to the non-guanylyl-cyclase-linked natriuretic peptide clearance receptor (NPR-C). Besides the clearance function exerted by NPR-C, an NPR-C-mediated inhibition of adenylyl-

cyclase was shown to be responsible for several *in vitro* effects of ANP. Because the ANP effect on TNF-alpha synthesis is shown to be mediated by cGMP, an insufficient amount of cGMP produced by CNP may indeed be responsible for the lack of TNF-alpha inhibition by this peptide. After demonstrating a specific inhibitory action of ANP on TNF-alpha production, the molecular mechanism of action needs to be investigated. In our study, Etanercept pretreatment also modified the effects of ANP on the serum creatinine levels although not much effect was seen on uric acid levels (Figs.1 & 2). ANP treatment attenuated the etanercept induced increase in creatinine levels and this could be beneficial for patients of heart failure (Gurantz *et al*, 2005). This can be attributed to the interaction between TNF-alpha and ANP as shown by previous studies. Because TNF-alpha is a transcriptionally regulated cytokine (Tendera *et al*, 1999), the effect of ANP on TNF-alpha mRNA has been determined. Previous studies done with Northern blot analysis revealed markedly reduced levels of TNF-alpha mRNA initiated by ANP. The levels of TNF-alpha mRNA are mainly controlled via activation of respective transcription factors (Kierner *et al*, 2000, Kierner *et al*, 2000). It was hypothesized that ANP decreases the transcription of TNF-alpha mRNA by interfering with two prominent transcription factors involved in TNF-alpha regulation, NF-kb and AP-1. Thus the modulatory effects of etanercept on ANP induced changes in heart rate and creatinine can be attributed to these factors.

Thus in conclusion, it can be stated based on the results of this study that etanercept was able to modulate the effects of ANP on the heart rate and serum creatinine levels considerably although the modification of the effects on serum uric acid levels was not appreciable.

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