SCIENCE AND TECHNOLOGY - Research Journal - Volume 3 - 1999
University of Mauritius, Réduit, Mauritius.

PLASMA ATRIAL NATRIURETIC PEPTIDE AS A NON-INVASIVE BIOCHEMICAL MARKER OF DYSPNOEA IN CONGESTIVE HEART FAILURE PATIENTS

by

A. H. SUBRATTY

Department of Health & Medical Sciences, Faculty of Science,

University of Mauritius, Réduit, Mauritius

(Received October 1998 – Accepted January 1999)

ABSTRACT

Plasma Atrial Natriuretic Peptide (ANP) levels on exercise were measured in 10 male patients suffering from congestive heart failure and 10 male subjects not presenting with any cardiac problems. A standardised 12min walking test was performed with ANP venous levels measured by radioimmunoassay pre and post exercise. Normal subjects aged 36 ± 6 yr manifested a non-significant increase of plasma ANP level from 5.2± 0.3 to 7.2 ± 0.2 pg/ml (P=0.15). The same exercise was also associated with a non-significant decrease from 64.6 ± 0.3 to 55.4 ± 0.2 pg/ml (P=0.34) in the Class II & III NYHA classification heart failure patients (mean age = 65 ± 5 yr). Our biochemical data showed a correlation (r=0.68, P=0.04) with a mean dyspnoea score assessed by a 10 graded visual analogue scale in the control group (mean score = 1) and an increased from 1.6 to 6.4 in the heart failure patients. In conclusion, as compared to normal adults, heart failure patients are characterised by a high ANP level at rest with a decrease in ANP levels on a dyspnoea-producing exercise.

Keywords : ANP, congestive heart failure, dyspnoea, visual analogue scale, exercise.

INTRODUCTION

Atrial natriuretic peptide (ANP) is synthesised and secreted by mammalian cardiocytes, and exerts extremely potent vasorelaxant and natriuretic/diuretic activities. The major physiological stimulus for the release of ANP is atrial stretch following a rise in atrial pressure. The process by which this mechanical event is translated into the release of the peptide appears to be dependent upon the activities of the phosphoinositol pathway and stimulation of protein kinase C. One of the determinants of right atrial pressure is the intravascular volume. In experimental studies plasma ANP has been shown to increase in response to acute volume expansion with intravenous saline and fall with volume depletion following administration of Furosemide (Wilkins *et al.* 1989).

A small but sustained rise in plasma ANP has been observed with increased dietary salt intake (Sanghella *et al*. 1987). Plasma ANP has been reported to be elevated in patients with congestive heart failure in proportion to the severity of the disease (Cody *et al*. 1987). Raised circulating levels are found in volume overload patients with chronic renal failure and levels fall during fluid removal during dialysis. Plasma ANP levels are also elevated in some patients with hypertension, most notably those with left ventricular hypertrophy. These studies suggest that ANP may play an important role in off-loading the heart in acute volume expansion, but the multifactorial regulation of sodium balance and blood pressure allow adequate compensation during prolonged ANP deficiency. Several studies have also reported an increasing plasma ANP level during dynamic exercise in healthy persons and in mild hypertension as well as in heart failure (Crozier, 1987).

The clinician's interest in atrial natriuretic peptide is focused on the insight it is believed to provide into the physiopathology of disease and its therapeutic potential in disorders associated with fluid overload and elevated blood pressure. A compound that combines natriuresis, diuresis, vasorelaxation and inhibition of the renin-angiotensin aldosterone system is an attractive candidate for pharmaceutical development; and may yet offer a novel therapeutic approach to the management of fluid retention and hypertension.

All reported studies have been accentuated towards investigating (i) the role of ANP as a natriuretic and vasorelaxant peptide and (ii) measurements of the peptide during dynamic (bicycle) exercise. A widely held view is that ANP may act as a circulating hormone with a role in regulating blood volume and blood pressure.

The response of plasma ANP to intravascular volume changes is appropriate for such a role and the profile of biological activity of ANP provide further support for this study. The present study was therefore designed to (i) compare levels of ANP in congestive heart failure while performing a daily activities test, and (ii) correlate dyspnoea scores as assessed using a visual analogue scale with measured levels of the peptide in the patients and control groups.

METHODOLOGY

Ten (10) male heart failure patients of Grade II and III severity according to the New York Heart Association Classification aged between 35-50yr participated in this study. Clinical evaluation included a resting electrocardiogram and standard chest X-ray. The patients were matched for age and sex with ten normal subjects. Upon inclusion into the study and oral consent obtained, 10ml of venous blood was collected from the antecubital fossa of the forearm. No tourniquet was used during blood collection to prevent stasis. Following blood collection, shortness of breath (dyspnoea) was assessed using a visual analogue scale in each participant while performing a 10 graded daily physical activities as described by Subratty *et al*.(1994). At the end of the daily activities test, 10ml of venous blood was collected again. Blood collected on ice was centrifuged at 3,000*g* for at least 5min and plasma obtained was used for the determination of ANP using a radioimmunoassay technique (Amersham, UK).

Measurement of plasma atrial natriuretic peptide

The assay of plasma ANP is based on the competition between unlabelled alpha-ANP (plasma ANP) and a fixed quantity of ^{125}I-labelled human ANP for a limited number of binding sites on an alpha-ANP specific antibody. To achieve greater sensitivity, a disequilibrium technique is used. Unlabelled human alpha-ANP (standard or sample) is pre-incubated with the antibody for 24h before addition of the ^{125}I-tracer. This technique reduces the number of binding sites available to the tracer, being dependent on the quantity of unlabelled human alpha-ANP present. This situation of "disequilibrium" leads to a sensitive dose response curve.

The antibody bound human alpha-ANP is then reacted with the Amerlex-M second antibody bound to the magnetisable polymer particles. Separation of the antibody bound fraction is centrifuged, followed by decantation of the supernatant. Measurement of the antibody bound fraction in the pellet enables the amount of labelled human alpha-ANP in the bound fraction to be calculated. The concentration of the unlabelled alpha-ANP in the sample is then determined by interpolation from

the standard curve. The radioactivity present in each tube is determined by counting for at least 60 seconds in a Gamma-scintillation counter.

Statistics

One way analysis of variance (ANOVA-test) and linear correlation analysis were used to assess dyspnoea scores and plasma ANP levels in the study populations. All data were analysed using Epi-info software. P values < 0.05 were taken as significant.

RESULTS

Our measurements did not show significant changes in plasma ANP levels at rest and on exercise for both the congestive heart failure patients and the control group ($P>0.05$). However, plasma ANP levels were significantly different ($P<0.05$) in the two groups at rest and on exercise as shown in Fig. 1. Furthermore, analysis showed a correlation between changes in dyspnoea scores and plasma ANP levels in the control group and the CHF patients.

The quality of life diagram (Fig. 2) shows changes in mean dyspnoea scores between the control and congestive heart failure patients pre and post exercise. It is to be noted that at rest the heart failure patients were already showing symptoms of shortness of breath. However, upon exercise a shift to the right in the mean dyspnoea scores further confirms the severity of dyspnoea in the patients which is reflected by the mean plasma ANP levels (Fig. 1).

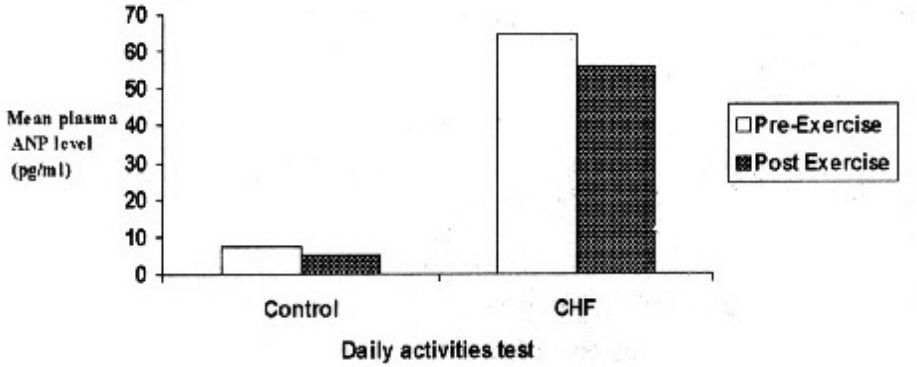

Fig. 1. Mean ANP levels in normal subjects and congestive heart failure patients pre and post exercise

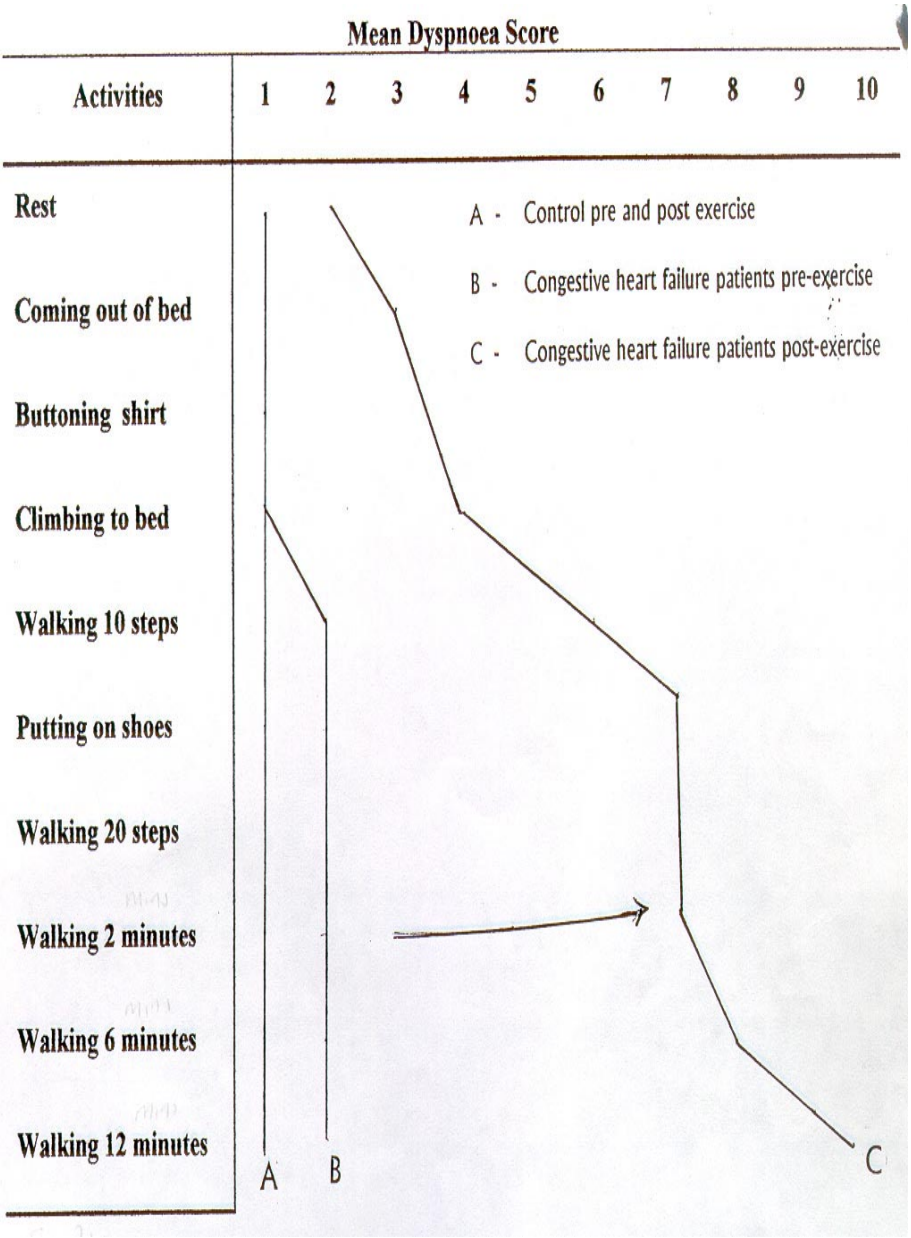

Fig. 2. Quality of life diagram showing changes in mean dyspnoea scores in normal and congestive heart failure subjects pre and post exercise.

DISCUSSION

Certain authors have reported that patients with chronic heart failure have raised circulating plasma levels of atrial natriuretic peptide compared to normal subjects (Mc Murray *et al.* 1988). Data from the literature show that both submaximal and maximal exertion produce increases in plasma ANP concentration (Weight *et al.* 1991). These increases are present throughout exercise but rapidly returned to pro-exercise values once exercise has stopped (Cornachelli *et al*. 1989). Hence the question that arises is whether the absence to detect significant changes in plasma ANP levels in the present study could be due to,

(i) The use of a mild exercise test in the form the daily activity test,
(ii) The production of precursor(s) of inactive fragments of ANP.

Chati *et al.* (1996) have reported that a downward shift in ANP levels in congestive heart failure patients could be due to a loss of biological activity of the peptide. Failure to increase ANP level on exercise in heart failure patients in our study could thus be due to an impaired mechanism of secretion of the peptide during a dyspnoea inducing activity. Our data are in agreement with similarly reported findings (Szekeres *et al.* 1993; MacGowan *et al.* 1996). However as far as we know this is the first study to show a relationship between severity of dyspnoea as assessed subjectively using a visual analogue scale and decrease in plasma ANP level in heart failure patients.

In conclusion, our results indicate that plasma atrial natriuretic peptide concentration could be used as a sensitive biochemical marker of cardiac impairment.

REFERENCES

CHATI, Z., MERTES, P.M., ALIOT, E. & ZANNAD, F. (1996). Plasma levels of atrial natriuretic peptide and of other vasoconstricting hormones in patients with chronic heart failure: relationship to exercise capacity. *International Journal of Cardiology* **57** (2), 135-142.

CODY, J.R., ATLAS, A. & LARAGH, J.H. (1986). Atrial natriuretic factor in normal subjects and heart failure patients. *Journal of Clinical Investigation* **78,** 1362-1374.

CORNACHELLI, A., DE GUILI, G. & CECONI, C. (1989). The course of ANF release during cardiopulmonary bypass. *Journal of Molecular and Cell Biology* **21** (suppl. IV).

CROZZIER, I.G. (1987). Haemodynamic effects of ANP infusion in congestive heart failure. *Lancet* **(II)**, 1242-1245.

MAC GOWAN, G.A., MURALI, S., LOFTUS, S. & URETSKY, B.F. (1996). Comparison of metabolic, ventilatory and neurohumoral responses during light forearm isometric exercise and isotonic exercise in congestive heart failure. *American Journal of Cardiology* **77** (5), 391-396.

Mc MURRAY, J., Mc FARLANE, L. & COUTIE, W. (1988). Atrial natriuretic factor attenuated prostaglandin E_2-stimulated plasma renin activity in humans. *Journal of Cardiovascular Pharmacology* **14**, 326-330.

SANGHELLA, G.A., MARKANHU, N.D. & SHORE, A.C. (1987). Plasma immunoreactive atrial natriuretic peptides and changes in dietary sodium intake. *Life Sciences,* 96-100.

SUBRATTY, A.H., MANRAJ, M. & BALIGADOO, S. (1994). A visual analogue scale for assessment of dyspnoea in congestive heart failure. *International Journal of Clinical Pharmacology, Toxicology & Therapeutics* **32** (5), 259-260.

SZEKERES, T., ARTNER-DWORZAK, E. & PUSCHENDORF, B. (1993). Correlation of atrial natriuretic peptide and cyclic guanosine monophosphate plasma concentrations in patients with heart disorders during rest and exercise. *European Journal of Clinical Chemistry & Clinical Biochemistry* **31** (2), 69-74.

TIKKANEN, T., TIKKANEN, I. & SARIOLA, H. (1989). Attenuated diuretic and natriuretic effects of ANP in rats with heart failure. *Hormone Metabolic Research* **21,** 185-188.

WEIGHT, L.M., ALEXANDER, D. & JACOBS, P. (1991). Strenuous exercise: analogous to the acute-phase response? *Clinical Science* **81**, 677-683.

WILKINS, M.R., STOTT, R. & LEWIS, H. Atrial natriuretic factor (1989). *Annals of Clinical Biochemistry* **26,** 115-118.