Original Article

Impact of N-acetylcysteine on Endothelial Function, B-type Natriuretic Peptide and Renal Function in Patients with the Cardiorenal Syndrome: A Pilot Cross Over Randomised Controlled Trial☆

Anthony C. Camuglia, MBBS (HonsI)[a], Micha T. Maeder, MD[b], Jennifer Starr[b], Catherine Farrington[b] and David M. Kaye, MD, PhD[a,b,*]

[a] *The Alfred Hospital, Commercial Road, Melbourne 8008, Victoria, Australia*
[b] *Heart Failure Research Group, Baker IDI Heart and Diabetes Institute, P.O. Box 6492, St Kilda Road Central, Melbourne 8008, Victoria, Australia*

Background: **Both heart and renal failure are characterised by increased systemic oxidative stress and endothelial dysfunction and occur in the cardiorenal syndrome (CRS). The aim of the present study was to assess the impact of N-acetylcysteine (NAC), a potent antioxidant, on endothelial function, B-type natriuretic peptide (BNP) and renal function in patients with CRS.**

Methods: **In a double blind, placebo controlled manner, we randomised nine stable outpatients with both heart failure (LVEF < 40% and NYHA class II or III) and renal failure (Cockroft Gault clearance of 20–60 ml/min) to placebo or NAC (500 mg orally twice daily) for 28 days followed by a wash out period (>7 days) and crossover to the other treatment.**

Results: **Eight patients completed the study and all data ($N = 9$) was used in the analysis. Mean forearm blood flow improved significantly with NAC with mean ratio of improvement of 1.99 (SEM: ±0.49) for NAC and 0.73 (SEM: ±0.23) for placebo with a p-value of 0.047. There was no significant difference in BNP ($p = 0.25$), renal function ($p = 0.71$) or NYHA class ($p = 0.5$). No deaths occurred during the trial.**

Conclusion: **In this pilot trial of patients with CRS, NAC therapy was associated with improved forearm blood flow. This may represent a general improvement in endothelial function and warrants further investigation of antioxidant therapy in these patients.**

(Heart, Lung and Circulation 2013;22:256–259)

Keywords. Heart failure; Endothelial dysfunction; Cardiorenal syndrome; Oxidant stress

Introduction

The cardiorenal syndrome (CRS) is a frequent complication in patients with chronic systolic heart failure of various aetiologies. The pathogenesis of the syndrome involves an interplay of several described mechanisms as well as yet poorly understood changes [1]. Type 2 CRS has been defined as the occurrence of chronic renal disease in the setting of, and likely driven by, chronic heart failure [2]. Neurohormonal changes in CRS include modulation of sympathetic nervous system activity and the renin–angiotensin–aldosterone system (RAAS) [3–5].

Heart failure and renal disease, both elements of the CRS, are associated with increased total body oxidative stress and endothelial dysfunction [6–9]. Endothelial dysfunction in the setting of heart failure has also been associated with increased morbidity [10]. Antioxidant therapy has a possible role in modifying this process. N-acetylcysteine (NAC) has been shown to improve endothelial function in end stage renal failure patients on dialysis with heart failure [11,12] as has the use of allopurinol in patients with heart failure [13]. N-acetylcysteine has also been demonstrated to improve cardiovascular function in a Syrian hamster heart failure model [14]. We hypothesised that antioxidant therapy with the potent antioxidant N-acetylcysteine would improve markers of endothelial, cardiac and renal function in patients with type 2 CRS.

Received 26 August 2012; received in revised form 24 October 2012; accepted 27 October 2012; available online 7 December 2012

☆ ClinicalTrials.gov number, NCT00532688.

* Corresponding author at: Heart Failure Research Group, Baker IDI Heart and Diabetes Institute, P.O. Box 6492, St Kilda Road Central, Melbourne 8008, Victoria, Australia.
E-mail address: david.kaye@bakeridi.edu.au (D.M. Kaye).

1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2012.10.012

Methods

Overview of Study Design

We performed a double blind crossover placebo controlled study investigating N-acetylcysteine (NAC) as an adjunctive therapy in patients with type 2 cardiorenal syndrome. We randomised nine stable outpatients with both heart failure (LVEF < 40% and NYHA functional class II or III) and renal failure (Cockroft Gault clearance of 20–60 ml/min) to placebo or NAC (500 mg orally twice daily) for 28 days followed by a wash out period (>7 days) and crossover to the other treatment for 28 days. The patients were recruited from the Heart Failure Clinic at the Alfred Hospital, Melbourne, Australia. The study protocol was approved by the Alfred Hospital Research and Ethics Committee. All study participants provided written informed consent.

Inclusion and Exclusion Criteria

Patients aged 18–75 years of age were eligible to be enrolled in the study. Inclusion criteria included presence of NYHA functional class II or III heart failure with LVEF of <40%. Patients must not have been admitted to a hospital for management of decompensated cardiac failure in the month preceding enrollment. Patients also had to have evidence of chronic renal failure with a glomerular filtration rate (GFR) (as estimated by the Cockroft Gault equation) of >20 ml/min and <60 ml/min (not on any form of dialysis).

Outcome Measures

Primary outcome measures were changes in endothelial function as measured by forearm plethysmography, changes in B-type natriuretic peptide (BNP) levels and change renal function as estimated by the Cockroft and Gault method. These variables were assessed and recorded at baseline, after the first 28 days and then again following the second 28 day period. Secondary outcomes were change in functional status (NYHA class) and death.

Endothelial Function Measurement

Endothelial function was assessed using forearm plethysmography utilising the DE Hokanson Plethysmograph (Bellevue, WA, USA) system of cuffs and transducers and using AD Instruments Chart Version 5 software. The procedure involved having participants rest in bed for 10 min before commencing measurements. The arm was elevated to 30 degrees to facilitate venous return flow. Forearm blood flow (FBF) was then assessed by inflating a pressure cuff around the upper arm to 50 mmHg to occlude venous return and recording the FBF by means of strain gauge plethysmograph around the forearm. This process was then repeated following 5 min of having the upper arm cuff inflated to suprasystolic pressure of 200 mmHg to induce an ischaemic state in the forearm with subsequent hyperaemic response. Forearm plethysmography has previously been validated as a method for assessing endothelial performance [15,16]. FBF was recorded as ml/min 100 ml^{-1} (forearm volume). The FBF measurements prior to and following induction of ischaemic reactive hyperaemia were compared and expressed as a percentage change. The flow figure used was a mean of three samples taken after hyperaemia developed. The percentage change after the interventional therapy (NAC or placebo) was compared with pre intervention percentage change by expression as ratio of improvement.

Table 1. *Baseline Patient Characteristics.*

Number of patients	9
Male	88.9% ($n = 8$)
Mean age (±SD)	60.4 (±8.9) years
Mean LVEF (±SD)	25.4 (±7.86)%
Mean Cockroft Gault GFR (±SD)	43.9 (±7.11) ml/min
Mean B type natriuretic peptide (±SD)	1250.67 (±860.2)
Mean systolic blood pressure (±SD)	104.44 (±25.2) mmHg
Mean diastolic blood pressure (±SD)	65.9 (±4.64) mmHg
Mean weight (±SD)	90.26 (±10.62) kg
Ischaemic cardiomyopathy	44.44% ($n = 4$)
Non Ischaemic cardiomyopathy	55.56% ($n = 5$)
Treatment with beta blockers	88.89% ($n = 8$)
Treatment with ACE inhibitor or angiotensin II receptor blocker	66.67% ($n = 6$)
Treatment with spironolactone	77.78% ($n = 7$)
Treatment with digoxin	55.56% ($n = 5$)
NYHA class II	44.44% ($n = 4$)
NYHA class III	55.56% ($n = 5$)

Statistical Analysis

The ratio of improvement for FBF with NAC and placebo were expressed with an attendant standard error of the mean (SEM) and the ratio of improvement of each group was compared using Student's *t*-test. Student's *t*-test was also used to compare changes in Cockroft Gault GFR, BNP and NYHA class between NAC and placebo treatment.

Results

Eight patients completed the study and all data ($n = 9$) was used in the final analysis. Baseline characteristics of the study participants are shown in Table 1. As shown in Figs. 1 and 2, mean forearm blood flow improved significantly with NAC versus placebo with a mean ratio of improvement from baseline of 1.99 (SEM: ±0.49) for NAC versus 0.73 (SEM: ±0.23) for placebo ($p = 0.047$).

There was no significant difference in the response of BNP or Cockroft and Gault estimated GFR (eGFR) to placebo versus NAC. The mean change in BNP with placebo was −7.53% (SEM: ±6.87) and was −5.74% (SEM: ±11.1) for NAC ($p = 0.25$). The mean change in eGFR with placebo was 1.95% (SEM: ±4.72) and was 0.26% (SEM: ±5.17) for NAC ($p = 0.71$). This data is also shown in Figs. 3 and 4. Mean change in NYHA class did not vary between NAC or placebo (mean improvement in NYHA class of 0.11% (SEM: ±0.26) for both placebo and NAC, $p = 0.5$). No deaths occurred during the trial.

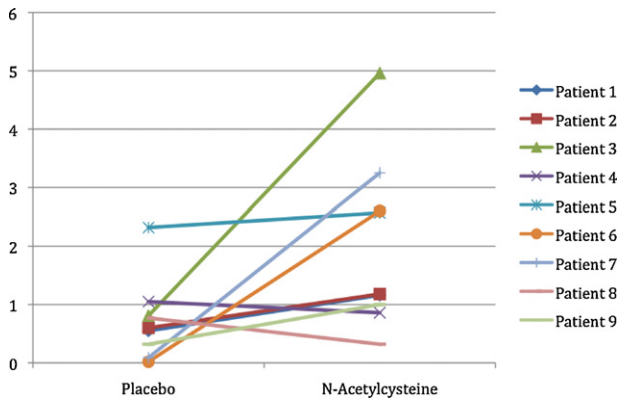

Figure 1. *Mean ratio of improvement in forearm blood flow with placebo vs NAC (individual data).*

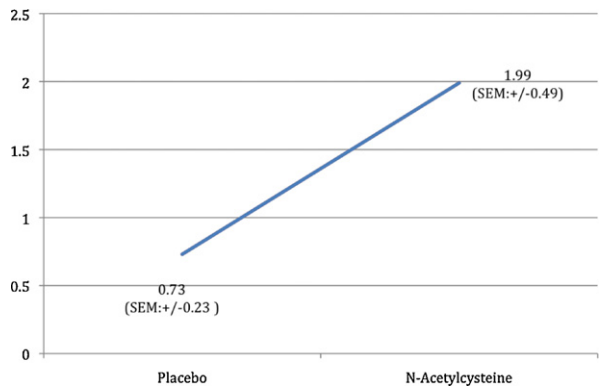

Figure 2. *Mean ratio of improvement in forearm blood flow with placebo vs NAC.*

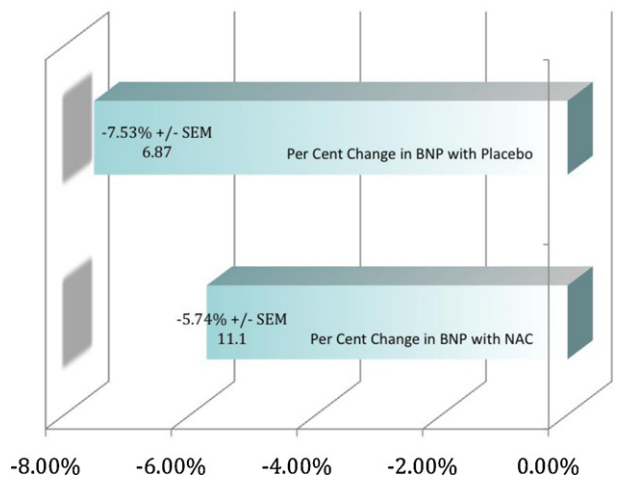

Figure 3. *Per cent change in BNP with placebo vs NAC.*

Discussion

The above results demonstrate a potential beneficial effect of antioxidant therapy with NAC on endothelial function in patients with type 2 CRS. This effect was shown in stable outpatients typical of patients with type 2 CRS. The ability to demonstrate a significant improvement in this primary outcome measure, despite our small sample size, likely indicates the potent potential effect of NAC on endothelial function in this patient population.

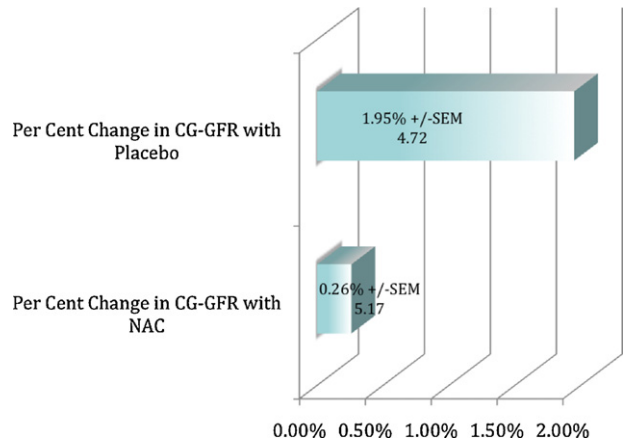

Figure 4. *Per cent change in Cockroft and Gault GFR with placebo vs NAC.*

Endothelial dysfunction is well recognised as a feature of the pathophysiology at play in heart failure [17,18]. Markers of endothelial dysfunction are correlated with relatively poorer outcomes [19,20]. Berrazueta and colleagues recently demonstrated a correlation between the degree of endothelial dysfunction in patients with heart failure and worsening prognosis utilising a forearm plethysmography technique similar to that employed by our group [10].

NAC is a potent antioxidant molecule with the potential to mediate important effects on endothelial function via its impact on reactive oxygen species at the endothelial cell membrane [21]. Endothelial availability of nitric oxide (NO) is reduced in patients with cardiac failure and it is likely that this plays a key role in the pathogenesis of endothelial dysfunction in this patient group [22,23]. Oxidative stress potentially participates in this process by facilitating the breakdown of NO [23].

The modification of this process of increased NO breakdown by NAC likely explains, at least in part, the mechanism by which endothelial function was improved by NAC within our study. However, oxidative stress has numerous other deleterious potential mechanisms by which endothelial function can be impaired [6,8]. NAC also has the potential to modulate these relationships, given its overall antioxidant effect and findings may also reflect this.

The key limitation of the current study is the small sample size. It may be partly for this reason that our pilot study did not demonstrate any significant effect on BNP, eGFR or NYHA class. More importantly, the small sample also meant that our study was underpowered to detect clinically meaningful changes in these parameters.

Our results add to a growing body of literature regarding the possible application of antioxidant therapies in patients with chronic heart failure. More specifically, our study suggests a possible role for NAC in the treatment of CRS. The significant improvement in endothelial function seen in this patient population in our study warrants further investigation.

Disclosures

This research was funded by a grant from the Alfred Hospital Small Projects Fund.

References

[1] Ronco C, Happio M, House A, Anavekar N, Bellomo R. Cardiorenal syndrome. J Am Coll Cardiol 2008;52:1527–39.
[2] Ronco C, McCullough P, Anker S, Anand I, Aspromonte N, Bagshaw S, et al. Acute Dialysis Quality Initiative (ADQI) consensus group cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. Eur Heart J 2010;31:703–11.
[3] Francis GS, Cohn JN, Johnson G, Rector TS, Goldman S, Simon A. Plasma norepinephrine, plasma renin activity, and congestive heart failure. Relations to survival and the effects of therapy in V-HeFT II. The V-HeFT VA Cooperative Studies Group. Circulation 1993;87:VI40–8.
[4] Hillege HL, Girbes ARJ, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, et al. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. Circulation 2000;102:203–10.
[5] Peterson M, Friberg P, Eisenhofer G, Lambert G, Rundqvist B. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. Eur Heart J 2005;26:906–13.
[6] Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury. Part I. Basic mechanisms and in vivo monitoring of ROS. Circulation 2003;108:1912–6.
[7] Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney Int 2004;65:1009–19.
[8] Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest 2005;115:500–8.
[9] Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, et al. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31:1352–6.
[10] Berrazueta J, Guerra-Ruiz A, Garcia-Unzueta MT, Toca GM, Las RS, de Adana MS, et al. Endothelial dysfunction, measured by reactive hyperaemia using strain-gauge plethysmography, is an independent predictor of adverse outcome in heart failure. Eur J Heart Fail 2010;12:477–83.
[11] Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. Circulation 2003;107:992–5.
[12] Sholze A, Rinder C, Beige J, Riezler R, Zidek W, Tepel M. Acetylcysteine reduces plasma homocysteine concentration and improves pulse pressure and endothelial function in patients with end-stage renal failure. Circulation 2004;109:369–74.
[13] Farquharson CAJ, Butler R, Hill A, Belch JJF, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation 2002;106:221–6.
[14] Cruz N, Quidgley J, Escobales N, Altieri P, Cresp M. Chronic treatment with N-acetylcysteine improves but does not prevent progression of cardiomyopahty in Syrian cardiomyopathic hamsters. J Cardiovas Pharmacol Ther 2011;16:197–204.
[15] Benjamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm blood flow and interpreting the responses to drugs and mediators. Hypertension 1995;25:918–23.
[16] Chin-Dusting JPF, Cameron JD, Dart AM, Jennings GL. Human forearm venous occlusion plethysmography: methodology, presentation and analysis. Clin Sci 1999;96:439–40.
[17] Drexler H, Hayoz D, Ṃnzel T, Hornig B, Just H, Brunner HR, et al. Endothelial function in chronic congestive heart failure. Am J Cardiol 1992;69:1596–601.
[18] Kubo S, Rector T, Bank A, Williams R, Heifetz S. Endothelium-dependent vasodilation is attenuated in patients with heart failure. Circulation 1991;84: 1589–96.
[19] Adlbrecht C, Hulsmann M, Strunk G, Berger R, Mortl D, Struck J, et al. Prognostic value of plasma midregional pro-adrenomedullin and c-terminal-pro-endothelin-1 in chronic heart failure outpatients. Eur J Heart Fail 2009;11: 361–6.
[20] Fischer D. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. Eur Heart J 2004;26:65–9.
[21] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of n-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 1989;6:593–7.
[22] Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation 1996;93:210–4.
[23] Hornig B, Arakawa N, Kohler C, Drexler H. Vitamin c improves endothelial function of conduit arteries in patients with chronic heart failure. Circulation 1998;97: 363–8.