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Does Oral *N*-Acetylcysteine Reduce Contrast-Induced Renal Injury in Patients With Peripheral Arterial Disease Undergoing Peripheral Angiography? A Randomized-Controlled Study

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Abstract

The nephroprotective role of *N*-acetylcysteine (NAC) against contrast-induced nephropathy (CIN) in patients undergoing peripheral arterial angiography remains unclear. A total of 40 patients undergoing peripheral arterial angiography were randomized to receive intravenous (iv) hydration only (group 1) or oral NAC in addition to iv hydration (group 2; ISRCTN: 35882618). Primary outcome was reduction in the elevation of urinary retinol binding protein (RBP), albumin−creatinine ratio (ACR), and serum creatinine (serC). Groups 1 and 2 had equivocal percentage reduction in RBP and ACR levels from baseline ($P = .80$ and .30). A significant reduction in serC was, however, observed with NAC by third postprocedure day ($P = .04$). One patient in the treatment arm developed CIN compared with 3 patients in the control group ($P = .33$). Equivocal changes in RBP and ACR levels by both treatments seem to indicate that either is equally effective in affording renal protection.

Keywords

N-acetylcysteine, nephroprotection, contrast-induced nephropathy, kidney

Introduction

Contrast-induced nephropathy (CIN) is a well-known complication of administration of iodinated contrast media. It is usually defined as an increase in the serum creatinine (serC) of 0.5 mg/dL (44.2 μmol/L) or a 25% increase from the baseline value within 48 hours of exposure to intravascular radiographic contrast media that is not attributable to other causes.[1,2] It is the third leading cause of hospital-acquired acute kidney injury (AKI).[3] The overall incidence of CIN is variable, depending on the renal biomarker used to define AKI and the presence of concomitant risk factors. For example, following coronary angiography, the reported incidence is between 2% and 50%, depending mainly on the presence of risk factors.[4]

N-acetylcysteine (NAC), which is traditionally used as an antidote for acetaminophen overdose, being an antioxidant theoretically, was first used with success in reducing CIN by Tepel in 2000 in patients with moderate degree of renal dysfunction after contrast-enhanced computerized tomography.[5] Since then, results of many randomized-controlled trials (RCTs) have been published evaluating its nephroprotective role, but the results have been mixed and controversial. Meta-analyses of these trials are fairly split between those that conclude that NAC protects against CIN and those that conclude that the evidence is insufficient to recommend NAC use. These different outcomes may be a result of the heterogeneity of patients, different inclusion criteria, and measurement methods for renal biomarkers, stage of chronic kidney disease, and differences in doses and routes of administration of NAC.[6]

In patients with peripheral arterial disease (PAD), only 2 RCTs have been published,[7,8] in which intravenous (iv) NAC was used whereas control participants received iv fluid hydration. Both trials suggested iv fluid hydration sufficient for renal protection. We compared the nephroprotective role of oral

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NAC + iv hydration with iv fluid hydration only in an RCT in patients with PAD.

Materials and Methods

The power calculation for this single-center RCT was based on a previous study done by our group.[9] We had observed that retinol binding protein (RBP) which is a sensitive urinary biomarker of renal damage, peaks to 699 μg/mmol, on the second postprocedure day in the control group. So, for this study to show that NAC would produce a 50% reduction in the urinary RBP level from 700 to 350 μg/mmol, 40 patients (half in each group) in total would be needed to give at least 80% power to show a significant difference at the 5% level. The study was approved by internal review board (ISRCTN: 35882618) and written informed consent was obtained from all patients.

A total of 40 patients undergoing peripheral angiography for PAD were recruited (Figure1). Patients with established renal failure on renal replacement therapy (dialysis) were excluded from the study. The patients were randomized using 1:1 to receive one of the following prophylactic treatments:

Group 1: Patients only received iv hydration (0.9% normal saline: 1 L iv infusion over a period of 12 hours before angiography and 1 L over 12 hours following the procedure).

Group 2: Patients received oral NAC 600 mg twice daily the day before the angiogram and 600 mg twice on the day of the angiogram along with iv fluids (as above).

Group assignment was carried out using a computer-generated randomization scheme. Patients in both groups had urine samples collected on the day before starting the prophylactic treatment and then at 24, 48, and 72 hours on the first, second, and third postprocedure day, respectively. This was done to measure urinary RBP and albumin−creatinine ratio (ACR), which are sensitive markers of acute renal injury. Serum samples were also taken at the above-mentioned timings to measure serC.

All types of diuretics and nonsteroidal anti-inflammatory drugs (except aspirin 75 mg/d) were discontinued 24 hours before the procedure. Patients taking metformin for diabetic control also discontinued on the day of angiography and the following 24 hours. A nonionic low-osmolality contrast agent Niopam 300 (Iopamidol, Bracco Ltd, United States) was used in all patients and the contrast volume used was documented.

The primary end point was a reduction in the urinary RBP levels and ACR from the baseline value within 72 hours of the primary angiography by NAC. Additional outcome measure was a reduction in the prevalence of contrast medium–induced nephropathy (defined as an increase in the serC concentration of ≥25% from the baseline value within the 72-hour period after primary angiography).

Statistical Analysis

Categorical variables were compared using Fischer exact test whereas the continuous variables were assessed by Mann-Whitney test. The relative percentage change in urinary or serum biomarkers of renal injury and their absolute levels for each day from baseline levels were compared. All tests were 2-sided and a $P < .05$ was considered significant. GraphPad InStat software (version 3.06) was used for analysis.

Results

The average age of patients was 75 ± 11 years. No significant age difference was noted in the 2 groups. The patient demographics are summarized in Table 1, which shows that both groups were comparable in their comorbidities and medications. The average amount of iodinated-contrast used was not statistically different for the 2 groups (75 ± 25 vs 70 ± 20 mL for groups 1 and 2, respectively, $P = .62$). Both groups had comparable baseline serC, urine RBP, and ACR levels (Table 2). Groups 1 and 2 had equivocal reduction in RBP and ACR levels ($P = .80$ and .30). Maximum percentage RBP and ACR reduction from baseline levels were seen by the third postprocedure day for NAC- and non-NAC-treated groups, but it did not reach statistical significance. A significant reduction in serC was observed with NAC by third postprocedure day ($P = .04$). One patient in the treatment arm developed CIN compared with 3 patients in the control group ($P = .33$). The absolute and relative percentage levels of these renal biomarkers are tabulated in Tables 2 and 3.

Discussion

To our knowledge, this is the first study to investigate the use of "oral" NAC along with iv hydration to reduce CIN in patients with PAD undergoing peripheral angiography. Patients with PAD have concomitant renal impairment, not solely due to renal artery stenosis.[10,11] A large study comprising of 5787 patients reported that patients with PAD having glomerular filtration rate (GFR) <30 mL/min per 1.73 m^2 tended to have a higher risk of presenting with tissue damage (ischemic ulceration or gangrene) compared with individuals having normal renal function (OR: 2.21; 95% CI: 0.64-2.98).[12] Therefore, using various renal protective therapies seems sensible. Using RBP and ACR reduction as an indicator of renal protection, we found that iv hydration alone was as good as in combination with oral NAC to protect against CIN, as observed by equivocal changes in levels of these biomarkers. Large reduction in the urinary renal biomarker levels, however, did not translate into a clinically beneficial outcome measure such as significant reduction in the prevalence of CIN. This questions the value of these sensitive biomarkers of AKI and their clinical relevance regarding CIN. Using a validated biomarker of renal injury like serC, percentage serC change, however, revealed that adding NAC does seem to offer renal protection.

Table 1. Patient Demographics (Number of Patients)

	Group 1, n = 19 (Intravenous Fluid Hydration)	Group 2, n = 21 (Oral *N*-Acetylcysteine + Intravenous Fluid Hydration)	*P*
Age			
Male	70 ± 14 yrs	75 ± 11 yrs	.90
Female	71 ± 14 yrs	74 ± 12 yrs	.90
Ischemic heart disease	6	4	.50
Hypertension	8	14	.20
Atrial fibrillation	1	4	.34
Chronic pulmonary obstructive disease	1	3	.60
Diabetes	3	5	.69
Coronary revascularization	3	1	.33
Ischemic heart disease	5	2	.22

Table 2. Percentage Average Change in Renal Biomarkers From Baseline

		First 24 Hours Percentage Change	First 48 Hours Percentage Change	First 72 Hours Percentage Change
Urine RBP	Control	21%	−23%	−27%
	NAC-treated	−11%	−53%	−56%
	P	.15	.47	.80
Serum creatinine	Control	−6%	8%	9%
	NAC-treated	−2%	−16%	−10%
	P	.21	.53	.04
Urinary ACR	Control	66%	−3%	−60%
	NAC-treated	−30%	−55%	−64%
	P	.05	.17	.30

Abbreviations: ACR, albumin−creatinine ratio; NAC, *N*-acetylcysteine; RBP, retinol binding protein.

Table 3. Absolute Levels of the Renal Biomarkers

		Day 0, Median (IQR)	Day 1, Median (IQR)	Day 2, Median (IQR)	Day 3, Median (IQR)
Urine RBP (μg/L)	Control	121 (100-162)	145 (115-178)	105 (66-259)	96 (68-107)
	NAC-treated	228 (124-306)	146 (126-259)	131 (31-139)	123 (70-305)
	P	.23	.80	.66	.43
Serum creatinine (μmol/L)	Control	88 (68-142)	87 (64-139)	156 (85-176)	107 (64-166)
	NAC-treated	97 (72-125)	75 (62-111)	71 (67-78)	94 (74-148)
	P	.64	.50	.05	.64
Urinary ACR	Control	2.4 (1.5-27.4)	4.45 (1.5-21.57)	3 (1.3-33.4)	4.4 (1.7-10)
	NAC-treated	5.1 (1.75-24.7)	1.7 (1.6-4.8)	3.7 (2.15-6.37)	5.95 (4.55-11.25)
	P	.33	.10	.05	.35

Abbreviations: ACR, albumin−creatinine ratio; IQR, interquartile range; NAC, *N*-acetylcysteine; RBP, retinol binding protein.

It is well known that a rise in serC occurs when two thirds of the renal functional reserve has been lost. The main purpose of using sensitive biomarkers like RBP is to identify AKI at an early stage before major renal damage has ensued so that early management can be started. Retinol binding protein has been used as a sensitive renal biomarker of AKI, particularly as an indicator of proximal tubule injury in preference to renal biomarkers such as β_2-microglobulin and β-*N*-acetyl-D-glucosaminidase (NAG) in earlier studies.[13,14] It is freely filtered by glomeruli and subsequently reabsorbed and catabolized by the proximal tubule. As CIN causes proximal tubular dysfunction in addition to its multifocal damage to the nephron, RBP can potentially be used as a marker of CIN. Retinol binding protein has, however, not been validated as a reliable predictor of CIN. In our previous study, significant elevation of RBP was observed following the use of iodinated contrast and was attributed to contrast-induced renal injury.[9] Assuming

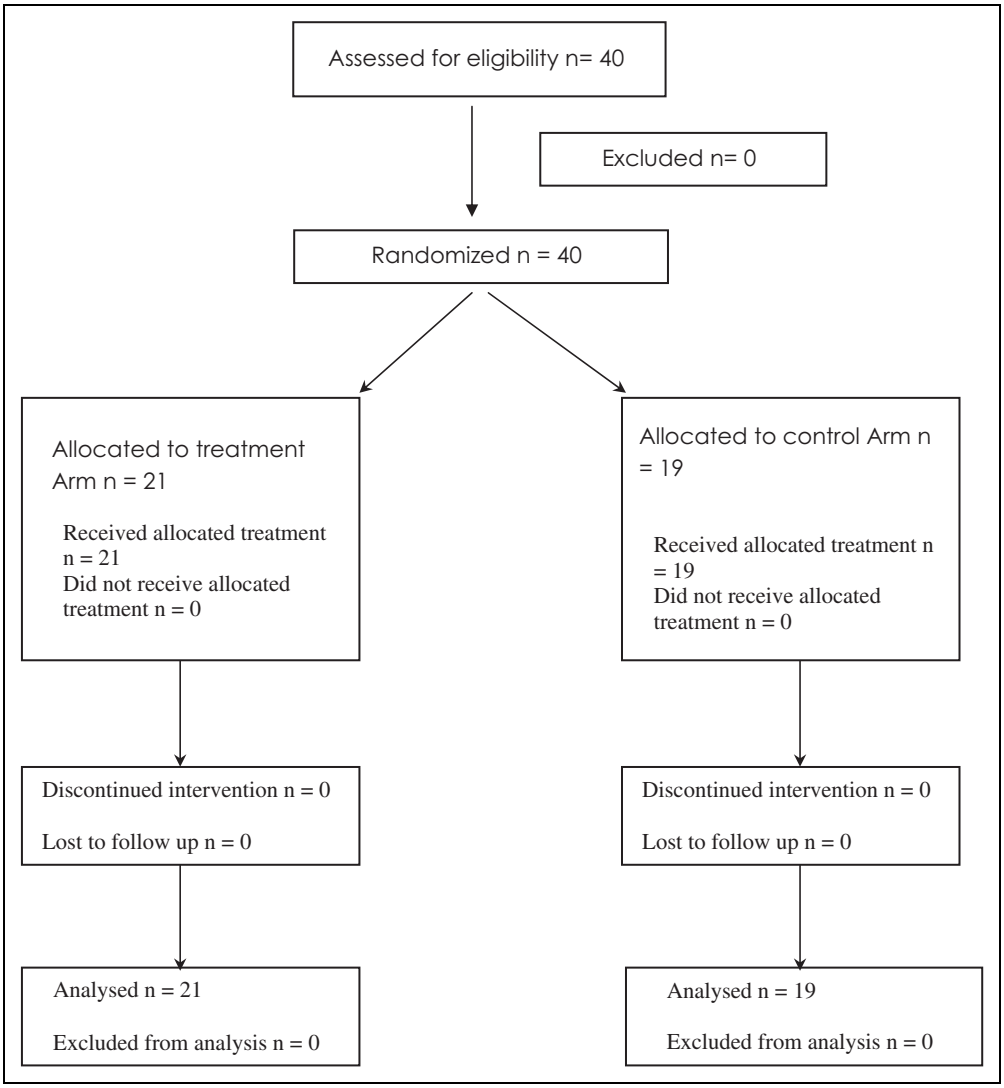

Figure 1. Patient flow in the trial (CONSORT flow diagram).

that it would be an indicator of CIN and taking into account its sensitive nature, we powered this study based on RBP reduction. Although this was achieved in this trial, the clinical relevance of this reduction was not evident. We did not observe a significant difference between the percentage reductions and absolute RBP levels of the 2 groups as can be seen from Tables 2 and 3. This is in accordance with our previous RCT, in which we did not observe any significant difference between the absolute RBP values of NAC- and non-NAC-treated patients.[9] Although this can be attributed to the multifactorial etiology of AKI and CIN, however the clinical relevance of RBP changes needs further exploration and validation if it were to be used as marker of CIN. From our results, it can be argued that besides the first day increase in RBP levels in the control group there was a decrease in the RBP levels over the 3 days in both groups, Is RBP a potential marker of renal injury? It can be explained by the fact that iv hydration alone or in combination with NAC resulted in reduced RBP levels that is renal damage. The fact that RBP reduction was more in the NAC group supports the fact that most likely it was due to additional renal protection by NAC. Had there been a control group in which none of the above prophylactic measures were taken, an increase in RBP levels may have been observed. Obtaining research ethical approval to include such a control group in the study would be a limiting factor. A control group that would be given iv hydration would be more practical. The above discussion holds true regarding our findings about ACR. In contrast, serC is a validated marker of renal damage. Serum creatinine forms the basis of the most commonly used definition of CIN.[1] A significant percentage reduction in serC from baseline by NAC compared with the control group was observed by the third postprocedure day. This highlights the potential of NAC as a nephroprotective agent. The inability of the significant reduction in serC to translate into a significant reduction

in the prevalence of CIN by NAC is most likely a type 2 error. This is, first, due to the small sample size. Second, the multifactorial etiology of CIN makes it a complex problem to diagnose and treat.[15-17]

Another noteworthy point is that all recruited patients were taking a statin as part of the best medical therapy for PAD in addition to aspirin. Perhaps this could have affected our results because statins have been shown to have nephroprotective effect in patients with PAD in addition to their cholesterol-lowering action.[18] Due to the small number of patients who developed CIN, small population size, and all patients on statin therapy, a regression analysis would not have provided any meaningful results about the impact of statin on the outcome. Future studies may focus on investigating the combined use of NAC, statin, and hydration for nephroprotection. A major limiting factor in organizing such a study would be to find patients for the control group, with PAD not taking statins.

Because of the above limitations associated with these markers, Vaidya et al have recently proposed that a combination of renal biomarkers should be used rather than individual biomarkers.[19] This is important because a single biomarker is rarely adequate to clearly define a particular pathologic state.[20,21] The sensitivity and specificity for diagnosis of AKI was made significantly greater by combining the urinary levels of kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), hepatocyte growth factor (HGF), and total protein using the logic regression model of $2.93 \times (\text{NGAL} > 5.72 \text{ and } \text{HGF} > 0.17) + 2.93 \times (\text{PROTEIN} > 0.22) - 2 \times (\text{KIM} < 0.58)$ than individual biomarkers. Future studies should focus on the correlation of different new renal biomarkers alone and in various combinations with definite clinical end points to ascertain their effectiveness in diagnosing AKI and CIN. This will prevent utilization of biomarkers, the changes which cannot be meaningfully translated into a clinical diagnosis.

Rashid et al and Kotlyar et al conducted a trial similar to ours using iv NAC for renal protection in patients undergoing peripheral angiography.[7,8] Both studies concluded that iv hydration alone was sufficient to reduce the prevalence of rising serC and creatinine clearance and that NAC did not confer any additional benefit. In terms of RBP and ACR-defined CIN and the actual prevalence of CIN being comparable between the 2 groups, our results are in agreement with the above trials but in light of the above discussed limitations of RBP and ACR, the significant change in serC reduction seems more reliable to suggest a potential nephroprotective role of NAC. The reduction in serC levels by NAC is not a new finding and has been reported earlier.[22] The precise mechanism by which NAC actually prevents reduction in the elevation of renal biomarkers or actual reduction in their levels needs further exploration as it may lead to the interpretation that reduction in serC by NAC is independent of its renal protective effect.

The importance of iv hydration cannot be ignored here because there is significant evidence, perhaps stronger than what is available regarding NAC, that periprocedural hydration and the use of low-osmolar or isoosmolar contrast media reduce the incidence of CIN,[23] but no regimen has been shown to prevent CIN completely.[24] In this trial, isoosmolar contrast was used and the doses were also small. The efficacy of NAC has been reported to increase in patients who receive smaller amounts of contrast media (between 75 and 117 mL) as suggested by the results of 3 studies.[5,25,26] This holds true for this study as well, where a mean contrast medium volume of around 70 mL was used. All of the above factors could have contributed to the beneficial effect of NAC to an extent that would overcome the potential limitation of lesser bioavailability of oral NAC compared with the iv route. Further investigation of this issue is also warranted.

Conclusions

Although iv hydration alone or in combination with NAC produced significant reductions in the sensitive biomarkers of renal injury, none of them appeared to significantly reduce the prevalence of CIN. As serC is an established and validated marker of renal injury, the observation that NAC significantly reduced the percentage serC levels indicates its potential nephroprotective action. The mechanism by which NAC reduces RBP, ACR, and serC needs investigation as it may be possible that NAC directly affects RBP and ACR levels independent of its nephroprotective action. Further clinical trials are required to assess the efficacy of novel biomarkers of renal injury by correlating them with clinically relevant end points. This is essential as it is also possible that a biomarker represents just another nonsurrogate marker whose modification may not translate into any clinically meaningful benefit.

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