

Acetylcysteine has No Mechanistic Effect in Patients at Risk of Contrast-Induced Nephropathy: A Failure of Academic Clinical Science

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Contrast-induced nephropathy (CIN) is a major complication of imaging in patients with chronic kidney disease (CKD). The publication of an academic randomized controlled trial (RCT; $n = 83$) reporting oral (N)-acetylcysteine (NAC) to reduce CIN led to > 70 clinical trials, 23 systematic reviews, and 2 large RCTs showing no benefit. However, no mechanistic studies were conducted to determine how NAC might work; proposed mechanisms included renal artery vasodilatation and antioxidant boosting. We evaluated the proposed mechanisms of NAC action in participants with healthy and diseased kidneys. Four substudies were performed. Two randomized, double-blind, placebo-controlled, three-period crossover studies ($n = 8$) assessed the effect of oral and intravenous (i.v.) NAC in healthy kidneys in the presence/absence of iso-osmolar contrast (iodixanol). A third crossover study in patients with CKD stage III (CKD3) ($n = 8$) assessed the effect of oral and i.v. NAC without contrast. A three-arm randomized, double-blind, placebo-controlled parallel-group study, recruiting patients with CKD3 ($n = 66$) undergoing coronary angiography, assessed the effect of oral and i.v. NAC in the presence of contrast. We recorded systemic (blood pressure and heart rate) and renal (renal blood flow (RBF) and glomerular filtration rate (GFR)) hemodynamics, and antioxidant status, plus biomarkers of renal injury in patients with CKD3 undergoing angiography. Primary outcome for all studies was RBF over 8 hours after the start of i.v. NAC/placebo. NAC at doses used in previous trials of renal prophylaxis was essentially undetectable in plasma after oral administration. In healthy volunteers, i.v. NAC, but not oral NAC, increased blood pressure (mean area under the curve (AUC) mean arterial pressure (MAP):

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There is uncertainty whether oral or intravenous acetylcysteine prevents contrast-induced nephropathy. A single small randomized controlled trial (RCT) in the year 2000 triggered a wave of > 70 clinical trials, 23 systematic reviews, and 2 large RCTs ultimately showing no effectiveness, but no mechanistic studies to determine whether (N)-acetylcysteine (NAC) might possibly work.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Does oral or intravenous acetylcysteine increase renal blood flow or reduce oxidative stress in patients with chronic kidney disease at risk of contrast nephropathy?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Oral acetylcysteine at doses previously used in RCTs has no reno-protective effects; it neither increased blood flow to the

kidneys nor protected against oxidative stress. Intravenous acetylcysteine did cause renal artery vasodilatation, increasing blood flow to the kidneys, in healthy volunteers but not in patients with kidney disease who are at risk of contrast nephropathy. Intravenous acetylcysteine offered no protection against oxidative stress.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study emphasizes the research and financial waste that may result when RCTs are initiated without mechanistic clinical studies to confirm potential benefit.

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mean difference 29 h·mmHg, $P = 0.019$ vs. placebo), heart rate (28 h·bpm, $P < 0.001$), and RBF (714 h·mL/min, 8.0% increase, $P = 0.006$). Renal vasodilatation also occurred in the presence of contrast (RBF 917 h·mL/min, 12% increase, $P = 0.005$). In patients with CKD3 without contrast, only a rise in heart rate (34 h·bpm, $P = 0.010$) and RBF (288 h·mL/min, 6.0% increase, $P = 0.001$) occurred with i.v. NAC, with no significant effect on blood pressure (MAP rise 26 h·mmHg, $P = 0.156$). Oral NAC showed no effect. In patients with CKD3 receiving contrast, i.v. NAC increased blood pressure (MAP rise 52 h·mmHg, $P = 0.008$) but had no effect on RBF (151 h·mL/min, 3.0% increase, $P = 0.470$), GFR (29 h·mL/min/1.73m², $P = 0.122$), or markers of renal injury. Neither i.v. nor oral NAC affected plasma antioxidant status. We found oral NAC to be poorly absorbed and have no reno-protective effects. Intravenous, not oral, NAC caused renal artery vasodilatation in healthy volunteers but offered no protection to patients with CKD3 at risk of CIN. These findings emphasize the importance of mechanistic clinical studies before progressing to RCTs for novel interventions. Thousands were recruited to academic clinical trials without the necessary mechanistic studies being performed to confirm the approach had any chance of working.

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Radiographic contrast has been used since the 1950s to enhance medical imaging in diagnostic and interventional procedures. Although risk from radiocontrast is generally low, patients with chronic kidney disease (CKD), particularly in the setting of diabetes or intravascular volume depletion, are at risk of developing contrast-induced nephropathy (CIN).^{1–4} There is no universally accepted definition for CIN⁵ and, partly as a result, its reported incidence varies from 3% to 19%.^{1,2,6,7} Importantly, CIN has been associated with increased length of hospital stay, adding \$10,000 on average to a US hospital admission,⁸ as well as increased morbidity and mortality.^{2,6,7,9,10} The underlying mechanisms of CIN are poorly understood.^{1,11} Reduced renal blood flow (RBF) due to afferent renal artery constriction, leading to ischemic kidney injury, as well as direct injury by oxygen free radicals have been proposed.^{12–14} Thus, preventative strategies, including intravenous (i.v.) hydration and bicarbonate, focus on maintaining RBF and reducing oxidative stress.¹¹ However, data supporting these strategies are limited.^{15,16}

In the year 2000, a small RCT ($n = 83$) reported less cases of CIN in patients undergoing diagnostic computed tomography contrast radiography after receiving oral (N)-acetylcysteine (NAC) 600 mg.¹⁷ NAC is a vasodilator¹⁸ and considered to be an antioxidant,¹⁹ supporting biological plausibility of efficacy. The results of the trial were accepted and led to a mass of papers on the role of NAC in CIN and the conduct of more than 70 randomized controlled trials (RCTs) testing oral (less frequently i.v.) NAC that recruited over 18,000 patients by 2020 (Figure 1).²⁰ The trials were followed by multiple systematic reviews calling for larger studies. Finally, two large RCTs of patients undergoing angiography compared oral NAC with placebo (ACT study²¹ $n = 2,308$ and the PRESERVE study²² $n = 4,993$) and showed no effect of oral NAC. After the ACT study, in 2013, an international consensus report stated that neither oral nor i.v. NAC should be administered for CIN prophylaxis.²³ However, systematic reviews continue to report that NAC shows promise for preventing CIN (for example, ref. 20). A 2019 National Institute for Health and Care Excellence (NICE)

review of the evidence found no evidence for benefit from NAC but recommended that more clinical research be performed.¹⁶

Unfortunately, the many RCTs on NAC renal prophylaxis performed to date have been done without any prior mechanistic studies of how NAC affects both healthy and damaged kidneys and without pharmacodynamic dose-finding studies. Systematic review of the literature revealed no studies that included mechanistic secondary analyses to explain the reported effects. To definitively identify the optimal role of NAC, if any, there is a need to determine how NAC affects kidneys in patients with CKD, to identify the ideal dose, route of administration, and outcome measure, based on its mechanism of action. Previous studies have used changes in serum creatinine to detect NAC's effect. However, if contrast causes renal vasoconstriction and NAC vasodilatation, NAC itself may cause a reduction in serum creatinine concentration,^{24,25} suggesting that changes in creatinine are not the best marker of NAC effect. Until mechanistic studies are done, there is a risk that NAC will be discarded without adequate testing, or that yet more time and money will be spent setting up yet more RCTs. The aim of this study was therefore to determine how NAC affects renal hemodynamics and oxidant status in healthy volunteers and in patients with CKD stage III (CKD3), a population at risk of CIN.

METHODS

Full details of the protocol have been published previously.²⁶ The studies were performed in the Wellcome Trust Clinical Research Facility and the Coronary Angiography Suite, Royal Infirmary of Edinburgh. Approval of the local research ethics committee and written informed consent of each subject were obtained. The investigations conformed to the principles outlined in the Declaration of Helsinki. This study was registered with European Clinical Trials Database (EudraCT 2006-017800-10) and ClinicalTrials.gov (NCT00558142).

Study design

The overall study comprised four substudies (Figure S1). Studies 1 and 3 were randomized, double-blind, double-dummy placebo-controlled, 3-period crossover studies, each recruiting 8 healthy volunteers to compare

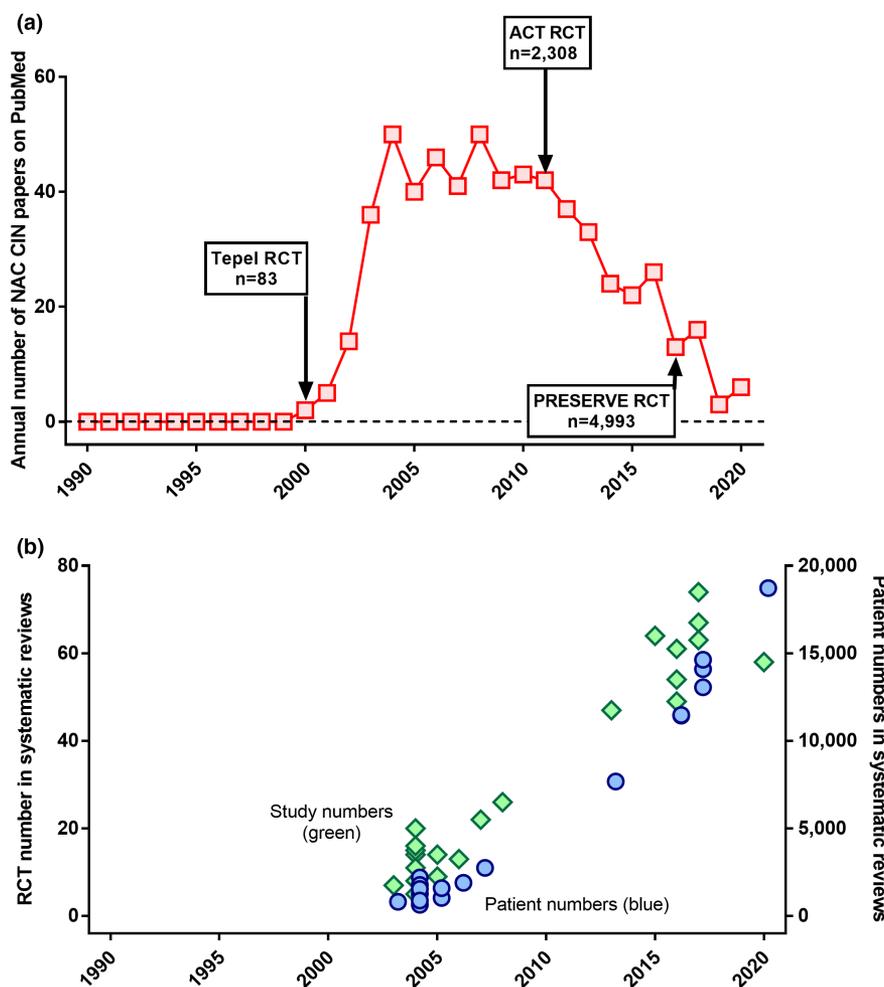


Figure 1 (a) Number of publications reporting clinical use of acetylcysteine for CIN and (b) number of RCTs and patients recruited in published systematic reviews and meta-analyses of studies assessing the effectiveness of NAC in CIN 1990–2020. There were no clinical or mechanistic studies of NAC published in the decade before Tepel’s publication in 2000. This paper was followed by a dramatic increase in the number of studies and systematic reviews, only beginning to fall after the publication of the negative ACT RCT in 2011. The largest meta-analysis (search performed September 21, 2018) reported 74 RCTs and recruitment of 14,635 patients in 2017.³⁹ In part (b), each dot/diamond represents a single published meta-analysis including all known participants (excluding systematic reviews addressing subpopulations of trials). The most recent large RCT (PRESERVE; $n = 4993$)²² was not included in any of the meta-analyses; the top right point (dark blue, $n = 19,628$) represents the sum of patients in the largest meta-analysis plus this RCT. Key: Green diamond: number of studies in each systematic review (left y-axis); Blue circles: number of participants in each systematic review (right y-axis). CIN, contrast-induced nephropathy; NAC, (N)-acetylcysteine; RCT, randomized controlled trial.

the effect of oral NAC, i.v. NAC, and placebo on renal and systemic hemodynamics with and without contrast, with at least a 2-week washout interval between study arms. In study 3, the protocol was as for study 1 but participants also received a single 100 mL dose of iodixanol (Visipaque 320, an iso-osmolar non-ionic radiocontrast agent used to show the coronary arteries) by i.v. injection. The protocol was replicated in study 2 (without contrast) in subjects with CKD3; estimated glomerular filtration rate (eGFR) 30 to < 60 mL/min/1.73 m²,²⁷ with a similar 2-week washout interval.

Study 4 was a 3-arm, randomized, double blind, double-dummy placebo-controlled study ($n = 22$ in each arm) comparing placebo, oral, and i.v. NAC in patients with CKD3 undergoing elective coronary angiography. A parallel group design was selected because multiple contrast administrations to patients with CKD3 were considered unethical. Dose and timing of iodixanol in study 4 was determined by the interventional cardiologist carrying out the procedure; these were therefore outside the control of the research protocol and varied between study arms. Iodixanol was selected because it has a low incidence of CIN complications.

Subjects

Healthy, non-smoking, male subjects over the age of 45 years who were not taking regular medicines were eligible for recruitment to studies 1 and 3. Subjects in study 1 were able to participate in study 3 provided that > 3 months had elapsed ($n = 4$). Male patients with stable CKD3, and such patients awaiting elective coronary angiography, were eligible for recruitment to studies 2 and 4, respectively; participants could do both studies with a 3-month interval ($n = 6$). Patients with CKD were allowed to continue their prescribed medications but omitted metformin and diuretic therapy from the day prior to the study as per local clinical guidelines. Exclusion criteria included clinically significant comorbidity, thyroid disease, asthma, atopy, or myasthenia gravis, and history of allergy or sensitivity to NAC or contrast medium.²⁶ Participants were enrolled by researchers from July 2008 to December 2014, follow-up was for 72 hours. The trial stopped when all planned participants had been recruited and studied. The study recruited only male volunteers. Previous experience has shown that regular timed voiding by female participants

receiving multiple infusions is difficult while maintaining volunteer privacy.²⁶

Interventions and randomization

Participants received all 3 interventions separately for studies 1–3, with the sequence in which they received the treatments randomized. Participants in study 4 were randomized to receive one treatment of i.v. NAC, oral NAC, or placebo. Placebo was i.v. 0.9% saline or oral lactose tablets; patients randomized to placebo received both. Randomization was done by the company supplying NAC/placebo capsules (Tayside Pharmaceuticals) using a random number table with a 1:1:1 allocation ratio (no blocking); the patients were allocated to study day (studies 1–3) or arm (study 4) by pharmacists. The investigators recording data in the clinical research facility were blind to allocation.

No definitive data exist to guide the optimal dosing regimen or route of administration of NAC. We chose a revised i.v. dosing regimen (100 mg/kg over 2 hours followed by 100 mg/kg over 5 hours) based on a regimen effective in treating paracetamol poisoning, and associated with a low rate of adverse reactions²⁸ (selection discussed in the methods paper²⁶). For the oral NAC regimen, we used 1,200 mg twice daily (b.i.d.) the day before and the day of the study (total dose 4.8 g, 53.3 mg/kg in a 90 kg study participant; double the dose used in the original paper¹⁷ and the dose often used in subsequent RCTs). All treatments and laboratory analyses were blind to subjects and investigators; placebo capsules were matched to oral NAC, whereas i.v. saline alone was administered as the placebo infusion. Visually it was not possible to distinguish the NAC and placebo infusions.

Hemodynamic measurements

Blood pressure and heart rate were measured with an appropriate size cuff using a calibrated oscillometric sphygmomanometer (PMS Instruments Ltd., Wokingham, UK). Consecutive measurements were taken at each time point until two consecutive measurements each of pulse, and systolic and diastolic blood pressure within 10 bpm or 10 mmHg of each other were achieved. The means of these two measurements were then used for analysis.

Clearance studies

Renal blood flow and GFR were formally measured by renal clearance of para-aminohippuric acid (PAH) and inulin, respectively, as previously described.²⁹ Well-hydrated participants arrived fasted, a standard light breakfast was given, and participants were asked to empty their bladders. Following loading doses of PAH and inulin, a maintenance infusion was given at 120 mL/h throughout the study. After a 2-hour equilibration period, the i.v. infusion of NAC or placebo was commenced, and volunteers were administered the third dose of oral NAC or placebo (having ingested 2 doses the previous day-self-reported compliance checked on arrival). Participants in study 3 received 100 mL of radiographic contrast i.v. after completion of the first 2-hour NAC/placebo infusion before starting the 5-hour infusion. Participants in study 4 received i.v. contrast (volume decided by the interventional cardiologist according to clinical need) during angiography at variable times after starting the NAC/placebo infusion. At the time the contrast was first given, the clock was reset, and samples were collected for 6 hours as the NAC was infused. After completion of the 5-hour NAC infusion, the infusion of PAH and inulin was continued until six hours after the contrast was given or the 2nd NAC infusion was started, if contrast was not given.

Blood pressure and pulse rate were recorded every 30 minutes throughout the study (every 60 minutes after contrast in study 4, due to presence of an arterial catheter entry site, usually in a radial artery) and blood samples obtained every hour (plus 15 and 30 minutes after contrast administration). Volunteers were asked to void urine every 120 minutes. The final dose of oral NAC/placebo was administered during the evening of the study day when the patients were usually home. Participants returned at 24 and 72 hours for repeat blood and

urine samples. Renal and systemic indices were calculated as previously described.²⁶

Outcome measures

The primary outcome for all four substudies was a change in RBF over 8 hours after administration of i.v. NAC/placebo. Changes in GFR, tubular function (through assessment of fractional excretion of sodium), blood and urine biomarkers of renal damage, plasma cysteine, cellular glutathione, oxidative balance, and systemic hemodynamic measurements were also assessed at multiple timepoints (**Table S1**).

Laboratory analyses

The PAH was measured by high-performance liquid chromatography with fluorescence detection (see **Supplementary Information** for details on all assays). Inulin was measured calorimetrically by reaction with resorcinol. Urine KIM-1 and NGAL were measured using enzyme-linked immunosorbent assay (ELISA; DY1750 and DY1757) and plasma cystatin C using DuoSet ELISA (DY1196; all R&D Systems). Serum and urine creatinine were measured using the hospital's clinical laboratory and by the Jaffe method, respectively. Plasma oxygen radical absorbance capacity (ORAC) was measured using Cell Biolabs kit. Total thiol (NAC, cysteine, and glutathione) concentrations (reduced + oxidized) were measured using a modified version of a published method.³⁰

Statistical analysis

The study was powered on the basis of a mean (SD) RBF in patients with CKD3 of 600 ± 100 mL/min.²⁹ Eight subjects in the crossover studies would allow a 16% change in RBF for an active arm compared with placebo to be detected at 80% power (alpha of 0.05). The parallel group study had a 90% power (alpha of 0.05) with $n = 22$ participants per arm to show a 30% change in RBF in patients with CKD.

A statistical analysis plan was written before the statistician had access to the trial data. All analyses used an intention to treat population. The primary analysis fitted random coefficient models to assess whether there were differences in the treatment effects (i.v. NAC vs. placebo and oral NAC vs. placebo), and whether these differences were constant with time. Because these models had convergence issues, the results from the secondary analysis were taken as the primary results (as per the statistical analysis plan). For the secondary analysis, the treatment effects were considered by calculating the area under the curve (AUC) for RBF from baseline to 8 hours after the i.v. NAC/placebo was administered for each time period. For studies 1–3, linear mixed models were fitted to the RBF AUC data with baseline RBF, treatment, and period as fixed effects and patients as a random effect. For study 4, a linear regression model was fitted with baseline RBF and treatment as covariates. These models were also fitted to the secondary outcomes. The point estimates and 95% confidence intervals for the adjusted difference in mean AUC for i.v. NAC vs. placebo and oral NAC vs. placebo are presented as treatment effects. All *P* values and 95% confidence intervals are 2-sided with no adjustment made for multiple comparisons. Analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA).

Role of the funding source and registration

The study funder had no role in the design of the study, data collection, analysis or interpretation, or writing of the report. All authors had full access to the study data and the corresponding author had responsibility for the decision to submit for publication. This trial was registered with European Clinical Trials Database (EudraCT number 2006-017800-10) and ClinicalTrials.gov (identifier NCT00558142) before any patient was recruited.

RESULTS

Eight healthy volunteers completed studies 1 (no contrast) and 3 (contrast), whereas 8 patients with stable CKD3 completed study

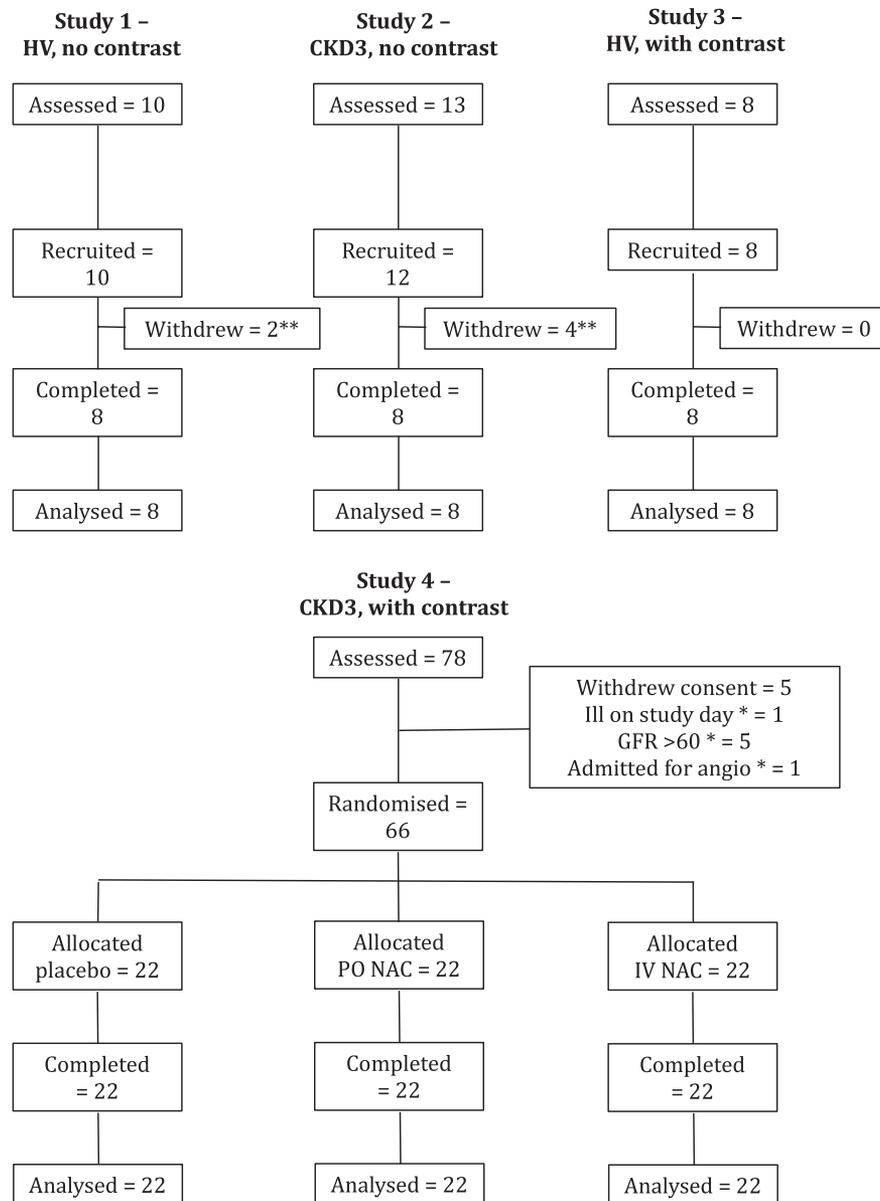


Figure 2 CONSORT flow diagram. Number analyzed refers to the RBF primary outcome. *One participant had a myocardial infarction on his way to the hospital, one underwent emergency coronary angiography just before the study day, 5 participants had eGFR > 60 at recruitment and were withdrawn, whereas 5 withdrew consent before the study day. **In study 1, one participant developed high blood pressure during the first study arm and was withdrawn, whereas one withdrew consent after the first study day. In study 2, two participants withdrew consent before the first study started, one withdrew consent after the first study day, and one had eGFR > 60 at recruitment. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HV, healthy volunteer; NAC, (N)-acetylcysteine; RBF, renal blood flow.

2 (no contrast; **Figure 2**). Sixty-six patients with stable CKD3 undergoing elective coronary angiography completed study 4. Two and three participants were withdrawn from studies 1 and 2 after randomization (see **Figure 2** for reasons), whereas one participant in study 2 had an eGFR > 60 at recruitment and was therefore excluded. Seven participants were withdrawn from study 4 after randomization and were replaced (**Figure 2**); 5 participants had eGFR > 60 at recruitment and were therefore excluded. All participants who completed the study were analyzed.

Patients with CKD were older than healthy volunteers and had higher serum creatinine concentrations and lower ORAC status

(**Table 1**). Patients with CKD undergoing elective angiography had higher systolic blood pressure at baseline (before administration of i.v. NAC/placebo). Baseline variables were similar at the start of each study period (for the crossover studies) and between study arms (for the parallel groups study; **Table S2**).

In study 4, patients receiving placebo, oral NAC, and i.v. NAC were administered median (interquartile range) contrast doses of 118 (84–221) mL, 115 (94–183) mL, and 138 (90–193) mL, a median of 118 (103–179) minutes, 142 (118–151) minutes, and 130 (105–173) minutes after the beginning of the i.v. NAC or saline placebo infusion.

Table 1 Study participant demographics at recruitment

	Study 1 Healthy volunteers No contrast	Study 2 CKD3 patients No contrast	Study 3 Healthy volunteers With contrast	Study 4 CKD3 patients With contrast
Age, year	58.4 ± 5.9	71.1 ± 4.8	56.9 ± 9.3	72.7 ± 6.3
Weight, kg	91.7 ± 19.1	85.5 ± 5.9	82.2 ± 11.0	88.8 ± 14.3
BMI, kg/m ²	27.7 ± 4.7	28.5 ± 2.6	25.8 ± 2.9	29.5 ± 4.3
Diabetes mellitus, <i>n</i>	0	5 (62.5%)	0	25 (37.9%)
Heart rate, bpm	55.5 ± 3.2	67.1 ± 15.3	67.8 ± 15.1	62.2 ± 10.2
Systolic BP, mmHg	135.1 ± 7.2	136.6 ± 13.7	134.3 ± 14.4	149.2 ± 22.0
Diastolic BP, mmHg	77.9 ± 10.4	74.4 ± 8.5	74.9 ± 10.3	71.7 ± 10.6
MAP, mmHg	97.0 ± 8.5	95.1 ± 8.0	94.7 ± 9.9	97.6 ± 11.1
Serum creatinine, µmol/L	77.4 ± 22.4	136.8 ± 22.2	80.3 ± 7.0	129.4 ± 19.7
eGFR	>60	46.3 ± 7.5	>60	48.4 ± 7.5

Data are displayed as mean ± SD.

BMI, body mass index; BP, blood pressure; CKD3, chronic kidney disease stage III; eGFR, estimated glomerular filtration rate; MAP, mean arterial pressure.

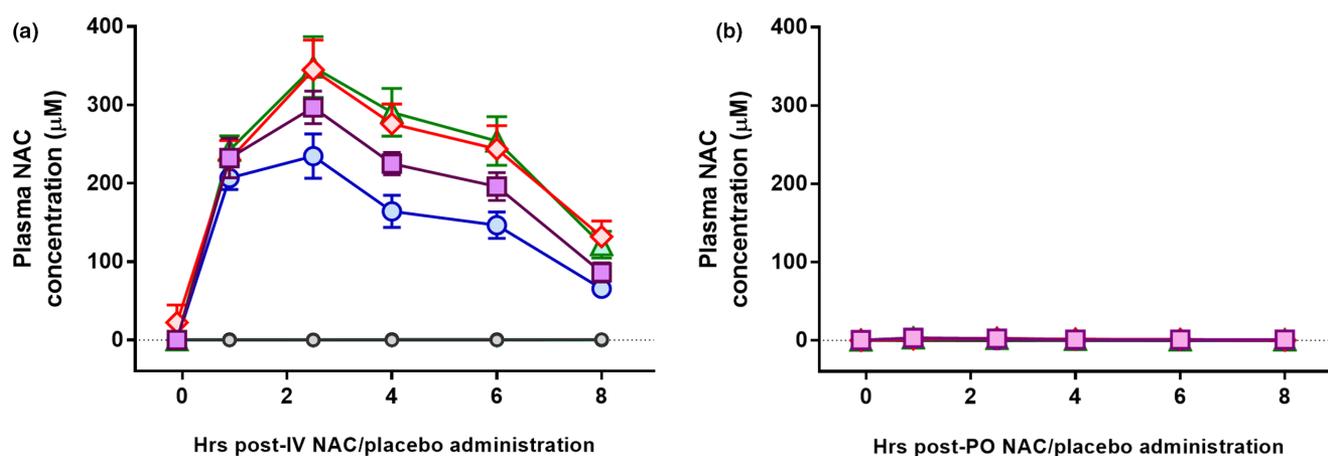


Figure 3 Pharmacokinetics of NAC. (a) IV NAC vs. placebo (only one set of placebo results is visible as all overlap), (b) oral NAC vs. placebo. Data are mean (±SD) plasma concentrations (µM). Study 1: healthy volunteers, without contrast (blue circles); study 2: patients with CKD3, without contrast (red diamonds); study 3: healthy volunteers, with contrast (purple squares); study 4: patients with CKD3, with contrast (green triangle). NAC concentrations in patients receiving placebo are shown with grey circles. Doses of NAC administered were i.v.: 200 mg/kg over 7 hours; oral: 1,200 mg b.i.d. for 2 days, starting the morning before the procedure. NAC, (N)-acetylcysteine.

Pharmacokinetics of oral and i.v. NAC

In healthy volunteers without contrast (study 1), plasma NAC concentration increased from baseline to a mean (SD) peak of 235 ± 27 µM at 2.5 hours following i.v. administration (AUC 1,278 (± 132) µM·h, $P < 0.0001$ vs. baseline for AUC; **Figure 3a**). Mean peak concentrations were 100-fold lower following oral administration (2.5 ± 0.7 µM at 1 hour, $P = 0.01$ vs. baseline; $P < 0.0001$ vs. i.v. NAC; **Figure 3b**). NAC concentrations after i.v. administrations were modestly higher in the other studies of patients with CKD3 and/or with contrast compared with healthy volunteers without contrast in study 1: study 2 (AUC 1,633 (± 124) µM·h, $P = 0.070$ vs. study 1), study 3 (AUC 1,946 (± 198) µM·h, $P = 0.014$), and study 4 (AUC 1,992 (± 355) µM·h, $P = 0.245$; **Figure 3a**).

Effects on NAC on systemic hemodynamics

NAC had modest effects on systemic hemodynamics. In the absence of contrast, administration of i.v. NAC, but

not oral NAC, increased heart rate (28 (16–40) h·bpm, $P < 0.001$ vs. placebo), systolic blood pressure (62 (26–98) h·mmHg, $P = 0.003$), and mean arterial pressure (MAP; 29 (6–53) h·mmHg, $P = 0.019$) of healthy volunteers (study 1), but only the heart rate (34 (10–57) h·bpm, $P = 0.010$) of participants with CKD3 (study 2; **Table 2**, **Figure 4**, **Figures S2**, **S3**).

When contrast was administered, i.v. NAC caused a larger increase in heart rate (46 (26–66) h·bpm, $P < 0.001$ vs. placebo) and blood pressure (MAP 59 (44 to 74) h·mmHg, $P < 0.001$) of healthy volunteers (study 3; **Table 2**). In patients with CKD3 undergoing angiography (study 4), i.v. NAC increased blood pressure (MAP 52 (14 to 90) h·mmHg, $P = 0.008$) but no clear increase in heart rate (26 (–2 to 53) h·bpm, $P = 0.064$). Oral NAC was associated with increased blood pressure (MAP 40 (2 to 79) h·mmHg, $P = 0.041$) in patients with CKD3 receiving contrast (**Table 2**).

Table 2 Changes in systemic and renal hemodynamics and oxidant status for participants receiving oral or i.v. NAC, with or without contrast

	No contrast				Contrast						
	Study 1: HV		Study 2: CKD3		Study 3: HV		Study 4: CKD3				
	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.		
Primary outcome											
AUC RBF, h mL/min			8 (100), 24 (100)			8 (100), 24 (100)			8 (100), 24 (100)	65 (98.5), 65 (98.5)	
i.v. NAC	9,590.8 (9,168.4, 10,013.2)			5,105.4 (4,714.0, 5,496.9)			8,529.4 (8,096.7, 8,962.1)			5,228.4 (4,935.4, 5,521.3)	
Oral NAC	9,088.6 (8,668.6, 9,508.6)			4,909.3 (4,517.8, 5,300.7)			7,648.2 (7,213.3, 8,083.2)			5,038.9 (4,740.0, 5,337.9)	
Placebo	8,876.5 (8,452.5, 9,300.5)			4,817.3 (4,426.0, 5,208.5)			7,612.8 (7,185.1, 8,040.6)			5,077.8 (4,785.8, 5,369.8)	
Treatment difference											
i.v. NAC – placebo	714.3 (254.1, 1,174.5)	0.006		288.2 (152.6, 423.8)	0.001		916.6 (351.9, 1,481.2)			150.6 (-263.9, 565.0)	0.470
Oral – placebo	212.1 (-239.0, 663.2)	0.325		92.0 (-42.2, 226.2)	0.159		35.4 (-539.1, 609.9)			-38.9 (-456.3, 378.6)	0.853
Secondary outcomes											
AUC SBP, h mmHg			8 (100), 24 (100)			8 (100), 24 (100)			8 (100), 24 (100)	66 (100), 66 (100)	
i.v. NAC	1,172.3 (1,145.1, 1,199.4)			1,203.4 (1,114.8, 1,292.0)			1,140.6 (1,117.3, 1,163.9)			1,174.6 (1,130.2, 1,219.0)	
Oral NAC	1,117.4 (1,090.6, 1,144.2)			1,155.6 (1,067.4, 1,243.7)			1,027.3 (1,004.2, 1,050.5)			1,161.1 (1,116.7, 1,205.6)	
Placebo	1,110.7 (1,083.9, 1,137.6)			1,159.5 (1,071.6, 1,247.5)			1,032.3 (1,009.4, 1,055.3)			1,099.6 (1,055.2, 1,144.0)	
Treatment difference											
i.v. NAC – placebo	61.5 (25.5, 97.5)	0.003		43.8 (-15.8, 103.4)	0.133		108.3 (79.9, 136.7)			75.0 (12.3, 137.8)	0.020

(Continued)

Table 2 (Continued)

	No contrast			Contrast								
	Study 1: HV			Study 2: CKD3			Study 3: HV			Study 4: CKD3		
	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.
Oral -placebo	6.7 (-28.4, 41.7)	0.680	8 (100), 24 (100)	-4.0 (-60.2, 52.2)	0.877	8 (100), 24 (100)	-5.0 (-33.7, 23.7)	0.704	8 (100), 24 (100)	61.5 (-1.3, 124.3)	0.055	65 (98.5), 65 (98.5)
AUC MAP, h mmHg												
i.v. NAC	834.7 (817.8, 851.6)		8 (100), 24 (100)	820.2 (762.3, 878.0)		8 (100), 24 (100)	807.7 (787.4, 828.0)		8 (100), 24 (100)	785.3 (758.2, 812.4)		65 (98.5), 65 (98.5)
Oral NAC	813.9 (797.3, 830.5)		8 (100), 24 (100)	790.5 (732.8, 848.1)		8 (100), 24 (100)	748.9 (728.5, 769.3)		8 (100), 24 (100)	773.5 (745.7, 801.2)		65 (98.5), 65 (98.5)
Placebo	805.3 (788.7, 821.9)		8 (100), 24 (100)	794.5 (736.9, 852.0)		8 (100), 24 (100)	748.4 (728.2, 768.7)		8 (100), 24 (100)	733.1 (706.1, 760.1)		65 (98.5), 65 (98.5)
Treatment difference												
i.v. NAC - placebo	29.4 (5.5, 53.4)	0.019	8 (100), 24 (100)	25.7 (-11.6, 63.0)	0.156	8 (100), 24 (100)	59.3 (44.2, 74.3)	<0.001	8 (100), 24 (100)	52.2 (13.9, 90.4)	0.008	65 (98.5), 65 (98.5)
Oral - placebo	8.7 (-14.7, 32.0)	0.447	8 (100), 24 (100)	-4.0 (-39.5, 31.5)	0.808	8 (100), 24 (100)	0.5 (-15.0, 15.9)	0.948	8 (100), 24 (100)	40.4 (1.6, 79.1)	0.041	65 (98.5), 65 (98.5)
AUC DBP, h mmHg												
i.v. NAC	666.3 (650.3, 682.4)		8 (100), 24 (100)	628.1 (582.5, 673.7)		8 (100), 24 (100)	642.2 (621.1, 663.2)		8 (100), 24 (100)	591.6 (569.7, 613.6)		65 (98.5), 65 (98.5)
Oral NAC	661.8 (646.0, 677.5)		8 (100), 24 (100)	608.2 (562.7, 653.7)		8 (100), 24 (100)	612.8 (591.5, 634.0)		8 (100), 24 (100)	570.8 (548.9, 592.6)		65 (98.5), 65 (98.5)
Placebo	652.5 (636.8, 668.2)		8 (100), 24 (100)	612.1 (566.6, 657.5)		8 (100), 24 (100)	604.9 (583.7, 626.1)		8 (100), 24 (100)	548.1 (526.3, 569.9)		65 (98.5), 65 (98.5)
Treatment difference												
i.v. NAC - placebo	13.9 (-8.8, 36.5)	0.215	8 (100), 24 (100)	16.1 (-13.8, 45.9)	0.258	8 (100), 24 (100)	37.3 (18.7, 55.8)	0.001	8 (100), 24 (100)	43.5 (12.5, 74.6)	0.007	65 (98.5), 65 (98.5)
Oral - placebo	9.3 (-12.9, 31.4)	0.390	8 (100), 24 (100)	-3.9 (-32.6, 24.9)	0.769	8 (100), 24 (100)	7.9 (-11.3, 27.1)	0.388	8 (100), 24 (100)	22.7 (-8.2, 53.5)	0.147	65 (98.5), 65 (98.5)
AUC Heart rate, h bpm												
i.v. NAC	450.2 (442.0, 458.3)		8 (100), 24 (100)	520.5 (494.4, 546.6)		8 (100), 24 (100)	503.6 (484.8, 522.3)		8 (100), 24 (100)	475.6 (456.3, 494.9)		65 (98.5), 65 (98.5)
Oral NAC	423.5 (415.4, 431.6)		8 (100), 24 (100)	476.6 (450.5, 502.7)		8 (100), 24 (100)	458.2 (439.3, 477.1)		8 (100), 24 (100)	444.9 (425.6, 464.2)		65 (98.5), 65 (98.5)

(Continued)

Table 2 (Continued)

	No contrast				Contrast				
	Study 1: HV		Study 2: CKD3		Study 3: HV		Study 4: CKD3		
	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.
Placebo	422.2 (414.1, 430.2)		486.9 (460.9, 512.9)		457.7 (439.1, 476.4)		450.0 (430.8, 469.2)		
Treatment difference									
i.v. NAC - placebo	28.0 (16.2, 39.8)	<0.001	33.6 (9.8, 57.3)	0.010	45.9 (25.8, 65.9)	<0.001	25.6 (-1.6, 52.7)	0.064	
Oral - placebo	1.4 (-10.3, 13.0)	0.801	-10.3 (-33.7, 13.1)	0.354	0.5 (-20.0, 21.0)	0.960	-5.1 (-32.3, 22.1)	0.709	
AUC GFR, h mL/min/1.73 m ²									65 (98.5), 65 (98.5)
i.v. NAC	982.2 (925.4, 1,039.0)		444.8 (415.7, 474.0)		891.2 (842.0, 940.4)		486.4 (460.4, 512.3)		
Oral NAC	900.0 (845.0, 955.0)		443.8 (415.0, 472.5)		806.7 (757.0, 856.3)		479.8 (453.1, 506.4)		
Placebo	952.9 (895.8, 1,010.0)		438.9 (410.5, 467.3)		744.4 (695.0, 793.8)		457.6 (431.7, 483.5)		
Treatment difference									
i.v. NAC - placebo	29.3 (-27.8, 86.4)	0.285	5.9 (-30.8, 42.6)	0.731	146.8 (77.1, 216.5)	0.001	28.7 (-7.9, 65.3)	0.122	
Oral - placebo	-52.9 (-102.6, -3.3)	0.038	4.8 (-30.8, 40.4)	0.770	62.3 (-8.7, 133.2)	0.080	22.1 (-15.2, 59.4)	0.240	
AUC EFF, h.%									65 (98.5), 65 (98.5)
i.v. NAC	83.4 (76.3, 90.5)		71.7 (61.5, 81.9)		82.7 (77.8, 87.6)		79.7 (73.9, 85.5)		
Oral NAC	80.7 (74.2, 87.2)		74.0 (63.7, 84.2)		83.0 (77.8, 88.2)		76.4 (70.5, 82.4)		
Placebo	87.2 (79.6, 94.7)		75.0 (64.7, 85.2)		80.4 (75.3, 85.6)		75.0 (69.1, 80.8)		
Treatment difference									
i.v. NAC - placebo	-3.8 (-13.3, 5.8)	0.414	-3.3 (-9.7, 3.2)	0.264	2.3 (-3.2, 7.7)	0.385	4.7 (-3.5, 13.0)	0.254	
Oral - placebo	-6.5 (-14.5, 1.6)	0.107	-1.0 (-7.2, 5.1)	0.698	2.6 (-3.7, 8.8)	0.393	1.5 (-6.9, 9.8)	0.727	

(Continued)

Table 2 (Continued)

	No contrast			Contrast		
	Study 1: HV		Study 2: CKD3	Study 3: HV		Study 4: CKD3
	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.
AUC UNaE, h micromol/min			7 (87.5), 17 (70.8)			8 (100), 23 (95.8)
i.v. NAC	1,797.8 (1,346.1, 2,249.5)		1,729.8 (1,165.3, 2,294.2)	2,170.5 (1,966.5, 2,374.5)		2,207.5 (1,836.1, 2,578.8)
Oral NAC	1,191.2 (754.6, 1,627.9)		1,292.5 (773.1, 1,812.0)	1,443.4 (1,243.5, 1,643.4)		1,363.8 (994.3, 1,733.3)
Placebo	1,469.4 (1,066.2, 1,872.6)		1,063.8 (560.9, 1,566.7)	1,321.5 (1,107.4, 1,535.6)		1,247.8 (880.4, 1,615.2)
Treatment difference						
i.v. NAC - placebo	328.4 (-276.8, 933.6)	0.258	666.0 (-98.2, 1,430.1)	849.0 (613.8, 1,084.3)	<0.001	959.7 (438.7, 1,480.7)
Oral - placebo	-278.2 (-903.7, 347.3)	0.349	228.7 (-495.4, 952.9)	121.9 (-88.6, 332.5)	0.224	116.0 (-414.9, 646.9)
AUC ORAC, h TEAC/mcg inulin			8 (100), 24 (100)	8 (100), 24 (100)		8 (100), 24 (100)
i.v. NAC	70.3 (48.5, 92.1)		56.3 (42.8, 69.7)	55.1 (50.0, 60.2)		48.3 (43.7, 52.9)
Oral NAC	73.3 (51.5, 95.1)		52.5 (39.3, 65.8)	54.4 (49.3, 59.5)		41.4 (36.8, 46.0)
Placebo	78.5 (56.7, 100.3)		62.0 (48.9, 75.0)	56.0 (51.0, 61.1)		41.0 (36.4, 45.6)
Treatment difference						
i.v. NAC - placebo	-8.2 (-30.3, 13.9)	0.426	-5.7 (-24.6, 13.2)	-0.9 (-7.6, 5.7)	0.765	7.3 (0.8, 13.8)
Oral - placebo	-5.2 (-27.3, 16.9)	0.610	-9.4 (-27.9, 9.1)	-1.7 (-8.4, 5.1)	0.599	0.3 (-6.2, 6.8)
AUC cysteine, h mg/L			8 (100), 24 (100)	8 (100), 23 (95.8)		8 (100), 24 (100)
						66 (100), 66 (100)

(Continued)

Table 2 (Continued)

	No contrast			Contrast		
	Study 1: HV	Study 2: CKD3	Study 3: HV	Study 4: CKD3		
	Estimate (95% CI)	P value	No. (%) of part., no. of obs.	Estimate (95% CI)	P value	No. (%) of part., no. of obs.
i.v. NAC	312.5 (275.6, 349.3)		376.9 (332.2, 421.7)	413.4 (356.3, 470.4)		431.0 (400.3, 461.7)
Oral NAC	85.8 (50.3, 121.3)		127.6 (84.3, 170.9)	85.4 (27.3, 143.5)		124.8 (94.2, 155.3)
Placebo	77.1 (41.5, 112.7)		105.8 (60.7, 150.8)	89.0 (31.0, 146.9)		117.9 (86.9, 148.9)
Treatment difference						
i.v. NAC - placebo	235.4 (182.8, 288.0)	<0.001	271.2 (205.9, 336.5)	324.4 (240.2, 408.5)	<0.001	313.1 (269.0, 357.2)
Oral - placebo	8.7 (-40.9, 58.4)	0.716	21.9 (-39.3, 83.0)	-3.6 (-90.0, 82.9)	0.930	6.9 (-36.9, 50.6)
AUC glutathione, h nmol/mg			8 (100), 24 (100)	8 (100), 24 (100)		8 (100), 24 (100)
i.v. NAC	266.0 (202.9, 329.0)		431.0 (379.4, 482.7)	263.8 (225.5, 302.1)		345.7 (317.0, 374.3)
Oral NAC	288.5 (225.3, 351.7)		375.9 (325.0, 426.8)	205.7 (166.9, 244.6)		335.8 (307.5, 364.2)
Placebo	295.2 (229.5, 361.0)		365.8 (315.9, 415.7)	259.2 (220.8, 297.7)		329.5 (301.1, 357.8)
Treatment difference						
i.v. NAC - placebo	-29.2 (-114.0, 55.6)	0.470	65.2 (-7.3, 137.8)	4.6 (-41.6, 50.7)	0.832	16.2 (-24.4, 56.7)
Oral - placebo	-6.7 (-91.3, 77.9)	0.867	10.1 (-60.8, 81.0)	-53.5 (-101.2, -5.8)	0.031	6.4 (-33.6, 46.3)

Comparisons are of mean area under the curve (AUC) values for i.v. NAC and oral NAC vs. placebo, from start of the NAC/placebo infusion until 8 hours later. AUC, area under the curve; CI, confidence interval; CKD, chronic kidney disease; DBP, diastolic blood pressure; EFF, effective filtration fraction; GFR, glomerular filtration rate; MAP, mean arterial pressure; no., number; obs., observations; ORAC, oxygen radical absorbance capacity; part., participants; RBF, effective renal blood flow; SBP, systolic blood pressure; UNaE, urine sodium excretion.

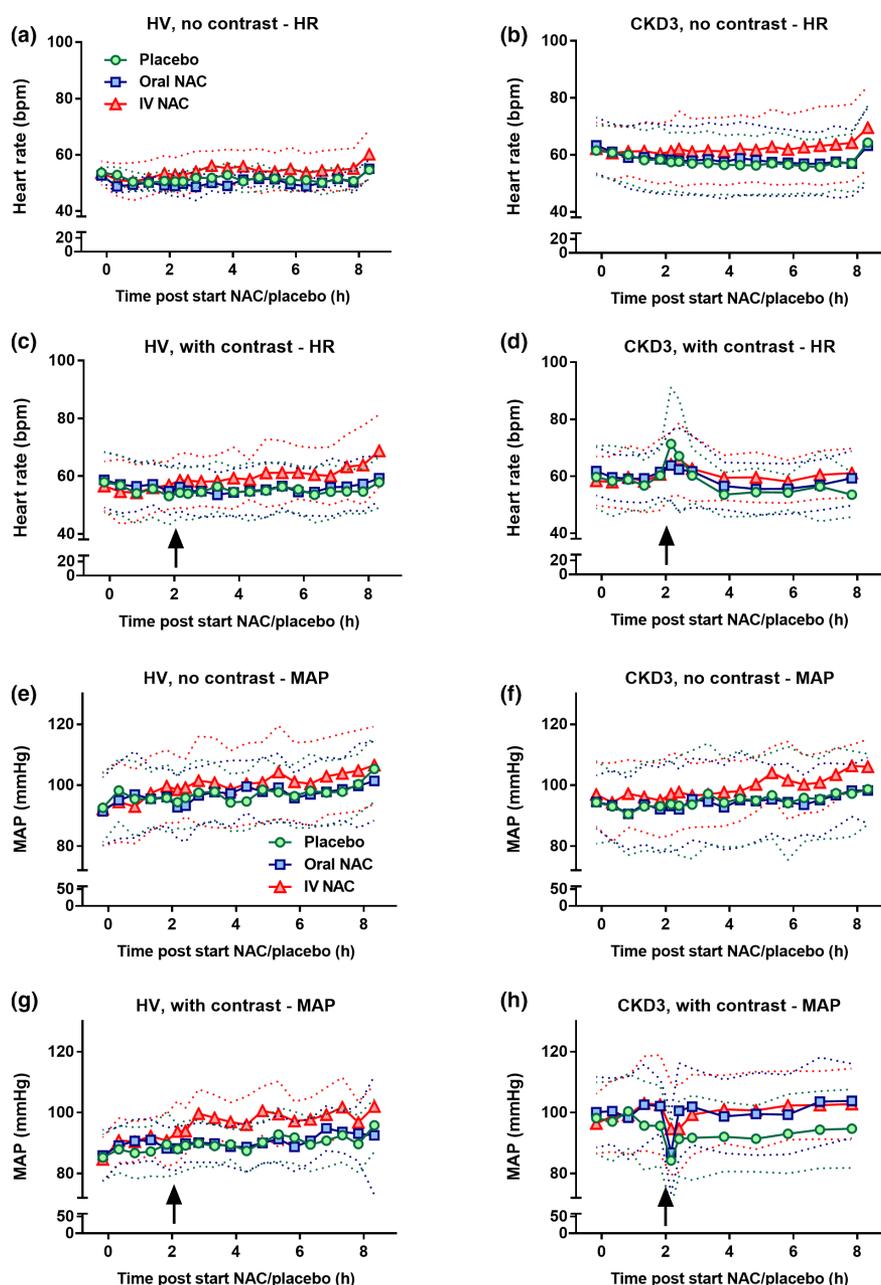


Figure 4 Systemic hemodynamics in (left column) healthy volunteers (HVs) and (right column) patients with CKD3, without (a, b, e, f) and with (c, d, g, h) contrast. a–d Mean (SD) heart rate (beats per min); (e–h): mean (SD) mean arterial pressure (MAP, mmHg). Participants received i.v. NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). Black arrows indicate the timing of the contrast administration in the appropriate groups. CKD3, chronic kidney disease stage III; HR, heart rate; NAC, (N)-acetylcysteine.

Effects on NAC on renal hemodynamics and function

Intravenous NAC increased RBF in healthy volunteers, in the absence (study 1; 714 (254 to 1,175) h mL/min, 8.0% increase, $P = 0.006$ vs. placebo) and presence (study 3; 917 (352–1,481) h·mL/min, 12.0% increase, $P = 0.005$) of contrast (Table 2, Figure 5a,c). Oral NAC had no such effect (without contrast: 212 (–239 to 663) h·mL/min, 2.4% increase, $P = 0.325$; with contrast: 35 (–539 to 610) h·mL/min, 0.5% increase, $P = 0.894$). Intravenous NAC increased renal blood flow in participants with CKD3 without contrast to a lesser degree than in healthy volunteers (study 2; 288 (153 to 424) h·mL/min, 6.0% increase, $P = 0.001$) but not

with contrast (study 4; 151 (–264 to 565) h·mL/min, 3.0% increase, $P = 0.470$; Figure 5b,d). The i.v. NAC increased the GFR of healthy volunteers receiving contrast (study 3; 147 (77–217) h·mL/min/1.73 m², 19.7% increase, $P = 0.001$), but not of patients with CKD3 (study 4; 29 (–8 to 65) h·mL/min/1.73 m², 6.3% increase, $P = 0.122$; Table 2, Figure 5e–h). In all four study groups, effective filtration fraction was unaffected by oral or i.v. NAC, whereas fractional excretion of sodium was increased by i.v. NAC only in patients receiving contrast (Table 2, Figures S4, S5).

In study 4, we assessed the effects of NAC on biomarkers of renal injury after contrast in patients with CKD3. Only six

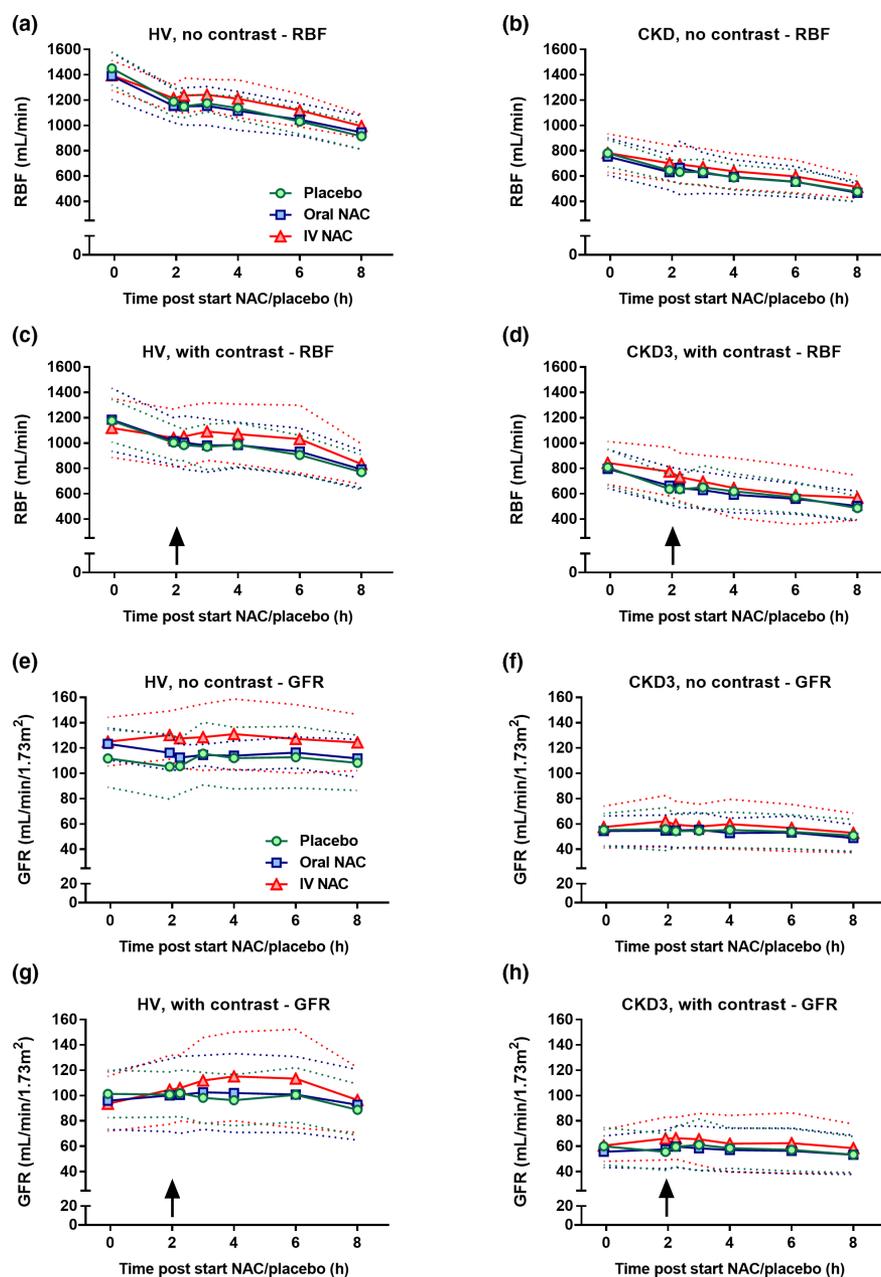


Figure 5 Renal blood flow (RBF) and glomerular filtration rate (GFR) in (left column) healthy volunteers (HVs) and (right column) patients with chronic kidney disease stage III (CKD3), without (**a, b, e, f**) and with (**c, d, g, h**) contrast. **a–d** Mean (SD, dotted lines) RBF (mL/min); **e–h** mean (SD, dotted lines) GFR (mL/min/1.73 m²). Participants received i.v. NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). Black arrows indicate the timing of the contrast administration in the appropriate groups.

patients (4 receiving placebo, 1 oral NAC, and 1 i.v. NAC) showed acute kidney injury at 72 hours (serum creatinine concentration > 170 $\mu\text{mol/L}$). None of the biomarkers consistently indicated injury after contrast (**Figure 6**). There were no statistically significant differences in concentration of serum creatinine or plasma cystatin C, or in the urinary KIM-1/creatinine or NGAL/creatinine ratios, between study arms (**Table S3**).

Effects of NAC on antioxidant status

Intravenous, but not oral, administration of NAC caused a marked increase in plasma cysteine as a breakdown product of acetylcysteine in all four studies (**Figure 7a–d**) but no increase in

white cell glutathione (formed from cysteine; **Figure S6**). Neither i.v. nor oral NAC increased ORAC in any of the studies (**Table 2, Figure 7e–h**).

Harms

In studies 1–3, 6, 4, and 2 participants reported adverse events. Two were serious adverse events, neither of which were considered to be associated with acetylcysteine (one episode of angina 3 days after the study day, requiring admission for angiography; one transient ischemic event at home between study days, admitted for 24 hours with full resolution). In study 4, there were 22 adverse events, with 6 considered to be serious (1 with myocardial

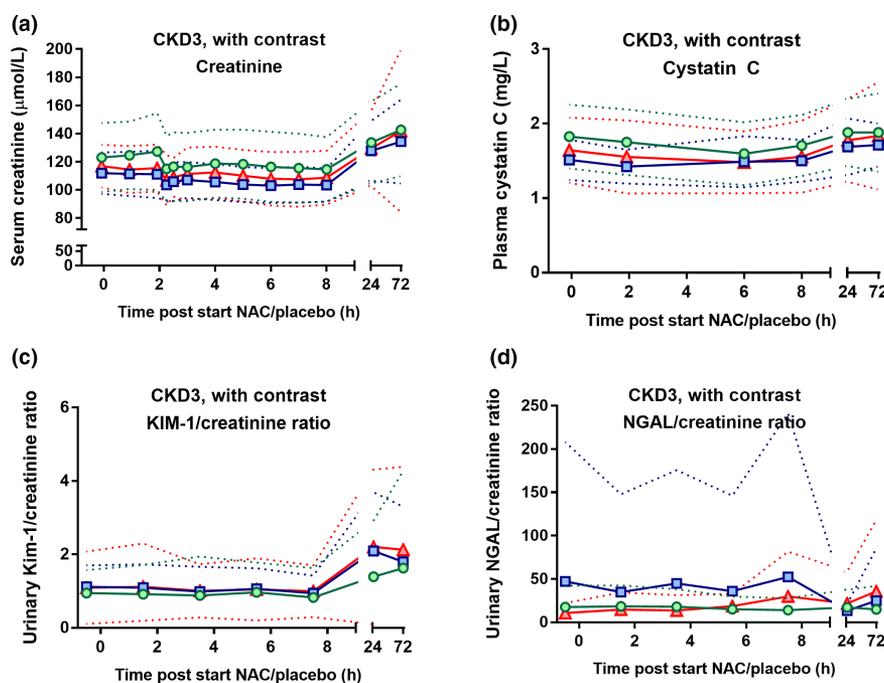


Figure 6 Blood and urinary renal biomarkers in patients with CKD3 receiving contrast. **(a)** mean (SD, dotted lines) serum creatinine concentration ($\mu\text{mol/L}$); **(b)**: mean (SD, dotted lines) plasma cystatin-C concentration (mg/L); **(c)**: mean (SD, dotted lines) urinary KIM-1/creatinine ratio; **(d)**: mean (SD, dotted lines) urinary NGAL/creatinine ratio. Participants received i.v. NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). CKD3, chronic kidney disease stage III; NAC, (N)-acetylcysteine; NGAL, neutrophil gelatinase-associated lipocalin.

infarction after recruitment before starting the study, 1 with angina during the study, 1 with exacerbation of heart failure during the study, 1 with bradycardia during angiography, 1 with angina secondary to coronary artery dissection during angiography, and 1 with episode of angina following the study day).

DISCUSSION

We show here that the oral NAC dose used in many reported RCTs results in barely detectable plasma NAC and cysteine concentrations and has no effect on renal hemodynamics or oxidant status, whereas i.v. NAC causes renal vasodilatation only in those patients with healthy kidneys who would not be expected to benefit. We found no mechanistic evidence that NAC can benefit patients at risk of CIN. Conducting unnecessary RCTs of interventions with no possibility of benefit leaves participants exposed only to any harms. It also has an opportunity cost for (at best) delaying RCTs of an intervention that might be effective and safe.

The last 2 decades have seen more than 18,000 patients recruited to over 75 RCTs of mostly low-dose oral NAC to prevent CIN, in turn meta-analyzed by more than 20 different groups. Two large RCTs, including over 7,000 patients, ultimately showed that oral NAC does not prevent CIN. The whole “enterprise” was started by a high impact journal publishing a single underpowered RCT performed by an academic research group. Unlike typical drug development in industry, there were no preclinical studies and no early phase clinical trials to clarify possible mechanisms and effective doses before the first phase III trial was performed.

In our study, we examined the potential mechanisms by which NAC may affect renal function that could be protective vs. CIN. We found that i.v. NAC increased heart rate and blood pressure in patients with CKD3 undergoing angiography—at relatively high risk for CIN—but had no effect on their renal hemodynamics, in particular, renal blood flow. By contrast, i.v. NAC did increase renal blood flow in healthy volunteers with normal renal function.

The total dose of oral NAC we used (1,200 mg b.i.d. for 2 days, 53.3 mg/kg for a 90 kg individual) is five to six-fold lower than the i.v. dose and resulted in a 100-fold lower blood NAC concentration around the time of contrast administration and no effect on renal hemodynamics. This blood concentration was barely detectable, in contrast to i.v. NAC. These findings indicate that one of NAC’s proposed mechanisms of protection against CIN—vasodilatation of the afferent arteries—is invalid in the patients at risk of developing CIN.

We also found that i.v. and oral NAC did not increase cellular glutathione concentrations or produce a clinically significant rise in plasma antioxidant capacity, as measured by ORAC. This suggests that NAC does not protect kidneys using its other proposed mechanism of action—that of boosting protective plasma antioxidant capacity.¹⁹ However, the status of NAC as a direct antioxidant has been challenged, particularly at clinically relevant concentrations³¹ and its potential to induce intracellular antioxidant effects might only relate to cells or individuals depleted of glutathione.³⁰ However, NAC delivery by both routes did increase thiol availability in plasma (NAC, cyst(e)ine) and intracellular (NAC, cyst(e)ine, GSH) compartments, as well as the MAP. These results suggest that a physiological effect may

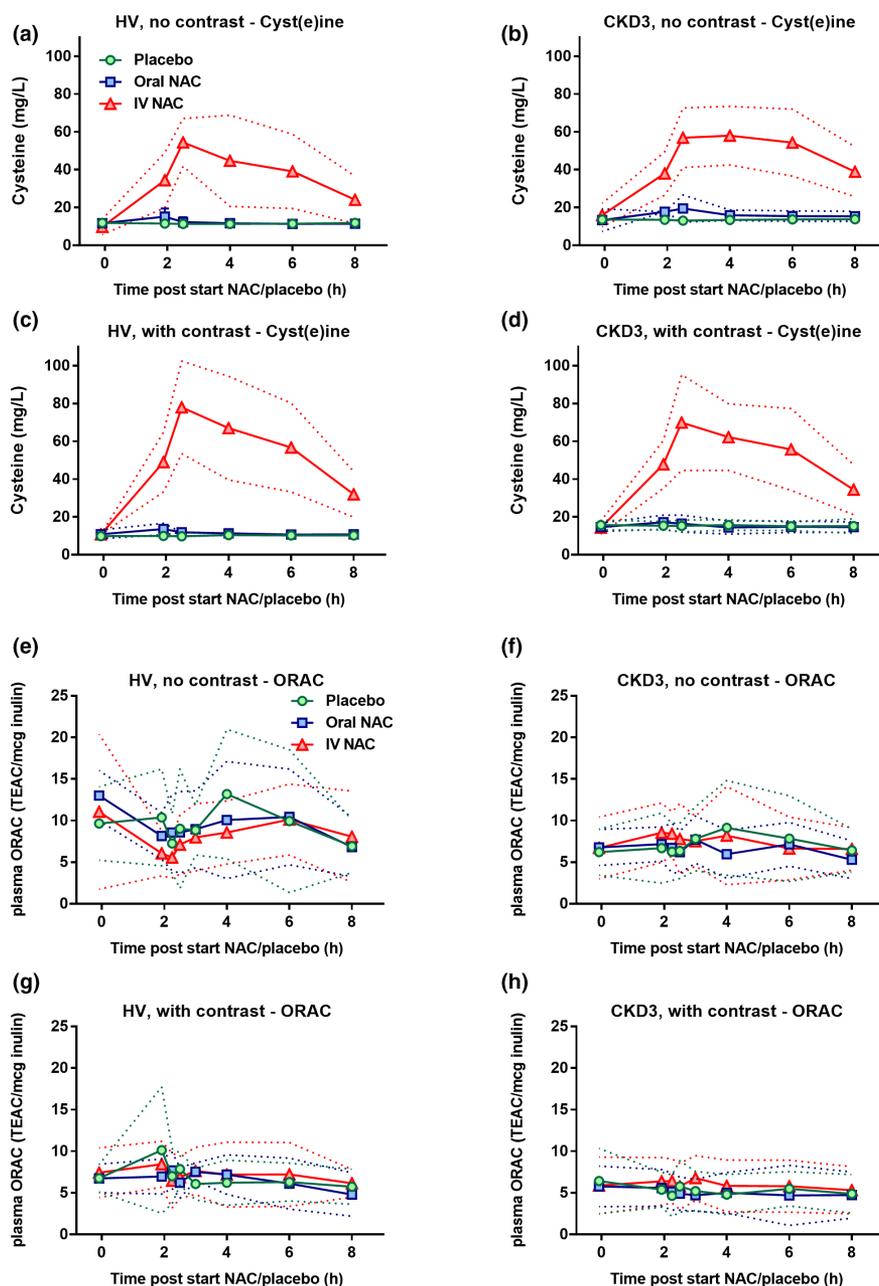


Figure 7 Plasma antioxidant status in (left column) healthy volunteers (HVs) and (right column) patients with CKD3, without (a, b, e, f) and with (c, d, g, h) contrast. **a–d** Mean (SD, dotted lines) plasma (de-acetylated) cystine concentration (mg/L); **e–h**: mean (SD, dotted lines) plasma oxygen radical absorbance capacity (ORAC, TEAC/ μ g inulin). Participants received i.v. NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). CKD3, chronic kidney disease stage III; HV, healthy volunteer; NAC, (N)-acetylcysteine; ORAC, oxygen radical absorbance capacity.

be conferred by the relatively small changes in thiol availability from oral NAC and that substantially higher plasma concentrations do not alter the extent of the MAP effect or protect against contrast-induced renal dysfunction. Further work is required to better understand the pharmacokinetic/dynamic effects of NAC and its metabolites.

Limitations

This study was not designed to assess the efficacy of NAC in preventing CIN—its purpose was to undertake the mechanistic

investigations that logically should have preceded the 70+ RCTs on > 18,000 participants that failed to show NAC was effective in preventing CIN. CIN was uncommon in the study - only 6 patients in study 4 showed a rise in serum creatinine concentration at 72 hours of > 170 μ mol/L, and there were no changes in plasma cystatin-C or urinary KIM-1 and NGAL. These are sensitive measures of acute kidney injury. In a recent meta-analysis,³² serum cystatin C had a sensitivity and specificity of 0.87 and 0.86, respectively, for diagnosing AKI. For urinary KIM-1, these figures have been estimated at 0.84 and 0.78, respectively,³³ and for

urine NGAL sensitivity and specificity are 0.82 and 0.90, respectively.³⁴ These figures will vary depending on the nature of acute or chronic kidney injury. A lack of change in these measures in the current study is likely because patients were asked to hydrate well before the procedure, modest amounts (study 3: 100 mL and study 4: median 130 mL) of an iso-osmolar contrast agent were used, and each patient received around 220 mL i.v. fluid per hour during the 9 hours surrounding the contrast administration.³⁵ The study recruited only male volunteers; however, we do not know of any reason why the results will not be as relevant for women as men.²⁶

This study was designed to assess mechanistic signals on (i) healthy volunteers and those with CKD, (ii) using oral or i.v. NAC, and (iii) with and without a contrast medium, on RBF and a range of secondary outcomes—NAC concentration, systemic hemodynamics (including heart rate and MAP), renal hemodynamics (including GFR), and antioxidant properties (including cysteine and ORAC). It was difficult to confidently assess the required sample sizes to be adequately powered for all these investigations and, with limited resources, we chose to recruit $n = 8$ to each of the three-period crossover studies and $n = 66$ to the 3-arm parallel groups study. That our first choice of statistical model—the random coefficients model—failed to converge on occasion indicated a lack of information, and hence too small a sample size. However, for all the important issues, we were able to rule out clinically important differences, and hence demonstrate that the putative mechanisms were unfounded and could not underpin the extensive suite of RCTs that overall failed to detect the benefit for NAC. They failed to detect benefit because that benefit is not there, which would have been clear and obvious if this study had been done first, and not last.

Baseline values for comparison were taken after participants had taken oral NAC for 24 hours, meaning that the effect of these first 2 doses of NAC was excluded from the statistical analysis. However, NAC was barely detectable at baseline in patients taking oral NAC, there was no evidence that oral NAC significantly affected glutathione or antioxidant status, and participants were similar at baseline whether they had received oral NAC or not (Table S2).

Implications

We have shown here that oral NAC could never have benefited patients at risk of CIN through the proposed mechanisms and that the initial finding was likely a false positive. The single study published in a high impact journal¹⁷ has resulted in an immense waste of funds (many millions of pounds at a cost of 1000–10,000 pounds to recruit one patient), time, and also opportunity, because the patients could have been recruited to trials more likely to show benefit. This story shows vividly the weaknesses of stand-alone small-scale academic RCTs that are not part of a development process. The initial RCT should not have started a series of clinical trials without careful evaluation of whether the medicine could ever have worked.

There have been improvements since the 1990s. Now all grant and ethics applications request information on RCTs that have gone before. Systematic reviews of evidence are more commonly done. However, in this case, there were no previous studies to

review and this requirement would therefore have made no difference. The problem was the wholesale acceptance of the result and the mass set-up of small RCTs worldwide, encouraged by the systematic review “industry.” It illustrates the weaknesses of systematic reviews, many of which suggested that oral NAC might prevent CIN. Two large RCTs showed that it offered no benefit. The study complements the discourse around research wastage³⁶ and the harms of unthinking systematic reviews.^{37,38}

In conclusion, this study shows that oral NAC is poorly absorbed and can offer no mechanistic benefit to kidneys affected by contrast, despite more than 18,000 patients being recruited to trials testing this intervention. Intravenous NAC did cause renal artery vasodilatation but only for healthy kidneys, not for kidneys in patients with CKD3 who are at higher risk of CIN. This study illustrates the dangers of designing clinical trials without clear proof of how an intervention might work and how the outcome should be measured.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.E., E.A.S., J.M.B.R., and I.L.M. wrote the manuscript. M.E., E.A.S., N.D., D.N.B., S.C., N.U., J.G., D.J.W., and I.L.M. designed the research. E.A.S., K.R., E.E.M., K.H., J.W., T.G., L.B., S.C., A.P.T., N.R.J., N.U., A.T., I.L.M., and M.E. performed the research. E.A.S., J.M.B.R., N.D., J.N., I.L.M., and M.E. analyzed the data. G.R. contributed new analytical tools. M.E. and E.S. are guarantors and accept full responsibility for the work and study conduct, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

DATA AVAILABILITY STATEMENT

The study protocol has been published.²⁶ A fully anonymized trial dataset will be made available to other researchers after the publication of the full trial report. Requests should first be directed to Michael Eddleston (Chief Investigator). Written proposals will be assessed and a decision made about the appropriateness of the use of data. A data sharing agreement will be put in place before any data will be shared.

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