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Potentially detrimental effects of N-acetylcysteine on renal function in knee arthroplasty

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Abstract

Ischaemia/reperfusion induces systemic inflammation and oxidative stress and thereby remote organ injury in the kidney. In a double-blind, placebo-controlled clinical trial of 30 patients undergoing knee arthroplasty with tourniquet, this study evaluated the effect of N-acetylcysteine (NAC) infusion on renal function by measuring urine alpha-1-microglobulin, N-acetyl-beta-D-glucosaminidase (NAG), glutathione-S-transferase-alpha and -phi and serum creatinine and cystatin C concentrations up to 24 h post-operatively. Compared to the baseline, urine alpha-1-microglobulin/creatinine increased in both groups and was higher in the NAC group than in the placebo group at tourniquet deflation and at 3 h thereafter. Urine NAG/creatinine increased at deflation and at 3 h thereafter in the NAC group and the ratio was higher than in the placebo group. The two sensitive indicators of proximal tubular damage and function used in the present study suggest that use of NAC in clinical setting of ischaemia/reperfusion injury may increase the risk of remote kidney injury.

Keywords: *N-acetylcysteine, ischemia, reperfusion, knee arthroplasty, tourniquet*

Introduction

Ischaemia/reperfusion (IR) induces systemic inflammation and oxidative stress. In addition to the reperfused vascular bed itself, IR may result in remote organ injury in the lungs, liver and/or kidney. Experimental studies indicate that reperfusion after tourniquet release of a limb may cause ischaemic changes in renal proximal tubuli and cortex [1,2]. Tourniquet used in knee arthroplasty serves as a clinical model to evaluate the effects of limb ischemia/reperfusion in humans.

N-acetylcysteine (NAC), a precursor of antioxidant glutathione, may protect organs against free oxygen radicals and lipid peroxidation [3,4]. In an experimental model of rhabdomyolysis, NAC improved kidney microcirculation and prevented renal damage [5]. In animals with acute renal failure, NAC enhanced renal medullary perfusion [6]. The aim of this double-blind placebo-controlled clinical trial was to evaluate possible beneficial effects of NAC-infusion on kidney function (remote organ damage object) in patients undergoing total knee arthroplasty with thigh tourniquet.

Subjects and methods

Patients

Thirty consecutive patients with normal renal function undergoing elective total knee arthroplasty were

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included in the study. Patients with renal insufficiency (serum creatinine concentration > 120 μmol/l) were excluded. The ethics committee of the hospital approved the prospective, double-blinded, placebo-controlled study protocol. The study was conducted in accordance with the Declaration of Helsinki and all patients gave written informed consent.

Intra-operative management

Intra-operative treatment was standardized. On the morning of the operation, patients received their normal beta blocking agents and nitrates and, as pre-medication, diazepam 10–15 mg by mouth 60 min before anaesthesia. Diuretics, angiotensin converting enzyme inhibitors or angiotensin receptor blocking drugs were not given. After pre-medication, the femoral vein of the operated leg was cannulated under local anaesthesia with a 20-cm catheter to measure common iliac vein pressure and to obtain blood samples. Common iliac vein pressure is comparable to central venous pressure [7]. Spinal anaesthesia with an indwelling catheter was induced with plain bupivacaine 5 mg/ml at a dose of 12.5–17.5 mg. An epidural catheter was inserted for post-operative pain management. A urine catheter was inserted after the onset of spinal anaesthesia for the measurement of urine output and for collecting urine samples. All patients received cefuroxim 1.5 g i.v. at induction for antibiotic prophylaxis. The operated limb was exsanguinated using a tight Esmarch bandage before the institution of the tourniquet. The cuff was pressurised to 300–350 mmHg. Tranexamic acid 1 g was injected intravenously before the release of the tourniquet.

Fluid therapy was standardized. Patients received Ringers' solution (Ringer-Acetat, B.Braun, Melsungen Germany) 8 ml/kg at the induction of anaesthesia, followed by an infusion of 10 ml/kg/h. Mean arterial pressure (MAP) was kept within 20% of pre-anaesthesia value and above 60 mmHg using fluid therapy if the common iliac vein pressure was below 6 mmHg and otherwise by ephedrine 5 mg i.v. Ten minutes before tourniquet deflation infusion of 500 ml of hydroxyethyl starch 6% solution (Hemohes 6%, B.Braun, Melsungen, Germany) was started. Blood loss after tourniquet release was measured and recorded. Packed red blood cells were transfused when needed to maintain the haematocrit above 25%.

Post-operative pain was treated using intratechal infusion of bupivacaine with morphine or fentanyl. Non-steroidal anti-inflammatory analgesics were not used during the trial.

Study protocol

The patients were randomly assigned (a sealed opaque envelope method) to receive either an i.v.-infusion of N-acetylcysteine (NAC group) or normal saline (placebo group). In the NAC group a loading dose of 150 mg/kg N-acetylcysteine (Parvolex®, Medeva Pharma Ltd, Leatherhead, UK) in 5% glucose was infused 30 min before the onset of tourniquet, followed by an infusion of 6.25 mg/kg/h. The patients in the placebo group received similar volumes of 5% glucose, respectively. The study drug infusions were discontinued at the end of surgery. The trial drugs were prepared by a nurse not participating in the study and the management of the patient, thus allowing the blinding.

The renal effects of tourniquet and reperfusion were evaluated using both traditional (serum creatinine and urea) and sensitive markers (serum cystatin C, urinary alpha-1-microglobulin, N-acetyl-beta-D-glucosaminidase, glutathione-S-transferase-alpha and -phi indexed to the urinary creatinine) of renal function. The stable metabolite of thromboxane A_2, thromboxane B_2 (TXB_2) and serum lactoferrin concentration were measured as markers of thromboxane production and neutrophil activation, respectively. The muscular injury of the operated leg was evaluated by measuring the release of myoglobin.

Urine samples for the measurement of alpha-1-microglobulin, N-acetyl-beta-D-glucosaminidase (NAG), glutathione-S- transferase-alpha and -phi (GST-alpha, -phi) and creatinine were collected at baseline, at the tourniquet deflation and at 3 and 24 h after the tourniquet.

Blood samples were obtained from the common iliac vein cannula or by cubital venipuncture at baseline, within the first minute after tourniquet deflation, and at 3 (common iliac vein) and 24 h (venipuncture) after tourniquet deflation. In the venous blood samples the following parameters were measured: cystatin C, creatinine, urea, TXB_2 and TXB_2 stimulated with calcium ionophore (measures the maximal capacity of TXB_2 production from the platelets), lactoferrin, lactate and myoglobin.

Analytic methods

Urine alpha-1-microglobulin was measured by radioimmunoassay [8] and NAG enzymatically by using 3-cresonsulphonphtalein-N-acetyl-beta-glucosamine as a substrate (Boehringer Mannheim Biochemica, Mannheim, Germany). Kinetic follow-up of the reaction method was adapted for a Kone Specific® random access analyser (Kone Instruments, Espoo, Finland). GST-alpha and GST-phi were analysed by using the commercial enzyme immunoassays (Nephkit™-Alpha and Nephkit™-Pi, Biotrin International Ltd., Dublin, Ireland). To eliminate the influence of variations in urine volume, alpha-1-microglobulin, NAG, GST-alpha and -phi were indexed to urine creatinine. Urine creatinine was determined by kinetic Jaffe reaction. The specificity of the reaction has been increased by measuring the speed of the

reaction of creatinine and picrinic acid in alkaline pH [9].

The hospital reference values for alpha1-microglobulin/creatinine ratio are 0.3 (0.04–0.70) mg/mmol (mean and range) and for GST-alpha and GST-phi/creatinine ratios 0.6 (0.10–1.9) μg/mmol and 2.4 (0.3–7.4) μg/mmol, respectively. These alpha-1-microglobulin/creatinine ratios are derived from 33 healthy individuals aged 23–63 years and GST-alpha and -phi/creatinine ratios from 38 healthy individual aged 18–46 years.

Serum cystatin C was analysed by using nephelometric assay and Dade Behring Nephelometer II and reagents (Marburg, Germany). Blood for the analysis of serum TXB_2 and calcium ionophore stimulated TXB_2 were collected and prepared as described earlier [10]. Serum lactoferrin was analysed by an ELISA method [11]. Standard methods were used to determine serum myoglobin, creatinine and urea and plasma lactate.

Statistics

The Kolmogorov-Smirnov test was used to test normal distribution of the data and non-parametric tests were used for non-normally distributed data. Differences between the groups and different time points were compared with the Mann-Whitney U-test. The changes in biochemical parameters from the pre-operative values within a group were analysed using the Wilcoxon test. Differences in demographic data between the treatment groups were compared with the Mann-Whitney U-test. Calculations were performed using SPSS 9.0 (SPSS Inc. Chicago, IL). *p*-values less than 0.05 were considered statistically significant. Data are presented as the median and 25^{th}, 75^{th} percentiles when appropriate.

Results

There was no difference between the two groups in the patients' baseline characteristics and surgical data (Table I). An optimal bloodless surgical field was achieved in all patients.

Compared to the baseline, urine alpha-1-microglobulin/creatinine ratio was increased at deflation and at 3 h after the tourniquet in both groups and it remained elevated up to 24 h after the tourniquet in both groups. There was a difference between the two study groups in the course of alpha-1-microglobulin excretion; the ratio was 2-times higher in the NAC group than in the placebo group at the tourniquet release and at 3 h thereafter (Figure 1).

In the NAC group, but not in the placebo group, the urine NAG/creatinine ratio increased at deflation and 3 h thereafter and returned to baseline at 24 h. Consequently, the NAG/creatinine ratio in the NAC group was higher than that in the placebo group at deflation and 3 h thereafter (Figure 2).

Table I. Patient characteristics and surgical data.

	N-acetylcysteine ($n=15$)	Placebo ($n=15$)
Age (years)	69 (62–77)	68 (64–73)
BMI (kg/m^2)	27 (24–30)	30 (27–30)
Patient gender, female/male	12/3	12/3
Tourniquet time (min)	105 (77–113)	100 (83–121)
Blood loss after tourniquet (ml)	200 (10–400)	200 (40–300)
Fluids given during the first 24 h (ml)	1800 (1500–2000)	2100 (1700–2300)

Median (25^{th}–75^{th} percentiles), BMI = Body mass index.

There were no differences between the study groups in urine GST-alpha/creatinine on GST-phi/creatinine ration at any time points. Urine myoglobin was higher in the NAC group than that in the placebo group at deflation (Table II). Serum cystatin C, creatinine and urea decreased comparably in both groups, most probably due to haemodilution (Table III).

Serum myoglobin and lactoferrin and plasma lactate increased after reperfusion, but there were no differences between the groups. Unstimulated TXB_2 remained stable throughout the study period in both groups. Compared to baseline, there was a slight increase in stimulated TXB_2 only in the NAC group at 3 h after deflation. Yet, at this or any other time points, there were no differences in stimulated TXB_2 between the study groups (Table III).

Discussion

In experimental studies, ischaemic changes in proximal tubules and renal cortex have been observed after tourniquet release [1,2]. In the present study, urine alpha-1-microglobulin/creatinine ratio, a marker of proximal tubular function [8], increased after the tourniquet deflation in both groups. The urine

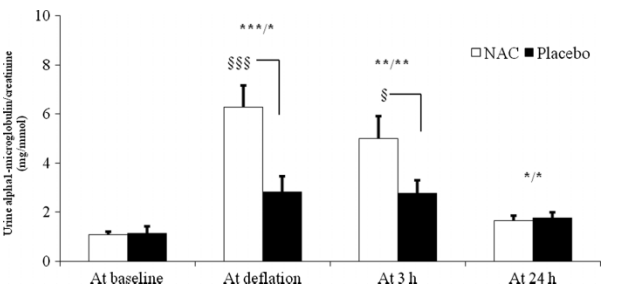

Figure 1. The urine alpha-1-microglobulin/creatinine ratio in the two study groups. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to the baseline within each study group. § $p<0.05$, §§§ $p<0.001$ between the study groups. NAC = N-acetylcysteine. At the deflation = within 1 min after tourniquet deflation, at 3 h = 3 h after the tourniquet, at 24 h = 24 h after the tourniquet.

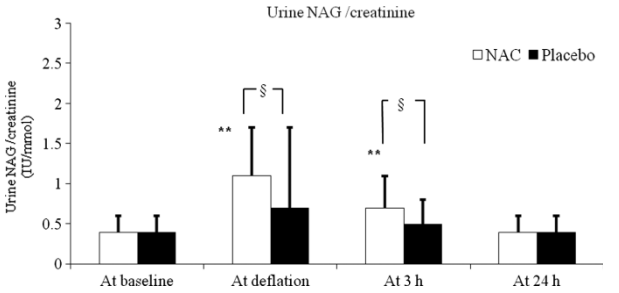

Figure 2. The urine NAG/creatinine ratio in the two study groups. ** $p < 0.01$, compared to the baseline in the NAC group. § $p < 0.05$ between the study groups. NAC = N-acetylcysteine. At the deflation = within 1 min after tourniquet deflation, at 3 h = 3 h after the tourniquet, at 24 h = 24 h after the tourniquet.

alpha-1-microglobulin/creatinine ratio was marginally elevated even at 24 h after the tourniquet. Surprisingly, supplementation of NAC aggravated the renal injury; the maximal urine alpha-1-microglobulin/creatinine ratio was 2-times higher in the patients having NAC-infusion compared to that in the placebo group. Also NAG/creatinine ratio, another sensitive marker of proximal tubular damage, was significantly increased in the NAC group but not in the placebo group.

Some other studies have reported results indicating that NAC may have harmful effects in some clinical situations. Decrease in cardiovascular performance in septic patients [12,13] and a trend towards increased mortality in multi-organ failure [14] have been described previously. Furthermore, in a human model of acute muscle injury induced by eccentric exercise, Childs et al. [15] have shown that combination of vitamin C and NAC possesses pro-oxidative properties. In that model serum-free iron increased after muscle injury, probably due to release from ferritin and especially from myoglobin. Transition metal iron catalyses auto-oxidation of sulphydryl group of NAC yielding hydrogen peroxide and superoxide, hydroxyl and sulphur-containing radicals. Accordingly, in our patients serum myoglobin increased after tourniquet deflation. The primary insult of tourniquet is muscle injury. Thus, it is tempting to speculate that the pro-oxidative mechanisms, suggested by Childs et al. [15] in their model of muscle injury, are operative also in NAC-induced increase in renal damage in the present study. As the present study in a clinical setting was not tailored for investigation of biochemical mechanisms of NAC, possible pro-oxidative action remains speculative and should be confirmed with another NAC intervention study. However, according to the present results, we find such an intervention unethical.

The optimal dose of NAC for prevention of the ischaemia/reperfusion syndrome has not been established and comparison of different clinical intervention studies with NAC is difficult because varying drug doses have been used. In the present study, as in all above-referred studies with adverse effects [12–14], a relatively high NAC loading dose and infusion was administered. In contrast, in arthroscopic knee surgery with tourniquet, low infusion dose of NAC without loading dose resulted in reduced reperfusion-induced lipid peroxidation after reperfusion [16]. In line with this, in a rodent endotoxin model, low dose of NAC protected against oxidative stress by directly scavenging oxygen radicals. On the contrary, higher doses decreased animal survival and increased oxidative stress by auto-oxidation of NAC sulphydryl groups [17]. To our knowledge, dose response of NAC on outcome or oxidative stress has not been evaluated in humans.

The primary aim of this study was to investigate renal damage and function between the study groups. Thus, in order to eliminate the confounding effect of underlying renal disease, only patients without known kidney disease and with pre-operative serum creatinine level within normal range were included in the study. Furthermore, careful attempts were made to avoid any other factors potentially leading to deteriorated renal function and, thus, confounding the interpretation of the results. The operation was undertaken in regional anaesthesia to avoid administration of any general anaesthetic agents that may affect the renal function. Hypotension was rigorously

Table II. Urine markers of renal function.

	At baseline	At tourniquet release	At 3 h after tourniquet	At 24 h after tourniquet
U-GST-α/crea (μg/mmol)				
NAC	0.7 (0.4–1.7)	1.3 (0.5–1.3)	0.4 (0.3–1.1)	0.1 (0.0–0.4)**
Placebo	0.5 (0.2–1.2)	0.9 (0.5–1.2)*	0.7 (0.2–1.1)	0.2 (0.0–1.2)
U-GST-π/crea (μg/mmol)				
NAC	0.5 (0.3–1.2)	0.7 (0.4–1.1)	0.6 (0.4–1.1)	0.2 (0.1–0.4)*
Placebo	0.5 (0.2–1.1)	0.9 (0.5–1.2)	0.7 (0.2–1.1)	0.2 (0.0–1.2)
U-Myoglobin (μg/l)				
NAC	6.0 (5.0–7.0)	7.0 (6.0–8.0)§§	6.5 (6.0–7.3)	
Placebo	6.0 (3.0–6.0)	6.0 (3.0–6.0)	6.0 (5.0–6.0)	

Median (25^{th}–75^{th} percentiles). * $p < 0.05$, ** $p < 0.01$ compared to the baseline in each group. §§ $p < 0.01$, between the groups. NAC = N-acetylcysteine, GST-α = glutathione-S-transferase-alpha, GST-π = glutathione-S-transferase-phi.

Table III. Plasma and serum markers of reperfusion and renal function. There were no differences between the groups.

	At baseline	At tourniquet release	3 h after tourniquet	At 24 h after tourniquet
S-Cystatin C (mg/l[1])				
NAC	0.8 (0.8–1.0)		0.7 (0.6–0.8)*	
Placebo	0.8 (0.8–1.1)		0.7 (0.6–0.8)**	
S-Creatinine (μmol/l)				
NAC	77 (65–93)			73 (55–76)**
Placebo	72 (65–80)			66 (56–72)**
S-Urea (mmol/l)				
NAC	4.6 (3.7–6.0)			4.0 (3.6–4.9)**
Placebo	5.9 (4.6–6.8)			4.5 (4.0–5.2)**
S- TXB_2 (pg/ml)				
NAC	160 (160–5920)	160 (60–6380)	540 (120–25000)*	
Placebo	1040 (154–6400)	760 (60–5600)	480 (152–7600)	
S-stim TXB_2 (pg/ml)				
NAC	100 (60–5200)	120 (50–4100)	320 (120–8480)	
Placebo	340 (220–5460)	2520 (280–5760)	420 (204–5280)	
S-Lactoferrin (ng/ml)				
NAC	259 (163–328)	320 (220–402)	479 (390–574) ***	
Placebo	289 (195–423)	322 (199–406)	456 (374–510)***	
P-Lactate (mmol/l)				
NAC	0.7 (0.6–0.8)	2.6 (2.3–3.1)***	0.7 (0.5–1.1)	
Placebo	0.7 (0.6–0.7)	3.6 (2.1–3.7)***	0.7 (0.2–3.9)	
S-Myoglobin (μg/l)				
NAC	48 (34–62)	60 (53–82)**	93 (59–149)**	74 (66–108)**
Placebo	35 (32–50)	58 (41–139)**	111 (69–156)**	64 (49–90)**

The data are median (25^{th}–75^{th} percentiles). S = Serum, P = Plasma. NAC = N-acetylcysteine, TXB_2 = Thromboxane B_2, stim TXB_2 = calcium ionophore stimulated tromboxane B_2, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the baseline in each group.

treated. And finally, proper fluid therapy was used and standardized in both groups. Of note, although haemodilution probably affected on serum concentrations of renal markers, urinary excretion of the measured markers, when indexed against urinary creatinine, should not have been influenced by fluid therapy. Furthermore, the unfavourable effect of NAC was based on two independent biochemical indices at two different time points. Taken together, the present results most probably reflect real changes in proximal tubular damage and function between the study groups.

There are some limitations in the present study. First, we did not measure the plasma NAC and its metabolite concentrations, so we are not able to evaluate the potential correlations of NAC concentration on renal parameters. Secondly, we did not measure free iron, reactive oxygen species generation or glutathione deficiency in erythrocytes. These are obviously shortcomings of the present study. However, the present study was not designed to evaluate the possible mechanism of NAC-induced remote kidney damage.

We conclude that, with sensitive indicators of renal function in a tightly controlled clinical setting of ischaemia/reperfusion, supplementation of NAC resulted in increased remote kidney injury. The present findings are in line with previous reports of NAC both in experimental [17] and human models [15]. According to the present trial, NAC infusion may not only remain ineffective but can even be harmful and thus should not be used, at least in higher doses, in patients having lower limb tourniquet for elective surgery.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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