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Original Article

N-acetylcysteine infusion reduces the resistance index of renal artery in the early stage of systemic sclerosis

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Aim: To evaluate resistance index (RI) changes in renal artery after N-acetylcysteine infusion in patients with systemic sclerosis.
Methods: In an open-label study 40 patients with systemic sclerosis (SSc) were treated with N-acetylcysteine (NAC) iv infusion over 5 consecutive hours, at a dose of 0.015 g·kg^{-1}·h^{-1}. Renal haemodynamic effects were evaluated by color Doppler examination before and after NAC infusion.
Results: NAC infusion significantly reduced RI in a group of sclerodermic patients with early/active capillaroscopic pattern, modified Rodnan Total Skin Score (mRTSS) <14 and mild-moderate score to the vascular domain of Medsger Scleroderma Disease Severity Scale (DSS). RI increased after NAC infusion in patients with late capillaroscopic pattern, mTRSS>14 and severe-end stage score to the vascular domain of DSS. In patients with reduction of RI after NAC infusion, diffusion capacity for carbon monoxide mean value was significantly higher than in those patients with an increase of RI. No significant differences in renal blood flow were found between patients with different subsets of SSc.
Conclusion: In patients with low disease severity NAC ameliorates vascular renal function.

Keywords: systemic sclerosis; N-acetylcysteine; color Doppler sonography; renal blood flow; resistance index

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrosis affecting the skin and the internal organs. Mortality and morbidity mostly depend on the involvement of lungs, heart, kidneys, and gastrointestinal tract[1].

Renal complications are common in SSc although they are not always clinically significant. Scleroderma renal crisis is the most important renal complication and occurs in 10%–15% of the patients with diffuse cutaneous SSc and seldom if ever in patients with limited cutaneous SSc[2]. In the early 1980s the use of angiotensin-converting enzyme inhibitors dramatically decreased the incidence of SSc renal crisis, which until then significantly contributed to disease's mortality. Other patterns of renal involvement can be found in patients with SSc and in all of them vascular abnormalities are documented.

Apart from scleroderma renal crisis, the majority of patients show less severe renal complications, more likely associated with reduced renal blood flow and consequent reduction in glomerular filtration rate[3]. Colour-flow Doppler ultrasonography is employed to evaluate renal vascular damage in SSc patients[4]. Doppler evaluation after prostacyclin infusion shows improved renal blood flow with a reduction of resistance index[5].

N-acetylcysteine (NAC), a sulfhydryl substance with a powerful antioxidant and cellular detoxifying action, is a precursor of the major antioxidant glutathione. This medical compound has been proposed about 50 years ago as a mucolytic agent for chronic pulmonary diseases and it is now used for the treatment of fibrotic lung disease, since it restores pulmonary glutathione's depleted levels, thus improving lung function[6]. It is also used in the treatment of Raynaud's phenomenon (RP) and SSc because of its oxygen free radicals scavenging and vasodilative properties[7–9].

NAC is particularly useful in improving myocardial func-

tion following an acute myocardial infarction[10, 11]. The prominent features of NAC are the enhancement of the glutathione system, elimination of peroxides and maintenance of ascorbate and α-tocopherol in their reduced states[12]. In patients with hypertension the glutathione system may be an effective way to decrease arterial pressure and to protect the kidney from damage. NAC treatment enhances the renal glutathione system, ameliorates renal function, markedly decreases arterial pressure and renal injury in salt-sensitive hypertension, and improves endothelial dysfunction by preventing the reduction of flow-mediated dilatation in dialysis patients[13, 14].

This drug also has a relevant action on radiocontrast-induced nephropathy, which includes vasodilation, enhancement of renal medullary blood flow and antioxidant properties[15].

The aim of our study was to evaluate the renal haemodynamic effects of NAC in different stages of SSc.

Materials and methods

We studied 40 patients (3 males and 37 females; mean age 49±15 years) who fulfilled the American Rheumatism Association (ACR) criteria for SSc[16]. All SSc patients were subsequently divided into limited (lcSSc) and diffuse (dcSSc) cutaneous SSc groups according to LeRoy[1]. Table 1 lists epidemiological and clinical features of the study population.

The subjects' written consent was obtained according to the Declaration of Helsinki and the design of the work conforms to standards currently applied in the country of origin.

Table 1. Epidemiological and clinical features of the study population.

Sex (female/male)	37/3
Age (mean±SD)	49±15
Disease duration, mean±SD years	11.4±7.1
Raynaud's phenomenon duration, mean±SD years	14.6±8.2
Subset (*n*) dcSSc/lcSSc	21/19
ANA pattern *n* (%)	
Speckled	10 (25)
Speckled and nucleolar	3 (7.5)
Nucleolar	16 (40)
Centromere	11 (27.5)
SSc-specific autoantibody *n* (%)	
Anti–topo I	17 (42.5)
ACA	13 (32.5)
None	10 (25)
DSS	6.4±3.1
SDAS	1.9±1.3
E/A <1 *n* (%)	12 (30)

dcSSc=diffuse cutaneous SSc; lcSSc=limited cutaneous SSc; ANA=antinuclear antibody; anti–topo I=anti–topoisomerase I; ACA=anticentromere antibody; Disease Severity Scale (DSS) according to Medsger *et al* (20); Scleroderma Disease Activity Score (SDAS) according to Valentini *et al* (19); E/A= E/A ratio evaluated to conventional transthoracic Doppler echocardiographic examination.

Exclusion criteria were: previous intravenous NAC infusion, arterial hypertension, pulmonary arterial hypertension, diabetes, liver or renal failure, therapy with dual or selective endothelin inhibitors and treatment with anti-hypertensive agents within 3 months before enrollment.

Clinical assessment

Patients underwent clinical assessment and the organs involvement was exhaustively investigated in each patient. Currently, modified Rodnan Total Skin Score (mRTSS) is the most used method to asses skin induration in SSc. It is determined at a standardized location of 17 different sites of the body with a standardized pinching method and it is scored from 0 to 3[17]. Therefore mRTSS value significantly correlates with disease activity index in univariate analysis[18]. Valentini *et al* have proposed to modify the original activity index by changing the method used to assess the TSS and using a value of mRTSS >14. Disease activity in SSc was measured using Valentini's Scleroderma Disease Activity Score (SDAS), which consists of 10 weighted variables: total skin score >14, scleroderma, digital necrosis, arthritis, total lung capacity <80%, erythrocyte sedimentation rate (ESR) >30, hypocomplementemia and change in cardiopulmonary, skin and vascular symptoms in the past month[19].

Disease severity was measured using Medsger Scleroderma Disease Severity Scale (DSS). The original scale assessed disease severity in 9 organs or systems, namely general health, peripheral vascular, skin, joint/tendon, muscle and gastrointestinal tract, lungs, heart and kidneys. Each organ/system is scored separately from 0 to 4 depending on whether there is no, mild, moderate, severe or end-stage involvement[20]. Our patients were divided on the basis of vascular domain: history of Raynaud's scored 1 (mild), digital pitting scars scored 2 (moderate), digital ulcerations scored 3 (severe) and digital gangrene scored 4 (end-stage).

Pulmonary function was tested by vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO).

Renal evaluation

All patients were free of clinical symptoms of renal damage. Urinalysis, 24-h protein excretion, serum creatinine concentration and Glomerular Filtration Rate (GFR) were within normal limits.

GFR was calculated using both equation 7 developed in the Modification of Diet in Renal Disease (MDRD) study and Cockroft-Gault formula[21].

MDRD formula does not require urine collection, using demographic and serum variables: $GFR=170\times[\text{serum creatinine concentration (mg/dL)}]^{-0.999}\times(\text{age})^{-0.176}\times[\text{serum urea nitrogen concentration (mg/dL)}]^{-0.17}\times[\text{albumin concentration (g/dL)}]^{0.318}\times(0.762 \text{ if the patient is female})\times(1.18 \text{ if the patient is black})$[22].

Creatinine clearance was also calculated using the Cockcroft and Gault formula: creatinine clearance=(140–age in yr)×weight (kg)×0.85 (if female) divided by serum creatinine concentration (mg/dL).

Medical treatment

NAC was administered as an iv infusion over 5 consecutive hours, at a dose of 0.015 g·kg^{-1}·h^{-1} according to our previous studies[8, 9].

Color Doppler examination

All patients underwent renal artery sonography before and after the 5-h treatment with NAC. Patients were examined in a supine position and studied by the same operator using a Toshiba Aplio equipment and a convex 3.5 MHz probe. All scans were initially performed in a gray-scale and then using a color Doppler module. After renal artery insonation the size of sample volume was optimised in each patient on the basis of the vessel's diameter.

Renal artery measurements

Renal artery measurements were: diameter expressed in cm, cross-sectional area in cm^2, time-averaged mean velocity in cm/s and renal flow volume (RFV) in mL/min. Resistance index (RI) measures the intrarenal arterial elasticity and compliance rate. Determining the peak systolic velocity (V_{max}) and the minimal diastolic velocity (V_{min}) during intrarenal Doppler scan, RI is calculated as $[1-(V_{min} \div V_{max}) \times 100]$. Resistance index was calculated as the average of 2 to 3 measurements in proximal segmental arteries (the first vessels branching off the main renal artery).

Nailfold videocapillaroscopy (NVC)

NVC was performed by the same operator in each patient before NAC infusion using an optical probe videocapillaroscopy equipped with magnification 100×contact lens and connected to an image analysis software (Studio Version 8, Pinnacle System, Milan, Italy).

Each subject underwent acclimation for a minimum of 15 min before the nailfold was examined and the room temperature was 20–22 °C. The nailfold (distal row) of the second, third, fourth and fifth finger was examined in each patient. Microvascular alterations, as detected by NVC in patients with SSc, have been re-classified in three different patterns according to Cutolo *et al*. The patterns identified within the "SSc pattern" include Early NVC pattern: few enlarged/giant capil- laries, few capillary haemorrhages, relatively well-preserved capillary distribution and no evident loss of capillaries; Active NVC pattern: frequent giant capillaries, frequent capillary haemorrhages, moderate loss of capillaries, mild disorganiza- tion of the capillary architecture and absent or mild ramified capillaries; Late NVC pattern: irregular enlargement of the capillaries, few or absent giant capillaries and haemorrhages, severe loss of capillaries with extensive avascular areas, disor- ganization of the normal capillary array and ramified/bushy capillaries[23].

Statistical methods

The statistical significance of the differences between the data was tested using SPSS statistical software (version 15). The coefficient of skewness and coefficient of kurtosis are used to evaluate normal distribution of data. Paired two tailed Stu- dent's *t*-test was performed to compare differences in obser- vation pre and post-infusion. Non-paired Student's test was performed for other numerical variables. *P* value less than 0.05 was considered significant.

Results

All data are normally distributed. Correlation before *N*-ace- tylcysteine treatment between epidemiological and clinical features (RP duration, disease duration, DSS, DLCO) and variables used for division into groups (capillaroscopic pat- tern, mRTTS, vascular score of DSS) have been shown in Table 2. RP duration mean was significantly (*P*<0.005) lower in patients with an early/active capillaroscopic pattern and with mild-moderate score than in those with a late capillaroscopic pattern and with severe-end stage vascular score (Table 2). Disease duration mean was significantly (*P*<0.005) lower in patients with an early/active capillaroscopic pattern and mild- moderate vascular score than in those with a late capillaro- scopic pattern and severe-end stage vascular score (Table 2). Patients with an early/active capillaroscopic pattern and mild- moderate score showed a significantly lower global DSS score when compared to those with a late capillaroscopic pattern and severe-end stage score (Table 2). Mean DLCO did not show significant differences in two groups of patients with dif- ferent capillaroscopic pattern and mRTSS (Table 2). Besides,

Table 2. Correlation before *N*-acetylcysteine treatment between epidemiological and clinical features (Raynaud's duration, disease duration, Disease Severity Scale, diffusion capacity for carbon monoxide) and variables used for division into subgroups (capillaroscopic pattern, mRTTS, vascular score of disease severity scale).

	Patients (*n*)	Raynaud's duration	Disease duration	DSS score	DLCO
Early/Active capillaroscopic pattern	22	9.8±7.7[b]	6.9±7.4[b]	3.7±0.8[b]	69.5±20.6
Late capillaroscopic pattern	18	19.4±8.5[b]	15.9±6.8[b]	8.9±1.5[b]	63.7±19
mRTSS<14	21	14.9±8.5	11.5±7.5	6.2±3	66.7±19.3
mRTSS>14	19	14.3±7.8	11.3±6.8	6.6±3.3	66.5±19.5
Mild-moderate[§]	20	8.9±7.9[b]	7.4±6.6[b]	3.5±0.6[b]	70.6±12.9[b]
Severe-end stage[§]	20	20.3±8.3[b]	15.3±7.6[b]	9.1±1.7[b]	57.9±20[b]

DSS: Disease Severity Scale; DLCO: diffusion capacity for carbon monoxide; mRTSS: modified Rodnan total skin score; [§]vascular score of disease severity scale; [b]*P*<0.05.

mean DLCO (Table 2) was significantly (*P*<0.05) higher in patients with mild-moderate vascular score when compared to those patients with severe-end stage vascular score (70.6±12.9 *vs* 57.9±20). No significant differences (Table 2) are shown before NAC treatment in patients with mRTSS<14 when compared to those with mRTSS>14.

RFV mean values after a five-hour infusion with NAC did not decrease significantly in all patients. A non-significant association between RFV and capillaroscopic pattern, mRTSS, vascular domain of DSS and DLCO was found. Therefore RFV increased not significantly in patients with early/active capillaroscopic pattern, mRTSS<14, mild-moderate score to vascular domain of DSS. Conversely RFV decreased not significantly in patients with late capillaroscopic pattern, mRTSS>14 and severe-end stage score (Table 3).

RI is significantly (*P*=0.002) higher before NAC treatment in patients with mild-moderate score to vascular domain of DSS when compared to patients with severe-end stage score (0.734±0.074 *vs* 0.667±0.047). In all 40 patients RI mean values after a five-hour infusion with NAC did not decrease significantly (Table 3). Nevertheless, mean RI was significantly (*P*<0.01) reduced (0.723±0.0730 versus 0.678±0.0695) in patients with an early/active capillaroscopic pattern when compared to patients with a late pattern, in whom it was found a statistically significant increase of mean RI (Table 4).

RI is significantly (*P*=0.03) higher before NAC treatment in patients with an early/active capillaroscopic pattern when compared to patients with a late pattern (0.723±0.073 *vs* 0.677±0.059). After NAC treatment no RI significant differences are present in patients with an early/active capillaroscopic pattern or mild-moderate score to vascular domain of DSS or with mRTSS<14 when compared to those with a late capillaroscopic pattern or severe-end stage score to vascular

domain of DSS or mRTSS>14.

After NAC treatment mean RI was significantly (*P*<0.005) reduced (0.713±0.079 *vs* 0.678±0.072) in patients with mRTSS<14 when compared to those with mRTSS>14, whose mean RI was significantly increased (Table 4). Besides, patients with an mRTSS<14 showed a significantly (*P*<0.001) lower SDAS when compared to those with mRTSS>14 (0.78±0.49 *vs* 3.13±0.48).

Mean RI was significantly decreased (*P*<<0.0001) in patients with mild-moderate score to vascular domain of DSS (0.734±0.074 *vs* 0.676±0.069), while it increased significantly in patients with severe-end stage score (Table 3).

DLCO mean (Figure 1) was significantly (*P*<0.005) lower in patients with RI increase than in those with reduction of RI (76.8±17.6 *vs* 60.6±18.1).
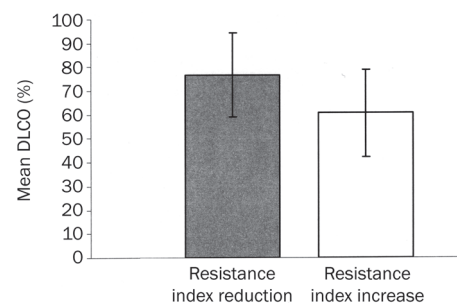

Figure 1. DLCO (mean±SD) in two groups of patients with reduction and increase of resistance index after NAC infusion.

In all patients we did not find any correlation between haemodynamic parameters (RFV, RI) and SSc subset or E/A ratio evaluated to conventional transthoracic doppler echocardiographic examination. No change of blood pressure during NAC infusion was observed.

Discussion

NAC is a sulfhydryl substance with a powerful antioxidant, precursor of glutathione and donor of nitric oxide. Free radicals scavenger activity and support of gluthatione synthesis are probably two principles of its anti-oxidant function.

In SSc patients NAC treatment ameliorates the ROS-induced damage and reduces peroxynitrite production by lung mac-

Table 3. Renal flow volume (mean±SD) and resistance index (mean±SD) before and after *N*-acetylcysteine treatment.

	Patients (*n*)	Pre-NAC	Post-NAC	*P*-value
RFV (L/min)	40	0.34±0.15	0.33±0.15	0.71
RI	40	0.699±0.0697	0.693±0.0650	0.65

RFV: renal flow volume; RI: resistance index

Table 4. Mean Resistance Index (mean±SD) before and after *N*-acetylcysteine treatment related to capillaroscopic pattern, mRTTS, and vascular score of disease severity scale.

	Patients (*n*)	pre-NAC RI	post-NAC RI	*P*-value
Early/Active capillaroscopic pattern	22	0.723±0.073	0.678±0.069	<0.0001
Late capillaroscopic pattern	18	0.677±0.059	0.707±0.058	0.008
mRTSS<14	21	0.713±0.079	0.678±0.072	0.002
mRTSS>14	19	0.679±0.056	0.706±0.053	0.04
Mild-moderate[§]	20	0.734±0.074	0.676±0.069	<0.0001
Severe-end stage[§]	20	0.667±0.047	0.709±0.058	<0.0001

RI: resistance index; mRTSS: modified Rodnan total skin score; [§]vascular score of disease severity scale.

rophages[9, 24]. Threrefore, up to the present there are no clinical studies that investigate the effects of NAC infusion on renal blood flow in patients with SSc or primary RP. Scorza *et al* have shown that prostacyclin infusion ameliorates renal blood flow and reduces the RI in SSc patients[5].

In our study we have evaluated renal haemodynamic effects before and after 5 h from a single NAC infusion with color Doppler examination. NAC infusion significantly reduced RI in a group of patients with early/active capillaroscopic pattern, mRTSS<14 and mild-moderate score to vascular domain of DSS. Conversely, RI was increased after NAC infusion in patients with late capillaroscopic pattern, mTRSS>14 and severe-end stage score to vascular domain of DSS. In addition, in patients with reduction of RI after NAC infusion, mean value of DLCO was significantly higher than in patients with an increase of RI.

RI measures the intrarenal arterial elasticity and compliance rate. It deteriorates more in vascular and interstitial nephropathies than in glomerular diseases, because glomeruli are only responsible for 10% of intraparenchimal flow resistances, whose control is mainly under the action of efferent and afferent arteries[25, 26]. The increase of total peripheral resistance of the kidney, determined by release of vasoconstrictive substances and remodeling of the microcirculation in course of vascular and interstitial nephropathies could explain the higher RI found in these diseases[27].

In patients with Lupus nephritis, RI may be considered a significant predictor of poor renal outcome, correlating with a chronicity index on the basis of sclerosis, atrophy and fibrosis of Bowman capsule, glomeruli and the tubulointerstitium. When a parenchimal or interstitial damage occurs the renal vascular bed is reduced because of lacking in arteriolar elasticity and compliance with increased RI[28]. Although the pathogenesis of scleroderma renal crisis is unknown, endothelial cell injury, leading to narrowing and obliteration of the lumen of the vessels, is the primary pathogenetic mechanism. Vascular damage with arteries wall thickening is present in SSc patients without scleroderma renal crisis[29]. Renal vascular resistance was increased in main, interlobar and cortical vessels of SSc patients with normal renal function when compared with normal controls[30]. RI and pulsatility index are increased in SSc patients with signs of renal involvement when compared to those without renal manifestations and healthy controls. Doppler indices of renal vascular resistance were closely related to disease duration, age and plasma renin activity[31]. In SSc patients elevated pulsatility index correlates with digital pitting scar, contracture of the phalanges, pulmonary fibrosis, DLCO's percentage and heart involvement[4].

RI was significantly improved by angiotensin II receptor blockers (ARBs). The reduction of RI after ARBs treatment was most evident in patients with hypertensive nephrosclerosis and/or with microalbuminuria or proteinuria[32].

NAC improves renal vasoconstriction induced by radiocontrast, angiotensin II, indomethacin and N^ω-nitro-*L*-arginine-methyl ester (*L*-NAME) in rats. NAC-induced renal vasodilation is greater after the most intense vasoconstrictive maneuvers[33].

NAC-related RI reduction may be mediated in part by vasodilation of the small and medium arteries not involved in the thickening process. RI may increase because of the involvement of endothelial cells, which release vasoconstrictive and vasodilator factors, such as angiotensin II, endothelin and NO, as a physiological answer to the friction of hematic flow on vascular wall (shear stress)[34, 35]. During endothelial dysfunction reduced responsivity to vasodilator impulses in small and medium size arteries is mediated by NO and acetylcholine, leading to the development of intimal hyperplasia, vascular lumen reduction, increase of vascular contractility and, in the end, ischemic and glomerular sclerosis, resulting in a RI increase. Similar vascular effects can be experimentally reproduced in rats by giving them NO inhibitors, such as *L*-NAME[36].

Rajkumar *et al* reported a link between vascular damage (an essential requirement in any scheme of SSc pathogenesis) and the formation of myofibroblasts from pericytes. As primitive mesenchymal cells, pericytes can give origin to several tissues, including cartilage and bone. Thus, both tissue fibrosis and ectopic calcification could be attributed to pericytes[37].

In SSc patients NAC induced renal arterial vasodilation only in early stage of disease when arteries are not involved by fibrosis. Vasodilative effects of NAC on renal artery are not present in late stage of SSc when arterial fibrosis, intimal hyperplasia, vascular lumen reduction are evident. For this reason NAC treatment should be started in early stage of disease, in association with other vasodilator drugs, because it may prevents progression of arterial renal fibrosis. The NAC as a vasodilator agent is effective only if introduced early, while the use in the late stage of the disease is restricted to its antioxidant effect. In late stage of disease NAC treatment for its negative effects in RI could be used only in combination with classic vasodilator drugs (prostacyclin analogues and ACE inhibitors). The indices of renal function (urinalysis, 24-h protein excretion, serum creatinine concentration and GFR) should be closely monitored if NAC therapy is started or continued in late stage of disease.

In conclusion, NAC is able to reduce RI after a single infusion in SSc patients with early/active capillaroscopic pattern, mRTSS<14 and mild-moderate score to vascular domain of DSS.

Author contribution

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