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Original article

Oral N-acetylcysteine in the treatment of Raynaud's phenomenon secondary to systemic sclerosis: A randomized, double-blind, placebo-controlled clinical trial

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ABSTRACT

Objective: To evaluate the safety and efficacy of oral N-acetylcysteine (NAC) on digital microcirculation blood flow in patients with Raynaud's phenomenon (RP) secondary to systemic sclerosis (SSc).

Methods: This was a randomized, double-blind, placebo-controlled trial in which 42 patients with SSc received oral NAC at a dose of 600 mg tid (21 patients, mean age 45.6 ± 9.5 years) or placebo (21 patients, mean age 45.0 ± 12.7 years) for four weeks. The primary endpoint was the change in cutaneous microcirculation blood flow before and after cold stimulation measured by laser Doppler imaging (LDI) at weeks 0 and 4. The frequency and severity of RP and the number of digital ulcers were also measured at weeks 0 and 4. The adverse events were recorded in the fourth week.

Results: There was no significant change in digital blood flow assessed by LDI before or after cold stimulus after four weeks of NAC or placebo. Both groups showed significant improvement in the frequency and severity of RP attacks, with no difference between the two groups. At the end of the study, the placebo group had three digital ulcers, while the NAC group showed no ulcers. NAC was well tolerated and no patient discontinued the treatment.

Conclusions: NAC orally at a dose of 1800 mg/day showed no vasodilator effect on hands' microcirculation after four weeks of treatment in patients with RP secondary to SSc.

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N-acetilcisteína oral no tratamento do fenômeno de Raynaud secundário à esclerose sistêmica: Ensaio clínico randomizado, placebo-controlado e duplo-cego

Palavras-chave:
Esclerose sistêmica
Fenômeno de Raynaud
Estresse oxidativo
N-acetilcisteína
Tratamento

RESUMO

Objetivo: Avaliar a segurança e a eficácia da *N*-acetilcisteína (NAC) por via oral sobre o fluxo sanguíneo da microcirculação digital em pacientes com fenômeno de Raynaud (FRy) secundário à esclerose sistêmica (ES).

Métodos: Este foi um estudo randomizado, duplo-cego e placebo-controlado, no qual 42 pacientes com ES receberam NAC oral na dose de 600 mg, três vezes ao dia (21 pacientes, idade média 45,6 ± 9,5 anos) ou placebo (21 pacientes, idade média 45,0 ± 12,7 anos) durante quatro semanas. O desfecho primário do estudo foi: melhora no fluxo sanguíneo da microcirculação cutânea antes e após estímulo frio avaliado pelo *laser Doppler imaging* (LDI) nas semanas 0 e 4. A frequência e a gravidade do FRy e o número de úlceras digitais também foram avaliados nas semanas 0 e 4. Os efeitos adversos foram registrados na quarta semana.

Resultados: Não houve mudança significativa no fluxo sanguíneo digital avaliado pelo LDI antes ou depois do estímulo frio após quatro semanas de NAC ou placebo. Ambos os grupos apresentaram melhora significativa na frequência e gravidade dos ataques de FRy, sem diferença entre os dois. O grupo placebo apresentou três úlceras digitais enquanto o grupo NAC não apresentou úlceras ao final do estudo. NAC foi bem tolerada e nenhum paciente descontinuou o tratamento.

Conclusões: NAC por via oral na dose de 1.800 mg/dia não demonstrou efeito vasodilatador sobre a microcirculação das mãos após quatro semanas de tratamento em pacientes com FRy secundário à ES.

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by microvascular damage and fibrosis of skin and internal organs. Raynaud's phenomenon (RP) is one of the most common and earliest manifestations of SSc. It is characterized clinically by reversible episodes of vasospasm, usually limited to the hands and/or feet, and triggered by exposure to cold or emotional stress. In patients with RP secondary to SSc, not only functional abnormalities but also structural changes are present in the microcirculation, making the vasospastic events more severe and possibly leading to complications such as ulceration or tissue necrosis.[1]

The pharmacological treatment of the peripheral vascular disease secondary to SSc includes the use of vasodilators such as calcium channel blockers, nitrates and prostanoids, and vasoconstriction inhibitors such as endothelin receptor antagonists and α-adrenergic blockers. These agents reduce the frequency and severity of RP in patients with SSc.[2–5] However, they are not always completely effective and new therapeutic options are desirable.

Oxidative stress mediated by an increased activity of free radicals has been implicated in the pathogenesis and progression of SSc.[6,7] Repeated episodes of ischemia and reperfusion observed in these patients cause activation of endothelial cells, an imbalance in the relation between vasoconstrictor and vasodilator substances and an increase in reactive oxygen species and other toxic products. This cascade of events contributes significantly to the vascular damage associated with the disease and can also activate fibroblasts and immune cells.[6,8]

N-Acetylcysteine (NAC) is a thiol-containing compound (containing sulfhydryl) with a powerful antioxidant action. As a source of sulfhydryl groups in cells, NAC directly fights against free radicals through its interaction with the hydroxyl radical and hydrogen peroxide.[9] NAC also acts indirectly by inducing the synthesis of glutathione, whose main function is the removal of free radicals and the defense against oxidative stress.[9–11] Due to these properties, NAC has been used not only as a mucolytic agent in a variety of respiratory diseases, but also in other conditions characterized by a reduced level of glutathione and by oxidative stress. NAC was shown to improve the microcirculation blood flow in smokers and promote coronary vasodilation, besides increasing the endothelium-dependent peripheral dilation in patients undergoing cardiac catheterization and improving the endothelial function in dialysis patients.[10,12–14]

In patients with SSc, some open studies on high-dose intravenous (IV) NAC showed a significant improvement in blood perfusion and reduction in the frequency and severity of RP and in the number of active digital ulcers after its administration.[15–17] However, the IV route in continuous infusion and the high cost of this treatment considerably restrict its use. Only one clinical trial evaluated oral NAC in patients with SSc, but the vascular involvement has not been evaluated.[18]

The present study aimed to evaluate the safety and efficacy of oral *N*-acetylcysteine on digital cutaneous microcirculation blood flow monitoring by laser Doppler imaging, and on clinical symptoms of RP in patients presenting RP secondary to SSc.

Materials and methods

Patients

Forty-two patients diagnosed according to the American College of Rheumatology classification criteria for SSc (1980),[19] or the LeRoy and Medger criteria for early SSc (2001) were selected.[20] Our patients were consecutively recruited from the Systemic Sclerosis Outpatient Clinic, Hospital São Paulo (UNIFESP). Inclusion criteria were age ≥18 years, at least six RP attacks per week, nail fold capillaroscopy with SD (scleroderma) pattern, disease duration ≤4 years from the first typical sign and symptom of SSc, excluding RP. Exclusion criteria were presence of active digital ulceration, current smoking, occupational exposure to cold environments and vibrating agents, uncontrolled hypertension or diabetes mellitus, and clinical evidence of proximal peripheral arterial disease. Three days before their inclusion in the study, patients discontinued oral vasodilators. Other medications remained unchanged throughout the study. All subjects completed a written informed consent obtained in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of UNIFESP, and was registered in the Australian New Zealand Clinical Trials Registry (ACTRN12610000114044).

Study design

This was a randomized, double-blind, placebo-controlled study conducted in 2009. To minimize the temperature variation, the inclusion of patients was interrupted during summer (January–March and December). Patients were randomized to receive 600 mg *N*-acetylcysteine or placebo in an identical capsule three times a day for four weeks. Randomization and blinding were performed by a staff member who was not participating in the study. Patients were evaluated at three time points: one screening visit (one or two weeks before randomization), at week 0 (randomization) and after four weeks of treatment.

Outcomes

The primary outcome was the change in digital cutaneous microcirculation blood flow using laser Doppler imaging (LDI) at time points 0 and after four weeks of NAC or placebo use. Secondary outcomes were the number of digital ulcers in the final evaluation and changes in the frequency and severity of RP attacks at 0 time point compared to week 4. Adverse events were recorded in the fourth week.

Evaluation of digital cutaneous microcirculation blood flow by laser Doppler imaging before and after cold stimulus

After acclimatization for 60 min in a laboratory under constant temperature of 24 ± 1 °C, the blood flow of the dorsum of the distal phalanx of the four fingers of the left hand (excluding the thumb) was evaluated with the use of a laser Doppler imaging unit (Moor LDI-VR, Moor Instruments, Axminster, UK) before and after cold stimulus (CS) exposure. All subjects were seated with the left arm placed on a flat surface at heart level. This device uses a low-power (2.0 mW) helium–neon red laser emission system operating at a wavelength of 633 nm with approximately 1 mm of penetration into the skin surface. The laser beam is directed to a selected area of skin by means of a mirror system located at a distance of 40 cm from the skin surface at an angle of 45°. All images were taken with a resolution of 256 × 256 pixels and speed of 4 pixels/millisecond with an acquisition time of 3 min and 15 s for each image (area of 10.4 cm × 16.2 cm). The blood flow of the dorsum of the four fingers was determined by establishing four regions of interest (ROI) on each finger, defined as an area encompassing the proximal interphalangeal joint and including the nail bed. The blood flow in the selected area was determined with the aid of the software MoorLDI V5.2 and expressed in arbitrary perfusion units (PU) in relation to an internal calibration standard device which is directly proportional to the product of the mean velocity by the concentration of blood cells. Mean fingertip blood flow (FBF) of four digits was considered for analysis. After measuring baseline FBF, patients underwent a cold stimulus (CS) (submersion of both hands in water at 15 °C for 1 min) (UNITEMP 116, Fanem, Brazil). Then, after CS the FBF was monitored for 30 min, including the time points: 1 min (T1), 4 min and 15 s (T4) 10 min and 45 s (T10), 17 min and 15 s (T17), 20 min and 30 s (T20) and 27 min (T27) after CS.

Clinical evaluation

The frequency and severity of RP attacks were recorded daily on a standard log book completed by the patient during the week previous to week 0 and during week 4. Patients were instructed to immediately record the event and the attack severity (in a scale with 0–10 range) whenever an RP attack occurred. The number of digital ulcers was recorded at baseline (week 0) and at the end of week 4. Adverse events were also recorded in week 4.

Statistical analysis

All results were expressed as mean ± standard deviation (SD). For comparison between placebo and NAC groups, the paired Student *t* test, Mann–Whitney test or chi-squared test were used. To compare the values of LDI and of severity and frequency of RP over time, ANOVA with repeated measures was used. For all analyses, the significance value <0.05 was considered. The statistical analysis was performed using SPSS statistical software (version 15.0 for Windows, SPSS Inc., Chicago, IL).

Results

Demographic data

Of the 42 patients with SSc included, 21 received oral NAC (mean age 45.6 ± 9.5 years) and 21 received placebo (mean age 45.0 ± 12.7 years) (Table 1). Seven patients (16.6%) had an early form of SSc according to LeRoy and Medsger criteria,[20] 14 (33.3%) limited cutaneous disease, and 21 (50%) diffuse cutaneous disease. According to Table 1, there were no differences in demographic and clinical characteristics between NAC and placebo groups. Before the start of the study, 28 patients were taking calcium channel blockers (nifedipine or amlodipine), eight were taking ACE inhibitors, and five were taking losartan. There was no significant difference in age, gender, RP duration and disease duration among patients with diffuse cutaneous form, limited cutaneous form, and those with early SSc (data not shown).

Digital cutaneous microcirculation blood flow and clinical outcomes

There was no significant difference between NAC and placebo groups in FBF values before (FBF 318.5 ± 204.5 vs. 224.5 ± 180.1 PU, respectively, $P = 0.122$) and after ($P = 0.432$ for T1, $P = 0.164$ for T4, $P = 0.269$ for T10, $P = 0.616$ for T17, $P = 0.344$ for T20, $P = 0.150$ for T27) cold stimulus on the baseline assessment. There was no significant difference in FBF values measured before and in each time point after CS when comparing the values of week 0 and week 4 in NAC group (Fig. 1A). In placebo group, there was no significant difference in FBF before and in each time point after CS when weeks 0 and 4 were compared (Fig. 1B). When patients with diffuse cutaneous, limited cutaneous and early SSc forms were evaluated in separate groups, also there was no significant difference in FBF values after the treatment with NAC or placebo (data not shown).

Both groups showed significant improvement in the frequency and severity of RP attacks after four weeks of treatment (Table 2), with no significant difference between the two groups. The mean reduction in the frequency of RP attacks per week was 5.9 in placebo group and 5.1 in NAC group ($P = 0.865$). The mean reduction in severity of RP attacks was 1.7 in placebo group and 3.0 in NAC group ($P = 0.074$). In the same line, there was no significant difference in the frequency of RP attacks ($P = 0.428$) and RP severity ($P = 0.716$) between NAC and placebo groups at baseline. Placebo group presented three new digital ulcers after four weeks of treatment, while NAC group showed no new ulcerations (Table 2).

Oral NAC was generally well tolerated, two patients had epigastric pain and a third exhibited an increase in menstrual flow. At the end of four weeks, no patient had discontinued the treatment. There were no adverse events in placebo group.

Discussion

This was the first double-blind placebo-controlled study to evaluate the efficacy of oral *N*-acetylcysteine for the treatment of RP secondary to SSc. Laser Doppler imaging, a method that allows an objective measure of the microvascular blood flow, was used to assess the response to treatment.[12] After four weeks, there was no significant change in fingertips blood flow before or after cold stimulus, both in NAC group and in placebo group. Interestingly, both groups showed significant improvement in RP attacks' frequency and in severity of RP, confirming the contribution of the placebo effect in clinical trials on patients with RP.[22,23] NAC was safe and well tolerated by almost all patients.

Several alternatives are suggested for the treatment of RP secondary to SSc.[4,5,23,24] Conventional vasodilator drugs

Table 1 – Clinical and demographic characteristics of patients with SSc.

	N-acetyilcysteine Group ($n = 21$)	Placebo Group ($n = 21$)	*P*
Age (years)	45.6 ± 9.5	45.0 ± 12.7	0.863
Gender M/F	21/0	20/1	1.000
Classification			0.350
Diffuse	9	12	
Limited	8	6	
Early	4	3	
Duration of RP (years)	5.5 ± 1.9	4.9 ± 1.6	0.275
Disease duration (years)	2.5 ± 1.2	2.8 ± 1.3	0.442
ANA	17	14	0.392
Microscars, resorption or digital amputation	8	6	0.578
Treatment of RP before inclusion			
Calcium channel blocker	16	12	–
ACE inhibitor	3	5	–
Losartan	2	3	–
Sildenafil	1	0	–
Pentoxifylline	2	2	–

RP, Raynaud phenomenon; ANA, positive antinuclear antibodies by indirect immunofluorescence on HEp-2 cells; ACE, angiotensin converting enzyme.

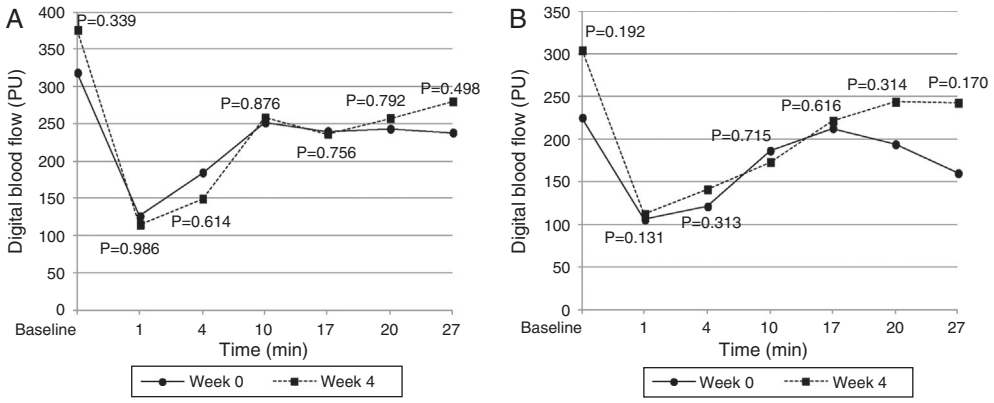

Fig. 1 – Mean fingertips blood flow in four phalanges (FBF) measured at baseline (week 0) and after four weeks of treatment with *N*-acetylcysteine (A) or placebo (B) before and at different time points after cold stimulus. P values correspond to a comparison between week 0 and week 4.

such as calcium channel blockers, α-adrenergic inhibitors, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, phosphodiesterase inhibitors, and nitrates (usually topical) are currently used for the treatment of Raynaud's phenomenon with heterogeneous results.[23–25] More recently, prostaglandin analogs and endothelin receptor antagonists (bosentan) showed promising results in the treatment and prevention of ischemic ulcers in SSc.[2,26] However, the IV route and the more expensive costs limit the prescription of these drugs for those patients with more severe disease.

Despite considerable evidence that oxidative stress is involved in the pathogenesis of SSc, few studies have evaluated the effects of oral antioxidants in the treatment of RP secondary to SSc. Probucol, a powerful antioxidant, has led to a reduction in the frequency and severity of RP attacks in a study of 40 patients with primary and secondary RP.[27] In contrast, another study showed no benefit after 10 weeks of treatment with a combination of antioxidants, micronutrients and allopurinol, or after three weeks of vitamin E, in patients with SSc.[28]

In conditions characterized by oxidative stress such as smoking and heart disease and in clinical situations in which glutathione levels are decreased, NAC appears to be effective as a complementary therapy.[9] In addition to its antioxidant properties, oral NAC also appears to have vasodilator action on the microvasculature, which could result in a beneficial effect on vascular changes and on episodes of vasospasm in patients with SSc. On the other hand, when added to standard therapy, NAC was able to preserve the vital capacity and DLco in patients with idiopathic pulmonary fibrosis.[29]

In SSc patients, high-dose intravenous NAC has been suggested as a valuable and effective treatment for RP. The efficacy and tolerability of a five-day continuous infusion of NAC in high doses was evaluated in an open label study of 22 patients with SSc.[15] There was a significant decrease in the frequency and severity of RP attacks and in the number of active ulcers after treatment. An improved digital perfusion, evaluated by plethysmography after treatment, also occurred in most patients.

More recently, two other studies have evaluated the efficacy of long-term treatment with IV NAC in patients with SSc, with positive results.[16,17] In our hospital, we also conducted a pilot study with high-dose IV NAC in three patients with SSc and active ischemic ulcers of the extremities.[30] Over the course of two months, all patients showed a decrease in diameter of at least one ulcer, and two of them showed complete healing of an ulcer. The present study evaluated the efficacy of oral NAC, instead of IV NAC. It is known that the intact NAC molecule has a low oral bioavailability. Following an oral dose, the majority of the NAC molecule is metabolized to other compounds, such as cysteine and inorganic sulfite, and the greater part of its activity and protective effects is credited to these metabolites.[9,31] However, we cannot exclude that the lower bioavailability of oral NAC might have influenced our results.

Our study has some limitations, such as its short duration and small sample size. The dose of NAC used may also have contributed to the observed lack of improvement in digital blood flow. The dose of 1.8 g per day was the same used in a clinical trial of oral NAC for the treatment of idiopathic pulmonary fibrosis.[29] The usual dosage of NAC as a mucolytic and

Table 2 – Number and severity of Raynaud's phenomenon attacks and number of digital ulcers before and after four weeks of treatment with *N*-acetylcysteine or placebo.

	Placebo Group			*N*-acetyilcysteine Group		
	Week 0	Week 4	P	Week 0	Week 4	P
Number of RP attacks/week	15.9 ± 12.7	10 ± 8.4 (−37%)	<0.000	12.3 ± 13.8	7.2 ± 4.5 (−41%)	<0.000
RP severity	8.5 ± 2.0	6.8 ± 2.1 (−20%)	<0.001	8.7 ± 1.1	5.7 ± 2.6 (−35%)	<0.000
Number of digital ulcers	0	3	–	0	0	–

antioxidant agent in chronic obstructive pulmonary disease is much lower (generally 600 mg per day). However, in other circumstances, such as acetaminophen toxicity, the NAC dosage is much higher.[32]

Our study was conducted before the publication of the new ACR/EULAR 2013 classification criteria for SSc.[33] We chose to include patients with a diagnosis of early SSc according to LeRoy and Medsger criteria, aiming to include patients with recent disease in whom a possible effect of NAC might be more important. Of the seven patients with early SSc, only three would meet the new ACR/EULAR 2013 criteria.

An important finding of this study was the improvement of clinical outcomes (number of attacks and RP severity) in both groups after treatment with NAC or placebo. This fact confirms the contribution of placebo effect in patients who are participants of clinical trials, which has been shown in previous studies in patients with RP.[3,34–36] In this context, our results reinforce the need to use more sensitive objective methods, such as LDI, for the evaluation of RP and of microcirculatory blood flow in therapeutic trials. In a previous study conducted by our group, the LDI technique proved to be a sensitive and objective method for the evaluation of digital blood flow in patients with RP secondary to SSc.[21] LDI has the advantage of being a noninvasive method that allows the measurement of blood perfusion over a wide area of skin surface, thereby providing more reproducible results.[21,37]

The development of new digital ulcers is an important outcome in clinical trials on RP secondary to SSc. In the present study, three patients in placebo group developed new digital ulcers, while new ulcers were not observed in NAC group. Despite this favorable observation, the short duration of the study and the small number of observed ulcers do not allow definitive conclusions about the ability of NAC to prevent the development of ulcers. Multicentric studies with a long-term follow-up are needed to better assess this issue.

In summary, the oral NAC dose of 1800 mg/day did not lead to a significant improvement in digital cutaneous microcirculation blood flow in this short-term study in patients with RP secondary to SSc. Additional studies with a longer duration of treatment and higher doses of NAC are needed to assess whether oral NAC may promote some benefit as an antioxidant therapy in the vascular involvement of SSc.

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Conflicts of interest

The authors declare no conflicts of interest.

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