Intravenous N-Acetylcysteine for Treatment of Raynaud's Phenomenon Secondary to Systemic Sclerosis: A Pilot Study

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ABSTRACT. ***Objective.*** To assess the efficacy and tolerability of N-acetylcysteine (NAC) in patients with Raynaud's phenomenon (RP) secondary to systemic sclerosis (scleroderma; SSc).

Methods. Twenty-two patients with RP secondary to SSc were enrolled in a multicenter, open clinical trial lasting 11 weeks and conducted in winter. Primary outcome measures were frequency and severity of RP attacks, and number of digital ulcers. Secondary outcome measure was improvement in digital cold challenge test assessed by photoelectric plethysmography. Patients received a continuous 5 day intravenous infusion of NAC starting with a 2 h loading dose of 150 mg/kg subsequently adjusted to 15 mg/kg/h.

Results. All 22 patients completed the 5 day infusion and 20 of them the posttreatment followup. Both frequency and severity of RP attacks decreased significantly compared to pretreatment values. Active ulcers were significantly less numerous at all followup visits (25.18% of baseline count on Day 33 from the beginning of infusion). In the cold challenge test mean recovery time fell by 69.56%, 67.70%, 71.42%, and 71.05% on Days 12, 19, 33, and 61 from the beginning of treatment. Side effects were minor, easily controlled, and reversible.

Conclusion. N-acetylcysteine appears to be safe for the treatment of RP secondary to SSc. These preliminary data warrant further controlled studies. (J Rheumatol 2001;28:2257–62)

Key Indexing Terms:
SCLERODERMA
RAYNAUD'S PHENOMENON
OXIDATIVE STRESS
N-ACETYLCYSTEINE

Raynaud's phenomenon (RP) affects over 90% of patients with systemic sclerosis (scleroderma, SSc) and represents the first symptom in most cases[1]. Although nothing more than a nuisance to some patients with SSc, recurrent and severe episodes of RP may involve disabling pain, digital ulceration, and loss of the digital tips.

Drug treatment of RP in SSc patients is symptomatic and centered on oral, intravenous, or transdermal administration of vasodilators[2,3]. However, currently available agents are effective only in a minor proportion of scleroderma patients[4-6] probably because of their inability to interfere with the pathogenic mechanism(s).

Poor understanding of the mechanisms underlying the exaggerated RP vasospasm has prevented development of more effective drugs. Although vessel wall abnormalities — well documented by widefield microscopy of the nailfold capillary bed[7] and histologic studies of skin biopsies[8,9] — are very likely implicated in the abnormal vasoconstriction and vasodilatation observed in RP[10-12], the factors responsible for the diffuse vascular lesions of scleroderma remain unclear. Likely candidates are immune and nonimmune mechanisms; among the latter, the main mediators of endothelial cell injury are free radicals, since microvascular endothelial cells have low capacity to synthesize the enzyme catalase, a free radical scavenger, and are thus particularly prone to reactive oxygen species (ROS) mediated injury[13].

With several recent studies reporting increased free radical activity in patients with SSc[14-17], it has been postulated that this abnormality might facilitate the vascular and nonvascular lesions of scleroderma. The corollary to this hypothesis is that treatment with a powerful antioxidant such as N-acetylcysteine (NAC) should ameliorate ROS induced damage. Since pharmacokinetic studies suggest that NAC can act *in vivo* as an effective antioxidant agent only when administered intra-

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Submitted October 18, 2000; revision accepted April 2, 2001.

venously at high dose[18], we assessed the safety and clinical effects of a continuous 5 day NAC infusion in patients with RP secondary to SSc.

MATERIALS AND METHODS

Patients. Patients with RP secondary to diffuse or limited SSc were recruited from outpatient clinics at 5 Italian university hospitals. Diagnosis of SSc was made according to the criteria of the American College of Rheumatology[19]. RP was defined as intermittent attacks of blanching and cyanosis of the digits upon exposure to cold or emotion. Patient inclusion criteria were: a minimum of 7 RP attacks per week — documented by the patients themselves in a diary in the 2 weeks preceding the study (washout period) — and/or one or more cutaneous ischemic lesions on fingers; age between 18 and 75 yrs; and negative pregnancy test for women of childbearing age. Patients were not informed of the attack rate requirement to participate in the study. Only patients who documented the required number of attacks in their diary at the end of the washout period were enrolled in the study. All patients gave written informed consent to participate in the study and the experimental protocol was approved by the ethical committees of the 5 centers. Exclusion criteria were: pregnancy; a history of bronchial asthma; hypertension treated with calcium channel blockers or angiotensin converting enzyme (ACE) inhibitors and not controlled with other drugs; or upper limb sympathectomy performed within the 12 mo preceding the study.

Administration of iloprost (carboprostacyclin) and alprostadil (prostaglandin E1) was not allowed in the 2 mo preceding the study and all other medications, except omeprazol, were discontinued starting 14 days before infusion. Patients were advised not to change their usual lifestyle. Protection from cold was encouraged, but no patient used electrically heated gloves or socks or chemical hand warmers.

Study design. This open multicenter study was conducted over a single winter period in 5 Italian university hospitals. It consisted of 3 phases: a washout period of 14 days during which all medications but omeprazol were suspended; a continuous 5 day treatment period (Day 0 to Day 5); and a followup period with visits scheduled on Days 5, 12, 19, 33, and 61.

Study medication and administration. N-acetylcysteine was supplied by Zambon Italia Srl (Bresso, Italy) in ampoules containing 5 g of the active drug. The drug was administered through a peripheral vein with the aid of a peristaltic pump, starting with a loading dose of 150 mg/kg diluted in 250 ml saline. After 2 h, the infusion rate was adjusted to 15 mg/kg/h for all 5 days. The daily dose was diluted in 1000 ml of saline solution. The infusions were done in hospital and patients were required to stay in the hospital for all 5 days.

Assessment of efficacy. Subjective assessment. A RP attack was defined as an episode of pallor followed by cyanosis on one or more digits precipitated by cold or emotion. Diaries were given to patients, who were instructed to record each attack at the time of the attack. Patients were also asked to score attack severity at the time of the attack on a 10 point scale, from 0: absence of attack, to 10: the most severe attack in terms of pain and effect on daily activities. Patients were not given a reference point and were not allowed to review previous scoring.

The mean weekly number of RP attacks was the arithmetic mean of the values obtained in each single patient, dividing the total number of attacks during a given period by the number of diary days and multiplying the result by 7. For a given period, the daily RP severity score was calculated as the arithmetic mean of the individual daily scores during that period. Each daily severity score was the mean of the severity of each single RP attack.

Daily measures were defined as available if records of at least 10 days from a 2 week period were observed.

Objective assessment. Digital ulcers, identified as a marginated lesion that resulted from destruction of the epidermis and dermis, were considered to be of ischemic origin, and thus were counted and their location was recorded. Fissures or paronychiae were considered to be less certainly secondary to disease in the small arteries and arterioles of the skin and were not recorded.

No patient underwent surgical debridement of infected ulcers. Healing referred to each ulcer and was defined as complete resolution of a finger lesion present at baseline. Digital ischemic ulcers were counted on Day 0 (baseline); the number of ulcers and appearance of new lesions were recorded on Days 5, 12, 19, 33, and 61.

Cold challenge test. On Day 0, baseline plethysmography was performed on all fingers before the infusion. After cold challenge, only one finger was tested. The same finger was evaluated at each followup visit.

Photoelectric plethysmography, a semiquantitative method to assess perfusion of digital arterioles, was performed after 30 min in a quiet room with a stable environmental temperature (21°C) with a Universal-Lichtreflex-Plethysmograf (Medizin-Elektronik, Eurasburg, Germany). Baseline values were recorded. One hand (wearing a disposable latex glove) was then immersed in cold water (10°C) for 3 min. After the glove was removed, photoelectric plethysmography was repeated until the plethysmographic curve regained baseline amplitude (recovery time).

Tolerability and safety. Any adverse effect noted during NAC infusion was reported at followup visits. Vital signs were measured during treatment and at each visit.

Laboratory variables were measured before the beginning of treatment, each day during the infusion period, and on Days 12, 19, 33, and 61. Hematology measures were hemoglobin, erythrocyte count, and leukocyte and platelet count. Serum chemistry included sodium, potassium, calcium, creatinine, alanine transaminase (ALT), and aspartate transaminase (AST). Urinalysis consisted of a dipstick test for protein, glucose, and blood.

All the investigators participated in several education sessions prior to the study to reach a consensus on the study design, on subjective and objective assessments, and on definition of ulcer and ulcer healing.

Statistical analysis. All continuous variables are shown as mean ± SD. Clinical outcome data were analyzed nonparametrically. Pre and posttreatment values for each variable were compared within groups using Wilcoxon's rank-sum test.

RESULTS

Fifty-two patients with scleroderma were screened at the participating centers. Demographic and clinical features of the 22 patients are shown in Table 1. All patients completed the 5 day NAC infusion and 19 days of followup without requiring vasodilator treatment. Two patients withdrew from the study after 3 weeks of followup because of worsening RP attacks and appearance of new skin ulcers, and resumed nifedipine treatment. Thus, all data referring to Day 61 account for the 20 patients who completed the followup.

Raynaud attacks. The frequency of attacks was estimated based on the total number of attacks recorded by the patients. Mean weekly frequency was 21.23 ± 14.88 during the washout period and subsequently fell by 49.45% from Day 5 to Day 19 (p = 0.0001), by 43.09% from Day 20 to Day 33

Table 1. Demographic and clinical characteristics of the 22 patients treated with NAC.

Age, yrs*	49.63 ± 14.79
Women	16
Men	6
Duration of Raynaud's phenomenon, yrs*	12.18 ± 8.36
Subset of scleroderma, n	
Limited	19
Diffuse	3

* Mean ± SD.

(p = 0.0003), and by 30.61% until Day 61 (p = 0.002). The improvement was statistically significant during the whole followup period. A reduction in the frequency of attacks exceeding 50% from baseline was recorded in 11 (50%) patients from Day 5 to Day 19 (Table 2).

The severity of each attack was subjectively estimated by patients using a 10 point scale. Daily mean severity score was 5.52 ± 2.04 at baseline. After NAC treatment, the mean percentage severity was significantly reduced compared to baseline values (38.22%, 46.74%, and 43.47% on Days 5–19, 20–33, and 34–61, respectively). The improvement was greater over Days 20–33 (p = 0.001), when the daily mean severity score was 2.58 ± 2.02 (Table 2).

Digital cutaneous lesions. Seventeen patients had digital ischemic ulcers when they entered the study (mean value 2.94 ± 1.63) (Table 3). After NAC infusion, 60%, 80%, 80%, and 60% of patients showed at least a 50% reduction in the number of lesions on Days 12, 19, 33, and 61, respectively, and complete healing of active ulcers in 40%, 53.3%, 53.3%, and 46.6% of cases, respectively.

Cold challenge test. Cutaneous blood flow was recorded at

Table 2. Frequency and severity of Raynaud's phenomenon in patients with scleroderma before and after treatment with NAC.

	Raynaud's Attacks			
	Patients, no.	Frequency/ wk	Patients, no.	Severity Score/Day
Baseline	22	21.23 ± 14.88	22	5.52 ± 2.04
Followup (Day +5 to +19)	22	10.73 ± 11.13	22	3.41 ± 3.86
Change from baseline		10.5 ± 8.91*		2.11 ± 3.44†
% change from baseline		–49.45%		–38.22%
Followup (Day +20 to +33)	22	12.08 ± 10.80	22	2.94 ± 1.91
Change from baseline		9.15 ± 8.26**		2.58 ± 2.02*
% change from baseline		–43.09%		–46.74%
Followup (Day +34 to +61)	22	14.07 ± 13.79	22	3.062 ± 2.12
Change from baseline		6.5 ± 6.57***		2.40 ± 1.82††
% change from baseline		–30.61%		–43.47%

* p = 0.0001, ** p = 0.0003, *** p = 0.0002, † p = 0.004, †† p = 0.0004, compared with baseline values.

Table 3. Number of ulcers and recovery time in patients with scleroderma before and after treatment with NAC.

	Patients, no.	Ulcers, no.	Patients, no.	Recovery Time, min.
Baseline	17	2.94 ± 1.63	14	16.1 ± 6.6
Followup (Day +12)	17	1.06 ± 1.22	14	4.9 ± 3.4
Change from baseline		1.86 ± 1.72		11.2 ± 6.22
% change from baseline		–63.26%*		–69.56%†
Followup (Day +19)	17	0.73 ± 0.96	14	5.2 ± 3.2
Change from baseline		2.2 ± 1.56		10.9 ± 6.28
% change from baseline		–74.82%**		–67.7%†
Followup (Day +33)	17	0.73 ± 1.10	14	4.6 ± 4.27
Change from baseline		2.2 ± 1.56		11.5 ± 5.54
% change from baseline		–74.82%**		–71.42%**
Followup (Day +61)	17	1.40 ± 1.54	14	4.66 ± 2.97
Change from baseline		1.53 ± 1.64		11.44 ± 5.59
% change from baseline		–52.04%**		–71.05%**

* p = 0.0033, ** p = 0.001, † p = 0.0015, compared with baseline values.

rest and after cold challenge by photoelectric plethysmography before the infusion and on Days 5, 19, 33, and 61. After treatment, plethysmographic curves showed greater amplitude in all patients at all time points (Figure 1). Mean recovery time at baseline was 16.1 ± 6.6 min, and decreased by 69.5%, 67.7%, 71.4%, and 70.5% on Days 12, 19, 33, and 61, respectively (Table 3).

Reduction in recovery time exceeding 50% was recorded in 85.71% of patients on Day 12; in 78.57% on Day 19; in 85.71% on Day 33; and in 92.85% on Day 61.

Two patients (9%) on Day 12 and 3 (13.6%) on Day 33 did not present RP after the provocation test.

Adverse events. The infusion of NAC was well tolerated and none of the side effects reported was so serious as to lead to interruption of treatment. The most frequent events reported during infusion were weight gain (31.81%), headache (22.72%), and epigastric pain (13.63%) (Table 4). During the loading dose, 13.63% of patients experienced flushing and scalp dysesthesia. Assessment of vital signs during treatment showed no clinically significant effects on blood pressure or heart rate. Two patients developed fluid retention with arterial hypertension that was successfully treated with diuretics. All side effects were reversible and disappeared at the end of infusion.

Table 4. Adverse reactions during drug infusion.

Adverse Reaction	Patients, n (%)
Weight gain	7 (31.81)
Headache	5 (22.72)
Peripheral edema	3 (13.63)
Arterial hypertension	2 (9.09)
Epigastric pain	3 (13.63)
Flushing	3 (13.63)
Itching	1 (4.54)
Scalp dysesthesia	1 (4.54)
Arthralgia	1 (4.54)
Diarrhea	1 (4.54)

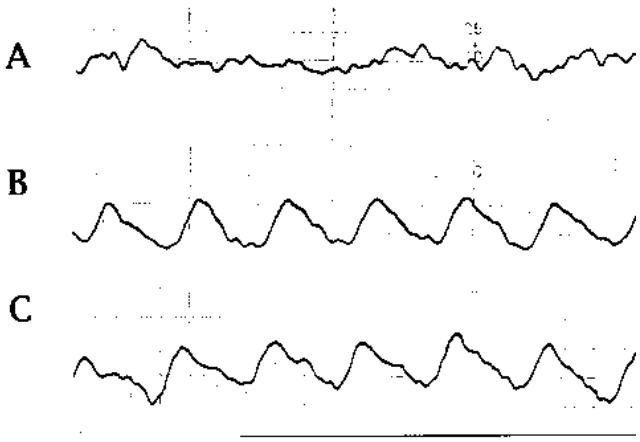

Figure 1. Representative plethysmographic curves: A. at baseline; B. on Day 12; C. on Day 33.

With reference to laboratory variables, a prolonged prothrombin time (without hemorrhagic events) was observed during infusion in all patients. Prothrombin activity reverted to baseline values 12 h after the end of NAC infusion.

DISCUSSION

Poor understanding of the pathogenesis of scleroderma vascular lesions makes treatment of Raynaud's phenomenon in patients with SSc difficult. Conventional treatment with calcium antagonists[5], and more recently intravenous prostanoids, is unsatisfactory[4] due both to their side effects and to the administration route. Furthermore, results with oral iloprost compared to placebo were disappointing in 2 studies[20,21] and of limited benefit in another[22]. The search for a more effective treatment must therefore continue.

It has been suggested that some of the clinical features of SSc may be ascribed to excessive oxidative stress, since ROS can induce endothelial cell dysfunction[23] and fibroblast proliferation[24], which are well known pathological features of scleroderma[25]. In this respect, recent direct[17] and indirect evidence[14-16] of enhanced oxidative stress in SSc patients has made antioxidant therapy in scleroderma theoretically attractive. However, few clinical studies have been undertaken to verify the clinical benefit of treatments aimed at reducing oxidative stress or augmenting antioxidant defenses.

Denton and colleagues showed that probucol, a powerful systemic antioxidant, may be useful for the symptomatic treatment of RP[26]. Although the drug was well tolerated, several side effects, including occasional cardiac arrhythmia, were documented, suggesting caution in its use. In a double blind, placebo controlled study oral NAC failed to produce significant improvement in most of the variables evaluated, although its effect on RP was not investigated[27].

Since, after oral administration, the bioavailability of NAC is less than 5% as a result of extensive first-pass metabolism in the body[18], we devised a protocol employing high dose intravenous NAC for the treatment of scleroderma.

Evaluation of the safety and side effects of this approach was our object in this preliminary study, which showed that a continuous 5 day infusion of NAC can be administered to scleroderma patients with minor, easily controlled adverse reactions. No patient needed to be withdrawn from the study as a consequence of these events.

Clinical studies are notoriously difficult in RP, and mostly rely on patients' self-report of symptoms, whose reliability depends both on patient education and on blinding of patients and investigators to the placebo response. Indeed, most clinical trials in RP show a 10–30% placebo effect[4,28,29], and sometimes the placebo group has an even larger improvement of most variables[20]. This finding and the fact that our patients stayed in hospital during all 5 days of treatment caution

against an optimistic interpretation of our data and reinforce the need for placebo controls. Further, our findings may be confounded by the study design, with an open nontreatment washout period to establish baseline values. High attack rates might be subjectively recorded due to enthusiasm to enter the study or to knowledge of no treatment. A better design would have been a patient blinded placebo treatment period (baseline) following a washout period, but this idea was discarded because it would have extended the trial beyond the cold weather months. In conclusion, open and uncontrolled studies such as this one do not allow reliable assessment of the clinical efficacy of drugs aimed at treating RP. These results show a promising trend, but do not allow firm conclusions.

Nonetheless, the variations we observed were impressive not only in terms of the reduction in the frequency and severity of RP attacks, but also with regard to the number of digital ulcers and to recovery time after the cold challenge. At variance with other therapeutic trials, we observed a statistically significant improvement in the response to a standard cold challenge test, which was reflected in the amelioration of the clinical measures. However, while the attack rate was worsening at 60 day followup, the results of the cold challenge test were still significantly different from baseline. We have no explanation for this finding other than that already outlined by Wigley and colleagues[4], who emphasized interpatient variability in the measurements and/or inadequacy of the cold challenge.

Interestingly, all patients expressed their preference for NAC over previous treatments (Table 5) and asked to be included in future clinical trials with this drug.

NAC might exert its therapeutic effects by virtue of its antioxidant properties, which appear to be related to at least 2 mechanisms, the nucleophilic properties of the molecule accounting for its free radical scavenger activity[30,31], and the capacity to support glutathione synthesis[32,33]. Alternatively, NAC could ameliorate RP attacks through its effects on microcirculation and tissue oxygenation. Recent data indicate that NAC markedly enhances the effectiveness of nitrocompounds in terms of vascular smooth muscle relaxation[34] and inhibition of platelet aggregation[35]. The vasodilator effects of NAC are partly mediated by its capacity to activate guanylate cyclase, the enzyme responsible for vasorelaxation, and to generate sulphydryl groups, which are required for relaxation of vascular smooth muscle and full activity of the endothelium derived relaxing factor through the generation of nitrosothiols[36-38]. The latter mechanism could be active at least during NAC infusion or soon after, as suggested by the flushing and scalp dysesthesia experienced by some patients during administration of the loading dose. Further, NAC interferes with the activation of nuclear factor-κB[39] and reduces *in vitro* cellular production of proinflammatory mediators (e.g., tumor necrosis factor-alpha, interleukin 1)[40] as well as the expression of vascular cell adhesion molecule-1[39,41] and endothelial-leukocyte adhesion molecule-1[42]. All these effects on the immune system make NAC particularly suitable for treatment of RP secondary to SSc.

We found that intravenous N-acetylcysteine can safely be used in patients with scleroderma, although a wider double blind, placebo controlled study is required to establish its place in the treatment of Raynaud's phenomenon.

Table 5. Drugs previously used in the treatment of Raynaud's phenomenon in 22 patients with scleroderma.

Drug	Patients, n (%)
Iloprost (carboprostacyclin)	22 (100)
Calcium channel blockers	22 (100)
Antiplatelet drugs	16 (72.7)
Angiotensin converting enzyme inhibitors	12 (54.5)
Alprostadil (PGE_1)	5 (22.7)

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