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Concise report

A phase 2 trial investigating the effects of the angiotensin II type 2 receptor agonist C21 in systemic sclerosis-related Raynaud's

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Abstract

Objective. Our main aim was to investigate the effect of a single oral dose of C21, a selective angiotensin II type 2 receptor agonist, on cold-induced vasoconstriction in SSc-related RP.

Methods. This was a phase IIa, randomized, double-blind, cross-over, single-dose, placebo-controlled, single-centre study. Twelve female patients with SSc (median age 58.5 years, median duration of RP 19.0 years) attended on four occasions: screening, treatment visits 1 and 2 (separated by 3–7 days) and follow-up. At the first treatment visit, patients were randomized to receive either a single oral dose of C21 (200 mg) or placebo, then the opposite treatment on the second visit. Forty min after each treatment, each patient underwent a standard hand cold challenge. The primary end point was the area under the curve (AUC) for rewarming for each finger (eight fingers) over 15 min. Secondary end points included the maximum finger temperature after rewarming (MAX). Statistical analyses were performed by multiplicative ANCOVA models.

Results. For all eight fingers combined, mean AUC for rewarming was higher after treatment with C21 than after placebo (geometric mean 20 046°C*s *vs* 19 558°C*s), but not significantly ($P = 0.380$) and MAX (at 15 min) was also higher (geometric mean 23.5°C *vs* 22.5°C; $P = 0.036$). C21 was well tolerated.

Conclusion. Despite the small trial size, a signal emerged suggesting that even in patients with established SSc, C21 may confer benefit for RP and deserves further investigation.

Trial registration. ClinicalTrials.gov, https://clinicaltrials.gov, NCT04388176.

Key words: angiotensin II type 2 receptor agonist, randomized controlled trial, RP, thermography, SSc

CLINICAL SCIENCE

Rheumatology key messages

- C21 is an agonist of the angiotensin II type 2 receptor, which contributes to vasodilation.
- C21 was associated with increased rewarming after a hand cold challenge compared with placebo.
- C21 deserves further investigation as a possible treatment for SSc-related Raynaud's phenomenon.

Introduction

SSc is an autoimmune disease characterized by vasculopathy and fibrosis of several organ systems. RP, painful discolouration of the fingers and toes in response to cold exposure or to emotional stress, is the commonest manifestation of SSc affecting >95% of patients [1], and is particularly severe in patients with SSc because of their underlying structural vasculopathy [2]. Although vasodilatory drugs such as calcium channel blockers, phosphodiesterase type 5 inhibitors and prostacyclin analogues are key components of therapy [3], these are

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only partially effective in many patients [4] and in most patients with SSc, RP is the symptom with most impact on quality of life [5]. New treatments are therefore badly needed.

Angiotensin II, the major effector peptide of the renin–angiotensin system, has potent vasoconstrictor effects mediated by the angiotensin II type 1 receptor (AT1R) [6]. Conversely, the angiotensin II type 2 receptor (AT2R) is considered the 'protective arm' of the renin–angiotensin system [7–9] and contributes to vasodilation, increasing endothelial nitric oxide (NO) synthase (eNOS) activity and thus increasing NO release [10]. In adult human skin, a complete renin–angiotensin system is present [11] and is upregulated with increased AT2R expression in SSc [12].

Compound 21 (C21) is a first-in-class, low molecular weight, orally available, specific, high-affinity AT2R agonist [13] with onset of action ~30 min following administration. Our aim was to investigate whether C21 has vasodilating properties in patients with established SSc-related vasculopathy, and specifically whether C21 can reduce cold-induced vasoconstriction in a controlled environment, assessing temperature response with thermography. Secondary objectives were to assess safety and tolerability of C21, and to examine the effects of C21 on finger temperature for 40 min following administration (prior to cold challenge).

Methods

Study design

This was a phase IIa, randomized, double-blind, cross-over, single-dose, placebo-controlled, single-centre study (ClinicalTrials.gov, NCT04388176). Patients attended four times: initial screening (up to 3 weeks before the first treatment visit), treatment visits 1 and 2 (3–7 days apart) and an end-of-study visit 1 (3–15 days after the last treatment). A cross-over design was applied to control for inter-individual variability in response to cold challenge. Randomization was in blocks of four.

At screening, baseline assessments included Raynaud's Condition Score (RCS), to assess the severity and impact of RP [14], nailfold capillaroscopy [15], modified Rodnan skin score (mRSS), and bilateral distal ring finger temperature (as measured by contact thermal probe).

At each treatment visit, patients arrived after an overnight fast and after abstaining from vigorous exercise, caffeine containing beverages and alcohol for 4 h. Patients acclimatized at 23°C for at least 20 min, after which they received a single oral dose of 200 mg of C21 or placebo. Thermography images were then captured every 10 min. Forty minutes after administration of the investigational medicinal product (IMP) a cold challenge was performed following a standard protocol [16]. In summary, both hands were immersed up to the metacarpophalangeal joints for 1 min in cool water (15°C), and thermography images (FLIR T540 thermal camera, FLIR Systems, Täby, Sweden) were captured every 15 s for 15 min. Analysis of the images was performed using Research IR Max (version 4.2; FLIR).

The study was approved by the East of England Cambridge South Research Ethics Committee and all patients signed written informed consent.

Outcome measures

The primary end point was defined as the mean area under the rewarming curve (over 15 min) of all eight fingers after cold challenge (AUC) as measured by thermography. Secondary end points were mean maximum skin temperature during rewarming (MAX), the mean gradient of rewarming in the first 2 min post-cold challenge (GRAD), and the mean difference in temperature between the dorsum of the hands and the fingers, distal dorsal difference (DDD), during 40 min following IMP administration. Safety was assessed by capturing adverse events, laboratory assessments (haematology and clinical chemistry) and Holter ECG recording for 30 min before and 180 min after IMP administration. *Post hoc* analyses included mean finger and hand temperature during the 40 min following IMP administration.

Patients

Patients were eligible for recruitment if they fulfilled the EULAR/ACR 2013 criteria for SSc [17], were aged 19–75 years and had an average frequency of RP attacks during the winter months (November–March) of at least 5/week. During the 4 weeks prior to screening, patients should not have been treated with immunosuppressive therapy other than stable doses of mycophenolate mofetil. Inhaled corticosteroids or stable doses of systemic corticosteroids corresponding to $<$10 mg prednisolone were also permitted. Vasoactive substances and mycophenolate mofetil were withheld for 3 days prior to the two treatment visits.

Patients with a mean bilateral finger temperature below 27°C after acclimatizing at an ambient temperature of 23°C for a period of 20 min at the time of screening were excluded from the study. Other exclusion criteria included smoking, pregnancy and breastfeeding. Women of childbearing age were asked to adhere to effective contraceptive methods.

Statistical analysis

The power calculation, based on previously reported cold challenge response variability [16], demonstrated that 14 subjects were required to detect a difference of 0.104 in the primary end point (logAUC) with a power of 90% (and $\alpha = 0.1$) assuming an intra-individual standard deviation of the differences of 0.126. To compensate for potential drop-outs, a total of 16 subjects were to be included.

Treatments were compared using an analysis of (co)-variance model [AN(C)OVA] adjusting for patient, period and treatment and including the baseline value of the period as a covariate if appropriate. The model for AUC,

MAX and GRAD was multiplicative, with data being logged prior to analysis. Model estimates were back-transformed giving the treatment contrast as a geometric mean ratio with 90% confidence intervals and associated, two-sided *P*-value. Of the exploratory end points finger and hand temperatures were compared using Student's paired *t*-test.

Results

The study was conducted from 3 January to 14 December 2020, and a total of 21 patients were screened (patient flow through study shown in Supplementary Fig. S1, available at *Rheumatology* online). Due to lock-down of the research facilities during the COVID-19 pandemic, the sponsor decided to stop recruitment when 12 patients had completed the study: it would have been very difficult to recruit further patients over the winter months. All were female and all had the limited cutaneous subtype of SSc, with a median age of 58.5 (range 35–69) years, a median duration of RP of 19.0 (range 0.4–41.0) years, and a median time since SSc diagnosis (since first non-Raynaud's manifestation) of 12.4 (range 0.4–39.0) years (Supplementary Table S1, available at *Rheumatology* online). Nine were anticentromere antibody positive and one was anti-topoisomerase positive (the other two patients were negative for SSc-specific autoantibodies). Five patients were on vasodilator therapy (three on nifedipine, one on amlodipine and one on sildenafil) and none was on immunosuppressant or systemic corticosteroid therapy. At screening, median RCS was 4.0 (range 1–10) and median mRSS was 1.0 (range 0–4). On nailfold capillaroscopy, median capillary density was 3.37/mm (range 0.45–6.41) and median capillary width 19.58 (range 11.45–42.00).

Response to cold challenge

Rewarming profiles after C21 and placebo are shown in Fig. 1, and results are summarized in Table 1. C21 showed a numeric advantage over placebo on the primary outcome (AUC) but the difference was not statistically significant (Table 1). MAX was significantly higher after treatment with C21 than after placebo (Table 1) and GRAD (over the first 2 min) was lower (i.e. temperature recovery was slower), although not significantly. At the end of the rewarming period the patients had recovered 62.7% and 47.5% of the drop during the cold challenge on the C21 and placebo days, respectively ($P = 0.084$). Mean temperature difference between the treatment periods gradually increased over time (Fig. 1).

Finger temperatures following IMP administration and prior to cold challenge

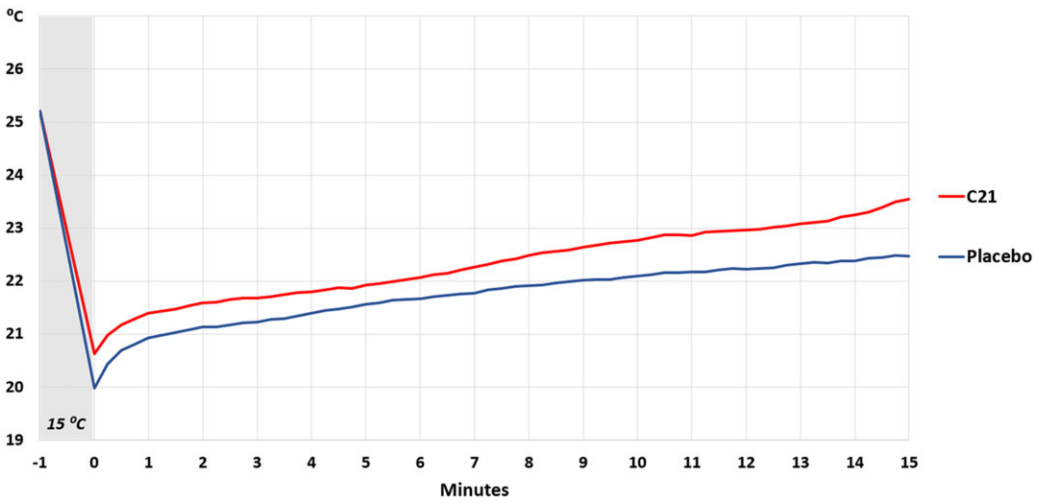
DDD at 10 min post-IMP dosing was greater (i.e. fingertips cooler compared with dorsum of hand) following C21

Table 1 Thermography: primary and secondary end points

Assessment	C21	Placebo	*P*
$AUC_{0-15min}$ (°C*s)[a]	20 046 (7.68)	19 558 (4.36)	0.380
MAX (°C)[a]	23.53 (8.49)	22.50 (3.73)	0.036
$GRAD_{0-2min}$ (°C/min)[a]	0.45 (39.4)	0.54 (42.3)	0.284
DDD_{10min} (°C)[b]	−3.40 (0.46)	−3.24 (0.30)	0.015

Values are means across all 12 patients, and for each patient results were averaged across eight fingers. [a]Geometric mean (CV %). [b]Mean (S.E.). Significance tests were performed by AN(C)OVA. AUC: area under the rewarming curve; DDD: distal dorsal difference; GRAD: the mean gradient of rewarming in the first 2 min post-col challenge; MAX: maximum skin temperature during rewarming.

Fig. 1 Rewarming profile after cold challenge in patients treated with C21 or placebo



compared with placebo (Table 1). *Post hoc* analyses demonstrated that the patients had higher baseline mean finger (and mean hand) temperatures on the C21 compared with the placebo administration day, but at the start of the cold challenge 40 min later there were no temperature differences between the treatment days (Supplementary Table S2, available at *Rheumatology* online).

Safety variables

There were no serious adverse events in the study. Five subjects reported eight adverse events (not considered related to treatment) during treatment with C21 and three subjects reported five adverse events (two, in one patient, were considered related to treatment—dizziness and flushing) during treatment with placebo. The majority of the events were of mild intensity. There were no clinically relevant notable changes in haematology, clinical chemistry or ECG during the study.

Discussion

Despite the small trial size, a signal emerged suggesting that even in patients with established SSc (and therefore with structural digital vascular disease), C21 may confer benefit for RP. Although the primary end point was not met, AUC for rewarming after a cold challenge was higher after C21 than after placebo, and MAX was significantly higher after C21. For both treatments, finger temperature was still increasing after 15 min of observation, suggesting that the full effect of C21 was not captured. C21 was well tolerated.

A treatment aimed at counterbalancing the vasoconstrictive arm of the renin–angiotensin system has a sound rationale for SSc-related RP. To date, treatments proposed and/or investigated for SSc-related RP because of their effects on the renin–angiotensin system have aimed to block the effects of angiotensin II. However, angiotensin converting enzyme inhibitors have not been found to be effective [4], and although one open label study suggested that the angiotensin II receptor blocker (ARB) losartan conferred benefit [18], ARBs are not widely prescribed. The novelty of C21 is that it works through a different mechanism, augmenting the 'protective' arm of the renin–angiotensin system via stimulation of AT2R. In a study using micro-dialysis to assess vascular tone, angiotensin II infusion caused a dose-dependent vasoconstriction, while AT2R inhibition with PD-123319 increased the vasoconstriction response, indicating a counter-regulatory role of the receptor [9]. Although AT2R agonists have been reported to cause subtle arterial relaxation *in vitro*, they do not generally lower systemic blood pressure *in vivo* [19], and this would be advantageous in those patients with SSc with a low baseline blood pressure. Such a lack of translation into antihypertensive effects of AT2R agonists may depend on overriding vasoconstrictive AT1R activity. This is supported by the finding that infusion of the selective AT2R agonist CGP42112 failed to reduce blood pressure in spontaneously hypertensive rats unless administered in the presence of a low dose of the AT1R antagonist candesartan [20].

The main outcome measures in this phase IIa study were related to temperature response to a standard cold challenge. These outcome measures have been shown, in a multicentre study [16], to be highly reliable and to correlate strongly with blood flow. Because RP is provoked primarily by cold exposure, it seems likely that a drug which improves rewarming after a cold challenge will confer benefit in patients with SSc-related RP by shortening attacks and rendering these less severe. Because of its rapid onset of action, there could be the option of preventative 'on demand' dosing prior to cold exposure. The fact that in our study a signal emerged even in patients with established SSc (long disease duration and a low nailfold capillary density) suggests that C21 could confer benefit even in patients with advanced structural vasculopathy.

The main limitation of our study was its small size (contributed to by the COVID-19 pandemic), that only a single dose was evaluated and that rewarming was still increasing after 15 min: future studies will include a longer observation period post-cold challenge. The higher baseline hand and finger temperatures during the C21 visits suggests that patients might not have been fully acclimatized, and therefore future studies should consider a longer acclimatization period: 20 min post-IMP administration (immediately prior to cold challenge) temperatures were similar in both groups.

In summary, C21 offers a potential new approach to therapy for SSc-related RP, with a sound therapeutic rationale. To confirm the utility, a larger Phase II trial incorporating a longer duration of treatment would be needed.

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Data availability statement

The Sponsor will share de-identified individual participant data collected during the trial with researchers who provide a methodologically sound proposal.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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