Utilizing venous occlusion plethysmography to assess vascular effects: A study with buloxibutid, an angiotensin II type 2 receptor agonist

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Abstract
Buloxibutid (also known as C21) is a potent and selective angiotensin II type 2 receptor (AT2R) agonist, in development for oral treatment of fibrotic lung disease. This phase I, open-label, pharmacodynamic study investigated vascular effects of buloxibutid in five healthy male volunteers. Subjects were administered intra-arterial infusions of buloxibutid for 5 min in ascending doses of 3, 10, 30, 100, and 200 μg/min, infused sequentially in the forearm. Infusions of sodium nitroprusside (SNP) solution in doses of 0.8–3.2 μg/min were administered as a positive control. Forearm blood flow (FBF) was measured by venous occlusion plethysmography. Safety and tolerability of intra-arterial administrations of buloxibutid were evaluated. Following infusion of buloxibutid in doses of 3–200 μg/min, the range of increase in FBF was 27.8%, 17.2%, 37.0%, 28.5%, and 60.5%, compared to the respective baseline. The largest increase was observed in the highest dose group. Infusions of SNP as a positive control, increased FBF 230–320% compared to baseline. Three adverse events (AEs) of mild intensity, not related to buloxibutid or SNP, were reported for two subjects. Two of these AEs were related to study procedures. There were no clinically relevant changes in arterial blood pressure during the study period. Intra-arterial infusion of buloxibutid in low, ascending doses increased FBF, indicating that buloxibutid may be effective in conditions associated with endothelial dysfunction. Venous occlusion plethysmography was found to be a useful method to explore pharmacodynamic vascular effects of novel AT2R agonists, while avoiding systemic adverse effects.

Study Highlights
WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
The angiotensin II type 2 receptor (AT2R) has been associated with a vasodilatory role. Buloxibutid is a first-in-class, potent and selective AT2R agonist with

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INTRODUCTION

The renin-angiotensin system (RAS) plays an important role in maintaining extracellular fluid volume, and there is substantial evidence supporting involvement of the RAS in the pathogenesis of cardiovascular and renal disease. Angiotensin II, the major effector peptide of the RAS, acts via two specific receptors, the angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R). The AT1R is mainly involved in blood pressure regulation through several different mechanisms related to vasoconstriction and fluid retention, whereas the AT2R mediates resolution and repair after tissue injury through anti-inflammatory, anti-fibrotic, and vasodilatory effects.1

With regard to vascular effects, AT2R agonists have been reported to cause nitric oxide (NO)-dependent arterial relaxation in vitro and local dose-dependent vasodilation when given by intra-arterial infusion to healthy volunteers.2 However, systemically administered AT2R agonists do not generally lower systemic blood pressure in vivo.1,3 Such a lack of translation into antihypertensive effects of AT2R agonists may depend on differential receptor expression and/or over-riding vasoconstrictive AT1R activity. The latter is supported by the finding that infusion of the selective AT2R agonist peptide CQ42112 failed to reduce blood pressure in spontaneously hypertensive rats unless administered in the presence of a low dose of the AT1R antagonist candesartan.4

Buloxibutid (also known as Compound 21 or C21), the first AT2R agonist in clinical development, is a low molecular weight, orally available, selective, high-affinity AT2R agonist acting via unique signaling pathways.1 Buloxibutid (100 mg per os, twice daily) is currently investigated in a phase II clinical study in patients with idiopathic pulmonary fibrosis in which preliminary efficacy results appear promising.5 In an earlier clinical study, buloxibutid (200 mg per os) was shown to cause vasodilation and accelerate skin rewarming after a cold challenge (without lowering systemic blood pressure) in patients with Raynaud’s phenomenon secondary to systemic sclerosis.6 In addition, there is a large number of animal studies documenting the therapeutic efficacy of buloxibutid in different models of cardiovascular, pulmonary, kidney, metabolic, and central nervous system disease.1

Early exploratory clinical studies to evaluate not only pharmacokinetics but also pharmacodynamics and mechanisms of action can be performed using small local doses or subtherapeutic systemic microdoses of novel drug candidates.7–10 Even if exposure is limited and there is no therapeutic intent or intent to evaluate clinical tolerability, such exploratory studies can provide valuable mechanistic information already in the initial phases of clinical drug development.9,10 In exploratory studies, microdose parenteral administration of an intended oral drug is an approach frequently taken to characterize new compounds and provide pharmacokinetic data, where microdose refers to the administration of a fraction of a therapeutic dose.7,9 Intra-target microdosing is a novel approach where an investigational drug is administered directly into a physical target such that only an equally
small fraction of total body mass is exposed to the drug for a limited duration.⁹

Venous occlusion plethysmography is a technique used to study vascular physiology in humans.¹¹ The underlying principle is that when venous return from the forearm is briefly interrupted while arterial inflow continues unimpeded, the forearm swells at a rate proportional to the rate of arterial inflow. This is achieved by inflating a cuff placed around the upper arm to well above venous pressure but below diastolic pressure. A second cuff around the wrist is inflated to supra-systolic pressures to exclude hand circulation.¹¹–¹³ Strain-gauges are placed around the widest part of the forearm. Increase in forearm volume results in a corresponding change in arm circumference and thus strain-gauge length, which can be detected as an alteration in electrical resistance of the gauge.¹¹ Forearm swelling rate is measured and expressed as forearm blood flow (FBF), typically mL per 100mL of forearm volume per minute (mL/100mL·min⁻¹), to allow for standardized and comparable measurements across individuals, by normalizing for limb size. Combined with brachial artery infusion of a vasoactive drug, venous occlusion plethysmography is considered a gold-standard method for assessing vascular drug effects, by measurement of FBF.¹¹,¹⁴

Sodium nitroprusside (SNP) is a potent and fast acting vasodilator and was used as a positive control in this study. SNP acts by release of NO, which stimulates guanylyl cyclase to produce cyclic GMP. The vasodilatory effect is dose-dependent and is caused by relaxation of vascular smooth muscle cells.¹⁵–¹⁸

The purpose of this exploratory pharmacodynamic clinical study was to study potential dose-dependent local effects on endothelial function, in terms of vasodilation, of buloxibutid in healthy subjects using plethysmography with intra-arterial drug infusion. Such an approach would also be useful for dose-finding and documenting target engagement in the early stages of development of new compounds of the angiotensin II type 2 receptor agonist (ATRAG) class.

**METHODS**

**Ethics statement**

The study (EudraCT 2021-000288-62; NCT05277922) was approved by the Swedish Ethical Review Authority (Dnr 2021-04501) and was conducted at CTC Clinical Trial Consultants AB (Uppsala, Sweden). Informed consent was provided by all subjects before participation in any study-related procedures. The study conduct was in accordance with the Declaration of Helsinki and in compliance with the International Council for Harmonization/Good Clinical Practice guidelines.

**Study design**

This was a phase I, open-label, single-center study investigating the effect of buloxibutid on FBF in healthy volunteer male subjects, by use of strain-gauge venous occlusion plethysmography.

Five healthy male volunteers were enrolled. Subjects were first screened and, if eligible, scheduled for the treatment visit. A standardized meal was served 90 min prior to the first FBF measurement. The investigational medicinal product (IMP) solutions were infused through a catheter placed in the brachial artery of the non-dominant arm. After a resting period of 25 min, baseline measurements of FBF were performed (schematic of study design is presented in Figure S1).

Ascending doses of buloxibutid (3, 10, 30, 100, and 200μg/min) were administered intra-arterially for 5 min to each subject with an infusion rate of 1 or 2 mL/min for the 200μg/min dose, corresponding to total doses of 15, 50, 150, 500, and 1000μg, respectively. Each infusion lasted for 5 min and was separated by a washout period of at least 15 min. Doses aimed to achieve local transient blood concentrations comparable to maximum concentration (Cmax) in prior oral studies (unpublished data, Vicore Pharma AB, Stockholm, Sweden) while taking similar studies into account.² Following a resting period, three doses of SNP, 0.8, 1.6, and 3.2μg/min, infused for 5 min, were used as a positive control. SNP doses were selected to attain vasodilatory effects reported in previously published studies.¹⁶–¹⁸

A follow-up phone call was made 7 to 10 days after the treatment visit.

**Study population**

Healthy male volunteers aged 18 to 50 years and with a body mass index of 18–30 kg/m² were included (inclusion and exclusion criteria are listed in Table S1). All subjects were in good health, as determined at the screening visit and with no history of clinically significant disease or disorder, including but not limited to vascular disorders. Subjects were nonusers of nicotine products and with no use of concomitant medication.

**Venous occlusion plethysmography**

Measurements of FBF were captured using a Conformité Européenne-marked automated strain-gauge venous occlusion plethysmography equipment providing on-line measurements (Bergenheim, Elektromedicin, Gothenburg, Sweden).
Subjects were placed resting in a comfortable supine position in a quiet room with normal room temperature (+20 to +25°C) with both forearms positioned above the level of the heart. Actual forearm volume was estimated by calculation, using circumferential measures. Cuffs were placed on the widest part of both forearms and smaller cuffs were placed around each wrist. The non-infused arm was used as a contemporaneous control to account for any minor changes in FBF affecting both arms, for example, emotional stress. A standard arterial cannula was used for brachial artery cannulation of the non-dominant arm. Normal saline was connected to maintain the patency of the arterial line.

Start- and stop times of infusions were recorded. Measurements of FBF were obtained in both arms simultaneously during the last 2 min and 40 s of each infusion by strain-gauge plethysmography. The individual doses of buloxibutid and SNP were separated by a washout period of at least 15 min. At the end of each washout period, FBF was measured and a baseline value for the next dose was recorded (Figure 1).

For each FBF measurement, an inflation pressure of 60 mmHg was used for intervals of ~7 s followed by ~8 s of deflation. This cycle was repeated approximately eight times for all baseline measurements as well as during each infusion. A mean value of the eight measurements was derived to represent FBF. The wrist cuff was inflated to 200 mmHg within 30 s before each measurement to exclude the hand circulation. Changes in forearm volume were measured using strain-gauges placed around the widest part of the forearms. Blood flow was expressed as mL per 100 mL of forearm volume per minute (mL·100 mL⁻¹·min⁻¹). FBF was calculated using the first two cardiac cycle pulses displayed by the plethysmograph, avoiding initial movement artifacts. Data were recorded, stored, and analyzed using PeriVasc Software (Ekman Biomedical Data AB, Gothenburg, Sweden).

After removal of the arterial catheter, a compression bandage was applied.

Safety assessments

At the screening visit, physical examination and assessments were performed, as well as medical history taken. Safety assessments during the study included recording of adverse events (AEs), vital signs (blood pressure and heart rate), 12-lead electrocardiograms (ECGs), and clinical laboratory measurements. Assessments were performed prior to first dose and before subjects left the clinic. Written instructions on how and when to contact the Investigator, if necessary, were provided to subjects before leaving the clinic. AEs were collected from the start of the study procedure at the treatment visit, until the follow-up telephone call. AEs were coded according to the Medical Dictionary for Regulatory Activities version 24.1.

Statistical analysis

Summaries and statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.). Descriptive statistics were computed for change from baseline after each dose. To assess dose dependency in FBF, an analysis of variance was applied with dose as a class variable and subject as a random variable. Data was logged prior to analysis and the result was back-transformed to generate a ratio of geometric means. Kenward-Roger’s approximation for degrees of freedom was used.

RESULTS

Study population

A total of eight subjects were screened and five subjects (Table 1) were included and dosed in the study. There were no withdrawals or replacements. All five subjects received all doses and were included in the analyses. The first subject was screened on April 28, 2022 and the last subject was dosed on May 11, 2022.

![Figure 1](https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.13735) Dosing and measurement flow-chart for buloxibutid infusions. Ascending doses of buloxibutid (3, 10, 30, 100, and 200 μg/min) were administered intra-arterially for 5 min to each subject, corresponding to total doses of 15, 50, 150, 500, and 1000 μg, respectively. Each infusion lasted for 5 min and was separated by a washout period of at least 15 min. Measurements of FBF were obtained using venous occlusion plethysmography in both arms simultaneously during the last 2 min and 40 s of each infusion. *Baseline measurement; FBF, forearm blood flow.
Mean FBF and relative response are summarized by increasing dose of buloxibutid and SNP in Table 2 (supplementary information on buloxibutid and SNP in Table S2). Buloxibutid was administered intra-arterially to the non-dominant arm while the dominant arm represents the no-intervention control.

The mean relative change in FBF (non-dominant arm) after increasing intra-arterial doses of buloxibutid compared to its respective baseline FBF was 27.8%, 17.2%, 37.0%, 28.5%, and 60.5% for the 3, 10, 30, 100, and 200 μg/min doses, respectively. The largest relative increase, 60.5%, was observed after the highest dose (200 μg/min).

After the lower doses (3–100 μg/min), there was no consistent pattern in terms of increase in mean FBF with increasing dose of buloxibutid.

In the control arm (dominant arm), the mean change from baseline FBF was 8.2%, 9.0%, 4.8%, 0.0%, and −0.4% for the 3, 10, 30, 100, and 200 μg/min doses, respectively. After the lower doses (3–100 μg/min), there was no consistent pattern in terms of increase in mean FBF with increasing dose of buloxibutid. After increasing intra-arterial doses of buloxibutid, the respective baseline FBF was 27.8%, 17.2%, 37.0%, 28.5%, and 60.5% for the 3, 10, 30, 100, and 200 μg/min doses, respectively.

Graphical presentation and comparison of FBF after infusion of buloxibutid and no-intervention control is shown in Figure 2 (mean FBF), and mean relative change of buloxibutid in Figure 3.

Geometric mean ratios and corresponding 95% confidence intervals for pairwise dose comparisons are presented in Table 3. In the buloxibutid infused arm, differences in FBF were observed for the 200, 100, and 30 μg/min doses versus 3 μg/min doses, but not for the 10 versus 3 μg/mL doses.
doses. Differences in FBF were also observed for the 200, 100, and 30 versus 10 μg/min doses, for the 200 versus 30 μg/min doses and for the 200 versus 100 μg/min doses.

**Vascular effects of SNP**

As a positive control, SNP was administered at three different dose levels (0.8, 1.6, and 3.2 μg/min) to the non-dominant arm (n = 5). Control represents dominant arm and has not received treatment. Baseline measurements (1–5) were performed prior to each dose. Measurements of FBF were obtained using venous occlusion plethysmography. Error bars represent standard deviations. FBF, forearm blood flow; n, number of subjects.

**Safety and tolerability**

There were three AEs reported by two subjects during the study (Table S3). One subject reported two AEs related to...
study procedures: transient presyncope symptoms during arterial catheter insertion prior to the first dose, and infusion site bruising. One subject reported a ligament sprain injury 5 days after the treatment visit. All AEs were mild in intensity and unrelated to buloxibutid or SNP.

No clinically significant changes in vital signs, ECGs or clinical laboratory values were observed.

**DISCUSSION**

**Evaluation of forearm blood flow**

The mean FBF, as measured by strain-gauge venous occlusion plethysmography, increased after intrarterial doses of buloxibutid. The largest mean increase from baseline prior to dose, 69.5%, was observed in the highest dose group (200 μg/min). Dose-dependent effects were observed in the dose range. In contrast, responses to SNP were uniform among subjects, suggesting a more consistent pharmacodynamic effect. Although AT2R expression is high in many fetal tissues, it is generally low in healthy adults but can increase in different pathological conditions. In healthy mice, it has been reported that arterial AT2R expression decreases with age. The variability in response to buloxibutid could be attributed to differences in receptor expression. Any assessment of whether the variability observed in our study is related to known background characteristics would, however, require a study with more subjects of different ages and backgrounds.

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<th>µg/mL</th>
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<th>30 vs. 3</th>
<th>30 vs. 10</th>
<th>100 vs. 3</th>
<th>100 vs. 10</th>
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<td>Ratio of LSMeans (95% CI)</td>
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<tr>
<td>Buloxibutid infused arm</td>
<td>0.9999 (0.8313; 1.2454) (1.0355; 1.2456) (1.0357; 1.2392) (1.0304; 1.2394) (1.0305; 0.9950) (1.2520; 1.5058) (1.2522; 1.5060) (1.2091; 1.2151) (1.0103; 1.4614)</td>
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<td>Control arm</td>
<td>1.1004 (0.9007; 1.2271) (1.0045; 1.1152) (0.9129; 1.1698) (0.9576; 1.0631) (0.8703; 0.9533) (0.7804; 1.1185) (0.9156; 1.0165) (0.7461; 0.9561) (0.7827; 1.1680)</td>
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Note: The table presents pairwise comparisons of response to buloxibutid doses. The natural logarithm of the maximum FBF value for each dose level and each subject is used as the dependent variable in the model. LSMeans differences ratios and corresponding 95% CIs are presented. Pairwise treatment comparisons are based on a mixed effects model with treatment as fixed effect(s) and subject as a random effect. Kenward-Rogers approximation for degrees of freedom has been used. Dominant arm represents control and has not received investigational medicinal product.

Abbreviations: CI, confidence interval; FBF, forearm blood flow; LSMeans, least square means.
dose-dependency could also be partly due to the small number of subjects. The standard deviations compared to mean values were relatively small, which supports measurements were consistent and reliable. However, given the small sample size, these measurements may not necessarily represent a broader population. For future studies of dose–response in FBF, a wider dose range of SNP, including a lower starting dose and higher maximum dose, could be considered. SNP doses up to 10 μg/min may be used for this purpose.21,22

For buloxibutid, the mean FBF at baseline, prior to administration of the first dose, was comparable in both arms, 2.1 (buloxibutid) and 1.9 mL·100 mL⁻¹·min⁻¹ (control), respectively. Mean baseline FBF measured at the end of each washout period increased in both arms over time with highest values of 2.7 (buloxibutid) and 2.4 mL·100 mL⁻¹·min⁻¹ (control), respectively. The relative increase in mean FBF was comparable for both arms. Due to baseline drift, the baseline FBF prior to infusion of the reference positive control, SNP, was slightly increased, as compared with baseline prior to buloxibutid infusion. The study was undertaken in a controlled environment to minimize variability, but absolute values may still vary due to, for example, circadian rhythm23 and sympathetic tone.13 Room temperature is known to alter FBF measurements.19 A minor increase in ambient temperature during the eight infusions could lead to a corresponding rise in body temperature, potentially influencing FBF through thermoregulatory vasodilation. In addition, baseline drift may be caused by increased vasodilation due to subjects becoming more relaxed and accustomed to the study setting, decreasing sympathetic tone over time. The baseline values for the three SNP infusions appeared to increase slightly with each subsequent dose in the treatment arm, while they remained stable in the control arm. The increasing trend in the treatment arm could suggest a possible drug carryover effect. However, the increase was relatively small for such a potent vasodilator, which implies that this effect, if present, is not pronounced. Although the washout periods were designed to be sufficient, one must consider that a contributing carryover effect cannot be completely ruled out. For future studies, a randomized treatment sequence or priming with vehicle could be used to prevent statistical bias. Increasing washout periods could also be considered but needs to be weighed against the overall time duration of the study for each subject.

Safety

There were three AEs in total in this study, reported by two subjects. All AEs were of mild intensity and unrelated to study drug (buloxibutid) or positive control (SNP). Two AEs, presyncope symptoms and infusion site bruising, were related to study procedures. The invasive aspect of plethysmography with intra-arterial dosing has been described as the major methodological disadvantage,12 and as being burdensome to the subjects.14 Although one subject did report procedure-related AEs, vasovagal reactions are not uncommonly seen in clinical studies with healthy volunteers and regular invasive procedures, such as venipuncture and venous catheters.24 Systemic blood pressure was unaffected in this study.

There is extensive experience with brachial artery infusion of vasoactive agents. The technique is considered safe and serious events are very rare.12,13 For local drug administration, it is essential to know that each dose has reached a plateau before the next dose is given, so as to not risk cumulative local or systemic effects occurring after recording has finished.13

Study design

This study used a microdosing approach to demonstrate proof of mechanism and investigate a pharmacodynamic biomarker of endothelial function (i.e., vasodilation). In microdose studies, a small fraction of the anticipated therapeutic dose is used.7 Exploratory microdose studies can, if taking an accepted approach and following regional guidance, be conducted prior to the traditional first-in-human (FIH) study evaluating safety and tolerability.7,9 It should be noted that this study was not an FIH study, and had been preceded by clinical studies where multiple oral daily doses of up to 200 mg daily had already been found to be well-tolerated (unpublished data, Vicore Pharma AB, Stockholm, Sweden). For comparison, the total dose of buloxibutid administered to each subject in this study was 1.715 mg, which for this drug is well below the maximum dose criteria in approaches recommended for microdose studies, as per the International Conference on Harmonization framework.7

Potential advantages of intra-arterial and other forms of intra-target microdosing studies have previously been discussed.9,25 Here, the methodology enabled us to perform bilateral simultaneous testing of both forearms, where each subject acted as their own control. At less than 50 mL/min, actual FBF is ~100-fold lower than cardiac output.11 Consequently, the systemic exposure and risk of systemic side effects was minimal. Dose selection was based on an assumed actual FBF of ~50 mL/min (i.e., absolute FBF measured in mL/min, not be equated with normalized FBF expressed as mL·100 mL⁻¹·min⁻¹) and aimed to achieve local transient blood concentrations...
comparable to $C_{\text{max}}$ in prior oral studies with human sub-
jects (unpublished data, Vicore Pharma AB, Stockholm,
Sweden). Considering local toxicity, temporary local in-
travascular concentrations in this study were lower than
1/20th of systemic $C_{\text{max}}$ achieved in monkeys without any
safety concerns (unpublished data, Vicore Pharma AB,
Stockholm, Sweden), thereby establishing an ample safety
margin for the subjects in terms of local concentrations in
the forearm.

The sample size of five subjects was determined by the
objective to investigate the effect of buloxibutid on FBF
measured by strain-gauge venous occlusion plethysmog-
raphy in healthy male subjects. Given the drug's novel
mechanism of action and the potential for low AT2R re-
ceptor expression in a healthy population, a small cohort
was selected to obtain an indication of vascular effects of
buloxibutid and to evaluate the usefulness of the method
for this purpose, while not unnecessarily exposing volun-
to invasive procedures. The limitations of this sample
size are recognized, particularly regarding the statistical
power and the low and potential variability in AT2R ex-
pression in healthy subjects.

**Implications of buloxibutid’s
vasodilatory effect**

Vasodilation by AT2R agonists is mediated by NO released
from the endothelium.\textsuperscript{2} The current results show that this
can be achieved in man with clinically relevant doses of
buloxibutid. The impaired release of NO is a defining fea-
ture of endothelial dysfunction, an instigator of vascular
disease, such as pulmonary artery hypertension\textsuperscript{26} and pul-
monary hypertension linked to pulmonary fibrosis.

Results of this study warrant further clinical studies of
buloxibutid in diseases where endothelial dysfunction is a
key factor in the pathogenesis.

**CONCLUSION**

Measurement of FBF can be a useful method for support-
ning proof of mechanism of an AT2R agonist. This method
could be used to document target engagement in vivo
of novel ATRAGs. In this study, a vasodilatory response
following infusion of buloxibutid was demonstrated.
Additional studies with more subjects and potentially a
broader range of doses would be helpful in exploring phar-
macodynamic dose–response. Combining intra-arterial
local microdosing in the forearm with venous occlusion
plethysmography is a straightforward, quick, and safe way
to investigate novel vasoactive agents in an early explora-
tory clinical study setting.

**AUTHOR CONTRIBUTIONS**

E.R.-H. wrote the manuscript. C.G., C.-J.D., T.B., F.S.,
E.R.-H, J.S., and H.Z. designed the research. E.R.H. and
F.S. performed the research. C.G., C.J.D., T.B., F.S., E.R.H.,
J.S., and H.Z. analyzed the data. J.S. and H.Z. contributed
new analytical tools.

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Sweden.

**CONFLICT OF INTEREST STATEMENT**

C.G. and C.J.D. are employees of, and hold shares/share
options in, Vicore Pharma AB. T.B. declares consultancy
fees from Vicore Pharma AB. All other authors declared
no competing interests for this work.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available
from the corresponding author upon reasonable request.

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**REFERENCES**

1. Steckelings UM, Widdop RE, Sturrock ED, et al. The angio-
tensin AT2 receptor: from a binding site to a novel therapeu-
pharmrev.120.000281

2. Schinzari F, Tesauro M, Rovella V, Adamo A, Mores N,
Cardillo C. Coexistence of functional angiotensin II type 2
receptors mediating both vasoconstriction and vasodilation
HJH.0b013e328349ae0d

3. Sumners C, de Kloet AD, Krause EG, Unger T, Steckelings UM.
Angiotensin type 2 receptors: blood pressure regulation and
doi:10.1016/j.coph.2015.01.004

4. Barber MN, Sampey DB, Widdop RE. AT2 receptor stimula-
tion enhances antihypertensive effect of AT1 receptor antago-
doi:10.1161/01.HYP.34.5.1112

5. Maher T, Ganslandt C, Batta R, et al. Interim results from AIR,
an open-label, single arm, 36-week ph 2 trial of C21 in subjects
with idiopathic pulmonary fibrosis. *Eur Respir J*. 2022;60(Suppl


16. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation.* 1993;87(5):1468-1474. doi:10.1161/01.CIR.87.5.1468


SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.