Supplemental oxygen does not modulate responses to acetylcholine or ascorbic acid in the forearm of patients with congestive heart failure

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ABSTRACT
Despite providing symptomatic relief in patients with congestive heart failure (CHF), supplemental oxygen (O₂) has been demonstrated to increase total peripheral resistance. The present study investigated the possibility that O₂ inhalation reduces nitric oxide (NO) bioavailability, using endothelium-dependent (acetylcholine) and -independent (phentolamine) vasodilators, and the antioxidant ascorbic acid. Ten patients (nine male and one female) with primary left ventricular failure participated in the study. Forearm venous occlusion plethysmography was used to study blood flow responses to acetylcholine and the α-adrenergic antagonist phentolamine during inhalation of either room air or 100% O₂, with and without the simultaneous infusion of ascorbic acid. Neither O₂ inhalation (3.9 ± 0.4 compared with 3.8 ± 0.3 ml·min⁻¹·100 ml⁻¹) nor ascorbic acid infusion (5.2 ± 0.4 compared with 5.5 ± 0.4 ml·min⁻¹·100 ml⁻¹) affected resting forearm blood flow. The percentage increase from basal blood flow after acetylcholine infusion was not altered by either O₂ inhalation or ascorbic acid infusion (room air, 140 ± 55%; O₂, 118 ± 46%; ascorbic acid, 147 ± 39%; ascorbic acid + O₂, 109 ± 31%). O₂ inhalation did, however, reduce the dilation induced by phentolamine (room air, 131 ± 24%; O₂, 80 ± 14%; P < 0.05). These data indicate that oxygen inhalation does not increase forearm vascular resistance. Secondly, preservation of reactivity to acetylcholine during O₂ inhalation suggests that degradation of NO by O₂-derived free radicals is not enhanced. Attenuation of phentolamine-induced vasodilation during O₂ inhalation, however, implies increased adrenergic activity, which may possibly exacerbate the detrimental effects of elevated sympathetic activity in CHF.

INTRODUCTION
Supplemental oxygen (O₂) is often administered to patients with congestive heart failure (CHF) to alleviate dyspnoea and hypoxia. However, inhaled O₂ has been shown to further increase total peripheral resistance in CHF patients [1–3] who already have increased vascular resistance and compromised cardiac function. The underlying mechanisms involved in the constrictor response to inhaled O₂ are unclear. A number of different mechanisms are responsible for the elevation in total peripheral resistance in CHF patients. These include increases in sympathetic vasomotor tone, circulating catecholamines and circulating humoral vasoconstrictors, including renin, angiotensin II, vasopressin and endothelin. In addition, endothelial and metabolic vasodilation appear to be impaired. Potentiation of any of these mechanisms may be responsible for the haemodynamic response to inhaled O₂.

Studies using microneurography during O₂ adminis-
tration suggest that its constrictor effects are independent of the sympathetic nervous system in both healthy subjects and CHF patients [1,4,5]. Inhalation of 100% \( O_2 \) has been shown to blunt flow-mediated dilation in response to forearm ischaemia, but not sublingual glyceryl trinitrate [3], in healthy subjects [4] and CHF patients [2]. These data suggest that endothelium-derived nitric oxide (NO) may play a role in the vasoconstrictor response to \( O_2 \). The bioavailability of NO is a function of the amount released and the presence of superoxide anions. Hyperoxia increases oxygen radical production [6,7] and, moreover, has been shown to inactivate NO [8]. Basal NO production is enhanced in CHF patients [9,10], possibly as a counter-regulatory response to constrictor mechanisms or to the increased oxidative stress seen in heart failure, or due to increased activity of inducible NO synthase [11,12].

Pretreatment with the \( O_2 \)-radical scavenger \( N \)-acetyl-cysteine has been shown to attenuate the effects of hyperoxia on indices of cardiac function and tissue oxygenation [13]. Ascorbic acid is an important antioxidant in human plasma, capable of scavenging \( O_2 \)-derived free radicals and sparing other important endogenous antioxidants from consumption [14]. In particular, ascorbic acid at concentrations approximating 10 mmol/l has been shown to be an important competitor of NO for superoxide [15]. Acute intra-arterial administration of ascorbic acid improves flow-mediated dilation in CHF patients [16] and forearm blood flow (FBF) responses to methacholine in both hypercholesterolaemic patients [17] and diabetic patients [18], supporting the concept that endothelial function is at least partially regulated by radicals.

We hypothesized that \( O_2 \)-induced vasoconstriction is mediated by a local mechanism involving decreased NO availability, as a result of either decreased NO production or increased NO consumption by \( O_2 \)-derived radicals. The latter effect, if operative, might be counteracted by acute administration of a suitable antioxidant. The present study compared the dilatory responses to the NO-mediated, endothelium-dependent dilator acetylcholine (ACh), the \( \alpha \)-adrenergic antagonist phentolamine, and the antioxidant ascorbic acid, in the presence and absence of inhaled \( O_2 \). We hypothesized that dilation in response to ACh, but not phentolamine, would be reduced by \( O_2 \) inhalation, and that ascorbic acid would restore the ACh response.

**METHODS**

**Subjects**

Ten patients (nine male and one female; all ex-smokers) with primary left ventricular failure participated in the study. Heart failure was due to either ischaemic heart disease (five patients) or dilated cardiomyopathy (five patients). All subjects gave their written informed consent for participation in the study, which was performed with the approval of the Alfred Hospital Ethics Committee, and in accordance with the Declaration of Helsinki (1989) of the World Medical Association. Inclusion criteria accepted patients with NYHA Class II–III CHF and a left ventricular ejection fraction of \(<30% \) at the time of the study (gated cardiac blood pool scan). All vasoactive medications were withheld for at least 12 h, and warfarin was withheld for 24 h. Patients with unstable angina, uncontrolled hypertension or any other condition that would preclude withholding vasoactive medications were excluded from the study. Patients taking antioxidant vitamin supplements and/or the antioxidant \( \beta \)-blocker carvedilol were also excluded.

**Study design**

Vasodilator responses to ACh and phentolamine were studied while patients breathed room air (study period 1). Then 100% \( O_2 \) was administered for 10 min, and these responses were repeated in the presence of \( O_2 \) (study period 2). The responses to ACh and phentolamine were again repeated in the absence and presence of \( O_2 \), with simultaneous infusion of ascorbic acid (study periods 3 and 4). The study protocol is shown schematically in Figure 1. Administration of room air and 100% \( O_2 \) was ordered, since wash-out time after \( O_2 \) administration would have extended the duration of the study beyond 2000

**Figure 1**  Schematic representation of infusion protocol during periods of room air breathing (open panels), 100% \( O_2 \) inhalation (shaded panels) and ascorbic acid infusion

Blood samples were taken (\( \downarrow \)) 10 min into each phase, immediately before ACh (Ach) infusion. A 10 min washout period was allowed between infusions of ACh (12 \( \mu g/min \)) and phentolamine (Ph; 50 \( \mu g/min \)), and a 45 min recovery period followed the cessation of \( O_2 \) administration.
levels tolerable by the subjects if this intervention was administered first. The study design described allowed multiple intra-arterial infusions during four experimental phases in a patient population that commonly experience dyspnoea in the supine position.

**Study protocol**
Under local anaesthesia and sterile conditions, the brachial artery was cannulated with a 3.0F catheter (Cook, Brisbane, Australia) to allow continuous monitoring of intra-arterial blood pressure (Biosensors International, Singapore), drug infusions and blood sampling. To enable sampling of venous blood draining from the forearm muscle bed, a second catheter (5.0F; Cook) was inserted retrogradely into a deep vein of the forearm. Correct positioning of the deep venous catheter was confirmed by blood gas measurement (O₂ saturation < 65%).

Venous occlusion plethysmography was used to measure FBF, with a mercury-in-silastic strain gauge recording for 10 s out of every 20 s. The hand circulation was excluded using a wrist cuff inflated to 200 mmHg, and venous return was occluded with an upper arm cuff inflated to 40 mmHg. Plethysmography data were digitized and recorded at 10 Hz using a MacLab data acquisition system (MacLab/2e; AD Instruments) connected to an Apple Macintosh SE (Apple Computer, Inc., Cupertino, CA, U.S.A.). Intra-arterial blood pressure was digitized at 500 Hz and a variable-threshold peak-detection technique was used to derive the R–R interval, and thus heart rate and systolic, diastolic and mean blood pressures, on a beat-to-beat basis.

ACh at 12 μg/min (Clinalfa AG, Läufelfingen, Switzerland) was administered via the brachial artery at an infusion rate of 2 ml/min. Phenolamine at 50 μg/min (Regitine; Ciba-Geigy, Sydney, Australia) was administered to assess smooth muscle relaxation via a non-endothelium-dependent, non-NO pathway. The doses of each drug given were chosen to achieve a submaximal increase in FBF and reduction in forearm vascular resistance (FVR) without causing systemic effects [19]. Concomitant infusion of ascorbic acid (David Bull Laboratories, Melbourne, Australia) at 24 mg/min along with ACh or phenolamine was used to assess the effect of an antioxidant on the response to each vasodilator in the presence and the absence of O₂. The dose and protocol for the administration of ascorbic acid were identical with those shown previously to augment endothelium-dependent dilation in smokers [20], hypercholesterolaemic patients [17] and diabetic patients [18]. This dose approximated a plasma concentration of 1–10 mmol/L, a concentration which has been shown to effectively compete with NO for superoxide in a rabbit model [15] and to protect human plasma from free-radical-mediated lipid peroxidation [21]. A 10 min washout period was allowed between each drug infusion, and a 45 min recovery period following cessation of O₂ inhalation, prior to ascorbic acid infusion.

Infrabrachial mean arterial pressure was recorded during measurement of basal flows and immediately following each drug infusion, for calculation of FVR and to monitor possible systemic effects.

Simultaneous arterial and venous samples were taken at each stage prior to infusion of vasodilator drugs (i.e. room air, O₂ inhalation, room air + ascorbic acid, O₂ + ascorbic acid). Blood gas analyses were performed on these samples. Lipid profiles were also determined from the first resting venous sample.

**Biochemical analyses**
Blood for lipid analysis was collected into EDTA tubes, and plasma was analysed within 5 days of collection for total, low-density-lipoprotein and high-density-lipoprotein cholesterol and for triacylglycerols using a Cobas-BIO centrifugal analyser (Roche Diagnostic Systems).

O₂ content was determined no later than 10 min after sampling, using an ABL 500 blood gas analyser (Radiometer, Copenhagen, Denmark) for measurement of arterial partial pressure of O₂ (PaO₂) and oxygen saturation. Oxygen content [in ml/100 ml (STP–dry)] was calculated as the sum of bound and dissolved oxygen: $(S/100) \times [Hb] \times 1.34 + \alpha \times PaO_2$, where $\alpha$ is the percentage saturation of haemoglobin, $[Hb]$ is the haemoglobin concentration in g/100 ml, and $\alpha$ is the solubility coefficient for oxygen in whole blood [0.0031 ml of O₂:ml⁻¹ blood:mmHg⁻¹ (STP–dry) at 37 °C], and PaO₂ is in mmHg.

**Statistical analysis**
Statistical analyses were performed using SPSS software (version 8.0 for Windows; SPSS Inc.). All measurements are presented as means ± S.E.M. unless otherwise indicated. Statistical analyses of responses to each drug with/without ascorbic acid and O₂ used analysis of variance (ANOVA) for repeated measures. Individual
means were compared using Fischer's least-significant-difference analysis. Statistical significance was accepted where \( P < 0.05 \).

**RESULTS**

**Subjects**

The clinical characteristics of the study group are shown in Table 1. Basal blood flow, blood pressure and heart rate did not change throughout the study (Figure 2).

**O\(_2\) inhalation**

O\(_2\) inhalation increased \( \text{PaO}_2 \) from 82 ± 3 to 232 ± 14 mmHg. Following cessation of O\(_2\) inhalation and commencement of ascorbic acid infusion, \( \text{PaO}_2 \) returned to control values (77 ± 3 mmHg), and then increased to 206 ± 18 mmHg during the second O\(_2\) inhalation period (Table 2). Despite being a known vasoconstrictor, O\(_2\) did not cause a measurable decrease in FBF (room air, 3.9 ± 0.4 ml min\(^{-1}\) 100 ml\(^{-1}\); O\(_2\), 3.8 ± 0.3 ml min\(^{-1}\) 100 ml\(^{-1}\)) or FVR (room air, 25.3 ± 3.8 units; O\(_2\), 24.2 ± 2.6 units). Mean arterial pressure (room air, 87 ± 4 mmHg; O\(_2\), 85 ± 5 mmHg) and heart rate (room air, 75 ± 4 beats/min; O\(_2\), 71 ± 5 beats/min) also remained unchanged.

**Ascorbic acid**

FBF (control, 5.2 ± 0.4 ml min\(^{-1}\) 100 ml\(^{-1}\); ascorbic acid, 5.5 ± 0.4 ml min\(^{-1}\) 100 ml\(^{-1}\)) and FVR (control, 18.4 ± 1.9 units; ascorbic acid, 16.6 ± 1.5 units) were not significantly altered during infusion of ascorbic acid.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Room air</th>
<th>( \text{O}_2 )</th>
<th>Ascorbic acid</th>
<th>Ascorbic acid + ( \text{O}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{O}_2 )  saturation (%)</td>
<td>96.0 ± 0.5</td>
<td>99.6 ± 0.1(^*)</td>
<td>95.1 ± 0.6</td>
<td>99.4 ± 0.1(^*)</td>
</tr>
<tr>
<td>( \text{PaO}_2 ) (mmHg)</td>
<td>82 ± 3</td>
<td>232 ± 14(^*)</td>
<td>77 ± 3</td>
<td>206 ± 18(^*)</td>
</tr>
<tr>
<td>( \text{O}_2 ) content (ml/100 ml)</td>
<td>17.7 ± 0.5</td>
<td>18.8 ± 0.6(^*)</td>
<td>17.5 ± 0.5</td>
<td>18.7 ± 0.5(^*)</td>
</tr>
<tr>
<td>( \text{PaO}_2 ) (mmHg)</td>
<td>40 ± 1</td>
<td>41 ± 2</td>
<td>42 ± 2</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 ± 0.01</td>
<td>7.45 ± 0.01</td>
<td>7.44 ± 0.01</td>
<td>7.44 ± 0.01</td>
</tr>
</tbody>
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**ACh**

In response to the intra-arterial infusion of ACh (room air), FBF increased from $3.0 \pm 0.3$ to $6.5 \pm 1.1 \, ml \cdot min^{-1} \cdot 100 \, ml^{-1}$, an increase of $140 \pm 55 \%$. The percentage increase in FBF from basal values in response to ACh did not change significantly upon inhalation of $100 \%$ $O_2$ ($118 \pm 46 \%$) or intrabrachial infusion of ascorbic acid ($147 \pm 39 \%$), or with both treatments ($109 \pm 31 \%$) (Figure 3, upper panel).

**Phentolamine**

Phentolamine increased FBF from $3.1 \pm 0.4$ to $6.3 \pm 0.4 \, ml \cdot min^{-1} \cdot 100 \, ml^{-1}$, an increase of $131 \pm 24 \%$. This dilator response was significantly reduced by $O_2$ to $80 \pm 14 \%$ (Figure 3, lower panel). The percentage increase in FBF in response to phentolamine was significantly lower during ascorbic acid infusion both in the absence ($81 \pm 12 \%$) and in the presence ($72 \pm 12 \%$) of $O_2$.

**DISCUSSION**

Increased oxidative stress is associated with CHF of various aetiologies [22–26]. Further production of free radicals and inactivation of NO during hyperoxia has been suggested as the mechanism responsible for $O_2$-induced constriction in CHF [1]. Similar mechanisms have been proposed for the response to $O_2$ seen in haemorrhagic shock [27] and cirrhosis [28,29]. Despite these findings, and previous studies in normal healthy individuals suggesting that $100 \%$ $O_2$ inhalation increases total peripheral resistance [30–32], in the present study we found that oxygen inhalation did not increase FVR. Furthermore, responses to both ACh and ascorbic acid were unaffected by oxygen inhalation, arguing against inactivation of NO in the forearm as an important effect of $O_2$ inhalation. Forearm responses to phentolamine were depressed during both $O_2$ inhalation and ascorbic acid infusion. These data are consistent with enhanced adrenergic activity.

**Reactivity to ACh**

The absence of an effect of $O_2$ inhalation on forearm vascular dilation in response to ACh is consistent with studies of isolated feline ophthalmociliary arteries, where the dose–response curve to ACh was not affected by changes in oxygen tension [33]. Furthermore, despite its capacity to reverse endothelial dysfunction in CHF [16] as well as other disease states [17,18,20,34,35], infusion of the antioxidant ascorbic acid did not affect responsiveness to ACh during inhalation of either room air or oxygen. These data may be explained by the fact that, in addition to inactivation of NO [8], superoxide and other $O_2^-$ derived free radicals have opposing effects on the NO system. For example, hydrogen peroxide and the hydroxyl radical evoke vasodilation by acting directly on vascular smooth muscle and also by stimulating the synthesis and release of NO [36]. Together, these data suggest that decreased NO bioavailability is not an important effect of $O_2$ inhalation in the forearm circulation of patients with cardiac failure.

**Reactivity to phentolamine**

The present study does not permit elucidation of the mechanisms underlying the reduced responsiveness to phentolamine. $O_2$ inhalation may increase the bioavailability of noradrenaline or augment $\alpha$-adrenoceptor sensitivity. The former could result from either an elevation of sympathetic nerve activity in the presence of $O_2$ or diminished inactivation of noradrenaline. Both possibilities appear unlikely, based on previous literature. Sympathetic nerve activity has been shown to be unchanged or diminished by $O_2$ [1,5,37]. Secondly, noradrenaline inactivation is likely to be enhanced rather than diminished in the setting of increased oxidant stress [38]. The possibility that $\alpha$-adrenoceptor sensitivity to noradrenaline is increased during $O_2$ inhalation remains. Indeed, a number of studies have shown, both in vivo [39] and in vitro [33], that noradrenaline constrictor responses are enhanced at higher $O_2$ tensions. $O_2$ inhalation, while beneficial with regard to the relief of dyspnoea and hypoxia, is not without side effects, including increased adrenergic sensitivity which may exacerbate the already elevated sympathetic activity associated with cardiac failure [40].

During breathing of room air, ascorbic acid also reduced the dilator response to phentolamine. This effect may relate to the inactivation of noradrenaline by the superoxide anion and the scavenger properties of ascorbic acid. The superoxide anion inactivates noradrenaline via oxidation to an inactive metabolite [38]. Singlet oxygen also depresses noradrenaline-induced contraction, in part via oxidant-induced receptor dysfunction [41]. Oxygen radicals can cause an endothelium-independent, oxidant-mediated decrease in noradrenaline responsiveness that may be related to defects in the mobilization of intracellular calcium from the smooth muscle sarcoplasmic reticulum [42]. Ascorbic acid is capable of preventing or delaying the oxidation of noradrenaline [43], and so, by increasing noradrenaline bioavailability, may result in reduced dilatation in response to submaximal phentolamine. $O_2$ inhalation in the presence of ascorbic acid did not reduce the dilator response to phentolamine further.

**Conclusion**

These data show that inhalation of $100 \%$ $O_2$ does not cause a significant increase in FVR. Secondly, preservation of ACh reactivity during $O_2$ inhalation suggests that degradation of NO by $O_2^-$-derived free radicals is not
enhanced in the forearm. Attenuation of phenolamine-induced vasodilation during O$_2$ inhalation, however, implies increased adrenergic activity, which may possibly exacerbate the detrimental effects of elevated sympathetic activity in CHF [40].

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REFERENCES


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