Desferrioxamine elevates pulmonary vascular resistance in humans: potential for involvement of HIF-1

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The mechanisms responsible for this slower component of the pulmonary vascular response to hypoxia remain unclear (13, 22), but one possibility is that the transcriptional regulator hypoxia-inducible factor (HIF)-1 is involved, as it is in some other homeostatic responses to hypoxia (18). Yu et al. (23) examined the kinetics of induction of increased levels of the subunit HIF-1α in isolated ferret lungs exposed to 8 h of hypoxia. They found that HIF-1α was not detectable at 0 h, increased at 1–2 h, peaked at 4 h, and remained elevated thereafter (23). Furthermore, the work of Yu et al. related the activity of HIF-1 to pulmonary vascular responses to hypoxia in mice. They showed that mice that are heterozygous for the HIF-1α gene, although apparently normally developed, have a substantially impaired chronic pulmonary vascular response to 1–6 wk of hypoxia compared with wild-type mice (24).

The reaction by which oxygen regulates HIF-1 has an absolute requirement for iron as a cofactor (10, 11). In a recent study by Ren et al. (17), it was demonstrated that 8 h of desferrioxamine (DFO) infusion increased erythropoietin production in humans, which is a response to sustained hypoxia that is known to be regulated by HIF-1 (21). Thus, if hypoxic pulmonary vasoconstriction is regulated by HIF-1, then it might be anticipated that iron chelation would induce a rise in pulmonary arterial pressure.

The objective of the present study was to examine whether DFO-induced iron chelation in humans could mimic hypoxia by inducing an increase in PVR with a similar time course. In study A, we characterized the human pulmonary vascular response to 4 h of isocapnic hypoxia by using Doppler echocardiographic measurement of tricuspid regurgitation. In study B, we used the same monitoring to examine the pulmonary vascular response to an 8-h DFO infusion.

METHODS

Subjects. Eleven healthy volunteers (3 women, 8 men) participated in study A. Nine healthy volunteers (5 women, 4 men) participated in study B. Subjects visited the laboratory once or twice before the experiment to become familiarized

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There is evidence to suggest that the human pulmonary circulation responds to sustained hypoxia by developing a progressively more intense constriction over a period of a few hours. In anesthetized patients, Fiser et al. (4) observed a gradual decline in pulmonary venous admixture during unilateral atelectasis over 90 min, suggesting that blood flow diversion by hypoxic pulmonary vasoconstriction was intensifying during this time. Exposure of healthy humans to an 8-h isocapnic hypoxia by using Doppler echocardiographic measurement of tricuspid regurgitation. In study B, we used the same monitoring to examine the pulmonary vascular response to an 8-h DFO infusion.

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with the procedure and to confirm that they were suitable for echocardiographic measurement of tricuspid regurgitation. Female subjects only participated during the first 14 days of their menstrual cycle. The appropriate protocols were explained to all participants, but they were naive about the exact purpose of the studies. Informed, written consent was obtained from all subjects on each experimental day. Ethical permission was obtained from the Central Oxford Research Ethics Committee.

Protocols for study A. Subjects undertook two protocols. Protocol A1 consisted of 4 h of isocapnic hypoxia with $\text{PETCO}_2$ maintained at 50 Torr and end-tidal carbon dioxide partial pressure ($\text{PETCO}_2$) maintained at each subject's normal value, followed by 2 h of isocapnic euoxia ($\text{PETCO}_2 = 100$ Torr). Protocol A2 consisted of 6 h of air breathing (control). In protocol A1, during hypoxia, echocardiography measurements were obtained every 30 min for the first 2 h and every 60 min for the remaining 2 h. During euoxia, echocardiography measurements were obtained every 30 min. In protocol A2, echocardiographic measurements were taken at the same times as in protocol A1.

Subjects were asked to refrain from alcohol and caffeine intake on experimental days. For every visit to the laboratory, subjects were allowed at least 30 min to settle before measurements were obtained. During the experiments, food and drinks were provided frequently but in small quantities in an effort to minimize the effects of digestion on cardiac variables. Subjects were free to watch television, read, write, or relax, according to their own preference.

Protocols for study B. Subjects undertook two protocols. Protocol B1 consisted of 8 h of DFO infusion (4 g/70 kg body wt). Protocol B2 consisted of 8 h of saline infusion (control). Both infusions were followed by 4 h of observation without infusion (early recovery). During the infusion, echocardiography measurements were made every 30 min for the first 2 h and every 60 min for the remaining 6 h. Measurements were obtained again every 30 min for the first 2 h of the recovery period and finally every 60 min until the end of the experiment. All subjects returned to the laboratory the next day for a final set of measurements at 24 h (late recovery) after the start of the infusion. In other respects, subjects were treated similarly to those who underwent study A.

Control of end-tidal gases (study A). Subjects undergoing study A were seated in a chamber in which the inspired gas partial pressures could be controlled so as to maintain desired end-tidal levels. Respired gas was sampled continuously via a nasal cannula and analyzed with a mass spectrometer. The inspired and end-expired values were recorded breath by breath on a computer. Computer-automated control of $\text{PETCO}_2$ and $\text{PETCO}_2$ was achieved by adjustment of the chamber gas composition every 5 min, as described elsewhere (7). During the control protocol for study A, subjects were seated in the chamber and breathed air.

DFO infusion (study B). DFO (Desfrol, Ciba-Geigy, Cheshire, UK) was dissolved in water (0.1 g/ml) and diluted with saline to a total volume of 50 ml. For the control infusion, 50 ml of saline were used. A vein in the back of the hand or the forearm was used for the infusions. The vein was cannulated aseptically, and an infusion pump was used to infuse the contents of the syringe at a constant rate over 8 h.

Echocardiographic measurements. Echocardiographic measurements were performed by using a Hewlett-Packard Sonos 5500 ultrasound machine with an S4 two-dimensional transducer (2–4 MHz). Heart rate and respiratory waveform were both recorded. Subjects were examined on a suitably modified couch while rolled slightly onto their left side. For each view, enough time was allowed for a consistent waveform and heart rate to appear before data from a series of consecutive heart cycles were stored to optical disk. At least three beats at end expiration were analyzed and averaged at a later time.

Tricuspid valve maximal pressure difference. The majority of people have detectable regurgitation through their tricuspid valve during systole. Doppler echocardiography is able to detect the jet and measure the velocity with which it travels back into the right atrium. On the assumption that the flow within the jet may be regarded as steady, Bernoulli's equation (Eq. 1) can be used to calculate the pressure difference between the right ventricle and right atrium

$$\Delta \text{P}_{\text{max}} = \rho v^2/2$$

where $\Delta \text{P}_{\text{max}}$ is maximal pressure difference, $\rho$ is the density of blood, and $v$ is the peak velocity of the jet. From this, systolic pulmonary arterial pressure (SPAP) is given by

$$\text{SPAP} = \text{RAP} + \Delta \text{P}_{\text{max}}$$

where RAP is right atrial pressure. If we assume RAP is constant, changes in $\Delta \text{P}_{\text{max}}$ will be equal to changes in SPAP. Furthermore, as it has been shown previously that SPAP and PVR correlate well within individual subjects (3), changes in $\Delta \text{P}_{\text{max}}$ can be used as an index of changes in PVR.

A standard technique was used for the measurement of $\Delta \text{P}_{\text{max}}$ (16). Two-dimensional echocardiography allowed visualization of the tricuspid valve in an apical four-chamber view, and then color Doppler format allowed for the regurgitant jet to be detected. After proper alignment with the Doppler beam, continuous-wave spectral analysis at a sweep speed of 50 mm/s was used to record the velocity profile of the jet. During analysis, the maximal velocity of the jet was measured by placing an electronic caliper tool at the most distal part of the envelope. $\Delta \text{P}_{\text{max}}$ values were then computed automatically.

Cardiac output. An apical five-chamber view was obtained, and by using color Doppler mode the flow through the aortic valve during systole was identified. Once this had been done, the velocity profile was obtained in pulsed-wave spectral mode at a display screen sweep speed of 100 mm/s. Doppler sampling of the flow was made just below the orifice of the aortic valve. An automated procedure was then used to calculate the velocity time integral (VTI) of the flow. VTI, measured in centimeters, represents the distance through which blood travels in the outflow tract with each ventricular contraction.

The diameter of the aortic valve was measured from a parasternal long axis view, and the area of the aortic valve (AoVarea) was calculated. The product of VTI and AoVarea gives the stroke volume (SV). Cardiac output ($Q$) can then be calculated by multiplying SV and heart rate (HR). These calculations are shown in Eq. 3

$$Q = SV \times HR = (\text{VTI} \times \text{AoVarea}) \times HR$$

Statistical analysis. Repeated-measures ANOVA was undertaken to determine whether there was an interaction between time and protocol. This analysis was undertaken separately for the data from study A (hypoxia vs. air-breathing control) and study B (DFO infusion vs. control infusion). Separate repeated-measures ANOVA were then performed on the data from just the control protocols to check that time did not have a significant effect in these protocols.

To determine the time at which steady state had been reached after the induction of hypoxia (study A) or the start of DFO infusion (study B), a family of linear models was used.
Gas control during study A. Figure 1 illustrates values for inspiratory and end-tidal PO$_2$ and PCO$_2$ for study A. For protocol A1, it may be seen that the steps into and out of hypoxia were achieved rapidly and that isocapnia was maintained with considerable precision throughout. For protocol A2, the end-tidal gases changed little over the 6-h period.

Pulmonary vascular response to hypoxia in study A. The changes in ΔP$_\text{max}$ during isocapnic hypoxia and the ensuing period of isocapnic euoxia (protocol A1) are presented in Fig. 2, together with data from the air-breathing control (protocol A2). ΔP$_\text{max}$ was significantly affected by hypoxia compared with euoxia ($P < 0.001$). No change in ΔP$_\text{max}$ was seen during the air-breathing control protocol. A progressive rise in ΔP$_\text{max}$ was observed during hypoxia that had not reached steady state by 3 h ($P < 0.001$). During the period of euoxia that followed 4 h of hypoxia, ΔP$_\text{max}$ had not reached steady state by 1.5 h of euoxia ($P < 0.02$).

Heart rate, stroke volume, and cardiac output responses to hypoxia in study A. Figure 3 shows heart rate, stroke volume, and cardiac output for the two protocols of study A. Heart rate was significantly and substantially elevated by hypoxia (repeated-measures ANOVA $P < 0.001$). Little change was seen in stroke volume, and so changes in heart rate were reflected in changes in cardiac output (Eq. 3). The rise in heart rate between 2 and 3 h coincides with the time at which most subjects ate a small lunch during both protocols. In Fig. 4 are plotted the absolute differences between the
measurements of heart rate, stroke volume, and cardiac output shown in Fig. 3, making the effects of hypoxia alone easier to appreciate.

**Study B: Pulmonary vascular response to DFO.** The changes in ΔPmax during DFO infusion and saline infusion are shown in Fig. 5. DFO induced a significant change in ΔPmax compared with control (P < 0.005). During DFO infusion, ΔPmax reached steady state by 3 h (P = 0.1) but not by 2 h (P < 0.02) and remained elevated until the end of the DFO infusion at 8 h. Although not significant, there appeared to be a decrease in ΔPmax in the early recovery period. At 24 h after the start of DFO infusion, ΔPmax had returned to control levels.

**Study B: Heart rate, stroke volume, and cardiac output responses to DFO.** Figure 6 shows heart rate, stroke volume, and cardiac output for study B. Stroke volume was lower at the commencement of the DFO infusions than at the beginning of the saline infusions and remained so throughout the 24 h of the study. This appears likely to be entirely a chance finding because the subjects and starting conditions for both protocols were identical. There was no statistically significant difference between the two protocols with regard to any of the three physiological variables in Fig. 6.

**DISCUSSION**

The main finding of study A was that the human pulmonary vascular response to hypoxia, as measured by use of Doppler echocardiography, increased progressively over at least 3 h and was several times greater after 4 h than after 30 min. The main finding of study B was that an 8-h infusion of DFO in humans induced a modest rise in tricuspid valve maximal pressure

![Fig. 3. Heart rate (A), stroke volume (B), and cardiac output (C) for study A. Closed symbols, measurements made under hypoxic conditions; open symbols, measurements made under euoxic conditions. Circles, protocol A1; diamonds, protocol A2. Data are means for 11 subjects; error bars are ± SE. Hypoxia induced a significant rise in heart rate (repeated-measures ANOVA P < 0.001) that was reflected in a rise in cardiac output.](image)

![Fig. 4. Mean differences (Δ) in heart rate (A), stroke volume (B), and cardiac output (C) between protocols A1 and A2 of study A. Closed symbols indicate the time points for which measurements were made under hypoxic conditions in protocol A1. Data are means for 11 subjects; error bars are ± SE. These data are derived from those in Fig. 3.](image)
gradient that had a very similar pattern over time to the more pronounced rise induced by hypoxia in study A. The findings suggest that HIF-1 may be involved in the pulmonary vascular responses to hypoxia over times of 0.5–4 h. We are unable to comment on responses over a shorter period than 0.5 h, because measurements were not made within this period, although others have seen very rapid responses (14). Because both the response to hypoxia and the response to DFO are likely to be dependent on the dose used, interpretation of the relative magnitudes of the pulmonary vascular responses seen in study A and study B is difficult to undertake without further studies.

**Echocardiographic assessment of SPAP.** Our results from study A may be compared with a very similar study in humans at the same level of (isocapnic) hypoxia in which pulmonary arterial pressures together with PVR were obtained directly by right heart catheterization (3). In that study, there was a progressive rise in SPAP over the first 2 h of isocapnic hypoxia, with SPAP remaining relatively constant over the rest of the hypoxic exposure. In the present study, ΔPmax also rose progressively over the first 2 h of exposure, but in this case a steady state was not obtained until at least 3 h had elapsed. The absolute values for SPAP from the previous study compare rather poorly with the absolute values for ΔPmax from the present study. One possible reason for this is that the subjects were seated in the former study, but lying down in the present study. The average change in SPAP over 4 h of hypoxia in the previous study was 17 Torr, and this compares closely with the average change in ΔPmax in the present study of 15 Torr. These data provide support for the use of changes in ΔPmax assessed by echocardiography as measures of changes in SPAP.

**Measures of pulmonary vascular tone.** None of the measures relating to the whole human lung (ΔPmax, SPAP, PVR) is a direct measure of the pulmonary vascular smooth muscle activity that is the variable of principal concern. In particular, there is a strong body of evidence supporting the notion that PVR varies substantially as a function of cardiac output in the absence of any underlying variations in pulmonary vascular smooth muscle activity. During exercise in humans, there is relatively little change in the arteriovenous pressure difference across the lung as cardiac output increases three- to fourfold (12). Similar results have been obtained for SPAP assessed by echocardiography (6). Quantitatively, these results suggest that
there is an approximately hyperbolic relationship between PVR and cardiac output when other factors that might affect pulmonary vascular tone remain constant.

A relationship between SPAP and PVR may be inferred from the effects of hypoxia on the pulmonary circulation from the data of Dorrington et al. (3) as

\[ SPAP = 11 \times PVR + 8 \]  \hspace{1cm} (5)

where PVR is in units of Torr \( \cdot \) min \( \cdot \) l \( \cdot \) \(^{-1} \). The presence of the intercept in this relation means that any given percentage change in PVR will result in smaller percentage change in SPAP. However, it should also be realized that this relationship was determined against a background of some increase in cardiac output over the course of the hypoxic exposure. Changes in cardiac output necessarily induce an alteration in the relationship between SPAP and PVR because for any particular PVR higher values of cardiac output will be associated with higher values of SPAP.

Interestingly, the combination of factors discussed in the preceding two paragraphs may mean that SPAP (or \( \Delta P_{max} \)) may be a better measure of underlying pulmonary vascular smooth muscle tone than PVR itself because it is less influenced by changes in cardiac output. At its most simple, we may consider SPAP to be related through an Ohm's law type of relationship to PVR and cardiac output such that SPAP = \( k \times Q \times PVR \), where \( k \) is a constant. This is precise if SPAP remains proportional to mean pulmonary arterial pressure. If PVR is related to cardiac output through a hyperbolic relationship as discussed above (\( PVR = m/Q \), where \( m \) is the underlying vascular tone), then substituting for PVR in the first relationship from the second yields \( SPAP = km \). Under these simple conditions, SPAP (or \( \Delta P_{max} \)) becomes a direct measure of pulmonary vascular smooth muscle tone (i.e., \( m \)) that is invariant with changes in cardiac output.

**DFO and pulmonary vascular tone.** DFO has been shown repeatedly to stabilize HIF in vitro by iron chelation and to induce HIF-regulated gene expression (21). Recently, an oxygen-sensitive mechanism for the degradation of HIF has been described, whereby a prolyl hydroxyxylases a proline residue in HIF, which tags the protein for proteosomal degradation (10, 11). This prolyl hydroxyxylase requires the presence of iron for its activity. In a human study, it has been shown that DFO may also induce HIF-dependent gene expression, in the sense that an 8-h infusion of DFO resulted in a dramatic increase in erythropoietin in the plasma (17). Thus, it would seem a reasonable proposition that DFO can stimulate other HIF-dependent gene expression in vivo in humans. In mice, it has recently been shown by Shimoda et al. (19) that partial deficiency of HIF-1α is sufficient to impair hypoxia-induced depolarization and reduction of membrane potassium currents within pulmonary artery smooth muscle cells. This evidence provides another possible link between DFO induction of HIF and increased PVR. An important factor in favor of such a link in our own study is that the time course of the pulmonary vascular response to DFO in study B is very similar to that to hypoxia in study A. This is illustrated in Fig. 7, where \( \Delta P_{max} \) for study A has been plotted against \( \Delta P_{max} \) for study B. In this figure, differences in the speed of response of \( \Delta P_{max} \) to the two stimuli would appear as deviations from linearity. Although the magnitude of the response to hypoxia is approximately four times greater than that to DFO, the correlation coefficient between the two responses is very high at 0.99.

HIF is now known to be a regulatory component in the expression of many genes. Among those that could have a role in regulating pulmonary vascular tone are endothelin-1 (8) and nitric oxide synthase (15).

Despite the above points in favor of DFO acting through a stabilization of HIF, it nevertheless remains possible that DFO may act in other ways. For example, it may reduce free radical production through lowering iron availability (1) or alternatively increase free radical production (20), possibly through an effect on the expression of heme oxygenase 1 (5). The latter effect may be a particular case of a more widespread regulatory effect of iron on gene expression, which could provide an alternative explanation for the effect of DFO in our experiments.

Finally, it is pertinent to consider whether DFO may be acting other than locally within the lung. The pulmonary vasculature is innervated by both sympathetic and parasympathetic nerves (9) and also potentially may be influenced by hormonal action. For example, a recent study has suggested that a progressive reduction in vagal tone may be responsible for the gradual elevation of heart rate in humans exposed to sustained hypoxia (2), and it is possible that such an alteration to the parasympathetic supply to the pulmonary vasculature could also modify PVR (9).

In summary, we have shown that the human pulmonary vascular responses to DFO infusion and hypoxia follow a very similar time course over 4 h but differ in their magnitudes. Taken together with the published
evidence that DFO infusion in humans can induce HIF-related erythropoietin gene expression, that some vasoactive mediators are under HIF control, and that HIF deficiency in mice impairs the electrophysiological responses to hypoxia of pulmonary artery smooth muscle cells, our results suggest that hypoxia might induce pulmonary hypertension at least in part via a HIF-related mechanism.

REFERENCES


