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Desferroxamine infusion increases cerebral blood flow: a potential association with hypoxia-inducible factor-1

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Abstract

Finding an effective means to improve cerebral perfusion during hypoxic/ischaemic stress is essential for neuroprotection. Studies in animal models of stroke have shown that desferroxamine activates HIF-1 (hypoxia-inducible factor-1), reduces brain damage and promotes functional recovery. The present study was designed to investigate the effects of desferroxamine infusion on the cerebral circulation in humans. Fifteen volunteers were enrolled in a randomized double-blind placebo-controlled crossover study. We measured cerebral blood flow velocity by transcranial Doppler ultrasonography in the middle cerebral artery, arterial blood pressure, end-tidal CO₂, as well as HIF-1 protein and serum lactate dehydrogenase concentrations in response to 8 h of desferroxamine compared with placebo infusion. Cerebrovascular resistance was calculated from the ratio of steady-state beat-to-beat values for blood pressure to blood flow velocity. We found that desferroxamine infusion was associated with a significant cerebral vasodilation. Moreover, decreased cerebrovascular resistance was temporally correlated with an increased HIF-1 protein concentration as well as HIF-1 transcriptional activation, as measured by serum lactate dehydrogenase concentration. The findings of the present study provide preliminary data suggesting that activators of HIF-1, such as desferroxamine, may protect neurons against ischaemic injury by dilating cerebral vessels and enhancing cerebral perfusion.

Keywords

cerebral blood flow; desferroxamine; hypoxia-inducible factor-1 (HIF-1); transcranial Doppler ultrasound

Introduction

Cerebral ischaemia is a common, morbid condition with severe life-threatening consequences. Unfortunately, there are currently no effective neuroprotective interventions to prevent ischaemic brain injury. Most of our research efforts have been directed at blocking specific

steps in apoptotic or excitotoxic cell death, whereas measures to improve perfusion have been limited. It is important to remember that neuroprotection is unlikely to work without improvements in flow and eventually recanalization. Therefore simple pharmacological measures that can safely enhance cerebral perfusion may have a significant impact on future clinical trials of stroke when used in conjunction with novel neuroprotective agents.

One possible approach to enhance cerebral perfusion may be via pharmacological activation of endogenous pathways responsible for enhancing oxygen and glucose delivery during hypoxic/ischaemic injury. HIF-1 (hypoxia-inducible factor-1) is considered to be a master regulator of oxygen homeostasis [1]. Recently, HIF-1 has been well-characterized, making it a very attractive pharmacological target to enhance cerebral perfusion [2,3]. HIF-1 is a heterodimeric transcription factor expressed in all tissues in response to hypoxia. It activates a repertoire of target genes, such as NOS (NO synthase), VEGF (vascular endothelial growth factor), EPO (erythropoietin), and glycolytic enzymes such as LDH (lactate dehydrogenase) [4], which function to protect oxygen- or glucose-deprived cells by activating a cascade of cellular, local and systemic responses aimed at increasing oxygen and glucose delivery to ischaemic organs. HIF-1 activation can also be achieved by means other than ischaemia or hypoxia.

Animal studies demonstrate that systemic administration of iron chelators such as DFO (desferrioxamine) results in HIF-1 activation as well ischaemic neuroprotection [5–8]. More recently, delayed administration of DFO has also been shown to reduce brain damage and promote functional recovery after transient focal cerebral ischaemia in the rat [9], suggesting that HIF-1 activation may also be involved in mechanisms underlying functional recovery after stroke. However, the mechanisms underlying DFO-mediated ischaemic tolerance and functional recovery, are unknown. Although HIF-1 activation may be one mechanism, DFO may have additional benefits via its radical scavenging and iron-chelating properties.

Results from prior studies suggest that DFO may be working at the vascular level. These studies have shown that systemic DFO increases forearm blood flow and improves vasoreactivity in patients with endothelial dysfunction [10]. DFO also prevents cerebral vasospasm in animal models of subarachnoid haemorrhage [11]. Moreover, starch-conjugated DFO, which is unable to cross the blood–brain barrier, can maintain its neuroprotective effects in animal models of ischaemia [12], suggesting a role for vascular endothelial cells as the site of DFO action. The results from these studies all support the notion that DFO may be mediating its effects within the vascular lumen and outside the blood–brain barrier.

Despite the availability of this clinically approved drug for use in humans, there are no prior clinical studies of DFO in humans. Therefore the present study was designed to examine the effects of DFO on cerebral blood flow and to determine whether there is a potential association between DFO-mediated enhanced cerebral perfusion and HIF-1 activation in humans.

Materials and Methods

Subjects

Fifteen subjects (58 ± 21 years, range 22–78 years) volunteered to participate in the study. Subjects were recruited from advertisements in the Partners Research Studies Bulletin and the Harvard Cooperative Program on Aging subject registry. All subjects were carefully screened with a medical history, physical examination and ECG to exclude any acute or chronic medical conditions. Subjects were non-smokers and refrained from alcohol for at least 12 h prior to the study. The study was approved by the Partners Healthcare Institutional Review Board, and followed institutional guidelines. All subjects gave written informed consent.

Experimental protocol

Study design—Each subject was admitted twice to the Clinical Research Center at the Brigham and Women's Hospital. Each admission was for 24 h and scheduled 30 days apart. Subjects were randomized to receive either DFO or PLB (placebo) during each admission. Subjects were instrumented as described below.

Instrumentation—Subjects reported to the Brigham and Women's Hospital Clinical Research Center at 07.00 hours prior to eating breakfast. Subjects were instrumented for heart rate (ECG) and beat-to-beat ABP [arterial BP (blood pressure)] monitoring (Finapres; Ohmeda Monitoring Systems) as previously described [13]. End-tidal CO₂ was measured using a VacuMed CO₂ analyser.

TCD (transcranial Doppler) ultrasonography (MultiDop X4; DWL-Transcranial Doppler Systems) was used to measure MCA (middle cerebral artery) mean BFV (blood flow velocity) at rest and in response to: (i) BP changes during a sit-to-stand protocol [13,14], and (ii) changes in end-tidal CO₂, as previously described [13]. The MCA signal was identified according to the criteria of Aaslid et al. [15] and recorded at a depth of 50–60 mm. A Mueller–Moll probe-fixation device was used to stabilize the Doppler probe for the duration of the study day. The envelope of the velocity waveform, derived from a fast-Fourier analysis of the Doppler frequency signal, was digitized at 500 Hz, displayed simultaneously with the ABP, ECG and end-tidal CO₂ signals, and stored for later off-line analysis. BFV is assumed to represent blood flow through the MCA because the diameter of the MCA has been shown to remain constant during manoeuvres that change cerebral blood flow [16]. Several previous studies using a variety of techniques [¹³³Xe, SPECT (single-photon emission computed tomography), MRI (magnetic resonance imaging)] have confirmed that relative changes in cerebral BFV are representative of changes in cerebral blood flow [17,18].

Study protocol—After instrumentation, two IV (intravenous) catheters were placed, one in each arm. D₅NS (dextrose/normal saline) was infused into the arm contralateral to the BP cuff at the rate of 70 ml/h (total intravenous infusion approx. 1 litre). The second IV was placed to minimize subject discomfort from multiple blood draws. Each subject underwent BFV measurements at rest, during the sit-to-stand protocol and in response to CO₂. Blood was drawn (10 ml) every 4 h in the following order: t_0 (baseline), t_1 (4 h after DFO or PLB), t_2 (8 h after DFO or PLB) and t_3 (4 h after stopping DFO or PLB). After the baseline study, the infusion was changed to either DFO in D₅NS (7.5 mg · kg⁻¹ of body weight · h⁻¹ for 8 h; maximum dose 4 g) or PLB (D₅NS) and continued for 8 h. All measurements were conducted 30 min after a meal. All meals were identical during and between study days. In addition, total fluids and the time of each study were matched between the two study days.

Standing protocol—The active sit-to-stand procedure, which produces immediate orthostatic hypotension without altering the spatial relation between the Doppler probe and the insonated vessels, was developed in our laboratory and has been previously described in detail [13,14]. After instrumentation, subjects sat in a straight-backed chair with their legs elevated at 90° in front of them on a stool. For each of three active stands, subjects rested in the sitting position for 5 min and then stood upright for 1 min. The initiation of standing was timed from the moment both feet touched the floor. Results were collected continuously during the final 1 min of sitting and 1 min of standing. The autoregulatory response to transient orthostatic hypotension was assessed by the ARI (autoregulatory index) using Tieck's method [19], as well as by determining the absolute and percentage changes in CVR (cerebrovascular resistance; $CVR=ABP/BFV$) for the MCA from the sitting position (average of 50 s data) to the BP nadir during standing (average of five values).

CO₂ vasoreactivity protocol—BFV in the MCA was measured continuously while subjects inspired a gas mixture of 5 % CO₂, 21 % O₂ and balance nitrogen for 2 min and then mildly hyperventilated to an end-tidal CO₂ of approx. 25 mmHg for 2 min. To determine cerebral vasoreactivity using this technique, the percentage change in MCA BFV was plotted against end-tidal CO₂ in response to room air, breathing 5 % CO₂ and mild hyperventilation. Cerebral vasoreactivity was measured as the slope of this relationship and expressed as the percentage change in cerebral blood flow per mmHg change in end-tidal CO₂.

Blood sample processing—Blood (10 ml) was collected from each subject in a sterile glass tube containing 100 i.u. of preservative-free heparin (Sigma) at each of the time points described. Of this, 2 ml was sent to the clinical laboratory at the Brigham and Women's Hospital for LDH measurement and 8 ml was used to isolate PBMCs (peripheral blood mononuclear cells) using the standard Ficoll–Hypaque (Histopaque; Sigma) density-gradient-centrifugation method. PBMCs were washed with PBS and immediately placed at –70°C and frozen for later analysis. Once PBMCs for all 15 subjects were collected, HIF-1 protein analysis was performed using TransAM HIF-1 kits (Active Motif), which is an ELISA-based assay with HIF-1 α -specific antibodies.

Data processing—All results were displayed and digitized in real-time at 500 Hz with commercially available data-acquisition software (Windaq; Dataq Instruments). BFV and BP waveforms were re-sampled at 1 Hz using a MATLAB program. Beat-to-beat R–R interval, ABP and BFV (reported as mean ABP and mean BFV) were determined from the R wave of the ECG and the maximum systolic and minimum diastolic of the ABP or BFV waveforms.

Steady-state beat-to-beat values for BFV and ABP were averaged across 5 min of rest at each time point for each individual. CVR was calculated as the ratio of steady-state beat-to-beat values for ABP to BFV. To compare individual responses, absolute changes in BFV, CVR and ABP were calculated for each time point as $t_1 - t_0$.

Statistical analysis

Repeated measures analyses of CVR and LDH average values were conducted using linear mixed-effects models [20]. Linear mixed-effects models extend multiple linear regression to allow for correlation between measurements from the same subject by specifying a within-subject correlation matrix. Autoregressive correlation structures were used for all outcomes. In addition to including treatment, time of measurement and the treatment by time interaction, all analyses adjusted for the baseline measurement and age group. The analysis of CVR included adjustment for both HIF and LDH, and the analysis of LDH included adjustment for HIF, treating these adjustment variables as time-varying covariates. Tests of treatment effect were conducted by first examining the treatment by time interaction and then the main effect of treatment if no interaction was found. All hypothesis tests were two-sided, and statistical significance was set at $P < 0.05$. Repeated measures analyses were conducted using SAS version 9 software (SAS Institute). Linear regression was used to compute the slope of the relationship between end-tidal CO₂ and BFV as a measure of cerebral vasoreactivity.

Results

Subject characteristics

Baseline subject characteristics are summarized in Table 1. Five of the subjects were hypertensive and on medication. There was no significant difference between their responses and those of the other ten subjects in the group.

Haemodynamic response to DFO infusion

Figure 1 summarizes the haemodynamic responses to 8 h of DFO infusion. DFO infusion was associated with a significant cerebral vasodilation as compared with PLB. CVR significantly declined in the DFO treatments at all three time points after infusion (-0.28 ± 0.09 compared with $0.05 \pm 0.05 \text{ mmHg} \cdot \text{s}^{-1} \cdot \text{cm}^{-1}$, $P = 0.01$; -0.26 ± 0.09 compared with $0.09 \pm 0.08 \text{ mmHg} \cdot \text{s}^{-1} \cdot \text{cm}^{-1}$, $P = 0.02$; and -0.29 ± 0.1 compared with $0.08 \pm 0.06 \text{ mmHg} \cdot \text{s}^{-1} \cdot \text{cm}^{-1}$, $P = 0.01$; at 4, 8 and 12 h respectively, DFO compared with PLB).

During these time points, DFO infusion was also associated with a corresponding decline in ABP and an elevation in BFV as compared with PLB. The differences in ABP between DFO and PLB were only significant at 8 h of infusion and 12 h (4 h after stopping the infusion; -3.2 ± 2.9 compared with $1.7 \pm 2.1 \text{ mmHg}$, $P = 0.18$; -3.9 ± 3.1 compared with $9.7 \pm 3.9 \text{ mmHg}$, $P = 0.03$; and -6.6 ± 4.8 compared with $7.7 \pm 3.1 \text{ mmHg}$, $P = 0.03$; at 4, 8 and 12 h respectively, DFO compared with PLB). ABP was not significantly different between the DFO and PLB treatments at 4 h of infusion.

As expected, blood flow changes in the MCA were only significantly different between DFO and PLB treatments at 4 h of infusion, a time when the ABP differences were not significantly different between the two treatments. At 4 h of infusion, BFV in the MCA increased in response to DFO and decreased in response to the PLB treatment (1.1 ± 1.4 compared with $-2.8 \pm 1.4 \text{ cm/s}$ at 4 h, $P = 0.02$, DFO compared with PLB). At 8 h and 12 h, when the BPs were significantly different, there were no significant differences between BFV responses to DFO and PLB.

The repeated measures analysis of CVR in response to DFO infusion found no significant treatment by time interaction for CVR; however, the main effect of treatment was significant ($P = 0.004$). That is, DFO infusion resulted in a significant decrease in CVR.

HIF-1 activation in response to DFO infusion

Figure 2 summarizes the changes in HIF-1 protein concentration as well as presumed HIF-1 activation, as indicated by changes in the serum LDH concentration, in response to 8 h of DFO infusion. DFO infusion increased and PLB infusion decreased HIF-1 protein concentrations, but the differences were not significantly different between the two treatments. However, DFO infusion did temporally correlate with LDH expression, which is one of the proteins regulated by HIF-1. LDH concentrations increased with DFO infusion as compared with PLB at 4 and 8 h of infusion (3.5 ± 5 compared with $-13 \pm 5 \text{ units/l}$, $P = 0.02$; 14 ± 8 compared with $-12 \pm 8 \text{ units/l}$, $P = 0.004$; and -1.5 ± 5 compared with $-2 \pm 12 \text{ units/l}$, $P = 0.9$; at 4, 8 and 12 h respectively, DFO compared PLB).

For the LDH only, repeated-measures analysis showed a significant treatment by time interaction ($P = 0.04$). That is, the increase in LDH concentration over the DFO infusion time was significantly higher than the change during PLB infusion.

Cerebral autoregulation and vasoreactivity in response to DFO infusion

Table 2 summarizes the changes in measurements of cerebral autoregulation using the sit-to-stand manoeuvre and CO_2 vasoreactivity in response to 8 h of DFO. Overall, DFO infusion did not alter measures of cerebral autoregulation or cerebral CO_2 vasoreactivity. However, given the overall vasodilated state of the cerebral vessels during DFO infusion, there was a greater reduction in BFV (lesser reduction in CVR) during the sit-to-stand manoeuvre at 8 h of DFO infusion compared with PLB.

Discussion

Finding an effective treatment for brain ischaemia is an immediate medical necessity. Neuroprotective strategies are unlikely to work without improvements in flow and eventually recanalization. The present study shows that a simple intervention, systemic infusion of DFO in healthy humans, was associated with significant cerebral vasodilation. The simultaneous increase in HIF-1 protein and LDH concentrations suggest a possible role for HIF-1 activation in this response.

Cerebral vasodilation and DFO

DFO infusion for 8 h resulted in a significant decrease in CVR at 4 and 8 h of infusion. We chose CVR as the primary haemodynamic-dependent variable because this variable accounts for the known effects of BP on cerebral blood flow. Given that DFO and PLB infusion were both associated with changes in BP, changes in BFV in response to DFO or PLB infusion cannot be considered independent of the BP effect. Although the effect of DFO infusion on CVR was significant at all time points, BFV increases were dampened by the decline in ABP and only reached significance at 4 h of infusion, the time point before ABP changes became significant. As the infusion progressed, ABP continued to decline, and at 8 h of infusion ABP was significantly reduced. These haemodynamic responses to DFO or PLB infusion were no different in the five subjects that were hypertensive as compared with the others. Moreover, none of these individuals were on β -blockers, which would affect their autoregulatory response during the sit-to-stand procedure.

It is possible that the observed cerebral vasodilation is solely an autoregulatory response to DFO-mediated hypotension. However, two observations challenge this hypothesis. First, at 4 h of DFO infusion, when there was no significant hypotension, there was already a significant vasodilation as well as a significant increase in cerebral BFV. Secondly, if this was just an autoregulatory response, one would expect a vasodilatory response sufficient to offset the hypotension and maintain flow, not one that leads to increased BFV. Interestingly, the infusion of PLB, which was equivalent to approx. 1 litre of IV fluid over the course of each study day, resulted in increased BP as well as CVR. BP most likely increased in response to the higher intravascular volume. However, the effect of DFO infusion on CVR was observed even in the face of increased intravascular volume (a similar 1 litre volume of infusion as the PLB), making our results even more compelling. The higher intravascular volume may also explain the observed decline in HIF-1 and LDH serum concentrations associated with PLB infusion.

DFO and HIF-1 activation

DFO infusion for 8 h also resulted in a tendency to simultaneously increase both HIF-1 concentrations and serum LDH levels, the latter suggesting increased HIF-1 transcriptional activity. HIF-1 activation triggers a number of cellular processes in response to decreased oxygen supply. One of these processes, targeted to improve cellular oxygen availability, includes those that adapt cellular energy metabolism to the hypoxic state by regulating glycolytic enzymes (LDH, phosphoglycerate kinase-1 and pyruvate dehydrogenase kinase) [21].

In the present study, although DFO infusion was associated with higher HIF-1 α concentrations as compared with the PLB, the difference was not statistically significant. This is most likely due to the short half-life of HIF-1 α under normoxic conditions. As the PBMCs are isolated under normoxia and DFO washed away, HIF-1 α is rapidly degraded, resulting in much lower concentrations in the final assay. However, although the actual HIF-1 concentrations were not statistically different, LDH serum concentrations, as a measure of HIF-1 transcriptional activation, were significantly higher in response to DFO as compared with PLB infusion. These

findings show that DFO-mediated cerebrovascular changes are temporally correlated with systemic HIF-1 transcriptional activation, suggesting a possible role for HIF-1 activation in DFO-mediated cerebral vasodilation.

DFO and vascular function: possible mechanisms

Although DFO infusion has been previously shown to alter vascular function in both humans and animals [10,11,22–24], the underlying mechanisms are still unknown. Some studies have suggested that the radical scavenging and iron-chelating functions of DFO are most likely involved, whereas others support DFO-mediated HIF-1 activation as the underlying mechanism (for a detailed review, see [25]).

The present study also provides preliminary support that DFO-mediated HIF-1 activation may be a critical step in modulating cerebral vascular function and enhancing cerebral perfusion, and that DFO-mediated ischaemic protection may also occur at the vascular, rather than just at the neuronal, level. Although the mechanisms underlying DFO-mediated cerebral vasodilation is unknown, there are some pathways worth discussing and exploring in future studies. One possibility is that the effects of DFO on cerebral perfusion is mediated via HIF-1. In fact, HIF-1-targeted genes are responsible for a number of cellular processes related to vascular remodelling, such as proliferation, migration, differentiation, extracellular matrix metabolism, pH adjustment, and regulation of enzymes, ligands, receptors and ion channels, which mediate either vasoconstrictor or vasodilator effects [26]. The specific gene products involved in these processes include direct and indirect HIF-1-dependent target genes, such as VEGF, [27] eNOS (endothelial NOS) [28], matrix metalloproteinases [29], angiopoietin-2 [30] and potassium channel Kv 1.5 [31], to name a few (for a detailed review, see [26]). VEGF and eNOS play synergistic roles in modulating vascular endothelial function [32–35].

Alternatively, the radical scavenging and iron-chelating functions of DFO may be responsible for improving endothelial function and enhancing cerebral perfusion. However, given that ferroxamine (preloaded DFO with Fe^{3+}) maintains the same level of protection as DFO, iron chelation is less likely to be the underlying mechanisms in the protective effects of DFO.

The findings of the present study should be discussed in light of the current debate over the role of HIF-1 in neuroprotection. Many previous studies have reported on the role of HIF-1 activation in neuroprotection using *in vitro*, as well as *in vivo*, models of ischaemia, and pharmacological agents as well as various HIF-1 α knockouts. Although some studies have shown that HIF-1 activation is neuroprotective [36–45], others show that HIF-1 is pro-apoptotic [46–52]. The answer may be somewhere in between and a direct consequence of the key regulatory role this remarkable protein plays in so many different homeostatic processes. It is possible that such a regulator may be protective in the early stages of injury when the neuron is viable and pro-apoptotic when the neuron is no longer salvageable. Despite the conflicting data over the role of HIF-1 in ischaemic neuroprotection, all studies of DFO in animal models of stroke have been promising.

DFO and cerebral autoregulation

Numerous methods have been used to estimate cerebral autoregulation. These include the thigh-cuff test, valsalva manoeuvre, lower-body negative pressure, antihypertensive medication administration and, most recently, the sit-to-stand protocol [14,53]. In the present study we chose the sit-to-stand protocol [13,14], a similar but more physiological stimulus than the thigh-cuff test, because it is much better tolerated by elderly subjects and simulates a common ADL (activity of daily living) that potentially threatens cerebral perfusion. In Table 2 we show that, although DFO infusion does not alter cerebral vasoreactivity to CO_2 , HIF-1 activation at 8 h is associated with a smaller percentage reduction in CVR, such that for the

same hypotensive stimulus there is a larger decline in cerebral BFV and a smaller vasodilatory effect. Therefore future clinical trials of DFO for ischaemic neuroprotection should be designed with this potential effect in mind and avoid simultaneous use of antihypertensives during DFO infusion. It is also important to note that the effects of DFO infusion on systemic BP are ideal features for a neuroprotective agent that can be used simultaneously with thrombolytic agents such as tissue plasminogen activators.

Summary and future directions

In the present study we have shown that, in humans, DFO infusion is associated with cerebral vasodilation and improved cerebral blood flow, and that this effect is temporally associated with HIF-1 activation. Our results, added to previous studies which show DFO-mediated HIF-1 activation reduces brain damage [5–8] and promotes functional recovery in animal models of stroke [9], pave the way for studying DFO and novel activators of HIF-1 as simple measures to enhance cerebral perfusion in future clinical trials of stroke and neuroprotection. Moreover, combining DFO treatment with imaging modalities such as CT (computed tomography) perfusion or MRI diffusion and perfusion weighted images in acute stroke will most likely show improvements in perfusion that might extend the window of opportunity for thrombolysis which has been a limiting factor in acute stroke intervention.

Study limitations

There are several limitations to the present study. First, although LDH is one of the glycolytic enzymes regulated by HIF-1, it is also regarded as a marker of toxicity; however, prior use of DFO in humans is not associated with any known toxicity or cell death. Therefore we propose that the elevations in serum LDH reflect increased intracellular LDH concentrations in response to HIF-1 transcriptional activation. LDH exhibits one of the largest increases in activity among the glycolytic enzymes after hypoxic stimulation of cells. Previous studies have also shown that, although serum concentrations do not reliably reflect tissue LDH concentrations, serum and tissue/cellular expressions of LDH are linked and positively correlated [54].

Secondly, the evidence that we present is correlational and does not functionally prove the significance of HIF-1-mediated cerebral vasodilatation in ischaemic neuroprotection. Although we expect that the serum levels of LDH are a marker of systemic HIF-1 activation, including HIF-1 activation in the central nervous system, we did not measure this directly. Direct measurements of HIF-1-activated proteins such as EPO and VEGF concentrations in the cerebral spinal fluid, although invasive, will be necessary to confirm our assumption of cerebral activation. Moreover, to prove definitively that DFO-mediated cerebral vasodilation is HIF-1-dependent, HIF-1 blockers would have to be administered simultaneously with DFO; however, no such agents are currently available for use in humans.

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Abbreviations

BFV	blood flow velocity
BP	blood pressure
ABP	arterial BP
CVR	cerebrovascular resistance
DFO	desferroxamine
D ₅ NS	dextrose/normal saline
EPO	erythropoietin
HIF-1	hypoxia-inducible factor-1
IV	intravenous

LDH	lactate dehydrogenase
MCA	middle cerebral artery
MRI	magnetic resonance imaging
NOS	NO synthase
eNOS	endothelial NOS
PBMC	peripheral blood mononuclear cell
PLB	placebo
VEGF	vascular endothelial growth factor

Figure 1. Cerebrovascular haemodynamic changes in response to 8 h of DFO compared with PLB infusion

●, DFO; ○, PLB. ABP, BFV and CVR (ABP/BFV) are all expressed as the absolute value of the variable minus the baseline value of that variable. Values are means $\pm$ S.E.M. * $P \leq 0.03$, for DFO compared with PLB.

Figure 2. DFO-mediated HIF-1 activation as shown by serum LDH concentrations (top panel) and by HIF-1 protein concentration in PBMCs (bottom panel)

All points are expressed as the absolute value of the variable minus the baseline value of that variable. Values are means $\pm$ S.E.M. * $P \leq 0.02$, for DFO compared with PLB.

Table 1
Baseline subject characteristics

Values are means (S.D.). The number in parentheses next to the medication refers to the number of subjects that were on the specific medication if more than one.

Characteristic	All subjects
<i>n</i>	15
Gender (male/female) (<i>n</i>)	8/7
Age (years)	58 (21)
Mean ABP (mmHg)	80 (18)
MCA baseline BFV (cm/s)	60 (19)
MCA CVR (mmHg · s ⁻¹ · cm ⁻¹)	1.5 (0.7)
Medication	Alendronate (2), levothyroxine (3), trazodone, hyzaar, hydrochlorothiazide, amitriptyline, sertraline hydrochloride, atorvastatin (2), lisinopril (3), allopurinol, fluticasone, albuterol, metformin, prednisone, aspirin, multivitamins

Table 2
Cerebrovascular haemodynamics during the sit-to-stand and CO₂ protocols

Values are means (S.D.). %Δ ABP, percentage change in mean ABP during the sit-to-stand manoeuvre; %Δ BFV, percentage change in mean BFV in the MCA during the sit-to-stand manoeuvre; %Δ CVR, percentage change in CVR (CVR=ABP/BFV); %Δ BFV/%Δ ABP, ratio of the percentage change in mean BFV in the MCA to the percentage change in the mean arterial pressure as a measure of blood flow regulation; del BFV/%Δ ABP, ratio of the percentage change in mean BFV in the MCA to the mean percentage change in arterial pressure as a measure of blood flow regulation; VR, cerebral vasoreactivity to CO₂. **P* < 0.05, DFO compared with PLB.

		Sit-to-stand						CO ₂	
Time (h)	Group	%Δ ABP	%Δ BFV	%Δ CVR	%ΔBFV/%Δ ABP	del BFV/%Δ ABP	VR		
0	DFO	-23 (3)	-16 (2)	-6.5 (4)	0.9 (0.2)	0.5 (0.1)		1.8 (0.3)	
	PLB	-24 (3)	-16 (3)	-8.8 (4)	0.6 (0.1)	0.4 (0.1)		1.8 (0.3)	
4	DFO	-22 (4)	-14 (3)	-8.0 (5)	1.0 (0.6)	0.7 (0.3)		2.1 (0.2)	
	PLB	-25 (3)	-14 (3)	-11.7 (4)	0.6 (0.1)	0.4 (0.1)		2.2 (0.3)	
8	DFO	-21 (3)	-16 (2)*	-4.6 (4)*	1.8 (0.7)	1.1 (0.5)		1.9 (0.2)	
	PLB	-21 (3)	-11 (3)	-10.5 (4)	0.7 (0.2)	0.5 (0.1)		2.2 (0.2)	
12	DFO	-23 (3)	-14 (2)	-7.4 (6)	0.7 (0.2)	0.5 (0.1)		2.1 (0.4)	
	PLB	-18 (3)	-15 (2)	-3.4 (4)	1.3 (0.5)	0.9 (0.3)		2.4 (0.5)	