Gene therapy with hypoxia-inducible factor 1 alpha in skeletal muscle is cardioprotective in vivo

Gabor Czibik a,c,1, Jørgen Gravning b,c, Vladimir Martinov a,b,c, Bushra Ishaq a,c, Eirunn Knudsen a, Havard Attramadal b,c, Guro Valen a,c,⁎

a Dept. of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
b Institute of Surgical Research, Rikshospitalet University Hospital, University of Oslo, Oslo, Norway
c Center for Heart Failure Research, University of Oslo, Oslo, Norway

A B S T R A C T

Aims: Gene therapy of a peripheral organ to protect the heart is clinically attractive. The transcription factor hypoxia-inducible factor 1 alpha (HIF-1α) transactivates cardioprotective genes. We investigated if remote delivery of DNA encoding for HIF-1α is protective against myocardial ischemia–reperfusion injury in vivo. Main methods: DNA encoding for human HIF-1α was delivered to quadriceps muscles of mice. One week later myocardial infarction was induced and four weeks later its size was measured. Echocardiography and in vivo pressure–volume analysis was performed. Coronary vasculization was evaluated through plastic casting. HL-1 cells, transfected with either HIF-1α or HMOX-1 or administered bilirubin or the carbon monoxide (CO) donor CORM-2, were subjected to lipopolysacharide (LPS)-induced cell death to compare the efficacy of treatments.

Key findings: After four weeks of reperfusion post infarction, animals pretreated with HIF-1α showed reduced infarct size and left ventricular remodeling (p < 0.05, respectively). Fractional shortening was preserved in mice pretreated with HIF-1α (p < 0.05). Invasive hemodynamic parameters indicated preserved left ventricular function after HIF-1α (p < 0.05), which also induced coronary vasculization (p < 0.05). HIF-1α downstream target heme oxygenase 1 (HMOX-1) was upregulated in skeletal muscle, while serum bilirubin was increased. Transfection of HL-1 cells with HIF-1α or HMOX-1 and administration of bilirubin or CORM-2 comparably salvaged cells from lipopolysacharide (LPS)-induced cell death (all p < 0.05).

Significance: HIF-1α gene delivery to skeletal muscle preceding myocardial ischemia reduced infarct size and postischemic remodeling accompanied by an improved cardiac function and vasculization. Similar to HIF-1α, HMOX-1, bilirubin and CO were protective against LPS-induced injury. This observation may have clinical potential.

© 2011 Elsevier Inc. All rights reserved.

Introduction

Ischemic heart disease is the world-wide leading cause of mortality (Yellon and Hausenloy 2007). Revascularization of the occluded artery by thrombolysis, percutaneous coronary intervention, or coronary artery bypass grafting remains the main forms of therapy to salvage ischemic myocardium. However, reperfusion per se causes damage evident as cell death, myocardial contractile dysfunction or arrhythmias (Tissier et al. 2008; Yellon and Hausenloy 2007).

The transcription factor hypoxia-inducible factor 1 (HIF-1) is a novel cardioprotective candidate. This heterodimeric HIF-1 transcription factor, consisting of the regulatory subunit HIF-1α, and the constitutively active subunit ARNT, coordinates gene programs leading to adaptive responses to hypoxia/ischemia from cellular to organism level (Czibik 2010). Gain-of-function studies indicate that HIF-1α enhances vasculization and ameliorates ischemic injury in the rabbit hind limb (Pajusola et al. 2005; Patel et al. 2005; Vincent et al. 2000) and rat and mouse heart (Kido et al. 2005; Shyu et al. 2002). Loss-of-function studies demonstrate that HIF-1α is an essential component of endogenous cardioprotection (Cai et al. 2008; Eckle et al. 2008). Of the HIF target genes, several have cardioprotective properties, such as adrenomedullin (Hamid and Baxter 2006), inducible nitric oxide synthase (iNOS) (Bolli et al. 1997) and heme oxygenase 1 (HMOX-1) (Yet et al., 2001; Yoshida et al. 2001). The latter is an inducible enzyme that catabolises heme...
to free iron, carbon monoxide (CO), and biliverdin that is promptly converted to bilirubin (Clark et al. 2000). Bilirubin is a potent antioxidant and CO is a vasodilator with antiapoptotic and anti-inflammatory properties. Local cardiac delivery of HMOX-1 or bilirubin is shown to be cytoprotective (Melo et al. 2002). CO may protect against cardiac ischemia–reperfusion injury (Fujimoto et al. 2004).

The possibility of using remote organs to protect the heart has been established through the concept of remote preconditioning (Przyklenk et al. 1993). Skeletal muscle can act as an endocrine organ, secreting products of delivered genes (Rizzuto et al. 1999). Initial work established an in vivo model of cardioprotection following delivery of DNA encoding for HIF-1α to the quadriceps muscle of mice (Czibik et al. 2009). Our hypothesis regarding the underlying mechanism of action is that HIF-1α-regulated cardioprotective genes are expressed locally and some of the products secreted to the circulation convey protective signals to the heart. However, protection of isolated hearts or cells in culture may not necessarily lead to protection in in vivo models of injury.

Failure to find cytoprotective substances cardioprotective in patients may be due to an unduly gap between experimental and clinical studies. Patients undergo prolonged periods of reperfusion, allowing complex effects to occur. Cardioprotection studies often focus on acute and local consequences of interventions, where some of the complex organ–blood cell interactions taking place over time can be lost. The discrepancy calls for follow-up studies with longer reperfusion (Yellon and Hausenloy 2007). Here we tested if peripher al HIF-1α treatment protects the heart in vivo with a prolonged reperfusion period and myocardial remodeling with a more faithful resemblance to the clinical setting.

Our findings indicate that remote HIF-1α gene pretreatment protects hearts in vivo. Increased cardiac vascularization and elevated levels of serum bilirubin illustrate cardiac implications of skeletal muscle HIF-1α gene delivery.

Methods

Animals

Male C57BL/6 mice (25–30 g) were used, and handled according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The study was approved by the local ethics committee for animal research.

Plasmid DNA

The pCEP4/HIF-1α derived from human HIF-1α cDNA sequence downstream of a cytomegalovirus promoter was purchased from ATCC, Johns Hopkins Special Collection.

Gene delivery

Animals were anesthetized with Hypnorm/Dormicum (1:1) 50 μl/10 g sc. 15 μg of naked DNA encoding for human HIF-1α was injected into the shaved right quadriceps muscle in a total volume of 50 μl saline and electroporated to enhance cellular DNA uptake as before (Czibik et al. 2009).

Study design

Mice were treated with pCEP4/HIF-1α plasmid or vehicle as sham one week before induction of ischemia (HIF-1α- or sham pretreatment), or peroperatively during ligation of the left coronary artery. Non-treated control (NTC) mice did not receive any treatment neither before nor during surgery. All mice underwent thoracotomy and coronary artery ligation. Mice were allocated to groups as indicated below (numbers in parentheses indicate total and surviving mice in respective group after 4 weeks):

1. HIF-1α pretreatment (n = 14/12)
2. Sham pretreatment (n = 14/12)
3. No treatment control (NTC; n = 19/11)
4. HIF-1α intraoperative treatment (n = 15/8)
5. Sham intraoperative treatment (n = 12/7)

Induced myocardial infarction

Mice were anesthetized with isoflurane, intubated and fixed on a heating pad. They were ventilated at a respiratory rate of 135/min with pure oxygen mixed with 1.5–2.0% isoflurane. After skin incision, the left pectoral muscles were prepared free and retracted, and the intrathoracic cavity opened through the fourth intercostal space. After exposing the heart, the left coronary artery was ligated 1.5 mm under the tip of the left auricle using a 6/0 silk suture to ensure a similar area at risk. The ligature was released after 45 min and successful reperfusion was observed upon the return of the red color. The intercostal space and the skin were closed using 5/0 polyester suture. Postoperative care is described in the online supplementary section. Animals were given buprenorphine for analgesia as detailed in the online supplementary section.

Transathoric echocardiography

Transthoracic echocardiography was performed using a high-resolution ultrasound system (Vevo 770, VisualSonics, Toronto, ON, Canada). The probe (RMV-707B, VisualSonics) was composed of a fixed-focused transducer operating at 30 MHz and performed a mechanical-sector scan in parasternal position. 2D-guided M-mode recordings and tissue Doppler of the left ventricle were obtained, and parameters were averaged between long-axis and short-axis views. All echocardiographic recordings were performed under anesthesia with 0.5% isoflurane. Data analysis was carried out blindly. A baseline echocardiograph prior to gene delivery was taken, followed by recordings one and four weeks after induction of ischemia. Data were normalized to the baseline value of each individual animal.

Pressure–volume analysis

After the final echocardiography, left ventricular function was evaluated by simultaneous pressure–volume analysis in vivo in a closed-chest model (Vinge et al. 2008). Recordings were obtained as previously described in anesthetized mice (1% isoflurane) by advancing a 1.4F micromanometer conductance catheter (SPR-719; Millar Instruments, Houston, TX) retrogradely into the left ventricle via the right carotid artery. Steady-state pressure–volume loops were recorded at heart rates stabilized above 450 beats/min.

Infarct size, remodeling

Mice were euthanized by cardiac excision. After rinsing and careful removal of pericardial connective tissue, hearts were weighed, fixed, embedded, and cryosectioned. Infarct size was determined in 12 μm sections stained with Masson’s trichrome (Michael et al. 1995). Images were scanned from just below the ligation site towards the apex, infarct areas designated as blue zones, and evaluated blindly using Adobe Photoshop CS2. Infarct percentages are related to the whole heart area in cross-sections. Four sections from each heart selected at different levels from apex to infarct site were used for quantification, and values from each heart were averaged for statistical analysis.

For evaluation of myocardial hypertrophy by the marker α-skeletal muscle actin, incubation with primary antibody (anti-skeletal
muscle actin, 1:50, rabbit polyclonal, Abcam) was performed overnight at 4 °C. Expression was visualized with the Vectastatin Elite ABC kit on haematoxylin-counterstained sections. Sections were evaluated blindly, and positive staining was scored with a system (0–3) reflecting signal intensity to estimate expression.

Casting of the coronary vasculature

To investigate if remote delivery of HIF-1α promoted cardiac vessel growth, additional mice were HIF-1α-, sham- or non-treated (n = 5–7 per group). Five weeks later hearts were excised and the aorta cannulated and retrogradely perfused with Krebs–Henseleit buffer to wash out vessels. A monomer-mix of a plastic replica and corrosion kit (Batson’s No. 17, Polysciences, Inc.) was injected to fill up the coronary circulation. Vascular casts were solidified in PBS at 4 °C for 4 h. Maceration solution was added at 50 °C for 3–4 h. Casts were imaged with an Olympus AX70 microscope at 40× original magnification, and the signal was quantified in Adobe Photoshop CS2 (total area of casts) and ImageJ (total number of pixels). Pixel density/cast area was plotted for statistical analysis.

Real-time PCR

RNA extraction, cDNA synthesis and real-time PCR are detailed in the online supplementary section.

Immunoblotting and bilirubin measurement

Proteins were prepared with Laemmli buffer as previously described (Russolepp et al. 2007). 30 μg of protein extracts/lane were separated on denaturing polyacrylamide gel followed by blotting to a nitrocellulose membrane (Amersham). Ponceau staining ascertained equal loading and transfer efficacy. After blocking with 3% bovine serum albumin, membranes were incubated with mouse anti-HMOX-1 antibody (Abcam) 1:250 overnight, and with mouse anti-rat horseradish peroxidase-conjugated secondary antibody (Dako) and developed using the ECL-kit (Amersham). The optical density of bands was measured with ImageJ software (NIH) and related to the optical density of Ponceau-staining. Serum was collected from additional HIF-1α and sham treated mice 5 weeks (7–10 of each) after DNA delivery. Heme oxygenase activity was indirectly estimated from total serum bilirubin level measured with a Modular Analytics SWA System (Roche).

Flow cytometry

Preparation of mononuclear cell suspensions from spleens, analysis of lymphocyte subsets in blood and spleens and that of intracellular cytokines in CD4 positive spleen cells is described in details in the online supplementary section.

Cell culture

HL-1-immortalized cardiomyocytes were seeded on gelatine/fibronectin-coated 6-well plates at a density of 5 × 10⁵ cells/well and cultured (Czibik et al. 2009). HL-1 cells were subjected to 150 μg/ml LPS for 24 h to model the inflammatory aspect of reperfusion, after performing dose–response studies to select a relevant concentration (approx 40% cell death). Cell viability was determined using Trypan blue (0.1% final concentration) exclusion assay and a total of 400 cells were evaluated under microscope by a blinded observer. Cells were either transfected with HIF-1α or HMOX-1 using Lipofectamine 2000 (Invitrogen) prior to LPS stimulation, or pre-incubated in 250 nM bilirubin for 2 h or the 10 nM of the CO-donor CORM-2 for 30 min. Both concentrations were selected after dose–response pilot studies (not shown). Results were related to LPS-treated, empty vector-transfected or vehicle-treated cells. Transfection efficacy was determined as described in the online supplementary section.

Statistics

Survival rate was calculated using the chi square test. T-test was used to compare infarct sizes, in vivo pressure–volume analysis and expression data. Kruskal–Wallis test was used for analysis of flow cytometry. ANOVA for repeated measurements was applied on echocardiographic data. ANOVA with Bonferroni posthoc test was used for HL-1 survival comparison. Differences were considered significant when p<0.05 and a tendency was defined as p = 0.05–0.08.

Results

Survival rate

The overall survival was 68%. Survival rate in both HIF-1α- and sham-pretreated group was 86%, in the non-treated control group 58%, in the intraoperative HIF-1α treatment group 53% and in the intraoperative sham-treatment group 58%. In HIF-1α- or sham-pretreated mice, survival rate tended to be higher than in NTCs and intraoperative sham groups (p = 0.07, for both), while the intraoperative HIF-1α group had a lower survival rate than either of the pretreatment groups (p < 0.05).

HIF-1α pretreatment reduced infarct size

Infarct size was estimated following Masson’s trichrome staining of myocardial sections where connective tissue stains blue and viable myocardium red. The infarcted areas were expressed as percentage of the corresponding whole heart section (Fig. 1). Infarct size in the HIF-1α-pretreated group was significantly lower than in any of the other groups (p < 0.05). There were no other differences between groups.

HIF-1α pretreatment reduced remodeling

Left ventricular remodeling was assessed in several ways (Fig. 2). Heart weight/body weight ratio (mg/g) post-infarction was attenuated by HIF-1α pretreatment compared to sham pretreatment...
There were no other significant differences between groups. When hypertrophy was evaluated by skeletal muscle actin, hearts of HIF-1α-pretreated mice had reduced expression compared to pretreated shams or NTCs (p < 0.05, Fig. 2B–C). Expression of skeletal muscle actin in both intraoperative groups was similar to that of the NTCs and higher than in the HIF-1α pretreatment group (p < 0.05, Fig. 2B).

**HIF-1α pretreatment improved contractile function**

Cardiac performance was monitored via transthoracic echocardiography (Fig. 3). Fractional shortening relative to baseline values decreased gradually in all groups (p < 0.05), except for HIF-1α-pretreated mice, where it was maintained around the baseline value. Furthermore, fractional shortening was higher in HIF-1α-pretreated than in the other groups (p < 0.05). Myocardial velocities were measured by tissue Doppler of the posterior wall, outside the infarcted area. Measurement of peak systolic velocity was maintained in HIF-1α-pretreated animals and deteriorated in the corresponding shams (p < 0.05), tended to be less reduced in HIF-1α-pretreated mice than in mice treated with HIF-1α intraoperatively (p = 0.064), but was not different from non-treated controls (p = 0.14; Fig. 3).

Preserved contractile function after HIF-1α- compared to sham pretreatment was found by in vivo pressure-volume analysis (Fig. 4). The two intraoperative groups were omitted for in vivo pressure-volume analysis. Cardiac output was higher in the HIF-1α-pretreated group than in sham-pretreated animals, and so was cardiac index (p < 0.05, Fig. 4). Further, ejection fraction was higher in HIF-1α-pretreated animals than in the corresponding sham animals (p < 0.05). Stroke work and stroke volume was higher in the HIF-1α-pretreated group compared to sham-pretreated animals (p < 0.05). There was no significant difference between either of these groups and the non-treated control group in any of these parameters (Fig. 4).

**HIF-1α pretreatment increased coronary vessel growth**

To investigate small vessel densities, replica casts of the coronary vasculature were made of hearts from HIF-1α-, sham-pretreated or NTC mice (Fig. 5). Quantitative analysis indicated that the coronary trees of HIF-1α-pretreated mice were denser than those of sham-pretreated animals (p < 0.05), and tended to be denser than coronary trees of NTC mice (p = 0.07; Fig. 5B).

**Immune cells were not activated by human HIF-1α**

To address the concern that human HIF-1α may induce an immune reaction, we undertook flow cytometry of peripheral blood and spleen cells. When peripheral blood was smeared, stained
with May-Grünwald/Giemsa, and cells differentially counted, no difference was found in the percentage of eosinophilic cells, lymphocytes, monocytes, or rod shaped or segmented neutrophils. Coulter counts revealed no changes in the absolute number of lymphocytes, monocytes, or rod shaped or segmented neutrophils. No difference was found in the percentage of eosinophilic cells. In the present work, HL-1 cells were less susceptible to LPS-induced injury not only when transfected with either HIF-1α or HMOX-1, but also when administered the CO-donor CORM-2 or bilirubin, where at least the latter was correspondingly elevated in serum of HIF-1α-pretreated mice. These results allow the speculation that the protective effect of HIF-1α was due to an increased production of HMOX-1 locally in the skeletal muscle, resulting in release of bilirubin into the blood stream where it acted upon the heart in an antiinflammatory mechanism. However, we do not provide direct evidence to exclusively support this hypothesis. The nature of the evidence presented here does not preclude a vital role for other HIF-1α-induced downstream cardioprotective mechanisms(s).

Bilirubin and CO protected HL-1 cells from LPS-induced injury

Following HIF-1α delivery in the skeletal muscle its target gene HMOX-1 was upregulated (Fig. 6A–B). Serum bilirubin levels reflecting HMOX activity were increased in mice treated with HIF-1α compared to sham mice (Fig. 6C). To verify if elevated bilirubin (and possibly CO) can protect against reperfusion injury and if these factors may be mediators of HIF-1α-induced remote cardioprotection, we used LPS to model the inflammation-induced injury of reperfusion. Transfection of HL-1 cells with either HIF-1α or HMOX-1 limited LPS-induced cell death (p<0.05, Fig. 6D). Similarly, administration of bilirubin or CORM-2 to HL-1 cells subjected to LPS-induced injury reduced cell death (p<0.05, Fig. 6D).

Discussion

Remote delivery of DNA encoding for HIF-1α preceding induction of myocardial infarction reduced infarct size and left ventricular remodeling, and preserved hemodynamic function in vivo. These effects were not observed when gene delivery took place in conjunction with induction of ischemia.

Myocardial ischemia ultimately leads to cell death unless resolved by reperfusion (Tissier et al. 2008). Our design of the experimental model aimed at closely mimicking a clinical situation of reperfusion. We chose HIF-1α as a therapeutic agent, as it regulates a number of genes that through various mechanisms (angiogenesis, increased oxygen transport and uptake, vasodilation, activation of cell survival pathways etc) protect against ischemic disorders (Czibik 2010). Remote delivery of DNA encoding for HIF-1α prior to induction of ischemia limited infarct size and maintained cardiac function. Intraoperative HIF-1α delivery failed to improve postschismic contractile function, suggesting that the active presence of HIF-1α or some of its target genes at the time of injury is decisive. HIF-1α gene delivery to skeletal muscle did not increase cardiac HIF-1α (not shown), which makes it an unlikely player to directly influence the myocardium, although its target gene(s) may do so through secretion.

The downstream cardioprotective factor HMOX-1 was upregulated in the HIF-1α-treated muscle. In another study from our laboratory, serum from HIF-1α-pretreated animals or transfer of conditioned media from cells transfected with HIF-1α to naïve cells, protected HL-1 cells from H2O2-induced injury, suggesting that the protective mechanism is blood-borne (Czibik et al. 2009). In the present work, HL-1 cells were less susceptible to LPS-induced injury not only when transfected with either HIF-1α or HMOX-1, but also when administered the CO-donor CORM-2 or bilirubin, where at least the latter was correspondingly elevated in serum of HIF-1α-pretreated mice. These results allow the speculation that the protective effect of HIF-1α was due to an increased production of HMOX-1 locally in the skeletal muscle, resulting in release of bilirubin into the blood stream where it acted upon the heart in an antiinflammatory mechanism. However, we do not provide direct evidence to exclusively support this hypothesis. The nature of the evidence presented here does not preclude a vital role for other HIF-1α-induced downstream cardioprotective mechanisms(s).

A peripheral treatment approach is attractive from a patient point of view. Remote gene delivery to the skeletal muscle was chosen based on the hypothesis that locally delivered HIF-1α may act through local transcription, translation and secretion of products. Bilirubin may be such a product. Intramuscular injection of DNA constructs followed by electroproporation to enhance DNA uptake has been used to deliver various factors (Gronevik et al. 2005; Rizzuto et al. 1999), but not to protect the heart. Both intramyocardial gene delivery of HIF-1α (Shyu et al. 2002) and cardiac-specific overexpression of HIF-1α (Kido et al. 2005) has previously led to increased cardiac vascularization. Gene therapy with HIF-1α promises to revascularize human ischemic limbs (Rajagopalan et al. 2007). Here, quantitative analysis of the coronary vasculature suggests an increased cardiac vascularization after peripheral HIF-1α pretreatment. Our findings demonstrate that remote gene delivery of HIF-1α promoted both cardiac vascularization and protection in vivo. Whether the increased vessel growth plays a direct role in HIF-1α-induced remote cardioprotection remains a subject for future investigations.

The present study used 45 min of ischemia followed by reperfusion, which resulted in a relatively small infarct size and moderate damage. The survival rates of animals in HIF-1α- and sham-pretreated mice were both 86%. This tended to be or was higher than in the other groups, suggesting that i) even sham pretreatment may hold a certain cardioprotective value in relation to the non-treated and the intraoperatively treated groups, and ii) that those mice who...
survived, were “the fittest”, potentially diminishing differences in infarct size and hemodynamic data between groups (i.e. as those who were less cardioprotected, suffered larger infarcts and tended to die easier). Accordingly, the hemodynamic data may be skewed in favor of NTC at the expense of the sham group. The paucity of experimental cardioprotection studies with longer periods of reperfusion and observation prevents us to make direct comparisons of potential survival benefits (Yellon and Hausenloy 2007). Although the survival rate in both HIF-1α- and sham-pretreated groups was high, the theoretical possibility remains that extracardiac deleterious effects of HIF-1α negatively influence survival. The smallest infarct size along with best viability and cardiac function in HIF-1α-pretreated mice, however, strongly argues for a profound cardioprotected state in this group.

Several cardiac patient subgroups could possibly benefit from remote gene therapy. Gene delivery of cardioprotective factors that are expressed prior to the advent of iatrogenic ischemia and reperfusion could afford myocardial protection in patients with preoperative reduced ejection fraction scheduled for open heart surgery. When any delay costs myocardium, a remote gene therapy-induced “preconditioned-like” state in patients with acute coronary syndromes undergoing myocardial infarction may save precious time for revascularization. In contrast with direct myocardial induction/delivery of HIF-1α that could alter myocardial metabolism and promote

---

**Fig. 4.** In vivo pressure–volume analysis of hearts of mice subjected to 45 min of myocardial ischemia and four weeks of reperfusion, see legend to Fig. 3 for details. Panel A, representative pressure–volume loops of HIF-1α- and sham-pretreated mice are shown. Note that there is a shift in volume between these two groups. Cardiac output (B) and index (C), ejection fraction (D), stroke work (E) and volume (F) were functionally preserved in HIF-1α-pretreated animals compared to sham-pretreated animals. Data are presented as individual values + mean (n = 9–10 in each group). * denotes p < 0.05.
pathological cardiac hypertrophy per se (Krishnan et al. 2009), remote treatment is probably exempt from this effect. Our results suggest that the peripheral approach to cardioprotection may be exploited therapeutically.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

Acknowledgements
We are grateful to Torun Flatebø, Julia Sagave, and Stian Weiseth for their invaluable technical assistance and to Göran Hansson and Henrik Isackson for critically reading the manuscript. The study was supported by grants from the Norwegian Research Council, Norwegian Health Association and the University of Oslo.

Appendix A. Supplementary data
Supplementary data to this article can be found online at doi:10.1016/j.lfs.2011.01.006.

Fig. 5. Representative plastic replica casting of the coronary vasculature of HIF-1α-, sham-, or non-treated control (NTC) mice are shown as explained in legend to Fig. 3. Original magnification: 40×. The lower panel shows quantification of coronary casts (individual values + mean; n = 5–7/group). * denotes p < 0.05.

Fig. 6. Expression of heme oxygenase-1 (HMOX-1) in mice with HIF-1α- or sham-pretreated skeletal muscle. A, Time course of HMOX-1 expression following HIF-1α treatment (real-time PCR). B, HMOX-1 protein expression and loading with Ponceau Solution, and the quantification of band density below. C, Serum bilirubin from mice pretreated with HIF-1α or sham at the time point corresponding to evaluation of heart function/infarction. D, Transfection with HIF-1α or HMOX-1 and administration of bilirubin or the CO donor CORM-2 reduce LPS-induced cell death in HL-1 cells. Values are mean ± SD of n = 7–11 per group (A–C), or individual values + mean of n=6–8 (D), * denotes p < 0.05 vs. sham (A–C) and vs. empty vector, LPS (D).