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Zusammenfassung des wissenschaftlichen Inhalts (Dr. M. Tiburcy):

Die zeitnahe Wiederherstellung der Herzdurchblutung ist die wichtigste Maßnahme, um den Schaden durch einen Herzinfarkt möglichst gering zu halten. Die Reperfusion des hypoxischen Herzmuskels kann jedoch auch zu zusätzlicher Zellschädigung im Sinne eines Reoxygenierungsschadens führen. Zentral für die Anpassung an eine Hypoxie ist das HIF1 System, das wichtige zelluläre Funktionen bei Sauerstoffmangel aufrechterhalten kann. Für die Entdeckung dieses Systems wurde 2019 der Nobelpreis für Physiologie und Medizin verliehen. In dieser Arbeit wurde der Effekt einer HIF1 stabilisierenden Substanz (Roxadustat) in einem humanen, präklinischen Modell von Herzmuskulatur, die durch Hypoxie und Reoxygenierung geschädigt wurde, getestet. Dabei zeigte eine kurzzeitige Gabe von Roxadustat im Anschluss an die Hypoxie einen protektiven Effekt vor einem Reoxygenierungsschaden mit deutlich verbesserter kontraktiler Funktion. Da sich Roxadustat bereits als Therapie der renalen Anämie im europäischen Zulassungsverfahren befindet, besteht die Hoffnung, dass die kardioprotektive Wirkung von Roxadustat zügig in eine kardioprotektive Therapie nach Herzinfarkt weiterentwickelt werden kann.

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Circulation

RESEARCH LETTER

Inhibition of Prolyl-Hydroxylase Domain Enzymes Protects From Reoxygenation Injury in Engineered Human Myocardium

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Reperfusion after revascularization of an acute myocardial infarction can contribute up to 50% of the resulting infarct damage.[1] Protection from reperfusion injury is an important strategy to prevent excessive damage. To date, clinical studies on reducing lethal reperfusion injury have been disappointing, despite promising preclinical data.[2] To improve preclinical drug development and close the apparent translational gap, human preclinical models of ischemia/reperfusion injury may be useful. To address this challenge, we have developed a novel model system of hypoxia followed by reoxygenation in engineered human myocardium (EHM). Hypoxia-inducible factor 1 (HIF-1) is the major switch for cellular adaptation to hypoxia. Its α-subunit HIF-1α is hydroxylated by prolyl-hydroxylase domain enzymes (PHDs) at 2 conserved proline residues that are located in the oxygen-dependent degradation domain in a strictly oxygen-susceptible manner leading to rapid proteasomal degradation (Figure [A]). Hypoxia or inhibition of PHDs stabilizes the HIF-1α protein that dimerizes with ARNT (aryl hydrocarbon receptor nuclear translocator) to activate target gene transcription—promoting metabolic adaptation, cell survival, mitochondrial function, erythropoiesis, and angiogenesis. In rodent models of myocardial infarction, inhibition of PHDs has been shown to stabilize HIF-1α, conferring not only protection when given preinsult but also in a more clinically relevant scenario when given immediately post insult.[3] Whether similar effects can be achieved in human myocardium is not known.

To monitor the effect of hypoxia and PHD inhibitors (PHDi) in human cardiomyocytes, we established a transgenic human embryonic stem cell line (Institutional Review Board approval obtained; ref. number: 1710-79-1-4-16) expressing the oxygen-dependent degradation (ODD) domain of HIF-1α fused to Firefly Luciferase (Luc) under the control of the CAG promoter. ODD-Luc reporter human embryonic stem cells responded to decreasing levels of ambient oxygen with an oxygen-dependent increase in bioluminescence (Figure [B]). High purity ODD-Luc cardiomyocytes (92±6% sarcomeric actinin-positive) were obtained by directed differentiation and metabolic selection as previously described.[4] ODD-Luc cardiomyocytes responded to the addition of the PHDi FG-2216 (EC_{50}: 250±3 μmol/L) and FG-4592 (EC_{50}: 110±3 μmol/L) in a concentration-dependent manner, demonstrating that tested PHDis are biologically active in human cardiomyocytes (Figure [C]). Consecutive experiments were performed with FG-4592 (Roxadustat), because this drug is a promising first-in-class PHD inhibitor for renal anemia patients and has recent regulatory approval in China. In line with the Luciferase reporter data, FG-4592 stabilized HIF-1α protein (Figure [D]). HIF-1α stabilization was followed by increased expression of prototypical HIF-1 target genes in human cardiomyocytes (Figure [E]) indicative of metabolic adaptation (GLUT1 [glucose transporter 1], LDHA [lactate dehydrogenase A]), angiogenic signaling (VEGFA [vascular endothelial growth factor]), and intact autoregulation (PHD2 [Prolyl hydroxylase domain-containing protein 2]).

Key Words: hypoxia ■ models, cardiovascular ■ myocardial infarction ■ reperfusion ■ stem cells ■ tissue engineering

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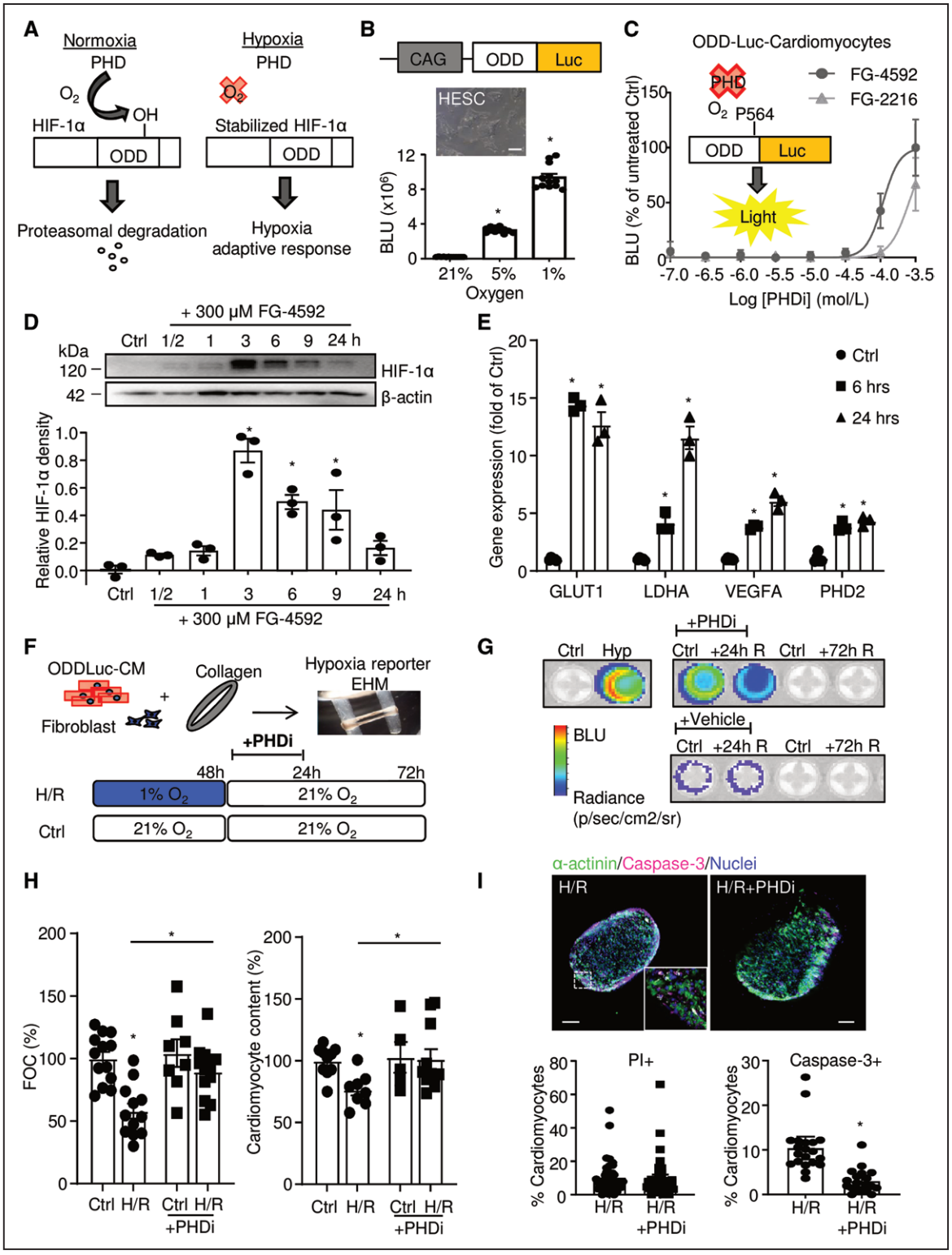

Figure. Inhibition of prolyl-hydroxylase domain enzymes activates HIF1 signaling and protects from simulated reoxygenation injury.
A, Schematic of the regulation of hypoxia inducible factor-1 (HIF-1) signaling. **B**, Human embryonic stem cell line (HESC) expressing firefly luciferase (Luc) fused to the oxygen-dependent degradation domain (ODD) of HIF-1α. Scale bar, 100 μm. Original ODD-Luc plasmid was a kind gift from the Kaelin laboratory. Bioluminescence (BLU) signal in response to indicated oxygen concentrations (n=3; *P<0.05). **C**, BLU signal in ODD-Luc HESC-derived cardiomyocytes (CMs) in response to prolyl-hydroxylase domain (PHD) inhibitors (PHDi; FG-4592 and FG-2216; 0.1–300 μmol/L; n=4). **D**, Time course of HIF1α protein stabilization (n=3; *P<0.05). **E**, Gene expression of HIF-1 target genes on treatment with 300 μmol/L FG-4592 for 6 or 24 hours (n=3; *P<0.05). **F**, Schematic of the engineered human myocardium (EHM) experiments to test the effect of posthypoxia administration of PHDi (300 μmol/L FG-4592 for 24 h). Cardioprotection is evaluated 72 hours postreoxygenation. **G**, Color-coded image of bioluminescence signal intensity (absolute radiance in p/sec/cm^2/sr) in the protein lysates of ODD-Luc EHM exposed to 21% O_2 (Ctrl), 1% O_2 (Hyp), or H/R with or without PHDi (300 μmol/L) at 24 hours and 72 hours reoxygenation (R) after hypoxia. **H**, EHM force of contraction (FOC; normalized to maximal FOC in Ctrl; n=8–13) and CM content (normalized to Ctrl; n=5–11; *P<0.05) at 72 hours postreoxygenation. (*Continued*)

Figure Continued. I, Immunostaining of α-actinin (green), active caspase 3 (magenta), and nuclei (blue) in EHM cross-sections 24 hours postreoxygenation. Note that active caspase-3-positive CMs will appear white (**inset**). Scale bar, 100 µm. Percentage of propidium-iodide (PI)–positive CMs (**left**) and active caspase-3$^+$ CMs (**right**; n>900 CMs per group; *P<0.05). Statistical testing was done by 1-way ANOVA (**B**, **D**, **E**), 2-way ANOVA (**H**) with Tukey multiple comparison test, and by 2-tailed unpaired Student t test (**I**). CAG indicates CMV enhancer chicken β actin promoter; *GLUT1*, Glucose transporter 1; H/R, Hypoxia/Reoxygenation; *LDHA*, Lactate dehydrogenase A; PHD2, Prolyl hydroxylase domain-containing protein 2 encoding RNA (transcribed from the *EGLN1* gene); and *VEGFA*, vascular endothelial growth factor A.

We then tested the effect of FG-4592 in EHM generated from 70% ODD-Luc cardiomyocytes and 30% human foreskin fibroblasts under serum-free conditions (Figure [F]).[4] Four-week-old EHMs were subjected to hypoxia (1% ambient O_2) followed by 24 hours of reoxygenation at 21% O_2. During reoxygenation EHM were treated with 300 µmol/L FG-4592 or vehicle (0.1% dimethyl sulfoxide) followed by measurement of force of contraction (FOC). We focused on modifying and controlling oxygen levels in this injury model to define the role of oxygen-sensing and signaling with the limitation that the complexity of ischemia/reperfusion in vivo is not fully recapitulated. Monitoring of Luc activity confirmed the biological effect of hypoxia and PHDi in EHM (Figure [G]). However, EHM showed a surprising tolerance to hypoxia alone (FOC after 24 hours: –17±11%, 48 hours: –38±6%, 72 hours: –45±4%). Reoxygenation injury was most pronounced after 48 hours of hypoxia with an additional decrease of FOC by 17%.

Interestingly, PHDi treatment significantly protected from contractile failure induced by hypoxia/reoxygenation (H/R) injury (H/R: 58±6% and H/R+PHDi: 90±6% of baseline FOC; n=8–13 EHM/group; P<0.05; Figure [H]). In line with improved function, transient PHD inhibition also preserved muscle mass (cardiomyocyte amount H/R: 77±5% of baseline and H/R+PHDi: 101±8% of baseline; n=8–11 EHM/group; P<0.05; Figure [H]). Because cell death occurs during H/R, we investigated whether the preservation of contractile force and cardiomyocyte number was attributable to the reduction of cardiomyocyte death. Although we did not observe an effect on cardiomyocyte necrosis 24 hours after reoxygenation based on propidium idodide staining, we found a reduction of activated caspase 3–positive cardiomyocytes, suggesting an antiapoptotic effect of HIF-1 (Figure [I]). We therefore propose a protective mechanism targeted mainly at cardiomyocytes in the area at risk, consistent with earlier data in the mouse.[3] Our data support—for the first time in a human model—the strategy to increase HIF-1 activity by PHDi for cardioprotection. The enhanced cardioprotective effect of PHDi in EHM regions with high structural maturation and relevant fatty acid utilization supports its potential for human adult hearts (Figure [I]), but the relative immaturity of cardiomyocytes remains a limitation. It is important to note that local application of FG-4592 into the coronary arteries after revascularization should reach similar drug concentrations as used in this study. Last, a transient stabilization of HIF-1α in the acute phase of reperfusion may be beneficial and sufficient for induction of cardioprotection, because chronic HIF-1α stabilization leads to adverse remodeling and heart failure.[5]

ARTICLE INFORMATION

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

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Disclosures

Dr Zimmermann is founder and, together with Dr Tiburcy, consultant of myriamed GmbH, which is offering tissue engineered-based drug screening services. The other authors report no conflicts.

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