MEDICAL SCIENCE MONITOR

Low-Dose 4-Hydroxy-2-Nonenal (HNE) Reperfusion Therapy Displays Cardioprotective Effects in Mice After Myocardial Infarction That Are Abrogated by Genipin

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Source of support: Departmental sources

Background: Revascularization is a successful therapeutic strategy for myocardial infarction. However, restoring coronary blood flow can lead to ischemia-reperfusion (I/R) injury. Low-dose 4-hydroxy-2-nonenal (HNE) therapy appears to play a key role in myocardial tolerance to I/R injury. We hypothesized that the positive effects of HNE on myocardial I/R injury may be UCP3-dependent.

Material/Methods: Adult male wild-type (WT) or UCP3 knockout (UCP3–/–) mice were pre-treated with the UCP inhibitor genipin or saline 1 h before ischemia and underwent 30-min coronary artery ligation followed by 24-h reperfusion. Mice were treated with intravenous HNE (4 mg/kg) or saline 5 min before reperfusion. Echocardiography was conducted to measure left ventricular end-diastolic posterior wall thickness (LVPWd), end-diastolic diameter (LVEDD), and fractional shortening (FS). Infarct size was measured by TTC staining. qRT-PCR and Western blotting were used to assess the expression of UCP3, UCP2, and the apoptosis markers cytochrome C and cleaved caspase-3.

Results: HNE improved survival at 24 h post-MI in wild-type mice ($p<0.05$) but not in UCP3–/– mice. HNE preserved LVEDD and FS in WT mice ($p<0.05$) but not in UCP3–/– mice. HNE reduced infarct size in WT mice ($p<0.05$) but not in UCP3–/– mice. HNE upregulated UCP3 expression ($p<0.05$) but did not affect UCP2 expression. HNE reduced apoptosis marker expression in WT mice ($p<0.05$) but not in UCP3–/– mice. HNE's positive effects were abrogated by genipin in an UCP3-dependent manner.

Conclusions: Low-dose HNE reperfusion therapy attenuates murine myocardial I/R injury in an UCP3-dependent manner. These effects are abrogated by genipin in an UCP3-dependent manner.

MeSH Keywords: **Cardiovascular Diseases • Myocardial Infarction • Reperfusion Injury**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/910494

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Background

Acute myocardial infarction with ST-segment elevation (STEMI), produced by an abrupt occlusion of a coronary artery, is a common cardiovascular emergency and a major cause of morbidity and mortality worldwide [1,2]. Urgent revascularization has been a successful therapeutic strategy in managing STEMI; however, the sudden restoration of coronary blood flow can lead to further myocardial damage through a process termed myocardial ischemia-reperfusion (I/R) injury [1,2]. Pre-clinical animal models have revealed several promising interventions (such as hypothermia, ischemic post-conditioning, and pharmacotherapy) for reducing myocardial I/R injury [2]. Unfortunately, clinical translation of these interventions has been largely unsuccessful [2].

Therefore, a better understanding of the mechanism(s) underlying myocardial tolerance to I/R are still needed. One mechanism that promotes myocardial tolerance to I/R is uncoupling of the mitochondrial electrochemical gradient by mitochondrial uncoupling proteins (UCPs) [3]. *In vitro*, UCP overexpression reduces cardiomyocyte death through maintaining mitochondrial structure and functionality under oxidative stress [4]. Moreover, the 2 UCP isoforms found in muscle tissue – UCP2 and UCP3 – promote cardiomyocyte tolerance to anoxia-reoxygenation stress *in vitro* [5]. However, *in vivo*, myocardial UCP3 mRNA levels are 5-fold higher than those of UCP2, and UCP2 does not play a significant role in cardioprotection against I/R injury in mice [3]. These combined findings suggest that UCP3 is the key UCP involved in myocardial tolerance to I/R injury.

During I/R, lipid peroxidation (the reaction of reactive oxygen species with unsaturated lipids) produces various aldehyde by-products, most notably 4-hydroxy-2-nonenal (HNE, 4-HNE) [6]. Interestingly, intravenous (i.v.) administration of low-dose HNE, which upregulates UCP3 but not UCP2 in murine cardiomyocytes *in vitro* [7], has been shown to ameliorate left ventricular recovery following myocardial I/R [8]. This evidence suggests that UCP3 upregulation by HNE may play a key role in myocardial tolerance to I/R injury.

On a related note, the aglycone genipin, a derivative of the fruit from *Gardenia jasminoides* [9], has been traditionally regraded as a UCP2-specific inhibitor [10]. Recent evidence reported by Kreiter et al. has revealed that genipin also displays potent inhibitory activity against UCP3 *in vitro* [11]. Notably, administration of genipin has been linked to negative outcomes following myocardial I/R, especially increased infarct size and decreased recovery of both LVDP and coronary flow [12]. This evidence suggests that UCP3 inhibition by genipin may reduce myocardial tolerance to I/R injury.

Integrating these previous findings, we hypothesized that low-dose HNE's positive effects on myocardial I/R injury may be dependent upon UCP3 expression. We also hypothesized that low-dose HNE's positive effects on myocardial I/R injury would be abrogated by genipin in a UCP3-dependent manner. Employing a murine model of STEMI in wild-type (WT) and UCP3–/– mice, we discovered that low-dose HNE reperfusion therapy improves post-I/R functional outcomes in an UCP3-dependent manner. We also found that these positive effects were abrogated by genipin in an UCP3-dependent manner. These findings suggest that low-dose HNE reperfusion therapy may be a potential therapeutic strategy for ameliorating post-STEMI myocardial I/R injury. These findings also suggest that genipin inhibits myocardial UCP3 activity *in vivo*.

Material and Methods

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. The procedures followed in this study were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals (eighth edition, National Institutes of Health (NIH), Bethesda, Maryland, USA).

Mice subjects

Male WT C57BL/6J mice (~8 weeks of age) and matching UCP3–/– mice on a C57BL/6J background were acquired from the Shanghai Laboratory Animal Center (Chinese Academy of Sciences). All mice were housed under temperature-controlled conditions with ad libitum access to water and standard chow.

Chemical reagents

HNE was purchased from Calbiochem (San Diego, CA, USA). Genipin was purchased from Wako Pure Chemicals (Japan). Triphenyltetrazolium chloride (TTC) and Zymosan A from Saccharomyces cerevisiae were both purchased from Sigma-Aldrich (St. Louis, MO, USA). Phthalo blue dye was purchased from Quantum Ink Company (Louisville, KY, USA).

Murine STEMI model

A schematic overview of the murine STEMI model is shown in Figure 1A. Construction of the murine STEMI model was performed as previously described, with minor modifications [13]. Briefly, mice were anesthetized with intraperitoneal (i.p.) pentobarbital (70 mg/kg). An effective dose of i.p. genipin (100 mg/kg) or volume-matched saline was administered 1 h prior to initiating ischemia [14]. The left descending coronary artery was
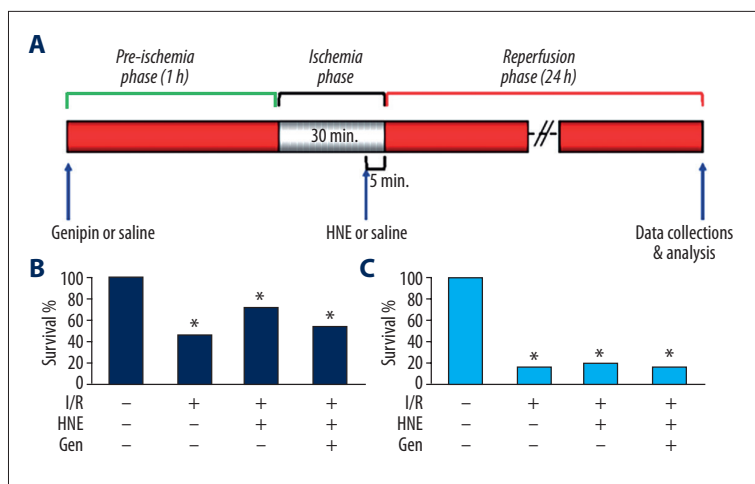
Figure 1. Schematic overview and survival analysis. (**A**) Schematic overview of experimental protocol. Blue arrows indicate key time points. Survival of (**B**) WT mice and (C) UCP3–/– mice in the various experimental groups at 24 h post-MI. The chi-square test was used for statistical comparisons between groups. * *p*<0.05 *vs.* sham group.

occluded with a 7.0 silk ligature and a small portion of polyethylene (PE10) tubing for a period of 30 min. After exactly 25 min of ischemia, a 50-μl bolus of low-dose HNE (4 mg/kg) or volume-matched saline was administered through the retro-orbital vein [8].

After exactly 30 min of occlusion, reperfusion was initiated through removal of the PE10 tubing compressing the artery. After 24 h of reperfusion, chest air was expelled. The mice were extubated and subjected to long-acting subcutaneous (s.c.) buprenorphine SR-LAB (0.5 mg/kg, Zoopharm, Windsor, CO, USA) and intramuscular (i.m.) gentamicin (0.7 mg/kg, Jiangsu Lianshui Pharmaceutical Co., Ltd., Lianshui, China). Sham mice were subjected to an identical protocol without arterial occlusion.

Survival

Post-MI survival rates were calculated based upon the number of mice that survived after the 24-h reperfusion phase.

Echocardiography

Left ventricular functional parameters were assessed via echocardiography immediately after the 24-h reperfusion phase as previously described [13]. Briefly, mice were subjected to isoflurane (2.5%) anesthesia. Echocardiography was conducted with a Vevo770 system (VisualSonics, Inc., Toronto, Canada) to measure left ventricular end-diastolic posterior wall thickness (LVPWd), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD). Left ventricular fractional shortening (FS) was calculated based on the following formula: (LVEDD-LVESD)/LVEDD×100%.

Infarct size

Infarct size was measured as previously described, with minor modifications [13]. Briefly, all mice subjects were placed under

deep i.p. pentobarbital anesthesia (100 mg/kg). The heart was quickly mounted on a Langendorff apparatus. Krebs-Henseleit buffer (37°C) was used to perfuse the coronary arteries and wash out the remaining blood. Then, the heart was then perfused over several minutes with 10% TTC (3 ml) dissolved in isotonic phosphate buffer (pH 7.4, 37°C). The ligature was re-tightened and 5% Phthalo blue dye (1 ml) was aortically-injected as a single bolus. The heart was then perfused over several minutes with saline to wash out the remaining dye.

The heart was immediately frozen and cut into transverse slices (~2 mm in thickness) from base to apex. The slices were fixed for 24 h in 10% neutral buffered formaldehyde; during the initial 30 min, a weight was placed on top of the slices to keep them flat. The infarcted area (IA), risk area (RA), and whole left ventricle were measured with computer morphometry (ImageJ, NIH).

Quantitative real-time RT-PCR (qRT-PCR)

qRT-PCR was performed as previously described, with minor modifications [7]. Briefly, total RNA extraction was performed with the RNeasy Fibrous Tissue Mini Kit (Qiagen China Co., Ltd., Shanghai, China). RNA concentrations were determined by assessing absorbance at 260 nm, and RNA purity was assessed with the 260 nm/280 nm absorbance ratio using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Boston, MA, USA). Total RNA samples were reverse-transcribed with the QuantiTect Reverse Transcription Kit (Qiagen). Resulting cDNA samples were amplified with primer pairs (Table 1) using the following protocol: 10 min of initial denaturation at 95°C, 40 15-s cycles of denaturation at 95°C, and 1 min of annealing at 60°C. Transcript levels were assessed by real-time PCR using SYBR Premix Ex Taq (TaKaRa, Dalian, China) in a 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Relative mRNA expression levels were calculated with glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) used as the control housekeeping gene [15].

Table 1. Primer sequences used for gene amplification.

Gene	Forward	Reverse
Ucp2	5'-GGCCTCTACGACTCTGTCAAACA-3'	5'-GCTCCCGATGCCTGCAT-3'
Ucp3	5'-CTGGAGGAGAGAGGAAATACAGAG-3'	5'-TGGCATTTCTTGTGATGTTGGGCC-3'
Gapdh	5'-TGCCCCCATGTTTGTGATG-3'	5'-TGTGGTCATGAGCCCTTCC-3'

Western blotting

Western blotting was performed as previously described, with minor modifications [13]. For whole-cell blots, isolated left ventricular myocardial tissue samples (n=4 per group) were homogenized and centrifuged at 12 000 g at 4°C for 10 min. For mitochondrial-fraction blots (UCP2, UCP3, and CoxIV), murine myocardial mitochondria were isolated by gradient ultracentrifugation, as previously described (16).

Total protein (75 μg) was separated on 4–20% gradient acrylamide gels and transferred to a nitrocellulose membrane. Membranes were then blocked with 5% non-fat dry milk in Tris-buffered saline and incubated overnight with the following monoclonal antibodies from Abcam China (Shanghai, China): anti-mouse UCP3 (diluted 1: 1000, ab10985), anti-mouse UCP2 (diluted 1: 1000, ab203244), anti-mouse CoxIV mitochondrial loading control (diluted 1: 2000, ab202554), anti-mouse cytochrome C (Cyt-c, diluted 1: 5000, ab133504), anti-mouse cleaved caspase-3 (CC-3, diluted 1: 500, ab13847), and anti-mouse β-actin loading control (diluted 1: 500, ab8226). Membranes were then incubated with the species-appropriate peroxidase-conjugated secondary antibody for 1 h and developed with enhanced chemiluminescence reagents (Pierce, Nantong, China). Densitometric analysis of the bands was performed with ImageJ.

Statistical analysis

All measurements are reported as means ± standard errors of the mean (SEMs). Continuous variables were analyzed using one-way analysis of variance (ANOVA) to calculate the main effect. The post hoc two-sided Dunnett's test or Tukey's test was then applied for pairwise comparisons. Discrete variables were analyzed using the chi-square test (or the Fisher's exact test when appropriate). The Bonferroni correction for post hoc analysis was applied for pairwise comparisons within a larger set of groups. Statistical differences were considered significant at a p-value of less than 0.05.

Results

HNE reperfusion therapy improves post-MI survival in an UCP3-dependent manner

Figure 1 displays the survival rates for WT mice (Figure 1B) and UCP3 knockout (UCP3–/–) mice (Figure 1C). As expected, the survival rates were 100% for both sham-operated WT mice and UCP3–/– mice. Notably, 71% of WT mice (17/24) survived with HNE reperfusion therapy as compared to only 46% of WT mice (11/24) treated with saline (p<0.05). Genipin partially abrogated the survival benefit of HNE reperfusion therapy in WT mice (54% with genipin $vs.$ 71% without genipin, p<0.05).

Consistent with previous research showing reduced post-MI survival for UCP3–/– mice [17], saline-treated UCP3–/– mice exhibited worse survival rates as compared to saline-treated WT mice (17% $vs.$ 46%). Notably, HNE reperfusion therapy failed to improve survival in UCP3–/– mice (21% $vs.$ 17%, p>0.05). Genipin had no survival effect upon HNE-treated UCP3–/– mice (17% $vs.$ 21%, p>0.05).

HNE reperfusion therapy improves post-MI left ventricular function in an UCP3-dependent manner

Figure 2 displays the left ventricular functional parameters for WT mice (Figure 2A–2C) and UCP3–/– mice (Figure 2D–2F) at 24 h post-MI. HNE reperfusion therapy in WT mice decreased LVPWd (12% decrease), improved LVEDD (42% increase), and improved left ventricular FS (58% increase) as compared to saline treatment (p<0.05). Genipin partially abrogated the functional benefits of HNE reperfusion therapy in WT mice (p<0.05).

Consistent with previous research showing reduced post-MI cardiac function for UCP3–/– mice (17), saline-treated UCP3–/– mice exhibited worse left ventricular functional parameters as compared to saline-treated WT mice (p<0.05). Notably, HNE reperfusion therapy failed to improve left ventricular functional parameters in UCP3–/– mice (p>0.05). Genipin had no functional effect upon HNE-treated UCP3–/– mice (p>0.05).
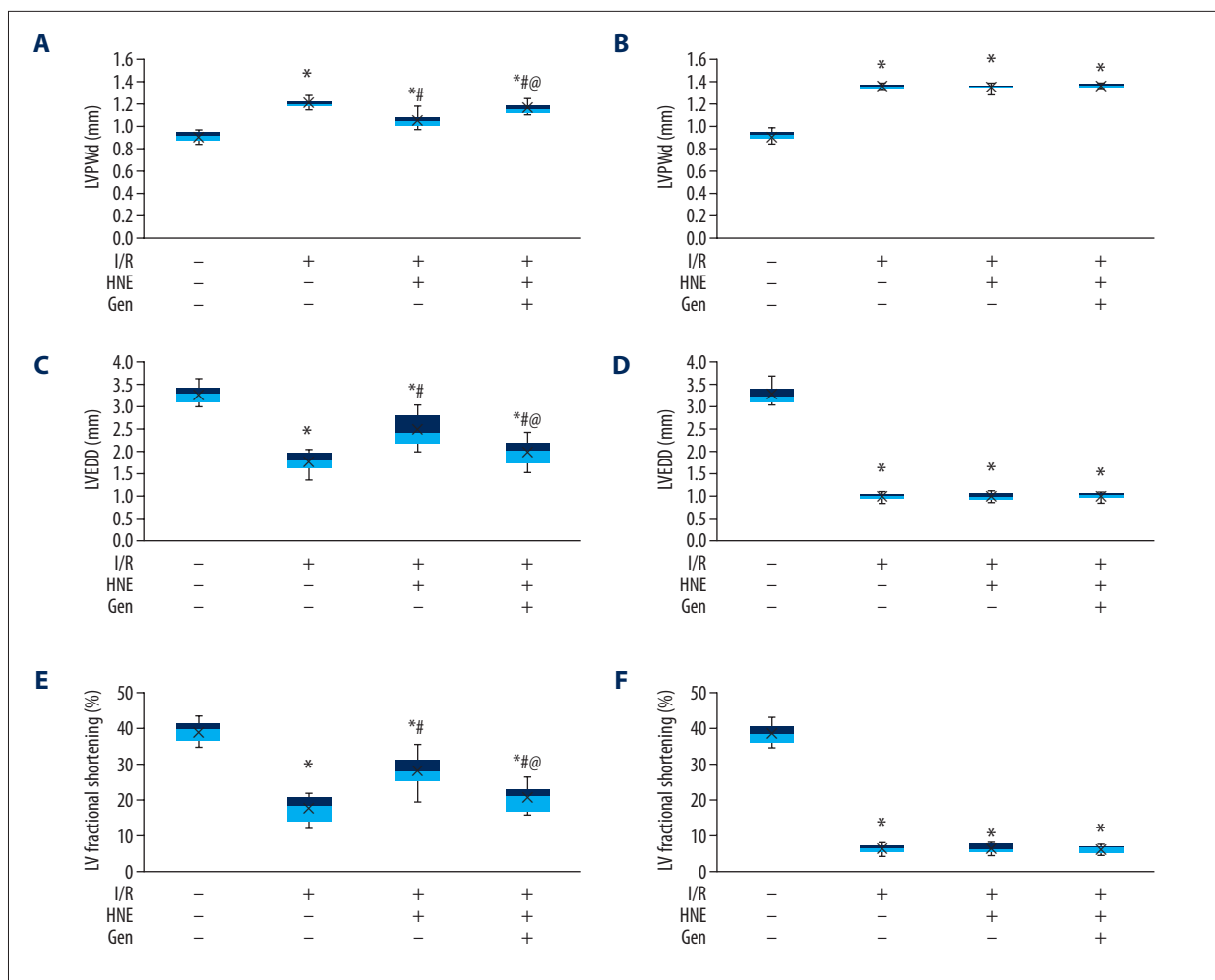
Figure 2. HNE improves post-MI left ventricular functional parameters. (**A, B**) Left ventricular end-diastolic posterior wall thickness (LVPWd) in (**A**) WT and (**B**) UCP3–/– mice. (**C, D**) Left ventricular end-diastolic diameter (LVEDD) in (**C**) WT and (**D**) UCP3–/– mice. (**E, F**) Left ventricular fractional shortening (FS) in (E) WT and (F) UCP3–/– mice. One-way ANOVA was applied to determine the main effect. Tukey's post hoc multiple comparisons test was then applied to compare the means of various groups. * $p<0.05$ *vs.* sham group, # $p<0.05$ *vs.* I/R group, @ $p<0.05$ *vs.* I/R+HNE group.

HNE reperfusion therapy reduces post-MI infarct size in an UCP3-dependent manner

Figure 3 displays the infarct size percentages (calculated as infarct area divided by risk area, IA/RA) for WT mice (Figure 3B) and UCP3–/– mice (Figure 3C) at 24 h post-MI. HNE reperfusion therapy in WT mice decreased infarct sizes (49% decrease) as compared to saline treatment ($p<0.05$). Genipin partially abrogated the benefit of HNE reperfusion therapy in WT mice (15% decrease with genipin *vs.* 49% decrease without genipin, $p<0.05$).

Consistent with previous research showing increased infarct sizes for UCP3–/– mice [17], saline-treated UCP3–/– mice exhibited larger infarct sizes as compared to saline-treated WT mice ($p<0.05$). Notably, HNE reperfusion therapy failed to reduce infarct sizes in UCP3–/– mice ($p>0.05$). Genipin had no impact upon infarct sizes in HNE-treated UCP3–/– mice ($p>0.05$).

HNE reperfusion therapy upregulates post-MI myocardial UCP3 expression

qRT-PCR and Western blot analyses demonstrated that UCP3 is expressed in the mouse myocardium (Figure 4A–4C). Consistent with previous research [18], we found that I/R upregulates UCP3 mRNA and protein expression ($p<0.05$). Notably, HNE reperfusion therapy further upregulated UCP3 mRNA and protein expression ($p<0.05$). As genipin directly inhibits UCP activity but does not affect UCP expression (11), genipin did not significantly impact UCP3 expression ($p>0.05$). As expected, all UCP3–/– mice groups displayed no discernable UCP3 expression.
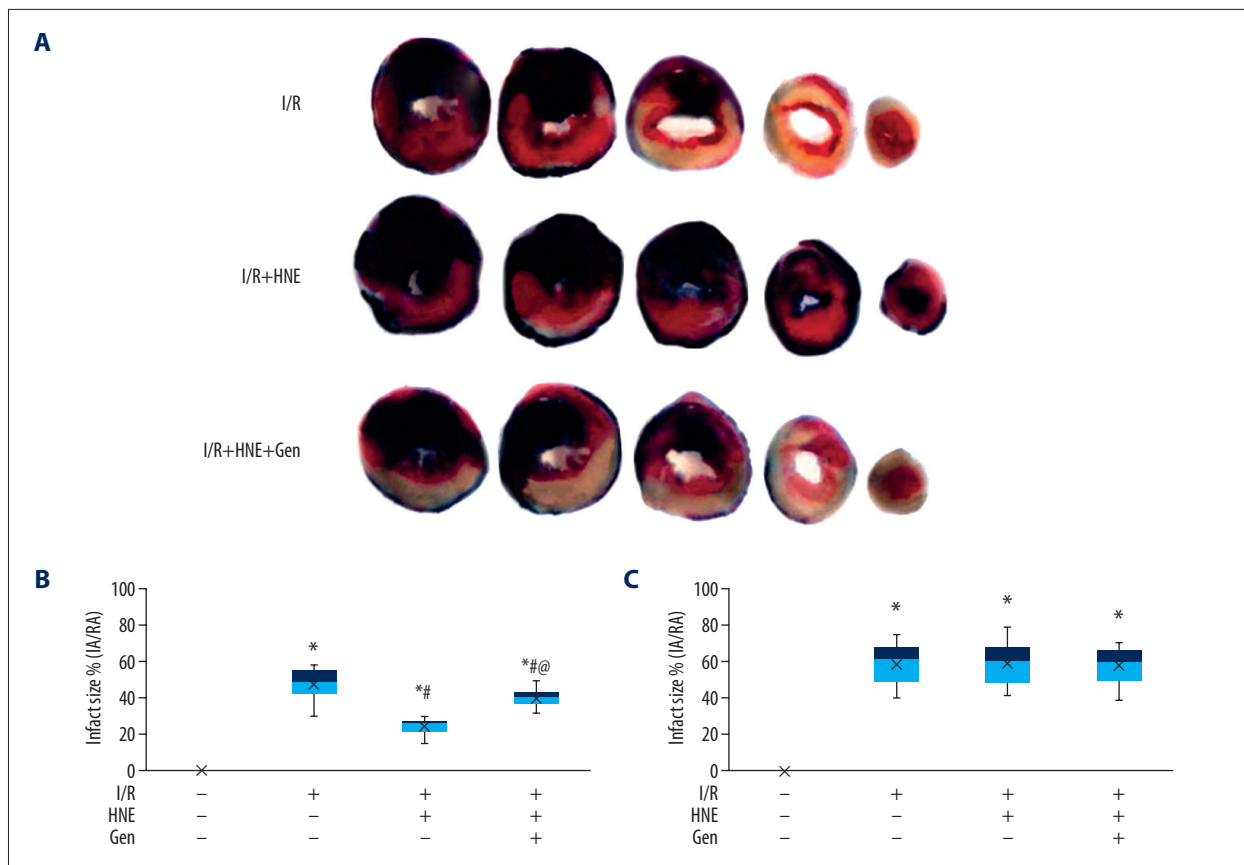
Figure 3. HNE reduces post-MI myocardial infarct size. (**A**) Representative TTC-stained WT murine heart sections. Non-risk areas stain blue, risk areas containing viable tissue stain red, and risk areas containing necrotic tissue appear white. (**B, C**) Myocardial infarct size (calculated as infarct area divided by risk area, IA/RA) in (**B**) WT and (C) UCP3–/– mice measured 24 h post-MI. One-way ANOVA was applied to determine the main effect. Tukey's post hoc multiple comparisons test was then applied to compare the means of various groups. * $p<0.05$ *vs.* sham group, # $p<0.05$ *vs.* I/R group, @ $p<0.05$ *vs.* I/R+HNE group.

HNE reperfusion therapy does not affect post-MI myocardial UCP2 expression

qRT-PCR and Western blot analyses demonstrated that UCP2 is expressed in the mouse myocardium (Figure 4D–4F); however, UCP2 expression was very minimal under sham conditions. Consistent with previous research [19], we found that I/R upregulates UCP2 protein expression ($p<0.05$) but does not impact UCP2 transcript expression ($p>0.05$). Notably, HNE reperfusion therapy does not affect UCP2 expression ($p>0.05$). Similar to its effects on UCP3, genipin did not significantly impact UCP2 expression ($p>0.05$).

HNE reperfusion therapy reduces post-MI apoptosis marker expression in an UCP3-dependent manner

Figure 4G–4I displays apoptosis marker expression for WT mice and UCP3–/– mice at 24 h post-MI. HNE reperfusion therapy in WT mice decreased cytochrome C and cleaved caspase-3 expression as compared to saline treatment ($p<0.05$). Genipin

partially abrogated these benefits of HNE reperfusion therapy in WT mice ($p<0.05$).

Consistent with previous research showing increased myocardial apoptosis in UCP3–/– mice [17], saline-treated UCP3–/– mice exhibited greater cytochrome C and cleaved caspase-3 expression as compared to saline-treated WT mice ($p<0.05$). Notably, HNE reperfusion therapy failed to reduce cytochrome C and cleaved caspase-3 expression in UCP3–/– mice ($p>0.05$). Genipin had no impact upon these markers in HNE-treated UCP3–/– mice ($p>0.05$).

Discussion

Here, we show that low-dose HNE reperfusion therapy (4 mg/kg, i.v.) improves survival, left ventricular function, infarct size, and apoptosis marker expression 24 h post-MI in WT mice. Our study design is clinically relevant to STEMI patients and interventional cardiologists; we administered i.v. HNE just prior to reperfusion
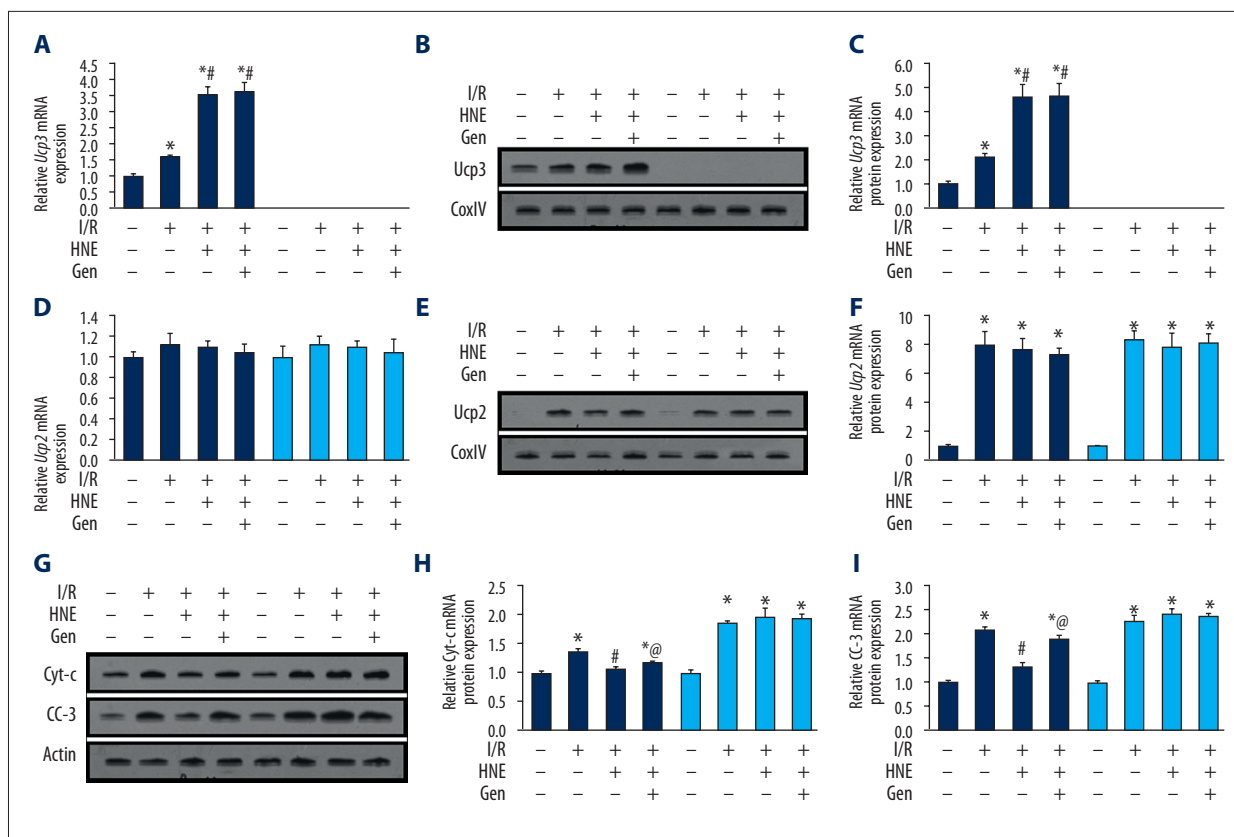
Figure 4. HNE upregulates post-MI myocardial UCP3 expression and reduces post-MI apoptosis marker expression. Data from WT mice are depicted in black bars, while data from UCP3–/– mice are depicted in gray bars. (**A**) qRT-PCR-derived UCP3 mRNA expression in WT and UCP3–/– mice. (**B**) Representative immunoblots of mitochondrial-fraction UCP3 expression from WT and UCP3–/– mice myocardium. (**C**) Densitometric analysis of UCP3 protein expression in WT and UCP3–/– mice. (**D**) qRT-PCR-derived UCP2 mRNA expression in WT and UCP3–/– mice. (**E**) Representative immunoblots of mitochondrial-fraction UCP2 expression from WT and UCP3–/– mice myocardium. (**F**) Densitometric analysis of UCP2 protein expression in WT and UCP3–/– mice. (**G**) Representative immunoblots of whole-cell cytochrome C (Cyt-c) and cleaved caspase-3 (CC-3) expression from WT and UCP3–/– mice myocardium. (H, I) Densitometric analysis of (**H**) Cyt-c and (**I**) CC-3 protein expression in WT and UCP3–/– mice. One-way ANOVA was applied to determine the main effect. Tukey's post hoc multiple comparisons test was then applied to compare the means of various groups. * $p<0.05$ *vs.* sham group, # $p<0.05$ *vs.* I/R group, @ $p<0.05$ *vs.* I/R+HNE group.

as could be conveniently performed in a catheterization lab setting. Several previous studies have demonstrated the cardioprotective effects of HNE against I/R injury in cultured cardiomyocytes or *in vivo* animal myocardial tissue [7,8]. However, this study is the first to take advantage of UCP3–/– mice to demonstrate that HNE's cardioprotective effects are UCP3-dependent. This finding is important since it shows the significance of UCP3 in mediating HNE's cardioprotective effects after MI. It also sheds light on potential applications for HNE therapy in other disease states that involve dysregulation of mitochondrial uncoupling. Secondarily, we also demonstrate that the UCP inhibitor genipin abrogates HNE's cardioprotective benefits in WT mice. Although recent research has shown the inhibitory effects of genipin on UCP3 *in vitro* [11], this study is the first to use UCP3–/– mice to demonstrate that the cardiotoxic effects of genipin under *in vivo* I/R conditions are UCP3-dependent.

Most of the early work in this field focused on the cardiotoxic effects of HNE [20,21]. However, more recent research has revealed that low levels of HNE appear to counteract I/R-induced oxidative stress through inducing an antioxidant response (mitohormesis) in cardiomyocyte mitochondria [22,23]. More specifically, Zhang et al.'s *in vitro* work determined that HNE is cytotoxic at concentrations above 20 µM but not so at lower concentrations [8]. Rather paradoxically, low doses of HNE (5 µM) primed cardiomyocytes to become resistant to high HNE levels through activating the NF-E2-related factor 2 (Nrf2) pathway and increasing intracellular levels of the potent antioxidant glutathione [8]. *In vivo*, low-dose i.v. HNE (4 mg/kg) activates Nrf2, elevated intramyocardial glutathione content, and improved post-MI left ventricular function in Langendorff-perfused hearts [8]. More recently, Lopez-Bernardo et al. showed that low-dose HNE activates Nrf2 and enhances UCP3

expression in mouse cardiomyocytes through promoting Nrf2 binding to the UCP3's promoter [7]. Notably, low-dose HNE increased proton leak across the inner mitochondrial membrane, decreased the maximal respiratory capacity, decreased the respiratory reserve capacity, and improved cardiomyocyte survival in a Nrf2/UCP3-dependent manner [7]. Integrating our *in vivo* findings, Nrf2-mediated UCP3 upregulation in cardiomyocytes following low-dose HNE therapy appears to play an important role in cardioprotection under I/R conditions.

With respect to the UCP inhibitor genipin, previous work has almost exclusively focused on the inhibitory action of genipin upon UCP2 activity in cultured cardiomyocytes or *in vivo* animal myocardial tissue [10,12]. Most notably, Moukdar et al. subjected Langendorff-perfused rat hearts to I/R and administered genipin 20 min prior to ischemia, wherein genipin pre-treatment produced increased infarct size as well as decreased left ventricular developed pressure (LVDP) and coronary flow [12]. Moukdar et al. attributed the effects of genipin to UCP2 inhibition [12]. However, recent *in vitro* research has revealed that the UCP-inhibitory activity of genipin is not exclusively limited to UCP2 [11]. Our current work substantiates the view that genipin acts as a myocardial UCP3 inhibitor *in vivo*, as the cardiotoxic effects of genipin under I/R conditions are UCP3-dependent.

Further work is needed to elucidate the mode of action of genipin on UCP3 activity in cardiomyocytes under anoxia-reoxygenation conditions *in vitro* and I/R conditions *in vivo*.

Conclusions

In conclusion, we provide novel evidence for a UCP3-mediated mechanism for HNE's cardioprotective effects against myocardial I/R injury. The treatment of reperfusion injury with low-dose HNE appears attractive considering its positive effects upon left ventricular function and infarct size, which are strong predictors of adverse outcomes following STEMI. Although our infarction model utilizes mice that display differences in coronary circulation and other physiological factors from humans, our study still serves as proof-of-concept for HNE's potential infarct-sparing effects. Given the favorable safety profile of low-dose HNE and previous evidence of its cardioprotective properties, low-dose HNE reperfusion therapy should be considered for larger-animal trials to further investigate its infarct-sparing effects.

Conflicts of interest

None.

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