Free Fatty Acid Receptor G-protein–coupled Receptor 40 Mediates Lipid Emulsion-induced Cardioprotection

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ABSTRACT

Background: We have previously shown that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity. However, the precise underlying mechanisms are not fully understood. Here we explored the hypothesis that free fatty acid receptor-1 or G-protein–coupled receptor 40 is expressed in the heart and that cardioprotective effects of lipid emulsion are mediated through G-protein–coupled receptor 40 in two animal models of ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity.

Methods: Langendorff-perfused male mouse hearts were subjected to ischemia/reperfusion with lipid emulsion alone (1%) or with G-protein–coupled receptor 40 antagonist (GW1100, 10 μM). Additionally, cardiotoxicity was achieved in male rats with bupivacaine bolus (10 mg/kg, IV) followed by lipid emulsion alone (20%, 5 ml/kg bolus, and 0.5 ml · kg^{-1} · min^{-1} maintenance, IV) or with GW1100 pretreatment (2.5 mg/kg, IV).

Results: G-protein–coupled receptor 40 is expressed in rodent hearts. GW1100 abolished lipid emulsion-induced cardioprotection against ischemia/reperfusion in mice because rate pressure product and left ventricular developed pressure were lower than lipid emulsion alone (rate pressure product: 2,186 ± 1,783 [n = 7] *vs.* 11,607 ± 4,347 [n = 8]; left ventricular developed pressure: 22.6 ± 10.4 *vs.* 63.8 ± 20; $P < 0.0001$). Lipid emulsion + GW1100 also demonstrated reduced LV dP/dt_{max} and LV dP/dt_{min} (dP/dt_{max} = 749 ± 386 *vs.* 2,098 ± 792, $P < 0.001$; dP/dt_{min} = −443 ± 262 *vs.* −1,447 ± 546, $P < 0.001$). In bupivacaine-induced cardiotoxicity rat model, GW1100 pretreatment had no significant effect on heart rate (HR) and ejection fraction after 30 min (HR: 302 ± 17 *vs.* 312 ± 38; ejection fraction: 69 ± 3% *vs.* 73 ± 4%). GW1100 pretreatment, however, prevented lipid-rescue, with no recovery after 10 min. In the control group, lipid emulsion improved HR (215 ± 16 at 10 min) and fully rescued left ventricle function at 10 min (ejection fraction = 67 ± 8%, fractional shortening = 38 ± 6%).

Conclusions: G-protein–coupled receptor 40 is expressed in the rodent heart and is involved in cardioprotection mediated by lipid emulsion against ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity. **(ANESTHESIOLOGY 2018; 129:154-62)**

WE and others[1–5] have shown that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion injury in rodents both *in vivo* and *ex vivo* through activation of intracellular signaling machinery. Recently, the protective effect of postischemic administration of lipid emulsion before aortic cross-unclamping on reperfusion injury was found in patients undergoing cardiac surgery as determined by a decrease in biomarkers of myocardial injury (cardiac troponin T and creatine kinase MB).[6] Lipid emulsion postconditioning represents a novel and clinically feasible cardioprotective strategy.

We and others[7–11] have also shown that lipid emulsion protects the heart against bupivacaine-induced cardiotoxicity. We proposed direct cardiac effects of lipid emulsion and discovered that the rescue by lipid emulsion is likely mediated through fatty acid oxidation pathway, because lipid emulsion did not resuscitate the hearts from bupivacaine overdose when the rats were pretreated with fatty acid oxidation inhibitor CVT-4325[8]. In a follow-up study, our group highlighted the involvement of G-protein–coupled opioid receptors in mediating the rescue action of lipid emulsion in resuscitating the heart.[9] Theories regarding the mechanism of intravenous lipid emulsion for bupivacaine cardiotoxicity include: (1) creation of an intravascular lipid sink into which the cardiotoxic drug is sequestered, (2) an improvement of impaired cardiac metabolism, and (3) restoration of cardiomyocyte function by increasing intracellular calcium.[12] The

What We Already Know about This Topic

- Previous studies have demonstrated that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity. Free fatty acid receptor-1 or G-protein–coupled receptor 40 is a major free fatty acid receptor in the body that is activated by medium and long chain fatty acids.

What This Article Tells Us That Is New

- This study demonstrates that G-protein–coupled receptor 40 is expressed in the rodent heart and is involved in cardioprotection mediated by lipid emulsion against ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity.

The abstract from this work was presented at the Best of Basic Science Session at the American Society of Anesthesiologists 2016 Meeting, October 22 to 26, 2016, Chicago, Illinois.

Submitted for publication June 6, 2017. Accepted for publication February 19, 2018. Corrected on May 9, 2018. From the Department of Anesthesiology and Perioperative Medicine, Division of Molecular Medicine, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, California.

 Anesthesiology 2018; 129:154-62

<zdoi;10.1097/ALN.0000000000002195>

lipid-sink theory, which suggested that increased intravascular lipid concentrations sequester drug from tissues, did not fully explain the beneficial effects of the intravenous lipid emulsion. Direct metabolic effects of lipid emulsion for its cardioprotective properties against bupivacaine-induced cardiac arrest have been reported.[8] Lipid emulsion has also been shown to have cardiotonic effects.[13]

Lipid emulsion is a mix of medium and long chain fatty acids. G-protein–coupled receptor 40, also known as free fatty acid receptor 1, is a free fatty acid receptor mainly expressed in pancreatic β-cells that is activated by medium- and long-chain fatty acids and regulates insulin secretion through an increase in cytosolic free calcium.[14,15] Despite the recent advances in the field, it is still unclear whether cardioprotection of lipid emulsion is mediated through a cell membrane receptor. Whether G-protein–coupled receptor 40 is expressed in the heart is largely unknown. Because G-protein–coupled receptor 40 is the main free fatty acid receptor in the body, it is a likely candidate receptor of the cellular effects of lipid emulsion.

Here we explored the hypothesis that the free fatty acid receptor G-protein–coupled receptor 40 is expressed in the rodent heart and the cardioprotective effects of lipid emulsion are mediated, at least in part, through G-protein–coupled receptor 40 in two animal models of ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity.

Materials and Methods

The protocols received institutional review and committee approval. The investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1996). Animals were randomly assigned to different experimental groups. Experimenters were not blinded to the experimental conditions.

Cardiac Ischemia/Reperfusion Injury Model in Mice

Langendorff Preparation. Male mice (2 to 3 months old, wild type, C57BL/6) were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), and heparin (200 U/kg) was injected to prevent blood coagulation. The heart was quickly removed; placed in ice-cold Krebs–Henseleit buffer solution: 11.1 mM glucose; 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 2 mM $CaCl_2$, pH 7.4; and bubbled with 95% O_2, 5% CO_2 at 37°C.

Experimental Protocol. We used the well established protocol to induce ischemia/reperfusion injury in isolated mouse hearts as shown by our group and others.[1,16,17] The heart was connected to the perfusion cannula *via* the aorta and perfused with Krebs–Henseleit buffer solution. Once equilibration was achieved, the aorta was clamped for 20 min to induce global normothermic (37°C) ischemia (the heart was immersed in the 37°C Krebs solution during ischemia), followed by reperfusion for 40 min with Krebs–Henseleit buffer (control), with additional 1% lipid emulsion (lipid emulsion group) or with 1% lipid emulsion together with the G-protein–coupled receptor 40 antagonist GW1100 (10 μM, lipid emulsion + GW1100) (fig. 1A). One group of mouse hearts (n = 6) was perfused on the Langendorff with GW1100 (10 μM) in Krebs–Henseleit buffer for 40 min without ischemia/reperfusion. GW1100 is a selective G-protein–coupled receptor 40 antagonist with a pIC_{50} of 6.[18] We selected the dose of GW1100 (10 μM) for our *ex vivo* experiments based on a study that demonstrated GW1100 to be a selective G-protein–coupled receptor 40 receptor antagonist at up to 10 μM.[18] The dose for *in vivo* experiments was calculated from the dose used in *ex vivo* experiments.

Cardiac Functional Measurements. A catheter (1.4F SPR-671; Millar, USA) connected to a pressure transducer was directly inserted into the left ventricle to measure left ventricular systolic pressure, left ventricular end-diastolic pressure, and heart rate. The left ventricle developed pressure was calculated as left ventricular developed pressure = left ventricular systolic pressure – left ventricular end-diastolic pressure and rate pressure product calculated by = heart rate × left ventricular developed pressure. The maximum rate of left ventricle pressure rise (dP/dt^{max}) and decline ($-dP/dt_{min}$) were directly calculated from the selected stable recordings. There were 6 to 8 mice/group, and data were expressed as mean ± SD.

Bupivacaine-induced Cardiotoxicity Model in Rats

Animals. Adult male Sprague–Dawley rats (250 to 300 g) were used. The rats were anesthetized intraperitoneally with a mixture of ketamine (80 mg/kg) and xylazine (8 mg/kg).

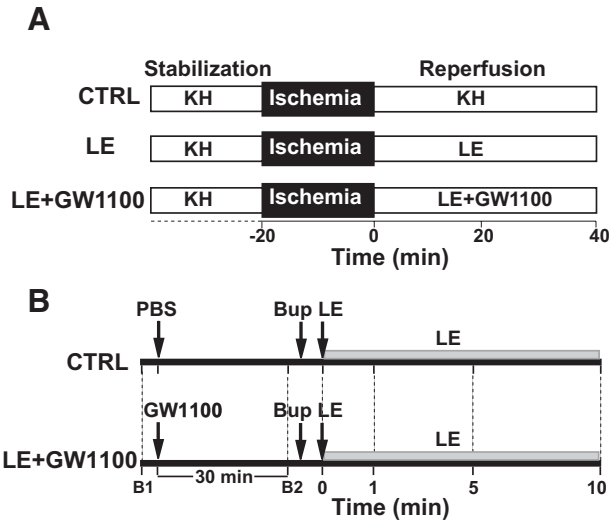

Fig. 1. Experimental protocols. (*A*) Cardiac ischemia/reperfusion injury model in male mice. (*B*) Bupivacaine (Bup)-induced cardiotoxicity model in male rats. B1 = baseline before GW1100 administration; B2 = baseline 30 min after GW1100; CTRL = control; LE = lipid emulsion; KH = Krebs–Henseleit buffer; PBS = phosphate-buffered saline.

Tracheostomy was performed using a 16-gauge angiocatheter, and the rats were ventilated with a ventilator. The femoral vein was accessed through a 24-gauge intravenous catheter. Body temperature was maintained at 37°C.

Treatments and Monitoring. The rats were pretreated with G-protein–coupled receptor 40-antagonist GW1100 (2.5 mg/kg, intravenous; Cayman Chemical, USA) 30 min before inducing asystole with one bolus of bupivacaine (10 mg/kg, IV over ~20 s, n = 7 rats). Resuscitation with lipid emulsion 20% (5 ml/kg bolus and 0.5 ml · kg^{-1} · min^{-1} maintenance) and chest compressions were initiated. HR (beats/min), ejection fraction (%), and fractional shortening (%) were measured using M-mode transthoracic echocardiography in the parasternal short axis view (VisualSonics [Canada] Vevo 2100 equipped with a 30-MHz linear transducer) before GW1100; 30-min after GW1100; and at 1, 5 and 10 min after lipid emulsion (fig. 1B). Control rats received phosphate-buffered saline followed by asystole with bupivacaine bolus (10 mg/kg, IV) and resuscitation with lipid emulsion 20% (5 ml/kg bolus and 0.5 ml · kg^{-1} · min^{-1} maintenance) along with chest compressions (n = 4 rats). Standard Lead II electrocardiograms were acquired under anesthesia continuously throughout the experiment.

Western Blot Analysis of the Hearts. Mouse and rat hearts were homogenized at 4°C in 150 mM NaCl, 50 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 1% Nonidet P-40, 0.1% SDS, and 0.5% sodium deoxycholate (pH 7.4). The samples were centrifuged at 12,000*g* for 10 min, and the supernatants were collected. Protein concentration was measured, and 100 μg of total protein was loaded on a 4 to 20% gradient Tris-HCl/SDS polyacrylamide gel, electrotransferred to nitrocellulose paper, blocked with 5% nonfat dry milk in 20 mM TBS with 0.1% Tween, and incubated with primary G-protein–coupled receptor 40 antibody (SC-32905, Santa Cruz Biotechnology, USA, 1:1,000 dilution) and vinculin antibody (Sigma [USA] catalog No. V3191, 1:20,000) overnight at 4°C. The blots were then indirectly labeled using secondary antibodies (IRDye 800CW anti-rabbit, LI-COR [USA] catalog No. 926-32211, 1:10,000; and IRDye 680RD anti-mouse, LI-COR catalog No. 926-68070, 1:10,000) for 2 h at room temperature and visualized with the Odyssey Imaging System (LI-COR).

Cardiomyocyte Isolation and Immunofluorescence Staining. The hearts were quickly removed and perfused through the aorta with the following solutions: (1) Ca^{2+}-free Tyrode solution containing 130 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 10 mM HEPES, 5.5 mM glucose (pH adjusted to 7.35 to 7.37 with NaOH) for 5 min; (2) Ca^{2+}-free Tyrode solution containing 160.4 U/ml collagenase type II (Worthington, USA) and 0.45 U/ml protease type XIV (Sigma) for ~15 min; and (3) Krebs solution containing 100 mM potassium glutamate, 10 mM potassium aspartate, 25 mM KCl, 10 mM KH_2PO_4, 2 mM $MgSO_4$, 20 mM taurine, 5 mM creatine base, 0.5 mM EGTA, 5 mM HEPES, and 20 mM glucose (pH adjusted to 7.2 with KOH) for 5 min. The solutions were oxygenated with 5% CO_2 and 95% O_2 before use and were maintained at 37 ± 1°C.

Freshly isolated cardiomyocytes were fixed in cold acetone for 10 min at −20°C. The isolated cells were incubated with 10% normal goat serum to block the background and were then stained with G-protein–coupled receptor 40 primary antibody (Santa Cruz catalog No. sc-32905, 1:50) in 1% normal goat serum and 0.2% Triton X-100 in phosphate-buffered saline at 4°C overnight. The cells were incubated with secondary antibody (Alexa Fluor 594 goat anti-rabbit, Thermo Fisher [USA] catalog No. A-11012, 1:1,000) at room temperature for 1 h, and the nuclei were stained with 4′,6′-diamino-2-phenylindole. After washing with phosphate-buffered saline + 0.1% Triton three times, the cells were mounted using ProLong Gold (Molecular Probes, USA) for imaging. Images were acquired with a confocal microscope (Nikon Eclipse E 400; Nikon, USA).

Statistical Analysis. Two-way repeated-measure ANOVA was used to evaluate the parameters over time (GraphPad Prism 7; GraphPad Software, Inc., USA). When significant overall differences were detected by the two-way ANOVA, pairwise *post hoc* comparisons were carried out between groups using the Bonferroni correction to adequately control our overall type 1 error rate at 0.05. All hypothesis tests were "two-sided." The sample size estimation was based on our previously published research work.[1–3,8,9] There were no missing, lost, or excluded data. *P* values of <0.05 were considered statistically significant. The values are expressed as means ± SD.

Results

Expression of G-protein–coupled Receptor 40 Protein in the Heart

Because the expression of G-protein–coupled receptor 40 in the heart tissue has not been demonstrated before, we evaluated the presence of G-protein–coupled receptor 40 in the mouse and rat heart tissue using Western immunoblotting. Fig. 2A shows that anti-G-protein–coupled receptor 40 antibody recognizes a single band at the expected molecular weight of G-protein–coupled receptor 40 protein in both mouse and rat hearts (31 kDa, *arrow*). Immunofluorescence staining of isolated mouse cardiomyocytes also demonstrated the presence of G-protein–coupled receptor 40 in cardiomyocytes at the cell membrane (fig. 2B). So, Western immunoblots and immunofluorescence staining of the hearts and cardiomyocytes confirmed the presence of G-protein–coupled receptor 40 protein in the heart.

G-protein–coupled Receptor 40 Antagonist Prevented Rescue of Ischemia/Reperfusion Injury by Lipid Emulsion

Next, we evaluated the role of G-protein–coupled receptor 40 in lipid emulsion-induced cardioprotection in the *ex vivo* ischemia/reperfusion mouse model. Lipid emulsion significantly improved rate pressure product from 2,966 ± 2,507

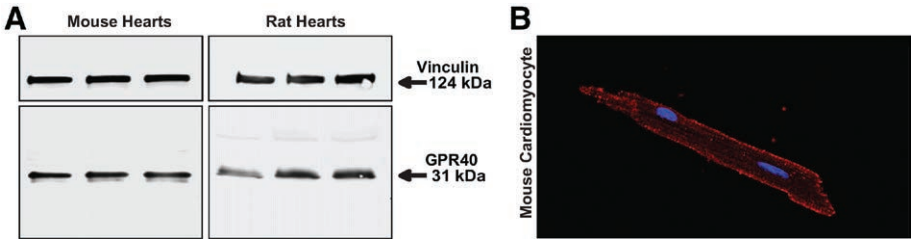

Fig. 2. Expression of G-protein–coupled receptor 40 (GPR40) in the rodent heart. (*A*) Western blot of the heart lysates from mice (*left*) and rats (*right*) showing a single band at the expected molecular weight for GPR40 (*arrow*) and vinculin (*arrow*). (*B*) Immunofluorescence stained representative isolated mouse heart cardiomyocyte showing GPR40 (*red*) and 4′,6′-diamino-2-phenylindole (*blue*) labeling.

(n = 6) in control to 11,607 ± 4,347 in the lipid emulsion group at the end of 40 min of reperfusion (n = 8, $P < 0.0001$). On the other hand, GW1100 prevented protective effect of lipid emulsion because the rate pressure product in lipid emulsion + GW1100 was significantly lower than lipid emulsion group (2,186 ± 1,783, n = 7, $P < 0.0001$). Left ventricular developed pressure was also lower in lipid emulsion + GW1100 compared to lipid emulsion alone (22.6 ± 10.4 in lipid emulsion + GW1100, 63.8 ± 20 in lipid emulsion, 14.5 ± 10 control, $P < 0.0001$ lipid emulsion + GW1100 *vs.* lipid emulsion alone). Lipid emulsion + GW1100 also showed much lower left ventricle dP/dt_{max} and left ventricle dP/dt_{min} compared to lipid emulsion alone (dP/dt_{max} = 749.1 ± 386 in lipid emulsion + GW1100, 2,098 ± 792 in lipid emulsion group, 640 ± 584 control $P < 0.001$ lipid emulsion + GW1100 *vs.* lipid emulsion alone; dP/dt_{min} = −443 ± 262 in lipid emulsion + GW1100, −1,447 ± 546 in lipid emulsion, −479 ± 457 in control, $P < 0.001$ lipid emulsion ± GW1100 *vs.* lipid emulsion, n = 6 to 8) (fig. 3). In fact, left ventricular developed pressure, rate pressure product, dP/dt_{max} and dP/dt_{min} in lipid emulsion + GW1100 group were not significantly different than control. Thus, inhibition of free fatty acid receptor G-protein–coupled receptor 40 with GW1100 completely abolishes lipid emulsion-induced cardioprotection against ischemia/reperfusion injury. A group of mouse hearts (n = 6) was perfused with GW1100 alone for 40 min without ischemia/reperfusion. The HR (beats/min) gradually decreased from 373 ± 55 at baseline to 281 ± 44 at 10 min ($P = 0.0471$ *vs.* baseline), 265 ± 50 at 20 min ($P = 0.0252$ *vs.* baseline), 266 ± 41 at 30 min ($P = 0.0176$ *vs.* baseline), and 263 ± 43 at 40 min ($P = 0.01$ *vs.* baseline).

G-protein–coupled Receptor 40 Antagonist Pretreatment Prevented Rescue of Bupivacaine-induced Cardiotoxicity by Lipid Emulsion

We also investigated the role of G-protein–coupled receptor 40 in the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion in the *in vivo* rat model. In controls, baseline HR, ejection fraction, and fractional shortening were 289 ± 17 beats/min, 62 ± 3%, and 34 ± 2%, respectively. Intravenous administration of bupivacaine resulted in asystole. Intravenously administered lipid emulsion gradually improved HR; 138 ± 51 at 1 min (47% recovery), 221 ± 16 at 5 min (76% recovery), and 215 ± 17 at 10 min (74% recovery). Left ventricle systolic function fully recovered at 5 min (ejection fraction = 68 ± 7%, fractional shortening = 39 ± 6%) and 10 min (ejection fraction = 67 ± 8%, fractional shortening = 38 ± 6%) after lipid emulsion. With G-protein–coupled receptor 40 antagonist pretreatment, HR and ejection fraction were unchanged before (HR = 302 ± 17, ejection fraction = 69 ± 3%) and 30 min after GW1100 (HR = 312 ± 38, ejection fraction = 73 ± 4%; $P = 0.36$ for HR and $P = 0.11$ for ejection fraction) excluding any significant effects of GW1100 on HR and ejection fraction. GW1100 pretreatment, however, prevented lipid emulsion rescue of bupivacaine-induced cardiac arrest because there was no recovery of cardiac function even after 10 min of lipid emulsion administration (fig. 4). Thus, inhibition of free fatty acid receptor G-protein–coupled receptor 40 with GW1100 completely abolishes lipid emulsion-induced cardioprotection against bupivacaine-induced cardiotoxicity.

Discussion

Our previous studies have shown that postischemic administration of lipid emulsion can protect the heart against ischemia/reperfusion injury in both the *in vivo* and *ex vivo* models.[1–3,8] Furthermore, we have also provided the evidence for direct cardioprotective effects of lipid emulsion in the rescue of bupivacaine-induced cardiotoxicity.[8,9] Here we demonstrate that free fatty acid receptor-1, also known as G-protein–coupled receptor 40, is expressed both in mouse and rat hearts. Furthermore, G-protein–coupled receptor 40 is involved in the cardioprotection conferred by lipid emulsion against cardiac ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity, because pretreatment with a selective G-protein–coupled receptor 40 antagonist, GW1100, prevented the rescue of lipid emulsion in both the models. The particular strengths of this study are the demonstration of G-protein–coupled receptor 40 expression in the heart and the use of two different animal models: the *ex vivo* mouse ischemia/reperfusion heart model and the *in vivo* rat bupivacaine-induced cardiotoxicity model, to investigate the mechanisms responsible for the cardioprotective properties of lipid emulsion.

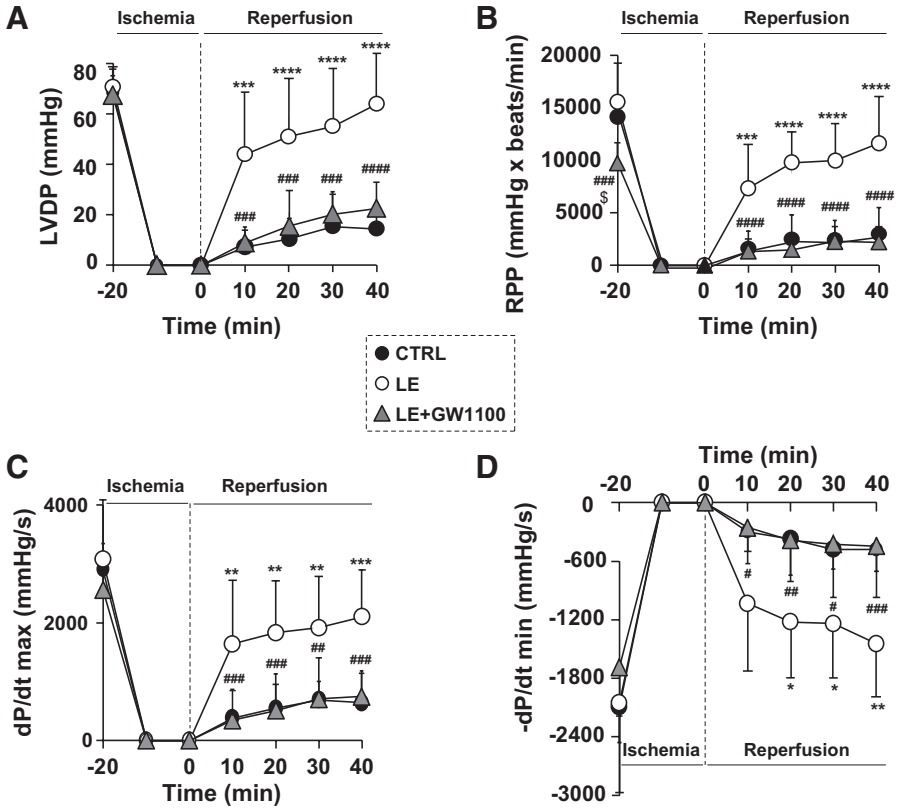

Fig. 3. G-protein–coupled receptor 40 antagonist prevents rescue of ischemia/reperfusion injury by lipid emulsion. (*A*) Left ventricular developed pressure (LVDP, mmHg). (*B*) Rate pressure product (RPP, mmHg x beats/min). (*C*) dP/dt_{max} (mmHg/s). (*D*) dP/dt_{min} (mmHg/s), from Langendorff perfused mouse hearts in control (CTRL, n = 6), lipid emulsion (LE, n = 8), and lipid emulsion + G-protein–coupled receptor 40 antagonist (n = 7) with ischemia/reperfusion injury as a function of time before ischemia, during ischemia, and reperfusion. The values are expressed as means ± SD. ****$P < 0.0001$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ####$P < 0.0001$; ###$P < 0.001$; ##$P < 0.01$; #$P < 0.05$; \$$P < 0.05$. For comparisons between groups: *LE compared to CTRL; #LE + GW1100 compared to LE; \$LE + GW1100 compared to CTRL.

Lipid emulsion has emerged as a novel and safe cardioprotective agent not just in preclinical studies[1–5,8,9] but also in a recently published randomized clinical trial.[6] The involvement of a cell membrane receptor/receptors in the intracellular effects of lipid emulsion has been largely unknown. Because lipid emulsion is an emulsion consisting of various components, it is likely that it might exert its cardioprotective effects *via* multiple cell membrane receptors. In that quest, our research group recently investigated the involvement of G-protein–coupled opioid receptors in mediating the rescue action of lipid emulsion in resuscitating the heart, because in the presence of opioid receptor antagonists, lipid emulsion failed to rescue bupivacaine-induced cardiac arrest.[9] Because lipid emulsion is rich in medium- and long-chain fatty acids, in the current study we explored whether intracellular effects of lipid emulsion are mediated *via* the main free fatty acid cell membrane receptor G-protein–coupled receptor 40 in the heart.

Acute ischemic tissue injury is one of the leading causes for perioperative organ failure. Ischemic injury to vital organs such as the heart causes significant morbidity and mortality worldwide each year. Early reperfusion is the key to salvage an ischemic organ. During the early stages of reperfusion, significant reversible and irreversible organ damage is initiated, a process referred to as reperfusion injury. The reperfusion injury is sometimes even more damaging than the ischemia itself because of oxidative damage caused by free radicals and calcium overload as a result of reintroduction of blood to the tissue.[19] Postconditioning has been used to protect the heart against ischemia/reperfusion injury. Cardioplegic arrest and cardiopulmonary bypass also trigger myocardial injury during cardiac surgery. Multiple strategies have been employed to protect the heart during the surgical requirement for global or regional ischemia.

We have reported that postischemic treatment with lipid emulsion inhibits the opening of mitochondrial permeability transition pore leading to cardioprotection through glycogen synthase kinase 3β phosphorylation *via* PI3K/Akt/ERK pathways.[1] Mitochondrial permeability transition pore is a large nonselective conductance pore located in the inner membrane of mitochondria.[20] The mitochondrial permeability transition pore remains closed during ischemia but opens during the reperfusion period.[21,22] Opening of the mitochondrial permeability transition pore is favored by events occurring during ischemia and reperfusion. Delaying the opening of the mitochondrial permeability transition

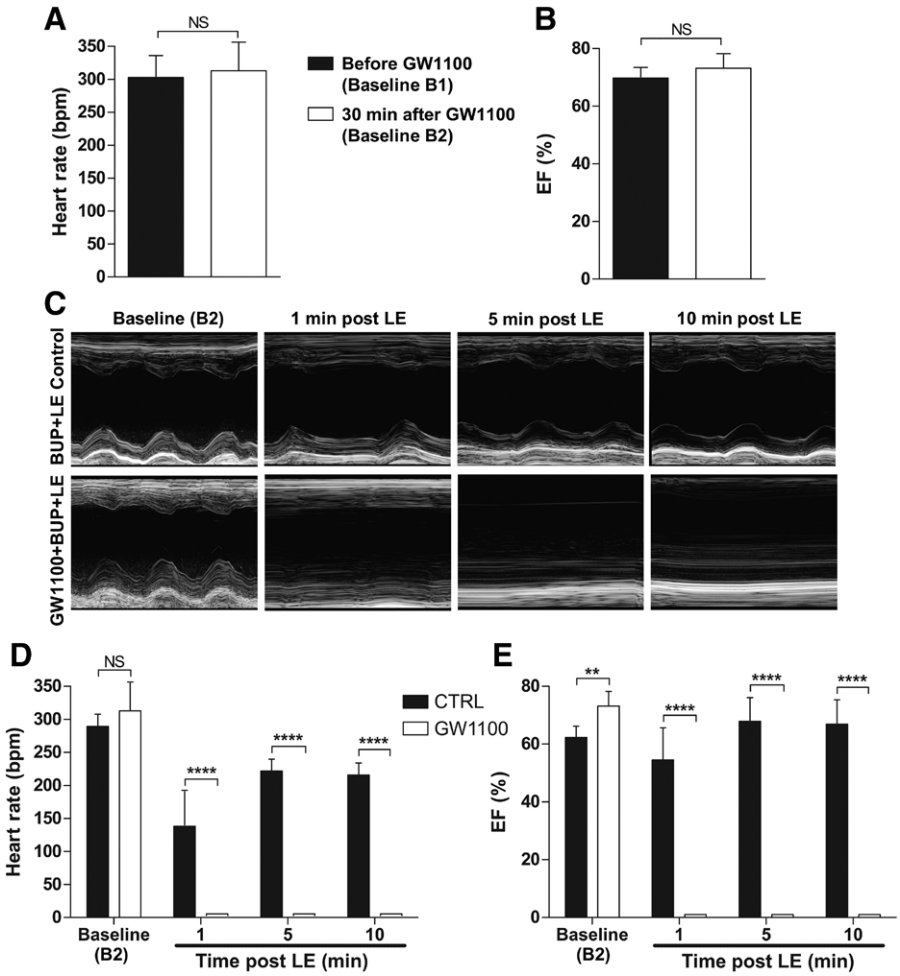

Fig. 4. G-protein–coupled receptor 40 antagonist pretreatment prevents rescue of bupivacaine (BUP)-induced cardiotoxicity by lipid emulsion. (*A*, *B*) Heart rate (*A*) and ejection fraction (EF, *B*) before and 30 min after GW1100 (n = 7). (*C*) M-mode transthoracic echocardiographic images from control (CTRL, lipid emulsion only, *upper panel*) and lipid emulsion + G-protein–coupled receptor 40 antagonist (*lower panel*) at baseline and 1, 5, and 10 min after lipid emulsion. (*D*, *E*) Heart rate (*D*) and EF (*E*) at baseline and 1, 5, and 10 min after lipid emulsion (n = 4 to 7/group). The values are expressed as means ± SD. $^{**}P < 0.01$; $^{****}P < 0.0001$. B1 = baseline before GW1100 administration; B2 = baseline 30 min after GW1100; LE = lipid emulsion; NS = not significant.

pore upon reperfusion has been a potential target to reduce myocardial injury.

Bupivacaine is a long-acting, lipophilic local anesthetic agent that is widely used in the perioperative period. Systemic toxicity from bupivacaine overdose can occur from accidental intravascular injection, drug overdose, or rapid absorption from the administration site.[7,23] Lipid emulsion has been successfully used in resuscitation from cardiac arrest as a result of bupivacaine toxicity in rat and canine models as well as in patients.[10,11,24–27] The precise molecular mechanisms of the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion are still not fully understood. The lipid-sink theory was considered to be the sole explanation for the rescue of lipid emulsion for many years, until recently when our group discovered that the rescue action of lipid emulsion is likely mediated through fatty acid oxidation pathway, because lipid emulsion could not resuscitate the hearts from bupivacaine overdose when the rats were pretreated with fatty acid oxidation inhibitor CVT-4325[8]. Since that discovery, multiple studies have favored the existence of a metabolic pathway for lipid emulsion rescue, in addition to the lipid-sink phenomenon. The dose-dependent recovery from cardiac pharmacotoxicity by lipid emulsion was also attributed to its cardiotonic effects.[13]

Taken together, lipid emulsion has emerged as an exciting prospect for conferring cardioprotection in various settings because its cardioprotection does not seem to be model-dependent. If lipid emulsion continues to show promise in larger animals and clinical studies, it may offer novel applications in organ protection, especially in the context of heart transplantation. Through the inhibition of mitochondrial permeability transition pore opening, lipid emulsion can help decrease reperfusion injury in human hearts after transplantation or other types of cardiac surgeries. In fact, in a recent clinical trial in cardiac surgery patients, postischemic administration of lipid emulsion was found to be safe and

was associated with significantly reduced myocardial injury as evidenced by a decrease in the markers of cardiac injury (creatine kinase MB and cardiac troponin T).[6]

In figure 5, using a schematic, we propose that lipid emulsion likely exerts its cardioprotection through activation of prosurvival signaling in the heart involving the well known protective reperfusion injury salvage kinase and survival activating factor enhancement pathways promoting glycogen synthase kinase 3β phosphorylation, leading to the inhibition of mitochondrial permeability transition pore opening.[1,3] Lipid emulsion may also fuel the β-oxidation and adenosine 5'-triphosphate synthesis in the mitochondria.[8] Furthermore, based on our previous work and our findings in this study, we also speculate that G-protein–coupled receptor 40 may mediate the cardioprotective effects of lipid emulsion *via* activation of reperfusion injury salvage kinase/survival activating factor enhancement pathways, resulting in glycogen synthase kinase 3β phosphorylation that in turn inhibits mitochondrial permeability transition pore[1–3,8] (fig. 5). Activation of G-protein–coupled receptor 40 by fatty acids has been shown to result in an increase in intracellular Ca^{2+} levels, an action mediated *via* $G\alpha_{q/11}$[28–30] that can result in inotropic effects in the heart. Activation of cardioprotective signaling pathways downstream of G-protein–coupled receptor 40 by lipid emulsion constitutes a clinically relevant strategy for cardioprotection. Identifying a receptor that lipid emulsion can interact with will allow us to better understand the mechanisms of lipid emulsion-induced cardioprotection. Identification of G-protein–coupled receptor 40 as the key receptor for the effects of lipid emulsion will also lead to development of more targeted therapeutic strategies with significant translational potential.

Conclusions

In conclusion, we demonstrate that G-protein–coupled receptor 40 is involved in the cardioprotection mediated by lipid

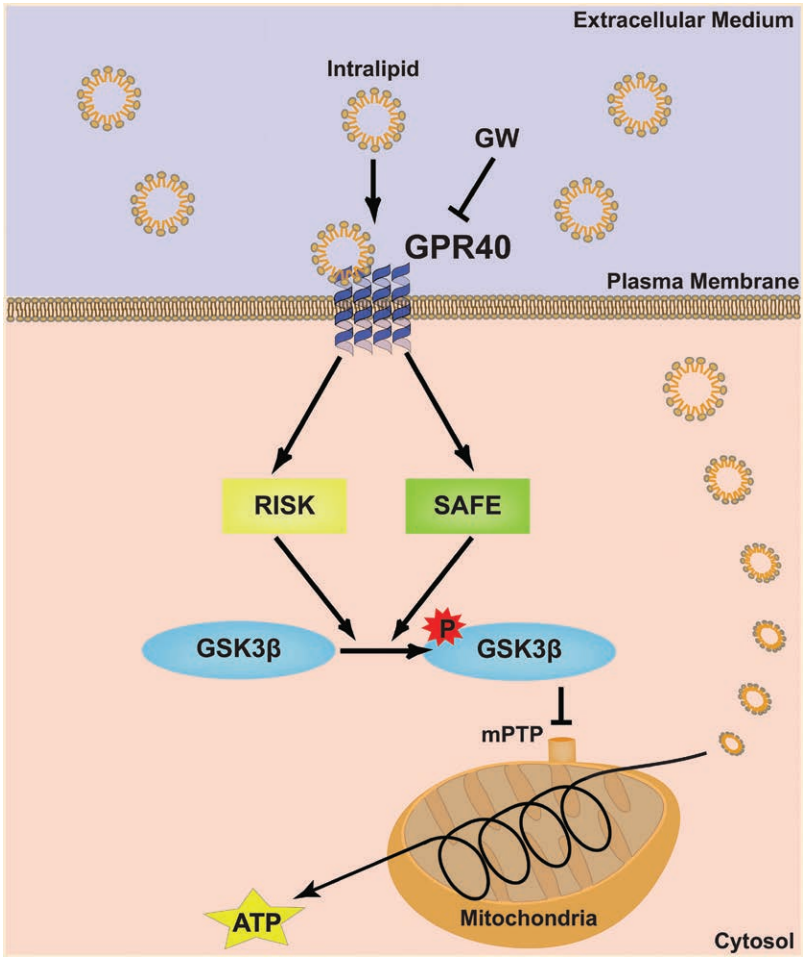

Fig. 5. Hypothetical scheme of mechanism of action of intralipid (lipid emulsion) for the rescue of ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity in the heart through activation of G-protein–coupled receptor 40 (GPR40). Glycogen synthase kinase 3β (GSK3β) phosphorylation-induced inhibition of the mitochondrial permeability transition pore (mPTP) is likely to be the common downstream cardioprotective mechanism in both ischemia/reperfusion injury[1,3] and bupivacaine-induced cardiotoxicity[8,9] involving reperfusion injury salvage kinase (RISK) and/or survival activating factor enhancement (SAFE) pathways. Lipid emulsion can also result in increased adenosine triphosphate (ATP) production. GW = GPR40 antagonist GW1100.

emulsion against cardiac ischemia/reperfusion injury and bupivacaine-induced cardiotoxicity. Discovery of G-protein–coupled receptor 40 as a potential receptor for intracellular effects of lipid emulsion opens up further avenues to help us understand lipid emulsion-induced cardioprotection. Acute ischemic tissue injury is one of the leading causes of organ failure. Lipid emulsion may play a role in organ protection and can potentially be used for the prevention of ischemia/reperfusion injury in human organ transplantation.

Research Support

Supported by National Institutes of Health (Bethesda, Maryland) grant No. R01HL131182 (to Dr. Eghbali) and by a Foundation for Anesthesia Education and Research (Schaumburg, Illinois) Mentored Research Training Grant (to Dr. Umar).

Competing Interests

The authors declare no competing interests.

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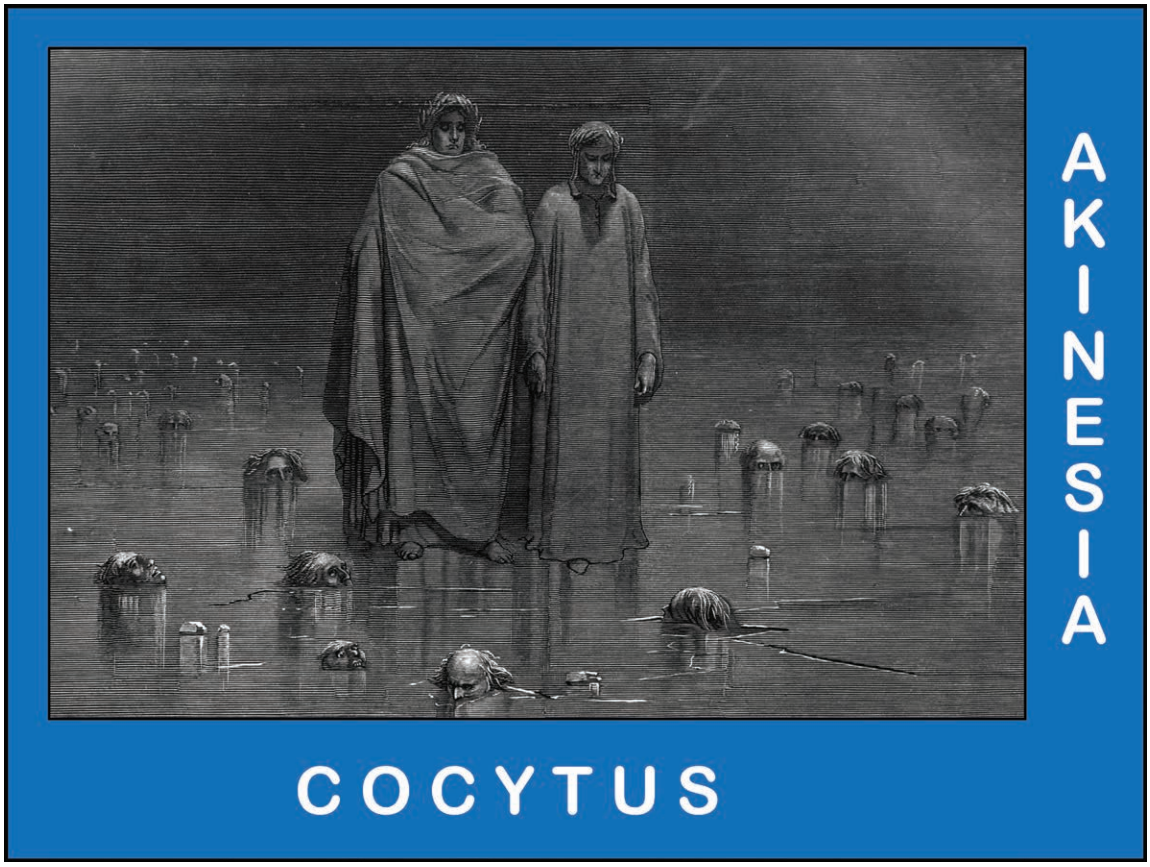
Streams of Unconsciousness IV: Akinesia Reflected in the Cocytus



For Dante's *Divine Comedy*, French illustrator Gustave Doré (1832 to 1883) depicted the souls of dead traitors as frozen into the Greco-Roman Underworld's River of Lamentation—the Cocytus (*above*). The poets walking across the icy Cocytus are Virgil and the shorter Dante. With the poetic license exercised by Dante, Hade's rivers Lethe, Styx, and Acheron can be understood to supply amnesia, hypnosis, and analgesia, respectively—later considered as hallmarks of general anesthesia. By immobilizing traitors in ice, the Cocytus, a fourth plutonic river, reflected akinesia, a fourth property of general anesthesia. A little more than a century after Doré published this image, immobility helped define the minimum alveolar concentration or MAC of anesthetic vapors. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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