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Free Fatty Acid Receptor GPR40 Mediates Lipid Emulsion-induced Cardioprotection

Soban Umar, M.D., Ph.D, Jingyuan Li, M.D., Ph.D, Kyle Hannabass, M.D., Mylene Vaillancourt, M.Sc, Christine M. Cunningham, BS, Shayan Moazeni, BS, Aman Mahajan, M.D., Ph.D, and Mansoureh Eghbali, Ph.D

Department of Anesthesiology and Perioperative Medicine, Division of Molecular Medicine, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA

Abstract

Background—We have previously shown that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion (I/R) 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 (GPR40) is expressed in the heart and cardioprotective effects of lipid emulsion are mediated through GPR40 in two animal models of I/R injury and bupivacaine-induced cardiotoxicity.

Methods—Langendorff-perfused male mouse hearts were subjected to I/R with lipid emulsion alone (1%) or with GPR40-antagonist (GW1100, 10 μ M). Additionally, cardiotoxicity was achieved in male rats with bupivacaine bolus (10mg/kg, IV) followed by lipid emulsion alone (20%, 5ml/kg bolus, and 0.5ml/kg/min maintenance, IV) or with GW1100 pre-treatment (2.5mg/kg, IV).

Results—GPR40 is expressed in rodent hearts. GW1100 abolished lipid emulsion-induced cardioprotection against I/R in mice since rate pressure product (RPP) and left ventricular developed pressure (LVDP) were lower than lipid emulsion alone (RPP: 2186 $\pm$ 1783 (n=7) vs. 11607 $\pm$ 4347 (n=8); LVDP: 22.6 $\pm$ 10.4 vs. 63.8 $\pm$ 20; p <0.0001). Lipid emulsion+GW1100 also demonstrated reduced LV dP/dtmax and LV dP/dtmin (dP/dtmax=749 $\pm$ 386 vs. 2098 $\pm$ 792, p <0.001; dP/dtmin=-443 $\pm$ 262 vs. -1447 $\pm$ 546, p <0.001).

In bupivacaine-induced cardiotoxicity rat model, GW1100 pre-treatment had no significant effect on heart rate and EF after 30-min (HR: 302 $\pm$ 17 vs. 312 $\pm$ 38; EF: 69 $\pm$ 3% vs. 73 $\pm$ 4%). GW1100 pre-treatment however prevented lipid-rescue, with no recovery after 10-min. In control-group, lipid emulsion improved HR (215 $\pm$ 16 at 10-min) and fully rescued LV function at 10-min (EF=67 $\pm$ 8%, FS=38 $\pm$ 6%).

Conclusions—GPR40 is expressed in the rodent heart and is involved in cardioprotection mediated by lipid emulsion against I/R injury and bupivacaine-induced cardiotoxicity.

Correspondence: Dr. Mansoureh Eghbali, UCLA School of Medicine, Dept. of Anesthesiology, BH-160CHS, Los Angeles, CA 90095-7115, Phone (310) 206-0345, meghbali@ucla.edu.

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Keywords

Free fatty acid receptor; Lipid emulsion; Bupivacaine; Cardiotoxicity; Ischemia/reperfusion injury

Introduction

We and other have shown that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion (I/R) injury in rodents both *in vivo* and *ex vivo* through activation of intracellular signaling machinery^{1–5}. Recently, the protective effect of post-ischemic administration of lipid emulsion prior to 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 have also shown that lipid emulsion protects the heart against bupivacaine-induced cardiotoxicity^{7–11}. We proposed direct cardiac effects of lipid emulsion and discovered that the rescue by lipid emulsion is likely mediated through fatty acid oxidation pathway, since lipid emulsion did not resuscitate the hearts from bupivacaine overdose when the rats were pre-treated with fatty acid oxidation inhibitor CVT-4325⁸. 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; i) creation of an intravascular lipid sink into which the cardiotoxic drug is sequestered, ii) an improvement of impaired cardiac metabolism, and iii) restoration of cardiomyocyte function by increasing intracellular calcium¹². The 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 reported⁸. Lipid emulsion has also been shown to have cardiotonic effects¹³.

Lipid emulsion is a mix of medium and long chain fatty acids. G-protein coupled receptor-40 (GPR40) also known as free fatty acid receptor-1 (FFAR-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 GPR40 is expressed in the heart is largely unknown. As GPR40 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 GPR40 is expressed in the rodent heart and the cardioprotective effects of lipid emulsion are mediated, at least in part, through GPR40 in two animal models of I/R injury and bupivacaine-induced cardiotoxicity.

Materials and methods

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 (NIH Pub. No. 85-23, Revised 1996). Animals were randomly assigned to different experimental groups. Experimenters were not blinded to experimental conditions.

1. Cardiac ischemia/reperfusion injury model in mice

Langendorff preparation—Male mice (2-3 month old, wild type, C57BL/6) were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and heparin (200 IU/kg) was injected to prevent blood coagulation. The heart was quickly removed and placed in ice-cold Krebs-Henseleit buffer solution (KH, in mM): glucose 11.1, NaCl 118, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 2 at pH 7.4 bubbled with 95% O₂/5% CO₂ at 37 °C.

Experimental protocol—We used the well-established protocol to induce I/R 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 KH 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 KH buffer (CTRL), with additional 1% lipid emulsion (Lipid emulsion group), or with 1% lipid emulsion together with the GPR40-antagonist, GW1100 (10µM, Lipid emulsion+GW1100) (Fig. 1A). One group of mouse hearts (n=6) was perfused on the Langendorff with GW1100 (10µM) in KH buffer for 40 min without I/R. GW1100 is a selective GPR40 antagonist with an pIC₅₀ of 6²⁷. We selected the dose of GW1100 (10µM) for our *ex-vivo* experiments based on a study that demonstrated GW1100 to be a selective GPR40 receptor antagonist at up to 10 µM²⁷. The dose for *in vivo* experiments was calculated from the dose used in *ex-vivo* experiments.

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

2. Bupivacaine-induced cardiotoxicity model in rats

Animals—Adult male Sprague-Dawley rats (250-300 g) were used. Rats were anesthetized intraperitoneally with a mixture of Ketamine (80 mg/kg) and Xylazine (8 mg/kg). Tracheostomy was performed using a 16-gauge angiocatheter and 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—Rats were pre-treated with GPR40-antagonist GW1100 (2.5 mg/kg, intravenous, Cayman Chemical, Ann Arbor, MI, USA) 30-min before inducing asystole with one bolus of bupivacaine (10 mg/kg, IV over ~20 seconds, n=7 rats). Resuscitation with lipid emulsion 20% (5 ml/kg bolus, and 0.5 ml/kg/min maintenance) and chest compressions were initiated. Heart rate (HR, beats per min) ejection-fraction (EF, %) and fractional shortening (FS, %) were measured using M-Mode transthoracic echocardiography in the parasternal short axis view (VisualSonics Vevo 2100 equipped with a 30-MHz linear transducer) before, 30-min after GW1100 and at 1, 5 and 10 min after lipid emulsion (Fig. 1B, Fig. 4). Control rats (CTRL) received phosphate buffered saline (PBS) 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/min 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 (mM): 150 NaCl, 50 Tris-HCl, 1 EGTA, 1 EDTA, 1 NaF, 1 PMSF, 1 Na3VO4, 1% NP-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 4-20% gradient Tris-HCl/SDS polyacrylamide gel, electrotransferred to nitrocellulose paper, blocked with 5% non-fat dry milk in 20 mM TBS with 0.1% Tween, and incubated with primary GPR40 antibody (SC-32905, Santa Cruz Biotechnology, Dallas, TX, USA, 1:1,000 dilution) and Vinculin antibody (Sigma cat# V3191, 1:20,000) overnight at 4°C. Blots were then indirectly labelled using secondary antibodies (IRDye 800CW anti-rabbit, Licor cat# 926-32211, 1:10,000 and IRDye 680RD anti-mouse, Licor cat# 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: (i) Ca²⁺-free Tyrode solution containing (in mM): 130 NaCl, 5.4 KCl, 1 MgCl₂, 0.33 NaH₂PO₄, 10 HEPES, 5.5 glucose (pH adjusted to 7.35–7.37 with NaOH) for 5 minutes, (ii) Ca²⁺-free Tyrode solution containing 160.4 U/ml Collagenase Type II (Worthington) and 0.45 U/ml Protease Type XIV (Sigma) for ~15 min; and (iii) Krebs solution containing (in mM): 100 K-glutamate, 10 K-aspartate, 25 KCl, 10 KH₂PO₄, 2 MgSO₄, 20 taurine, 5 creatine base, 0.5 EGTA, 5 HEPES, 20 glucose (pH adjusted to 7.2 with KOH) for 5 minutes. The solutions were oxygenated with 5% CO₂ and 95% O₂ prior to 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 GPR40 primary antibody (Santa Cruz cat# sc-32905, 1:50) in 1% NGS and 0.2% Triton X-100 in PBS at 4°C overnight. Cells were incubated with secondary antibody (Alexa fluor 594 Goat anti-rabbit, ThermoFisher cat# A-11012, 1:1,000) at room temp for 1h and nuclei were stained with DAPI. After washing with PBS + 0.1% Triton

three times, the cells were mounted using ProLong Gold (Molecular Probes) for imaging. Images were acquired with a confocal microscope (Nikon Eclipse E 400).

Statistical analysis—Two-way repeated measures ANOVA was used to evaluate the parameters over time (GraphPad Prism 7). 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 <0.05 were considered statistically significant. Values are expressed as mean ± SD.

Results

Expression of GPR40 protein in the heart

As the expression of GPR40 in the heart tissue has not been demonstrated before, we evaluated the presence of GPR40 in the mouse and rat heart tissue using Western immunoblotting. Fig. 2A shows that anti-GPR40 antibody recognizes a single band at the expected molecular weight of GPR40 protein both in mouse and rat hearts (31 kDa, arrow). Immunofluorescence staining of isolated mouse cardiomyocytes also demonstrated the presence of GPR40 in cardiomyocytes at the cell membrane (Fig. 2B). So, Western immunoblots and immunofluorescence staining of the hearts and cardiomyocytes confirmed the presence of GPR40 protein in the heart.

GPR40 antagonist prevented rescue of ischemia/reperfusion injury by lipid emulsion

Next, we evaluated the role of GPR40 in lipid emulsion-induced cardioprotection in the *ex vivo* I/R mouse model. Lipid emulsion significantly improved RPP from 2966 ± 2507 (n=6) in CTRL to 11607 ± 4347 in lipid emulsion group at the end of 40 min reperfusion (n=8, $p < 0.0001$). On the other hand, GW1100 prevented protective effect of lipid emulsion since the RPP in lipid emulsion+GW1100 was significantly lower than lipid emulsion group (2186 ± 1783 , n=7, $p < 0.0001$). LVDP 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 CTRL, $p < 0.0001$ lipid emulsion+GW1100 vs. lipid emulsion alone). Lipid emulsion+GW1100 also showed much lower LV dP/dtmax and LV dP/dtmin compared to lipid emulsion alone (dP/dtmax= 749.1 ± 386 in lipid emulsion+GW1100, 2098 ± 792 in lipid emulsion group, 640 ± 584 CTRL $p < 0.001$ lipid emulsion+GW1100 vs. lipid emulsion alone; dP/dtmin= -443 ± 262 in lipid emulsion+GW1100, -1447 ± 546 in lipid emulsion, -479 ± 457 in CTRL, $p < 0.001$ lipid emulsion+GW1100 vs. lipid emulsion, n=6–8) (Fig. 3). In fact, LVDP, RPP, dP/dtmax and dP/dtmin in lipid emulsion+GW1100 group were not significantly different than CTRL. Thus, inhibition of free fatty acid receptor GPR40 with GW1100 completely abolishes lipid emulsion-induced cardioprotection against I/R injury. A group of mouse hearts (n=6) was perfused with GW1100 alone for 40 min without I/R. The HR (bpm) 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).

GPR40 antagonist pre-treatment prevented rescue of bupivacaine-induced cardiotoxicity by lipid emulsion

We also investigated the role of GPR40 in the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion in the *in vivo* rat model. In controls, baseline HR, EF and FS were 289 ± 17 beats/min, $62 \pm 3\%$ and $34 \pm 2\%$ 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). LV systolic function fully recovered at 5-min (EF= $68 \pm 7\%$, FS= $39 \pm 6\%$) and 10-min (EF= $67 \pm 8\%$, FS= $38 \pm 6\%$) after lipid emulsion. With GPR40 antagonist pre-treatment, HR and EF were unchanged before (HR= 302 ± 17 , EF= $69 \pm 3\%$) and 30-min after GW1100 (HR= 312 ± 38 , EF= $73 \pm 4\%$; $p=0.36$ for HR and $p=0.11$ for EF) excluding any significant effects of GW1100 on HR and EF. GW1100 pre-treatment however prevented lipid emulsion rescue of bupivacaine-induced cardiac arrest as there was no recovery of cardiac function even after 10 min of lipid emulsion administration (Fig. 4). Thus, inhibition of free fatty acid receptor GPR40 with GW1100 completely abolishes lipid emulsion-induced cardioprotection against bupivacaine-induced cardiotoxicity.

Discussion

Our previous studies have shown that post-ischemic administration of lipid emulsion can protect the heart against I/R injury in both the *in-vivo* and *ex-vivo* models^{1,8,2,3}. 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 GPR40 is expressed both in mouse and rat hearts. Furthermore, GPR40 is involved in the cardioprotection conferred by lipid emulsion against cardiac I/R injury and bupivacaine-induced cardiotoxicity, as pre-treatment with a selective GPR40 antagonist, GW1100 prevented the rescue of lipid emulsion in both the models. The particular strengths of this study are the demonstration of GPR40 expression in the heart and the use of two different animal models, the *ex-vivo* mouse I/R heart model and the *in vivo* rat bupivacaine-induced cardiotoxicity model, to investigate the mechanisms responsible for the cardioprotective properties of lipid emulsion.

Lipid emulsion has emerged as a novel and safe cardioprotective agent not just in pre-clinical studies^{8,1,2,9,3-5} but also in a recently published randomized clinical trial⁶. The involvement of a cell membrane receptor/receptors in the intracellular effects of lipid emulsion has been largely unknown. As 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, as in the presence of opioid receptor antagonists, lipid emulsion failed to rescue bupivacaine-induced cardiac arrest⁹. As 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 GPR40 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 due to oxidative damage caused by free radicals and calcium overload as a result of re-introduction of blood to the tissue¹⁸. Post-conditioning has been used to protect the heart against I/R 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 post-ischemic treatment with lipid emulsion inhibits the opening of mitochondrial permeability transition pore (mPTP) leading to cardioprotection through glycogen synthase kinase-3 (GSK-3 β) phosphorylation *via* PI3K/Akt/ERK pathways¹. mPTP is a large non-selective conductance pore located in the inner membrane of mitochondria¹⁹. The mPTP remains closed during ischemia, but opens during the reperfusion period^{20,21}. Opening of the mPTP is favored by events occurring during ischemia and reperfusion. Delaying the opening of the mPTP 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^{7,22}. 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^{10,11,23–26}. 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, since lipid emulsion could not resuscitate the hearts from bupivacaine-overdose when the rats were pre-treated with fatty acid oxidation inhibitor CVT-4325⁸. 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 cardioprotective effects¹³.

Taken together, lipid emulsion has emerged as an exciting prospect for conferring cardioprotection in various settings as 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 mPTP 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, post-ischemic 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 (CK-MB, C-TnT)⁶.

In Figure 5, using a schematic, we propose that lipid emulsion likely exerts its cardioprotection through activation of pro-survival signaling in the heart involving the well known protective RISK and SAFE pathways promoting GSK-3 β phosphorylation, leading to the inhibition of mPTP opening^{1,3}. Lipid emulsion may also fuel the β -oxidation and ATP synthesis in the mitochondria⁸. Furthermore, based on our previous work and our findings in this study we also speculate that GPR40 may mediate the cardioprotective effects of lipid emulsion *via* activation of RISK/SAFE pathways resulting in GSK-3 β phosphorylation, that in turn inhibits mPTP^{1,8,2,3} (Fig. 5). Activation of GPR40 by fatty acids has been shown to result in an increase in intracellular Ca²⁺ levels, an action mediated via G α_q /11^{28,29,30} that can result in inotropic effects in the heart. Activation of cardioprotective signaling pathways downstream of GPR40 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 GPR40 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 GPR40 is involved in the cardioprotection mediated by lipid emulsion against cardiac I/R injury and bupivacaine-induced cardiotoxicity. Discovery of GPR40 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 for organ failure. Lipid emulsion may play a role in organ protection and can potentially be used for the prevention of I/R injury in human organ transplantation.

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References

1. Rahman S, Li J, Bopassa JC, Umar S, Iorga A, Partownavid P, Eghbali M. Phosphorylation of GSK-3 β mediates intralipid-induced cardioprotection against ischemia/reperfusion injury. *Anesthesiology*. 2011; 115:242–53. [PubMed: 21691195]
2. Li J, Iorga A, Sharma S, Youn J-Y, Partow-Navid R, Umar S, Cai H, Rahman S, Eghbali M. Intralipid, a clinically safe compound, protects the heart against ischemia-reperfusion injury more efficiently than cyclosporine-A. *Anesthesiology*. 2012; 117:836–46. [PubMed: 22814384]
3. Li J, Ruffenach G, Kararigas G, Cunningham CM, Motayagheneh N, Barakai N, Umar S, Regitz-Zagrosek V, Eghbali M. Intralipid protects the heart in late pregnancy against ischemia/reperfusion injury via Caveolin2/STAT3/GSK-3 β pathway. *J Mol Cell Cardiol*. 2017; 102:108–16. [PubMed: 27847332]
4. Lou P-H, Lucchinetti E, Zhang L, Affolter A, Schaub MC, Gandhi M, Hersberger M, Warren BE, Lemieux H, Sobhi HF, Clanachan AS, Zaugg M. The mechanism of Intralipid®-mediated cardioprotection complex IV inhibition by the active metabolite, palmitoylcarnitine, generates

- reactive oxygen species and activates reperfusion injury salvage kinases. *PloS One*. 2014; 9:e87205. [PubMed: 24498043]
5. Ma L-L, Ge H-W, Kong F-J, Qian L-B, Hu B-C, Li Q, Xu L, Liu J-Q, Xu Y-X, Sun R-H. Ventricular hypertrophy abrogates intralipid-induced cardioprotection by alteration of reperfusion injury salvage kinase/glycogen synthase kinase β signal. *Shock Augusta Ga*. 2014; 41:435–42.
 6. Zhou R-H, Yu H, Yin X-R, Li Q, Yu H, Yu H, Chen C, Xiong J-Y, Qin Z, Luo M, Tan Z-X, Liu T. Effect of intralipid postconditioning on myocardial injury in patients undergoing valve replacement surgery: a randomised controlled trial. *Heart Br Card Soc*. 2017; doi: 10.1136/heartjnl-2016-310758
 7. Weinberg GL. Lipid emulsion infusion: resuscitation for local anesthetic and other drug overdose. *Anesthesiology*. 2012; 117:180–7. [PubMed: 22627464]
 8. Partownavid P, Umar S, Li J, Rahman S, Eghbali M. Fatty-acid oxidation and calcium homeostasis are involved in the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion in rats. *Crit Care Med*. 2012; 40:2431–7. [PubMed: 22647409]
 9. Partownavid P, Sharma S, Li J, Umar S, Rahman S, Eghbali M. Involvement of Opioid Receptors in the Lipid Rescue of Bupivacaine-Induced Cardiotoxicity. *Anesth Analg*. 2015; 121:340–7. [PubMed: 25993392]
 10. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ. Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. *Anesthesiology*. 1998; 88:1071–5. [PubMed: 9579517]
 11. Weinberg G, Ripper R, Feinstein DL, Hoffman W. Lipid emulsion infusion rescues dogs from bupivacaine-induced cardiac toxicity. *Reg Anesth Pain Med*. 2003; 28:198–202. [PubMed: 12772136]
 12. Mottram AR, Valdivia CR, Makielski JC. Fatty acids antagonize bupivacaine-induced I(Na) blockade. *Clin Toxicol Phila Pa*. 2011; 49:729–33.
 13. Fettiplace MR, Akpa BS, Ripper R, Zider B, Lang J, Rubinstein I, Weinberg G. Resuscitation with lipid emulsion: dose-dependent recovery from cardiac pharmacotoxicity requires a cardiotonic effect. *Anesthesiology*. 2014; 120:915–25. [PubMed: 24496123]
 14. Itoh Y, Hinuma S. GPR40, a free fatty acid receptor on pancreatic beta cells, regulates insulin secretion. *Hepatol Res Off J Jpn Soc Hepatol*. 2005; 33:171–3.
 15. Schnell S, Schaefer M, Schöfl C. Free fatty acids increase cytosolic free calcium and stimulate insulin secretion from beta-cells through activation of GPR40. *Mol Cell Endocrinol*. 2007; 263:173–80. [PubMed: 17101212]
 16. Bopassa JC, Eghbali M, Toro L, Stefani E. A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2010; 298:H16–23. [PubMed: 19880667]
 17. Jin Z-Q, Goetzl EJ, Karliner JS. Sphingosine kinase activation mediates ischemic preconditioning in murine heart. *Circulation*. 2004; 110:1980–9. [PubMed: 15451787]
 18. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev*. 2008; 88:581–609. [PubMed: 18391174]
 19. Baines CP. The mitochondrial permeability transition pore and ischemia-reperfusion injury. *Basic Res Cardiol*. 2009; 104:181–8. [PubMed: 19242640]
 20. Halestrap AP, Kerr PM, Javadov S, Woodfield KY. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochim Biophys Acta*. 1998; 1366:79–94. [PubMed: 9714750]
 21. Lemasters JJ, Qian T, He L, Kim J-S, Elmore SP, Cascio WE, Brenner DA. Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. *Antioxid Redox Signal*. 2002; 4:769–81. [PubMed: 12470504]
 22. Hicks SD, Salcido DD, Logue ES, Suffoletto BP, Empey PE, Poloyac SM, Miller DR, Callaway CW, Menegazzi JJ. Lipid emulsion combined with epinephrine and vasopressin does not improve survival in a swine model of bupivacaine-induced cardiac arrest. *Anesthesiology*. 2009; 111:138–46. [PubMed: 19512878]
 23. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, Strichartz G, Feinstein DL. Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. *Reg Anesth Pain Med*. 2006; 31:296–303. [PubMed: 16857549]

24. Foxall G, McCahon R, Lamb J, Hardman JG, Bedford NM. Levobupivacaine-induced seizures and cardiovascular collapse treated with Intralipid. *Anaesthesia*. 2007; 62:516–8. [PubMed: 17448066]
25. McCutchen T, Gerancher JC. Early intralipid therapy may have prevented bupivacaine-associated cardiac arrest. *Reg Anesth Pain Med*. 2008; 33:178–80. [PubMed: 18299100]
26. Lin EP, Aronson LA. Successful resuscitation of bupivacaine-induced cardiotoxicity in a neonate. *Paediatr Anaesth*. 2010; 20:955–7. [PubMed: 20849501]
27. Briscoe CP, Peat AJ, McKeown SC, Corbett DF, Goetz AS, Littleton TR, McCoy DC, Kenakin TP, Andrews JL, Ammala C, Fornwald JA, Ignar DM, Jenkinson S. Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. *Br J Pharmacol*. 2006; 148(5):619–28. [PubMed: 16702987]
28. Briscoe CP, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, Ellis C, Elshourbagy NA, Goetz AS, Minnick DT, Murdock PR, Sauls HR Jr, Shabon U, Spinage LD, Strum JC, Szekeres PG, Tan KB, Way JM, Ignar DM, Wilson S, Muir AI. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J Biol Chem*. 2003; 278(13):11303–11. [PubMed: 12496284]
29. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature*. 2003; 422(6928):173–6. [PubMed: 12629551]
30. Kotarsky K, Nilsson NE, Flodgren E, Owman C, Olde B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochem Biophys Res Commun*. 2003; 301:406–410. [PubMed: 12565875]

What We Already Know about This Topic

Previous studies have demonstrated that intralipid (lipid emulsion) protects the heart against ischemia/reperfusion (I/R) injury and bupivacaine-induced cardiotoxicity. Free fatty acid receptor-1 or G-protein coupled receptor-40 (GPR40) 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 GPR40 is expressed in the rodent heart and is involved in cardioprotection mediated by lipid emulsion against I/R injury and bupivacaine-induced cardiotoxicity.

Figure 1. Experimental protocols

A) Cardiac ischemia/reperfusion (I/R) injury model in male mice. KH stands for Krebs-Henseleit buffer. B) Bupivacaine-induced cardiotoxicity model in male rats. B1 denotes the baseline before GW1100 administration and B2 is the baseline 30 min after GW1100. LE, lipid emulsion; Bup, bupivacaine.

Figure 2. Expression of 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 DAPI (blue) labeling.

Figure 3. GPR40 antagonist prevents rescue of ischemia/reperfusion injury by lipid emulsion
 A. Left ventricular developed pressure (LVDP, mmHg); B. Rate pressure product (RPP, mmHg*beats/min); C. dP/dt max (mmHg/s), D. dP/dt min (mmHg/s), from Langendorff perfused mouse hearts in CTRL (n=6), lipid emulsion (LE, n=8) and lipid emulsion+GPR40 antagonist (n=7) with I/R injury as a function of time before ischemia, during ischemia, and reperfusion. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ##### $p < 0.0001$; ### $p < 0.001$; ## $p < 0.01$; # $p < 0.05$; \$ $p < 0.05$. Values are expressed as mean $\pm$ SD.

Figure 4. GPR40 antagonist pre-treatment prevents rescue of bupivacaine-induced cardiotoxicity by lipid emulsion

Heart Rate (A) and EF (B) before and 30 min after GW1100 (n=7). C. M-Mode Transthoracic echocardiographic images from CTRL (lipid emulsion only, upper panel) and lipid emulsion+GPR40 antagonist (lower panel) at baseline, and 1, 5, 10 min after lipid emulsion. Heart rate (D) and EF (E) at baseline, and 1, 5, 10 min after lipid emulsion (n=4-7/group, **** $p < 0.0001$; ** $p < 0.01$; NS: not significant). LE, lipid emulsion; BUP, bupivacaine; EF, ejection fraction. Values are expressed as mean $\pm$ SD.

Figure 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-3 beta (GSK-3β) 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 stands for GPR40 antagonist GW1100.

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