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Inhibition of Fatty Acid Synthase with C75 Decreases Organ Injury after Hemorrhagic Shock

Michael Kuncewitch, MD^{*}, Weng Lang Yang, PhD^{*,#}, Asha Jacob, PhD^{*,#}, Adam Khader, MD^{*}, Matthew Giangola, MD^{*}, Jeff Nicastro, MD, FACS^{*}, Gene F. Coppa, MD, FACS^{*}, and Ping Wang, MD^{*,#}

^{*}Department of Surgery, Hofstra North Shore-LIJ School of Medicine, Manhasset, NY

[#]Center for Translational Research, The Feinstein Institute for Medical Research, Manhasset, NY

Abstract

Background—Hemorrhagic shock is the primary cause of morbidity and mortality in the intensive care units in patients under the age of 35. Several organs including the lungs are seriously affected due to the hemorrhagic shock and inadequate resuscitation. Excess free fatty acids have shown to trigger inflammation in various disease conditions. C75 is a small compound that inhibits fatty acid synthase, a key enzyme in the control of fatty acid metabolism that also stimulates fatty acid oxidation. We hypothesized that C75 treatment would be protective against hemorrhagic shock.

Methods—Adult, male, Sprague-Dawley rats were cannulated with a femoral artery catheter and subjected to controlled bleeding. Blood was shed to maintain a mean arterial pressure of 30 mm Hg for 90 min, then resuscitated over 30 min with a crystalloid volume equal to twice the volume of shed blood. Fifteen minutes into the 30 min resuscitation, the rats received either intravenous infusion of C75 (1 mg/kg BW) or vehicle (20% DMSO). Blood and tissue samples were collected 6 h after resuscitation (i.e., 7.5 h after hemorrhage) for analysis.

Results—After hemorrhage and resuscitation, C75 treatment decreased the increase in serum free fatty acids by 48%, restored adenosine triphosphate (ATP) levels, and stimulated carnitine palmitoyl transferase-1 (CPT-1) activity. Administration of C75 decreased serum levels of markers of injury (AST, lactate, and LDH) by 38%, 32%, and 78%, respectively. Serum creatinine and blood urea nitrogen (BUN) were also significantly decreased by 38% and 40%, respectively. These changes correlated with decreases in neutrophil infiltration in the lung, evidenced by decreases in Gr-1-stained cells and myeloperoxidase activity and improved lung histology. Finally, administration of C75 decreased pulmonary mRNA levels of COX-2 and IL-6 by 87% and 65%, respectively.

Please address correspondence, proofs, and reprint requests to: Ping Wang, MD, Professor and Vice Chairman for Research, Department of Surgery, Hofstra North Shore-LIJ School of Medicine, Head, Center for Translational Research, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Tel: (516) 562-3411, Fax: (516) 562-2396, pwang@nshs.edu.

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Conclusions—Administration of C75 after hemorrhage and resuscitation decreased the increase in serum FFA, decreased markers of tissue injury, downregulated the expression of inflammatory mediators, and decreased neutrophil infiltration and lung injury. Thus, the dual action of inhibiting fatty acid synthesis and stimulating fatty acid oxidation by C75 could be developed as a promising adjuvant therapy strategy to protect against hemorrhagic shock.

Introduction

Trauma is the primary cause of death among people under 35 years of age and over five million injury related deaths are seen every year. About 30% of these deaths can be attributed to hemorrhagic shock [1]. Hemorrhagic shock caused by the loss of circulatory volume impairs adequate oxygen delivery to ischemic tissues and decreases oxidative phosphorylation, which leads to a decrease in intracellular storage of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) [2–4]. Consequently, all energy-dependent processes including active membrane transport are disrupted which affects membrane-bound organelles such as mitochondria [5]. The decreased ATP levels after hemorrhage have been correlated with intracellular edema and mitochondrial damage, leading to cellular injury and death [6,7].

C75 is a small molecule originally designed as a fatty acid synthase inhibitor [8]. C75 modulates lipid metabolism via two targets: fatty acid synthase and carnitine palmitoyl transferase-1 (CPT-1). Fatty acid synthase is the primary enzyme responsible for *de novo* synthesis of fatty acids, which catalyzes the NADPH-dependent condensation of malonyl-CoA and acetyl-CoA to produce palmitate. CPT-1 is the rate limiting enzyme responsible for mitochondrial fatty acid oxidation and energy production. C75 blocks fatty acid synthase, thereby, inhibiting fatty acid synthesis and stimulating simultaneously CPT-1, which increases mitochondrial fatty acid oxidation and subsequent energy production. This dual-action appears to be the key to the effects of C75 on fatty acid metabolism [9].

C75 has been reported to cause reversible weight loss in lean mice, diet-induced obese mice, and leptin-deficient (ob/ob) mice [8,10]. These studies suggest that the dual action of C75 as a fatty acid synthase inhibitor and as a CPT-1 agonist might have therapeutic implications [11]. Therefore, we hypothesized that the administration of C75 decreases production of free fatty acids, which leads to an increase in energy production and a subsequent decrease in tissue injury and inflammation after hemorrhagic shock.

Materials and Methods

Experimental Animals

Male, adult (3–4 months) Sprague-Dawley rats (weighing 275–325 g) were purchased from Charles River Laboratories (Wilmington, MA). They were housed under 12 h light/dark cycle and fed standard Purina rat chow diet. After acclimation to the environment for 5 days, the rats were fasted for 10 h prior to the model of hemorrhage. Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Feinstein Institute for Medical Research and conducted in accordance to the *Guide for the Care and Use of Laboratory Animals*.

Rat Model of Hemorrhagic Shock

The model of hemorrhagic shock in rats for this study was described previously [12,13]. Briefly, rats were anesthetized with isoflurane inhalation, and the right femoral vein and artery and the left femoral artery were cannulated with PE50 tubings. The right arterial catheter was used for monitoring of blood pressure and heart rate via a Blood Pressure Analyzer (BPA) (Digi-Med, Louisville, KY), the left arterial catheter was used for blood withdrawal, and the venous catheter was used for fluid resuscitation. The rats were bled to 30 mm Hg and maintained for 90 min with either blood withdrawal or infusion with small volumes of Ringer's lactate. At the end of 90 min, the rats were resuscitated with two times the shed blood volume with Ringer's lactate (i.e., crystalloid resuscitation) over 30 min. The rats were not heparinized, and the shed blood was not used for resuscitation. Sham-operated rats underwent a similar procedure but were neither bled nor resuscitated.

Treatment with C75

At 15 min after the initiation of resuscitation, rats received either intravenous infusion of 1 mL vehicle (20% dimethyl sulfoxide [DMSO] in normal saline) or 1 mg/kg C75 (α -methyline- γ -butyrolactone; Sigma-Aldrich, St Louis, MO) in vehicle for over a period of 45 min. Blood and tissue samples were harvested at 6 h after the initiation of resuscitation (i.e., 7.5 h after hemorrhage). C75 was diluted in tissue culture grade DMSO prepared in sterile normal saline and filter sterilized prior to administration in animals.

Determination of Serum Fatty Acids

Serum content of fatty acids was determined by using a free fatty acid quantification kit (BioVision, Mountain View, CA) according to the instructions from the manufacturer.

Determination of Renal ATP Levels and CPT-1 Activity

Kidney tissue (25 mg) was homogenized in assay buffer and centrifuged at 13,000 g to remove insoluble material. The collected supernatant was then deproteinized by perchloric acid precipitation and KOH neutralization. The resultant supernatant was subjected to an ATP assay based on instructions provided by the kit (BioVision, Mountain View, CA). CPT-1 activity was measured using a rapid spectrophotometric assay as described previously [14]. Briefly, frozen kidney tissue (200 mg) was homogenized in buffer (0.25 mol/L sucrose, 1 mmol/L ethylene diaminetetraacetic acid, 0.1% ethanol, and protease inhibitors) at a ratio of 1:5 (w/v) and centrifuged at 12,000 g for 5 min at 4°C. The collected supernatant was assayed spectrophotometrically for the release of CoA-SH from Palmitoyl CoA. The activity was defined as nanomoles of CoA-SH released per minute per gram tissue.

Determination of Serum Markers of Organ Injury

Serum levels of aspartate aminotransferase (AST), lactate, lactate dehydrogenase (LDH), creatinine, and blood urea nitrogen (BUN) were determined by using assay kits according to manufacturer's instructions (Pointe Scientific, Lincoln Park, MI).

Lung Pathohistology

Lung tissues were fixed in 10% buffered formalin, paraffin-embedded, and cut into 5 μ m sections. The sections were stained with hematoxylin and eosin and examined under a light microscope. A histologic injury score was created, measuring differences in alveolar septal thickening, intra-alveolar hemorrhage, hyalin deposits, and neutrophil infiltration [15,16].

Lung Gr-1 Staining and Myeloperoxidase (MPO) Activity

Paraffin-embedded lung tissue was deparaffinized and immunostained with Gr-1 antibody and detected with NovaRED substrate (Vector Labs, Burlingame, CA) as previously described [17]. Lung tissues were homogenized in KPO₄ buffer containing 0.5% hexadecyl-trimethyl-ammonium bromide. MPO activity was measured from the supernatant as reported previously [18,19].

Lung IL-6 and COX-2 mRNA Expressions

Total RNA was extracted from the lungs using Tri Reagent (Molecular Research Center, Cincinnati, OH). RNA (4 μ g) was reverse-transcribed to cDNA and analyzed by real time PCR using primers specific for rat IL-6 (NM_012589) and rat COX-2 (NM_017232). Rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. The primer sequences are the following: IL-6 forward: 5'-AGG GAG ATC TTG GAA ATG AGA AAA-3' and reverse: CAT CAT CGC TGT TCA TAC AAT CAG-3'; GAPDH forward: 5'-ATG ACT CTA CCC ACG GCA AG-3' and reverse: 5'-CTG GAA GAT GGT GAT GGG TT-3'. Each cycle consisted of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C.

Statistical Analysis

All data are expressed as mean $\pm$ SE (n=6) and analyzed by one way analysis of variance (ANOVA) and compared using Student Newman Keul's (SNK) test for multiple comparisons. The differences in values were considered significant if $P < 0.05$.

Results

C75 Attenuated Serum Fatty Acids after Hemorrhagic Shock

C75 modulates fatty acid metabolism by inhibiting fatty acid synthase and simultaneously stimulating CPT-1. As shown in Figure 1A, free fatty acid content in the serum increased markedly in hemorrhaged rats. Treatment with C75 significantly attenuated these levels by 48%.

C75 Restored Renal ATP and Increased Fatty Acid Oxidation

After hemorrhage and resuscitation, high energy phosphates are decreased in the lungs, liver, kidneys, and intestine, but the time course and the extent of decline varies from organ to organ [20]. At 6 h after hemorrhage and resuscitation, renal ATP levels were significantly decreased by 25%. Treatment with C75 restored these levels to sham values and significantly increased from vehicle (Fig. 1B). CPT-1 activity, measured by the release of CoA-SH from Palmitoyl CoA, decreased markedly by 53%; C75 treatment increased these levels by 74% from vehicle and restored to 82% of the sham values (Fig. 1C).

C75 Did not Decrease Mean Arterial Pressure (MAP)

In hemorrhaged rats, MAP increased significantly during fluid resuscitation regardless of the treatment with C75, suggesting that C75 treatment after hemorrhage does not cause any hypotension (Fig. 2).

C75 Attenuated Systemic Markers of Tissue Injury

Serum levels of AST, lactate, and LDH were significantly increased by 624%, 100%, and 800%, respectively after hemorrhagic shock. Treatment with C75 decreased these levels by 38%, 32%, and 78%, respectively (Fig. 3). Likewise, serum levels of creatinine and BUN also significantly increased by 166% and 238%, whereas C75 treatment decreased these levels by 38% and 40% (Fig. 4).

C75 Improved Integrity of Lung Histology

Lung tissues from vehicle-treated animals after hemorrhage showed alveolar septal thickening, intra-alveolar hemorrhage, hyalin deposits, and neutrophil infiltration (Fig. 5B) compared to Shams (Fig. 5A). Treatment with C75 improved lung histologic integrity (Fig. 5C). Lung injury score significantly increased after hemorrhage and treatment with C75 exhibited a significant decrease in these values (Fig. 5D).

C75 Attenuated Lung Neutrophil Infiltration

In hemorrhaged rats, lung tissue from vehicle-treated animals showed neutrophil infiltration as evidenced by intensity of Gr-1 staining (Fig. 6B) in comparison to Shams (Fig. 6A). Treatment with C75 decreased markedly the Gr-1 stain indicating a decrease in neutrophil infiltration (Fig. 6C). Similarly, MPO activity, another measure of neutrophil infiltration, also significantly increased after hemorrhage, whereas C75 treatment markedly decreased these activities (Fig. 6D).

C75 Decreased Lung IL-6 and COX-2 mRNA Expression

As a measure of inflammation, COX-2 and IL-6 mRNA expression, was assessed in lung tissues. IL-6 and COX-2 mRNA levels increased after hemorrhage while treatment with C75 significantly decreased IL-6 and COX-2 levels by 65% and 87% (Fig. 7).

Discussion

The pathophysiology of hemorrhagic shock is based on multiple factors. The deprivation of cellular energy and subsequent cellular damage play an important role in the outcome. Restoring cellular energy during resuscitation can decrease tissue damage and improve survival [21]. In the current study, we showed that C75 blunted the increase in serum free fatty acids and restored cellular energy, i.e., increased ATP levels by increasing fatty acid oxidation apparently via CPT-1 stimulation. This effect correlated with a significant decrease in systemic markers of organ injury, a decrease in neutrophil infiltration as evidenced by decreased Gr-1 stained cells and a decrease in MPO activity in the lungs. In addition, C75 treatment significantly improved histologic appearance of the lung and decreased the inflammatory factor IL-6 and COX-2. These results suggest that targeting

lipid metabolism by C75 to restore cellular energy could be protective against hemorrhagic shock.

During shock, catecholamines stimulate the breakdown of lipids in adipose tissue, resulting in the release of free fatty acids into the plasma at an increased rate. Although some of the free fatty acids are cleared by the liver, increased fatty acids cause an inhibitory effect on oxidation of glucose and decrease total energy production [22]. Long chain fatty acids enter into mitochondria via CPT-1 [9]. Glucose-induced expression of acetyl CoA carboxylase increases the production of malonyl CoA, thereby inhibiting mitochondrial entry of long chain fatty acids via CPT-1 and promotes lipid synthesis and storage in the cytosol [23]. C75, instead of inhibiting fatty acid synthesis by acting on acetyl CoA carboxylase inhibits fatty acid synthase leading to an increase in malonyl CoA. Malonyl CoA is an allosteric inhibitor of CPT-1. C75, however, stimulates CPT-1 even in the presence of high malonyl CoA, leading to an increase in fatty acid oxidation and subsequent energy production. C75 treatment of rodent adipocytes, hepatocytes, and human breast cancer cells increased fatty acid oxidation and ATP levels by stimulating CPT-1 activity even in the presence of increased levels of malonyl CoA [11]. Administration of C75 decreased food intake and body weight in rodents [8]. C75 treatment in diet-induced obese mice showed significant weight loss and increased energy production due to fatty acid oxidation. These studies indicate that the dual action of C75 as an inhibitor of fatty acid synthase and as a CPT-1 agonist might have therapeutic implications [11]. In our study, C75 ameliorated the hemorrhagic shock induced by an increase in free fatty acids in serum. We also demonstrated that CPT-1 activity is significantly increased by the presence of C75 and that ATP levels were restored to sham values. Collectively, these data suggest that the mode of action of C75 in hemorrhagic shock is mediated at least in part by the stimulation of fatty acid oxidation by the mitochondria. Because C75 inhibits fatty acid synthase, the decrease in free fatty acid content in the serum of C75-treated animals could also be due to the direct inhibition of fatty acid synthase.

To further elucidate whether altering the fatty acid metabolism can decrease tissue inflammation associated with hemorrhagic shock, we examined neutrophil infiltration in the lungs, tissue histopathology, as well as expression of IL-6 and COX-2 mRNA in the lung. Our results showed significant decreases in lung inflammation as evidenced by decreases in the above mentioned parameters; however, whether altering the fatty acid metabolism by C75 or as yet unidentified independent function of C75 is the reason for this decrease in lung inflammation is not known. Prior studies have shown that C75 was synthesized as a small molecule inhibitor of fatty acid synthase and that it interacts with the two enzymatic targets, fatty acid synthase and CPT-1 [9]. It is possible that C75 could have functions other than inhibiting fatty acid synthase or promoting fatty acid oxidation. Based on our data that C75 treatment decreased serum free fatty acids and increased CPT-1 activity and ATP levels in the kidneys, however, we suggest that the C75 effect in hemorrhagic shock is mediated at least in part by the alteration of the fatty acid metabolism. FAs are the major energy source for the kidneys [24]. Therefore, we measured ATP levels only in the kidneys. We also observed concomitant decreases in markers of tissue injury from various organs and of inflammation. Whether the observed benefit in inflammatory parameters is due to a direct effect of altering the fatty acid metabolism or due to other independent effects of C75 yet to

be identified, is not known. Future studies can address the exact mechanism of C75 in hemorrhagic shock.

The consequence of hypoperfusion post resuscitation is a decrease in the supply of oxygen which leads to a decrease in oxidative phosphorylation and ATP formation. Thus, during hemorrhagic shock, all energy-dependent processes including membrane transport are compromised severely resulting in an osmotic imbalance leading to cellular edema. Replenishing ATP by means of resuscitation in hemorrhagic shock, however, has been ineffective due both to its inability to pass the cell membrane in large quantities as well as its short half-life in circulation [25–31]. Administration of glutamine (a precursor of ATP), lipid encapsulated ATP, pyruvate, and crocetin have been effective in restoring energy and decreasing cellular damage after hemorrhagic shock in animal models [5,32–34].

Mitochondria consume greater than 90% of the cellular oxygen [35]. Even at optimal delivery of oxygen, mitochondria in patients undergoing resuscitation do not fully utilize the available oxygen. This impairment in oxygen utilization is due to uncoupling of the electron transport chain causing cytopathic hypoxia [36]. The majority of oxygen consumption by the electron transport chain will be mediated by cytochrome c oxidase. Cytochrome c oxidase contributes to the production of reactive oxygen species in the mitochondria. These changes in redox state leads to an inflammatory response and tissue injury [37]. Because C75 was administered immediately after reperfusion/resuscitation, the protective effects seen in the study could be associated with the prevention of reperfusion injuries. In fact, our study showed that C75 treatment significantly decreased markers of systemic injury, decreased neutrophil infiltration to the lungs, and decreased lung MPO activity. Furthermore, C75 administration decreased inflammatory responses by decreasing the expression of IL-6 and COX-2 mRNA. The changes in all these parameters indicate protection from reperfusion injuries; however, we recognize that one limitation of our study is that we only examined the effect of C75 given during reperfusion/resuscitation.

Mitochondrial fatty acid oxidation supplies more than 50% of the energy for the adult heart, while glucose only plays a minor role in energy production in the heart [38]. Palmitic acid is the first fatty acid produced during fatty acid synthesis and is the precursor for long chain fatty acids [39]. In this regard, our study showed that hemorrhagic shock presumably accelerated fatty acid synthesis in the tissue and released high amounts of palmitate in the circulation; C75 treatment decreased these values. In the model of fixed pressure hemorrhage employed in our study, hemodynamic parameters, such as mean arterial pressure, heart rate, and cardiac output, were restored by resuscitation with crystalloids alone. While restoration by intravenous fluids after hemorrhagic shock tends to normalize hemodynamic parameters, ongoing hypoperfusion in organs such as the liver, lungs, and kidneys leads to ischemia. Inappropriate resuscitation causes a systemic inflammatory response and tissue injury, leading to multi-organ dysfunction and death. In this regard, both treatment with C75 and with vehicle improved the decrease in mean arterial pressure after hemorrhagic shock suggesting that the treatment did not cause any adverse effect in hemodynamic parameters (Fig 2). Whether C75 treatment affects myocardial energy production during hemorrhagic shock was not addressed in our study.

Our prior studies in hemorrhagic shock showed significant increases in serum markers of tissue injury and cytokine levels at 4 h after hemorrhagic shock and resuscitation [40]. In the current study, we examined the effect of C75 at 6 h after hemorrhagic shock (i.e., 7.5 h after the beginning of hemorrhage). We have not examined organ function at later time points, nor did we assess the histologic changes from any other organs. While there were increases in serum levels of AST, lactate, LDH, creatinine, and BUN after hemorrhagic shock, the shock-related tissue injury is relatively low in the fixed pressure model of hemorrhage. Therefore, it is possible that the histologic appearances of other organs may be unchanged. Future studies are needed for such conclusion.

Our study has several limitations. The major challenge in setting up a model of hemorrhagic shock is mimicking the clinical condition while achieving reproducibility and standardization. The experimental model employed in our study is the fixed-pressure hemorrhage, where the animals are anesthetized and a controlled amount of blood volume removed to maintain a certain pressure to achieve the desired hypotension [41]. A primary advantage of this model is reproducibility and standardization, but the model has minimal clinical relevance [42]. Furthermore, the animals received two times the shed blood volume of Ringer's lactate, and some would argue that three times the volume resuscitation would be a better model. Our prior studies utilized four times and even equal volume of resuscitation to vary the severity of the hemorrhagic shock [12,13,40]. Another limitation is that the treatment was initiated during the early phase of reperfusion, which has less clinical significance. Nevertheless, based on this initial finding using one dose and one time point, we demonstrated the beneficial effect of C75 in decreasing inflammatory complications associated with hemorrhagic shock. Also, we have not addressed the functional effect of C75 on attenuating hemorrhage-induced mortality. The model of hemorrhagic shock used in this study was reported previously by us [12,43]. In those studies, the mortality in the vehicle group was 50% in the first 24 h, 29% by 48 h, and remained 29% at 12 days [40]. Likewise, it is not known about the toxicity or complications associated with the use of C75 at the doses required for clinical use; animal studies using doses up to 20 mg/kg BW of C75 delivered intraperitoneally have been reported presumably without any toxicity or complications [44]. While the fixed pressure hemorrhage with crystalloid resuscitation employed in this study has limited clinical relevance, it is highly reproducible and easy to standardize the model. Future studies with more clinically relevant models are needed to determine the dose response, therapeutic window, and long-term effects of C75 in hemorrhagic shock.

In summary, our studies show that C75 treatment blunted the increase in free fatty acids after hemorrhagic shock. C75 resulted in restoration of cellular energy and a decrease in tissue damage. Thus, modulation of lipid metabolism by C75 may be a promising therapeutic strategy for patients with hemorrhage complications.

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References

1. Cothren CC, Moore EE, Hedegaard HB, Meng K. Epidemiology of urban trauma deaths: a comprehensive reassessment 10 years later. *World J Surg.* 2007; 31:1507–11. [PubMed: 17505854]
2. Chang CG, Van Way CW 3rd, Dhar A, Helling T Jr, Hahn Y. The use of insulin and glucose during resuscitation from hemorrhagic shock increases hepatic ATP. *J Surg Res.* 2000; 92:171–6. [PubMed: 10896818]
3. Helling TS, Wogahn BM, Olson SA, Evans LS, Reddy BR, VanWay C 3rd. The effect of prostaglandin E1 on liver adenine nucleotides and cytoplasmic enzymes in a porcine model of normothermic hepatic ischemia. *Hepatology.* 1995; 22:1554–9. [PubMed: 7590675]
4. Van Way CW, Dhar A, Reddy R, Evans L, Wogahn B, Helling TS. Changes in adenine nucleotides during hemorrhagic shock and reperfusion. *J Surg Res.* 1996; 66:159–66. [PubMed: 9024829]
5. Zakaria el R, Ehringer WD, Tsakadze N, Li N, Garrison RN. Direct energy delivery improves tissue perfusion after resuscitated shock. *Surgery.* 2005; 138:195–203. [PubMed: 16153427]
6. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K⁺ channel in the mitochondrial inner membrane. *Nature.* 1991; 352:244–7. [PubMed: 1857420]
7. Van Way CW 3rd, Dhar A, Morrison DC, Longorio MA, Maxfield DM. Cellular energetics in hemorrhagic shock: restoring adenosine triphosphate to the cells. *J Trauma.* 2003; 54:S169–76. [PubMed: 12768121]
8. Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, et al. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science.* 2000; 288:2379–81. [PubMed: 10875926]
9. Kuhajda FP, Landree LE, Ronnett GV. The connections between C75 and obesity drug-target pathways. *Trends Pharmacol Sci.* 2005; 26:541–4. [PubMed: 16169094]
10. Thupari JN, Kim EK, Moran TH, Ronnett GV, Kuhajda FP. Chronic C75 treatment of diet-induced obese mice increases fat oxidation and reduces food intake to reduce adipose mass. *Am J Physiol Endocrinol Metab.* 2004; 287:E97–E104. [PubMed: 14736702]
11. Thupari JN, Landree LE, Ronnett GV, Kuhajda FP. C75 increases peripheral energy utilization and fatty acid oxidation in diet-induced obesity. *Proc Natl Acad Sci U S A.* 2002; 99:9498–502. [PubMed: 12060712]
12. Wu R, Cui X, Dong W, Zhou M, Simms HH, Wang P. Mechanisms responsible for vascular hyporesponsiveness to adrenomedullin after hemorrhage: the central role of adrenomedullin binding protein-1. *Ann Surg.* 2005; 242:115–23. [PubMed: 15973109]
13. Wu R, Dong W, Zhou M, Cui X, Simms HH, Wang P. A novel approach to maintaining cardiovascular stability after hemorrhagic shock: beneficial effects of adrenomedullin and its binding protein. *Surgery.* 2005; 137:200–8. [PubMed: 15674202]
14. Bieber LL, Abraham T, Helmrath T. A rapid spectrophotometric assay for carnitine palmitoyltransferase. *Anal Biochem.* 1972; 50:509–18. [PubMed: 4630394]
15. Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. *Clin Chest Med.* 1982; 3:35–56. [PubMed: 7075161]
16. Matsuda A, Wu R, Jacob A, Komura H, Zhou M, Wang Z, et al. Protective effect of milk fat globule-epidermal growth factor-factor VIII after renal ischemia-reperfusion injury in mice. *Crit Care Med.* 2011; 39:2039–47. [PubMed: 21666453]
17. Giangola MD, Yang WL, Rajayer SR, Nicastro J, Coppa GF, Wang P. Growth arrest-specific protein 6 attenuates neutrophil migration and acute lung injury in sepsis. *Shock.* 2013; 40:485–91. [PubMed: 23881260]
18. Cui T, Miksa M, Wu R, Komura H, Zhou M, Dong W, et al. Milk fat globule epidermal growth factor 8 attenuates acute lung injury in mice after intestinal ischemia and reperfusion. *Am J Respir Crit Care Med.* 2010; 181:238–46. [PubMed: 19892861]
19. Koike K, Moore EE, Moore FA, Read RA, Carl VS, Banerjee A. Gut ischemia/reperfusion produces lung injury independent of endotoxin. *Crit Care Med.* 1994; 22:1438–44. [PubMed: 8062567]

20. Keller ME, Aihara R, LaMorte WW, Hirsch EF. Organ-specific changes in high-energy phosphates after hemorrhagic shock and resuscitation in the rat. *J Am Coll Surg*. 2003; 196:685–90. [PubMed: 12742196]
21. Van Way CW 3rd, Dhar A, Morrison D. Hemorrhagic shock: a new look at an old problem. *Mo Med*. 2003; 100:518–23. [PubMed: 14601444]
22. Wolfe RR, Shaw JH, Durkot MJ. Energy metabolism in trauma and sepsis: the role of fat. *Prog Clin Biol Res*. 1983; 111:89–109. [PubMed: 6867023]
23. Yan J, Young ME, Cui L, Lopaschuk GD, Liao R, Tian R. Increased glucose uptake and oxidation in mouse hearts prevent high fatty acid oxidation but cause cardiac dysfunction in diet-induced obesity. *Circulation*. 2009; 119:2818–28. [PubMed: 19451348]
24. Portilla D. Energy metabolism and cytotoxicity. *Semin Nephrol*. 2003; 23:432–8. [PubMed: 13680532]
25. Chaudry IH. ATP-MgCl₂ and liver blood flow following shock and ischemia. *Prog Clin Biol Res*. 1989; 299:19–31. [PubMed: 2657790]
26. Chaudry IH. The effect of ATP on survival in intestinal ischemia shock, hemorrhagic shock, and endotoxin shock in rats. *Circ Shock*. 1982; 9:629–31. [PubMed: 6983934]
27. Chaudry IH, Clemens MG, Baue AE. The role of ATP-magnesium in ischemia and shock. *Magnesium*. 1986; 5:211–20. [PubMed: 3523059]
28. Chaudry IH, Stephan RN, Dean RE, Clemens MG, Baue AE. Use of magnesium-ATP following liver ischemia. *Magnesium*. 1988; 7:68–77. [PubMed: 3294518]
29. Ehringer WD, Chiang B, Chien S. The uptake and metabolism of fructose-1,6-diphosphate in rat cardiomyocytes. *Mol Cell Biochem*. 2001; 221:33–40. [PubMed: 11506184]
30. Ehringer WD, Niu W, Chiang B, Wang OL, Gordon L, Chien S. Membrane permeability of fructose-1,6-diphosphate in lipid vesicles and endothelial cells. *Mol Cell Biochem*. 2000; 210:35–45. [PubMed: 10976756]
31. Ehringer WD, Su S, Chiang B, Stillwell W, Chien S. Destabilizing effects of fructose-1,6-bisphosphate on membrane bilayers. *Lipids*. 2002; 37:885–92. [PubMed: 12458624]
32. Dhar A, Cherian G, Dhar G, Ray G, Sharma R, Banerjee SK. Molecular basis of protective effect by crocetin on survival and liver tissue damage following hemorrhagic shock. *Mol Cell Biochem*. 2005; 278:139–46. [PubMed: 16180099]
33. Sharma P, Walsh KT, Kerr-Knott KA, Karaian JE, Mongan PD. Pyruvate modulates hepatic mitochondrial functions and reduces apoptosis indicators during hemorrhagic shock in rats. *Anesthesiology*. 2005; 103:65–73. [PubMed: 15983458]
34. Yang R, Martin-Hawver L, Woodall C, Thomas A, Qureshi N, Morrison D, et al. Administration of glutamine after hemorrhagic shock restores cellular energy, reduces cell apoptosis and damage, and increases survival. *JPEN J Parenter Enteral Nutr*. 2007; 31:94–100. [PubMed: 17308249]
35. Rushing GD, Britt LD. Reperfusion injury after hemorrhage: a collective review. *Ann Surg*. 2008; 247:929–37. [PubMed: 18520219]
36. Fink MP. Cytopathic hypoxia. Mitochondrial dysfunction as mechanism contributing to organ dysfunction in sepsis. *Crit Care Clin*. 2001; 17:219–37. [PubMed: 11219231]
37. Cairns CB, Moore FA, Haenel JB, Gallea BL, Ortner JP, Rose SJ, et al. Evidence for early supply independent mitochondrial dysfunction in patients developing multiple organ failure after trauma. *J Trauma*. 1997; 42:532–6. [PubMed: 9095123]
38. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev*. 2005; 85:1093–129. [PubMed: 15987803]
39. Kingsbury KJ, Paul S, Crossley A, Morgan DM. The fatty acid composition of human depot fat. *Biochem J*. 1961; 78:541–50. [PubMed: 13756126]
40. Wu R, Dong W, Qiang X, Ji Y, Cui T, Yang J, et al. Human vasoactive hormone adrenomedullin and its binding protein rescue experimental animals from shock. *Peptides*. 2008; 29:1223–30. [PubMed: 18403050]
41. Kawasaki T, Fujimi S, Lederer JA, Hubbard WJ, Choudhry MA, Schwacha MG, et al. Trauma-hemorrhage induces depressed splenic dendritic cell functions in mice. *J Immunol*. 2006; 177:4514–20. [PubMed: 16982888]

42. Fulop A, Turoczi Z, Garbaisz D, Harsanyi L, Szijarto A. Experimental models of hemorrhagic shock: a review. *Eur Surg Res.* 2013; 50:57–70. [PubMed: 23615606]
43. Cui X, Wu R, Zhou M, Dong W, Ulloa L, Yang H, et al. Adrenomedullin and its binding protein attenuate the proinflammatory response after hemorrhage. *Crit Care Med.* 2005; 33:391–8. [PubMed: 15699844]
44. McCullough LD, Zeng Z, Li H, Landree LE, McFadden J, Ronnett GV. Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. *J Biol Chem.* 2005; 280:20493–502. [PubMed: 15772080]

Figure 1. C75 attenuated serum free fatty acids, restored ATP and stimulated CPT-1 after hemorrhage and resuscitation

A. Serum samples were collected 6 h after resuscitation from Sham, Vehicle, and C75-treated groups for measurement of circulating free fatty acids. B. Renal ATP levels were measured using an assay kit and the data are shown in percentages. C. CPT-1 activity was measured by a rapid spectrophotometric assay with activity expressed as nmol/min/g tissue. Data presented are means $\pm$ SE (n= 6/group) and compared by one-way ANOVA and Student Newman Keul's (SNK) test. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. Vehicle.

Figure 2. C75 did not alter mean arterial pressure after hemorrhage and resuscitation
Recordings of mean arterial pressure (MAP) during the 90-min ischemia and the first 50-min of resuscitation in Sham, Vehicle and C75 treatment. Representative recordings from a single animal from different groups are shown.

Figure 3. C75 attenuated markers of tissue injury after hemorrhage and resuscitation
 Serum samples were measured for AST (A), lactate (B) and LDH (C) from Sham, Vehicle, and C75-treated groups. Data presented are means $\pm$ SE ($n = 6/\text{group}$) and compared by one-way ANOVA and SNK method. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. Vehicle.

Figure 4. C75 decreased parameters of renal function parameters after hemorrhage and resuscitation

Serum samples were measured for creatinine (A) and BUN (B) from Sham, Vehicle and C75-treated groups. Data presented are means $\pm$ SE (n= 6/group) and compared by one-way ANOVA and SNK method. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. Vehicle.

Figure 5. C75 improved pulmonary cellular architecture after hemorrhage and resuscitation Lungs were harvested from Sham (A), Vehicle (B), and C75-treated (C) groups at 6 h after resuscitation. Representative photomicrographs of tissue sections stained with hematoxylin and eosin are shown at 200 $\times$ magnification. D. Histologic injury score was calculated as described in Materials and Methods. Data presented are means $\pm$ SE (n= 6/group) and compared by one-way ANOVA and SNK method. * P < 0.05 vs. Sham; # P < 0.05 vs. Vehicle.

Figure 6. C75 attenuated neutrophil infiltration after hemorrhage and resuscitation

Lung tissue sections were stained with anti-Gr1 antibody in sham (A), vehicle (B), and C75-treated groups (C). Brown staining signifies Gr-1 antigen on the surface of neutrophils.

Representative images are shown at 400× magnification. Pulmonary tissue lysates were analyzed for myeloperoxidase (MPO) activity (D). Data presented are means $\pm$ SE (n= 6/group) and compared by one-way ANOVA and SNK method. * P < 0.05 vs. Sham; # P < 0.05 vs. Vehicle.

Figure 7. C75 reduced IL-6 and COX-2 mRNA expressions after hemorrhage and resuscitation
Total RNA from lung tissues were analyzed by real time reverse transcription-polymerase chain reaction for IL-6 (A) and COX-2 (B). GAPDH was used for internal housekeeping control. Data were normalized to GAPDH and are presented as means $\pm$ SE (n= 6/group) and compared by one-way ANOVA and SNK method. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. Vehicle.