Resveratrol Improves Survival and Prolongs Life Following Hemorrhagic Shock

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Resveratrol has been shown to potentiate mitochondrial function and extend longevity; however, there is no evidence to support whether resveratrol can improve survival or prolong life following hemorrhagic shock. We sought to determine whether (a) resveratrol can improve survival following hemorrhage and resuscitation and (b) prolong life in the absence of resuscitation. Using a hemorrhagic injury (HI) model in the rat, we describe for the first time that the naturally occurring small molecule, resveratrol, may be an effective adjunct to resuscitation fluid. In a series of three sets of experiments we show that resveratrol administration during resuscitation improves survival following HI ($p < 0.05$), resveratrol and its synthetic mimic SRT1720 can significantly prolong life in the absence of resuscitation fluid (<30 min versus up to 4 h; $p < 0.05$), and resveratrol as well as SRT1720 restores left ventricular function following HI. We also found significant changes in the expression level of mitochondria-related transcription factors Ppar-α and Tfam, as well as Pgc-1α in the left ventricular tissues of rats subjected to HI and treated with resveratrol. The results indicate that resveratrol is a strong candidate adjunct to resuscitation following severe hemorrhage.
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INTRODUCTION

Injury is a leading cause of death in people under the age of 45 and approximately 15% to 20% of deaths due to trauma are preventable (1,2). Hemorrhage is reported as the most common cause of preventable death following trauma (3,4). Severe hemorrhage results in whole body oxygen and nutrient deprivation that may lead to organ dysfunction and death. An early and effective hemorrhage control is a determining factor in the outcome following severe hemorrhage (2). In humans, although a variety of fluid resuscitation methods are employed, an optimal resuscitative approach to hemorrhagic shock is still unsettled (5,6). The use of agents that may extend life of cells under adverse conditions such as hypoxia and decreased nutrient availability following hemorrhage may also play an important role in changing the outcome following severe hemorrhage (7).

Studies using animal models of hemorrhagic injury (HI) have demonstrated increased systemic inflammation, mitochondrial dysfunction and reduced endoplasmic reticulum stress with concomitant decrease in organ function including cardiac function, despite fluid resuscitation (8–11).

The hypoxic condition following hemorrhage as well as tissue reperfusion following resuscitation are conducive to the production of oxidative damage in most organs (12–15). Mitochondria is likely to be a major focal point for the generation of reactive oxygen species (16–19). In addition, oxygen insufficiency results in decreased mitochondrial function resulting in altered cellular energetics. Consistent with these physiological changes following ischemia and reperfusion, antioxidants and agents that preserve mitochondrial function have been shown to reduce organ functional decline and restore cellular homeostasis in such injuries (20–22). Our laboratory and that of others have demonstrated that the natural antioxidant resveratrol improves organ function following severe hemorrhage (23–25). Resveratrol has been shown to enhance mitochondrial function as well as biogenesis, and has been implicated in the extension of life span in several species (26–29). However, determining the effect of resveratrol in reducing mortality or prolonging survival following hemorrhagic shock is important toward initiating clinical trials to use this agent as an adjunct to resuscitation fluid following severe hemorrhage.

Our study tests, for the first time, whether resveratrol can improve survival

following hemorrhagic shock in a rat model.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250–350g) were obtained from Charles River Laboratory (Wilmington, MA, USA). The experiments performed in this study including surgical procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at GRU. All animals were housed in GRU animal facility during the experiments.

Hemorrhage Procedure

The animals were fasted overnight (water allowed *ad libitum*) prior to the sham or hemorrhagic injury (HI) procedure. Hemorrhagic procedure was as described before with some modification (23). The animals were anesthetized with 2. 5% isoflurane (Henry Schein, Dublin, OH, USA) and were placed on Plexiglas plate in a supine position. Isoflurane was administered in oxygen using an anesthetic vaporizer. Midline laparotomy (5 cm) was performed in both sham and HI animals to induce soft tissue trauma. Incision was closed aseptically in two layers with sutures. Two femoral arteries and one femoral vein were cannulated (PE-50 tubing), one artery was hooked up to a Blood Pressure Analyzer (Digi-Med; Micro-Med Inc., Louisville, KY, USA) to monitor mean arterial pressure (MAP) and bleeding was performed through the other artery. Femoral vein was cannulated to administer fluids. Surgical sites were bathed with bupivacaine. Sham animals were not subjected to bleeding or resuscitation. Upon awakening, the animals in HI groups were bled rapidly within the first 10 min to a MAP of 40 ± 5 mmHg. The bleeding was continued for 45 min, maintaining the low MAP, until 60% of circulatory blood volume was withdrawn. The animals were maintained in the state of shock by maintaining MAP at 40 ± 5 for another 45 min. During this time not more than 40% of the shed blood volume in the form of Ringer lactate was given to

maintain the blood pressure. Resuscitation was performed with Ringer lactate for one hour on animals in the respective groups. Vehicle (dimethyl sulfoxide [DMSO]; 120 μL/mouse) or drug was administered at 10 min from the onset of resuscitation or immediately after shock period when there was no resuscitation.

10-d Survival Study

In this group of animals, resuscitation was carried out by Ringer lactate, four times the volume of shed blood and the rats were observed for 10 d. Resveratrol (10 mg/kg body weight) or vehicle (DMSO) was administered at 10 min after the start of resuscitation. Weight was monitored every day and left ventricular function was determined by measuring positive and negative maximal dP/dt in animals that survived until the end of the study. One animal in resveratrol treated group was inadvertently euthanized at d 9.

4-h Survival Study

The animals in this group did not receive resuscitation fluid following the shock period. Resveratrol (10 mg/kg body weight), SRT1720 (2 mg/kg body weight) or vehicle (DMSO) was administered intravenously at the end of the shock period. As death cannot be an endpoint as per the IACUC policy, animals were euthanized when their mean arterial pressure dipped below 30 mmHg. This allowed us to collect blood from these animals before euthanasia, to test lactate and pH levels.

2-h Study

The animals in this group were subjected to a less aggressive resuscitation regimen by administering Ringer lactate, two times the volume of shed blood. Resveratrol (10 mg/kg body weight), SRT1720 (2 mg/kg body weight) or vehicle was administered 10 min following the start of resuscitation. After resuscitation, the animals were observed for 2 h, at which point the left ventricular function was measured. The animals were then euthanized and left ventricular tissue was removed for molecular analysis.

Cardiac Contractility

Cardiac maximum and minimum contractility were assessed by measuring positive and negative dP/dt. Briefly, animals were anesthetized in supine position with isoflurane (2. 5%). The surgical site was bathed in bupivacaine. The right carotid artery was exposed and a PE-50 tubing was inserted into the left ventricle through the right carotid artery. Positive and negative maximum dP/dt (+dP/dt and –dP/dt) were assessed using a Heart Performance Analyzer (HPA) (Digi-Med).

Blood Analysis

Analysis of hematocrit, hemoglobin, lactate and pH were measured using a portable blood gas analysis instrument (Opti-Med, Atlanta, GA, USA) and appropriate cartridge as per the manufacturer's directions; 150 μL blood was used for each measurement.

Real-time Polymerase Chain Reaction

The TaqMan primer/probe set for real-time PCR analysis of transcription factor A, mitochondrial (*Tfam*) (Rn00580051_m1; cat. #4331182), peroxisome proliferator-activated receptor α (*Ppar-α*) (Rn00566193_m1; cat. #4331182), *Ppar-γ*, coactivator 1α (*Pgc-1α*) (Rn01453111_m1; cat. #4331182) and *β-actin* (Rn00667869_m1; cat. #4331182) were Life Technologies (Thermo Fisher Scientific Inc., Waltham, MA, USA) products. Total RNA was isolated from heart tissue using Total RNA isolation mini kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol (Qiagen Inc, Valencia, CA, USA) and purity verified by measurement of absorbance ratios 260/280 nm in Take3 Micro-Volume plate, (BioTek Instruments Inc., Winooski, VT, USA). 200 ng of total RNA isolated was reverse transcribed to cDNA using SuperScript VILO cDNA synthesis kit (Life Technologies [Thermo Fisher Scientific]). Quantitative real-time PCR was performed using Agilent Technologies Stratagene Mx3000P real-time PCR machine with the TaqMan primer/probe set for *Tfam*, *Ppar-α*, and *Pgc-1α*, and normalized to *β-actin*. The 20 μL reac-

tion mixture constituted 10 μL 2× master mix containing ROX reference dye, 1 μL primer/probe set, 8 μL RNase-DNase free water and 1 μL cDNA as PCR template. The thermal cycling conditions were 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. As a negative control, cDNA synthesis was carried out without reverse transcriptase enzyme in the RNA sample, thereby precluding any amplification due to contaminating genomic DNA. Results are expressed after normalizing to the values obtained for samples in sham group.

Western Blot Analysis

The heart tissues were homogenized in Pierce RIPA lysis buffer (cat. #89901; Thermo Scientific [Thermo Fisher Scientific]) containing 25 mmol/L Tris-HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktail (cat. #P2714; Sigma Aldrich, St. Louis, MO, USA) in a D1000 handheld homogenizer (Benchmark Scientific, Sayreville, NJ, USA). Tissue lysates were centrifuged at 14,000g for 10 min and the supernatant saved for protein estimation and analysis. Protein aliquots were combined with 4× Laemmli buffer (cat. #161-0747; Bio-Rad, Hercules, CA, USA) and resolved on a 10% SDS polyacrylamide gel, transferred to PVDF membrane, blocked using 5% (w/v) nonfat dried milk or 5% BSA in Tris-buffered saline containing 25 mmol/L Tris-HCl (pH 7. 4), 0.13 mol/L NaCl, 0.0027 mol/L KCl and 0.1% Tween 20 for 1 h at room temperature (RT) and then incubated with respective antibodies overnight at 4°C or for 1 h at RT. The membranes were probed with antibodies to Ppar-α (cat. #ab8934; Abcam, Cambridge, MA, USA), Tfam (cat. #sc-23588; Santa Cruz Biotechnology, Dallas, TX, USA), Pgc-1α (cat. #sc-13067; Santa Cruz Biotechnology) and Gapdh (cat. #2118; Cell Signaling Technology, Danvers, MA, USA). The membranes were subsequently washed and incubated with horseradish peroxidase conjugated secondary antibody for 1 h at RT and developed using enhanced
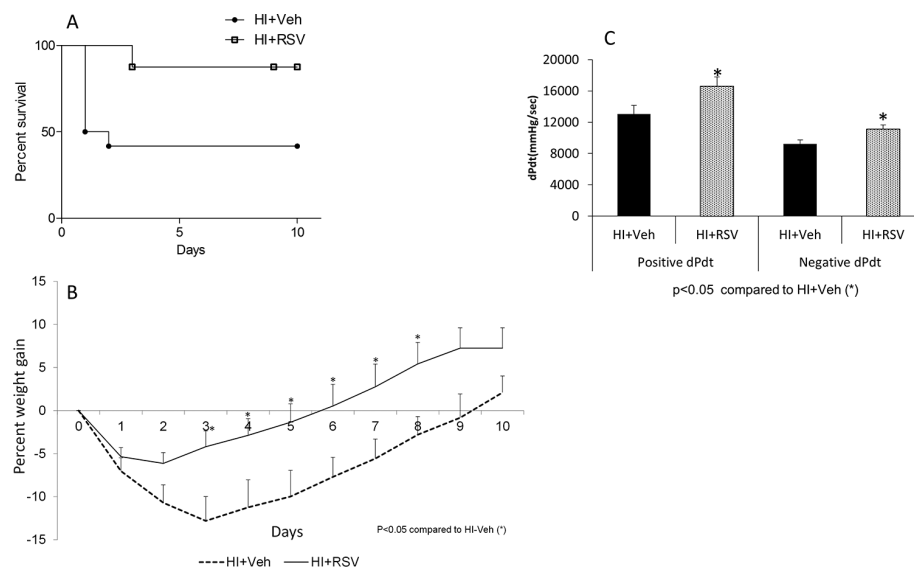
chemiluminescence (cat. #34080; Thermo Scientific [Thermo Fisher Scientific]). Protein bands developed on X-ray films were quantified using the ImageJ software (Wayne Rasband, NIH, Rockville, MD, USA).

Statistics

Survival analysis was performed by Kaplan-Meier plot and significance between survival curves was determined using GraphPad software (La Jolla, CA). Multigroup comparisons were carried out and significance determined by one-way ANOVA followed by Tukey test using GraphPad software. Two-group comparisons for significance were done by unpaired t test using GraphPad software.

All supplementary materials are available online at www.molmed.org.

RESULTS

Resveratrol Improves Survival Following Hemorrhagic Shock

Rats were subjected to soft tissue trauma, hemorrhage and resuscitation.

Figure 1. Resveratrol (RSV) improves survival after HI. (A) Kaplan-Meier survival curves for HI + Veh (n = 12) and HI + RSV (n = 8) groups. $p < 0.05$. (B) Weight gain/loss for HI + Veh (n = 5) and HI + RSV (n = 7), Mean ± SE; * indicates $p < 0.05$ compared with weight in the Veh treated group on the respective day. (C) dP/dt 10 d following HI; (n = 4-6); Bars represent mean ± SE. *indicates $p < 0.05$ compared with the Veh control.

Ten minutes into resuscitation either resveratrol or vehicle was administered to the animals and monitored for 10 d. As shown in Figure 1A, mortality rate was significantly reduced in animals that received resveratrol. There was a significant improvement in MAP and heart rate at 2 h following resuscitation in the animals that received resveratrol (Supplementary Figures 1A, B). Seven of the 12 animals in the control group died, compared to one of the eight animals died in the resveratrol-treated group. Most of the animals that died following hemorrhage in the control group did not survive beyond 24 h.

In addition to accounting for mortality, we also monitored the weight gain of all the animals in each of the groups for the duration of the study. As shown in Figure 1B, there was a significant difference in weight gain in the resveratrol-treated animals as compared with the control animals, among those that survived till the end of the study. It is noteworthy that the curve of means of the weight of the two groups of animals remained separated till the tenth day. In
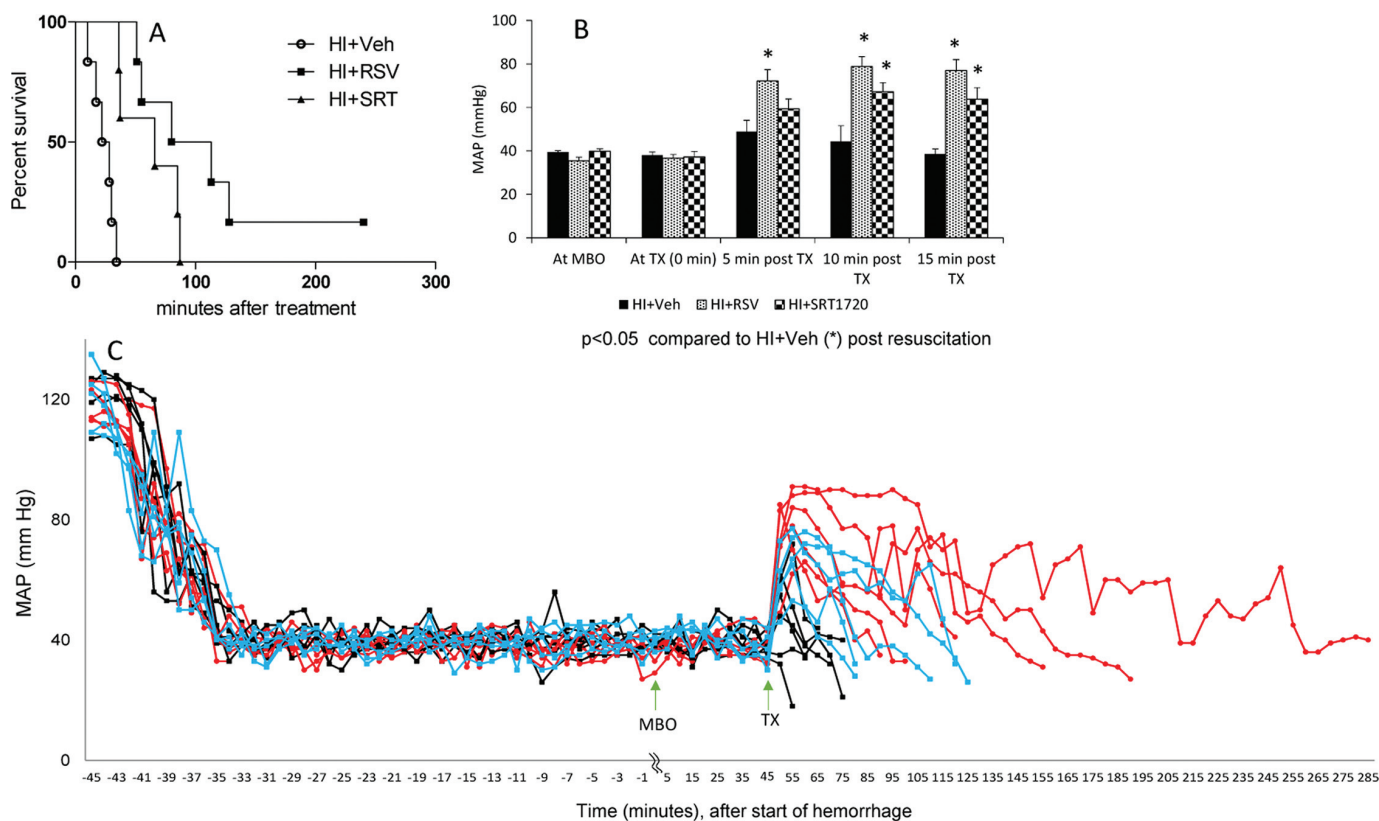
Figure 2. Resveratrol (RSV) and SRT1720 prolongs life after HI without resuscitation. (A) Kaplan-Meier survival curves for HI + Veh, HI + RSV and HI + SRT1720 (n = 5-6). *p < 0.05 versus HI + Veh, both curves. (B) Mean arterial pressure (MAP) at maximum bleed out (MBO), treatment (TX) with Veh, RSV or SRT1720, 5 min after TX, 10 min after TX and 15 min after TX in HI + Veh, HI + RSV and HI + SRT1720 (n = 5-6). Bars represent mean ± SE. *p < 0.05 versus HI + Veh. (C) MAP from the onset of hemorrhage to immediately prior to euthanasia in individual animals in HI + Veh, HI + RSV and HI + SRT1720 groups. (n = 5-6). Black lines: HI + Veh; blue lines: HI + SRT1720; red lines: HI + RSV.

the survived animals, a significantly higher left ventricular function as measured by dP/dt and heart rate was observed in the resveratrol-treated group when compared with vehicle treated group (Figure 1C and Supplementary Figure 1C).

Resveratrol Prolongs Life in the Absence of Resuscitation Fluid

To determine whether resveratrol is effective in prolonging survival in the absence of resuscitation, we conducted a 4-h survival study without performing resuscitation. In this study, in a subset of animals, we administered either resveratrol or vehicle with 500-600 μL of Ringer lactate immediately after the shock period. No fluid was given thereafter. The animals that received resveratrol survived longer (Figures 2A–C). We found a sustained deterioration of MAP in the rats that received only vehicle, whereas the rats that received resveratrol had a prolonged maintenance of MAP (Figures 2B, C).

SRT1720 Extends Life after Severe Hemorrhage

Based upon the published knowledge that one mechanism by which resveratrol exerts its metabolic action is by activation of the NAD$^+$-dependent enzyme SIRT1, we tested the effect of SRT1720, a specific activator of SIRT1. SRT1720 was administered in a manner similar to that of resveratrol and found a significant delay in mortality, though not to the extent seen in the rats that received resveratrol (Figures 2A–C). This indicates that SRT1720 target SIRT1 may be important in the restoration of organ function following hemorrhagic shock.

SRT1720 Improved Cardiac Contractility

To further verify the effect of SRT1720, we used a less aggressive resuscitation model than the one we used for 10-d survival study to test whether SRT1720 may improve cardiac function as measured by + and –dP/dt. SRT1720 or resveratrol was administered 10 min into resuscitation and the rats were euthanized 2 h after the end of resuscitation. Consistent with our previous observation, resveratrol also demonstrated significant improvement in + and –dP/dt. We observed improved MAP, heart rate, left ventricular function and blood lactate in

animals treated with resveratrol or SRT1720 (Figures 3A–D).

Molecular Mediators in the Heart

We tested the gene expression and protein expression of *Ppar-α* and *Tfam* by real-time PCR and Western blot. There was a significant decrease in the transcript level of both *Ppar-α* and *Tfam* following HI, and the levels were restored by resveratrol (Figures 4A, B). The Western blots and their semiquantitation demonstrated a marked increase in both Ppar-α and Tfam proteins in the heart tissue of resveratrol treated animals compared with vehicle-treated animals as shown in Figures 5A, B and D, E, respectively. However, in our experiments, Tfam protein levels did not decrease following HI. As Pgc-1α is a cofactor for Ppar-α, we tested the change in mRNA transcript (Figure 4C) and protein level (Figures 5C, F) of Pgc-1α and found significant alteration following HI and restoration in resveratrol treated rats.

DISCUSSION

Hemorrhagic injury is among the major causes of death due to trauma. Severe hemorrhage leads to whole body hypoxia and nutrient deprivation. One of the major cellular processes affected by severe hemorrhage is mitochondrial function. Cells rely on mitochondrial oxidative phosphorylation for most of their energy needs. The reduced tissue availability of oxygen in hemorrhagic shock adversely affects oxidative phosphorylation and cellular energetics. We hypothesized that agents that improve mitochondrial function may promote cellular function and extend life following hemorrhagic injury. In previous studies, our laboratory and others have observed improved organ function in rats treated with resveratrol following hemorrhagic injury (23,30). Resveratrol, a phytochemical, is a naturally occurring antioxidant and free radical scavenger. During the past decade, investigators have demonstrated that the sirtuin family of proteins, especially sirtuin 1 (SIRT1), are among the intracellular targets for resveratrol (31), although other
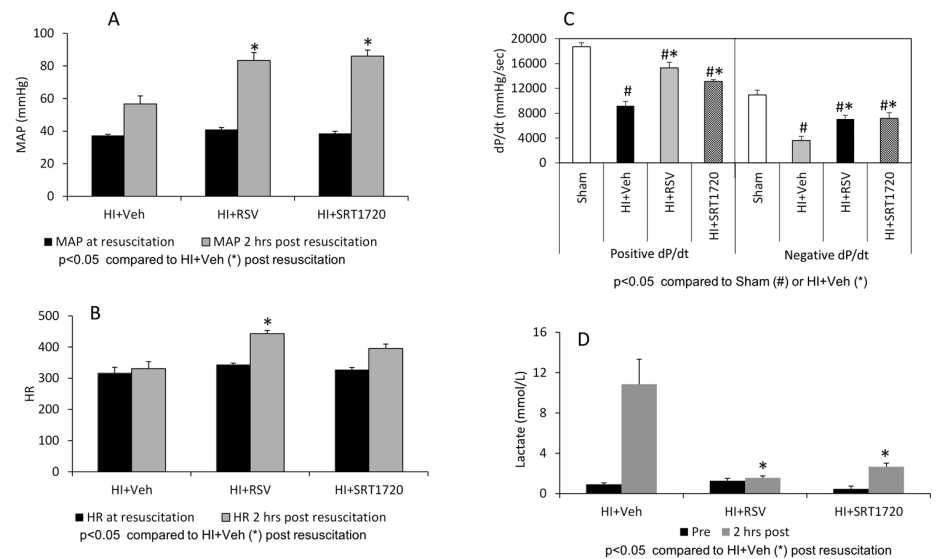

Figure 3. Hemodynamics at 2 h after resuscitation: mean arterial pressure (MAP) (A) and heart rate (HR) (B) at the start of resuscitation and at 2 h after resuscitation in HI + Veh, HI + resveratrol (RSV) and HI + SRT1720; n = 5-6; bars represent mean ± SE; * indicates $p < 0.05$ compared with the respective value at resuscitation. (C) ±dP/dt in sham group and at 2 h after resuscitation in HI + Veh, HI + RSV and HI + SRT1720 groups; (n = 4-5); bars represent mean ± SE. $p < 0.05$ compared with sham (*) or HI + Veh (#). (D) Lactate levels before HI and at 2 h after resuscitation in HI + Veh, HI + RSV and HI + SRT1720; n = 5-6; bars represent mean ± SE; $p < 0.05$ versus HI + Veh; * indicates $p < 0.05$ compared with preresuscitation.
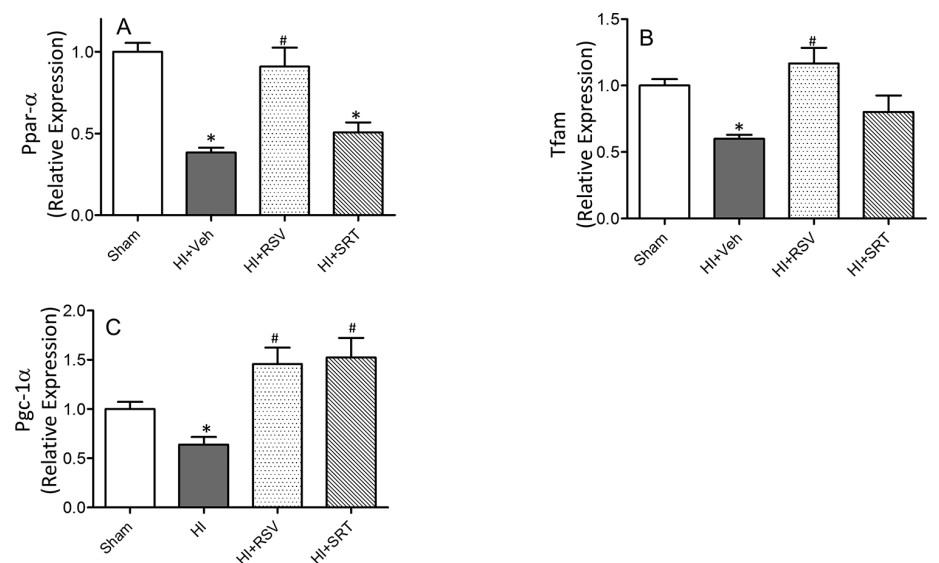

Figure 4. Effect of resveratrol (RSV) and SRT1720 on *Tfam, Ppar-α* and *Pgc-1α* expression following HI. HI down regulated the mRNA expression of *Ppar-α* (A) mitochondrial transcription factor *Tfam* (B) and *Pgc-1α* (C) in the heart and the levels restored by RSV treatment. The values are normalized to sham; n = 4-6. Bars represent mean ± SE. $p < 0.05$ compared with sham (*) or HI + Veh (#).
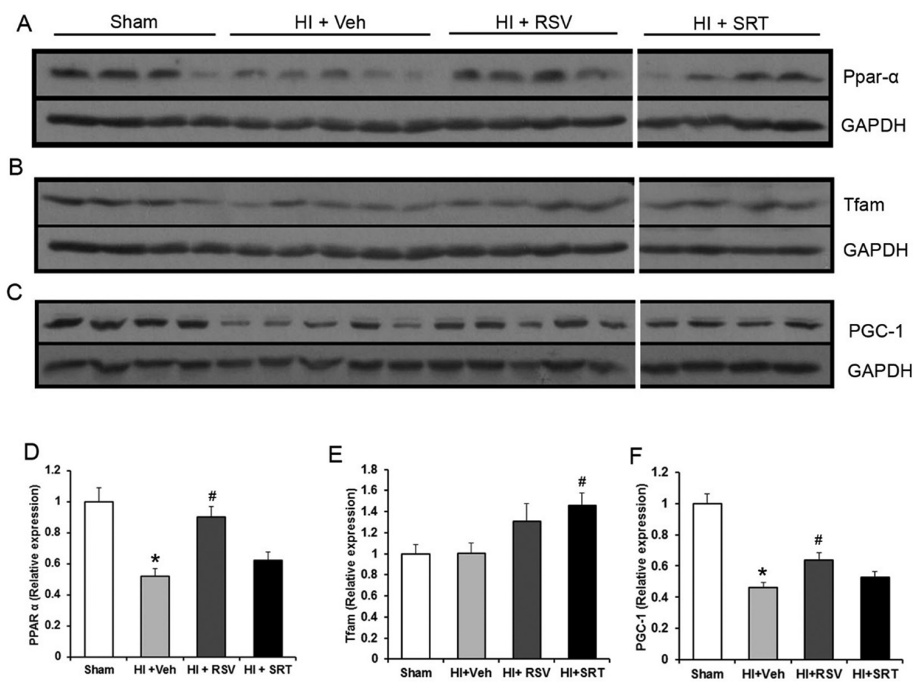
Figure 5. Effect of resveratrol (RSV) and SRT1720 (SRT) on Tfam, Ppar-α and Pgc-1α protein level following HI. HI significantly downregulated Ppar-α (A,D) and Pgc-1 expressions (C,F) and the levels were restored significantly with RSV. RSV and SRT augmented Tfam expression levels (B,E). Western blots from three experiments were used in the semiquantitation. Bars represent mean + SE. n = 4–6. *p* < 0.05 compared with sham (*) or HI + Veh (#).

targets are also described (32,33). Our previously published study demonstrated augmented expression of SIRT1 in rats subjected to trauma and severe hemorrhage and treated with resveratrol (23).

Though resveratrol has been shown to improve organ function and mitochondrial function, determining the effect of resveratrol on survival following HI may result in better strategies in the management of hemorrhagic shock and has not been investigated before. In our first experiment in this study, young rats were subjected to soft tissue trauma and severe hemorrhage, and resveratrol or vehicle was administered at 10 min following the start of resuscitation. We found a significantly increased mortality in the rats that were not treated with resveratrol, six of the 12 rats in this group died within 24 h and one died on the d 3, whereas only one of the eight resveratrol treated rats died during the 10-day observation period. We also observed a significant weight gain in the rats that re-

ceived resveratrol and survived for the duration of the study, demonstrating the beneficial effect of an early intervention with this naturally occurring small molecule. The results show a profound influence of resveratrol on survival when administered as a single dose as an adjunct to the resuscitation fluid. We had previously shown that rats that were administered resveratrol along with resuscitation fluid following hemorrhagic injury had significantly elevated ATP and decreased cytosolic cytochrome c levels in the left ventricular tissue and improved cardiac contractility, indicating a positive influence on mitochondrial homeostasis and organ function (23).

In our 10-day survival experiment, most of the mortality observed in animals that were not treated with resveratrol was found to be within 24 h. The left ventricular contractility measured in surviving animals at the end of the study showed a persisting effect on hemodynamics as observed by decreased dP/dt

and heart rate in this group of animals. This once again demonstrates the significance of early intervention following HI in providing a lasting effect not only for survival, but also for organ function.

We further wanted to test whether resveratrol can extend survival in the absence of resuscitation. One of the objectives of this experiment was to determine the effectiveness of resveratrol in the maintenance of life in a field setting following hemorrhage, in the absence of resuscitation. In clinical situations where blood transfusion or resuscitation is not possible, whether resveratrol can extend life until medical help becomes available will be of significant clinical advantage. Secondly we wanted to determine the physiological effect of resveratrol in the absence of resuscitation fluid, to further understand whether this molecule can preserve hemodynamics for a prolonged period in the absence of exogenous fluid supplementation. To address these questions, we administered resveratrol immediately after shock period in a subset of rats and found that the rats that received resveratrol survived for a significantly longer period than those that received only vehicle. It is noteworthy that all the resveratrol-treated animals had distinctly longer survival time than the longest survived animal in the untreated group (Figure 2C). The difference in mean arterial pressure between the two groups was evident from five minutes after the administration of resveratrol (Figure 2B). At the time of euthanasia there was a significant improvement in both lactate and blood pH in resveratrol-treated rats (Supplementary Figure 2). It is not clear whether this is a direct or indirect (prolonged survival and therefore more time to reach metabolic balance) effect of resveratrol.

Various studies have shown that SIRT1, an NAD$^+$-dependent deacetylase, is a molecular target of resveratrol (27,34,35). We therefore tested whether administration of SRT1720, a small molecule activator of resveratrol, mimics the life-extending effect of resveratrol in nonresuscitated rats. The administration of SRT1720 resulted in a significant improvement in survival

time, though the effect seemed to be less than that of resveratrol. These results indicate that at least part of the effect of resveratrol is through activation of SIRT1.

In our first experiment, we followed a well standardized and well described soft tissue trauma and hemorrhage procedure with an aggressive resuscitation method (8,36,37) and found that resveratrol can improve survival following HI. In the second experiment, we demonstrated that resveratrol can prolong life in the absence of resuscitation and SIRT1720 can partially mimic the effect of resveratrol. In the third experiment, the effect of SRT1720 on organ function following the hemorrhage was further confirmed. In the third experiment, we used a less aggressive resuscitation model compared with the model used for the 10-d study. There was significant improvement in left ventricular function at the end of 2 h following resuscitation in rats that received resveratrol as well as SRT1720. As we have done in a previous study (23) in a small group of three rats, we administered sirtinol, a known SIRT1 inhibitor, five minutes prior to resveratrol administration and found a marked drop in left ventricular performance (+dP/dt = 6432 ± 1294 and –dP/dt = 3104 ± 307; mean ± SE) as compared to that obtained in resveratrol-treated animals. These experiments demonstrate that activation of SIRT1 is an important molecular step in restoring organ function following hemorrhagic shock.

It may be speculated that the salutary effect of SRT1720 is due to its effect on mitochondrial function through SIRT1 (38,39). One of the mechanisms by which resveratrol improves mitochondrial function is likely due to allosteric activation of SIRT1 (31). SIRT1, an NAD$^+$-dependent deacetylase, plays a significant role in cellular physiology by deacetylating target proteins. Among the target proteins of SIRT1 are transcription factors such as FOXO3, p53 and NFκb, and the transcriptional cofactor Pgc-1α. The proteins Tfam and Ppar-α are transcription factors critical to mitochondrial biogenesis and function, and their expression level in left ventricular tissue was restored by resveratrol and markedly by SRT1720, following HI. We also found that the level of Ppar-α and Tfam was restored or augmented, respectively, following resveratrol treatment (Figures 4, 5). Pgc-1α is a cofactor for Ppar-α and the decreased level of Pgc-1α following HI and the increase with treatment show the possible downregulation of the Pgc-1α–Ppar-α–mediated pathway following HI and amelioration when treated with resveratrol. This is one of the critical pathways in the homeostasis of cellular energetics. Previously, we have shown that resveratrol can restore total ATP and decrease cytosolic cytochrome C levels in the heart following HI (23). We, and subsequently others, have also shown a declining SIRT1 level in the heart following HI and restoration by resveratrol (23) or estradiol (40). Further studies using genetically modified mice might address the role of these factors in isolation, in a reductionist approach, in resveratrol-induced restoration of cellular homeostasis. Nevertheless, the results from these studies and those reported in the present study provide a mechanistic basis for the salutary effect of resveratrol on survival presented in this report emphasizing the significance of mitochondrial function in restoring organ function and improving survival following HI.

In a previous study by Kentner *et al.*, administration of Tempol early during the resuscitation phase significantly improved survival in Sprague Dawley rats (41). The 3-d survival study also showed that when the antioxidant was administered during the later part of resuscitation, the benefit was markedly less. The study therefore demonstrated the beneficial effect of early intervention in hemorrhagic shock (41). On the basis of the results of this study, we may speculate that the time of administration of resveratrol also played an important role in survival advantage observed in our study.

Oxidative stress is largely recognized as a pathogenic hallmark of hemorrhagic/ reperfusion shock (42,43). Therefore the effect of resveratrol as an antioxidant is very pertinent in this model. In addition to resveratrol and tempol, several other agents that are known to reduce oxidative stress have also been tested in hemorrhagic shock/resuscitation models, in shorter term studies (24,44,45). In various models of oxidant stress, it has been reported that resveratrol inhibits the expression and activity of NADPH oxidase, enhances activity of several antioxidant enzymes, including that of the SOD and scavenges reactive oxygen free radicals (ROS) (46). Juan *et al*. reported that resveratrol augments the expression of heme oxygenase-1 (HO-1) in human aortic smooth muscle cells, reducing ROS (47). The effect of resveratrol on lipid peroxidation, NADPH oxidase and xanthine oxidase are also well studied in different models (48,49). In view of these results, the complex hit involved in hemorrhagic shock involves alterations in multiple different pathways that lead to cellular dysfunction and cell death leading to organ dysfunction.

CONCLUSION

In summary, for the first time, we show that when resveratrol is used as an adjunct to resuscitation fluid following HI, there is significant improvement in survival. In addition, in the absence of resuscitation, resveratrol extended life significantly making this small molecule a strong candidate in the treatment of severe hemorrhagic condition.

DISCLOSURE

The authors declare they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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