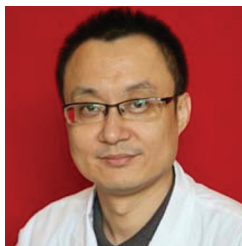# Selective Inhibition of SIRT2 Improves Outcomes in a Lethal Septic Model

T. Zhao[1], H.B. Alam[1], B. Liu[1], R.T. Bronson[2], V.C. Nikolian[1], E. Wu[3], W. Chong[*,4] and Y. Li[*,1]

W. Chong

Y. Li

[1]Department of Surgery, University of Michigan Hospital, Ann Arbor, MI, USA; [2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA; [3]Department of Neurosurgery, Baylor Scott and White Health Care, Temple, TX, USA; [4]Department of Emergency, the First Hospital of China Medical University, Shenyang, China

Abstract: *Background*: Seven isoforms of histone deacetylase Class III have been reported - Sirtuin (SIRT) 1-7. We recently demonstrated that EX-527, an inhibitor of SIRT1, reduces mortality in a mouse model of lethal-cecal-ligation-and-puncture (CLP)-induced septic shock. Our present study was aimed at determining whether selective inhibition of SIRT2, with AGK2, would decrease animal death and attenuate the inflammatory response in a septic model.

Methods: Experiment I: C57BL/6J mice were intraperitoneally given either AGK2 (82 mg/kg) in dimethyl sulfoxide (DMSO) or DMSO alone, and 2 h later subjected to CLP. Survival was monitored for 240 hours. Experiment II: mice treated the same way as Experiment I, were grouped into (i) DMSO vehicle, and (ii) AGK2, with sham mice (operating but without any treatment) serving as controls. Peritoneal fluid and peripheral blood were examined at 24 and 48 hours for cytokine production. Samples of blood at 48 h were also allocated to assess coagulability using Thrombelastography (TEG). Morphological changes of bone marrow were evaluated from long bones (femurs and tibias) with hematoxylin and eosin (H&E) staining. Bone marrow atrophy was quantified by a blinded pathologist. Experiment III: cytokines in supernatant of the cultured normal primary splenocytes were measured after the cells were stimulated by lipopolysaccharide and treated with or without AGK2 (10 µM) for 6 hours.

Results: AGK2 significantly reduced mortality and decreased levels of cytokines in blood (TNF-α: 298.3±24.6 *vs* 26.8±2.8 pg/ml, p=0.0034; IL-6: 633.4±82.8 *vs* 232.6±133.0 pg/ml, p=0.0344) and peritoneal fluid (IL-6: 704.8±67.7 *vs* 391.4±98.5 pg/ml, p=0.033) compared to vehicle control. Also, AGK2 suppressed the TNF-α and IL-6 production in the cultured splenocytes (TNF-α: 68.1±6.4 *vs* 23.9±2.8 pg/ml, *p*=0.0009; IL-6: 73.1±4.2 *vs* 49.6±3.0 pg/ml; *p*=0.0051). The TEG data showed that the mice subjected to CLP displayed prolonged fibrin formation and fibrin cross-linkage time, slower clot formation, decreased platelet function, and clot rigidity. AGK2 treatment was associated with dramatic improvements in fibrin cross-linkage and clot formation times, without a significant impact on the clot initiation parameters or platelet function. Additionally, AGK2 significantly attenuated the bone marrow atrophy (58.3±6.5 *vs* 30.0±8.2%, *p*=0.0262).

Conclusion: Selective inhibition of SIRT2 significantly improves survival, and attenuates sepsis-associated "cytokine storm", coagulopathy, and bone marrow atrophy in a mouse model of lethal septic shock.

Keywords: Bone marrow atrophy, mouse, SIRT2 inhibitor, septic shock, survival.

INTRODUCTION

Histone acetylation is a fundamental epigenetic modification that plays a role in immune signaling. The process of acetylation is controlled by two classes of competing proteins: histone acetyltransferasees (HATs) and histone deacetylases (HDACs). By modulating the structure of chromatin, acetylation of histone controls the accessibility of proteins (e.g. transcription factors) to DNA and the subsequent process of gene transcription. Histone acetylation provides an "open" chromatin structure, favoring activation of gene transcription. Histone deacetylation compacts chromatin structure, favoring gene silencing. Many non-histone proteins necessary for essential cell functions, such as α-tubulin, heat shock protein (HSP) 90, and some receptors, can also be reversibly modified by acetylation [1].

*Address correspondence to these authors at the (W. Chong) Department of Emergency, The First Hospital of China Medical University, 155 North Nanjing Street, Shenyang, Liaoning 110001, China; Tel: 86-13358860636; E-mail: chongweixiena@yahoo.com and (Y. Li) Department of Surgery, University of Michigan at Ann Arbor, North Campus Research Complex, Rm. 363N, Bldg. 26, 2800 Plymouth Road, Ann Arbor, MI 48109, USA; Tel: 734-763-0848; E-mail: yqli@med.umich.edu

Eighteen HDAC enzymes have been identified and divided into Zn^{2+} dependent (Class I, II, and IV) and Zn^{2+} independent, nicotinamide adenine dinucleotide (NAD+)-dependent (Class III) groups. Class III HDACs, act through a nicotinamide adenine dinucleotide (NAD^+)-dependent mechanism. Class III HDACs are made up of the Sirtuins enzymes which include seven members (SIRTs 1–7) homologous to the yeast HDAC silent information regulator 2.

SIRT2 resides primarily in the cytoplasm, but it can shuttle to the nucleus where it has been shown to be involved in many pathologic and physiologic processes, including bacterial infection [2], genomic instability [2], carcinogenesis [3], cell cycle progression [4], and arthritis [5]. SIRT2 functions as a α-tubulin deacetylase and has a role in oligodendroglial differentiation [6, 7]. It is suggested that myelinogenesis, myelin-axon interaction, and brain aging are all affected by the selective availability of SIRT2 to oligodendroglial cells. Knockdown of SIRT2 with small interfering RNA can increase the acetylation of alpha-tubulin and expression of myelin basic protein in oligodendrocyte precursors [6]. In the nucleus, SIRT2 controls the cell cycle and acts as a H4K16 deacetylase. SIRT2-deficient mouse embryonic fibroblasts accumulate acetylated H4K16 during mitosis and exhibit a delay in S-phase entry [8]. In a model of Parkinson's disease, inhibition of SIRT2 can rescue alpha-synuclein toxicity and modify inclusion morphology [9]. SIRT2 can also regulate adipocyte differentiation by deacetylating forkhead box protein O1 (FOXO1) [10]. Given the wide array of actions associated with the SIRT2 isoform, exploration in its role in septic shock provides an opportunity to explore a potential targeted therapy.

We recently demonstrated that EX-527, an inhibitor of SIRT1, can promote survival in a rodent model of lethal CLP-induced septic shock [11]. Unlike SIRT1, which has been extensively studied because of its association with caloric restriction, biological and therapeutic roles of SIRT2 still remain to be explored. The aim of present study was to determine whether selective inhibition of SIRT2 with AGK2 could also attenuate inflammatory responses and improve survival in a lethal septic model.

MATERIALS AND METHODS

Cells and Reagents

Mice primary splenocytes were prepared. Briefly, spleens were harvested and homogenized through a Corning® 70 μm cell strainer (Sigma Aldrich, St. Louis, MO). Red blood cells were lysed using Lysis Buffer (Sigma Aldrich, St. Louis, MO). Isolated primary splenocytes were then cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 U/ml streptomycin (Invitrogen, Grand Island, NY) at 37°C and 5% CO_2. For some experiments, the cells were incubated with 1 μg/mL lipopolysaccharide (LPS)

(Sigma Chemical Co, St. Louis, MO) or 10 μM AGK2 (EMD Millipore, Billerica, MA).

Sepsis Model: Cecal Ligation and Puncture (CLP)

This protocol was approved by the Institutional Animal Review Committee. Male C57BL/6J mice (4-6 weeks) were purchased from The Jackson Laboratory (The Jackson Laboratory, Bar Harbor, ME) and housed for 3 days before experiments. The CLP murine model [12], modified by our laboratory, was used to induce fecal peritonitis. Peritoneal cavity was opened after the mice inhaled isoflurane anesthesia. Cecum was exposed, and ligated with a 5-0 suture below the ileocecal valve. Two holes were then punctured through and through with a 20 gauge needle. The punctured cecum was pressed to expel a small amount of fecal material and put back to the peritoneal cavity. Abdominal incision was closed with 4-0 silk suture. Animals were resuscitated by subcutaneous injection of 1 mL of saline. Sham-operated animals were operated in the same manner, without the CLP.

Administration of AGK2 and Experimental Design

Experiment I: Using C57BL/6J mice (n = 9/group), intraperitoneal injections of either AGK2 (82 mg/kg, calculated based on findings of previous studies) in dimethyl sulfoxide (DMSO) or DMSO alone were performed 2 hours prior to CLP. Survival was monitored for 240 hours.

Experiment II: Sham mice (laparotomy without CLP) were treated with identical intraperitoneal injections as Experiment I, grouped into two control groups: (i) DMSO vehicle and (ii) AGK2/DMSO (n=10-14/group).

Blood and peritoneal fluid samples were collected to measure cytokines at 24 or 48 h. Blood at 48 h was also analyzed for the coagulation status using Thrombelastography (TEG). Blood samples from cardiac puncture and peritoneal fluid from lavage with 1 mL of normal saline were collected. Femurs and tibias were harvested (n=5-6/group) at 48 h and morphological changes in bone marrow was determined *via* H&E staining. Atrophy of bone marrow was quantified by a blinded pathologist.

Cytokine Measurements

Experiment III: To assess cytokine production, normal primary splenocytes were incubated with LPS in the presence or absence of AGK2 (10 μM) for 6 h (n=4/group). The Quantikine Enzyme-Linked Immunosorbent Assay (ELISA) Kit (R&D Systems, Minneapolis, MN) was used to measure concentrations of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 in the plasma, peritoneal fluid, and cell culture supernatant.

Thromboelastography (TEG)

Several TEG parameters, such as *Split Point* (*SP*), *Reaction time* (*R*), *Clot formation time* (*K*), *Alpha Angle* (*α*), and *Maximum Amplitude* (*MA*), were analyzed as previously described [13]. Briefly, samples of whole

blood were collected into heparin-rinsed tubes 48 h after CLP. From each sample, 340 μl was taken and added to a heparinase-coated tube. Thromboelasto-graphy using the TEG® 5000 Thrombelastograph® Hemostasis Analyzer System (Haemonetics Corpo-ration, Braintree, MA) was performed according to manufacturer's instructions.

Histologic Analysis

To determine morphological changes of bone marrow by H&E staining, bones of femur and tibia were harvested 48 h after CLP. Samples were fixed in 10% buffered formalin, embedded in paraffin, sliced into 5-μm sections, and stained with H&E. Bone marrow atrophy was examined by the pathologist who was blinded to the sample's groups. The degree of atrophy was graded and evaluated as previously described [14].

Statistical Analysis

Results were represented as mean ± standard error of the mean (SEM). Kaplan-Meier method was taken to compare outcomes of survival. Log-rank test was used to analyze survival differences. One way analysis of variance (ANOVA) followed by Bonferroni post-hoc test was used for differences among three or more groups. Student's *t*-test was used for comparison of the differences between two groups. All analysis was carried out using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). *P*-values of 0.05 or less were considered significant.

RESULTS

AGK2 Significantly Improves Survival in a Mouse Model of CLP-Induced Septic Shock

C57BL/6J mice were intraperitoneally given either AGK2 (82 mg/kg) in dimethyl sulfoxide (DMSO) or DMSO alone, and 2 h later subjected to CLP. All mice from the vehicle DMSO alone group died in less than 3 days whereas the majority (55.6%) of mice treated with AGK2 survived for the duration of the 240 hours following CLP (55.6% *vs* 0% survival, *p*<0.0001; Fig. **1**).

AGK2 Decreases Levels of Cytokine in Blood and Peritoneal Cavity

Blood samples and peritoneal fluid were obtained after CLP. Levels of TNF-α and IL-6 were determined by ELISA. Normally no TNF-α and IL-6 were detected in blood and peritoneal fluid. CLP induced sepsis significantly increased the levels of the cytokines in circulation and within the peritoneal fluid. Mice treated with AGK2 has significantly decreased the levels of cytokines in circulation (TNF-α: 298.3±24.6 *vs* 26.8±2.8 pg/ml, *p*=0.0034; IL-6: 633.4±82.8 *vs* 232.6±133.0 pg/ml, *p*=0.0344; Figs. **2**, **3**) and within peritoneal fluid compared to DMSO vehicle (IL-6: 704.8±67.7 *vs* 391.4±98.5 pg/ml, *p*=0.033; Fig. **3**).
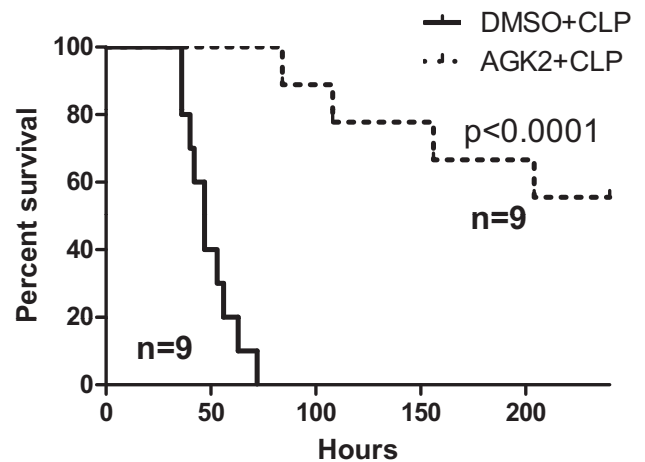

Fig. (1). AGK2 significantly improves survival in CLP-induced lethal septic model. C57BL/6J mice were intraperitoneally administered 82 mg/kg AGK2 dissolved in DMSO or vehicle DMSO, and 2 h later subjected to CLP (n = 9 animals/group). Treatment with AGK2 significantly improved long-term survival compared to DMSO vehicle group (55.6% *vs* 0% survival, *p* < 0.0001). CLP: cecal ligation and puncture; DMSO: dimethyl sulfoxide.
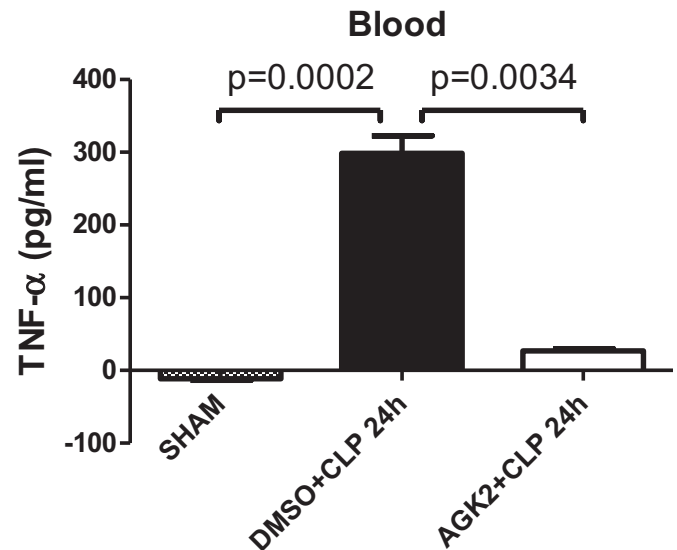

Fig. (2). AGK2 attenuates TNF-α levels in blood *in vivo*. Blood samples were collected at 24 h after CLP and assayed for TNF-α levels by ELISA (means ± SEM, n = 4-6 animals/group). TNF-α: tumor necrosis factor-α; CLP: cecal ligation and puncture; ELISA: enzyme-linked immunosorbent assay.

AGK2 Decreases Levels of Cytokine in Cultured Splenocyte Supernatant

Using ELISA, levels of TNF-α and IL-6 were measured in the supernatant of the cultured mouse primary splenocytes treated with LPS in the presence or absence of AGK2 for 6 hours. Sham group of normal primary splenocytes (without any treatment) served as a control, and revealed expectedly low concentrations of TNF-α and IL-6. LPS stimulated an increase of TNF-α and IL-6 in the supernatant of the cells. AGK2
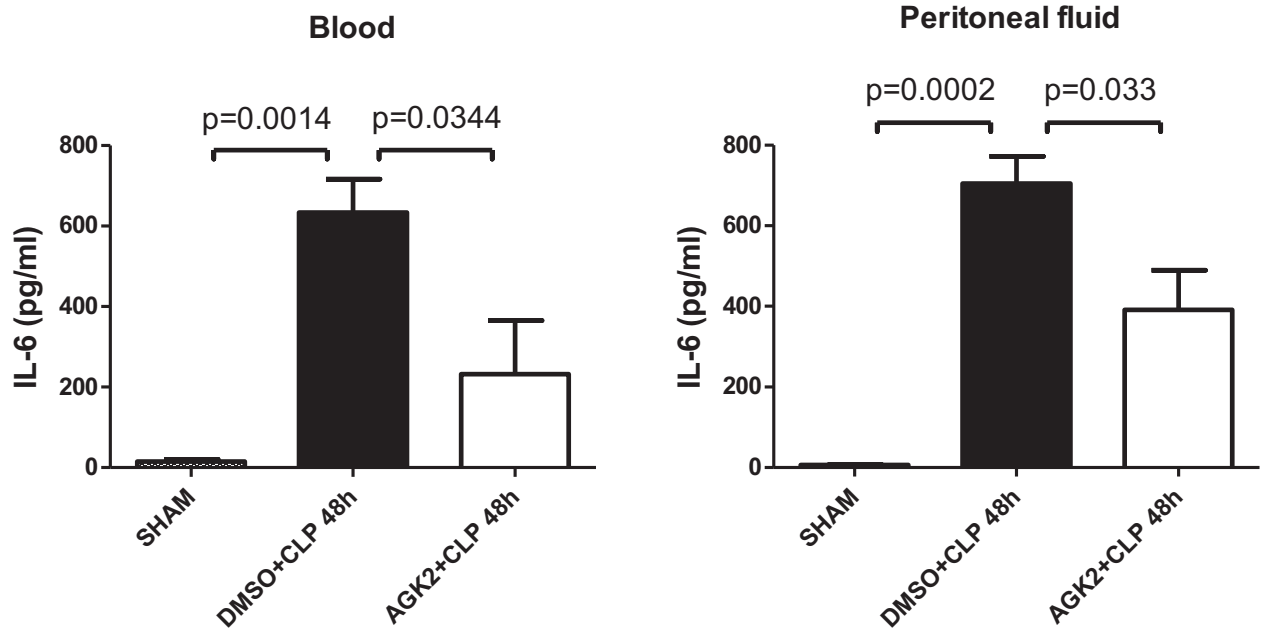
Blood

Peritoneal fluid

Fig. (3). AGK2 attenuates IL-6 levels in blood and peritoneal fluid *in vivo*. Blood and peritoneal fluid were collected at 48 h after CLP and determined for IL-6 levels by ELISA (means ± SEM, n = 4-6 animals/group). CLP: cecal ligation and puncture; IL-6: interleukin-6; ELISA: enzyme-linked immunosorbent assay.
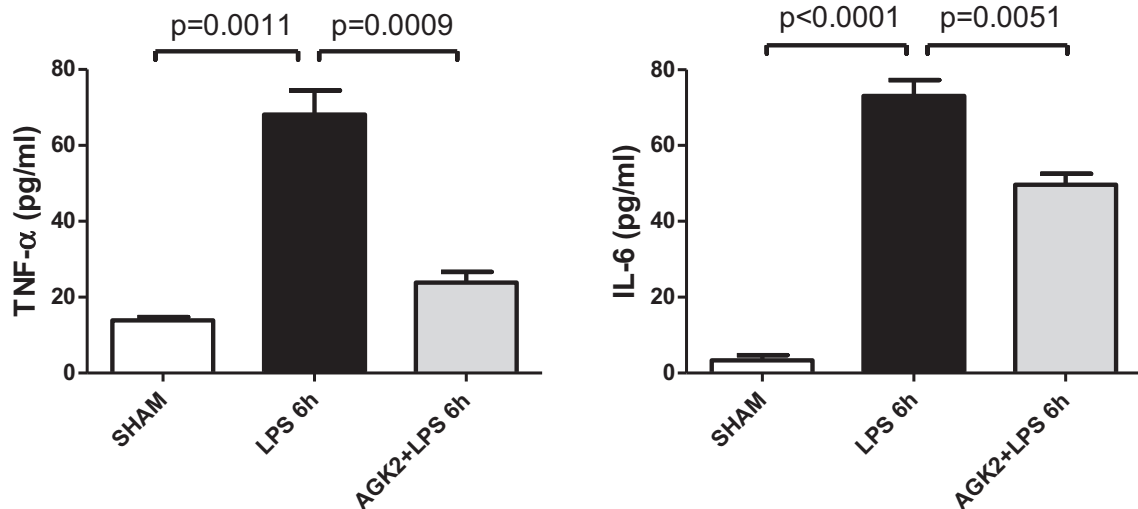

Fig. (4). AGK2 decreases levels of cytokine in cultured splenocyte supernatant. Levels of TNF-α and IL-6 in culture supernatant of mice primary splenocytes were measured by ELISA at 6 h after LPS treatment in the absence or presence of AGK2. Untreated primary splenocytes served as control (means ± SEM, n = 4/group). TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; ELISA: enzyme-linked immunosorbent assay; LPS: lipopolysaccharide.

treatment significantly decreased the LPS-induced TNF-α and IL-6 production (TNF-α: 23.9±2.8 *vs* 68.1±6.4 pg/ml, *p*=0.0009; IL-6: 49.6±3.0 *vs* 73.1±4.2 pg/ml; *p*=0.0051; Fig. **4**).

Effect of AGK2 Treatment on Thrombelastograph Parameters in the Septic Shock Model

Whole blood samples were collected 48 h after CLP, and subjected to the TEG® 5000. Thrombelastograph® Hemostasis Analyzer System for analysis. As shown in Fig. (**5**), the DMSO treated mice displayed prolonged *K*, slower *Angle*, and decreased *MA* compared to sham animals (Fig. **5A**). However, selective inhibition of

SIRT2 with AGK2 significantly improved *K* (17.0±3.5 *vs* 6.2±1.0 min, *p*=0.0392) and *Angle* (10.3±2.5 *vs* 32.3±3.8 degree, *p*=0.0012; Fig. **5B**), without a significant effect on the clot initiation parameters (*SP*: 8.1±1.1 *vs* 7.0±0.9 min; *R*: 11.5±2.2 *vs* 8.8±1.0 min) and *MA* (28.7±6.0 *vs* 47.5±4.3 mm, *p*=0.0514; Fig. **5C**).

AGK2 Reduces Atrophy of Bone Marrow in the Septic Shock Model

Bone samples of femur and tibia were collected 48 h after CLP, processed and stained with H&E. Normal cell composition and histology of bone marrow were noted in the sham group. The ratio of bone marrow
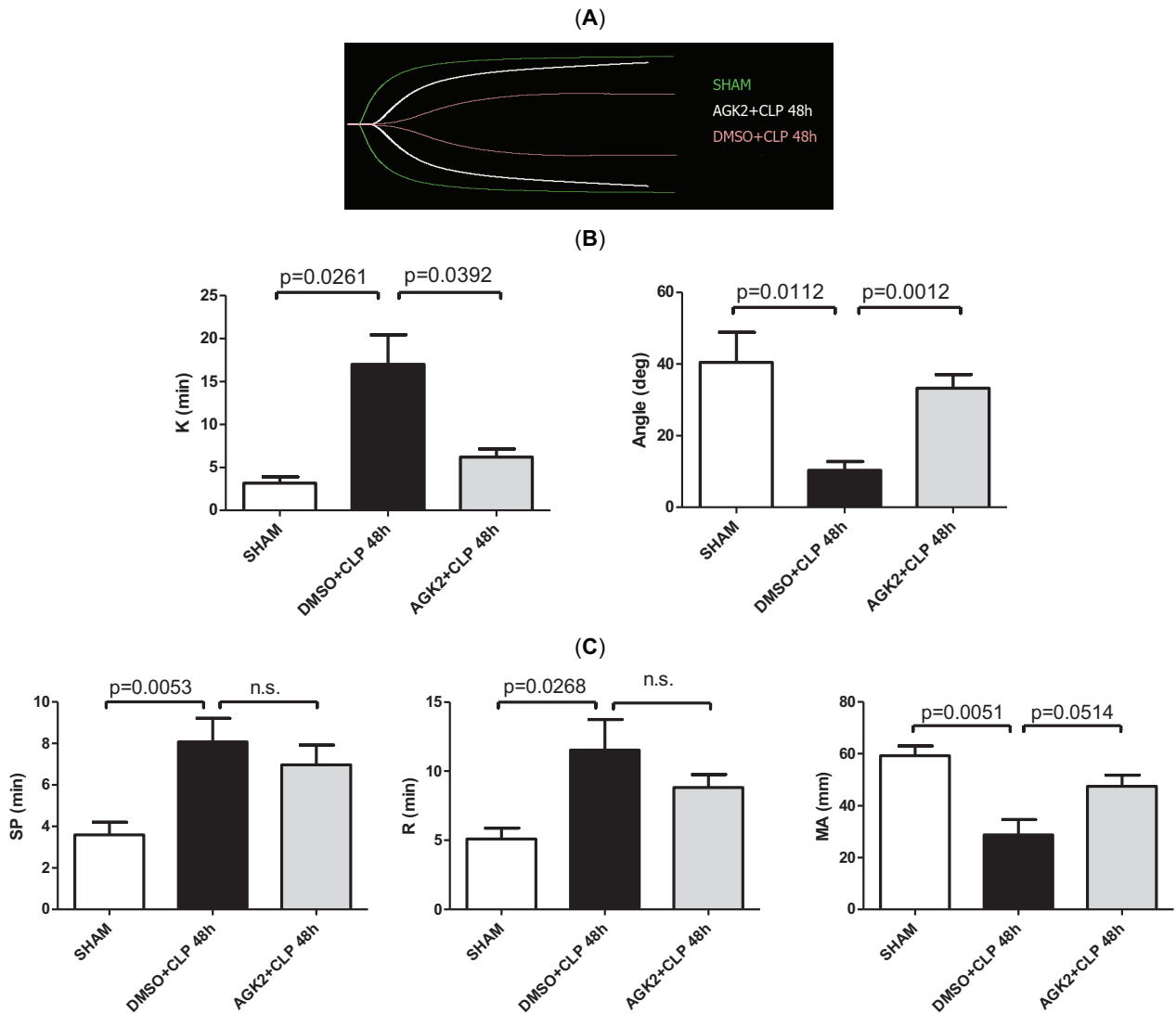
Fig. (5). Effect of AGK2 treatment on Thrombelastograph parameters. (**A**) Representative TEG® tracings for animals of different groups. The *SP*, *R*, *K*, *Angle* and *MA* values were recorded in TEG® 5000 Thrombelastograph® Hemostasis Analyzer System. Reaction time (*R*) is the time from sample placement until TEG® tracing amplitude reaches 2 mm, and Split Point (*SP*) is the time from sample placement to first divergence of the trace. *SP* and *R* represent the initial fibrin formation rate. *K* measures the time from clotting initiation (*R*) until clot formation reaches amplitude of 20 mm, and it reflects the time for development of fixed degree of viscoelasticity during clot formation. *Angle* is formed by the slope of initial TEG® tracing, and it denotes the speed at which solid clot forms. Maximum Amplitude (*MA*) is the greatest amplitude of TEG® tracing, and it reflects platelet function and absolute strength of the fibrin clot. TEG: thromboelastography. (**B**) AGK2 restores fibrin cross-linkage and clot formation speed. (**C**) AGK2 does not affect clot initiation parameters and platelet function significantly. Values were expressed as mean ± SEM; n = 5-8 animals/group.

cells to veins was increased 48 h after CLP in the DMSO vehicle group, with bone marrow cell depletion and venous dilation (0±0 *vs* 58.3±6.5 %). AGK2 treatment significantly attenuated the bone marrow depletion (Fig. **6A**, magnification 40x) and atrophy (Fig. **6B**) (58.3±6.5 *vs* 30.0±8.2%, *p*=0.0262).

DISCUSSION

In the present study, we have investigated effects of AGK2 on animal survival, pro-inflammatory cytokine production, coagulopathy, and bone marrow atrophy in a lethal CLP model. We discovered that selective inhibition of the Class III HDAC SIRT2 isoform with

AGK2 significantly promotes long-term survival, attenuates sepsis-associated cytokine production, reduces coagulopathy, and decreases bone marrow atrophy.

Sirtuins are a family of enzymes that are found in organisms such as plants, bacteria, and animals. It has been reported that sirtuins suppress the formation of toxic extra-chromosomal rDNA circles and improve longevity in yeast [15]. It is not clear, however, whether the increase of longevity is due to overexpression of sirtuin in metazoans or because of different genetic background [16, 17]. Studies have shown that sirtuins may mediate caloric restriction without having overt
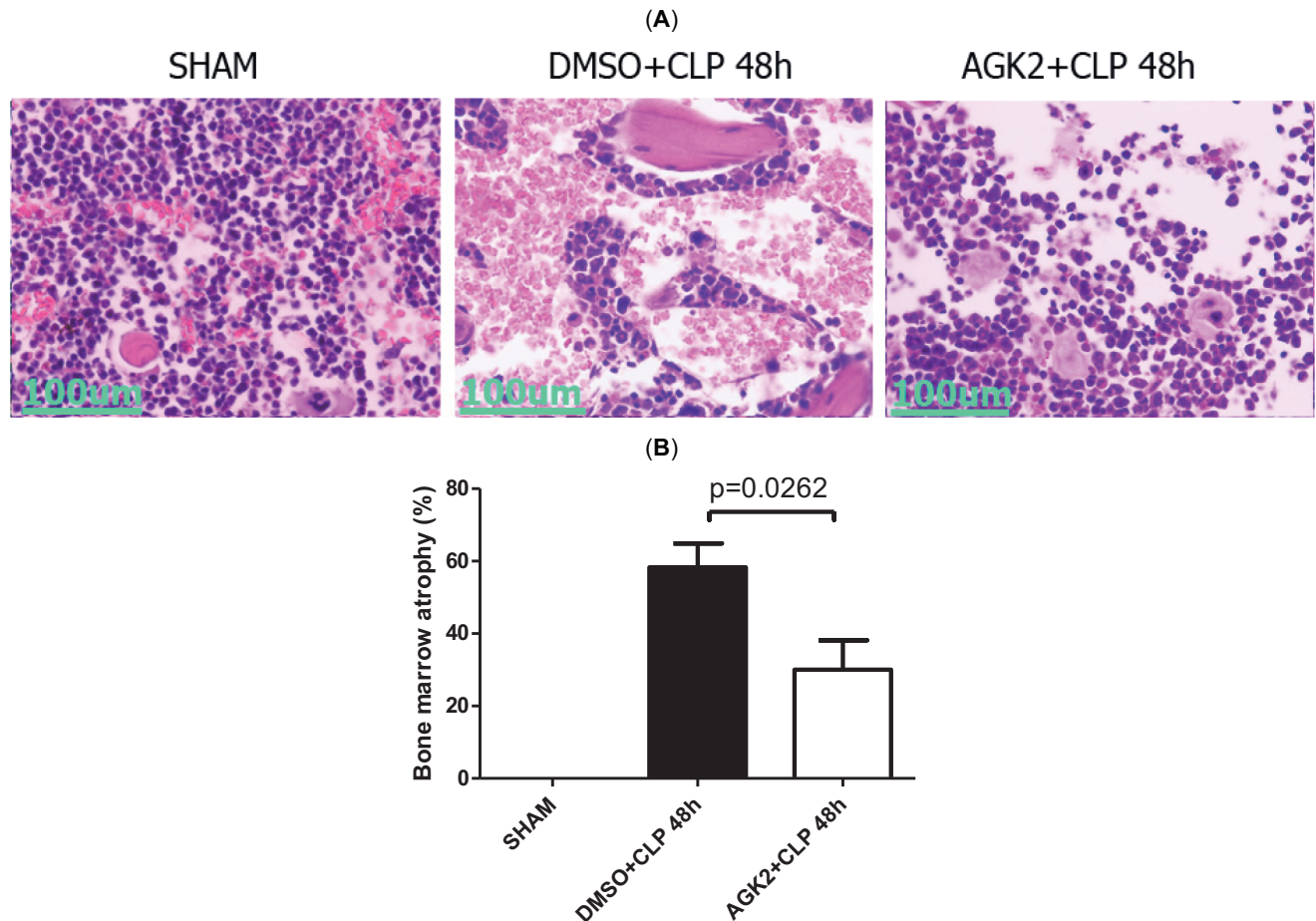
(A)

(B)

Fig. (6). AGK2 decreases bone marrow atrophy markedly in the lethal septic model (H&E, magnification 40×). (A) Tissue samples of long bones (femur and tibia) were harvested at 48 h after CLP. Samples were processed and stained with H&E. Representative images were chosen from different experimental groups. **(B)** Semiquantitative pathology scores for bone marrow atrophy were graded according to the diameter proportion of veins to bone marrow cells (means ± SEM, n = 5-6 animals/group). CLP: cecal ligation and puncture; H&E: hematoxylin and eosin.

metabolic evidence of malnutrition [18, 19]. As caloric restriction is explored in its association with improved overall health in multiple models, the role of sirtuins in debilitating illnesses, such as septic shock, which may already cause emaciation, must be explored.

Normally SIRT2 is in the cytoplasm, and deacetylates α-tubulin at lysine 40 [7]. During G2/M SIRT2 transiently translocates into the nucleus, deacetylates histone H4 at lysine 16, regulating chromatin condensation and gene transcription [8]. Moreover, reduction of SIRT2 expression in adipocytes has been associated with increased FOXO1 acetylation and subsequent adipogenesis [10]. FOXO transcription factors participate in diverse pathways such as DNA repair, the cell cycle, apoptosis, metabolism, and aging [20].

The diversity of SIRT2 substrates makes this HDAC isoform quite complicated. Inhibition of SIRT2 has been associated with dopaminergic cell survival-advantage in Parkinson's disease models has been shown to protect cells from toxicity related to alpha-synuclein expression, and has been associated with decreased H_2O_2-induced apoptosis [9, 21]. The mechanism of

action related to SIRT2 inhibition is largely unknown. This study shows that SIRT2 plays a role in three important pathways: cytokine production, coagulopathy, and bone marrow atrophy.

First, we found that the inhibition of SIRT2 attenuates the production of pro-inflammatory cytokines such as TNF-α and IL-6 in blood, peritoneal fluid, and splenocytes in a model of sepsis. Cytokine production is regulated by various pathogens, including LPS, *via* pathogen-associated molecular patterns (PAMP). In splenocytes, LPS can bind to Toll-like receptor-4 (TLR-4), and in turn activate the transcriptional factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) *via* phosphorylation, leading to translocation of NF-κB into the nucleus where it may regulate the production of many inflammatory cytokines, including TNF-α and IL-6 [22]. In primary neuronal cells, we have demonstrated that treatment with valproic acid, a Class I and II HDAC inhibitor, induces acetylation and activation of NF-κB, protecting cells from hypoxia-induced apoptosis [23]. Recently, Rothgisser *et al*. showed that SIRT2 may regulate NF-κB activity by deacetylating p65 Lys (310). Interestingly, knock-out of Sirt2 (Sirt2$^{-/-}$) has been

shown to increase expression of a subset of NF-κB target genes, not including TNF-α and IL-6 [24]. It still remains unknown what is the molecular basis of the relationship between phosphorylation and acetylation of NF-κB.

We have previously found that suberoylanilide hydroxamic acid (SAHA), a pan-HDAC inhibitor, suppresses LPS/TLR4 signaling in LPS-stimulated macrophages through multiple potential mechanisms. SAHA induces hyperacetylation of HSP90 and inhibits the function of the chaperone protein, which resulted in dissociation and degradation of IL-1 receptor associated kinase 1 (HSP90's client protein) and led to a decrease in translocation of NF-κB into the nucleus and attenuation of gene expression of key pro-inflammatory cytokines [25]. Similarly, AGK2 may affect all of these gene expression through NF-κB-related pathways. However, more investigations with technologies of genomics and proteomics should be performed to determine the functions of SIRT2 and molecular mechanisms underlying the inhibition of SIRT2.

Second, inhibition of SIRT2 attenuated a CLP-induced progressive coagulopathy. In the pathogenesis of sepsis, there is significant cross talk between the inflammatory and coagulation cascades. Severe systemic inflammation is also associated with intravascular fibrin deposition and thrombin formation, known to be a cause of coagulopathies related to disseminated intravascular coagulation, multiple organ dysfunction syndromes, and consumptive coagulopathy [26, 27]. Therefore, anti-inflammatory properties of SIRT2 inhibition may play a role in the attenuation of coagulopathy seen in this model. The direct effect of SIRT2 inhibition on the coagulation components *in vitro* needs to be further elucidated.

Third, inhibition of SIRT2 significantly decreased the post-CLP bone marrow atrophy. Bone marrow is crucial for myelopoiesis and B cell generation, and is associated with maturation of T lymphocytes to a lesser degree [28, 29]. In CLP-induced sepsis, there is a significant decline of the percentage of Grl$^+$-myeloid cells in the bone marrow, accounting for four-fifth of the decrease in viable cells yield in the marrow. It is likely that during severe sepsis myeloid cells are recruited to the inflammatory sites, leading to depletion of the bone marrow [30]. By inhibiting the systemic inflammatory cytokines and decreasing the recruitment of marrow cells to distant sites, AGK2 may prevent bone marrow from depletion and exhaustion. We have previously shown that selective inhibition of HDAC6 can attenuate stress responses and prevent immune organ atrophy in a mouse model of lethal septic shock [14]. Also in these animals, HDAC6 inhibition can increase the monocyte count, decrease the percentage of granulocytes, restore the lymphocyte population, and decrease the ratio of granulocyte-to-lymphocyte [31]. It is understandably easy to interpret that AGK2 could function in a similar fashion to HDAC6 inhibitors.

Evidence regarding the effects of SIRT2 on expansion of hematopoietic progenitor cells will require further exploration. Future directives may consider evaluating the attenuated bone marrow suppression following SIRT2 inhibition and determining if this is related to a latent stress response that could be elucidated *via* leukocyte composition in circulation.

The present study has some limitations that must be acknowledged. Whether the SIRT2 inhibition directly affects coagulation components *in vitro* requires further investigations. Furthermore, we only examined selected cytokines and limited pathways for logistical reasons. Without a doubt, more mechanisms and molecules are affected by the inhibition of SIRT2 and will provide substantial opportunities for new developments and experimentation to better understand the pathways at hand.

In summary, we have revealed that selective inhibition of SIRT2, a Class III histone deacetylase, can significantly improve survival, attenuate sepsis-associated cytokine production and coagulopathy, and reduce bone marrow atrophy in a lethal CLP model. Although the exact molecular and cellular mechanisms are unclear, SIRT2 may represent a potential therapeutic target to treat lethal sepsis in the future.

ABBREVIATIONS

ANOVA	=	One Way Analysis of Variance
CLP	=	Cecal Ligation and Puncture
DMSO	=	Dimethyl Sulfoxide
ELISA	=	Enzyme-Linked Immunosorbent Assay
FOXO1	=	Forkhead Box Protein O1
HDAC	=	Histone Deacetylase
HDACI	=	HDAC Inhibitors
HSP	=	Heat Shock Protein
H&E	=	Hematoxylin and Eosin
IL	=	Interleukin
LPS	=	Lipopolysaccharide
SAHA	=	Suberoylanilide Hydroxamic Acid
SIRT	=	Sirtuin
TEG	=	Thromboelastography
TNF	=	Tumor Necrosis Factor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 2007; 26: 5541-52.

[2]   Eskandarian HA, Impens F, Nahori MA, *et al.* A role for SIRT2-dependent histone H3K18 deacetylation in bacterial infection. Science 2013; 341: 1238858.

[3]   Seo KS, Park JH, Heo JY, *et al.* SIRT2 regulates tumour hypoxia response by promoting HIF-1alpha hydroxylation. Oncogene 2015; 34: 1354-62.

[4]   Serrano L, Martinez-Redondo P, Marazuela-Duque A, *et al.* The tumor suppressor SirT2 regulates cell cycle progression and genome stability by modulating the mitotic deposition of H4K20 methylation. Genes Dev 2013; 27: 639-53.

[5]   Lin J, Sun B, Jiang C, Hong H, Zheng Y. Sirt2 suppresses inflammatory responses in collagen-induced arthritis. Biochem Biophys Res Commun 2013; 441: 897-903.

[6]   Li W, Zhang B, Tang J, *et al.* Sirtuin 2, a mammalian homolog of yeast silent information regulator-2 longevity regulator, is an oligodendroglial protein that decelerates cell differentiation through deacetylating alpha-tubulin. J Neurosci 2007; 27: 2606-16.

[7]   North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol Cell 2003; 11: 437-44.

[8]   Vaquero A, Scher MB, Lee DH, *et al.* SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. Genes Dev 2006; 20: 1256-61.

[9]   Outeiro TF, Kontopoulos E, Altmann SM, *et al.* Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. Science 2007; 317: 516-9.

[10]   Jing E, Gesta S, Kahn CR. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. Cell Metab 2007; 6: 105-14.

[11]   Zhao T, Li Y, Liu B, Bronson RT, Halaweish I, Alam HB. Histone deacetylase III as a potential therapeutic target for the treatment of lethal sepsis. J Trauma Acute Care Surg 2014; 77: 913-9.

[12]   Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009; 4: 31-6.

[13]   Zhao T, Li Y, Liu B, *et al.* Histone deacetylase inhibitor treatment attenuates coagulation imbalance in a lethal murine model of sepsis. Surgery 2014; 156: 214-20.

[14]   Zhao T, Li Y, Bronson RT, Liu B, Velmahos GC, Alam HB. Selective histone deacetylase-6 inhibition attenuates stress responses and prevents immune organ atrophy in a lethal septic model. Surgery 2014; 156: 235-42.

[15]   Sinclair DA, Guarente L. Extrachromosomal rDNA circles--a cause of aging in yeast. Cell 1997; 91: 1033-42.

[16]   Burnett C, Valentini S, Cabreiro F, *et al.* Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and Drosophila. Nature 2011; 477: 482-5.

[17]   Viswanathan M, Guarente L. Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. Nature 2011; 477: E1-2.

[18]   Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol 2005; 6: 298-305.

[19]   Kumar R, Mohan N, Upadhyay AD, *et al.* Identification of serum sirtuins as novel noninvasive protein markers for frailty. Aging Cell 2014; 13: 975-80.

[20]   Calnan DR, Brunet A. The FoxO code. Oncogene 2008; 27: 2276-88.

[21]   Nie H, Hong Y, Lu X, *et al.* SIRT2 mediates oxidative stress-induced apoptosis of differentiated PC12 cells. Neuroreport 2014; [Epub ahead of print].

[22]   Xu X, Yin P, Wan C, *et al.* Punicalagin inhibits inflammation in LPS-induced RAW264.7 macrophages *via* the suppression of TLR4-mediated MAPKs and NF-kappaB activation. Inflammation 2014; 37: 956-65.

[23]   Li Y, Yuan Z, Liu B, *et al.* Prevention of hypoxia-induced neuronal apoptosis through histone deacetylase inhibition. J Trauma 2008; 64: 863-70; discussion 870-1.

[24]   Rothgiesser KM, Erener S, Waibel S, Luscher B, Hottiger MO. SIRT2 regulates NF-kappaB dependent gene expression through deacetylation of p65 Lys310. J Cell Sci 2010; 123: 4251-8.

[25]   Chong W, Li Y, Liu B, *et al.* Histone deacetylase inhibitor suberoylanilide hydroxamic acid attenuates Toll-like receptor 4 signaling in lipopolysaccharide-stimulated mouse macrophages. J Surg Res 2012; 178: 851-9.

[26]   Levi M, Schultz M, van der Poll T. Disseminated intravascular coagulation in infectious disease. Semin Thromb Hemost 2010; 36: 367-77.

[27]   Semeraro N, Ammollo CT, Semeraro F, Colucci M. Sepsis-associated disseminated intravascular coagulation and thromboembolic disease. Mediterr J Hematol Infect Dis 2010; 2: e2010024.

[28]   Tobon GJ, Izquierdo JH, Canas CA. B lymphocytes: development, tolerance, and their role in autoimmunity-focus on systemic lupus erythematosus. Autoimmune Dis 2013; 2013: 827254.

[29]   Liu H, Zhou J, Cheng P, Ramachandran I, Nefedova Y, Gabrilovich DI. Regulation of dendritic cell differentiation in bone marrow during emergency myelopoiesis. J Immunol 2013; 191: 1916-26.

[30]   Ayala A, Herdon CD, Lehman DL, Ayala CA, Chaudry IH. Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators. Blood 1996; 87: 4261-75.

[31]   Zhao T, Li Y, Liu B, Halaweish I, Mazitschek R, Alam HB. Selective inhibition of histone deacetylase 6 alters the composition of circulating blood cells in a lethal septic model. J Surg Res 2014; 190: 647-54.