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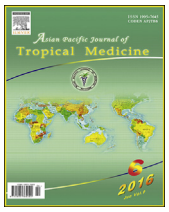

Original research http://dx.doi.org/10.1016/j.apjtm.2016.04.006

Protection mechanism of deacetylase inhibitor on spleen of rats with severe hemorrhagic shock

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ARTICLE INFO

Article history:
Received 15 Feb 2016
Received in revised form 16 Mar 2016
Available online 15 Apr 2016

Keywords:
Hemorrhagic shock
HDACIs
Apoptosis
Histone acetylation

ABSTRACT

Objective: To explore the protection and molecular mechanism of histone deacetylase inhibitors (HDACIs) on the spleen of rats with hemorrhagic shock.

Methods: A total of 60 SPF male SD rats were selected for the modeling of severe hemorrhagic shock using the method of arterial and venous cannulation with the time-divided bleeding. The measurement of mean arterial blood pressure and blood lactic acid was used to verify the modeling. The modeled rats were randomly divided into shock group, shock + suberoylanilide hydroxamic acid (SAHA) group, shock + autogenous transfusion group and shock + SAHA + autogenous transfusion group. Three hours after the treatment, the spleen of rats was collected and TUNEL method was employed to detect the apoptosis of spleen cells in each group. The statistical analysis was performed. Afterwards, real-time PCR and western blot were employed to detect the expression of BCL-2, BAX and caspass3 in the spleen of rats in each group.

Results: A total of 53 rats had successful modeling of severe hemorrhagic shock, with success rate of 88%. Cell apoptosis in the severe hemorrhagic model group was the most serious. After the intervention of HDACIs and the autogenous transfusion, the tissue injury was a bit recovered. Cell apoptosis was least in the shock + SAHA + autogenous transfusion group ($P < 0.05$). After the intervention of HDACIs and the autogenous transfusion, the relative expression of BCL-2 was significantly increased ($P < 0.05$), with highest relative expression of BCL-2 in shock + SAHA + autogenous transfusion group ($P < 0.05$). After the intervention of HDACIs and the autogenous transfusion, the relative expression of BAX was significantly decreased ($P < 0.05$), with lowest relative expression of BAX in the intervention group of single HDACIs. The change in the expression of caspass3 was similar to BAX, namely the relative expression of caspass3 was significantly decreased after the intervention of HDACIs and the autogenous transfusion ($P < 0.05$).

Conclusions: HDACIs and autogenous transfusion can all protect the spleen injury because of the severe hemorrhagic shock. Its molecular mechanism may be related to the regulation on the expression of BCL-2/BAX and caspass3, which may affect the apoptosis process of cells.

1. Introduction

The histone deacetylase inhibitor (HDACI) is a family of compounds, including butyrate, phenyl butyrate, hydroxamic acid TSA, valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA). With the function of intervention and histone deacetylase, HDACIs could increase the degree of acetylation in the histone of cells to increase the expression of genes such as *p21*, inhibit the proliferation of tumor cells and induce the cellular differentiation and apoptosis [1,2]. Thus HDACIs were regarded as a type of new cancer therapeutic drug. Hidehiko K *et al.* reported that several HDACIs had the Phases I, II and III clinical trials [3]. These clinical trials mainly included the hematological malignancies such as the chronic myeloid leukemia. Meanwhile, the previous studies also reported that

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Peer review under responsibility of Hainan Medical College.
Foundation project: It was supported by the National Natural Science Foundation of China (No. 81160230); the Natural Science Foundation of Jiangxi Province of China (No. 20114BAB205003).

HDACIs had the potential therapeutic effect in the inflammatory, immunologic, traumatic, neurological and cardiac diseases [4–6].

The researches also found that the hemorrhagic shock could induce the ratio imbalance of histone acetyltransferase/histone deacetylase and thus affect the acetylation of histone or non-histone. HDACIs could correct such imbalance to improve the function of tissues and organs, namely protecting the tissues and organs [7,8]. Gonzales ER *et al.* adopted VPA and 2-methyl-2-pentenoic acid (2M2P) in the rat model of hemorrhagic shock. Compared with the control group after 12 h, 70% of rats in VPA treatment group and 12% of rats in 2M2P treatment group survived, while no rat survived in the control group [9]. Tom Lin *et al.* compared the effect of three HDACIs (VPA, trichostatin A and SAHA) in the treatment of SD rats with hemorrhagic shock. The lung cells of rats in the control group had no histone acetylation, while rats in the resuscitation group showed the state of over-acetylation. These results indicated that the mechanism of HDACIs on the protection of rat model of hemorrhagic shock was extremely complicated [10]. With the increase in the risk factors of traffic accidents and natural disasters, the incidence of hemorrhagic shock is also increased. Accordingly, the study on the mechanism of HDACIs in the hemorrhagic shock will be of critical significance.

In this study, the method of arterial and venous cannulation with the time-divided bleeding was employed to build the model of severe hemorrhagic shock. The modeled rats were randomly divided into four groups, namely the shock group, shock + SAHA group, shock + autogenous transfusion group and shock + SAHA + autogenous transfusion group. The spleen of rats was separated and the apoptosis of spleen cells was detected in each group. The statistical analysis was performed. Afterwards, real-time PCR and western blot were employed to detect the expression of *BCL-2*, *BAX* and *caspass3* in the spleen of rats in each group, in order to specify the mechanism of HDACIs in the hemorrhagic shock and the synergistic effect of emergency usage and transfusion.

2. Materials and methods

2.1. Materials

2.1.1. Laboratory animals

A total of 60 specific pathogen free (SPF) male SD rats, with the weight of (350 ± 50) g, were purchased from Department of Laboratory Animal Science, Nanchang University. They were given the standard pellet diet in the standard animal cage, with 5 rats in each cage. Rats were given the diet and drinking freely during the experiment, with the good ventilation in the feeding room and natural lighting day and night. The room temperature was maintained at (18–25) °C. The design scheme, operation procedure and animal ethics of this study had been reviewed and approved by Medical College of Nanchang University.

2.1.2. Reagents and instruments

The lactate assay kit was purchased from Nanjing Jiancheng Bioengineering Institute; SAHA from Selleck (America); Tunel assay kit from Vazyma (America); RNA extraction kit from Ambion (America); reverse transcription kit from Promega (America); GoTaq® qPCR Master Mix from Promega (America); ReadyPrep protein extraction kit from Bio-Rad (America); BCA protein assay kit from Vazyme Biotech (China); BCL-2, BAX, caspass3 monoclonal antibody and horseradish peroxidase (HRP) labeled secondary antibodies from Beijing Zhongshan Jinqiao Biotechnology; PVDF membrane (0.22 μm) from Millipore (America); ECL chemiluminescence assay kit from Millipore (America).

The micro-injection pump was purchased from B. Braun Melsungen (Germany); the biological and functional experimental system from Chengdu Techman Software Co., Ltd.; the quantitative DNA analyzer was Qubit Fluorometer; UV2450 UV spectrophotometer from Shimadzu (Japan); the fluorescence quantitative PCR was Applied Biosystems 7500.

2.2. Methods

2.2.1. Modeling and grouping [11]

The method of arterial and venous cannulation with the time-divided bleeding was employed to build the model of severe hemorrhagic shock. SPF SD rats had 7 d of adaptive feeding, with the fasting 6 h before the experiment. Rats were given the intraperitoneal injection of 2% pentobarbital under the sterile condition for the anesthesia (30 mg/kg). The skin on the right groin and back was prepared for the disinfection. An oblique incision was made in the lower abdomen and then the microscopy technique was performed to separate the artery and vein. PE50 catheter was placed and fixed in the femoral artery and vein. The method of arterial and venous cannulation with the time-divided bleeding was employed to build the rat model of severe shock that the total amount of blood loss occupied 60% of the total blood volume. 50 000 IU penicillin was injected to prevent from the infection. The mean arterial pressure was maintained at (45 ± 5) mmHg. The measurement of blood lactic acid was employed to verify the model.

After the verification, the modeled rats were randomly divided into four groups, namely the shock group, shock + SAHA group, shock + autogenous transfusion (5 mL, heparin anticoagulation, and half an hour of transfusion) and shock + SAHA + autogenous transfusion. SAHA was diluted into 1 mL (0.1% DMSO as the solvent) according to the dose of 8 mg/kg and it was pumped in the vein for 10 min through the micro-injection pump. After 3 h, the spleen tissue of rats was separated and TUNEL test was performed. The remained tissues were stored in the liquid nitrogen quickly.

2.2.2. TUNEL to detect the cell apoptosis

The spleen tissue was fixed with 4% formaldehyde. The tissue sections were placed in the staining jar to be washed with dimethylbenzene twice, with 5 min each time. Afterwards, they were washed with anhydrous ethanol twice, with 3 min each time. Then they were washed with 95% and 75% ethanol respectively, with 3 min each time. After being reacted in 20 μg/mL DNase-free proteinase K at (20–37) °C for 15 min, they were washed with PBS for 3 times. The preparation of TUNEL solution and the staining were performed according to the instruction manual of assay kit. The counting was done under the high power field and the fluorescence analysis software was employed to analyze the experimental data. Image-Pro Plus 6.0 was used to count the positive cells. The specific steps were: open 200× field photo-measure-count/size-image-flatten background-20 px, balance the background of photo by 20 px and then count automatically.

2.2.3. Real-time PCR

The spleen tissue was taken out from the liquid nitrogen and then the total RNA was extracted (according to the instruction manual of RNA extraction kit). Qubit Fluorometer was employed to detect the concentration and purity of RNA. The total RNA was transcripted reversely into cDNA according to the instruction manual of reverse transcription kit. Real-time PCR was employed to detect the related genes. The mRNA sequence of *Bcl-2* gene could be referred to NCBI database and then the real-time PCR primers could be designed. All primers were synthesized by Shanghai Sangon Biotech, with the specific sequence shown as follows: Bcl-2 (NM_000633.2). For: GGTGAACTGGGGGAGGATTG; Rev: GCATGCTGGGGCCATATAGT; BAX (NM_017059); For: GGATGGCCTCCTTTCCTACTTC; Rev: ACCTGAGGTTTATTGGCACCT; CASP3 (NM_012922); For: AAGTGACCATGGACAACAACG; Rev: GCCTCCACTGGTATCTTCTGG; ACTB (NM_031144); For: ACCCGCGAGTACAACCTTCTT; Rev: TCATCTTTTCACGGTTGGCCT.

By measuring the Ct value of each gene amplification, Ct value was negatively related to the starting copy number of DNA. The double ΔCt method was adopted to calculate the relative expression of target gene: the mean of three parallel repeated experiments was treated as the CT value of each sample, ΔCT = CT (target gene) − CT (reference), ΔΔCT = ΔCT (sample) − ΔCT (control). Therefore, the relative expression of target gene = 2 − ΔΔCT and the relative expression of control group was 2^0 = 1 [12]. The reverse transcription system was (20 μL): 5 × GoTaq® qPCR Master Mix 4 μL; 1 μL reverse transcriptase; 1 μg RNA template (1 μg RNA). The reaction conditions included: Initial denaturation at 95 °C for 10 min; denaturation at 95 °C for 10 s; the annealing temperature was set according to Tm value of primers for 20 s, with the extension at 72 °C for 33 s and 40 cycles.

2.2.4. Western blot

The spleen tissue was ground to be powder in the liquid nitrogen. ReadyPrep protein extraction kit was used to extract the histone. After being put on the ice, the probe-type ultrasound was used to produce the short impact with the appropriate frequency on the ice. The lysis mixture was centrifuged at 4 °C and 13000 r/min for 20 min. The supernatant was transferred to the new centrifuge tube. BCA kit was employed to detect the protein concentration.

SDS-PAGE electrophoresis was performed on the protein samples. The gel was soaked in the transfer buffer for 10 min of equilibrium. It was installed with the transfer 'sandwich', 100 V and (45–60) min. After the transfer, PVDF membrane was washed with TBS for (10–15) min. The primary antibody with the appropriate degree of dilution was added (diluted with TBST containing 1% (w/v) skimmed milk powder). It was incubated at the room temperature for 2 h and then the membrane was washed with TBST for 3 times, with (5–10) min each time. The membrane was incubated with the secondary antibody (1:10000, HRP-labeled) that was diluted with TBST containing 0.05% (w/v) skimmed milk powder. It was washed with TBST for 3 times, with (5–10) min each time. It was exposed and then photographed to save the experimental results. Quantity one v4.62 was used to measure the gray value of molecular band (trace tracking). The semi-quantitative value of target protein/reference protein was treated as the quantitative foundation. The statistical analysis was performed as well.

2.2.5. Statistical analysis

The experimental data was treated with SPSS17.0. The results were expressed by $\bar{x} \pm SD$. The *t* test was employed for the comparison between groups and the analysis of variance for the comparison among groups. Difference is considered significant at $P < 0.05$.

3. Results

3.1. Modeling of rat with severe hemorrhagic shock

The blood loss volume of rat (mL) = 0.06 × weight (g) + 0.77. During the modeling, 5 rats died because of the excessive loss of blood. The mean arterial blood pressure and blood lactic acid were monitored to verify the model. The results indicated that the mean weight of rats after the modeling was (325.6 ± 17.6) g, which had no significant difference from that of (328.6 ± 20.6) before the modeling ($P > 0.05$). Arterial pressure (mmHg) of rats after the modeling was (48.2 ± 3.3), which was significantly decreased than that of (112.68 ± 2.85) mmHg before the modeling ($P < 0.01$). The content of blood lactic acid was (7.38 ± 0.67) mM before the modeling; while it was (13.44 ± 1.04) mM after the modeling, which was increased by 82% than that in the control group ($P < 0.05$). Therefore, 55 rats had the successful modeling of severe hemorrhagic shock, with the success rate of 92%.

3.2. Comparison of spleen cell apoptosis of rats between groups

The spleen tissue of each group was stained according to the instruction manual of assay kit. Then the fluorescence detection was performed and Image-Pro Plus 6.0 was employed to count the positive cells. According to the results of statistics, the number of positive cells in the control (shock) group, shock + SAHA group, shock + BLOOD transfusion group and shock + SAHA + BLOOD transfusion group was 70.75, 43.83, 40.17 and 24.25, respectively. It could be indicated that the cell apoptosis was most serious in the hemorrhagic model. After the intervention of HDACIs and the autogenous transfusion, the tissue injury was a bit recovered. Where, the cell apoptosis was least in the shock + SAHA + autogenous transfusion group ($P < 0.05$).

3.3. Study on difference in expression of apoptosis-related genes in spleen tissue of rats in each group

Real-time PCR and western blot were employed to detect the expression of *BCL-2*, *BAX* and *caspass3* in the spleen of rats at mRNA and protein levels in each group, with results shown in Table 1. Taking the expression in the control group as 1,

Table 1
Relative expression of real-time PCR.

	Control	Shock + SAHA	Blood trans	Blood trans + SAHA
Bcl-2	1	1.83 ± 0.52*	1.62 ± 0.38*	2.11 ± 2.16**
BAX	1	0.26 ± 0.96*	0.63 ± 0.58**	0.71 ± 0.36*
caspass3	1	0.74 ± 0.48*	0.81 ± 0.55*	0.75 ± 0.41*

*vs Control, $P < 0.05$; **vs Shock + Blood trans, $P < 0.05$.

compared with the control group, after the intervention of HDACIs and the autogenous transfusion, the relative expression of *BCL-2* was significantly increased ($P < 0.05$), where the relative expression of *BCL-2* was highest in the shock + SAHA + autogenous transfusion group ($P < 0.05$). According to the further analysis on the change in the expression of *BAX* gene, it could be indicated that after the intervention of HDACIs and the autogenous transfusion, the relative expression of *BAX* was significantly decreased ($P < 0.05$), where the relative expression of *BAX* was increased in the shock + autogenous transfusion and shock + SAHA + autogenous transfusion group was increased compared with that in the intervention group of single HDACIs ($P < 0.05$). The change in the expression of *caspass3* was similar to *BAX*, namely the relative expression of *caspass3* was significantly decreased after the intervention of HDACIs and the autogenous transfusion ($P < 0.05$) (see Table 2).

Table 2
Results of western blot (gene/β-actin).

	Control	Shock + SAHA	Shock + Blood trans	Shock + Blood trans + SAHA
Bcl-2	0.83 ± 0.21	1.33 ± 0.50*	1.22 ± 0.68*	2.21 ± 1.67**
BAX	1.33 ± 0.52	0.66 ± 0.56*	0.68 ± 0.48*	0.69 ± 0.46*
caspass3	1.19 ± 0.47	0.75 ± 0.29*	0.76 ± 0.55*	0.55 ± 0.51*

*vs Control, $P < 0.05$; **vs Shock + Blood trans, $P < 0.05$.

According to the results of analysis at the protein level, the intervention of HDACIs and the autogenous transfusion could all increase the expression of *BCL-2*, where the relative expression of *BCL-2* gene was highest in the SAHA + autogenous transfusion group ($P < 0.05$). After the intervention of HDACIs and the autogenous transfusion, the relative expression of *BAX* was significantly decreased ($P < 0.05$), but there was no significant difference between shock + SAHA group, shock + autogenous transfusion group and shock + SAHA + autogenous transfusion group. The change in the expression of *caspass3* was similar to *BAX*, namely the relative expression of *caspass3* was significantly decreased after the intervention of HDACIs and the autogenous transfusion ($P < 0.05$). As a whole, the change in the expression of *BCL-2*, *BAX* and *caspass3* was similar at mRNA and protein levels.

4. Discussion

The hemorrhagic shock could induce the ratio imbalance of histone acetyltransferase/histone deacetylase and thus affect the acetylation of histone or non-histone. HDACIs could correct such imbalance to improve the function of tissues and organs, namely protecting the tissues and organs. According to the previous literature, the treatment of HDACIs could increase the survival rate of animals with hemorrhagic shock, namely inhibiting the cell apoptosis and protecting the survival and function of cells. But the mechanism of apoptosis inhibition was different. In the process of studying the molecular mechanism of HDACIs, VPA could promote the histone acetylation and thus increase the survival rate of rats with life-threatening bleeding [13,14]. VPA could increase the expression of *BCL-2* gene and protein through β-catenin pathway to protect the neurons. Nikolaos Zacharias *et al.* proposed that such mechanism was related to phosphoinositol3-kinase and the reverse serine threonine kinase (AKT); then Bcl-2 was used as the anti-apoptosis factor to increase the survival rate of animals with hemorrhagic shock [15–17]. Accordingly, HDACIs could activate the AKT pathway to induce the apoptosis inhibition; meanwhile HDACIs could increase the protein acetylation, reduce BAD of cells, increase the expression of *Bcl-2* and inhibit the cell apoptosis, which would greatly improve the survival and function of renal cells of rats with hemorrhagic shock [18–20].

The inhibition against the cell apoptosis is the important biological effect of HDACIs. But its molecular mechanism on the cells is not only to inhibit the cell apoptosis, but also stress, regulate, promote or inhibit the transcription and expression of cytokines. According to the published literature, there had been no treatment protocol of transfusion resuscitation in HDACIs treatment of animals with hemorrhagic shock. But in this study, it's regarded that the transfusion resuscitation was the most important method to correct the hemorrhagic shock and its resuscitation effect was irreplaceable.

To study the protection mechanism of HDACIs and specify the correlation between HDACIs and transfusion, in this study, the time-divided bleeding method was employed to build the rat model of hemorrhagic shock and the modeled animals were divided into four groups, namely the shock group, shock + SAHA group, shock + autogenous transfusion group and shock + SAHA + autogenous transfusion group. At first, it was to study the relationship between the protection and apoptosis of spleen cells of rats with hemorrhagic shock after the treatment of autogenous transfusion and SAHA. According to the results, it could be indicated that the cell apoptosis was most serious in the hemorrhagic model group (namely the control group). After the intervention of HDACIs and the autogenous transfusion, the tissue injury was a bit recovered. Where, the cell apoptosis was least in the shock + SAHA + autogenous transfusion group ($P < 0.05$). It showed that SAHA and autogenous transfusion could all protect the injured tissues, which was in accordance with the previous findings. Gonzales ER *et al.* used VPA and 2M2P to treat the rats with hemorrhagic shock. After 12 h, compared with the control group, 70% of rats in VPA group and 12% of rats in 2M2P group were survived, but no rat survived in the control group. It indicated that the acetylation of histones H2A, H3, and H4 was related to the survival. The cytotoxicity factors showed that VPA could significantly improve the hepatocellular function of rats with hemorrhagic shock [9,21,22]. In this study, it's regarded that the significance of SAHA in the treatment of hemorrhagic shock was the survival of animals with shock and the protection of organs (liver, spleen, lung, kidney, heart and brain); while the anti-apoptosis was one of important mechanisms. Though many researches had reported such perspective, the previous literature did not discuss its protection of organs with the autogenous transfusion. In this study, it could be observed that the single autogenous transfusion or SAHA intervention treatment could lead to the serious apoptosis, but the apoptosis of spleen tissues was improved in SAHA + transfusion group, which could provide the significant theoretical foundation for the clinical treatment of hemorrhagic shock.

Tom Lin *et al.* compared the effect of VPA, trichostatin A and SAHA as the resuscitation drugs in the treatment of SD rats with hemorrhagic shock. The cDNA biological chip detected that 57 genes had the abnormal expression, where the expression of 44 genes was increased and the expression of 13 genes was decreased. These genes with the abnormal expression involved

the functions of metabolism, growth, proliferation, differentiation, transformation and signaling of cells, which indicated that the protection mechanism of HDACI on rats with hemorrhagic shock was extremely complicated [10,23,24]. The increased expression of apoptosis inhibition protein BCL-2 in the acetylation could reduce the apoptosis, while the acetylation could reduce the expression of pro-apoptosis protein BAX and caspass 3, but the autogenous transfusion had the positive effect on the expression of BAX and caspass 3 [25–27]. According to the results of this study at mRNA and protein levels, after the intervention of HDACIs and the autogenous transfusion, the relative expression of BCL-2 was significantly increased ($P < 0.05$), where the relative expression of BCL-2 was highest in the shock + SAHA + autogenous transfusion group ($P < 0.05$). After the intervention of HDACIs and the autogenous transfusion, the relative expression of BAX was significantly decreased ($P < 0.05$), where the relative expression of BAX was lowest in the intervention group of single HDACIs. The change in the expression of caspass3 was similar to BAX, namely the relative expression of caspass3 was significantly decreased after the intervention of HDACIs and the autogenous transfusion ($P < 0.05$). It indicated that the tissues with ischemia reperfusion during the transfusion might be the potential factor to cause the cell apoptosis. As the spleen tissue is of critical significance for the recovery of immunologic function, the protection of spleen function will be necessary for the prevention and treatment of hemorrhagic shock. In conclusion, it can be regarded that SAHA can protect the spleen tissue of rats with severe hemorrhagic shock, which mainly induce the increased expression of BCL-2 and the decreased expression of pro-apoptosis protein BAX and caspass 3. The SAHA with autogenous transfusion can jointly protect the spleen tissue.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Mai A, Rotili D, Tarantino D, Nebbioso A, Castellano S, Sbardella G, et al. Identification of 4-hydroxyquinolines inhibitors of p300/CBP histone acetyltransferases. *Bioorg Med Chem Lett* 2009; **19**(4): 1132-1135.

[2] Zhang H, Dai X, Qi Y, He Y, Du W, Pang JJ. Histone deacetylases inhibitors in the treatment of retinal degenerative diseases: overview and perspectives. *J Ophthalmol* 2015; **2015**: 250812.

[3] Hidehiko K, Tatsuo N. GCN5 and BCR signaling collaborate to induce pre-mature B cell apoptosis through depletion of ICAD and IAP2 and activation of caspase activities. *Gene* 2008; **419**(1–2): 48-55.

[4] Zhang B, West EJ, Van KC, Gurkoff GG, Zhou J, Zhang XM, et al. HDAC inhibitor increases histone H3 acetylation and reduces microglia inflammatory response following traumatic brain injury in rats. *Brain Res* 2008; **1226**: 181-197.

[5] Degano AL, Park MJ, Penati J, Li Q, Ronnett GV. MeCP2 is required for activity-dependent refinement of olfactory circuits. *Mol Cell Neurosci* 2014; **59**: 63-75.

[6] Colussi C, Illi B, Rosati J, Spallotta F, Farsetti A, Grasselli A, et al. Histone deacetylase inhibitors: keeping momentum for neuromuscular and cardiovascular diseases treatment. *Pharmacol Res* 2010; **62**: 3-10.

[7] Sailhamer EA, Li Y, Smith EJ, Shuja F, Shults C, Liu B, et al. Acetylation: a novel method for modulation of the immune response following trauma/hemorrhage and inflammatory second hit in animals and humans. *Surgery* 2008; **144**(2): 204-216.

[8] Shults C, Sailhamer EA, Li Y, Liu B, Tabbara M, Butt MU, et al. Surviving blood loss without fluid resuscitation. *J Trauma* 2008; **64**(3): 629-638.

[9] Gonzales ER, Chen H, Munuve RM, Mehrani T, Nadel A, Koustova E. Hepatoprotection and lethality rescue by the histone deacetylase inhibitor valproic acid. *J Trauma* 2008; **65**(3): 554-565.

[10] Halaweish I, Nikolian V, Georgoff P, Li Y, Alam HB. Creating a 'prosurvival phenotype' through histone deacetylase inhibition: past, present, and future. *Shock* 2015; **44**(Suppl. 1): 6-16.

[11] Deng GR, Wu BH, Gao X, Dong YY, Miao X, Huang W, et al. Effect of histone deacetylase inhibitor on the survival of rats with severe hemorrhagic shock. *Shandong Med J* 2015; **55**(29): 26-28.

[12] Mortarino M, Franceschi A, Mancianti F, Bazzocchi C, Genchi C, Bandi C. Quantitative PCR in the diagnosis of *Leishmania*. *Parassitologia* 2004; **46**(1–2): 163-167.

[13] Lin T, Chen H, Koustova E, Sailhamer EA, Li Y, Shults C, et al. Histone deacetylaseas therapeutic targetin a rodent model of hemorrhagic shock: effect of different resuscitation strategies on lung and liver. *Surgery* 2007; **141**(6): 784-794.

[14] Cheng X, Liu Z, Liu B, Zhao T, Li Y, Alam HB. Selective histone deacetylase 6 inhibition prolongs survival in a lethal two-hit model. *J Surg Res* 2015; **197**(1): 39-44.

[15] Zacharias N, Sailhamer EA, Li Y, Liu B, Butt MU, Shuja F, et al. Histone deacetylase inhibitors prevent apoptosis following lethal hemorrhagic shock in rodent kidney cells. *Resuscitation* 2010; **82**(1): 105-109.

[16] Reddy MA, Natarajan R. Recent developments in epigenetics of acute and chronic kidney diseases. *Kidney Int* 2015; **88**(2): 250-261.

[17] Speir RW, Stallings JD, Andrews JM, Gelnett MS, Brand TC, Salgar SK. Effects of valproic acid and dexamethasone administration on early bio-markers and gene expression profile in acute kidney ischemia-reperfusion injury in the rat. *PLoS One* 2015; **10**(5): e0126622.

[18] Matthews GM, Mehdipour P, Cluse LA, Falkenberg KJ, Wang E, Roth M, et al. Functional-genetic dissection of HDAC dependencies in mouse lymphoid and myeloid malignancies. *Blood* 2015; **126**(21): 2392-2403.

[19] McDermott J, Jimeno A. Belinostat for the treatment of peripheral T-cell lymphomas. *Drugs Today (Barc)* 2014; **50**(5): 337-345.

[20] Foss F, Advani R, Duvic M, Hymes KB, Intragumtornchai T, Lekhakula A, et al. A Phase II trial of Belinostat (PXD101) in patients with relapsed or refractory peripheral or cutaneous T-cell lymphoma. *Br J Haematol* 2015; **168**(6): 811-819.

[21] Guo W, Tsujimura K, Otsuka IM, Irie K, Igarashi K, Nakashima K, et al. VPA alleviates neurological deficits and restores gene expression in a mouse model of Rett syndrome. *PLoS One* 2014; **9**(6): e100215.

[22] Zhang X, Zhang X, Huang T, Geng J, Liu M, Zheng J. Combination of metformin and valproic acid synergistically induces cell cycle arrest and apoptosis in clear cell renal cell carcinoma. *Int J Clin Exp Pathol* 2015; **8**(3): 2823-2828.

[23] Imesch P, Dedes KJ, Furlato M, Fink D, Fedier A. MLH1 protects from resistance acquisition by the histone deacetylase inhibitor trichostatin A in colon tumor cells. *Int J Oncol* 2009; **35**(3): 631-640.

[24] Samuni Y, Flores-Santana W, Krishna MC, Mitchell JB, Wink DA. The inhibitors of histone deacetylase suberoylanilide hydroxamate and trichostatin: a release nitric oxide upon oxidation. *Free Radic Biol Med* 2009; **47**(4): 419-423.

[25] Pang WJ, Xiong Y, Wang Y, Tong Q, Yang GS. Sirt1 attenuates camptothecin-induced apoptosis through caspase-3 pathway in porcine preadipocytes. *Exp Cell Res* 2013; **319**(5): 670-683.

[26] Wu J, Zhu Y, Xu C, Xu H, Zhou X, Yang J, et al. Adenovirus-mediated p53 and *ING4* gene co-transfer elicits synergistic antitumor effects through enhancement of p53 acetylation in breast cancer. *Oncol Rep* 2016; **35**(1): 243-252.

[27] Duan WX, He MD, Mao L, Qian FH, Li YM, Pi HF, et al. NiO nanoparticles induce apoptosis through repressing SIRT1 in human bronchial epithelial cells. *Toxicol Appl Pharmacol* 2015; **286**(2): 80-91.