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Defying Death

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In this issue of Critical Care Medicine, Li et al.¹ have provided a combination of in vivo and in vitro data to show that suppression of Histone Deacetylase (HDAC) activity by a pan-HDAC inhibitor, valproic acid (VPA), and blockade of IKK/NF- κ B signaling during resuscitation, can ameliorate the microvascular endothelial pro-inflammatory responses in multiple organs. They subjected mice to pressure-controlled hemorrhagic shock (mean arterial pressure of 30 mm Hg $\times$ 90 minutes) and then resuscitated them either with albumin alone or albumin supplemented with VPA (300 μ g/g) or IKK inhibitor BAY11-7082 (400 μ g per mouse). They harvested lung, kidney and liver tissues 1 and 4 hours later for analyses. They performed additional mechanistic studies using human umbilical vein endothelial cells (HUVEC). Overall, their data demonstrated that inhibition of HDAC activity (and IKK/NF- κ B signaling) during resuscitation resulted in an attenuation of endothelial inflammatory response induced by hemorrhage/resuscitation.

Worldwide, injuries kill nearly 6 million people annually and cause harm to millions more, accounting for 10% of global mortality². Despite numerous advancements in trauma care, a recent multinational trial of more than 20,000 patients³ once again demonstrated that majority of deaths still occur within the first few hours after injuries, with hemorrhage being the number one cause of *preventable* deaths. Resuscitation fluids are routinely administered in an attempt to replace the lost intra-vascular volume, but they have no inherent pro-survival properties⁴. In severely bleeding patients, early aggressive crystalloid resuscitation has not been shown to provide any survival benefit⁵, and may even worsen the outcomes⁶. We also know that cellular damage suffered during shock is further exaggerated by inappropriate resuscitation⁴. Therefore, contemporary trauma care now favors “damage control resuscitation” that promotes minimal use of crystalloids, rapid hemorrhage control, and early administration of blood products⁷. However, use of blood products is not practical (need for blood banking, cross match etc.) in the pre-hospital setting where most of the deaths occur, and this is even more challenging in the battlefield environment. This has fueled an initiative to develop novel life-preserving strategies that could serve as a bridge to definitive care.

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Modulating the functions of pre-existing cytoprotective proteins using commonly available pharmacological methods is one such approach. Life threatening insults, including hemorrhage and sepsis, disturb the normal balance of acetylation in the cells resulting in the suppression of gene transcription and down-regulation of pro-survival mechanisms, which can be promptly reversed by the administration of an HDAC inhibitor, such as VPA⁸. Although nuclear histones have historically been considered the main target of HDAC, we now know that non-histone proteins involved in key cellular functions such as cell cycle, stress response, cytoskeleton dynamics, signaling, repair/healing/remodeling, communication, and proliferation are equally involved⁹. In fact, the data from numerous pre-clinical studies were compelling enough to justify an ongoing phase I trial of VPA in trauma patients (ClinicalTrials.gov identifier NCT01951560), and plans are underway to start a phase 2 trial funded by the US Department of Defense in the near future. VPA treatment appears to be equally beneficial when hemorrhage is complicated by concurrent traumatic brain injury, as it decreases the size of brain lesion and enhances the rate of neurological recovery¹⁰.

Despite these promising results, there are concerns about the high dose of VPA that is required to exert the HDAC inhibiting effect that potentially could have adverse effects. The authors used a dose of 300 mg/kg, which is 6-10 fold higher than what is typically used in humans for the treatment of seizures. Previous dose optimization studies in rodents indeed support this high dose. However, emerging data from larger animal models (swine) seem to suggest that a dose of only 150 mg/kg is adequate to induce acetylation and be cytoprotective¹¹. Unpublished data from the phase I clinical trial (currently enrolling subjects in the 150mg/kg cohort) also suggests that VPA in doses as low as 120mg/kg can clearly induce acetylation in humans. 18 different HDACs have been described in humans that differ markedly in their cellular location and organ distribution⁸. An exciting area of active investigation is the development and testing of the next generation of isoform-specific HDACI that are not only much more potent, but can also be chosen selectively based upon the disease process and the target organs.

These inter-species differences must be kept in mind while reading this current study and when designing future experiments. Some other limitations of this study include the use of a controlled model of hemorrhage, absence of concurrent injuries, lack of long-term monitoring and organ function. Despite these limitations, it is a well designed and carefully executed experiment that improves our understanding of how HDAC inhibitors alter key cellular processes to create an anti-inflammatory and pro-survival environment.

Keeping the injured alive is especially challenging in the austere pre-hospital setting/ battlefield environment where medical resources are limited. This study adds to the growing body of literature to show that pharmacological interventions targeting specific cellular mechanisms can be used to defy death. There are many features that make acetylation an attractive therapeutic target: it is *rapid*, completely *reversible*, and can be altered by drugs that are *already in clinical use*. Many studies, including this paper, have shown that treatment with HDAC inhibitors represents a novel and promising approach for not only treatment of lethal hemorrhage, but also sepsis, TBI, and polytrauma. Early administration of these cytoprotective agents can maintain life and thus serve as a bridge to definitive care

(e.g. control of the bleeding source). How these agents exert their beneficial effect in such diverse lethal insults is a complex question, but newer studies such as this work, are rapidly improving our understanding of the underlying mechanisms. The investigators in this paper focused on IKK/NF- κ B intracellular signaling pathway. NF- κ B is a well-described transcription factor that controls the expression of multiple pro-inflammatory mediators. It is activated early in hemorrhagic shock, which can lead to an exaggerated systemic inflammatory response, and subsequent immune mediated cellular damage. The fact that HDAC inhibition attenuated the inflammatory mediators within an hour in this study is in line with the emerging consensus that in rapidly lethal conditions, direct (and immediate) modulation of existing cellular proteins is the dominant mechanism as opposed to alteration of gene transcription and creation of new proteins (slow process).

With some additional research, including appropriately designed clinical trials the use of potent pharmacological agents (including but not limited to HDACI) is likely to become an integral component of trauma care in the future.

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