Rev Med (São Paulo). 2016 abr.-jun.;95(1):91-102.

doi: http://dx.doi.org/10.11606/issn.1679-9836.v.95i2p91-102

The viability of using epigenetic drugs as a treatment of patients in sepsis - a translational perspective*

A viabilidade da utilização de drogas epigenéticas como tratamento de pacientes com sepse – uma perspectiva translacional

Carolina Reis Bonizzio[1]*, Maria Clara Andrade da Luz[1]*, Thamara Rodrigues da Costa[1]*, Francisco Garcia Soriano[2]*

Bonizzio CR, Luz MCA, Costa TR, Soriano FG. The viability of using epigenetic drugs as a treatment of patients in sepsis - a translational perspective / A viabilidade da utilização de drogas epigenéticas como tratamento de pacientes com sepse – uma perspectiva translacional. Rev Med (São Paulo). 2016 abr.-jun.;95(2):91-102.

ABSTRACT: Sepsis is a serious and potentially lethal clinical condition characterized by dysregulated immune and systemic inflammatory responses (SIRS) to an infection. Although sepsis has a high mortality rate (reaching 25% in Europe and North America), the clinical interventions available are still limited. In the bottom of this exacerbation of the immune response, that evolves to immunosuppression and immune paralysis, lies epigenetic mechanisms. In sepsis, the balance between activated and repressed immune related genes is at lost, and to recover that epigenetic based drugs promises to be the future of sepsis treatment. Histone deacetylase inhibitors (HDAC's inhibitors) are drugs based in the epigenetic mechanism of acetylation and deacetylation of histones, and they have already been tested - phases three and four of clinical trials - as treatment for other diseases, such as multiple myeloma, and cutaneous t-cell lymphoma. Furthermore, experimental studies in sepsis models shows that HDAC's inhibitors are a promising suppressor of the exacerbated inflammatory response. Therefore, as the recent works shows, epigenetic drugs should be considered a viable sepsis therapy in the future. The focus of this review is to present the most recent scientific advances in the basic and clinical areas of epigenetic as a sepsis treatment, opening opportunities for the use of epigenetic in treating this condition.

Keywords: Sepsis; Epigenetic; Histone deacetylase inhibitor.

RESUMO: Sepse é uma condição clínica grave e potencialmente letal caracterizada por desreguladas respostas imunes e inflamatórias sistêmicas (SIRS) a uma infecção. Embora a sepse tenha uma alta taxa de mortalidade (atingindo 25% na Europa e América do Norte), as intervenções clínicas disponíveis ainda são limitadas. Por trás dessa exacerbação da resposta imunológica, que evolui para a imunossupressão e paralisia imune, residem mecanismos epigenéticos. Na sepse, o equilíbrio entre genes imunes ativados e reprimidos relacionados é perdido, e reaver drogas baseadas na epigenética promete ser o futuro do tratamento da sepse. Inibidores da histona desacetilase (inibidores de HDAC) são drogas baseadas no mecanismo epigenético de acetilação e desacetilação de histonas, e eles já têm sido testados - fases três e quatro de ensaios clínicos - como tratamento para outras doenças, tais como o mieloma múltiplo, e linfoma cutâneo de células T. Além disso, os estudos experimentais em modelos de sepse mostram que os inibidores de HDAC são promissores supressores da resposta inflamatória exacerbada. Portanto, como os trabalhos recentes mostraram, drogas epigenéticas poderiam ser consideradas uma viável terapia para a sepse no futuro. O foco desta revisão é apresentar os mais recentes avanços científicos nas áreas básicas e clínicas de epigenética como um tratamento da sepse, abrindo oportunidades para o uso da epigenética no tratamento desta condição.

Descritores: Sepse; Epigenética; Inibidor de histona desacetilase.

*Joint first authors.

Artigo desenvolvido na Disciplina Optativa "Abordagem Prática da Escrita Científica" sob coordenação da Revista de Medicina do DC-FMUSP.

1. Universidade de São Paulo, Faculdade de Medicina, São Paulo, Brazil. E-mails: carolbonizzio@hotmail.com, claraa.luz@gmail.com, thamara.rod100@gmail.com
2. Universidade de São Paulo, Faculdade de Medicina Professor Associado da Disciplina de Emergências Clínicas do Departamento de Clínica Médica, São Paulo, Brazil. E-mail: gsoriano@usp.br

Corresponding author: Thamara Rodrigues da Costa. University of São Paulo, São Paulo Medical School, Brazil. Av. Dr. Arnaldo, 455. Cerqueira César - São Paulo, SP, Brasil. CEP: 01246-903. E-mail: thamara.rod100@gmail.com

INTRODUCTION

Sepsis is a serious and potentially lethal clinical condition characterized by dysregulated immune and systemic inflammatory responses (SIRS) to an infection. There is an exacerbated and unbalanced production of mediators and inflammatory cells, leading the patient to a hemodynamic instable state, multiple organ dysfunction and death[1].

Although the huge effort by international societies (Society of Critical Care Medicine and the European Society of Intensive Care Medicine) in developing a coordinated bundle care to the treatment of sepsis, the mortality of this condition is still very high, reaching impressive 25% in Europe and North America. The treatment for sepsis is so far been based on antibiotic therapy, life support and monitoring. However, it's low efficiency justifies an incessant search for alternative therapies able to complement the current one[2].

Since in sepsis we found the rising expression of specific genes during the inflammatory and immune responses, epigenetic – which is a form of DNA expression modulation - came out as a potential treatment option according to the last ten years studies. Drugs based in epigenetic mechanisms, such as acetylation of histones, have already been tested as a possible treatment for other conditions. Studies based on sepsis physiopathology, and experimental studies shows a promising future for epigenetic as a treatment for sepsis[3]. Therefore, the focus of this review is to present the most recent scientific advances in the basic and clinical areas of epigenetic as a sepsis treatment.

Sepsis Physiopathology - Basic Background

Pathogenic microorganisms, when invading tissue or blood, forces the host must start an orderly and balanced inflammatory response against infection. Any microorganism can lead to sepsis in a health person or with susceptible clinical conditions (underlying diseases, degree of immunosuppression, basal pattern of inflammatory response).

The first step for an inflammatory response is the recognition of a molecular pattern present on the cell wall or membrane of the microorganism, or a toxin produced by it. In bacteria, the most common cause of sepsis, the surface molecules lipid A (gram negative) and teichoic acid (gram positive) are recognized by Toll-like receptors on antigen presenting cells. These cells become activated and produce cytokines that have distal and local effects. The distal effect refers to attract cells able to phagocyte microorganisms (macrophages), and specialized cells to coordinate and help fight infection (lymphocytes). The local effect of cytokines relates to the constant activation of inflammatory cells nearby, amplifying and perpetuating the inflammatory state until the situation is controlled. The major cytokines produced in this phase are TNF-alpha, interleukin-1 and PAF[4].

In sepsis, these cytokines can be produced in excess, what makes their systemic effects very detrimental frequently greater than the toxicity of the bacteria itself. Patients with sepsis may present hemodynamic instability and multiple organ failure due to the large vasodilator power and direct cellular toxicity of these cytokines. The presence of a distributive shock only increases perfusion damage to organs, especially the kidneys. Other organs also affected are lungs, liver, brain, and intestine due to presence of inflammatory infiltrate.

The clinical manifestations are nonspecific, which demand attention from professional: fever or hypothermia, tachycardia, tachypnea, leukocytosis or leukopenia, metabolic acidosis, organ failure, decreased saturation, confusion, among others[4].

Epigenetic - Definitions

Epigenetics can be defined as the "inheritance of variation (-genetic) above and beyond (epi-) changes in the DNA sequence"[5]. Cells can change genes availability for transcription using the epigenetic machinery, adapting phenotypes to different environments, without changing the DNA sequence. The changes in the gene availability are made through biochemical variations, such as DNA methylation, histone modification, and microRNA (miRNA).

These mechanisms can increase or decrease the gene products – mRNA transcription, and finally protein translation. DNA methylation is the result of the attachment of methyl groups to cytosine bases in the DNA sequence, made by enzymes called DNA methylases. It decreases gene transcription into mRNA, and can be transmitted through mitosis or meiosis. Histones are responsible for winding and coiling of DNA into nucleosomes and then chromatin. ꝛ Histones tails can suffer methylation, acetylation, ubiquitination or phosphorylation, which alter the way DNA will coil around the histone. In conclusion, the tightness which chromatin is condensed defines the availability of gene sequences. The acetylation and deacetylation of histones are the regulators of gene expression most used in pharmacology, these reactions are controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Inhibitors of HDACs, used in cancer treatments, has been promising for inflammatory diseases, such as sepsis. Finally, miRNA are small, noncoding RNAs that regulates different elements in transcription and translation.

Severe sepsis leads to an immune dysfunction. At first, there's an exacerbation of the immune response, which evolves to profound immunosuppression and immune paralysis. In the bottom of this condition lie epigenetics

mechanisms described previously. The gene expression in mild and severe inflammation is known to be a balance between activation of genes and repression of others, which leads to homeostasis. Sepsis physiopathology shows us that this balance between activated and repressed genes is at lost. A better understanding of epigenetics mechanisms leads to an important source of possible treatments for sepsis, therefore the importance of knowing them, and their applicability.

METHODS

Our main objective in this article was to demonstrate the most recent advances by the scientific community in reaching a new perspective of treatment in sepsis. To this end, we looked for experimental and observational studies in animals and in humans that evidenced epigenetic mechanisms in the pathophysiology of sepsis and possible drugs for therapy.

We selected articles from 2010 to 2016, using the keywords "Epigenetic Sepsis" in the databases PubMed, Web of Science and Clinical Trials. PubMed was the database where we have found the majority of the studies included in our revision (total of 13 articles), followed right after by Web of Science (total of 6 articles). No results were found in Clinical Trials. Therefore, our analysis of epigenetic drugs in humans could not be directly related to sepsis treatment, but it had to be adapted to an evaluation of their toxicity in others diseases.

The new keywords tested were "histone deacetylase inhibitor". Our choice was based on findings of Eleonora Ciarlo in the review "Epigenetics in sepsis: targeting histone deacetylases". The author concludes that inhibiting histones deacetylases is the main epigenetic mechanism being studied in sepsis so far. The keyword was tested in Clinical Trials and Web of Science databases, but not PubMed's because in this last one we already have found a lot of articles.

Only clinical trials on phase 3 or 4 were included in this review because it is when, after passing numerous toxicity tests, drugs become available for trading. It is our chance to demonstrate how reliable epigenetic drugs are becoming in medical treatment.

Other inclusion and exclusion criteria used are enumerated above (Image 1).

These criteria were part of our strategy for picking up the most suitable articles. Our selection process consisted in three steps: reading the studies' title, abstract and entire text. As demonstrated in the next flowcharts, we reached a total of 26 articles for this review.

Inclusion criteria

1. Articles which epigenetic and sepsis were the main focus.
2. Experimental which epigenetic drugs were analysed as a therapy for sepsis.
3. Publication between 2010 and 2016.
4. Clinical trials on phases 3 or 4 which analysed epigenetic drugs as a therapy for other diseases.
5. Articles in English.

Exclusion criteria

1. Articles that describe epigenetic mechanisms in sepsis that aren't accessible to epigenetic drugs.
2. Review articles.
3. Clinical trials that had no results publicated, except from those that were testing drugs of the experimental studies in sepsis selected in this review.
4. Studies that repeated information obtained in previous articles include.

Image 1. Inclusion and exclusion criteria

Flowcharts

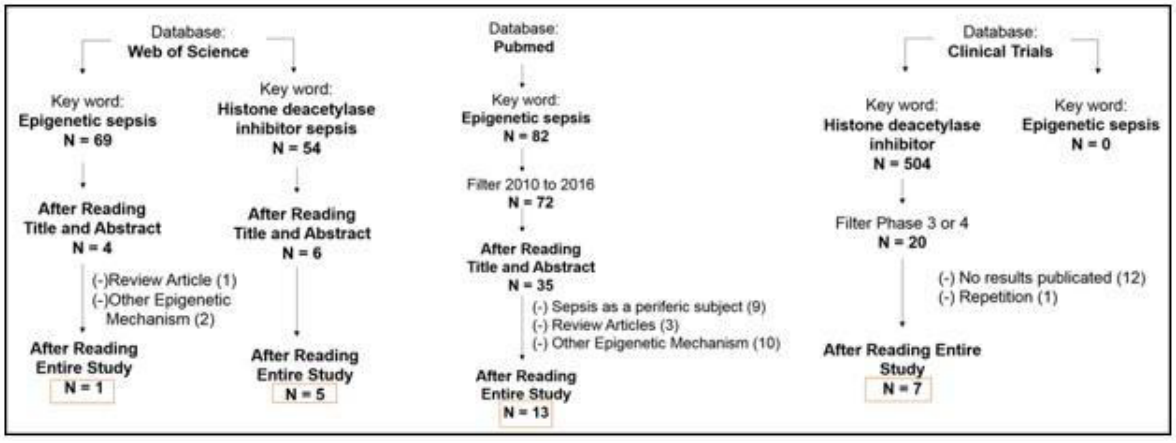

Image 2. Method's flowchart

RESULTS

From the twenty-six selected works in this systematic review, nineteen were observational and seven clinical trials. In observational studies, the most common sepsis model used was the CLP animal model, totalizing five articles, which used *in vivo* model only. This model consist in reproducing a systemic inflammatory process in mice by a proliferation stimulus of its own intestinal flora bacteria after a section in intestine cecal portion inside abdominal cavity. Four *in vivo* articles used other models, such as LPS-induced acute lung injury. Studies that used *in vitro* and *in vivo* models were the second most common, counting six articles. We found three studies which used *in vitro* model only. They reproduce a cellular inflammatory status by exposing them to a surface bacteria toxin, the LPS.

One observational cohort in humans was also selected, due its importance in the understanding of epigenetic in sepsis physiopathology in humans. Five studies were selected for a better understanding of epigenetic mechanisms in sepsis physiopathology as well, even though they didn't tested any drug. The drugs most tested in the observational studies were Trichostatin A (TSA) and Suberoylanilide hydroxamic acid (SAHA), also known as Vorinostat. They are both histone deacetylase inhibitors, and were found in four studies each. Although the majority of substances tested were HDAC inhibitors, other epigenetic mechanisms were also found, such as a DNA methylation inhibitor (Table 1).

As mentioned earlier, no sepsis study using epigenetic drugs was found in humans. Therefore, for better understanding the potential toxicity of these drugs, we selected twenty clinical trials using HDAC's inhibitors in the treatment of other diseases, mainly cancer (Table 2). Despite only four studies had published results, we also included clinical trials with no results in this review because most of them were testing drugs we found in experimental articles in sepsis.

Table 1. Epigenetic drugs mechanisms

Drugs	Mechanism	Observational Studies
Trichostatin A (TSA)	Histone deacetylase inhibitor	4
Suberoylanilide hydroxamic acid (SAHA) or Vorinostat	Histone deacetylase inhibitor	4
Sodium butyrate (SB)	Histone deacetylase inhibitor (class I)	3
Tubastatin A	Selective histone deacetylase 6 inhibitor	2
Inhibitor 5-Aza 2-deoxycytidine (Aza)	DNA methylation inhibitor	1
CG200745	Histone deacetylase inhibitor	1
Valproic Acid (Valproate)	Histone deacetylase inhibitor	1
LNAME	Inhibition of nitric oxide activity	1
Compound 9a	Histone deacetylase inhibitor	1
Curcumin	Inhibition of TREM-1	1
Protein C	Histone acetylation/ deacetylation regulation	1

Table 2. Epigenetic drugs and their application

Drugs	Diseases	Clinical Trials (N = 20)
Vorinostat	Multiple Myeloma	2
	Myelodysplastic syndrome	1*
	Cutaneous T-Cell Lymphoma	2
	Non-Small Cell Lung Cancer	1*
	Pruritus	1
	Mesotelioma	1
Panobinostat	Chronic Myeloid Leukemia in Blast Crisis	1
	Chronic Myeloid Leukemia in Chronic Phase	1
	Cutaneous T-Cell Lymphoma	2
	Myelodysplastic syndrome	1
	Multiple Myeloma	2*
	Hodgkin's Lymphoma	1
Valproate	Atrofia muscular espinal	1**
Theophylline	Bronchiectasis	2**
Sodium butyrate	Schizophrenia	1**

*Results available.
** Study included despite not having results available.

DISCUSSION

The epigenetic research for use in many therapeutic areas has proved increasingly promising nowadays. However, basic research in the area are still scarce, compared to its potential research, especially considering major syndromes, such as sepsis. The objective of this work is to look in the anthology scientific works that, through epigenetic mechanisms, enable the search for drug treatments that can treat sepsis, through attenuation of inflammatory process or other physiological characteristics related to pathophysiological complex. This analysis was based on other underlying diseases with inflammatory mechanisms, such as cancer and lung diseases, in order to correlate the usual mechanisms to sepsis and prevalent diseases in the population.

Observational and Experimental Studies

The experimental studies that aims to describe the role of epigenetic mechanisms in sepsis physiopathology, shows how this mechanism can produce a certain phenotype which leads to sepsis. Although the authors don't test any substance, they suggest the possibility of using it for epigenetic modification. Comparing monocytes from healthy donors to septic patients was showed histones modifications in many promoters' genes regions. As for instance, MHC class II locus and its corresponding master regulator CIITA, which can be associated to immune innate suppression[11]. Literature reported epigenetic modifications in macrophages, in the experimental model called "trained immunity". Mice injected with live C. Albicans and primary human monocytes, showed induced gene expression through histone modifications, involved in glucose metabolism. These changes resulted in aerobic glycolysis by AKt-mTOR-HIF-1α pathway, which creates a proinflamatory phenotype[12].

A murine model of acute lung injury-induced sepsis, described the implication of downregulation of Tie2/angiopoietin (Tie2/Ang) and vascular endothelial growth factor, which play important roles in the pathogenesis of sepsis. Gene expression downregulations due to histone modifications (histone acetyl-lysine modifications, Di- and tri-methylation of histone H3 lysine 4, H3K27m3, H3K9m2, H3K9m3, and H4K20m3 specifically), showed that a decrease in transcription-permissive histone modifications in those genes are systemic. The study suggests that these modifications could be modulated by histones acetylases and deacetylases drugs[13]. To understand the role of H3K4 methyltransferase Ash1l, in the regulation of innate immune response, a study used Ash1l-silenced mice injected with LPS. They found that Ash1l suppressed IL-6 and TNF production in TLR-triggered macrophages, thus protecting mice from sepsis[14]. Epigenetic mechanisms has been shown to take part on sepsis physiopathology and the studies highlight how these aspects can be targets as new therapies in sepsis.

As mentioned earlier, the drugs most tested in the observational studies were Trichostatin A and SAHA, histone deacetylase inhibitors (HDACi). By analyzing experimental works that seek, through epigenetic modifications, enable the production medicines in the treatment of sepsis, we found that many mechanisms are used for the inhibition of HDAC, which breaks the balance between HAT and HDAC. Inhibition of HDAC prevents these enzymes act in the removal of acetyl groups from DNA strand, thus preventing its chromatin condensation (6). Thus, it enables characteristic genotypes can fight pathophysiological mechanisms of sepsis, such as inflammation. In fact, many substances have been tested in several diseases to analyze the epigenetic response, as treatment with suberoylanilide hydroxamic acid (SAHA), an HDACi, which improved the survival of mice in LPS and CLP models, as shown by Ting Zhao et al.[7]. Its mechanism of action involves the factor-kB-inhibition by the substance, as well as the hypoxia-inducible factor-1a in inflammatory-mediated pathways, leading to reduced production of proinflammatory cytokines. PTX3 molecule (protein pentraxin 3) amount increased after accelerated lung injury in model mice with high tidal volume ventilation, which was counteracted by SAHA reducing their levels in the lung and blood[8]. Rats lethal shock induced by LPS with SAHA (50 mg / kg dissolved in dimethyl sulfoxide) before and after sepsis induced by LPS, it was found that SAHA inhibits inflammatory infiltration in the lungs of animals.

TSA and SAHA were also compared, in a study which investigated if septic brain is epigenetically modulated by HDACs. The study showed that inhibition of HDACs attenuated neuronal apoptosis both in vitro (primary hippocampal neuronal) and in vivo (CLP rats). Administration of TSA or SAHA improved the spatial learning and memory disorders of CLP rats[16]. Sodium butyrate was also an important drug tested in the studies. Aiming the to evaluate the effects of a microinjection of sodium butyrate (SB, HDACi) into cerebral ventricle on aversive memory in rats submitted to the sepsis (CLP induction in two groups, one evaluated 24h after the CLP induction and other 10 days after). This study showed that HDAC activity was increased in the sepsis group in hippocampus and cortex in 24 h after CLP induction; and in prefrontal cortex and hippocampus 10 days after sepsis. Results showed that SB administration reverses only in prefrontal cortex and hippocampus at 10 days[17].

Ting Zhao et al.[9] showed that tubastatina A administration of the murine CLP model reduces thymic atrophy during severe sepsis, further reducing apoptosis of splenic follicles by inhibition of HDAC6. Moreover, with treatment as tubastatina A led to low levels of corticosterone in the plasma and an increase of ACTH and since the level of cortisol can be forecast sepsis, tubastatina The

attenuates the response to stress-induced sepsis, protecting also against one of adrenal insufficiency. In addition, Li Y et al.[10] shows that the inhibition of HDAC6 improves survival in murine models of sepsis by PLC, also using tubastatina A, which decreased clearance of bacteria in the circulation in vivo inhibition RAW264.7 macrophages of apoptosis, reduction of pulmonary injury and in the case of LPS model for sepsis, decreased levels of inflammatory cytokines such as TNF-α and IL-6 in this case LPS models of sepsis induction. TSA were tested in an *in vitro* study (THP-1 cells cultivated and submitted to LPS), that analyze the effect of tolerance induction with low doses of LPS in the cytokine production, and in epigenetic regulation. The study showed that low doses of LPS reduced cytokines production, and the activity of HAT. This effect was Nitric Oxide dependent. The NOS inhibition (with LNAME) decreased histone acetylation and blocked the effect of tolerance in reducing cytokine release. TSA abolished the effect of LPS as well, in the case of IL-10[15].

Table 3. Observational studies evaluated

Study Design	Study Goal	Epigenetic Mechanism	Drug Tested	Markers	Results
In vitro. THP-1 cells, cultivated and submitted to LPS.	The effect of tolerance induction with low doses of LPS in the cytokine production, and in epigenetic regulation. It also analyses the role of Nitric Oxide in the LPS-induced chromatin modifications and its effects in the cytokine production, compared with those produced by HDAC inhibition.	Histone acetyl transferases/histone deacetylases (HAT/ HDAC) activity, nitrosylation of HDAC-2 and -3, expression of acetylated histones H3 and H4.	Trichostatin (TSA) and LNAME.	Cytokines IL-6 and IL-10 production, HAT/ HDAC activity, nitrosylation of HDAC-2 and -3, expression of acetylated histones H3 and H4.	Low doses of LPS reduces cytokines production, and the activity of HAT. This effect is Nitric Oxide dependent. The NOS inhibition (with LNAME) decreased histone acetylation and blocked the effect of tolerance in reducing cytokine release. TSA abolished the effect of LPS as well, in the case of IL-10.
In vivo (LPS-induced acute lung injury in mouse).	Efficacy of the DNMT inhibitor Aza, the HDAC inhibitor TSA, and the combination of Aza and TSA (Aza+TSA) as therapy for acute lung injury.	DNA methylation or demethylation and histone deacetylation or acetylation.	DNA methyl transferase (DNMT) inhibitor 5-Aza 2-deoxycytidine (Aza), and the HDAC inhibitor Trichostatin A (TSA).	LPS–TLR4–MAPK pathway, 38MAPK phosphorylation, and TNFα.	Combinatorial Aza+TSA therapy is more efficacious than therapy with them separately in preventing the release of LPS-induced chemokines and cytokines from macrophages.
Observational cohort in Humans (monocytes isolated from healthy donors and patients with sepsis).	Histone modification analysis of human CD14++ CD16- monocytes from patients suffering from sepsis.	Histone modifications H3K4me3, H3K27me3 and H3K9ac.	None.	Genomic locations and its distribution of H3K4me3, H3K27me3 and H3K9ac between healthy donors and patients with sepsis with a association to the promoter regions of many genes.	Alterations in the MHC class II locus and its corresponding master regulator CIITA, resulting in reduced activity of the later.. Alterations of the promoter regions of IL-1β, IL1 Receptor 2, was found, resulting in down-regulation of IL-1β and up-regulation of IL1R2, meaning a suppression of innate immunity.
In vivo (murine model of acute lung injury-induced sepsis)	Implication of downregulation of Tie2/angiopoietin (Tie2/Ang) and vascular endothelial growth factor receptor-ligand systems (VEGFR/VEGF) genes in the pathogenesis of sepsis-related microvascular leak and multiple organ dysfunction syndrome.	Histone acetyl-lysine modifications, Di- and tri-methylation of histone H3 lysine 4, H3K27m3, H3K9m2, H3K9m3, and H4K20m3.	None.	Tissue albumin levels, mRNA levels of Tek receptor and its main cognate agonist/ligand, Angpt1, Angpt2, Vegfα, and Flt1 (Vegfr1).	Changes in permissive epigenetic histone modifications at Angpt1, Tek, and Kdr in the lung, kidney, and liver. ALI-induced changes in repressive epigenetic histone modifications at these genetic loci were relatively minor. Alterations in Angpt1, Tek, and Kdr during sepsis may be modulated through targeting histone acetylases/deacetylases.

Table 3. Observational studies evaluated (Cont.)

Study Design	Study Goal	Epigenetic Mechanism	Drug Tested	Markers	Results
In vivo (murine model of CLP).	Evaluated the effects of a microinjection of sodium butyrate (SB, HDACi) into cerebral ventricle on aversive memory in rats submitted to the sepsis.	Acetylation of histones (HDAC) activity and histone deacetylase inhibitors (HDACi).	Sodium butyrate (SB, HDACi).	HDAC activity in hippocampus, cortex, prefrontal and striatum.	HDAC activity was increased in the sepsis group in hippocampus and cortex in 24 h after CLP induction; and in prefrontal cortex and hippocampus 10 days after sepsis. SB administration reverses this only in prefrontal cortex and hippocampus at 10 days.
In vivo (Mice injected with live *C. albicans), In vitro* (primary human monocytes).	The study aim is to understand the mechanism of trained immunity in monocytes or macrophages, through histone modifications.	Histone methylation and acetylation patterns (H3K4me3 and H3K27ac).	None.	Histone modifications and RNA sequencing analysis, glucose, lactate, NAD+ and NADH concentration, cellular O2 consumption and AMPK, mTOR, AKT and actin.	Blocking of the mTOR–HIF-1α pathway, responsible for the metabolic shift induced by β-glucan, by chemical inhibitors inhibited trained immunity. The study identified glycolysis as a fundamental process in trained immunity.
In vivo (CLP in BALB/c mice)	The aim was to explore how HDAC can contribute to sepsis-associated inflammation and apoptosis.	Acetylation of histones (HDAC1, HDAC2, and HDAC3).	CG200745, a HDAC inhibitor, or valproic acid, a inhibitor of class I HDACs.	HDAC1, HDAC2, and HDAC3. Bcl-2. Blood levels of TNF, IL-1b, and MCP-1.	Sepsis increased histone H3 and H4 acetylation in lungs. CG200745 apoptosis was suppressed in lungs and spleens of septic mice, but it failed to inhibit cytokines and lung inflammation. Valproic acid also showed antiapoptotic but not anti-inflammatory effects in septic mice.
In vivo (CLP rats), *in vitro* (primary hippocampal neuronal).	Histone deacetylases were implicated in neurodegeneration and cognitive functions. The study investigate if septic brain is epigenetically modulated by HDACs.	Acetylated histone 3 (AcH3), acetylated histone 4 (AcH4), cytoplasmic HDAC4.	Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA).	Hippocampal acetylated histone 3 (AcH3) and acetylated histone 4 (AcH4), cytoplasmic HDAC4 and nuclear HDAC4, cytoplasmic and nuclear HDAC2. Bax and Bcl-XL.	Inhibition of HDACs attenuated neuronal apoptosis both in vitro and in vivo. Administration of HDACs inhibitors (TSA or SAHA) also improved the spatial learning and memory disorders of CLP rats.
In vivo (Ash1l-silenced mice injected with LPS), *in vitro* (peritoneal macrophage).	To understand the role of H3K4 methyltransferases and demethylases in regulation innate inflammatory immune responses, it was silenced 14 enzymes involved in methylation in mouse peritoneal macrophages with specific small interfering RNAs (siRNAs), and then measured LPS-induced IL-6 production.	Histone methyltransferase Ash1l (a H3K4 methyltransferase).	None.	IL-6, TNF and IFN-β.	Ash1l, a H3K4 methyltransferase, suppressed interleukin-6 (IL-6), and tumor necrosis factor (TNF) production in Toll-like receptor (TLR)-triggered macrophages, protecting mice from sepsis.

Table 3. Observational studies evaluated (Cont.)

Study Design	Study Goal	Epigenetic Mechanism	Drug Tested	Markers	Results
In vivo (G. mellonella larvae).	The role of HDACs and HATs in epigenetic reprogramming during metamorphosis, wounding and infection in the lepidopteran model host Galleria mellonella.	Acetylation of histones by HATs and removal of acetyl groups by HDACs.	Suberoylanilide hydroxamic acid (SAHA) and sodium butyrate.	Expression of genes encoding components of HATs and HDACs, and expression of MMP, p38 MAP kinase, IMPI and galiomycin.	Pathogenic bacteria can interfere with the regulation of HDACs and HATs in insects, manipulating host immunity and development. The histone acetylation/deacetylation in insects mediates transcriptional reprogramming during metamorphosis and in response to wounding and infection.
In vivo (CLP murine model)	To demonstrate that selective inhibition of HDAC6 with tubastatin A displays dramatically better survival outcomes compared with inhibition of HDAC1, 2, and 3 in the lethal cecal ligation and puncture (CLP)	Inhibition of HDAC6	Tubastatin A	Cellular apoptosis measurement	Blunting of the stress responses, attenuated thymic and bone marrow atrophy and decreased apoptosis in splenic follicles.
In vitro (RAW264.7 cells) and in vivo (Polymicrobial sepsis was induced by CLP in mice)	To investigate the protective mechanisms of a newly synthetized HDAC inhibitor, Compound 9a, in sepsis, particularly with regard to MAPK signalling associated with inflammation.	Inhibition of HDAC1, after trying with HDAC1, HDAC2 and HDAC3	Compound 9a	ALT, AST, creatinine, BUN and LDH.	It inhibited MAPK-mediated inflammatory responses and attenuated the organ damage.
In vivo (mouse primary splenocytes)and in vitro (RAW 264.7 murine macrophages)	To determine if selective inhibition of HDAC6 has a substantial advantage for sepsis treatment.	Inhibition of HDAC6	Tubastatin A	TNF-α and IL-6 and inhibits cell apoptosis of RAW264.7 macrophages	It improves survival and decreases cytokine levels in peritoneal fluid and circulation.
In vivo (mice in LPS model)	To use high-throughput methods to investigate the multidimensional impact of SAHA treatment on gene expression profiles at an early stage of LPS-induced shock	Attenuation of inflammatory mediators including (TNFR1 and 2, TRAF6, TLR-2, PTX3, MyD88, CCL3)	SAHA	TLR-4, TNF-a, IL-1b	It attenuates inflammation and decreases neutrophil infiltration in the lungs
In vivo (mice in LPS model) and in vitro (primary bone marrow derived macrophages)	To investigate the effects of curcumin on the expression of TREM-1 in vitro in primary bone marrow derived macrophages and in vivo in a septic lung injury model	It attenuates methylation and acetylation of histone 3 and 4 in the TREM-1 promoter	Curcumin	IL-6, IL-10 and TNF-a	Reduction septic lung injury

Table 3. Observational studies evaluated (Cont.)

Study Design	Study Goal	Epigenetic Mechanism	Drug Tested	Markers	Results
In vitro (Human monocyte derived macrophages)	To investigate novel targets for the anti-inflammatory action of APC in human macrophages	Effect on HDACs activity	Protein C	Comparison between two samples (LPS/INFc and LPS/INFc/APC)	APC down-regulates the mRNA level of IL-1b, IL-8, MCP-1 and MIP-1b and the protein expression of MCP-1, and MIP-1b in macrophages
In vivo (CLP model in mice)	To demonstrate that HDAC inhibitors effectively attenuate LPS-induced inflammation in vitro and in vivo	It decreased the MPO activities in lung homogenates, moreover it downregulated the expression of ICAM-1 and E-selectin	Trichostatin A (TSA) and sodium butyrate (SB)	IL-6	HDACs control injury from inflammation
In vivo (CLP sepsis model)	To characterize the coagulation abnormalities in a lethal CLP model using TEG, and to assess the effects of SAHA treatment on these disturbances	It restores fibrin cross-linkage time in a lethal, moreover it improves platelet function	SAHA	TNF-a and IL-6	It prevented coagulopathy
In vitro (blood samples collected from sepsis subjects with septic shock and multiorgan failure and healthy controls)	To demonstrate that TLR4 stimulation and human sepsis activate pathways that couple NAD and its sensor SIRT1 with epigenetic reprogramming	SIRT1 Deacetylates RelA/p65 at Lysine 310 at Promoters to Limit Gene Transcription of Proinflammatory Cytokine Genes. Moreover, it activates RelB transcription	None	TNF-a	It represses transcription of TNF-a and IL-1

Clinical Trials Studies

In clinical trials the most studied drugs nowadays are Panobinostat and SAHA, especially for the treatment of hematological malignancies such as types of lymphoma and leukemia (see Table). However, the drugs theophylline, sodium butyrate and valproate are being tested in the treatment of other conditions in which gene expression regulation is also a pathophysiological factor. These diseases are spinal muscular atrophy, bronchiectasis and schizophrenia.

In bronchiectasis study, experimental findings that justify the use of HDAC resemble to sepsis' because it evaluates theophylline effect over inflammatory process. In this clinical trial, theophylline is a possible target-therapy for bronchiectasis due to its anti-inflammatory results of previous studies for the treatment of COPD and asthma. The hypothesis is that theophylline has no inhibitory action, but directly increases the activity of histone deacetylases by downregulating the expression and production of interleukins such as IL-8, enhancing corticosteroids' effect[27].

On the other hand, other clinical trial evidenced that the use of valproate as an inhibitor of histone deacetylase, led to increased SMN 1(survival motor neuron 1) expression, which is deleted gene in 95% of patients with Spinal Muscular Atrophy[28].

Therefore, it is important to consider that the final effect of epigenetic drugs may not be predictable. Not necessarily, these drugs will provide less gene expression in all genetic code. Actually, there is a risk of over expression, which side effects are yet unknown, justifying further investigation for each situation.

Studies with oncologic patients, the only with results, analyzed the use of SAHA and Panabinostat drugs. In general, almost all participants in control groups and groups exposed to new combination therapy had some type of adverse event. In relation to the most common serious adverse events (see Table 4), the control group showed a lower percentage of patients with the outcome, suggesting that the tested HDAC's could lead to further complications. However, most studies did not show statistical analysis of the data, not allowing affirm that difference between the groups is significant. Moreover, it is important to consider that these studies were conducted in oncologic patients with a higher degree of clinical fragility.

Table 4. Adverse Events

Drug	Disease	Adverse Events		Serious Adverse Events		Most common Serious Adverse Events	Reference
		Experimental	Control	Experimental	Control		
Panobinostat/ LBH589	Multiple Myeloma	53/55 (96.36%)	-	39/55 (70%)	-	Pneumonia Thrombocytopenia Pyrexia	23
Panobinostat/ LBH589	Multiple Myeloma	379/381 (99.48%)	366/377 (97.08%)	228/381 (59.84%)	157/377 (41.64%)	Diarrhoea Pneumonia	24
Vorinostat/ SAHA	Non-Small Cell Lung Cancer	114/124 (91.94%)	117/124 (94.35%)	63/124 (50.81%)	45/124 (36.29%)	Febrile neutropenia Neutropenia	25
Vorinostat/ SAHA	Myelodysplastic Syndrome	91/91 (100.00%)	89/91 (97.80%)	47/91 (51.65%)	8/91 (8.79%)	Anemia Pneumonia Thrombocytopenia	26

Table 5. Progression-free survival

Drug	Disease	Progression-free Survival		Unit	Reference
		Experimental	Control		
Panobinostat/LBH589	Multiple Myeloma	164.0 (107.0 to 204.0)	-	Days	23
Panobinostat/LBH589	Multiple Myeloma	11.99 (10.32 to 12.94)	8.80 (7.56 to 9.23)	Months	24
Vorinostat/ SAHA	Non-Small Cell Lung Cancer	4.3 (0.03 to 13.4)	5.5 (0.03 to 13.8)	Months	25
Vorinostat/ SAHA	Myelodysplastic Syndrome	455 *	311 (182 to 640)	Days	26

*The upper limit of the confidence interval cannot be estimated.

To compare these drugs efficiency in studies, we chose the parameter "progression-free survival" (Table 5). Analyzing the table, in almost all clinical trials the use of HDAC in experimental group led to increase disease-free time. The progression of the disease in each study was assessed differently, in most cases considering the result of imaging and laboratory. Just as mentioned before, the studies showed no statistical analysis, being impossible to assert significant difference. According to these clinical trials, HDAC's inhibitors have a toxic action in neoplasia because they are able to promote accumulation of cytotoxic misfolded protein aggregates and dysregulate neoplasic cell function, leading to its death[24].

CONCLUSION

After all those works analyses, it is clear that septic patients have epigenetic alterations. When exposed to an infection the expression changes in immune related genes, creates a phenotype that results in an exacerbated pro-inflammatory cytokines release and inflammatory cells production, characterizing sepsis.

The new findings in epigenetic alterations in sepsis physiopathology, shed a light in a new possible treatment based on epigenetic drugs. Although studies *in vitro* or *in vivo* pointed the use of HDAC's inhibitors as a promising suppressor of the exacerbated inflammatory response in sepsis models, we still have to be careful with their use in humans for mainly two reasons:

Firstly, severe adverse effects, like cytopenias, observed in cancer patients from HDAC's Clinical Trials should be considered in the treatment of sepsis treatment as well. The balance between immune and inflammatory response must our final goal for having success in sepsis treatment. Secondly, no clinical parameters for sepsis were totally evaluated in the experimental studies selected, which we strongly recommend for next researches.

As no studies in humans using epigenetic drugs for the treatment of sepsis were found, we are able to affirm that the translational between basic and clinical areas is still not finished. The use of epigenetic drugs in other diseases, specially in studies on phases three or four, shows that toxicity in humans have been overcome. Therefore, if the development of new studies in this area pursues, epigenetic drugs should be considered as viable sepsis therapy.

REFERENCES

1. Vachharajani V, Liu T, McCall C. Epigenetic coordination of acute systemic inflammation: potential therapeutic targets. Expert Rev Clin Immunol. 2014;10(9):1141-50. doi: 10.1586/1744666X.2014.943192.
2. Rhodes A, Phillips G, Beale R, Cecconi M, Chiche J, De Backer D, et al. The surviving sepsis campaign bundles and outcome: results from the International Multicentre Prevalence Study on Sepsis (the IMPreSS study). Intensive Care Med. 2015;41(9):1620-8. doi: 10.1007/s00134-015-3906-y.
3. Ciarlo E, Savva A, Roger T. Epigenetics in sepsis: targeting histone deacetylases. Int J Antimicrob Agents. 2013;42:S8-S12. doi: 10.1016/j.ijantimicag.2013.04.004.
4. Pereira Junior GA, Marson F, Abeid M, Ostini FM, Souza SH, Basile-Filho A. Pathogenetic mechanisms of sepsis and their therapeutics implications. Medicina, Ribeirão Preto. 1998;31:349-62.
5. Bonasio R, Tu S, Reinberg D. Molecular signals of epigenetic states. Science. 2010;330(6004):612-616. doi: 10.1126/science.1191078.
6. Menditi KBC, Hye CK. The role of histones proteins in hematological neoplasias. Rev Bras Cancerol. 2007;53(4):453-60.
7. Zhao T, Li Y, Liu B, Wu E, Sillesen M, Velmahos G, et al. Histone deacetylase inhibitor treatment attenuates coagulation imbalance in a lethal murine model of sepsis. Surgery. 2014;156(2):214-20. doi: 10.1016/j.surg.2014.04.022.
8. Li Y, Liu B, Gu X, Kochanek A, Fukudome E, Velmahos G, et al. Creating a "pro-survival" phenotype through epigenetic modulation. J Surg Res. 2012;172(2):199. doi: 10.1016/j.surg.2012.06.036.
9. Zhao T, Li Y, Bronson R, Liu B, Velmahos G, Alam H. Selective histone deacetylase-6 inhibition attenuates stress responses and prevents immune organ atrophy in a lethal septic model. Surgery. 2014;156(2):235-42. doi: 10.1016/j.surg.2014.03.033.
10. Li Y, Zhao T, Liu B, Halaweish I, Mazitschek R, Duan X, et al. Inhibition of histone deacetylase 6 improves long-term survival in a lethal septic model. J Trauma Acute Care Surg. 2015;78(2):378-85. doi: 10.1097/TA.0000000000000510.
11. Weiterer S, Uhle F, Lichtenstern C, Siegler B, Bhuju S, Jarek M et al. Sepsis induces specific changes in histone modification patterns in human monocytes. PLOS ONE. 2015;10(3):e0121748. doi: 10.1371/journal.pone.0121748.
12. Cheng S, Quintin J, Cramer R, Shepardson K, Saeed S, Kumar V, et al. mTOR- and HIF-1 -mediated aerobic glycolysis as metabolic basis for trained immunity. Science. 2014;345(6204):1250684. doi: 10.1126/science.1250684
13. Bomsztyk K, Mar D, An D, Sharifian R, Mikula M, Gharib S, et al. Experimental acute lung injury induces multi-organ epigenetic modifications in key angiogenic genes implicated in sepsis-associated endothelial dysfunction. Crit Care. 2015;19(1). doi: 10.1186/s13054-015-0943-4.
14. Xia M, Liu J, Wu X, Liu S, Li G, Han C, et al. Histone Methyltransferase Ash1l Suppresses Interleukin-6 Production and Inflammatory Autoimmune Diseases by Inducing the Ubiquitin-Editing Enzyme A20. Immunity. 2013;39(3):470-81. doi: 10.1016/j.immuni.2013.08.016.
15. Rios E, de Lima T, Moretti A, Soriano F. The role of nitric oxide in the epigenetic regulation of THP-1 induced by lipopolysaccharide. Life Sci. 2016;147:110-16. doi: 10.1016/j.lfs.2016.01.041.
16. Fang J, Lian Y, Xie K, Cai S, Wen P. Epigenetic modulation of neuronal apoptosis and cognitive functions in sepsis-associated encephalopathy. Neurol Sci. 2013;35(2):283-8. doi: 10.1007/s10072-013-1508-4.
17. Steckert A, Comim C, Igna D, Dominguini D, Mendonça B, Ornell F, et al. Effects of sodium butyrate on aversive memory in rats submitted to sepsis. Neurosci Lett. 2015;595:134-8. doi: 10.1016/j.neulet.2015.04.019.
18. Kim S, Baek K, Park H, Jung Y, Lee S. Compound 9a, a novel synthetic histone deacetylase inhibitor, protects against septic injury in mice by suppressing MAPK signalling. Brit J Pharmacol. 2016;173(6):1045-57. doi: 10.1111/bph.13414.
19. Yuan Z, Syed M, Panchal D, Rogers D, Joo M, Sadikot R. Curcumin mediated epigenetic modulation inhibits TREM-1 expression in response to lipopolysaccharide. Int J Biochem Cell Biol. 2012;44(11):2032-43. doi: 10.1016/j.biocel.2012.08.001.
20. Pereira C, Bachli E, Schaer D, Schoedon G. Transcriptome analysis revealed unique genes as targets for the anti-inflammatory action of activated protein C in human macrophages. PLoS ONE. 2010;5(10):e15352. doi: 10.1371/journal.pone.0015352.
21. Zhang L, Jin S, Wang C, Jiang R, Wan J. histone deacetylase inhibitors attenuate acute lung injury during cecal ligation and puncture-induced polymicrobial sepsis. World J Surg. 2010;34(7):1676-83. doi: 10.1007/s00268-010-0493-5.
22. Liu T, Yoza B, El Gazzar M, Vachharajani V, McCall C. NAD+-dependent SIRT1 deacetylase participates in epigenetic reprogramming during endotoxin tolerance. J Biol Chem. 2011;286(11):9856-64. doi: 10.1074/jbc.M110.196790.
23. Richardson P, Schlossman R, Alsina M, Weber D, Coutre S, Gasparetto C, et al. PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. Blood. 2013;122(14):2331-7. doi: 10.1182/blood-2013-01-481325.
24. Novartis (Novartis Pharmaceuticals). Panobinostat or placebo with bortzomib and dexamethasone in patients with relapsed multiple myeloma (PANORAMA-1). ClinicalTrials.gov [Internet]. 2015 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT01023308.

25. Merck Sharp & Dohme Corp. A clinical trial of vorinostat (MK0683, SAHA) in combination with FDA approved cancer drugs in patients with advanced Non-small Cell Lung Cancer (NSCLC)(0683-056). ClinicalTrials.gov [Internet]. 2015 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT00473889.

26. National Cancer Institute (NCI). Azacitidine with or without lenalidomide or vorinostat in treating patients with higher-risk myelodysplastic syndromes or chronic myelomonocytic leukemia. ClinicalTrials.gov [Internet]. 2016 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT01522976.

27. The First Affiliated Hospital of Guangzhou Medical University. The role of theophylline plus low-dose formoterol-budesonide in treatment of bronchiectasis. ClinicalTrials.gov [Internet]. 2015 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT01769898.

28. All India Institute of Medical Sciences, New Delhi. Valproate and levocarnitine in children with spinal muscular atrophy. ClinicalTrials.gov [Internet]. 2015 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT01671384.

29. Smith RC, Nathan Kline Institute for Psychiatric Research. Sodium butyrate for improving cognitive function in schizophrenia. ClinicalTrials.gov [Internet]. 2016 [cited 2016 Feb 17]. Available from: https://clinicaltrials.gov/ct2/show/NCT02654405.