Effect of a Histone Deacetylases Inhibitor of IL-18 and TNF-Alpha Secretion in Vitro

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Abstract

**BACKGROUND:** Interleukin-18 (IL-18) and Tumor Necrosis Factor-alpha (TNF-α) are proinflammatory cytokines that increased the development of Th1 immune response, but have a different type of regulation of the gene expression. Whereas TNF-α has an inducible expression, IL-18 is translated as an inactive protein and required proteolytic cleavage by Casp-1 in inflammasome complexes.

**AIM:** To investigate the effect of the histone deacetylases inhibitor Suberoylanilide Hydroxamic Acid (SAHA) on the gene expression and secretion of both cytokines, IL-18 and TNF-α, according to their contribution to the cancer development and anticancer immunity.

**METHODS:** Isolated peripheral blood mononuclear cells (PBMC) were stimulated with LPS and C3bgp with or without SAHA. Cytokine production was assessed by ELISA at 6 and 24h.

**RESULTS:** IL-18 and TNF-α secretion was significantly increased at 6h and 24h in response to stimulation. TNF-α production from stimulated PBMC was downregulated by SAHA at 6 and 24h. Treatment with SAHA does not inhibit the secretion of IL-18 significantly either at 6 or 24h of stimulation.

**CONCLUSION:** The inhibition of histone deacetylases by SAHA does not influence the inflammasome-dependent production of immunologically active IL-18. In contrast, the production of proinflammatory TNF-α in cultures was mediated by the activity of HDAC class I and class II enzymes.

Introduction

The inducible cytokine release is regulated at several levels started from chromatin remodelling allowing gene expression and finalised with protein secretion. Before transcription, the gene region must be accessible to the transcriptional factors binding. The key role in this process has the histone modification mainly acetylation and deacetylation. Two classes of enzymes drive the acetylation status of the chromatin. Acetyltransferases open the chromatin conformation by histone acetylation and allow the transcriptional process. Histone deacetylases (HDACs) compress the chromatin structure triggering the gene silence by deacetylation [1][2]. HDACs are divided into four classes. Of them, class I are resident to the nucleus, where they act as histone modifiers and repressors of the transcription. Histone deacetylases class II is moving between the nucleus and cytoplasm. They can regulate the gene expression also by the changes in acetylation/deacetylation status of other proteins [3][4].

Recently, a new class of small organic molecules-HDAC inhibitors (HDI), which abolish the action of HDACs, are intensively studied, especially about cancer and inflammatory diseases treatment [5][6]. They regulate the expression up to 10% of the cellular genes by affecting enzymes including in chromatin remodelling complex and recruiting of the transcription factors [7]. Although the histone acetylation is linked to an increased transcription; HDI also can increase the expression of some genes by the still unclear mechanism. Suberoylanilide Hydroxamic Acid (SAHA) is the HDI interacting with...
class I and class II histone deacetylases [4]. SAHA was the first HDI approved by U. S. Food and Drug Administration for the treatment of some malignant disease as CTCL [8].

During the early phase of the immune response, binding of microbial antigens (especially those referring to pathogen-associated molecular patterns-PAMPs) to pattern recognition receptors (PRR) of the immune cells, activate intracellular signalling pathways, which in turn lead to alteration in cell behaviour and gene expression. As a consequence soluble mediators are synthesised and secreted by the activated immune cells including proinflammatory and immunoregulatory cytokines like TNF-α and IL-18. Tumor necrosis factor-alpha (TNF-α) was first discovered as a mediator of chronic inflammation which drives the cancer development [9]. TNF-α is a pro-inflammatory cytokine, but it is also included in Th1 polarisation. It's inducible gene expression after recognition of PAMPs by PRR lead to the synthesis of an inactive protein (pro – IL-18). Pro – IL-18 is converted into a biologically active molecule after processing of membrane-bound TNF-α by constitutive expressed enzyme [10], mainly by the activated macrophages/monocytes. Interleukin-18 (IL-18) is also a proinflammatory cytokine, but it is included in Th1 polarisation. Its inducible gene expression after recognition of PAMPs by PRR lead to the synthesis of an inactive protein (pro – IL-18). Pro – IL-18 is converted into a biologically active IL-18 by another activation pathway in inflammasomes through caspase 1-mediated cleavage [11]. IL-18 is involved in the development of successful antitumor immunity through its ability to induce IFN-γ secretion [12]. Unlike TNF-α, the mechanisms regulating IL-18 processing and secretion remains not well understood.

In this regard, our study was designed to investigate the SAHA effect on protein synthesis and release of TNF-α and IL-18 from stimulated healthy human PBMC.

**Methods**

**Isolation of PBMC**

Peripheral venous blood was taken by venipuncture from 10 healthy donors after the approval of the Ethics Board of Medical Faculty, Trakia University. Each volunteer was informed and signed informed consent. The samples (10 ml) were collected in sterile tubes with EDTA. Peripheral blood mononuclear cells (PBMC) were harvested after density gradient centrifugation over Histopaque-1077.

**In vitro culturing**

PBMC (1 x 10⁶ cells/ml) cultures were prepared as described previously by Dobreva et al. [13]. They were stimulated with: 30 μg/ml C3 binding glycoprotein, (C3bgp) [14]; or 1 μg/ml Lipopolysaccharide (LPS) from Escherichia coli (Sigma-Aldrich-Merck, Darmstadt, Germany). PBMC cultures were incubated at 37°C for 6 and 24h. After incubation the separated supernatants were stored at -70°C.

**HDAC inhibition**

SAHA (Sigma-Aldrich-Merck, Darmstadt, Germany) (5 μM) was used for the inhibition of histone deacetylases. The inhibitor was added one h before stimulation.

**Cytokine evaluation**

Assessment of IL-18 and TNF-α was performed by ELISA, under to the manufacturer's instructions. For the detection of TNF-α, R&D Systems Qantikine ELISA kit (Minneapolis, MN 55413, USA) was used. IL-18 production was measured using commercially available kits purchased from MBL International Corporation (Woburn, MA 01801, USA). The colour reaction was measured as OD units and expressed in pg/ml. The sensitivity of the ELISA kits was 12 pg/ml for IL-18 and 15 pg/ml for TNF-α.

**Statistical analysis**

The data was presented as means and standard error of the mean. Evaluation of the statistical differences between cultures was performed by Student’s t-test. Differences were significant when the P value was equal or less than 0.05.

**Results**

**TNF-α production was suppressed by SAHA**

Results presented in Figure 1 demonstrated that C3bgp and LPS increased significantly TNF-α production at 6 and 24 h in comparison with nonstimulated controls (p < 0.05). The addition of SAHA to stimulated cultures leads to significantly decreased TNF-α production at 6 and in higher degree at 24 h. TNF-α quantity secreted by PBMC cultured with HDAC inhibitor was 4 to 6-fold less than in cultured PBMC without SAHA at six h and 5 to 8 fold less at 24 h.
SAHA did not modulate significantly IL-18 production

The addition of C3bgp and LPS leads to significantly more IL-18 production in comparison with nonstimulated cultures (p < 0.05). Moreover, we did not observe significant differences between 6 and 24 h in the secretion of IL-18 from stimulated cultures. The inhibition of HDAC slightly decreased IL-18 production. However, we did not detect significant differences between stimulated cultures treated with SAHA and cultures without SAHA as shown in Figure 2.

Discussion

IL-18 and TNF-α are proinflammatory cytokines, with a different effect on the acquired immunity and different production and regulatory mechanism as well. Whereas TNF-α gene expression and secretion is mediated through the TLR signalling pathway, IL-18 required proteolytic processing by Casp-1 in inflammasome before its secretion [11]. In the current study, we evaluated the effect of the histone deacetylases inhibitor SAHA on the gene expression and secretion of both cytokines. IL-18 and TNF-α, according to their contribution to the cancer development and anticancer immunity.

TNF-α is a main proinflammatory cytokine identified for the first time because of its rapid cytolytic effect on some experimental cancers [15]. Recently, new studies showed that TNF-α has protumorigenic activity and is involved in all key points of tumorigenesis – tumour promotion, malignant transformation, tumour cell proliferation, angiogenesis and malignant cell spreading [9]. This investigation showed that the addition of the HDAC inhibitor SAHA downregulated the production of TNF-α released by the stimulated mononuclear cells. The same results were obtained from other authors [16]. Today is accepted that histone deacetylation is linked to the decreased gene transcription. Nevertheless, there was experimental evidence demonstrating that the treatment with HDI downregulated the expression of some proinflammatory cytokines by a mechanism which currently is being studied extensively. For example, Takada et al. demonstrated that HDI SAHA did not affect the binding of NF-kB transcription factor to the promoters of the target genes, but inhibited IκBα kinase activation, IκBα phosphorylation and translocation of p65 to the nucleus [17]. It is widely accepted that TNF-α production in response to LPS is mediated by TLR4 followed by the activation of NF-kB. Our study demonstrated that SAHA downregulated TNF-α production after stimulation with LPS. Therefore, our results are in accordance with decisions of the other investigators [17][18] that the downregulating effect of SAHA on the proinflammatory cytokine production is mediated by the suppression of NF-kB transduction pathway. Previously we showed that C3bgp activated JNK and p38 intracellular transduction pathways [19]. Furthermore, our results showing that SAHA inhibited C3bgp-mediated TNF-α production are in concordance with the study of Ajizian et al., demonstrating that the suppression of p38 MAPK leads to downregulated TNF-α production [20] and with the study of Choo and coauthors, showing that SAHA affected p38 activation [21].

Currently, it is widely accepted that IL-18 drives the Th1 immune response, because induces IFN-γ secretion from T cells and natural killer cells [12]. There is evidence that treatment with IL-18 of experimental animals has significant antitumor action [11]. Moreover, in vivo IL-18 administration in experimental mice inoculated with tumour cell line stimulated IFN-γ production and IL-12 independent antitumor response [22]. Its elevated levels have been...
observed in several types of cancers, especially in advanced cancers with metastasis [23][24][25]. Our study indicated that SAHA did not influence significantly IL-18 production from PBMC. There are few studies about SAHA and its effect on IL-18 secretion. However, our results contradict the study of Choo et al., which demonstrated that SAHA inhibits the production of IL-18 in E11 and THP-1 cell lines in a dose-dependent manner [26]. One explanation for this discrepancy may be the different cell sources of IL-18. Our experiments were done with PBMC from healthy donors. In their study Choo et al., used E11 cell line of human rheumatoid synovial cells, transformed with simian virus 40 large T antigen expression vector or a THP-1 monocytic cell line derived from a patient with acute myeloid leukaemia. Moreover, it is well known that the tumour cell lines had specific regulation of the gene expression in comparison with normal human cells [27].

Recently, it is widely accepted that chronic inflammation has a crucial role in the tumour-promoting and survival, regardless of its origin. In this process, proinflammatory cytokines play a key role [28]. Many studies indicated that SAHA suppresses proinflammatory cytokines like IL-12, IFN-γ, TNF-α and IL-1β expression [13][16]. However, not always the inhibition of IL-12 and IFN-γ has positive effects, because of their immunoregulatory function as cytokines triggering cell-mediated anticancer immunity. Our study has shown that treatment with SAHA leads to decreased TNF-α and unmodified IL-18 production by PBMC-a surprising effect on the synthesis and secretion of both cytokines. Considering the protumorigenic activity of TNF-α, the downregulation of its production is a desirable effect of treatment with various histone deacetylase inhibitors including SAHA. However, IL-18 like IL-12 has antitumorigenic activity because of its property to induce Th1 cell-mediated type of the immune response. Therefore, under immunosuppression triggered by SAHA, an unaffected IL-18 production may have a crucial role in the realization of antitumor immunity. Thus, this SAHA-specific modulation of the TNF-α and IL-18 production may provide a further clinical advantage for the treatment of various malignant conditions.

In summary, our study showed that HDAC inhibitor SAHA downregulated TNF-α production and did not affect IL-18 secretion from activated PBMC. Therefore, we conclude that the production of TNF-α is mediated by HDAC class I and class II enzymes, but they are not involved in inflammasome-dependent regulation of biologically active IL-18 secretion. This different regulation of both cytokines by histone deacetylases and their inhibitors may be used in the development a new approach in the therapy of certain types of cancers.

References

1. Li N, Zhao D, Kirschbaum M, Zhang C, Lin CL, Todorov I, Kandeel F, Forman S, and Zeng D. HDAC inhibitor reduces cytokine storm and facilitates induction of chimerism that reverses lupus in anti-CD3 conditioning regimen. PNAS. 2008; 105:4796–4801. https://doi.org/10.1073/pnas.0712051105 PMid:18347343 PMCID:PMC2290749

2. Roger T, Lugin J, Le Roi D, Goy G, Mombelli M, Koessier T, Ding XC, Chanson AL, Raymond MK, Miconnet I, Schrenzel J, Francois P, and Calandra T. Histone deacetylase inhibitors impair innate immune response to Toll-like receptor agonist and to infection. Blood. 2011; 117:1205–1217. https://doi.org/10.1182/blood-2010-05-28471 PMid:20956800

3. Shakespear MR, Hailili MA, Irvine KM, Fairlie DP, and Sweet MJ. Histone deacetylases as regulators of inflammation and immunity. Trends in Immunology. 2011; 32:335–343. https://doi.org/10.1016/j.it.2011.04.001 PMid:21570914

4. Hailili MA, Andrews MR, Labzin LJ, Schroder K, Matthias G, Cao C, Lovelace E, Reid RC, Le GT, Hume D, Irvine KM, Matthias P, Fairlie DP, and Sweet MJ. Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the Toll-like receptor 4 agonist LPS. J Leuk Biol. 2010; 87:1–11. https://doi.org/10.1189/jlb.0509363 PMid:20200406

5. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006; 5:769–784. https://doi.org/10.1038/nrd2153 PMid:16955068

6. Wang Z, Chen C, Finger SN, Kwajah SMM, Jung M, Schwarz H, Swanson N, Lareu RR, and Raghunath M. Suberoylanilide hydroxamic acid: a potential epigenetic therapeutic agent for lung fibrosis? Eur Respir J. 2009; 34:145–155. https://doi.org/10.1183/09031936.0004808 PMid:19224893

7. Tifon CE, Adams JE, Fits van der L, Wen S, Townsend PA, Ganesan A, Hodges E, Vermeer MH, and Packham G. The histone deacetylase inhibitors vorinostat and romidepsin downmodulate IL-10 expression in cutaneous T-cell lymphoma cells, Br J Pharmacol. 2011; 162:1590–1602. https://doi.org/10.1111/j.1476-5381.2010.01188.x PMid:21985455 PMCID:PMC3657296

8. Ververs K, Hiong A, Karagiannis TC, Liciardi PV. Histone deacetylase inhibitors (HDACis): multitargeted anticancer agents. Biologics: Targets and Therapy. 2013; 7:47–60. PMid:23459471 PMCID:PMC3584656

9. Wu Y and Zhou BP. TNF-α/NFκB/Snail pathway in cancer cell migration and invasion. British Journal of Cancer. 2010; 102:639–644. https://doi.org/10.1038/ajbc.2010.55 PMid:20873533 PMCID:PMC2837572

10. Bell JH, Herrera AH, Li Y, Walcheck B. Role of ADAM17 in the ectodomain shedding of TNF-α and its receptors by neutrophils and macrophages. J Leukoc Biol. 2007; 82:173–176. https://doi.org/10.1189/jlb.0307193 PMid:17510296

11. Fabbri M, Carbotti G, and Ferrini S. Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP. J Leuk Biol. 2015; 97:665–675. https://doi.org/10.1189/jlb.5RU0714-360RR PMid:25548255

12. Dinarello CA. IL-18: A TH1 inducible, proinflammatory cytokine and new member of the IL-1 family, J Allergy Clin Immunol. 1999; 103:11–24. https://doi.org/10.1016/s0091-6749(99)70518-x

13. Dobrevska ZG, Grigorov BG, Stanilova SA. Suppression of IL-12p40-related regulatory cytokines by suberoylanilide hydroxamic acid an inhibitor of histone deacetylases. Immunopharmacol Immunotoxicol. 2010; 38:281–285. https://doi.org/10.1080/08928973.2010.1188940 PMid:27240992

14. Zhelev Z, Stanilova S, Carpenter B. Isolation, partial characterization and complementary inhibiting activity of a new glycoprotein from Cuscuta europaea. Biochem Biophys Res Commun. 1994; 202:86–94. https://doi.org/10.1016/bbrc.1994.1911

15. Balkwill F. Tumour necrosis factor and cancer. Nature Reviews
Cancer. 2009; 9:361-371. https://doi.org/10.1038/mrc2628 PMid:19343034

16. Leoni F, Zaiiani A, Bertolini G, Porro G, Pagani P, Pozzi P, Dona G, Fossati G, Sozanni S, Azam T, Bufler P, Fantuzzi G, Goncharov I, Kim SH, Pomerantz BJ, Reznikov LL, Siegmund B, Dinarello CA, and Mascagni P. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. PNAS. 2002; 99:2995-3000. https://doi.org/10.1073/pnas.052702998 PMid:11867742 PMcid:PMC122461

17. Takada Y, Gillenwater A, Ichikawa H, and Aggarwa BB. Suberoylanilide Hydroxamic Acid Potentiates Apoptosis, Inhibits Invasion, and Abolishes Osteoclastogenesis by Suppressing Nuclear Factor-kB Activation. J Biol Chem. 2006; 281:5612-5622. https://doi.org/10.1074/jbc.M507213200 PMid:16377638

18. Bode KA, Schroder K, Hume DA, Ravasi T, Heeg K, Sweet MJ, and Dalpke HA. Histone deacetylase inhibitors decrease Toll-like receptor-mediated activation of proinflammatory gene expression by impairing transcription factor recruitment. Immunology. 2007; 122:596-606. https://doi.org/10.1111/j.1365-2567.2007.02678.x PMid:17635610 PMCid:PMC2266046

19. Dobreva ZG, Stanilova SA. The immunomodulatory activity of C3 binding glycoprotein (C3bgp) is mediated by the complement receptor type III and mitogen-activated protein kinase signal transduction pathways. Immunopharmacol & Immunotoxicol. 2007; 29:549-562. https://doi.org/10.1080/08923970701691017 PMid:18075864

20. Ajizian SJ, English BK, Meats EA. Specific Inhibitors of p38 and Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase Pathways Block Inducible Nitric Oxide Synthase and Tumor Necrosis Factor Accumulation in Murine Macrophages Stimulated with Lipopolysaccharide and Interferon-γ. J Infect Dis. 1999; 179:939–44. https://doi.org/10.1086/314659 PMid:10068590

21. Choo QY, Ho PC, Tanaka Y, and Lin HS. The histone deacetylase inhibitors MS-275 and SAHA suppress the p38 mitogen-activated protein kinase signaling pathway and chemotaxis in rheumatoid arthritis synovial fibroblastic E11 cells. Molecules. 2013; 18:14085-14095. https://doi.org/10.3390/molecules181114085 PMid:24241152

22. Osaki T, Peron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, Lotze MT, and Tahara H. IFN-g-inducing factor/IL-18 Administration Mediates IFN-g and IL-12-Independent Antitumor Effects. J Immunol. 1998; 160:1742-1749. PMid:9469432

23. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, Carrascal T, Walsh P, Reznikov LL, Kim SH, Novick D, Rubinstein M, and Dinarello CA. IL-18 regulates IL-1β-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. PNAS. 2000; 97:734-739. https://doi.org/10.1073/pnas.97.2.734 PMid:10639148 PMcid:PMC15399

24. Fernandes JV, Cobucci RNO. Jatobá CAN. Fernandes TAAM, Azevedo JVV, Araújo JMG. The Role of the Mediators of Inflammation in Cancer Development. Pathol Oncol Res. 2015; 21:527-534. https://doi.org/10.1007/s12253-015-9913-z PMid:25740073

25. Lissoni P, Brivio F, Rovelli F, Fumagalli G, Malugani F, Vaghi M, Secondino S, Bucovec R, Gardani GS. Serum concentrations of interleukin-18 in early and advanced cancer patients: enhanced secretion in metastatic disease. J Biol Regul Homeost Agents. 2000; 14:275-277. PMid:11215816

26. Choo QY, Ho PC, Tanaka Y, and Lin HS. Histone deacetylase inhibitors MS-275 and SAHA induced growth arrest and suppressed lipopolysaccharide-stimulated NF-κB p65 nuclear accumulation in human rheumatoid arthritis synovial fibroblastic E11 cells. Rheumatology. 2010; 49:1447–1460. https://doi.org/10.1093/rheumatology/keq108 PMid:20421217

27. Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. Gene Expression Profiles in Normal and Cancer Cells. Science. 1997; 276:1268-1272. https://doi.org/10.1126/science.276.5316.1268 PMid:9157888

28. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454:444-452. https://doi.org/10.1038/nature07205 PMid:18650914