Biomedicine & Pharmacotherapy 101 (2018) 145–154

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Inhibition of class IIa histone deacetylase activity by gallic acid, sulforaphane, TMP269, and panobinostat

Sin Young Choi[a], Hae Jin Kee[a,*], Li Jin[a,b], Yuhee Ryu[a], Simei Sun[a], Gwi Ran Kim[a], Myung Ho Jeong[a,**]

[a] Heart Research Center of Chonnam National University Hospital, Gwangju 61469, Republic of Korea
[b] Jilin Hospital Affiliated with Jilin University, 4 Nanjing Street, Chuanying, Jilin, 132011, China

ARTICLE INFO

Keywords:
HDAC enzyme activity assay
Class IIa histone deacetylase
HDAC8
Gallic acid
TMP269
Piceatannol
Sulforaphane

ABSTRACT

Histone deacetylase (HDAC) inhibitors are gaining increasing attention as potential therapeutics for cardiovascular diseases as well as cancer.

We recently reported that the class II HDAC inhibitor, MC1568, and the phytochemical, gallic acid, lowered high blood pressure in mouse models of hypertension. We hypothesized that class II HDACs may be involved in the regulation of hypertension. The aim of this study was to determine and compare the effects of well-known HDAC inhibitors (TMP269, panobinostat, and MC1568), phytochemicals (gallic acid, sulforaphane, and piceatannol), and anti-hypertensive drugs (losartan, carvedilol, and furosemide) on activities of class IIa HDACs (HDAC4, 5, 7, and 9).

The selective class IIa HDAC inhibitor, TMP269, and the pan-HDAC inhibitor, panobinostat, but not MC1568, clearly inhibited class IIa HDAC activities. Among the three phytochemicals, gallic acid showed remarkable inhibition, whereas sulforaphane presented mild inhibition of class IIa HDACs. Piceatannol inhibited only HDAC7 activity. As expected, the anti-hypertensive drugs losartan, carvedilol, and furosemide did not affect the activity of any class IIa HDAC.

In addition, we evaluated the inhibitory effect of several compounds on the activity of class l HDACs (HDAC1, 2, 3, and 8) and class IIb HDAC (HDAC6). MC1568 did not affect the activities of HDAC1, HDAC2, and HDAC3, but it reduced the activity of HDAC8 at concentrations of 1 and 10 μM. Gallic acid weakly inhibited HDAC1 and HDAC6 activities, but strongly inhibited HDAC8 activity with effectiveness comparable to that of trichostatin A. Inhibition of HDAC2 activity by sulforaphane was stronger than that by piceatnnaol.

These results indicated that gallic acid is a powerful dietary inhibitor of HDAC8 and class IIa/b HDAC activities. Sulforaphane may also be used as a dietary inhibitor of HDAC2 and class IIa HDAC. Our findings suggest that the class II HDAC inhibitor, MC1568, does not inhibit class IIa HDAC, but inhibits HDAC8.

1. Introduction

Histone deacetylases (HDACs) are a family of enzymes that remove acetyl groups from ε-*N*-acetyl lysine amino acid on a histone and many non-histone proteins including p53, signal transducers and activators of transcription (STAT3), E2F1, heat shock protein 90 (Hsp90), and NF-κB [1]. Deacetylation by HDACs causes chromatin compaction and transcription repression, whereas acetylation of histones facilitates chromatin access and induces activation of gene transcription.

HDACs are divided into four classes depending on structure. Class I HDACs include HDAC1, HDAC2, HDAC3, and HDAC8 which are mainly distributed in the nucleus. Class IIa HDACs include HDAC4, HDAC5, HDAC7, and HDAC9 which can translocate from the nucleus to the cytoplasm. Class IIb HDACs include HDAC6 and HDAC10. HDAC11 is a class IV HDAC. These HDACs are all zinc-dependent. HDAC expression and activity are dysregulated in various diseases including asthma [2], chronic obstructive pulmonary disease (COPD) [3], cancer [4], cardiac hypertrophy [5], and neurodegenerative and psychological disorders [6]. Thus, HDAC inhibitors could be a potential therapeutic target for many diseases.

To date, vorinostat (SAHA), romidepsin (depsipeptide), panobinostat (LBH589), and belinostat (PXD101) HDAC inhibitors have been

* Corresponding author at: Heart Research Center of Chonnam National University Hospital, 42 Jebong-ro, Dong-gu, Gwangju 61469, Republic of Korea.
** Corresponding author at: FACC, FAHA, FESC, FSCAI, FAPSIC, Director of Heart Research Center Nominated by Korea Ministry of Health and Welfare, Chonnam National University Hospital, 42 Jebong-ro, Dong-gu, Gwangju 61469, Republic of Korea.
E-mail addresses: sshjkee@empas.com (H.J. Kee), myungho@chollian.net (M.H. Jeong).

https://doi.org/10.1016/j.biopha.2018.02.071
Received 29 November 2017; Received in revised form 2 February 2018; Accepted 19 February 2018

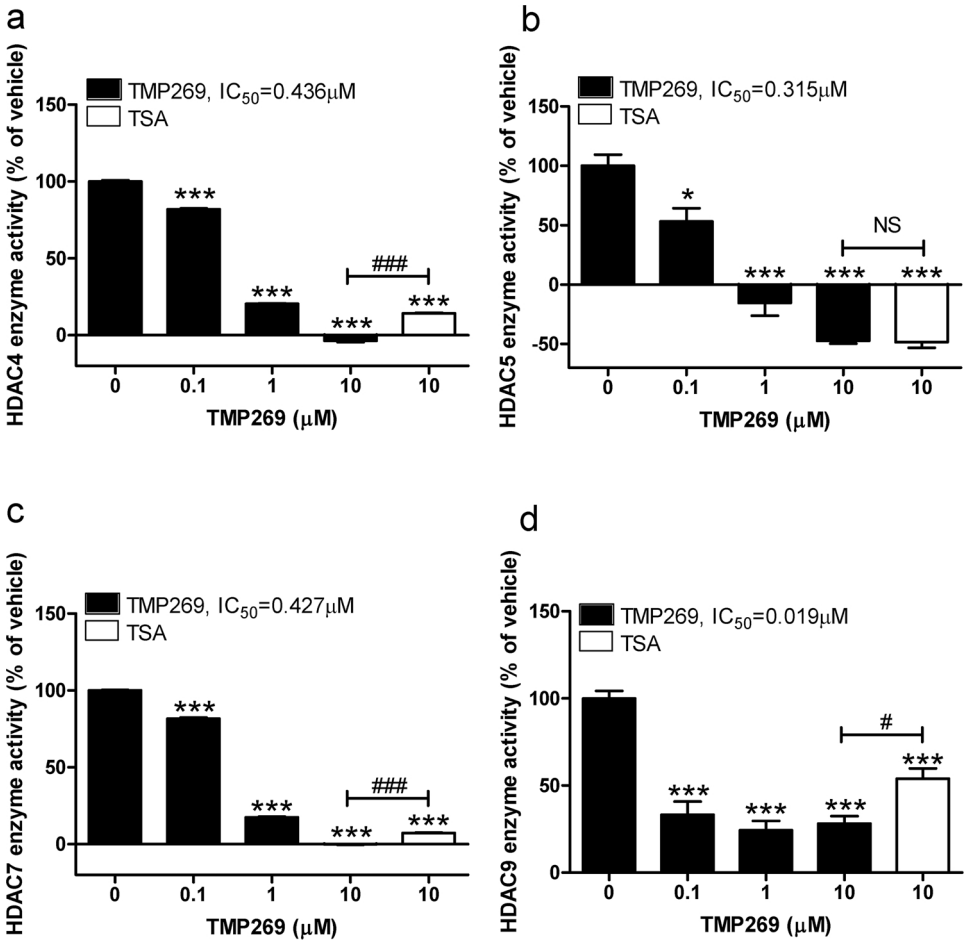

Fig. 1. TMP269 inhibits HDAC4, HDAC5, HDAC7, and HDAC9 enzyme activities.
a–d, HDAC4 (a), HDAC5 (b), HDAC7 (c), and HDAC9 (d) enzyme activities were measured in the cell-free system. TMP269 was tested at different concentrations (0.1–10 μM). TSA (10 μM) was used as the reference compound. * $p < 0.05$ and *** $p < 0.001$ versus vehicle group. # $p < 0.05$ and ### $p < 0.001$ versus TSA-treated group. NS indicates not significant. Data represent the means ± SE of at least three independent experiments.

approved by the United States Food and Drug Administration (FDA) for the treatment of cutaneous T cell lymphoma (CTCL) and peripheral T cell lymphoma (PTCL). Chidamide is approved in China for the treatment of PTCL. HDAC inhibitors are divided into four classes: hydroxamate, cyclic peptide, benzamide, and aliphatic acids. Hydroxamate includes trichostatin A (TSA), vorinostat, panobinostat, and belinostat. TSA is the most intensively studied pan-HDAC inhibitor. Depsipeptide belongs to the cyclic peptide class. MS-275 (etinostat) and MGCD0103 (mocetinostat) are benzamides which generally target class I HDACs. Valproic acid, sodium butyrate, and phenyl butyrate are aliphatic acids. Zinc-dependent HDAC inhibitors have common pharmacophores composed of cap group, linker, and zinc-binding domain.

Hypertension is a major leading risk factor in cardiovascular diseases. In hypertension, aortic stiffness is usually increased and vascular smooth muscle cells (VSMCs) contribute to vascular stiffness [7]. Therefore, we used VSMCs to test the degree of acetylation of histones in this study. Lemon et al. reported HDAC6 catalytic activity induced in deoxycorticosterone acetate (DOCA)-salt hypertensive rats [8]. We have demonstrated increased HDAC6 and HDAC8 activities in DOCA-salt hypertensive rats. Interestingly, valproic acid treatment inhibited both enzyme activities in DOCA-salt hypertension [9]. Recently, we have identified that tubastatin A, a selective HDAC6 inhibitor, did not affect high blood pressure in angiotensin II-induced hypertensive mice, implying that HDAC6 enzyme activity is not associated with the development of hypertension [10]. MC1568 is a class IIa/b HDAC inhibitor. We have demonstrated that MC1568 lowers angiotensin II-induced hypertension [11]. This result suggests that class IIa HDACs might have a critical role in hypertension.

Gallic acid is a trihydroxybenzoic acid found in many plants. Especially, black tea has high amounts of gallic acid [12]. Piceatannol is a metabolite of resveratrol and is found in red wine. Sulforaphane is organosulfur compound found in broccoli sprouts. Sulforaphane has been reported to inhibit HDAC activity in human colorectal and prostate cancer cells [13,14].

Gallic acid, piceatannol, and sulforaphane have been shown to negatively regulate hypertrophy [15–17]. Gallic acid was reported to reduce hyperglycemia-induced cytokine secretion and NF-κB activity in human monocytes (THP-1 cells) through downregulation of histone acetyltransferase and upregulation of HDAC2, indicating that gallic acid has a potential for the treatment of diabetes [18]. Furthermore, we have demonstrated that gallic acid reduces hypertension in spontaneously hypertensive rats [19] and in mice with *N*-nitro-L-arginine methyl ester-induced hypertension [20]. However, the inhibitory HDAC enzyme activity of phytochemicals, including gallic acid, piceatannol, and sulforaphane, has not yet been investigated in a cell-free system.

Here, we examined the class IIa HDAC enzyme activity of HDAC inhibitors, phytochemicals, and anti-hypertensive agents in a cell-free system. We found that gallic acid mildly inhibited HDAC1, HDAC4, and HDAC6. Gallic acid showed strong suppression of HDAC5, HDAC7, HDAC8, and HDAC9 enzyme activities. Sulforaphane attenuated class IIa HDAC and HDAC2 enzyme activities. TMP269 and panobinostat completely inhibited class IIa HDACs. Piceatannol inhibited HDAC7 enzyme activity. We demonstrate that gallic acid is a new dietary inhibitor of class IIa/b HDACs as well as HDAC8.