

Lacosamide at therapeutic concentrations induces histone hyperacetylation in vitro

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SUMMARY

Inhibition of histone deacetylases (HDACs) and subsequent hyperacetylation of histone proteins lead to altered gene expression associated with therapeutic drug effects, but also with teratogenicity. The only US Food and Drug Administration (FDA)-approved antiepileptic drug that has been consistently shown to induce histone hyperacetylation is valproic acid. More recently, lacosamide was reported to interfere with histone modifications, but histone hyperacetylation was not demonstrated. In the current study we evaluated the effects of lacosamide on histone acetylation in vitro. MDA-MB-231 (triple-negative breast cancer) cells and human placental BeWo cells were exposed for 16 hours to 5–20 $\mu\text{g/ml}$ (20–80 μM) lacosamide. Histone acetylation was evaluated by western blot analysis. We additionally measured HDAC1 activity in the presence of lacosamide. At 5, 10, and 20 $\mu\text{g/ml}$, lacosamide enhanced histone acetylation in BeWo cells by 1.7-fold ($p > 0.05$), 3.4-fold ($p < 0.05$), and 3.0-fold ($p > 0.05$), respectively. Histone H3 acetylation and total histones H3 and H4 levels were not significantly modified ($p > 0.05$). The magnitude of change in histone acetylation in MDA-MB-231 cells was smaller ($p > 0.05$). In contrast to valproic acid, lacosamide did not inhibit HDAC1. Our findings suggest that the effects of lacosamide on gene expression, and the related potential antitumor activity and teratogenicity, may differ from those of valproic acid.

KEY WORDS: Histone deacetylase, Lacosamide, Valproic acid, Pregnancy, Cancer therapy.

Lacosamide, a synthetic derivative of the amino acid D-serine, is a third-generation antiepileptic drug (AED) approved for use in focal epilepsy since 2008. Unlike older-generation sodium-blocking AEDs that modulate fast inactivation, lacosamide selectively enhances slow activation of voltage-gated sodium channels.¹ Lacosamide additionally binds to collapsin response mediator protein 2 (CRMP2), which mediates neuroprotective and neuroregenerative

responses. Lacosamide is generally well tolerated in the majority of adult patients,² with a suggested serum reference range of 10–40 μM (2.5–10 $\mu\text{g/ml}$).²

Recently, lacosamide has been shown to reduce in the rat brain the expression of histone deacetylase (HDAC)1,³ a member of a family of proteins that remove acetyl groups from lysine on histones.⁴ HDACs regulate the degree of DNA compaction and play a key role in gene expression and cell differentiation.⁴ One of the most established HDAC inhibitors is valproic acid,⁵ which, based on this activity, is being evaluated for its therapeutic effects in patients with solid and hematologic tumors, HIV, and other medical conditions (clinicaltrials.gov). However, histone hyperacetylation induced by valproic acid and some of its analogs and derivatives has additionally been associated with teratogenicity.^{6–8}

In 2004 we demonstrated that HDAC inhibition is not shared by commonly used AEDs other than valproic acid, which were approved at that time.⁹ In the current study, we evaluated the effects of lacosamide on histone acetylation

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and its HDAC1 inhibition potency in vitro. Valproic acid was used as the positive control.

METHODS

Cell culture

The human placental trophoblastic choriocarcinoma BeWo cells were cultured in F-12 Ham medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO₂ humidified incubator. The triple-negative breast cancer MDA-MB-231 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1% penicillin, 1% streptomycin, and 1% Glutamax. HeLa cervical carcinoma cells were cultured in DMEM, supplemented with 10% fetal calf serum, Glutamax, and 1% penicillin/streptomycin. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂/95% O₂. Glutamax was obtained from ThermoFisher Scientific (Waltham, MA, U.S.A.). All other cell culture reagents were from Biological Industries Ltd. (Beit Haemek, Israel).

Lacosamide (Cayman chemical, MI, U.S.A.) was dissolved in dimethylsulfoxide (DMSO), diluted in medium, and added to cells at a final concentration of up to 0.01% DMSO. Sodium valproate (Merck KGaA, Darmstadt, Germany) was dissolved in the culture medium. Cells (70% confluence) were incubated with 5, 10, or 20 µg/ml lacosamide (20, 40, or 80 µM, respectively, representing the higher range of measured lacosamide concentrations in many patients²) or 166 µg/ml sodium valproate (equivalent to 144 µg/ml valproic acid; 1 mM, twice the inhibition constant (K_i) for HDAC inhibition⁹) in culture medium for 16 hours. Controls were prepared by incubating cells for the corresponding period in culture medium containing 0.1% DMSO.

Analysis of histone acetylation

Histone acetylation studies were conducted as we described recently,¹⁰ with slight modifications. Briefly, histones were extracted using histone extraction kit (Abcam, Cambridge, UK), according to the manufacturer's instructions. Protein concentrations were determined by a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.). Samples were mixed with sodium dodecyl sulfate (SDS) sample buffer and underwent SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Stacking and separating gels were made of 5% and 17.5% acrylamide, respectively. Each lane was loaded with a 10 µg protein sample. Following electrophoresis, the proteins from the gels were transferred to nitrocellulose membranes using a Mini Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and 5% skim milk powder (Difco, Franklin Lakes, NJ, U.S.A.) and probed for 1 hour at room temperature with primary antibodies (Abcam, Cambridge, UK) against β-actin (1:1,000), histone H3 (1:1,000),

histone H4 (1:1,000), acetylated histone H3 (1:500), and acetylated histone H4 (1:20,000). The blots were incubated for 1 hour with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (Jackson ImmunoResearch, West Grove, PA, U.S.A.) at 1:10,000 (for acetylated histones) or 1:5,000 (for total histones) dilutions and developed by enhanced chemiluminescence. Assays in MDA-MB-231 cells were repeated 3 times. Assays in BeWo cells were repeated twice (n = 6 per each treatment group).

HDAC enzymatic assay

The activity of HDAC1 was measured using HDAC1 Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI, U.S.A.), according to manufacturer's instructions. For this analysis, we serially diluted in the HDAC1 Inhibitor Screening assay buffer a commercial liquid lacosamide preparation (Vimpat solution for Intravenous use; UCB, Inc., Brussels, Belgium). Human recombinant HDAC1 was incubated in a 96-well microplate with 200 µM acetylated substrate in assay buffer containing or lacking lacosamide (total volume 170 µl). An additional negative control was incubation of the substrate without HDAC1. Trichostatin A (TSA) at 12.35 µM and valproic acid at the indicated concentrations served as the positive controls. Following incubation at 37°C for 30 minutes, the reaction was stopped by addition of 40 µl TSA-containing developer. The plate was incubated for an additional 15 minutes at room temperature, and the newly formed fluorophore was detected on a fluorometric reader (SpectraMax iD3; Molecular Devices, San Jose, CA, U.S.A.; excitation at 350 nm, emission at 450 nm). Fluorescence was measured by an independent lab and the raw data were translated to percent HDAC inhibition as per the kit manufacturer's instructions. Results are reported as percentage HDAC1 activity as compared to control (activity in the absence of inhibitors).

Cell proliferation

Analysis was conducted using the XTT (2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) assay kit (Biological Industries Ltd.), according to the manufacturer's instructions.

Statistical analysis

The Kruskal-Wallis test followed by Dunn's post-test (InStat; GraphPad, La Jolla, CA, U.S.A.) was used to determine the statistical significance (p < 0.05) of the differences between experimental groups. Data are presented as mean and standard deviation.

RESULTS

Lacosamide's effect on histone acetylation

In the human placental cell line BeWo, histone H4 acetylation was significantly increased to 166 ± 154%

of control at 5 $\mu\text{g/ml}$ ($p > 0.05$), $343 \pm 81\%$ at 10 $\mu\text{g/ml}$ ($p < 0.05$), and $303 \pm 81\%$ at 20 $\mu\text{g/ml}$ lacosamide ($p > 0.05$; Fig. 1A,C). Histone H3 acetylation and total histones H3 and H4 levels were not affected by lacosamide (Fig. 1C). The magnitude of lacosamide's effect (at 10 μM and 20 μM) on histone H4 acetylation was comparable to that of 1 mM valproic acid. In MDA-MB-231 cells, lacosamide enhanced histone H4 acetylation to a lesser and statistically nonsignificant extent, up to 136% of control at 10 $\mu\text{g/ml}$ ($p > 0.05$; Fig. 1B). The effect of valproic acid on histone acetylation in MDA-MB-231 cells was also smaller than in BeWo cells. In contrast to the shared effect of lacosamide and valproic acid on histone acetylation, only valproic acid directly inhibited HDAC1, reducing its activity by up to 72% (Fig. 1D).

Lacosamide's effect on cell proliferation

After 72 hours of incubation, lacosamide slightly but significantly ($p < 0.05$) reduced, as compared to the vehicle, the proliferation of HeLa cells, but not that of MDA-MB-231 cells (Fig. 2).

DISCUSSION

Valproic acid has been the only AED demonstrated to significantly affect histone acetylation.⁹ Although carbamazepine was suggested to exert HDAC inhibitory activity,¹¹ the related change in histone acetylation was described as moderate and its magnitude was not reported. In addition, data on the antineoplastic effects of carbamazepine treatment in patients with malignant glioma are not consistent.¹² Here, we establish the histone-modifying activity of an additional AED, lacosamide, at therapeutic concentrations.

The effect of lacosamide on histone acetylation was demonstrated particularly in BeWo cells. These cells were selected based on valproate-induced histone hyperacetylation in human placental tissue,¹⁰ and as a part of a project aimed at characterizing the effects of AEDs on the human placenta. Significant histone hyperacetylation was observed at 10 $\mu\text{g/ml}$ lacosamide, a therapeutically relevant concentration. Although the effect of 5 $\mu\text{g/ml}$ was not statistically significant, it reflects a trend, suggesting that concentrations lower than 10 $\mu\text{g/ml}$ might as well induce histone

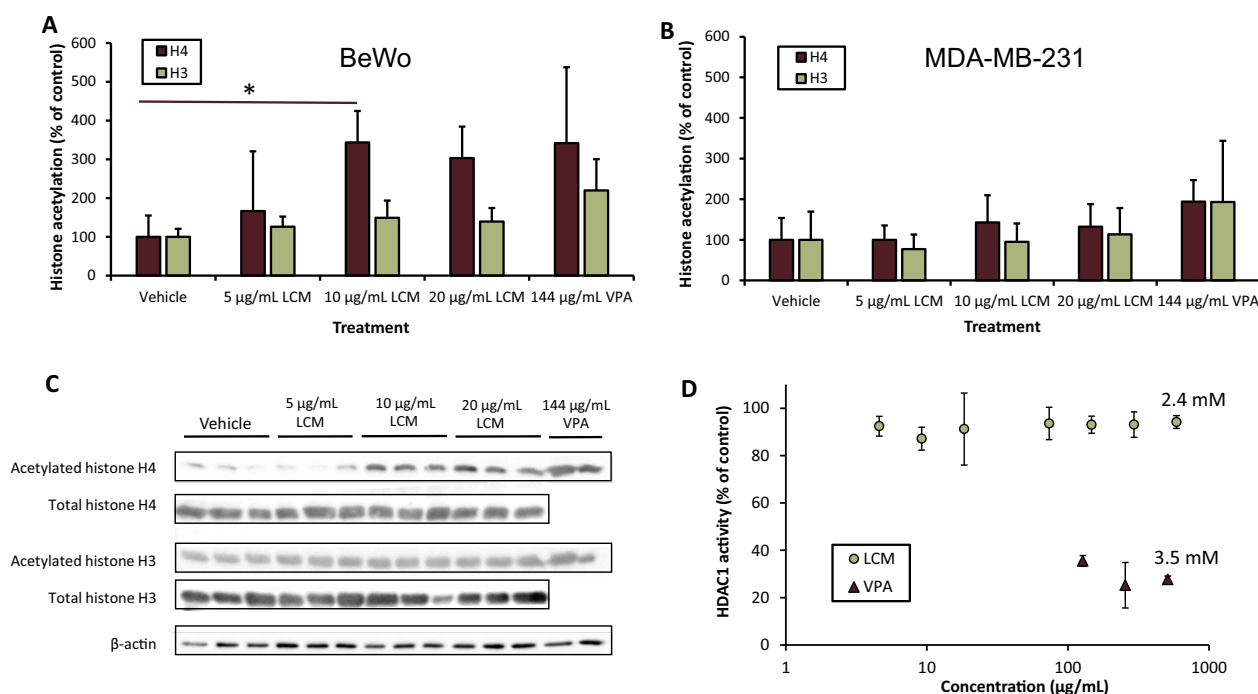


Figure 1.

Lacosamide induces histone hyperacetylation without directly inhibiting HDAC1 activity. **(A)** Histone acetylation in BeWo cells. **(B)** Histone acetylation in MDA-MB-231 cells. **(C)** Representative images demonstrating the effects of lacosamide on total and acetylated histones H3 and H4 in BeWo cells. Cells were exposed to lacosamide for 16 hours. Histone acetylation was evaluated by western blotting. Results are presented as percent of control (mean \pm standard deviation [SD]); $n = 6$ /treatment group, except for the valproic acid group of BeWo cells ($N = 3$; the group was not included in the statistical comparisons of BeWo cells). * $p < 0.05$, Kruskal-Wallis test. **(D)** HDAC1 activity (percent of control). Analyses were conducted twice, in triplicate. Valproic acid was used at final concentrations of 51–510 $\mu\text{g/ml}$ (0.35–3.5 mM). Also indicated are the maximal molar concentrations of each compound. LCM, lacosamide; VPA, valproic acid. *Epilepsia Open* © ILAE

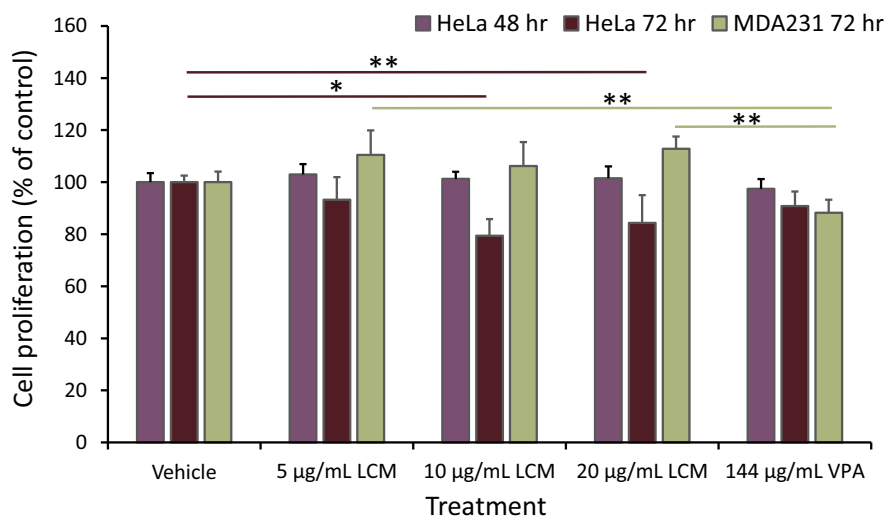


Figure 2.

Lacosamide reduces the proliferation of HeLa cells. HeLa cells were incubated with lacosamide for 48 or 72 hours. MDA-MB-231 cells were exposed to lacosamide for 72 hours. Cell proliferation was measured by the XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. Results are presented as mean \pm SD (n = 6/group). *p < 0.05, **p < 0.01, Kruskal-Wallis test. LCM, lacosamide; VPA, valproic acid.

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hyperacetylation in placental cells. MDA-MB-231 cells represent human triple-negative breast cancer, an aggressive subgroup of these tumors, which accounts for approximately 15-20% of newly diagnosed breast cancer cases worldwide.¹³ These cells were used as a model for evaluating the potential antineoplastic lacosamide effects on extracerebral tumors but were less sensitive than HeLa cells to the antiproliferative effects of both lacosamide and valproic acid.

The mechanism(s) by which lacosamide induces histone hyperacetylation have yet to be clarified. Our results indicate that lacosamide does not modify the cellular levels of total histones H3 and H4 and is not a direct inhibitor of HDAC1, although inhibition of other HDAC isoforms cannot be ruled out. Hence, the downstream effects of lacosamide might be different than those of other HDAC inhibitors including valproic acid, which inhibit HDAC1 and other HDACs.⁴ Previously, intraperitoneal treatment of rats with 30 mg/kg lacosamide was associated with reduced HDAC1 protein expression in the cerebral cortex.³ However, histone acetylation was not evaluated in that study. Additional potential effects of lacosamide might include enhanced expression of histone acetyltransferases (which mediate histone acetylation), or HDAC inhibition by lacosamide's metabolites.

The antiproliferative effect of lacosamide in HeLa cells (Fig. 2), although mild, is in line with recent data from glioma cell lines, although it has been attributed to CRMP2 inhibition.¹⁴ Others have demonstrated lacosamide-induced interference of cell cycle and inhibition of cell migration.¹⁵ These findings suggest that lacosamide should be further evaluated as an antineoplastic agent.

Lacosamide is being increasingly prescribed to pregnant women with epilepsy, although data on its teratogenicity are sparse.¹⁶ The drug label states that "oral administration of lacosamide to pregnant rats (20, 75, or 200 mg/kg/day) and rabbits (6.25, 12.5, or 25 mg/kg/day) during the period of organogenesis did not produce any teratogenic effects. However, the maximum doses evaluated were limited by maternal toxicity in both species and embryo/fetal death in rats." In those studies, lacosamide's area under the curve (AUC) was approximately 2-fold higher (rats) or similar (rabbit) to that obtained in humans at the maximum recommended human dose of 400 mg/day.¹⁷ A recent report described 3 cases of maternal exposure to lacosamide at a median daily dose of 400 mg, without major or minor congenital malformations in the offspring. Normal developmental milestones were achieved in all newborns.¹⁸ Notably the concentrations chosen in our study, based on plasma data, might not represent those obtained in each target tissue of lacosamide.

It is notable that the value of these findings is not transferable to lacosamide's antiepileptic effects considering that no central nervous system (CNS) cell lines have been used and that the antiepileptic effects of this drug can be very rapid.

In conclusion, lacosamide at therapeutic concentrations significantly affects histone acetylation in vitro. However, our data suggest that despite the shared effects on histone proteins of lacosamide, valproic acid, some of valproic acid's analogs and derivatives, and other HDAC inhibitors, the downstream effects of these changes may vary across compounds. Further studies are required to establish the effects of lacosamide on tumors. Although direct HDAC

inhibition has also been associated with teratogenicity, the effects of lacosamide on histone proteins differ from those of valproic acid, and to date preclinical studies with lacosamide have not demonstrated structural teratogenicity. Whether lacosamide interferes with cognitive development is as yet unknown. Given the complexity of treating pregnant women with AEDs, data from pregnancy registries are urgently required to direct lacosamide use in women of childbearing age.

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DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

- Bialer M, Johannessen SI, Levy RH, et al. Progress report on new antiepileptic drugs: a summary of the Ninth Eilat Conference (EILAT IX). *Epilepsy Res* 2009;83:1–43.
- Svendsen T, Brodtkorb E, Baftiu A, et al. Therapeutic drug monitoring of lacosamide in Norway: focus on pharmacokinetic variability, efficacy and tolerability. *Neurochem Res* 2017;42:2077–2083.
- Bang SR, Ambavade SD, Jagdale PG, et al. Lacosamide reduces HDAC levels in the brain and improves memory: potential for treatment of Alzheimer's disease. *Pharmacol Biochem Behav* 2015;134:65–69.
- Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nat Rev Drug Discov* 2014;13:673–691.
- Göttlicher M, Minucci S, Zhu P, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001;20:6969–6978.
- Eyal S, Yagen B, Shimshoni J, et al. Histone deacetylases inhibition and tumor cells cytotoxicity by CNS-active VPA constitutional isomers and derivatives. *Biochem Pharmacol* 2005;69:1501–1508.
- Gurvich N, Berman MG, Wittner BS, et al. Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo. *FASEB J* 2005;19:1166–1168.
- Phiel CJ, Zhang F, Huang EY, et al. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J Biol Chem* 2001;276:36734–36741.
- Eyal S, Yagen B, Sobol E, et al. The activity of antiepileptic drugs as histone deacetylase inhibitors. *Epilepsia* 2004;45:737–744.
- Rubinichik-Stern M, Shmuel M, Bar J, et al. Adverse placental effects of valproic acid: studies in perfused human placentas. *Epilepsia* 2018;59:993–1003.
- Beutler AS, Li S, Nicol R, et al. Carbamazepine is an inhibitor of histone deacetylases. *Life Sci* 2005;76:3107–3115.
- Gefroh-Grimes HA, Gidal BE. Antiepileptic drugs in patients with malignant brain tumor: beyond seizures and pharmacokinetics. *Acta Neurol Scand* 2016;133:4–16.
- von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol* 2012;30:1796–1804.
- Moutal A, Villa LS, Yeon SK, et al. CRMP2 phosphorylation drives glioblastoma cell proliferation. *Mol Neurobiol* 2018;55:4403–4416.
- Rizzo A, Donzelli S, Girgenti V, et al. In vitro antineoplastic effects of brivaracetam and lacosamide on human glioma cells. *J Exp Clin Cancer Res* 2017;36:76.
- Meador KJ, Pennell PB, May RC, et al. Changes in antiepileptic drug-prescribing patterns in pregnant women with epilepsy. *Epilepsy Behav* 2018;84:10–14.
- Vimpat label. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022253s039,022254s030,022255s0221bl.pdf. Revised 11/2017, Accessed May 26, 2018.
- Lattanzi S, Cagnetti C, Foschi N, et al. Lacosamide during pregnancy and breastfeeding. *Neurol Neurochir Pol* 2017;51:266–269.