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Valproic acid neuroprotection in 6-OHDA lesioned rat, a model for parkinson's disease

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Abstract

Background: Valproic acid (VPA) a long standing anti-epileptic and anti-manic drug has been recently investigated as a neuroprotective molecule, in relation to its action as an inhibitor of histone deacetylases (HDACs), favoring relaxed configuration of chromatin and thus promoting gene transcription.

Methods: In the present study, chronic administration of VPA added to the diet, was tested for neuroprotection in a rat model of Parkinson's disease. The model consists of multiple injections of the dopaminergic toxin, 6-hydroxydopamine (6-OHDA), unilaterally in the striatum with consequent degeneration of the dopaminergic neurons originating the nigro-striatal pathway. This model of neurodegeneration is widely used as a reliable animal model for Parkinson's disease (PD).

Results: Chronic VPA administration significantly reduced degeneration of dopaminergic neurons in the substantia nigra, and of dopaminergic terminals in the striatum, in rats subjected to the unilateral lesion of the nigrostriatal pathway. VPA treatment was also able to increase α-synuclein expression in the substantia nigra and to counteract the lesion-dependent decrease of the protein in the substantia nigra itself and in the striatum.

Conclusions: Present data, which follow previous results obtained in the rotenone rat model of nigrostriatal degeneration, allow to propose VPA as a treatment to be tested for its effectiveness in other animal models of parkinsonism, in view of possible translation to patients.

Background

Animal models of Parkinson's disease (PD) have been studied for many years to understand the etiology of the disease and to test putative therapeutic interventions [1-4]. In particular, the dopaminergic toxin, 6-hydroxydopamine (6-OHDA) has been widely used to lesion the nigro-striatal pathway and to reproduce in rodents main neuropathologic features of PD [5,6]. Multiple intrastriatal injections of this neurotoxin result in rapid loss of dopaminergic terminals in the striatum itself followed by slower (3-4 weeks) and partial retrograde degeneration of dopaminergic neurons in the substantia nigra [7]. This lesion reproduces in the rat model Parkinson-like conditions and is particularly useful when chronic pharmacologic treatments are devised to counteract the relatively slow and partial degeneration of dopaminergic nigral neurons [7].

Valproic acid (VPA), a long standing anti-epileptic and anti-manic drug, has recently revealed neuroprotective properties possibly related, among the several other targets, to its action as histone deacetylase (HDAC) inhibitor [8-10]. In particular, VPA neuroprotection has been demonstrated in several culture models: spontaneous age-related death and excitotoxic damage of cortical neurons [11,12], beta-amyloid toxicity in hippocampal neurons [13], non-depolarizing conditions and exposure to 6-OHDA in cerebellar granule neurons [14,15], ischemic conditions in cultured hippocampal slices [16]. In vivo, VPA was proven neuroprotective in several animal models of acute brain damage and neurodegenerative diseases: transient cerebral ischemia in rats [17], animal models of Huntington's disease [18,19] and Alzheimer disease [20,21], as well as in clinical trials on patients affected by spinal muscular atrophy [22]. Following reports on culture-based studies showing that HDAC inhibitors protect against dopaminergic neurotoxicity [15], we have recently demonstrated that VPA added to the diet protects nigral dopaminergic neurons in the rotenone rat model of PD [23]. We provide here evidence that the same treatment significantly protects dopaminergic neurons of the substantia nigra in the 6-OHDA model of multiple striatal toxin injections and that neuroprotection is related to the maintenance of increased a-synuclein expression in the substantia nigra. The positive results of VPA treatment in two different types of Parkinson-like degeneration suggest the opportunity to test such treatment in other animal models of the disease in view of possible translation to patients.

Methods

Male Wistar rats from Harlan Nossan, weighing 200–220 grams were used. All animals were maintained on a 12:12 h light/dark cycle and given food and water ad libitum. The experiments were carried out in agreement with the Italian and European Community laws on the use of animals and protocols were approved by a local bioethical committee. After acclimation (age of rats around 11-12 weeks) , rats of the VPA groups were fed for 4 weeks with standard chow added with 2% sodium valproate (Sigma, St Louis, MO). As previously reported [23], VPA feeding resulted in approximate daily intake of 1.4g/kg body weight, which has been shown to result in mean plasma concentration of 42.6 mg/L, close to the human therapeutic window [24]. Animals

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anesthetized with Zoletil (20 mg/Kg) were placed in a David Kopf stereotaxic instrument and four injections of 6-OHDA (7 μg free base/2μl each, dissolved in saline containing 0.02% ascorbic acid) were made at four locations into the left striatum according to the procedures previously described [7]. Injection were made through a 5 μl Hamilton syringe to the needle of which a thin (50-70 μm in diameter) glass capillary had been fixed with wax, in order to minimize penetration damage to the tissue. Coordinates of the injections (in mm) were the followings: AP: +1.3, +0.4, -0.4 -1.3 with respect to bregma; ML: 2.6, 3.0, 4.2, 4.5 with respect to midline; DV: 5.2 below the dura. After each injection, needle was left in place for 2 min and then slowly retracted. Equivalent injections of saline were made in the contralateral striatum. After surgery, rats survived for further 4 weeks during which the same previous feeding regimens were maintained. For western blot analysis, rats were killed by decapitation, the brains were sliced with a tissue chopper, samples of the substantia nigra and the striatum were dissected under the stereomicroscope on a cold plate and frozen in dry ice. Samples were homogenized in 50 mM Tris buffer containing 5 mM EDTA, 0.1% SDS, a protease inhibitor cocktail and standard western blot analysis was performed, using antibodies against tyrosine hydroxylase (TH, Sigma) and a-synuclein (clone P4D1, Santa Cruz) [15]. In the same homogenates, b-actin content was determined in parallel as reference protein with a specific antibody (Sigma). Densitometric results were statistically evaluated by one way ANOVA followed by Bonferroni's tests. For immunohistochemistry, rats were deeply anesthetized and perfusion-fixed through heart with 4% paraformaldehyde in 100 mM phosphate buffer pH 7.4. Brains removed from the skull were postfixed overnight in the same fixative and left in 18% sucrose until sunk. Frozen free-floating sections, 40 μm thick, were processed for immunohistochemistry using a TH monoclonal antibody (TH-16, Sigma, 1:5,000) and a HRP-linked secondary antibody. Reaction was visualized using diaminobenzidine as chromogen (DAB).

Results & Discussion

After 4 weeks from 6-OHDA injections into the left striatum, this region appeared shrunken and largely devoid of TH immunoreactivity in normally-fed rats while damage was less evident in VPA-fed rats (Figure 1). In the substantia nigra of normally-fed (control) rats immunoreactivity of TH-positive dopaminergic neurons (Figure 2A) was almost completely lost in the 6-OHDA injected side (Figure 2B) while these neurons appeared significantly spared in the injected side of VPA-treated rats (Figure 2C). Partial sparing of 6-OHDA lesioned dopaminergic neurons in VPA-fed rats was confirmed by western blot analysis for TH immunoreactivity in homogenates of substantia nigra and quantitative analysis based on band densitometry (Figure 3A). While TH content was significantly decreased by more than 60% in the substantia nigra of control rats, this decrease was substantially recovered in VPA-fed rats (Figure 3A). A similar trend of reduced TH loss in VPA-fed animals was also observed in homogenates of striata (Figure 3B). Western blot analysis of substantia nigra homogenates revealed that VPA treatment increased the level of expression of a-synuclein with respect to controls and that the decrease of this protein in the substantia nigra of control rats injected with 6-OHDA was completely restored in the same region of VPA-fed rats (Figure 4A). Also regarding a-synuclein,

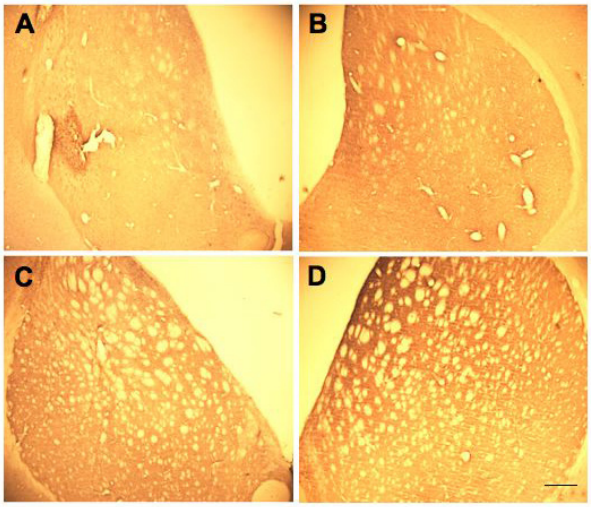

Figure 1. Effect of VPA treatment on 6-OHDA injection at the lesion site in the striatum. TH immunoreactivity in the 6-OHDA injected (A, left) and contralateral (B, right) striatum of a normally-fed rat, 4 weeks after toxin injection. Equivalent pictures of the striatum in a VPA-fed rat (C, left; D, right). Calibration bar, 500 μm.

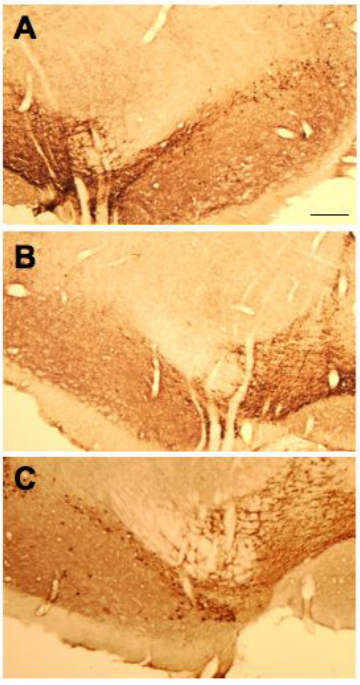

Figure 2. VPA neuroprotection from 6-OHDA toxicity in the substantia nigra. Immunohistochemistry for TH in a control substantia nigra (A), in the same region of a normally-fed 6-OHDA-lesioned rat (B) and of a VPA-fed 6-OHDA-lesioned rat (C). Calibration bar: 500 μm.

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striatal homogenates showed trends of variation similar, albeit not statistically significant, to those described in the substantia nigra (Figure 4A). Following previous results obtained with the rotenone model for PD [23], our novel demonstration of neuroprotection in the 6-OHDA model of the disease suggests that VPA may have a general neuroprotective role in PD. Our data supporting the beneficial effect of VPA on 6-OHDA lesion are presently based on qualitative immunohistochemistry for TH in both substantia nigra and striatum as well as on quantitative densitometry of western blots from tissue extracts. To better quantify the neuroprotective effect, future studies should include cell counts in the substantia nigra and high resolution analysis of TH-positive terminals in the striatum. From our previous results [23], we know that the regimen of VPA administration adopted here is able to effectively inhibit HDAC and to increase histone acetylation state in the substantia nigra and the striatum, at the time when 6-OHDA injection is made. While VPA is a relatively unspefic HDAC inhibitor, its prevalent effect is against class I HDACs which are highly expressed in brain [25]. Due to the numerous molecular targets of VPA [9], the link between its neuroprotective action and the increase of hystone acetylation is still correlative. It will be important for future research to test VPA in other models of PD, in particular those obtained through genetic manipulation of mice or targeted delivery of mutated genes to the rat brain using the viral vector technology [2]. This research could hopefully open novel therapeutic perspectives for patients. Previous results of limited clinical trials did not demonstrate any beneficial effect of VPA in PD patients [26] and suggested that reversible parkinsonism and cognitive impairment could be developed by patients treated with high doses of VPA for epilepsy [27]. However, a recent report based on three cases of PD patients demonstrated that daily doses of 500-1000 mg of VPA ameliorated compulsive behaviors associated with dopaminergic medications prescribed to patients and allowed to reduce drug dosages without worsening PD symptoms [28]. Thus VPA could act as a neuroprotectant against degeneration of dopaminergic neurons in parkinsonism (present results and reference 23) and fight at the same time compulsive behaviors due to treatments with levo-DOPA and dopaminergic agonists [28]. Taken together, these results may bring to devise novel trials for PD patients based on VPA treatment, particularly in the case that additional positive data derive from experiments based on VPA administration in genetic animal models.

The molecular targets of VPA are multifarious [9] and it is likely that multiple cellular pathways are involved in its neuroprotective effects. A specific molecular determinant of this neuroprotection emerging from the present study, is the VPA-related increase of a-synuclein levels in the substantia nigra, and the consequent protection from the decrease caused by the neurotoxic lesion. Previous results demonstrated a similar effect of VPA on a-synuclein expression in mice brain and linked the increased expression to neuroprotection from excitotoxicity [10]. This novel concept of VPA-mediated neuroprotective role of a-synuclein, fits with other recent studies on a-synuclein neuroprotection in culture and in vivo, including PD-like models of neurodegeneration [9,10,29].

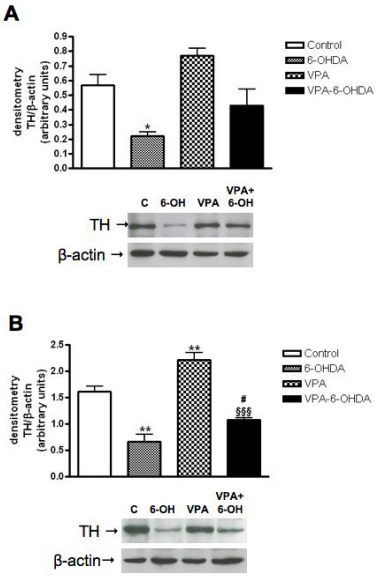

Figure 3. VPA neuroprotection from 6-OHDA toxicity in substantia nigra and striatum. Western Blot analysis and relative densitometries of TH and β-actin, as reference protein, expression in substantia nigra (A) and striatum (B) from control, 6-OHDA-treated, VPA-treated and VPA+6-OHDA-treated animals. In the graphic, the level of TH was normalized for the β-actin content in each sample and the data were expressed as arbitrary units. Each bar represents the mean ± S.E. of 3–6 samples from different animals. A: *p<0.05 with respect to control; B: **p<0.01 with respect to control, #p<0.05 compared to 6-OHDA, Bonferroni's test after ANOVA.

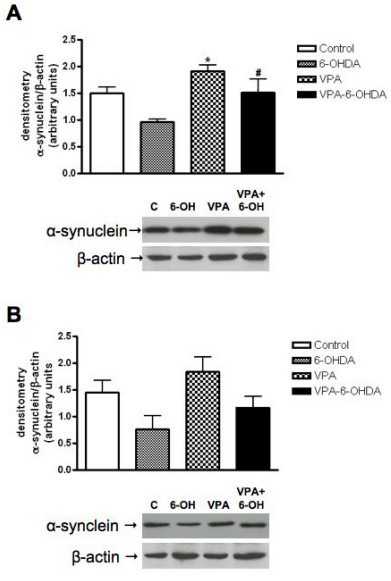

Figure 4. Western Blot analysis and relative densitometries of α-synuclein and β-actin, as reference protein, expression in substantia nigra (A) and striatum (B) from control, 6-OHDA-treated, VPA-treated and VPA+6-OHDA-treated animals. The level of α-synuclein was normalized for the β-actin content in each sample and the data were expressed as arbitrary units. Each bar represents the mean ± S.E. of 3–6 samples from different animals. *p<0.05 with respect to control, #p<0.05 with respect to 6-OHDA, §§§p<0.01 with respect to VPA; Bonferroni's test after ANOVA.

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Conclusion

In conclusion, the demonstration that a chronic treatment with VPA added to the diet is neuroprotective in the rat against different types of nigrostriatal degeneration, allows to propose this treatment as a model to be tested for its effectiveness in other Parkinson-related animal studies in view of possible translation to patients. Present, as well as previous [9,10,15,23] data suggest the interest of focusing this research on the effects of VPA on histone-regulated transcriptional activity and on a-synuclein expression in brain regions relevant to PD.

Competing interests

The authors declare that they have no competing interests.

Author's contribution

BM participated in designing experiments, carried on wester blot analysis and contributed to writing the paper. DM participated to animal treatment, surgery and immunohistochemical experiments. AC participated in designing experiments, did surgery and wrote first draft of the paper.

Author's information

BM is assistant professor and AC full professor at the Department of Biology, University of Bologna. DM was a master thesis student and is presently a PhD student at the University of Bologna.

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References

1. Ulusoy A, Decressac M, Kirik D, Bjorklund A: **Viral vector-mediated overexpression of alpha-synuclein as a progressive model of Parkinson's disease**. *Prog Brain Res* 2010; **184**;(89-111.
2. Magen I, Chesselet MF: **Genetic mouse models of Parkinson's disease The state of the art**. *Prog Brain Res* 2010; **184**;(53-87.
3. Cannon JR, Greenamyre JT: **Neurotoxic in vivo models of Parkinson's disease recent advances**. *Prog Brain Res* 2010; **184**;(17-33.
4. Pienaar IS, Gotz J, Feany MB: **Parkinson's disease: insights from non-traditional model organisms**. *Prog Neurobiol* 2010; **92**;(4.);558-71.
5. Blandini F, Armentero MT, Martignoni E: **The 6-hydroxydopamine model: news from the past**. *Parkinsonism Relat Disord* 2008; **14 Suppl 2**;(S124-9).
6. Deumens R, Blokland A, Prickaerts J: **Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway**. *Exp Neurol* 2002; **175**;(2.);303-17.
7. Kirik D, Rosenblad C, Bjorklund A: **Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat**. *Exp Neurol* 1998; **152**;(2.);259-77.
8. Gottlicher M, Minucci S, Zhu P, Kramer OH, Schimpf A, Giavara S*, et al.*: **Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells**. *EMBO J* 2001; **20**;(24.);6969-78.
9. Monti B, Polazzi E, Contestabile A: **Biochemical, molecular and epigenetic mechanisms of valproic acid neuroprotection**. *Curr Mol Pharmacol* 2009; **2**;(1.);95-109.
10. Leng Y, Chuang DM: **Endogenous alpha-synuclein is induced by valproic acid through histone deacetylase inhibition and participates in neuroprotection against glutamate-induced excitotoxicity**. *J Neurosci* 2006; **26**;(28.);7502-12.
11. Jeong MR, Hashimoto R, Senatorov VV, Fujimaki K, Ren M, Lee MS*, et al.*: **Valproic acid, a mood stabilizer and anticonvulsant, protects rat cerebral cortical neurons from spontaneous cell death: a role of histone deacetylase inhibition**. *FEBS Lett* 2003; **542**;(1-3.);74-8.
12. Mark RJ, Ashford JW, Goodman Y, Mattson MP: **Anticonvulsants attenuate amyloid beta-peptide neurotoxicity, Ca2+ deregulation, and cytoskeletal pathology**. *Neurobiol Aging* 1995; **16**;(2.);187-98 98
13. Hashimoto R, Hough C, Nakazawa T, Yamamoto T, Chuang DM: **Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation**. *J Neurochem* 2002; **80**;(4.);589-97.
14. Mora A, Gonzalez-Polo RA, Fuentes JM, Soler G, Centeno F: **Different mechanisms of protection against apoptosis by valproate and Li+**. *Eur J Biochem* 1999; **266**;(3.);886-91.
15. Monti B, Polazzi E, Batti L, Crochemore C, Virgili M, Contestabile A: **Alpha-synuclein protects cerebellar granule neurons against 6-hydroxydopamine-induced death**. *J Neurochem* 2007; **103**;(2.);518-30.
16. Rekling JC: **Neuroprotective effects of anticonvulsants in rat hippocampal slice cultures exposed to oxygen/glucose deprivation**. *Neurosci Lett* 2003; **335**;(3.);167-70.
17. Ren M, Leng Y, Jeong M, Leeds PR, Chuang DM: **Valproic acid reduces brain damage induced by transient focal cerebral ischemia in rats: potential roles of histone deacetylase inhibition and heat shock protein induction**. *J Neurochem* 2004; **89**;(6.);1358-67.
18. McCampbell A, Taye AA, Whitty L, Penney E, Steffan JS, Fischbeck KH: **Histone deacetylase inhibitors reduce polyglutamine toxicity**. *Proc Natl Acad Sci U S A* 2001; **98**;(26.);15179-84.
19. Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL*, et al.*: **Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila**. *Nature* 2001; **413**;(6857.);739-43.
20. Qing H, He G, Ly PT, Fox CJ, Staufenbiel M, Cai F*, et al.*: **Valproic acid inhibits Abeta production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models**. *J Exp Med* 2008; **205**;(12.);2781-9.
21. Eleuteri S, Monti B, Brignani S, Contestabile A: **Chronic dietary administration of valproic acid protects neurons of the rat nucleus basalis magnocellularis from ibotenic acid neurotoxicity**. *Neurotox Res* 2009; **15**;(2.);127-32.
22. Weihl CC, Connolly AM, Pestronk A: **Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy**. *Neurology* 2006; **67**;(3.);500-1.
23. Monti B, Gatta V, Piretti F, Raffaelli SS, Virgili M, Contestabile A: **Valproic acid is neuroprotective in the rotenone rat model of Parkinson's disease: involvement of alpha-synuclein**. *Neurotox Res* 2010; **17**;(2.);130-41.
24. Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P*, et al.*: **Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis**. *J Neurosci* 2004; **24**;(29.);6590-9.
25. Contestabile A, Sintoni S, Monti B (2012) Histone deacetylase (HDAC) inhibitors as potential drugs to target memory and adult hippocampal neurogenesis. Curr Psychopharmacol 1:
26. Nutt J, Williams A, Plotkin C, Eng N, Ziegler M, Calne DB: **Treatment of Parkinson's disease with sodium valproate: clinical, pharmacological, and biochemical observations**. *Can J Neurol Sci* 1979; **6**;(3.);337-43.
27. Armon C, Shin C, Miller P, Carwile S, Brown E, Edinger JD*, et al.*: **Reversible parkinsonism and cognitive impairment with chronic valproate use**. *Neurology* 1996; **47**;(3.);626-35.
28. Hicks CW, Pandya MM, Itin I, Fernandez HH: **Valproate for the treatment of medication-induced impulse-control disorders in three patients with Parkinson's disease**. *Parkinsonism Relat Disord* 2011; **17**;(5.);379-81.
29. Manning-Bog AB, McCormack AL, Purisai MG, Bolin LM, Di Monte DA: **Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration**. *J Neurosci* 2003; **23**;(8.);3095-9.

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