HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neurodegenerative disease of the central nervous system induced by human T-lymphotropic virus type 1. As a potential therapeutic approach, we previously suggested reducing the proviral load by modulating lysine deacetylase activity using valproic acid (VPA) and exposing virus-positive cells to the host immune response. We conducted a single-center, 2-year, open-label trial, with 19 HAM/TSP volunteers treated with oral VPA. Proviral load, CD38/HLA-DR expression, and CD8+ lysis efficiency were not significantly affected by VPA. Mean scores of HAM/TSP disability did not differ between baseline and final visit. Walking Time Test improved rapidly after VPA discontinuation. We conclude that long-term treatment with VPA is safe in HAM/TSP. (Blood. 2011;118(24):6306-6309)

Methods

VPA given orally (20 mg/kg per day) was planned to be given for 24 months when the trial started. Clinical assessment was performed every 3 months by the same observer. DSS was used as a measure of global neurologic disability, 30-meter Walking Time Test (WTT) quantified gait performance, manual muscle score, and modified Ashworth score were applied to 9 muscle groups in both legs, and urinary disability was assessed by a short questionnaire. Overall analysis of neurologic disability test was performed based on observed cases and also using the last observation carried forward method. Worsening of neurologic disability was defined as: significant mean scores or time differences between M0 and M24 or M0 and the last assessment visit before treatment discontinuation and individually by the increase DSS score of 1 or 0.5 point regarding baseline DSS (< 5.5 or ≥ 5.5, respectively) or a WTT variation rate > 20%. The study was approved by the Local and Regional Research Ethics Committee, and all procedures were carried out in accordance with the Declaration of Helsinki.


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Figure 1. CTL lysis efficiency and CD38/HLA-DR expression in HAM/TSP subjects treated with VPA. (A) Before and after VPA treatment (eg, days −6, +29, and +42 are illustrated), the rate of CD8 cell-mediated lysis of HTLV-1–infected cells was estimated as described previously. CD8 lymphocytes were selected by MACS and titrated back into the CD8-depleted fraction at different ratios. Reconstituted cell populations were cocultivated at 37°C for 18 hours, fixed, and analyzed by FACS for Tax, CD4, and CD8 expression. The proportion of Tax+ CD4 cells surviving coculture was plotted against the proportion of CD8 cells present. Two independent experiments were performed with 3 HAM/TSP patients 1 through 3. (B) As previously described, a mathematical model was then used to analyze the data. The model describes the onset of Tax expression in CD4 cells and the lysis of Tax+ CD4 cells by CD8 cells. The model was solved analytically and then fitted to the data using nonlinear regression. The rate of lysis of CD4 Tax+ cell, “CD8 cell lysis efficiency,” is estimated. CD8 cell lysis efficiency (expressed as the proportion of Tax-expressing CD4 cells killed per CD8 cell per day) was calculated for each HAM/TSP patient tested. All assays were done in duplicate, and the results are presented as the mean CD8 cell lysis efficiency. Indicated values result from experiments performed in duplicate at days −6, +10, +15, +29, +35, +42, and +56. (C) The median absolute change in lysis efficiency between consecutive time points was plotted against initial rate of lysis efficiency for control patients (●) and VPA treated patients (○). (D) Expression of CD38 and HLA-DR in CD4 cells before (at month −1; upper plots) and after (at month +1: lower plots) initiation of VPA treatment. PBMCs were labeled with the MultiTEST CD4 FITC/CD38 PE/CD3 peridinin chlorophyll protein/anti–HLA-DR allophycocyanin and analyzed with a FACSaria (BD Biosciences). After gating of CD3 cells (left panels: peridinin chlorophyll protein), the frequencies of FITC-labeled CD4* cells expressing either CD38 (middle plots: PE) or HLA-DR (right panels: allophycocyanin) were determined. (E) FACS analysis of PBMCs isolated from 5 patients before (month −1; M-1) and after (at 3 months: M3) initiation of VPA treatment: percentages of CD4+ or CD8+ (CD3− CD4−) cells expressing CD38 or HLA-DR.

The efficiency of CD8+ cell–mediated lysis before and after VPA treatment was performed as described by Mosley et al. The proportions of CD4+ or CD8+ cells expressing CD38 or HLA-DR were determined by flow cytometry using the MultiTEST assay from BD Biosciences.
Results and discussion

A major concern with VPA is that it may impair the anti-viral immune response. Indeed, the rate of CD8\(^+\) cell-mediated lysis of Tax-expressing cells ex vivo is halved in short-term cultures complemented with a 5mM dose of VPA.\(^8\) Although this concentration is indeed above the levels that can be achieved in patients (estimated \(\sim 1-2\text{mM}\)), the risk of long-term treatment with lower doses cannot be predicted. Because the CTL response is an important factor in the immune control of HTLV-1 infection,\(^2\) we addressed this question directly by measuring the efficiency of CD8\(^+\) cell-mediated lysis. The cytotoxic response was evaluated by an assay in which the number of Tax\(^+\) cells surviving autologous CD8\(^+\) killing is measured by FACS after overnight culture.\(^9\) The percentages of surviving Tax\(^+\) CD4\(^+\) cells were then plotted against the proportion of CD8\(^+\) cells present (duplicate assays are...
shown in Figure 1A and □). Lysis efficiency, which was estimated by fitting a mathematical model to these data, was calculated and expressed as the proportion of Tax-expressing CD4+ cells killed per CD8+ cell per day (Figure 1B). To assess whether these variations were within the normal range observed in untreated patients, the initial rate of lysis efficiency was plotted against the median absolute change in lysis efficiency between consecutive time points. Lysis efficiency variations observed during VPA treatment (□) were not significantly different from those in untreated patients (Figure 1C, P = 0.07, Wilcoxon-Mann-Whitney 2-tailed test). We conclude that CD8+ cell-mediated lysis efficiency fluctuates throughout treatment but remains within the normal range, indicating that the subject’s CTL immune response against HTLV-1 is not significantly suppressed by VPA. Likewise, the CTL response is not significantly exacerbated and must not be a concern regarding HAM/TSP progression.

To support this conclusion, we next assessed T-cell activation as defined by expression of CD38 and HLA-DR. PBMCs from 5 patients before and 3 months after initiation of VPA treatment were analyzed by FACS (illustrated for patient 4 on the plots of Figure 1D and recapitulated for 5 patients on Figure 1E). It appeared that there is no significant difference in the proportions of CD4+ or CD8+ cells expressing CD38 and HLA-DR.

The initial objective of VPA treatment in HAM/TSP patients was to permanently reduce the PVL with the aim of attenuating collateral damages to the central nervous system. However, long-term administration of VPA did not achieve this goal. The PVL determined by real-time PCR10 after 12 and 24 months or the last quantification before treatment discontinuation was similar to before treatment (Figure 2A). Based on last observation carried forward analysis, no significant differences were found in mean neurologic scores and WTT (Figure 2B). No patient’s DSS score increased significantly over the study. Eight of 19 patients stopped VPA treatment before M24 (Figure 2C). Three patients were withdrawn because of a significant increase in the rate of variation of WTT. WTT improved rapidly after treatment discontinuation. Gait impairment worsening was probably related to severe drowsiness and tremor side effects experienced by 3 patients. The main clinical side effects of VPA were drowsiness (52%), tremor (47%), digestive symptoms (37%), vertigo (26%), and alopecia (10%), and their frequencies tended to decrease over the trial course. No significant biologic side effect was documented. Walking deterioration in a few patients may be associated with VPA side effects and is quickly reversible after treatment discontinuation.

In conclusion, we have shown that CTL response is preserved on VPA treatment of HAM/TSP patients. Although clinical symptoms are not improved, our data clearly indicate that long-term treatment with VPA is safe. This report is important in view of the recent evidence in STLV-1 infected baboons in which combined treatment with VPA and azidothymidine efficiently decreases PVL. If effective in human, long-term administration of VPA and azidothymidine may reduce the risk of HAM/TSP. Safety is also crucial for maintenance therapy of acute ATL where molecular clearance was observed with VPA combined with azidothymidine plus IFN-α.

References


Acknowledgments

This work was supported by the Fonds National de la Recherche Scientifique, the Télécie, the Belgian Foundation against Cancer, the Sixth Research Framework Program of the European Union (project INCA LSHC-CT-2005-018704), the Neoangio excellence program and the Partenariat Public Privé INCA of the Direction générale des Technologies, de la Recherche et de l’Energie/DG06 of the Walloon government, the Action de Recherche Concertée Glyvir of the Communauté française de Belgique, the Fonds spéciaux pour la Recherche of the University of Liège, the Synbiofor program of GxABT (University of Liège), the Centre anticancéreux près University of Liège, and the Plan Cancer of the Service Public Fédéral. M.B., N.G., and S.R. (Postdoctoral Researchers) and L.W. (Research Director) are members of the FRS-FNRS.

Authorship

Contribution: S.O., A.S., and D.S. performed clinical evaluation of VPA treatment; G.B., O.V., and A.L. measured proviral loads; N.G. and C.B. performed CTL lysis assay; M.B. performed the FACS analysis of CD38 HLA-DR; B.A. modeled data; S.O., A.L., S.R., R.C., and L.W. designed, coordinated, and supervised studies; and S.O. and L.W. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Safety of long-term treatment of HAM/TSP patients with valproic acid

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