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Comparative Study between Valproic Acid Combined with Conventional Chemotherapy Versus Conventional Chemotherapy Alone in Egyptian Acute Myeloid Leukemia Patients

Hashem Neanaa[1], Nahla AM Hamed[1*], Ahmad Raafat[2], Iman Diab[3] and Ahmed Shehata[1]

[1]*Departments of Clinical Hematology, Alexandria University, El-Gaish Rd, Egypt*

[2]*Faculty of Medicine and Faculty of Science, Alexandria University, El-Gaish Rd, Egypt*

[3]*Medical Biochemistry, Alexandria University, El-Gaish Rd, Egypt*

[*]**Corresponding author:** Nahla AM Hamed, Departments of Clinical Hematology, Alexandria University, El-Gaish Rd, Egypt, Tel: +20 101605847; E-mail: drhamedn@hotmail.com

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Abstract

It has been postulated that inhibition of Histone Deacetylase (HDAC) can restore normal acetylation of histone proteins and transcription factors, and to be of benefit in the treatment of cancer. So, this study aimed at assessing the response to histone deacetylase inhibitor valproic acid combined with conventional chemotherapy versus conventional chemotherapy alone in Egyptian Acute Myeloid Leukemia (AML) patients. Thirty newly diagnosed AML patients were divided into 2 groups. Group 1 consisted of 15 AML patients received conventional chemotherapy while group 2 consisted of 15 AML patients received valproic acid 40 mg/kg for 7 days and conventional chemotherapy. Ten healthy persons of matched age and sex were considered group 3 (controls). Serum histone deacetylase activity, vascular endothelial growth factor, basic fibroblast growth factor, tumor necrosis factor α, glutathione S transferase and nuclear factor κB in nuclear extract before and after chemotherapy were measured in all patients and controls. Results revealed better clinical response with no side effects with valproic acid than conventional chemotherapy alone (p=0.021). This was associated with statistically significant decrease in histone deacetylase activity in patients receiving valproic acid compared to the other AML group (p=0.00019). There was significant negative correlation between age and HDAC activity at initial presentation in patients receiving valproic acid (p=0.039) while no significant correlation was detected with the other studied laboratory parameters. Our results revealed that valproic acid in the tested dose was safe and associated with better therapeutic response when used in combination with conventional chemotherapy.

Keywords: HDAC; Valproic acid; Angiogenesis; Acute leukemia

Introduction

Acute Myeloid Leukemia (AML) is a myeloid malignancy characterized by deregulated proliferation, increased self-renewal and limited differentiation of myeloid blasts. AML is typically diagnosed in elderly patients and the standard treatment is mainly chemotherapy. Most patients relapse and perish from the disease or its associated complications. Aggressive chemotherapeutic treatment can only be used in a minority of patients; hence, there is a great need, for effective targeted therapy with less toxicity and better tolerability [1].

Reduced or abnormal acetylation of numerous Histone Deacetylase (HDAC) has been identified in leukemia, lymphoma and solid tumor cell lines [2]. Aberrant recruitment of HDAC activity has been reported in Acute Promyelocytic Leukemia (APL) cell lines. Furthermore, resistance to the differentiating actions of all-trans-retinoic acid in an APL patient was overcome by co-treatment with an HDAC inhibitor [3]. Another example is acute myelogenous leukemia associated with the chromosomal translocation t(8;21) [3]. In both of these cases, transcriptional repression appears to be mediated by recruitment of HDAC to the transcriptional repressor complex [3].

HDAC inhibition has therefore been postulated to restore normal acetylation of histone proteins and transcription factors, and to be of benefit in the treatment of cancer [2]. Histone deacetylase inhibitors (HDACi) are a class of drugs that alter the acetylation status of both histone and non-histone proteins, thereby affecting cellular functions of neoplastic cells, such as transcriptional activation, cell proliferation, immune responses, cell differentiation, survival and angiogenesis [1]. Early phase clinical assessment indicated that treatment with HDAC is, may be effective in t(8,21) AML patients [4].

Among the different HDAC inhibitors currently undergoing clinical testing for myeloid malignancies, is Valproic Acid (VPA) [5]. VPA is a powerful HDAC inhibitor. VPA has a wide range of effects on AML cells. It has antiproliferative and proapoptotic effects and it can induce differentiation. Indirect effects are mediated through increased antileukemic immune reactivity. However, patients are heterogeneous with regard to both susceptibility to VPA and molecular mechanisms mediating the antileukemic effects. Low response rates have been seen when VPA has been used as monotherapy in AML [6].

Aim of the Work

To assess histone deacetylase activity in newly diagnosed acute myeloid leukemia patients and its relation to important mediators and to compare the influence of adding the histone deacetylase inhibitor, valproic acid to conventional chemotherapy on response and report any observed side effects.

Material and Methods

This study was performed on 30 newly diagnosed AML patients admitted to Hematology unit at Alexandria Main University hospital. The patients were divided into 2 groups according to the treatment protocol used, 15 patients each. Group 1 received conventional chemotherapy, 7+3 protocol (daunorubicin 45 mg/m^2 for 3 days and cytarabine 100 mg/m^2 for 7 days) [7] while group 2 received valproic acid 40 mg/kg for 7 days and conventional chemotherapy. Ten healthy persons of matched age and sex were also included (group 3, controls).

All patients in this study were subjected to: thorough history taking and detailed clinical examination, routine investigations including complete blood picture, bone marrow aspiration, cytochemical staining (myeloperoxidase and sudan black B) and cytogenetic analysis [8]. In addition, the following parameters were measured at diagnosis and after induction chemotherapy: serum Histone Deacetylase activity (HDAC) [9] and serum Vascular Endothelial Growth Factor (VEGF) by ELISA [10], serum basic Fibroblast Growth Factor (bFGF) by a competitive enzyme immunoassay, R & D systems-UK, [11], serum tumor necrosis factor-α (TNF-α) by Peprotech ELISA Development protocol technique [12], Nuclear Factor-κB (NF-κB) [13] in nuclear extracts using ELISA principle, in which NF-κB, was captured by a double-stranded oligonucleotidic probe containing the consensus binding sequence for NF-κB and serum glutathione S transferase activity by Continuous Spectrophotometric technique [14]. Written consent was taken from all patients. The study was approved by the local Ethics Committee.

Statistical analysis

Data were analyzed using SPSS version 9. Qualitative data were compared using chi square while quantitative data were compared using t test (for 2 means) and ANOVA test (for more than 2 means). Least Significant Difference (LSD) was used when F-value is significant to detect the presence of significance between each 2 groups. Paired t test was used to compare histone deacetylase activity before and after treatment. Correlations between histone deacetylase activity versus different studied parameters in group 2 were done using Pearson's correlation. p was considered significant if <0.05.

Results

Parameter	Group 1	Group 2	Controls	p value
Age (years) Mean±SD	33.27 ± 9	35.07 ± 13.8	34.6 ± 10.65	F=0.966 p=0.377
Sex				
Male (%)	10 (66.7%)	7 (46.6%)	5 (50%)	X2=1.35
Female (%)	5 (33.3%)	8 (53.3%)	5 (50%)	p=0.509
Response to therapy				
Responders (CR+PR)				P=0.021*
Non responders	9 (60.0%)	14 (93.3%)		
	6 (40.0%)	1 (6.7%)		
Group 1: AML patients received conventional chemotherapy, Group 2: AML patients on valproic acid and conventional chemotherapy. P is significant if <0.05				

Table 1: Demographic data of the three studied groups.

Parameter	Group 1	Group 2	Controls	Test of significance P value
VEGF (pg/ml)	446.96 ± 325.66	286.27 ± 141.095	51.60 ± 10.21	F=9.834* p=0.0001
bFGF (pg/ml)	16.267 ± 2.9147	21.10 ± 6.119	4.240 ± 0.49	F=49.884* P=0.0001
NF□B (OD/25µg NE)	1.64 ± 0.229	1.63 ± 219	0.21 ± 0.06	F=196.434* P=0.0001
TNF-α (pg/ml)	165.20 ± 24.31	161.87 ± 17.59	22.50 ± 6.67	F=212.302* P=0.0001
GST (O.D)	1.77 ± 0.107	1.65 ± 0.217	1.24 ± 0.18	F=29.454* P=0.0001
Group 1: AML patients received conventional chemotherapy, Group 2: AML patients on valproic acid and conventional chemotherapy. Values are expressed as mean ±SD; LSD: showed significant difference between all groups (1,2)*, (1,3)* and (2,3) for VEGF and bFGF, *: P is significant if <0.05				

Table 2: Biochemical data of the three studied groups at presentation.

Table 1 shows the demographic data of the three studied groups. No statistically significant difference was present between both groups regarding bone marrow blasts (t=0.95, 0.353). Both AML groups were matched for age, sex and tumor burden. Better response (11 complete remission, CR, 3 partial remission, PR and 1 refractory) was obtained in group 2 who received valproic acid and conventional chemotherapy than group 1 (7 CR, 2 PR and 4 refractory) who received only conventional chemotherapy (p=0.021). In group 1, normal cytogenetics were present in 10 cases (66.6%), t(15,17) in 2 cases (13.3%), t(8,21) in 1 case (6.7%), 46xx1p+ in 1 case (6.7%), and 45 xy-7 in 1 case (6.7%) while in group 2, normal cytogenetics were present in 10 cases (66.6%), t(16,16) in 1 case (6.7%), t(15,17) in 1 cases (6.7%),

and 46xy-20 in 1 case (6.7%). Table 2 shows the studied laboratory parameters in the three studied groups. There was statistically significant difference between both AML groups as regards VEGF and bFGF while TNFα, GST and NFκB were non significantly differed between both AML groups. Table 3 shows comparison between serum histone deacetylase activity before and after chemotherapy in the two AML groups. Statistically significant decrease in serum histone deacetylase activity was observed in those receiving valproic acid while statistically significant increase was present in AML group treated by conventional chemotherapy alone. Table 4 shows the correlation between histone deacetylase activity before and after treatment and the different studied laboratory parameters. There was significant negative correlation between age and HDAC activity at initial presentation (r=-0.538, p=0.039*) while no significant correlation was present with the other studied laboratory parameters.

HDAC activity (OD)	Group 1		Group 2	
	Before	After	Before	After
Mean ±S.D	0.21 ± 0.06	0.34 ± 0.05	0.30 ± 007	0.21 ± 0.1
P value	0.001*		0.00019*	
Group 1: AML patients received conventional chemotherapy, Group 2: AML patients on valproic acid and conventional chemotherapy, P is significant if <0.05				

Table 3: HDAC activity before and after chemotherapy.

Parameter	HDAC before		HDAC after	
	r	p	r	p
Age (years)	-0.538	0.039*		
BM Blast (%)	0.409	0.130	0.499	0.058
VEGF (pg/ml)	-0.313	0.257	-0.319	0.246
bFGF (pg/ml)	-0.252	0.364	-0.485	0.067
NFκB (OD/ 25 µg nuclear extract)	-0.166	0.554	-0.173	0.537
TNF-α (pg/ml)	-0.455	0.088	-0.203	0.467
GST (OD)	-0.344	0.209	-0.027	0.925
*Correlation is significant at the 0.05 level (2-tailed)				

Table 4: Correlation between HDAC before and after treatment with clinical and laboratory variables in group 2.

Discussion

Valproic acid (VPA) is a short chain fatty acid that had an antiepileptic and mood-stabilizing activity [5]. It inhibits growth and induces differentiation of murine B and human T-lymphoblastic cells [15]. VPA seems to exert its anticancer activity by inducing proteosomal degradation of HDAC2 [5].

HDAC inhibitors are likely to act synergistically with drugs acting via different mechanisms, such as shifting the balance of pro- and anti-apoptotic genes, inducing reactive oxygen species and inhibiting angiogenesis. HDAC inhibitors can synergize with many anti-cancer agents, including gemcitabine, paclitaxel, cisplatin, etoposide and doxorubicin, as well as the HSP90 inhibitor 17-AAG, the proteasome inhibitor bortezomib and the DNA methylation inhibitor 5-azacytidine [16].

Tang et al. [17] also reported the capability of valproic acid to overcome multidrug resistance phenotype in AML cell lines, and to synergy with Ara-C. In vitro data indicate that HDAC inhibitors are equally effective in killing proliferating and non-proliferating tumor cells.

The used dose of valproic acid in our study was 40 mg/kg which is matching with the dose used in Atmaca study [18] and is less than the minimum dose used in psychosis. A study done by Chavez-Blanco et al. [19] showed that magnesium valproate at a dose between 20 and 40 mg/kg inhibits deacetylase activity and hyperacetylates histones in tumor tissues.

The most serious adverse events of valproic acid are liver failure and teratogenicity. These adverse events are dose dependent so dose reduction was sufficient to reduce these side effects in most patients [20]. Neurologic side effects such as sedation, dizziness and tremor as well as mild gastrointestinal toxicities usually occur early during treatment. No hepatic toxicity or Neurological side effects were reported in any of our studied patients with the selected dose. However, in a separate phase I study, encephalopathy was seen in AML patients treated with valproic acid plus low-dose decitabine (20 mg/m^2/d for 10 days) [21].

Better clinical response was obtained in group 2 (93.3%) who received valproic acid and conventional chemotherapy than group 1 (60%). This was associated with significant decrease in HDAC postvalproic acid treatment (group 2) while HDAC was significantly increase postconventional chemotherapy (group 1) than its pretreatment level.

Valproic acid (VPA) can induce in vitro differentiation of primary AML blasts. Hematological improvement was observed in (24%) of non-responders or relapsed AML patients treated with All Trans-Retinoic Acid (ATRA) combined with valproic acid [22]. Another clinical study showed that VPA/ATRA combination results in transient control of AML evolved from a myeloproliferative disorder but not in patients with a primary or MDS-related AML [23,24].

The combination of epigenetic therapy in leukemia appears to be safe and active, and was associated with transient reversal of aberrant epigenetic marks in a previous study [25]. In our study, HDAC did not appear to produce its effect via the angiogenic factors (VEGF, b-FGF, TNFα), drug metabolizing enzymes GST or the proteasome inhibitor NFk since we did not find significant correlation between any of these parameters and HDAC before and after treatment in both groups. Sakajiri et al. [26] reported that HDAC inhibitors downregulate cyclin D1 levels, upregulate the cell cycle inhibitors p21 and p27 and inhibit the production of the angiogenic cytokine VEGF.

The genes encoding for proteins involved in angiogenesis including hypoxia-inducible factor 1a and its target vascular endothelial growth factor (VEGF), VEGF receptor VEGFR-1 and 2 and CXC chemokine receptor 4 were downregulated by HDAC inhibitors whereas gene encoding suppressor for angiogenesis such as p53, von Hippel Lindau, thrombospondin-1 and neurofibrin-1 were upregulated by HDAC inhibitors in different cancer and endothelial cells [27].

Conclusion

From the previous study, we can conclude that the use of valproic acid in combination with conventional chemotherapy is safe and produce better therapeutic response than the use of conventional chemotherapy alone so we recommend future studies including also resistant and relapsed cases.

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