Valproic acid combined with 13-cis retinoic acid and 1,25-dihydroxyvitamin D3 in the treatment of patients with myelodysplastic syndromes

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ABSTRACT

Valproic acid (VPA), an inhibitor of histone deacetylases, inhibits the growth of leukemia cells and induces their differentiation *in vitro*. In the present study, VPA in combination with two differentiating agents, 13-cis retinoic acid and 1,25-dihydroxyvitamin D3, was given to 19 previously untreated patients with MDS or CMML. Eight patients had to discontinue treatment before week 16 due to toxicity. According to international working group criteria, three patients (16%) responded to treatment. No correlation between VPA serum level, histone acetylation or clinical response was observed.

Key words: valproic acid, 13-cis retinoic acid, myelodysplastic syndromes.

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Patients with myelodysplastic syndromes (MDS) face impaired quality of life due to cytopenias and finally die with worsening pancytopenia and transformation to acute leukemia. Although some responses can be achieved with growth factors, immunosuppressive therapies, demethylation agents and cytotoxic drugs, in most cases supportive care remains the mainstay of MDS treatment. Valproic acid (VPA) is a potent inhibitor of histone deacetylases (HDAC). It can modify the structure of chromatin allowing recruitment of transcription factors to restore epigenetically suppressed genes.[1] VPA has been shown to possess antiproliferative activity and to overcome the differentiation block in leukemia blast cells.[2,3] VPA concentrations consistent with normal clinical dosage are believed to achieve the inhibition of HDAC.[2,4]

Retinoids and 1,25-dihydroxyvitamin D3 (VD3) have also been shown to inhibit the proliferation accompanied by induction of differentiation in several leukemia cell lines.[5] Although the single-agent activity of a retinoid and VD3 has been modest *in vivo*, the synergistic antileukemia activity of these drugs and inhibitors of HDAC *in vitro* suggest that reasonable responses could be achieved with combination therapy.[6-8] In the present study, the effect of VPA in combination with a retinoid and VD3 in the treatment of patients with MDS was investigated. VPA was used at a maximum tolerated dosage whereas retinoid and VD3 were used at a low dosage to minimize their possible side effects. The retinoid chosen was 13-cis retinoid acid since its efficacy seems to be one of the best documented in MDS.[5]

Design and Methods

Patients

Altogether nineteen patients with MDS or chronic myelomonocytic leukemia (CMML) were recruited into the present MDS study by the Finnish leukemia group in 15 hospitals. Each patient provided written informed consent. The protocol was approved with the ethical committees of all participating centres. The eligibility criteria were: diagnosis of MDS or CMML based on World Health Organization (WHO) classification criteria,[9] aged between 50 and 90

Table 1. Clinical and hematological characteristics of the patients at the beginning of the study.

Patient No	Gender	Age (years)	WHO subtype[9]	Time from diagnosis (weeks)	Cytogenetics	IPSS subgroup[18]
1	F	84	RCMD	1	-7	Int-2
2	M	78	RAEB-1	1	Normal	Int-1
3	F	74	RAEB-2	156	del(7), -7	High
4	F	74	CMML-1	1	Normal	Low
5	M	75	RAEB-2	8	Normal	Int-2
6	M	69	RAEB-1	1	Complex	Int-2
7	F	75	5q-	164	5q-	Int-1
8	F	81	RAEB-2	6	Complex	High
9	F	76	CMML-1	104	Normal	Low
10	F	73	RARS	260	Normal	Low
11	F	58	RAEB-2	42	+8	High
12	M	59	RAEB-1	12	Normal	Int-1
13	F	59	RCMD	5	Normal	Int-1
14	F	76	RAEB-2	4	Normal	Int-2
15	F	79	CMML-2	15	t(1;3), +8	Int-2
16	F	78	RAEB-1	12	Complex	Int-2
17	M	73	CMML-1	32	Normal	Int-1
18	M	84	RAEB-2	7	t(13;19), del(5)	Int-2
19	F	70	RAEB-1	165	5q-, inv(3)	Int-2

WHO: World Health Organization; RCMD: refractory cytopenia with multilineage dysplasia; RAEB: refractory anemia with excess of blasts; 5q-, MDS associated with isolated del(5q); CMML, chronic myelomonocytic leukemia; RARS: refractory anemia with ringed sideroblasts; IPSS: International Prognostic Scoring System; int: intermediate.

Table 2. Response to therapy and outcome.

Patient	Maximum/ median* serum VPA concentration during treatment (μmol/L)	Treatment duration (weeks)	Reason to off-study before week 16	Response	Change in the acetylation of histone H3	Survival from the start of treatment (months)
1	530/ND	7	Toxicity	SD	ND	3, dead
2	579/480	16		SD	No change	26, dead
3	622/510	11	Toxicity	SD	No change	7, dead
4	606/573	16		SD	Decrease	33, alive
5	485/345	16		HI-P	Increase	8, dead
6	412/358	8	PD	PD	No change	13, dead
7	371/163	8	Toxicity	SD	ND	29, dead
8	530/ND	4	Toxicity	SD	ND	6, dead
9	487/388	16		SD	Decrease	24, alive
10	617/526	10	Toxicity	SD	No change	24, alive
11	518/491	16		SD	No change	25, alive
12	454 /415	10	PD	PD	ND	22, dead
13	611/499	13	Toxicity	HI-H	Increase	19, alive
14	401/ND	2	PD	PD	ND	7, dead
15	583/518	11	Toxicity	SD	Increase	21, alive
16	565/ND	2	Toxicity	SD	ND	10, dead
17	601/523	16		HI-H, HI-N	No change	17, alive
18	562/522	16		PD	Increase	7, dead
19	559/520	10	PD	PD	Increase	8, dead

*SD: stable disease; PD: progressive disease; HI: hematological improvement; P: platelet; H: hemoglobin; N: neutrophil; ND: not determined; *median of at least three serum VPA concentrations measured within minimun of 8 weeks time period .*

years and unsuitable for aggressive chemotherapy or stem cell transplantation. Patients with less than 10 % blasts in bone marrow fulfilled additional criteria: symptomatic anemia requiring red cell transfusions, platelets $<50\times10^9$/L in two measurements or neutrophils $<1.0\times10^9$/L with an infection requiring intravenous antibiotics. Exclusion criteria were: any previous treatment for MDS (including growth factors) or secondary MDS. Patients' clinical characteristics are shown in Table 1.

Study protocol

Patients received 2 oral administrations of equal doses of VPA (Deprakine®, Sanofi-Synthelabo). Dose escalation was performed during the first two weeks to achieve the serum concentration of 500-700 μmol/L, which is the upper therapeutic range for the treatment of seizures. Serum VPA level was monitored weekly until the target VPA concentration was achieved and thereafter once a month. 13-cis retinoid acid (Roaccutan®, Roche) was administered at a dosage of 10 mg twice a day and VD3 1 μg (Etalpha®, Leo Pharma) once a day. Programmed duration of treatment was 16 weeks. Treatment response was assessed according to revised international working group (IWG) criteria for MDS.[10] Treatment toxicity was scored according to the WHO criteria. Comparisons were performed using a two-tailed Student´s t-test, Fisher´s exact probability test and χ^2 test (SPSS 13.0 version). p values of <0.05 were considered statistically significant.

Analysis of histone H3 acetylation

Acetylation of histone H3 was analyzed by Western blot using an antibody for human acetyl-histone H3 from Upstate Biotechnology. Histones were extracted from peripheral blood and/or bone marrow mononuclear cells for analysis.[11] Western blotting was performed as previously described.[3] The equivalence of gel loading in Western blotting was confirmed by Ponceau S protein stain (Sigma-Aldrich).

Results and Discussion

Response to treatment

Some clinical trials with VPA alone or in combination with all-trans retinoic acid have been reported in MDS[12-14] and AML.[4,14-17] In the pilot study of Kuendgen *et al.*,[17] patients with MDS or AML secondary to MDS were treated with VPA with or without all-trans retinoic acid resulting in remarkable response rate of 40 %.[12] In the follow-up study of 43 patients, an even higher response rate of 52 % was observed in those MDS patients with a normal blast count, while for the patients with excess blasts and CMML response rates were 6% and 0% respectively.[13] Besides these reports, in another study only 1 out of 7 high risk MDS patients had hematological improvement with VPA.[14]

In the present study, 15 patients with MDS and 4 patients with CMML with a median age of 73 years were recruited. In contrast to previous studies, none of

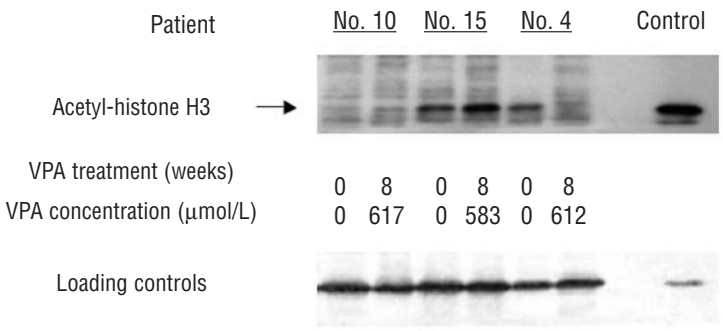

Figure 1. Representative example of histone H3 acetylation analysed from the bone marrow samples of 3 patients. After 8 weeks of VPA treatment there was no change (patient 10), an increase (patient 15) or a decrease (patient 4) in the acetylation status of histone H3. The serum VPA concentrations of patients are shown at the same time points. OCI/AML-2 cell line treated with 1 mM valproate for 48 hours was used as a positive control.[3]

the patients had received any treatment other than supportive therapy for MDS. The majority of the patients were recently diagnosed (Table 1). According to the International Prognostic Scoring System (IPSS), 8 patients (42 %) were categorized as low or int-1 risk subgroups and 11 as int-2 or high subgroups.[18] Seven patients continued treatment for 16 weeks (Table 2) and the median duration of the treatment was 11 weeks (range 2-16). Toxicity was the cause for the disruption of medication in 8 cases and 4 patients were withdrawn from the study due to a progressive disease. Three patients were unable to continue the medication for more than 4 weeks.

According to IWG criteria, 3 patients (16%) responded to treatment (Table 2) and the median time to response was 8 weeks (range 4-12). All the responses were hematological improvements. No complete or partial responses were observed. Hemoglobin concentration increased from 90 g/L to 116 g/L in patient number 13, whereas there was a rise in both hemoglobin and neutrophil values in patient number 17 (from 105 g/L to 123 g/L and from 0.46×10^9/L to 1.48×10^9/L, respectively). Patient number 5 responded to the treatment with an increase in platelet value from 67×10^9/L to 105×10^9/L. Furthermore, his peripheral blood and bone marrow blast cells decreased from 4% to 0% and from 19% to 7%, respectively. A reduction of the blasts with VPA has also been reported in some cases of AML.[4,13,15-17] All 3 responders had a normal karyotype. Two of the responders (patients 13 and 17) belonged to the low risk (int-1) and 1 (patient 5) to the high risk (int-2) category of IPSS. During VPA treatment, the disease remained stable in 11 patients but progressed in 5. Because of the low number of patients, prognostic factors were not determined. In contrast to earlier reports, the results of the present study showed that clinical responses can also be expected among patients with CMML (patient 17). The modest response rates of the present study compared to those reported using VPA monotherapy[12] does not support the idea that combining a low dose 13-retinoic and VD3 with VPA would result in any additional improvement.

Toxicity

Cheilitis and dry skin, well-known side effects of retinoids, were observed in 12 patients but were the reasons for discontinuing of treatment only in 1 patient. The most common side effect of the treatment was fatigue (10 patients) which led to an interruption of VPA treatment in 5 patients. Other severe toxicities leading to patients withdrawing from the study were grade 3 elevations of transaminases (n=1) and pneumonitis (n=1). Dizziness, headache, mental confusion, tremor, urinary incontinence, hypotonia and gastrointestinal side effects were also reported. There was no association between toxicity, IPSS subgroup and age. An increase in triglycerides known to be caused by retinoids was noticed in 17 out of 19 patients (*data not shown*). This demonstrated that the low dosage of 13-cis retinoid acid had some biological relevance. Since no hypercalcemia was observed, it might have been possible to use higher doses of VD3 in the combination treatment.

VPA serum level and induction of histone acetylation

A target VPA concentration of 500-700 μmol/L was achieved in 13 patients (Table 2). The mean VPA concentration was 525 μmol/L among non-responders (n=16) and 566 μmol/L among responders (n=3) with no statistically significant difference (p=0.415). In addition, no differences were observed in mean VPA concentrations between the patients who discontinued the treatment due to toxicity (n=8) and those who continued treatment for 16 weeks (n=7) (554 μmol/L vs. 512 μmol/L).

Histone H3 acetylation was analysed using Western blotting in 13 patients at week 8 or 16 (Figure 1). Histone acetylation increased in 5 patients (38 %), decreased in 2 patients and in 6 patients there was no change (Table 2). This finding that only part of patients induced histone acetylation with VPA, has also been observed in an AML trial.[19] Although there was no statistical correlation between histone H3 acetylation, VPA concentration or clinical response in the present study, a VPA-induced increase in histone H3 acetyla-

tion was shown in 2 out of the 3 responders. A third patient who responded had no change in histone H3 acetylation. It is, therefore, possible that, at least in some cases, a different mechanism other than inhibition of histone deacetylation could mediate the therapeutic effects of VPA.

Although VPA induced some hematological improvements in 2 patients with low-risk MDS and decreased the blast count in 1 patient with high-risk MDS, the overall response rate of 16 % suggests only a minor therapeutic benefit of VPA, a first-generation HDAC inhibitor, in the management of the patients in this disease group. However, combining VPA with chemotherapy or demethylating agents might be worth studying in future trials because of the promising synergistic effect observed between these agents *in vitro*.[3,20]

Authors' Contributions

The conception and design of the study were from TS and TT. TS made analysis and drafting the article. All the authors participated to the treatment of the patients, made equal contribution to the revision of the article, and provided final approval of the present version of the manuscript. In particular, E-RS gave a technical and logistic support for the study.

Conflicts of interest

The authors reported no potential conflicts of interest.

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