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Dextromethorphan Protect the Valproic Acid Induced Downregulation of Neutrophils in Patients with Bipolar Disorder

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Objective: Valproic acid (VPA) is an anticonvulsant and commonly long term used as a mood stabilizer for patients with mood disorders. However its chronic effects on the hematological changes were noticed and need to be further evaluated. In this study, we evaluated, in Taiwanese Han Chinese patients with bipolar disorders (BD), the chronic effects of VPA or VPA plus dextromethorphan (DM) on the hematological molecules (white blood cell [WBCs], red blood cells [RBCs], hemoglobin, hematocrit, and platelets).
Methods: In a 12-week, randomized, double-blind study, we randomly assigned BD patients to one of three groups: VPA plus either placebo (VPA+P, n = 57) or DM (30 mg/day, VPA+DM30, n = 56) or 60 mg/day (VPA+DM60, n = 53). The Young Mania Rating Scale and Hamilton Depression Rating Scale were used to evaluate symptom severity, and the hematological molecules were checked.
Results: Paired *t* test showed that the WBC, neutrophils, platelets and RBCs were significantly lowered after 12 weeks of VPA+P or VPA+DM30 treatment. VPA+DM60 represented the protective effects in the WBCs, neutrophils, and RBCs but not in the platelets. We further calculated the changes of each hematological molecules after 12 weeks treatment. We found that combination use of DM60 significantly improved the decline in neutrophils induced by the long-term VPA treatment.
Conclusion: Hematological molecule levels were lower after long-term treatment with VPA. VPA+DM60, which yielded the protective effect in hematological change, especially in the neutrophil counts. Thus, DM might be adjunct therapy for maintaining hematological molecules in VPA treatment.

KEY WORDS: Bipolar disorder; Dextromethorphan; Neutrophils; Blood platelets; Erythrocytes; Valproic acid.

INTRODUCTION

Valproic acid (VPA) is conventionally used to treat epilepsy. VPA is also extensively used as a first-line mood stabilizer in patients with bipolar disorder (BD) for months and years. Thus, the chronic side effects of VPA needed to be closely observed. In addition, accumulating evidence suggests that VPA treatment significantly may reduce the levels of hemoglobin [1], platelets [2,3] and neutrophils [4]. In young new BD patients, chronic VPA treatment caused thrombocytopenia [2]. In female but not the male patients with BD, a significant negative correlation between serum VPA level and platelet count was found [3]. Moreover, a 13-year-old Caucasian boy were demonstrated having mild anemia 12 months after the introduction of VPA therapy [1]. A 56-year-old white woman with tonic-clonic seizure and treated with VPA, developed severe neutropenia after 2 days of VPA therapy [4]. Thus, keeping the blood concentration of VPA at a safe therapeutic level (ca. 80 μg/ml) and closely monitoring

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the hematological toxicity during VPA treatment are noticed [3]. Furthermore, in addition to controlling blood levels of VPA, adjunct therapy combined with long-term VPA treatment to prevent hematological deterioration might be helpful.

In addition, because prior studies on hematological changes in patients treated with VPA were case reports and prospective observation studies rather than intervention studies, they did not control for confounding factors or analyze the time effects of VPA on the hematological changes. Thus, time effects and adjunct treatments require investigation.

Moreover, our previous study, in two medical centers National Cheng Kung University (NCKU) Hospital and National Defense Medical Center, showed that combination of dextromethorphan (DM) with VPA provided the therapeutic benefit in the plasma brain-derived neurotrophic factor (BDNF) levels of BD [5]. DM is a dextrorotatory opioid derivative that does not act on opioid receptors, it has been used as an antitussive drug with few side effects for more than 60 years. Previously study showed that femtomolar concentrations of dextromethorphan protected mesencephalic dopaminergic neurons in neuron-glia culture [6]. Thus, combination of DM not only provided benefit in plasma BDNF expression but also may has the neuronal protective effects [5] of BD. Moreover, DM also represented a significant cardiovascular protective effects. Study showed that low-dose of DM could reduce the blood pressure and enhances vascular protection through NADPH oxidase inhibition in rat [7]. Thus, we hypothesized that DM might has the hematological protective effects and that VPA plus DM would be more beneficial in the changes of hematology than would VPA-only for BD patients.

In the present study, we evaluated the chronic effects of long-term VPA-only therapy on hematological changes in a 12-week follow-up study. In NCKU Hospital, we collected and analyzed the related parameters in patients with BD. In addition, the protective effects of combination of DM (30 to 60 mg/day) with VPA in the changing of hematology were also evaluated.

METHODS

Patient Selection

We recruited the Taiwanese Han Chinese BD patients from the Department of Psychiatry at NCKU Hospital, Tainan. The NCKU Institutional Review Boards (IRB) for the Protection of Human Subjects approved the study protocol (IRB no. HR-95-110). After the study had been completely described to the participants, they all signed written informed consent forms.

The inclusion criteria were: (A) being a psychiatric inpatient or outpatient who sought psychiatric care or had been referred for psychiatric evaluation because suspected of having a mood disorder; (B) a diagnosis of BD-I or BD-II based on the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria and confirmed by the Chinese version of the modified Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L); (C) being between 18 and 65 years old; (D) a Hamilton Depression Rating Scale (HDRS) score of at least 18 or a Young Mania Rating Scale (YMRS) score of at least 14 at the screening stage; (E) able to communicate in Mandarin Chinese or Taiwanese; (F) a signed informed consent form; and (G) attendance and compliance with the terms of the study ensured by the patient or the patient's caregiver.

The modified version of SADS-L, a semi-structured interview with good inter-rater reliability [8,9], was used as the gold standard. The diagnoses of a mood disorder were made based on DSM-IV criteria, except for BD-II, for which the 4-day duration criterion for hypomania was replaced with a 2-day criterion because the 4-day minimum might not be evidence-based [10]. The 2-day criterion has been supported by several studies [11].

Exclusion criteria were: (A) being pregnant or breast-feeding an infant; (B) having taken DM within 1 week before the first dose of the double-blind study medication; (C) a major mental illness other than BD, e.g., alcoholism or an illegal substance use disorder; (D) having a poorly controlled clinically significant medical condition, e.g., cardiac, hepatic, or renal disease; (E) having undergone electroconvulsive therapy within 4 weeks before the first dose of the double-blind study medication; (F) total aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, or creatinine levels three times higher than normal.

Study Design

Patients first underwent one week of daily open-label VPA-only treatment (500－1,000 mg daily [50－100 μg/ml

in plasma]). They were then randomly assigned to VPA plus placebo (VPA+P), VPA+30 mg/day of extended-release DM (VPA+DM30), and VPA+60 mg/day of extended-release DM (VPA+DM60) treatment groups. A concomitant benzodiazepine (preferably lorazepam [1−4 mg]) was used for nighttime sedation, agitation, or insomnia during the study. Risperidone (1−6 mg/day) and fluoxetine (20 mg/day) were permitted during symptomatic mood episodes, and an anticholinergic drug was used for extrapyramidal syndrome.

Measurement of Mood State

The severity of current symptoms was assessed using the YMRS and HDRS.

Blood Sample Assessment

Ten milliliters of whole blood was drawn from the antecubital vein of each patient during the initial visit and again at 12 weeks. WBCs, neutrophils, eosinophils, basophils, monocytes, lymphocytes, RBCs, and platelets were counted, and hemoglobin and hematocrit levels measured.

Statistical Analysis

Data are means ± standard error of the mean. Paired *t* tests were used to evaluate hematological molecular changes before (visit) and after 12 weeks of treatment. The changes of each hematological molecules after 12 weeks treatment between groups were analyzed by One- Way ANOVA with Tucky *post-hoc* test. Significance was set at $p < 0.05$. Prism 5 (GraphPad Software, La Jolla, CA, USA) and SPSS 22 (IBM Co., Armonk, NY, USA) were used to analyze the data.

RESULTS

Initially, 255 BD patients from NCKU hospital were enrolled and randomly assigned to one of the three different treatment groups, but 89 patients (34.9%) dropped out during the 12-week trial (dropout rate: VPA+P, n = 29 [33.7%]; VPA+DM30, n = 29 [34.1%]; VPA+DM60, n = 31 [36.9%]) (Fig. 1). The reasons for dropping out were: (1) attempted suicide or poor tolerance of side effects (VPA+P, n = 4 VPA+DM30, n = 1; VPA+DM60, n = 1); (2) insufficient therapeutic response (VPA+DM60, n = 2); (3) lost to follow-up for unknown reason (VPA+P, n = 11; VPA+DM30, n = 11; VPA+DM60, n = 13); (4) refused treatment (VPA+P, n = 7; VPA+DM30, n = 3; VPA+DM60, n = 1); (5) violation of protocol and poor drug compliance (VPA+P, n = 1; VPA+DM60, n = 2); and (6) administrative reasons (VPA+P, n = 5; VPA+DM30, n = 9; VPA+DM60, n = 12).

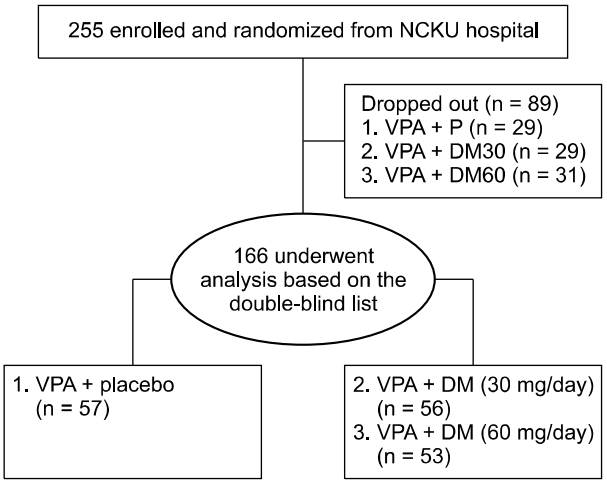

Fig. 1. Enrollment, randomization, and follow-up in National Cheng Kung University (NCKU) Hospital.
DM, dextromethorphan. VPA, valproic acid.

Finally, 166 BD patients (65.1%) completed the 12-week double-blind study (VPA+P, n = 57; VPA+DM30, n = 56; VPA+DM60, n = 53) (Fig. 1). There were no differences in demographic data between these three groups (Table 1). Neither sex nor BD-type differences (BD-I vs. BD-II) were significantly different between groups. Nor were the mean age, height, body weight, plasma levels of VPA at week 2 and week 12, or HDRS and YMRS scores significantly different at visit (Table 1). We also checked the blood DM and its metabolites dextrorphan (DX) concentration. We found that the slow-release DM30 and DM60 yielded 5-800 ng/ml of DM (4.8−15.5 ng/ml of mean concentration) and DX (10.3−24.1 ng/ml of mean concentration) in the blood (Table 1).

VPA+DM60 Improved the Long Term VPA Induced Downregulation of WBCs and Neutrophils

A paired *t* test showed that after 12 weeks of VPA+P, or VPA+DM30 treatment, WBCs and neutrophils counts were significantly downregulated, but not in the group given VPA+DM60 (Table 2). To further verify the effects of DM, We calculated the changes of each hematological molecules after 12 weeks treatment. We found that com-

Table 1. Demographic data

Variable	VPA+P (n = 57)	VPA+DM30 (n = 56)	VPA+DM60 (n = 53)	χ^2, F	p value
Sex (F/M)	29/28	30/26	23/30	$\chi^2 = 1.20$	0.55[a]
BD-I/BD-II	21/34	25/30	26/24	$\chi^2 = 1.74$	0.42[a]
Age (yr)	30.6 ± 1.4	30.0 ± 1.5	29.5 ± 1.3	$F = 0.15$	0.87[b]
Height (cm)	165.6 ± 1.1	164.2 ± 1.3	167.6 ± 1.2	$F = 1.92$	0.15[b]
Body weight (kg)	61.5 ± 1.6	65.3 ± 2.2	65.9 ± 2.5	$F = 1.27$	0.28[b]
Plasma VPA (μg/ml, W2)	58.8 ± 4.3	69.5 ± 4.7	72.9 ± 3.9	$F = 2.81$	0.06[b]
Plasma VPA (μg/ml, W12)	52.8 ± 6.1	54.7 ± 5.6	57.0 ± 5.6	$F = 0.13$	0.88[b]
Plasma DM (ng/ml, W12)	0.0 ± 0.0	4.8 ± 1.5	15.5 ± 3.5***	$F = 13.24$	< 0.0001[b]
Plasma DX (ng/ml, W12)	0.0 ± 0.0	10.3 ± 1.3	24.1 ± 4.0***	$F = 25.13$	< 0.0001[b]
HDRS at visit	16.9 ± 0.8	16.5 ± 0.8	15.4 ± 0.9	$F = 0.89$	0.41[b]
YMRS at visit	10.6 ± 0.7	10.9 ± 0.7	12.2 ± 0.7	$F = 1.38$	0.26[b]

Values are presented as mean ± standard error of the mean.
VPA, valproic acid; W, week; DM, dextromethorphan; DX, dextrorphan; F, female; M, male; YMRS, Young Mania Rating Scale; HDRS, Hamilton Depression Rating Scale.
[a]χ^2 tests and [b]one-way ANOVA were used to analyze differences between groups. ***$p < 0.0001$ vs. VPA+P group.

Table 2. White blood cells and related molecules before and after 12 weeks of treatment in each group

Blood cell counts in per cmm	VPA+P (n = 57)			VPA+DM30 (n = 56)			VPA+DM60 (n = 53)		
	Visit	Week 12	p value	Visit	Week 12	p value	Visit	Week 12	p value
WBCs	6,435.7 ± 238	5,887.1 ± 193*	0.018	6,705.5 ± 245	6,221.6 ± 190*	0.035	6,442.0 ± 174	6,564.8 ± 242	0.59
Neutrophils	3,864.2 ± 211	3,261.1 ± 173**	0.004	4,131.0 ± 193	3,543.9 ± 144**	0.006	3,847.4 ± 120	3,893.8 ± 180	0.82
Eosinophils	163.1 ± 20.1	166.6 ± 17.4	0.77	156.6 ± 16.6	166.4 ± 30.0	0.72	153.5 ± 14.3	171.4 ± 16.1	0.06
Basophils	36.0 ± 2.8	34.3 ± 2.3	0.51	32.4 ± 1.8	28.6 ± 1.8	0.05	34.6 ± 2.1	31.7 ± 1.9	0.22
Monocytes	483.5 ± 27.1	485.0 ± 20.2	0.96	463.2 ± 22.5	469.7 ± 17.3	0.77	498.9 ± 21.9	487.2 ± 24.4	0.65
Lymphocytes	1,897.3 ± 58.4	1,939.5 ± 58.8	0.45	1,883.4 ± 89.3	1,976.5 ± 73.3	0.22	1,904.5 ± 77.5	1,920.0 ± 69.5	0.84

Values are presented as mean ± standard error of the mean.
WBCs, white blood cells; VPA, valproic acid; DM, dextromethorphan.
All measurement are cell counts in one cubic millimeters (cmm); *$p < 0.05$, **$p < 0.01$ vs. visit within same group (paired t test).

bination use of DM60 improved the decline in WBC (Fig. 2A, $F = 2.595$, $p = 0.078$) and neutrophil (Fig. 2B, $F = 3.258$, $p = 0.041$) counts induced by the long-term VPA treatment.

RBCs, Hemoglobin, Hematocrit, and Platelets, were Lower in Patients Long Term treated with VPA+P

A paired t test showed that after 12 weeks of VPA+P, or VPA+DM30 treatment, RBCs, hemoglobin, and hematocrit were significantly downregulated, but not in the group given VPA+DM60 (Table 3). However, the VPA induced downregulation of platelet counts (Table 3, $p < 0.0001$), were not protected in VPA+DM-treated patients.

To further verify the effects of DM, We calculated the changes of each hematological molecules after 12 weeks treatment. We found that combination use of DM60 improved the decline in RBC (Fig. 2C, $F = 2.172$, $p = 0.117$) counts but not the platelet counts (Fig. 2D, $F = 0.005$, $p = 0.995$) induced by the long-term VPA treatment.

DISCUSSION

This pilot study investigated the effects of DM on hematological changes induced by chronic VPA treatment. WBCs and their differential counts (neutrophils, eosinophils, basophils, monocytes, and lymphocytes), RBCs, hemoglobin, hematocrit, and platelet counts were measured before and after VPA treatment. We found that 12 weeks of VPA-alone treatment had induced significantly lower levels of WBCs, neutrophils, RBCs, hemoglobin, hematocrit, and platelets, in adult Taiwanese Han Chinese BD patients. In contrast, VPA+DM60 treatment protected against VPA-induced hematological changes. Of notice, BD patients treated with VPA+DM60 significantly im-

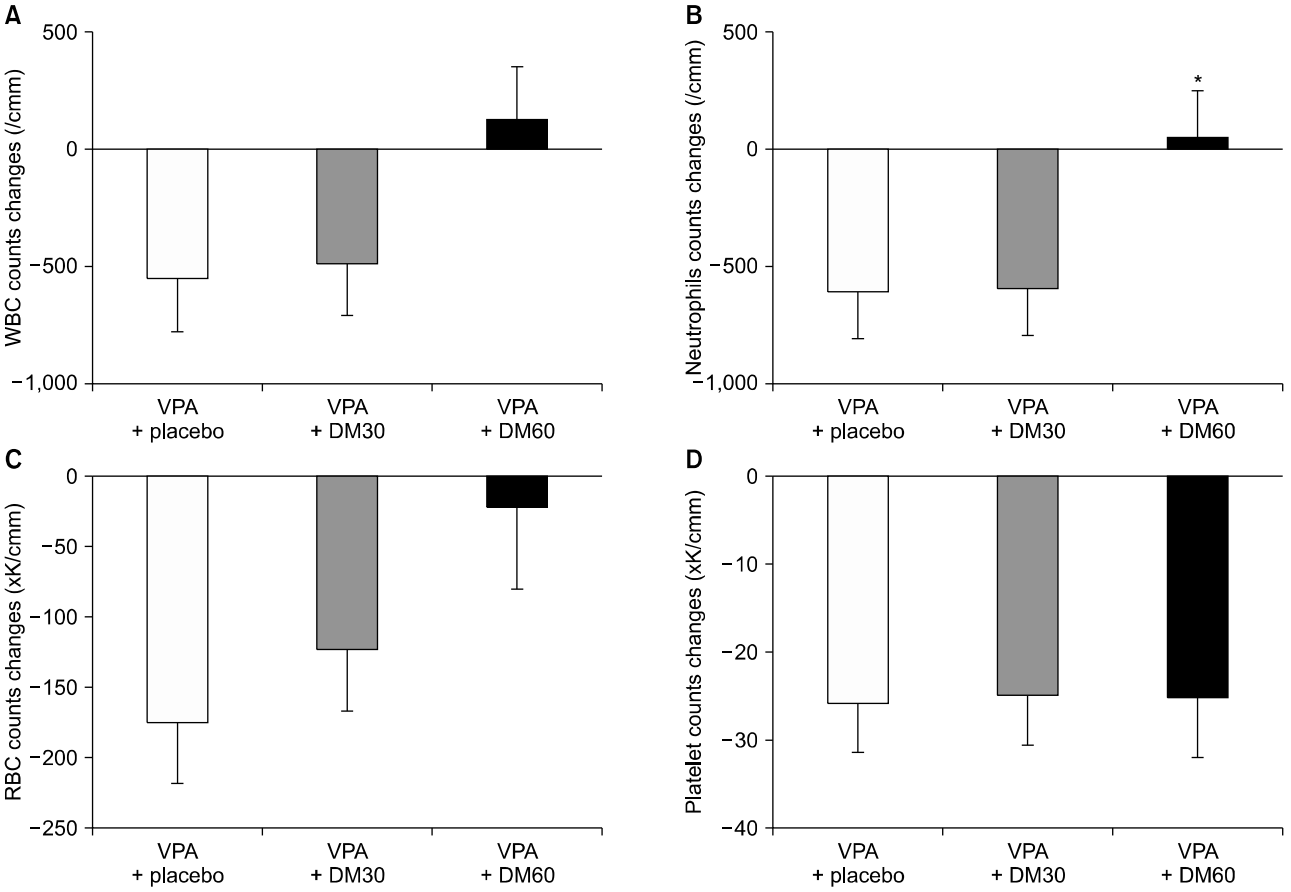

Fig. 2. The changes of hematological molecules in patients with bipolar disorder (BD) after 12 weeks treatment with VPA+Placebo (n = 57), VPA+DM30 (n = 56) and VPA+DM60 (n = 53). The changes of (A) white blood cell (WBC) counts, (B) neutrophils counts, (C) red blood cell (RBC) counts and (D) platelet counts were calculated and analyzed between groups. All data are mean ± standard error of the mean. All measurement are cell counts in one cubic millimeters (cmm); *$p < 0.05$, vs. VPA+Placebo group (One-Way ANOVA with Tucky *post-hoc* test).
DM, dextromethorphan. VPA, valproic acid.

Table 3. Platelets, red blood cells and related molecules before and after 12 weeks of treatment in each group

Blood cell counts in per cmm	VPA+P (n = 57)			VPA+DM30 (n = 56)			VPA+DM60 (n = 53)		
	Visit	Week 12	p value	Visit	Week 12	p value	Visit	Week 12	p value
Platelets (×10^3/cmm)	251.5 ± 8.4	225.7 ± 7.4***	< 0.0001	249.9 ± 7.1	225.1 ± 6.4**	< 0.0001	261.2 ± 9.0	231.0 ± 7.2**	0.001
RBCs (×10^6/cmm)	4.73 ± 0.1	4.56 ± 0.1***	< 0.0001	4.58 ± 0.1	4.45 ± 0.1**	0.007	4.61 ± 0.1	4.57 ± 0.1	0.51
Hemoglobin (gm/ml)	14.3 ± 0.2	14.0 ± 0.2**	0.010	14.0 ± 0.2	13.8 ± 0.2*	0.04	13.9 ± 0.2	13.9 ± 0.2	0.94
Hematocrit (%)	42.2 ± 0.7	41.0 ± 0.5**	0.005	41.1 ± 0.5	40.3 ± 0.5*	0.012	40.7 ± 0.7	41.0 ± 0.5	0.50

Values are presented as mean ± standard error of the mean.
RBCs, red blood cell; VPA, valproic acid; DM, dextromethorphan.
Most of measurement are cell counts in one cubic millimeters (cmm) or protein weight (gm) in one milliliter (ml). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. visit within same group (paired t test).

proved the long term VPA induced downregulation of neutrophil counts.

The common therapeutic range recommended for VPA is 50−125 μg/ml [12]. In clinic, monitoring the therapeutic blood concentration of VPA is recommend to prevent the toxic effects of VPA. However, the toxic and therapeutic effects of VPA are considered poorly correlated with its concentrations in children's blood [13]. The therapeutic s concentrations of VPA also vary in different adult patients [14]. Thus, the pharmacokinetic, ther-

apeutic and toxic effects of VPA should be carefully monitored in different patients. VPA is highly protein-bound (87−95%) with low clearance [15,16]. In humans, 30−50% of VPA is metabolized by glucuronidation and by β oxidation in the mitochondria, and about 10−20% is metabolized by cytochrome P450 (CYP)-mediated oxidation [17-19]. Studies had showed that the metabolism of VPA and DM can be metabolically and pharmacokinetically affected by the same enzymes: CYP2C9 and CYP3A4; that multiple human cytochromes contribute to the biotransformation of DM, including the enzymes CYP2C9, CYP2C19, CYP2D6, and CYP3A [20,21]; and that VPA inhibits CYP2C9, CYP3A4 [22] or induces CYP3A4 gene expression *in vitro* [23] which support the possible drug interaction of these two drugs. Thus, when combination of DM with VPA, their pharmacokinetic interactions and effects should be carefully monitored.

Our study found that in weeks 2 and 12, blood VPA concentrations of 22 of 57 (22/57, 38.6%) VPA+P patients were > 80 μg/ml. When combination of DM, 14 of 56 (14/56, 25%) VPA+DM30 and 16 of 56 (16/56, 28.6%) VPA+DM60 patients had such high concentrations. The differences of blood VPA concentration between the VPA+P and the VPA+DM groups were nonsignificant, and no blood VPA levels were toxic (≥ 150 μg/ml) in VPA+DM groups. In addition, the slow-release DM30 and DM60 yielded 5−800 ng/ml of DM and DX in the blood. Thus, the low doses of DM did not cause the obvious pharmacokinetic interaction with VPA.

VPA is a class I selective histone deacetylase inhibitor and inhibits hematopoiesis in developing rodents [24]. In mouse and Xenopus frog embryos, 48−72 hours of exposure to VPA significantly inhibits the formation of RBCs [24], and chronic high doses of VPA significantly inhibit bone marrow production [25] and reduce platelet [2,3], WBCs [4], and RBCs [1] counts. VPA can, however, elevate neutrophil counts [26]. Because prior studies had small samples and did not do pre- and post-treatment comparisons of blood cell counts, they do not clearly tell us about the effects of VPA on hematological changes in clinic BD subjects. In this study, the randomized research design revealed the chronic effects of VPA in the hematology changes. Our data indicated the importance of blood cell count monitoring, even during the normal therapeutic VPA ranges. In addition, the combination of slow-release formula DM 60 mg/day can alleviate the toxic effects of VPA in hematology, especially in the neutrophils counts.

This is the first finding of DM in hematological changes. DM was reported acting as a noncompetitive Nmethyl-D-aspartate (NMDA) receptor antagonist [27,28] and sigma-1 receptor agonist [29,30]. DM and its metabolite DX binding at sigma-1 receptors with high affinities at nanomolar (nM) range [31] and with lower affinities on NMDA receptors at micromolar (μM) range [27,28,30] *in vitro* and in rodent brain. Previous studies had showed that in RBCs [32] and neutrophils [33], NMDA receptor activity were found. And the sigma-1 receptors were also found in human plasma [34], leukocytes [35] and lymphocytes [36]. In the current study, the levels of DM and DX in our patients taking with slow-release DM60 are in ng/ml range (Table 1). Thus, in our participants, DM may insufficient to block NMDA receptors. DM may affect the blood cell through the sigma-1 receptor pathway. In addition, we previously showed that femtomolar concentrations of DM protected mesencephalic dopaminergic neurons in a neuron-glia culture [6]. However, the mechanism that DM uses to protect against hematological changes in human subjects requires additional study. To verify the effects and mechanisms of DM on the hematology, *in vitro* or *in vivo* experiment with pharmacological approach is needed in the future.

Our study has some limitations. First, the longer following up duration was needed in the further study. Although we did the 12 weeks observation, however, the longer observation period and more subjects enrolled will provide the more evident results. Second, medication permitted in the study might affect the hematology and obscure the changes in blood cells. Due to medical ethics, appropriate medications must be given to the subject's symptoms. We tried our best to limit concomitant treatment medication to only three drug such as lorazepam, risperidone and anticholinergic. Other medications, such as traditional herbal medicines, are excluded as much as possible during initial screening and treatment evaluation. However, we could not exclude the possible interaction induced by these adjunctive drugs with treatment group. Therefore, *in vitro* or *in vivo* studies to verify the drug effects of VPA and VPA+DM are needed.

Taken together, our data support our hypothesis that combination of such low-dose of DM with VPA is safe [5,37] and protect VPA-induced reductions of blood cell

counts. We suggested that using low dose of DM with VPA might as an ideal adjunct therapy to protect the peripheral blood cell counts in BD.

Chronic VPA-only treatment significantly lowered blood-cell counts in Taiwanese Han Chinese BD patients. Regular monitoring of blood cell counts is important during VPA treatment. VPA+DM60 treatment did not affect the pharmacokinetics of VPA, but it did significantly benefit the therapeutic effects and protect against the reduction of blood cell counts caused by VPA. With the therapeutic advantages both in plasma BDNF expression [5] and hematology, DM may be considered as an adjunct treatment with the VPA in BD treatment.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Designed the study and wrote the protocol: Ru -Band Lu, Jau-Shyong Hong, Shiou-Lan Chen. Managed the literature searches and analyses: Shiou-Lan Chen, Yun-Hsuan Chang. Collected and followed up the clinic subjects: Tzu-Yun Wang, Sheng-Yu Lee, Shiou-Lan Chen, Po-See Chen, Yen-Kuang Yang. Wrote the first draft of the manuscript: Shiou-Lan Chen. All authors contributed to and have approved the final manuscript.

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