Clostridium butyricum MIYAIRI 588 as Adjunctive Therapy for Treatment-Resistant Major Depressive Disorder: A Prospective Open-Label Trial

Tsuyoshi Miyaoka, MD, PhD, Misako Kanayama, MD, Rei Wake, MD, PhD, Sadayuki Hashioka, MD, PhD, Maiko Hayashida, MD, PhD, Michiharu Nagahama, MD, Shihoh Okazaki, MD, Satoko Yamashita, MD, Shoko Miura, MD, Hiroyuki Miki, MD, Hiroyuki Matsuda, MD, Masahiro Koike, MD, Muneto Izuhara, MD, Tomoko Araki, MSc, Keiko Tsuchie, MSc, Ilhamuddin Abdul Azis, MD, Ryosuke Arauchi, MSc, Rostia Arianna Abdullah, MD, Arata Oh-Nishi, PhD, and Jun Horiguchi, MD, PhD

Aim: Up to 60% of depressed patients do not obtain sufficient relief from a course of antidepressant therapy, and these treatment-resistant major depressive disorder (TRD) patients are at increased risk for relapse, chronicity, persistent psychosocial impairments, and suicide. Probiotics actively participate in treatment of neuropsychiatric disorders. However, the role of gut microbiota in brain disorders and depression remains unclear. We performed a prospective study to evaluate the effects of *Clostridium butyricum* MIYAIRI 588 (CBM588).

Methods: This was an 8-week open-label study to evaluate the efficacy and safety of CBM588 in combination with antidepressants in adult patients diagnosed with TRD according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*. Forty antidepressant-treated inpatients were included. Patients were randomized to adjuvant treatment with CBM588 (n = 20) or control (n = 20). The primary endpoint was the change in the 17-item Hamilton Depression Rating Scale score from baseline to week 8. Secondary end points were changes in the Beck Depression Inventory and the Beck Anxiety Inventory scale scores from baseline to week 8. The Systematic Assessment of Treatment Emergent Events—General Inquiry was used to assess adverse effects.

Results: CBM588 (60 mg/d) in combination with antidepressants (flvoxamine, paroxetine, escitalopram, duroxetine, and sertraline) provided significant improvement in depression. All patients completed the trial, and 70% responded to treatment; the remission rate was 35.0%. No serious adverse events occurred.

Conclusions: These preliminary data suggest that CBM588 in combination with antidepressants is effective and well tolerated in the treatment of TRD. Further studies using a larger, double-blind, parallel-group design are warranted to confirm these findings.

Key Words: butyrate, *Clostridium butyricum* MIYAIRI (CBM588), flora balance in the gut, probiotics

(*Clin Neuropharm* 2018;41: 151–155)

Despite the progressive development of dozens of antidepressant agents, more than half of all patients treated with antidepressant monotherapy fail to experience a remission of their major depressive episode.[1] Thus, developing safe, well-tolerated, and effective treatments that would help bring about remission in patients with treatment-resistant major depressive disorder (TRD) is of paramount importance. In light of studies suggesting the potential efficacy, safety, and tolerability of probiotics as an adjunct to standard antidepressants,[2] they may represent a unique opportunity for novel treatment development in major depressive disorder.[3]

There is increasing, but largely indirect, evidence pointing to an effect of commensal gut microbiota on the central nervous system (CNS), suggesting an interaction between the intestinal microbiota, the gut, and CNS in what is recognized as the microbiome-gut-brain axis.[4,5] Furthermore, recent evidence indicates that the absence and/or modification of the gut microflora resulted in specified comorbidity between functional gastrointestinal disorders and CNS disease, such as traumatic brain injury,[6,7] psychological stress,[8] and Parkinson disease.[9–11] Regulating the flora balance in the gut is achieved by the intake of probiotics, which is believed to be effective in lifestyle-related disease.[12,13] In recent years, it has been reported that probiotics confer a benefit on the development and function of the brain in the host.[8,14] There is abundant evidence demonstrating that *Lactobacillus* and *Bifidobacterium* attenuated anxiety, prevented the chronic psychological stress, reduced apoptosis in several brain regions, and improved learning and memory in mice.[15,16] Thus, it proposed that gut microbiota could be associated with brain function as well as neurological diseases via the gut-brain axis.

Clostridium butyricum, a Gram-positive, spore-forming, and obligate anaerobic rod bacterium, is found in the feces of 10% to 20% of healthy humans.[17] *C. butyricum* MIYAIRI 588 (CBM588) is a specific phenotype of the strain *C. butyricum*. CBM588 spores orally administered to rats can germinate and grow in the intestinal tract.[18] CBM588 produces short-chain fatty acids such as butyrate, acetate, and propionate. There are many reports suggesting that short-chain fatty acids have potential beneficial effects; in particular, butyrate has a proliferative effect on intestinal mucosal cells[19] and anti-inflammatory effects[20,21] in animals. The organism has been approved since 1968 in Japan as a probiotic for the treatment and prevention of antimicrobial-associated diarrhea and other causes of diarrhea in humans and animals.[22,23] Furthermore, butyrate is not restricted to the intestinal tract but can be disseminated systemically and detected in the rat brain.[24] Butyrate in the rat brain can exert neuroprotective effects on neurodegenerative disorders and improve behavioral deficits via the inhibition of histone deacetylasses.[25]

The aim of this prospective, open-label, fixed-dose, exploratory study was to evaluate changes in depressive symptoms, in patients with TRD who were treated adjunctive CBM588. In addition, the safety and tolerability of CBM588 were assessed.

Department of Psychiatry, Shimane University School of Medicine, Izumo, Japan.
Address correspondence and reprint requests to Tsuyoshi Miyaoka, MD, PhD, Department of Psychiatry, Shimane University School of Medicine, 89-1 Enyacho, Izumo 693-8501, Japan; E-mail: miyanyan@med.shimane-u.ac.jp
Conflicts of Interest and Source of Funding: The authors have no conflicts of interest to declare.
This trial was registered with UMIN (number 000028341).

DOI: 10.1097/WNF.0000000000000299

METHODS

Study Design

This study was an 8-week prospective open-label evaluation of CBM588 (60 mg/d) in patients with TRD. This study was approved by the Helsinki Committee (institutional review board) of the Department of Psychiatry of the Shimane University School of Medicine. Before participation, all subjects gave written informed consent according to institutional guidelines and the recommendations of the Declaration of Helsinki.

Patient Selection

Forty patients experiencing symptoms of TRD according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*,[26] were enrolled in this study. Diagnosis of TRD was based on chart reviews and defined as an inadequate or nonresponse to 2 or more 8-week trials with 2 different classes of antidepressants. Subjects were randomized to receive either CBM588 or control (1:1). For at least 3 of these 8 weeks, doses were required to be at or near the highest recommended therapeutic dose. Patients with a 17-item Hamilton Depression Rating Scale (HAMD-17)[27] total score of 16 or greater at the end of the screening phase were eligible to participate in this study.

Patients were excluded if they met the criteria for an Axis I diagnosis of delirium, dementia, or other cognitive disorder, bipolar disorder, schizophrenia or other psychotic disorder, or a clinically significant Axis II diagnosis of obsessive-compulsive, schizoid, schizotypal, paranoid, antisocial, or histrionic personality disorder. Patients were also excluded if they acknowledged substance abuse or dependence within the past 6 months, or if they were pregnant, were nursing, or posed a significant risk of suicide during the study period. Patients with chronic deteriorating illnesses such as diabetes, human immunodeficiency virus, gastrointestinal disease, and seizure disorders were also excluded. The subjects have been taking other medications, and the dosage was stable. All patients provided informed consent before participation in the study.

Study Treatments

All patients had been taking a stable dose of antidepressant medication for at least 1 month before baseline screening and entry into the open-label CBM588 treatment phase of the study. Patients continued to take the same dose of antidepressant medication for the duration of their involvement in the protocol. All patients were taking selective-serotonin reuptake inhibitor or serotonin-noradrenalin reuptake inhibitor medications, including fluvoxamine, paroxetine, escitalopram, sertraline, duloxetine, and milnacipram.

After baseline assessment with the aforementioned instruments, CBM588 treatment was initiated according to the following titration schedule: 20 mg orally twice daily for the first week and 20 mg orally three times daily from weeks 2 to 8. We chose 60 mg/d of CBM588 referring to a previous study.[22] Patients were forbidden to take any new psychotropic medications during the study. These included benzodiazepines, barbiturates, narcotics, or herbal supplements with putative psychotropic or analgesic effects.

Efficacy Endpoints

Baseline severity of MDD was assessed by using validated translations of the HAMD-17, the Beck Depression Inventory (BDI),[28] and the Beck Anxiety Inventory (BAI).[29] The primary end point for clinical effectiveness was determined by the mean change in HAMD-17 at the end of the 8-week trial; treatment response was defined as a reduction of 50% or greater in HAMD-17 total score; and remission was defined as a HAMD-17 total score of 7 or less at the end of treatment. Secondary end points for clinical effectiveness included mean change in BDI and BAI scores at the end of the trial.

Safety Endpoints

The incidence of adverse events was recorded, and a physical examination including vital signs, electroencephalogram, electrocardiogram and clinical laboratory tests was carried out at the beginning or end of this study. Adverse effects were assessed at the end of the 8-week trial by using the Systematic Assessment of Treatment Emergent Events (SAFTEE)—General Inquiry.[30] The SAFTEE is a technique for the systematic assessment of adverse effects in clinical trials developed by National Institute of Mental Health. It is a questionnaire that rates the current severity of a wide range of somatic, behavioral, and affective symptoms in general and specific inquiry formats. It is designed to report adverse health events, regardless of whether or not they are suspected to be drug related, to reduce the underreporting of unanticipated events compared with known or expected events.[30]

Statistical Analysis

Mean changes in HAMD-17, BDI, and BAI scores and vital signs were assessed using the Mann-Whitney test. All statistical assessments were 2-tailed and evaluated at the 0.05 level of significance. All data analysis was performed using Statistical Package for the Social Science Windows, Version 15.0 (SPSS Inc., Chicago, Ill). Descriptive statistics considered the mean ± SD. Mann-Whitney test was conducted on the groups to determine statistical significance. We completed analysis for multiple comparisons using Bonferroni adjustment with correction for correlation between observations. Significance for adjusted differences was set at $P < 0.05$.

RESULTS

Patients

Forty-five patients with TRD were screened. Five patients were excluded because of the presence of psychotic symptoms (n = 2), alcohol abuse (n = 1), and bipolar disorder (n = 1), and 1 refused to provide written informed consent. A total of 40 patients (16 men and 24 women) were therefore included in this study. Subjects were randomized to receive either CBM588 (n = 20) or control (n = 20) (1:1). The demographics of these patients, baseline characteristics, medications, and dosage are shown in Table 1. Baseline characteristics are shown in Table 1. The baseline characteristics showed no material differences. All patients were receiving 60 mg/d of CBM588 at the time of the final visit (week 8).

Improvement in Depressive Symptoms

Figure 1 describes the mean change in end points of primary and secondary effectiveness at the end of the 8-week trial with adjunctive CBM588. Over this period, patients who received CBM588 had reduced median HAMD-17, BDI, and BAI (Fig. 1) scores ($P < 0.001$). A positive response was defined as a reduction of 50% or greater in the HAMD-17 total score; 70.0% patients were considered to be responders. Remission was defined as a HAMD-17 score of 7 or less at the end of treatment. The remission rate was 35.0%.

TABLE 1. Baseline Demographics and Disease Characteristics: Intent-to-Treat

Demographic/Disease Characteristic	CBM588 (n = 20)	Control (n = 20)	*P*
Sex, n (%)			
Male	8 (48)	8 (48)	ns
Female	12 (52)	12 (52)	ns
Age, mean (SD), y	44.2 (15.6)	41.9 (14.2)	ns
Medications			
Antidepressants, n (%)	20 (100)	20 (100)	
Flvoxamine, n (%)	1 (5)	1 (5)	ns
Paroxetine, n (%)	5 (25)	5 (25)	ns
Sertraline, n (%)	3 (15)	4 (20)	ns
Duloxetine, n (%)	5 (25)	4 (20)	ns
Escitalopram, n (%)	5 (25)	4 (20)	ns
Milnacipram, n (%)	1 (5)	2 (10)	ns
Disease duration, mean (SD), y	5.1 (14.2)	3.9 (12.7)	ns
Baseline HAMD-17, mean (SD)	1.9 (2.8)	31.8 (4.0)	ns
Baseline BDI, mean (SD)	43.8 (4.3)	41.4 (4.8)	ns
Baseline BAI, mean (SD)	32.8 (5.8)	33.3 (4.7)	ns

ns indicates not significant.

Safety and Tolerability

Table 2 shows the adverse effects that were determined by the SAFTEE—General Inquiry. No serious adverse events were noted in any patient during this study. Laboratory parameters were within the normal range at baseline and remained in the reference range for the whole sample throughout the 8-week trial. There were no reports of serious adverse effects attributable to the study drug. The adverse effects were mild and transient (headache) in 2 cases. CBM588 was well tolerated overall, with no severe or serious adverse effects recorded during the study. None of the adverse effects was treatment limiting. No subject exited the study due to drug-related adverse events.

DISCUSSION

The results of this prospective, open-label, 8-week, fixed-dosed trial of adjunctive CBM588 corroborate the current literature as to the effectiveness and safety of the use of CBM588 in TRD. All patients completed the trial, and 70.0% of patients responded to treatment. The remission rate was 35.0%. We observed more than a 50% reduction in HAMD-17 scores, BDI scores, and BAI scores at the end of the 8-week trial, regardless of the type of antidepressant drug used. Our findings indicate that adjunctive CBM588 elicited a greater magnitude of treatment effectiveness despite comparatively more severe TRD in patients who had failed to achieve an adequate response to previous antidepressant therapy. To our knowledge, this is the first study to report the use of CBM588 in combination with antidepressants in patients with TRD. Significant improvements were seen across the spectrum of symptoms in TRD, as measured by HAM-D-17, BDI, and BAI rating scales.

CBM588 in combination with antidepressants was well tolerated in patients with TRD and was not associated with any unexpected adverse events. The incidence of adverse effects was low, and they were generally mild. Two patients experienced a single

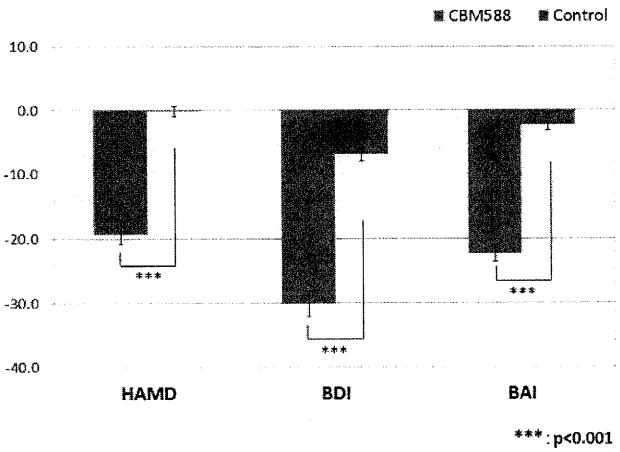

FIGURE 1. Mean changes in HAMD-17, BDI, and BAI scores at each assessment from baseline to week 8.

TABLE 2. Definite, Probable, and Possible Adverse Reactions of Study Intervention by Week 4

	CBM588 (n = 20)	Control (n = 20)
Psychological	0	0
Neurological	2	0
Gastrointestinal	0	1
Genitourinary	0	0
Musculoskeletal	0	0
Dermatological	1	0
Respiratory	0	0
Cardiovascular	0	0
Infection	0	0
Ear, nose, and throat	0	0
Hematological	0	0
Endocrine	0	2
Other	0	0
Overall	3	3

Data are number of participants (number of events). Participants could report more than 1 category of event.

episode of headache that did not lead to study withdrawal. There were no serious adverse events.

Given the debilitating nature of TRD, it is imperative to seek new treatments that improve the current mainstays of psychiatric practice. Combination pharmacotherapy (antidepressant + antipsychotic) is often the first-line treatment for this patient population, and electroconvulsive therapy is usually reserved for more severe cases. Although these treatment options have some utility in this patient population, they also present very real drawbacks. The newer generation antipsychotics often prescribed for this condition are known to increase a patient's risk of obesity, diabetes, and metabolic syndrome,[31] and patients with mental illness already have an approximately 2-fold higher all-cause mortality rate relative to the general population. Electroconvulsive therapy is associated with adverse cognitive effects, financial burden, and is, unfortunately, associated with substantial social stigma.

In human, existing evidence suggests that depression is associated with increased permeability of the gut wall,[32,33] increased immune and inflammatory activation,[34] and gut disorders such as irritable bowel syndrome.[35] These underlying biological factors support a proposal made by Logan and Katzman[36] that probiotics could be used as a treatment for low mood. Despite accumulating evidence of the effect of microbiota on behavior in animal models, data from humans are rather limited. Two studies in healthy volunteers showed no major effects of probiotics on anxiety and depression scores.[15,16,37] A very recent pilot study in patients with major depression showed that depression improved in both the placebo and probiotic groups, although the improvement appeared to be greater in the latter.[3]

Recent evidence also suggests that impaired neuroprotection is highly involved in the pathogenesis of major depression.[38] For example, decreased neuronal survival and disordered neurogenesis in the hippocampus have been repeatedly found in patients with major depression and are now considered to be potential pathophysiological factors and therapeutic targets for major depression.[39,40]

Liu et al[41] reported the use of *C. butyricum* as a safe and economical therapeutic option against mental disorders, especially its ability to effect the gut microbiota-butyrate-brain axis, to prevent and treat mental disorders in mice. The possibility that dietary *C. butyricum* can regulate gut microbiota and lead to changes in the fecal butyrate concentration that raised the butyrate in concentration the brain will further encourage exploration aimed at understanding the mechanisms underlying mental disorders recovery. These results open new avenues for viable therapeutic options against mental disorders by gut microbiota modulation. Therefore, the administration of *C. butyricum* may become an adjuvant therapy for patients with mental disorders.[42] In this regard, CBM588 has powerful anti-inflammatory and neuroprotective effects and is a potential new agent for treatment of major depression. Hence, we propose that CBM588 may exert potential antidepressant effects through its robust neuroprotective activities, which include neurogenesis, antioxidation, antiglutamate excitotoxicity, and direct regulation of proinflammatory agents.[42] Based on these findings from animal and human studies with CBM588, several lines of evidence support the hypothesis that CBM588 may ameliorate major depression through anti-inflammatory activity and neuroprotection (neurogenesis, antioxidation, and antiglutamate excitotoxicity).[42]

It will be interest to know the clinical outcome after CBM588 discontinuation and the possible difference of outcomes when used as augmentation to antidepressants with different mechanisms of action. The study of this subject is going on now in our laboratories.

This study had several limitations. First, the open-label design, small patient population, and absence of a placebo-control group make it difficult to draw conclusions with substantial clinical confidence. Second, although other antidepressants may provide more effective antidepressant treatment than the antidepressants used,[43] they were not available in Japan at the time this study was carried out. Third, because patients with depression often require long-term treatment to achieve remission, studies longer than 8 weeks in duration are warranted. Fourth, as fixed doses of CBM588 and antidepressants were prescribed, the specific contribution of each drug to the improvement in symptoms cannot be evaluated. However, the preliminary findings indicate that CBM588 in combination with an antidepressant shows promising efficacy and is well tolerated in the treatment of patients with TRD.

CONCLUSIONS

Our results suggested that probiotic therapy using CBM588 might be useful complementary with minimal adverse effects for the treatment in patients with TRD. Future larger scale investigations can be conducted according to the results of the current study.

ACKNOWLEDGMENT

Part of this work was supported by Grant-in-Aid for Scientific Research on Priority Areas numbers 13770544 and 50284047 from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Trivedi MH, Rush AJ, Ibrahim HM, et al. The Inventory of Depressive Symptomatology, Clinician Rating (IDS-C) and Self-Report (IDS-SR), and the Quick Inventory of Depressive Symptomatology, Clinician Rating (QIDS-C) and Self-Report (QIDS-SR) in public sector patients with mood disorders: a psychometric evaluation. *Psychol Med* 2004;34:73–82.
2. Latalova K, Hajda M, Prasko J. Can gut microbes play a role in mental disorders and their treatment? *Psychiatr Danub* 2017;29:28–30.
3. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *Nutrition* 2016;32:315–320.
4. Burcelin R, Serino M, Chabo C, et al. Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. *Acta Diabetol* 2011;48:257–273.
5. Dinan TG, Cryan JF. Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterol Motil* 2013;25:713–719.
6. Bansal V, Costantini T, Kroll L, et al. Traumatic brain injury and intestinal dysfunction: uncovering the neuro-enteric axis. *J Neurotrauma* 2009;26:1353–1359.
7. Hang CH, Shi JX, Li JS, et al. Alterations of intestinal mucosa structure and barrier function following traumatic brain injury in rats. *World J Gastroenterol* 2003;9:2776–2781.
8. Savignac HM, Tramullas M, Kiely B, et al. Bifidobacteria modulate cognitive processes in an anxious mouse strain. *Behav Brain Res* 2015;287:59–72.
9. Dutkiewicz J, Szlufik S, Nieciecki M, et al. Small intestine dysfunction in Parkinson's disease. *J Neural Transm* 2015;122:1659–1661.
10. Kim JS, Sung HY. Gastrointestinal autonomic dysfunction in patients with Parkinson's disease. *J Mov Disord* 2015;8:76–82.
11. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol* 2015;21:10609–10620.
12. Camilleri M. Probiotics and irritable bowel syndrome: rationale, putative mechanisms, and evidence of clinical efficacy. *J Clin Gastroenterol* 2006;40:264–269.
13. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470–1481.

14. Mikelsaar M, Zilmer M. Lactobacillus fermentum ME-3 — an antimicrobial and antioxidative probiotic. *Microb Ecol Health Dis* 2009;21: 1–17.
15. Messaoudi M, Violle N, Bisson JF, et al. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes* 2011;2:256–261.
16. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr* 2011;105:755–764.
17. Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol Immunol* 1984;28:975–986.
18. Sato R, Tanaka M. Intestinal distribution and intraluminal localization of orally administered *Clostridium butyricum* in rats. *Microbiol Immunol* 1997;41:665–671.
19. Ichikawa H, Kuroiwa T, Inagaki A, et al. Probiotic bacteria stimulate gut epithelial cell proliferation in rat. *Dig Dis Sci* 1999;44:2119–2123.
20. Okamoto T, Sasaki M, Tsujikawa T, et al. Preventive efficacy of butyrate enemas and oral administration of *Clostridium butyricum* M588 in dextran sodium sulfate-induced colitis in rats. *J Gastroenterol* 2000;35:341–346.
21. Yin L, Laevsky G, Giardina C. Butyrate suppression of colonocyte NFκB activation and cellular proteasome activity. *J Biol Chem* 2001;276: 44641–44646.
22. Seki H, Shiohara M, Matsumura T, et al. Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. *Pediatr Int* 2003; 45:86–90.
23. Takahashi M, Taguchi H, Yamaguchi H, et al. The effect of probiotic treatment with *Clostridium butyricum* on enterohemorrhagic *Escherichia coli* O157:H7 infection in mice. *FEMS Immunol Med Microbiol* 2004;41: 219–226.
24. Varela RB, Valvassori SS, Lopes-Borges J. Sodium butyrate and mood stabilizers block ouabain-induced hyperlocomotion and increase BDNF, NGF and GDNF levels in brain of Wistar rats. *J Psychiatr Res* 2015;61: 114–121.
25. Hyeon JK, Rowe M, Ren M, et al. Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. *J Pharmcol Experiment Therapeutics* 2007;321:892–901.
26. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. 4th Edition, Text Revision 2000.
27. Hamilton M. Rating depressive patients. *J Clin Psychiatry* 1980;41:21–24.
28. Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. *Arch Gen Psychiatry* 1961;4:561–571.
29. Beck AT, Epstein N, Brown G, et al. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol* 1988;56: 893–897.
30. Levine J, Schooler NR. SAFTEE: a technique for the systematic assessment of side effects in clinical trials. *Psychopharmcol Bull* 1986;22: 343–381.
31. Baptista T, Zarate J, Joober R, et al. Drug induced weight gain, an impediment to successful pharmacotherapy: focus on antipsychotics. *Curr Drug Targets* 2004;5:279–299.
32. Maes M, Kubera M, Leunis J-C, et al. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J Affect Disord* 2012;141: 55–62.
33. Berk M, Williams LJ, Jacka FN, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 2013; 11:200.
34. Maes M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;35:664–675.
35. Found G, Loundou A, Hamdani N, et al. Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. *Eur Arch Psychiatry Clin Neurosci* 2014;264: 651–660.
36. Logan AC, Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. *Med Hypotheses* 2005;64:533–538.
37. Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. *Eur J Clin Nutr* 2007;61: 355–361.
38. Pae CU, Marks DM, Hsn C, et al. Does minocycline have antidepressant effect? *Biomed Pharmacother* 2008;62:308–311.
39. Perera TD, Coplan JD, Lisanby SH, et al. Antidepressant-induced neurogenesis in the hippocampus of adult nonhuman primates. *J Neurosci* 2007;27:4894–4901.
40. Czeh B, Lucassen PJ. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur Arch Psychiatry Clin Neurosci* 2007;257:250–260.
41. Liu J, Sun J, Wang F, et al. Neuroprotective effects of *Clostridium butyricum* against vascular dementia in mice via metabolic butyrate. *Biomed Res Int* 2015;41294:12.
42. Sun J, Wang F, Ling Z, et al. *Clostridium butyricum* attenuates cerebral ischemia/reperfusion injury in diabetic mice via modulation of gut microbiota. *Brain Res* 2016;1642:180–188.
43. Sanchez C, Bogeso KP, Ebert B, et al. Escitalopram versus citalopram: the surprising role of the R-enantiomer. *Psychopharmacology (Berl)* 2004;174: 163–176.