ORIGINAL ARTICLE

Design and rationale of a 16-week adjunctive randomized placebo-controlled trial of mitochondrial agents for the treatment of bipolar depression

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Objective: Bipolar disorder places a significant burden on individuals, caregivers and family, and the broader community. Current treatments are believed to be more effective against manic symptoms, leaving a shortfall in recovery during the depressive phase of the illness. The current study draws on recent evidence suggesting that, in addition to increased oxidative load, alterations in mitochondrial function occur in bipolar disorder.

Methods: This 16-week study aims to explore the potential benefits of N-acetylcysteine (NAC) alone or in combination (CT) with selected nutraceuticals believed to enhance mitochondrial function. The study includes adults diagnosed with bipolar disorder currently experiencing an episode of depression. Participants are asked to take NAC, CT, or placebo in addition to any usual treatments. A post-discontinuation visit is conducted 4 weeks following the treatment phase.

Results: The primary outcome of the study will be mean change on the Montgomery-Asberg Depression Rating Scale. Secondary outcomes include functioning, substance use, mania ratings, and quality of life. Blood samples will be collected at baseline and week 16 to explore biochemical alterations following treatment.

Conclusion: This study may provide a novel adjunctive treatment for bipolar depression. Analysis of biological samples may assist in understanding the therapeutic benefits and the underlying etiology of bipolar depression.

Trial registration: Australian and New Zealand Clinical Trial Registry ACTRN12612000830897.

Keywords: Mitochondria; bipolar disorder; depression; acetylcysteine; oxidative stress

Introduction

Given the failure of almost all modern trials of antidepressants to demonstrate adequate efficacy in bipolar disorder (BD) and the limited efficacy of mood stabilizers (anticonvulsants and atypical antipsychotics) in the depressive phase of the disorder, the treatment of bipolar depression is widely acknowledged as the major unmet need in the disorder. Yet, individuals with BD spend three times longer in the depressive phase than the manic phase.[1] Together with depressive mixed states, it is the most lethal phase of the disorder, as it is associated with substantial suicide risk.[2] Bipolar depression is highly disabling, and causes marked occupational and social impairment. The three largest and most methodologically rigorous modern trials of antidepressants in BD (paroxetine, bupropion, and agomelatine) have all been negative.[3-5]

The etiology of BD remains to be fully elucidated. Therefore, current research is increasingly focusing on novel pathways. For example, new treatments are being sought that focus specifically on pathophysiological aberrations seen in BD. To this end, there is a growing body of evidence that points to a critical role for oxidative stress and mitochondrial dysfunction in BD. The aim of this study is to develop a novel intervention for the depressive phase of BD which specifically targets these pathways. In particular, two parallel strategies will be studied: a definitive exploration of the use of N-acetylcysteine (NAC), an agent that has robust antioxidant effects, enhances neurogenesis, reduces

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inflammation, and reverses mitochondrial toxicity; and a combination of agents that synergistically target mitochondrial dysfunction.[6,7]

Mitochondria are responsible for energy generation, and are particularly active in oxygen-rich and highly energy dependent tissues, such as the brain. Impaired energy metabolism triggers pro-apoptotic signaling, oxidative damage, and excitotoxicity, and impedes mitochondrial DNA repair. These processes can interact and potentiate one another, which, in turn, results in a continuation of energy depletion. Due to high levels of oxygen metabolism in brain tissue, mitochondria are themselves highly susceptible to oxidative stress.[8] This then leads to a vicious cycle whereby mitochondrial dysfunction leads to further oxidative stress, in turn causing further damage to the mitochondrion. As such, interventions that improve mitochondrial function by sustaining adenosine triphosphate (ATP) levels could improve neuronal dysfunction and reduce loss. Importantly, interventions that buffer intracellular energy levels and offer neuroprotection may significantly impede the progression of neurological damage, which is an important demonstrated feature of BD.[9]

There is accumulating evidence demonstrating alterations in mitochondrial homeostasis and, subsequently, function in BD. For example, there is a far higher prevalence of both BD and depression in people with mitochondrial disease than in the general population.[10] Changes in brain energy levels and markers of energy metabolism are altered in depression and BD, also implicating a role of mitochondrial changes in these illnesses. For example, resting energy expenditure and maximal aerobic capacity (VO$_2$ max) of individuals during the manic phase have been shown to be increased when compared with controls and euthymic participants.[11] There is also an established body of data from SPECT studies showing increased blood flow in mania and diminished uptake in depression.[12] Increased phosphomonoester and decreased ATP and phosphocreatine (PCr, a high energy phosphate) levels have been documented in unmedicated patients with depression. ATP and PCr appear to normalize following acetyl-L-carnitine (ALC) treatment, and this correlates with improvement on Hamilton Depression Rating Scale (HAM-D) symptom scores.[13] Furthermore, Kato et al.[14] reported that frontal lobe PCr metabolite levels are decreased in patients with severe depression. Subsequent studies suggested that such findings were also seen in patients with bipolar depression in the left frontal region, especially in bipolar II disorder,[15] and correlated with HAM-D ratings of depression.[16] Furthermore, photic stimulation-induced reduction of PCr in the occipital cortex was exaggerated in patients with lithium-resistant BD.[17] In addition, there is mounting evidence showing genetic links between BD and mitochondrial dysfunction.[18-20]

The current study has two broad aims. First, we wish to establish further evidence of the efficacy of NAC in the management of BD.[21,22] As we have previously reported a significant reduction in depressive symptoms associated with NAC in people with BD,[21-23] a second positive placebo-controlled trial would provide sufficient evidence to consider NAC as a first-line therapy. Second, we aim to develop a combination treatment consisting of agents with known efficacy in mitochondrial disorders. We are using a "systems biology theoretical approach," which suggests that greater efficacy might be attainable using simultaneous and synergistic combinations of agents. These agents will be used adjunctively to augment existing treatments, potentially resulting in better outcomes for people with BD, specifically those experiencing the depressive phase of the illness.

We aim to test this using a 16-week treatment trial that builds on the evidence of mitochondrial dysfunction in BD and will explore the development of a rational, pathophysiologically based intervention for this disorder. This novel project aims to target underlying mitochondrial dysfunction in BD with a purpose-designed, multi-component nutrient formula.

A number of pharmacological treatments and nutritional supplements have previously been used to treat conventional mitochondrial disorders. These include antioxidants (ubiquinone, idebenone, vitamin C, vitamin E, and menadione), agents that specifically improve lactic acidosis (dichloroacetate and dimethylglycine), agents that correct secondary biochemical deficiencies (carnitine and creatine), respiratory chain cofactors (nicotinamide, thiamine, riboflavin, pantothenic acid, pyridoxine, and ubiquinone), and hormones (growth hormone and corticosteroids).[24] While there are few robust findings (reflecting the nascence of the studies), this literature, to some extent, informs potential treatment options.

N-acetylcysteine

Robust evidence demonstrates the efficacy of the redox modulator and glutathione precursor NAC as a treatment of BD. Our research group conducted the first Stanley Medical Research Institute-sponsored randomized, placebo-controlled trials of NAC in both schizophrenia and BD. In the 6-month schizophrenia trial (n=140), adjunctive NAC was associated with a significant advantage over placebo on the Clinical Global Impression (CGI) scale, the Positive and Negative Symptoms Scale total score, and the Barnes Akathisia Scale.[25] In the 6-month BD trial (n=75), adjunctive NAC in participants with either BD I or II disorder was associated with significantly superior outcomes on the MADRS. Similar findings were evident on measures of functioning and quality of life, with effect sizes consistently in the large range on almost all outcomes measured.[23] In both trials, adverse effects did not significantly differ between the NAC and placebo groups.

Ubiquinone (coenzyme Q10)

This compound is an endogenous biological chemical with some evidence for its use in mitochondrial disorders.[8] Ubiquinone links mitochondrial complex I and III, transferring electrons between the units,[26] and

supplementation may improve the efficiency of this process. Unpublished pilot data on the use of a proprietary compound containing ubiquinone demonstrated improvement in mood symptoms in a non-clinical cohort of a placebo-controlled trial. A number of studies examining the effects of ubiquinone in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) have demonstrated significant reductions in serum lactate and pyruvate and improvement in brain ATP synthesis and symptomologies at a dosage of 150 mg/day.[27-30] The lack of research examining the potential benefits of ubiquinone for treatment of BD is surprising. With evidence of benefits in several other related pathologies[31-33] and its benign tolerability profile, ubiquinone appears to be an ideal candidate for further research in this area.

Alpha-lipoic acid

Alpha-lipoic acid (ALA) is suggested to have antioxidant properties and play a crucial role in mitochondrial dehydrogenase reactions. Furthermore, ALA has been shown to be beneficial in many oxidative stress models, such as HIV activation, radiation energy, and diabetes (through inhibition of glycation reactions).[34]

Acetyl-L-carnitine

ALC is another compound with potential utility in mitochondrial disorders. Carnitine is responsible for transport of fatty acids across the mitochondrial membrane, and plays a role in energy generation via acetyl-coenzyme A. Excitotoxicity and disrupted energy metabolism share free radical-induced oxidative damage as a cause; ALC has shown potential to correct this.[35] The usefulness of ALC in mood disorders has been suggested by a treatment trial of 204 patients with dysthymia, where ALC was equivalent to amisulpride.[36] ALC as an adjunct to psychosocial intervention was reported to be useful in the treatment of attention deficit/hyperactivity disorder in 63 boys with fragile X syndrome.[37] Increased phosphomonoester and decreased ATP and PCr levels have been implicated in depression. These levels were increased and normalized following ALC treatment, and this increase correlated with trends for improvements in symptoms based on HAM-D scores in two unmedicated patients.[13] The prevalence of metabolic syndrome in BD has been described as "alarmingly high,"[38] and increased levels of both lactate and glucose are observed in the cerebrospinal fluid of patients with BD.[39] L-Carnitine has shown some efficacy in glucose disposal, storage, and uptake in metabolically impaired and in normal healthy adults.[40]

Retinyl palmitate (vitamin A equivalent)

Retinal palmitate is a highly bioavailable fat-soluble compound and is a common form of vitamin A used in nutraceutical formulas. Vitamin A plays a major role in physiological processes including vision and reproduction,

in addition to its specific role in cell differentiation and growth, and, importantly, immune function. Retinol assists in the modulation of the PKC signaling pathway in mitochondria. While its antioxidant profile is well established, there is also evidence that retinol may be an electron carrier.[41]

Vitamin D (cholecalciferol, D3)

Vitamin D is an antioxidant that may be useful in enhancing mitochondrial function in BD. It plays a predominant role in the regulation of calcium and phosphate metabolism,[42] which may be particularly important, given the alterations seen in BD. Furthermore, vitamin D is believed to play a role in the production of melatonin and may be implicated in seasonal affective disorder.[43] Deficiency of vitamin D has been associated with low mood in a cross-sectional study of older adults.[44] Vitamin D deficiency is prevalent in psychiatric disorders, and has been linked to the symptoms of depression and schizophrenia.[45] Furthermore, it has been reported that the risk of developing psychotic symptoms is lower in women in the highest quartile of vitamin D levels, compared with those in the lowest.[46]

Ascorbic acid (vitamin C)

Ascorbic acid (in the form of calcium ascorbate dihydrate as used in the current study) has possible efficacy in mitochondrial myopathy,[47] and has a theoretical mechanism of action as an antidepressant in BD through the suppression of vanadium, which is elevated during manic episodes.[48,49] Ascorbic acid (vitamin C) is also a mediator in L-carnitine production.[50] Dual administration may strengthen the availability of L-carnitine in addition to its intrinsic properties.

Tocopherol (vitamin E)

The main function of tocopherol (vitamin E) is to scavenge free radicals and reduce lipid peroxidation, which improves mitochondrial membrane integrity. Studies have suggested that tocopherol and ubiquinone are more efficient when used together.[51] As such, ascorbic acid and tocopherol could be useful cofactors in a mitochondrial formulation.

B-group vitamins

Other cofactors associated with improved mitochondrial function include B vitamins, which often work in synergy, and thereby are often best administered as a complex. The water-soluble B vitamins play essential roles in mitochondrial energy production and cellular function by a variety of means. Vitamin B1 (thiamine) functions as a coenzyme in the oxidative decarboxylation of both pyruvate and alpha-ketoglutarate. The use of thiamine in the treatment of some forms of pyruvate dehydrogenase deficiency has been well established.[52] This use is postulated to improve aerobic glycolysis by enhancing pyruvate decarboxylation. Vitamin B2 (riboflavin) is

required synergistically with other B vitamins for mitochondrial respiration.[53] Vitamin B3 (niacin) acts as a precursor for both nicotinamide adenine dinucleotide (NAD/NADH) and nicotinamide adenine dinucleotide phosphate.[54] Pantothenic acid (vitamin B5) is required for the synthesis of coenzyme A, which, among other roles, is important in the tricarboxylic acid (TCA) cycle. In a study of immortalized T-cell lines, pantothenic acid was shown to prevent loss of mitochondrial membrane potential and also to reestablish ATP synthesis. Levels of catalase, glutathione peroxidase and glutathione reductase were also restored following pantothenic acid treatment.[53]

Vitamin B6 (pyridoxine) is also involved in mitochondrial energy production, and has important effects on antioxidant status, being involved with aminotransferase activity.[53,55] The major role of NADH is to transfer electrons from metabolite intermediates to the respiratory chain. Complex I accepts electrons from NADH and passes them to ubiquinone. Nicotinamide increases the cellular NADH and NAD concentration and thereby enhances substrate availability for complex I.

Vitamin B7 (biotin) is part of the B-group complex and is responsible for a variety of biological activities, including promotion of immune and metabolic function, as well as cellular growth. It is a coenzyme for five mitochondrial carboxylases and is essential for growth, development, and normal mitochondrial and cellular functions, including fatty acid oxidation and gluconeogenesis.[53] Reductions in biotin result in the loss of mitochondrial complex IV, which leads to increased production of oxidative species by the mitochondria.

Vitamin B9 (folic acid) is commonly deficient in hospitalized psychiatric patients.[56] Deficiency in folic acid impairs synthesis of tetrahydrobiopterin (BH4), which is essential for the synthesis of serotonin and other monoamines involved in the pathogenesis of affective disorders.[57] Together with vitamin B12 (in the form of cyanocobalamin in the current study), folate plays an essential role in mitochondrial energy production through one-carbon transfer pathways.[53] B12 is also often deficient in hospitalized psychiatric patients,[58] and works synergistically with folic acid.[59] A common finding in vitamin B12 deficiency is pernicious anemia, which may cause a derivative secondary mania.[60-62] Even in the absence of clinical features of pernicious anemia, neurological signs are often present and, in such cases, may be resolved through B12 supplementation.[62-64]

Although there are many mitochondrial-enhancing agents to consider as potential treatments for BD, for reasons of practicality a number of key agents can be chosen. Additionally, many mitochondrial agents have overlapping mechanisms of action as antioxidants. NAC has been selected as an antioxidant in this trial as it has the best-established efficacy in mental health, and ALC and ubiquinone, for their critical roles in mitochondrial bioenergetics. The cofactor nutrient agents ascorbic acid, α-tocopherol, thiamine, niacin, folic acid, and

cyanocobalamin were chosen not only because of their individual benefits, but also due to their synergistic effects when administered conjunctively with other mitochondrial agents. Dhitavat et al.[65] found a synergistic effect against oxidative stress with combined folic acid, tocopherol, and carnitine administration, which provided superior protection against amyloid-beta induced reactive oxygen species in human neuroblastoma cells. In this combination, folic acid was used to maintain endogenous glutathione, a role provided by NAC in our combination. Importantly, all agents used in the present clinical trial are known to have benign adverse event profiles, with adverse effect rates not differing significantly from placebo in previous studies. Finally, the combination has been formulated as not to interact or deteriorate during storage, allowing maximal effects from all components.

Methods

Study design

The study is a 16-week, multi-site, randomized, double-blind, parallel-group trial of a combination therapy (CT), NAC alone, or placebo in the depressive phase of BD. Participants receive 16 weeks of daily treatment, adjunctive to treatment as usual, with assessment visits at baseline, weeks 4, 8, 12, 16, and 20 (washout), and a phone interview at week 2 (Table 1). Three study sites, in Geelong, Melbourne, and Sydney, Australia, are recruiting participants. The trial is being conducted in accordance with good clinical practice guidelines, and research and ethics committee approval was sought before the start of the study. All components of the active treatment are well tolerated by humans at the doses proposed for use in this study and are currently available for purchase without prescription in Australia.

Trial allocation, sequence generation, and blinding

Pack number allocation to treatment arm is randomly assigned using permutated block randomization. The computer-generated randomization plan has been developed by an independent researcher utilizing four-to-a-block design. Disposable blister packs containing the trial medications (Medico Paks) are allocated by trial clinicians sequentially. Packs are identical so as to conceal treatment allocation and blinding. To facilitate the double-blinding process, the trial medications (CT, NAC only, and placebo) are dispensed by an independent pharmacist in identical numbers and capsule forms in sealed containers. The placebo containers are specially treated with subtherapeutic levels of NAC dust to produce its characteristic smell (as manufactured in our previous study). The statistician and trial clinicians are blinded to group allocation. Participants are assigned randomly and consecutively to treatment with CT, NAC, or placebo (1 : 1 : 1 design) in a double-blind fashion. All participants will remain on treatment as usual (TAU) for the duration of the trial.

Table 1 Visit schedule

Visit	Visit 1a	Visit 1b*	Phone call	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Phase	Screening	Baseline			Treatment phase			Washout
Week/scale	Week 0	Week 0	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20
Informed consent	X	-	-	-	-	-		-
MINI-Plus 5.0	X	-	-	-	-	-		-
Concomitant medication	X							
Entrance criteria	X	-	-	-	-	-		-
SAPAS		-		X				
Family history		X	-	-	-	-		-
Psychiatric history		X	-	-	-	-		-
Demographic information		X						
Habits	-	X		X	X	X	X	X
Adverse events	-	-	X	X	X	X	X	X
Alcohol/substance use	-	X		X	X	X	X	X
MADRS with SIGMA	X	X		X	X	X	X	X
BDRS	-	X		X	X	X	X	X
HAM-A	-	X		X	X	X	X	X
YMRS	-	X		X	X	X	X	X
LIFE-RIFT	-	X		X	X	X	X	X
SOFAS	-	X		X	X	X	X	X
Q-LES-Q (short form)	-	X		X	X	X	X	X
CGI-BP severity	-	X		X	X	X	X	X
CGI-BP Improvement				X	X	X	X	X
PGI	-	-		X	X	X	X	X
Food Frequency Questionnaire	-			X				
Qualitative data collection	X	X	X	X	X	X	X	X
Adherence to trial medication			X	X	X	X	X	X
Blood sampling (30 ml)	-	X	-	-	-	-	X	-

BDRS = Bipolar Depression Rating Scale; CGI-BP = Clinical Global Impression for Bipolar Disorder; HAM-A = Hamilton Anxiety Rating Scale; LIFE-RIFT = Longitudinal Interval Follow-up Evaluation – Range of Impairment Functioning Tool; MADRS = Montgomery-Asberg Depression Scale; MINI-Plus = Mini-International Neuropsychiatric Interview-Plus; PGI = Patient Global Impression; Q-LES-Q = Quality of Life Enjoyment and Satisfaction Questionnaire; SAPAS = Standard Assessment of Personality – Abbreviated Scale; SIGMA = Structured Interview Guide for the Montgomery-Asberg Depression Rating Scale; SOFAS = Social and Occupational Functioning Assessment Scale; YMRS = Young Mania Rating Scale.
* Screening and baseline visits may be conducted together or as two separate visits, depending on the participant's needs.

Treatment regimen

The treatment regimen, including the composition of each capsule and the agents included in each group, is shown in Table 2. Each participant is required to take a total of 10 capsules/day (five in the morning and five in the evening). To clarify the inclusion of ubiquinone and ALC twice, and the addition of other agents that are not listed in the rationale above, we are using a commercially available product (kindly supplied by Bioceuticals). The manufacturers have provided a product that best fits our rationale.

As the trial is double-blind, each participant will consume the same amount of capsules at each dose time point (twice daily) in all groups. For example, the NAC-alone group is only required to take four capsules/day to fulfill the dose of NAC. However, for blinding purposes, we have included six placebo capsules, matched to the CT group, to ensure concealment.

Study group

Two hundred and twenty-five participants (aged 18 years and over) meeting DSM-IV-TR criteria for BD (I, II, or NOS) on a structured clinical interview (Mini-International Neuropsychiatric Interview-Plus) with a current acute depressive episode (MADRS score $\geqslant$ 20) will be

recruited. Participants are recruited through their case clinicians, advertisement, or via private clinicians (e.g., family physicians or specialists). Potential participants are contacted, briefly screened, and a preliminary interview scheduled. All participants are required to provide written informed consent before enrollment.

Inclusion criteria

To be included, the participants are required to meet DSM-IV-TR criteria for BD (I, II, or NOS), have a current episode of depressive illness with a MADRS score equal to or greater than 20, have the capacity to consent to the study and comply with study procedures, and be using effective contraception if female, sexually active, and of childbearing age. Participants currently under any form of therapy for their BD (psychotropic medication or psychological therapy) are required to have been on a stable dose of that therapy for at least one month prior to randomization. Participants are also required to nominate a current treating physician, and are not eligible to enter the study until one is identified. If there is a delay of $>$ 7 days between screening and baseline assessments, or between baseline assessment and medication commencement, the MADRS will be administered again to ensure the participant still meets eligibility criteria ($>$ 20).

Table 2 Treatment group composition and dose

Capsule/component	Total/capsule	Total dose/day	Total no. capsules/day
Group 1: combination treatment			
Capsule 1: N-acetylcysteine	500 mg	2,000 mg	4
Capsule 2: acetyl-ʟ-carnitine	500 mg	1,000 mg	2
Capsule 3:			2
Ubiquinone (coenzyme Q10)	75 mg*	200 mg	
d-alpha-Tocopherol (vitamin E)	3.36 mg (5 IU)*	40.32 mg (60 IU)	
Alpha-lipoic acid	75 mg	150 mg	
Magnesium (as orotate 500 mg)	32 mg	64 mg	
Capsule 4:			2
Ubidecarenone (coenzyme Q10)	25 mg*		
d-alpha-Tocopherol (vitamin E)	16.8 mg (25 IU)*		
Thiamine hydrochloride	50 mg	100 mg	
Riboflavin (vitamin B2)	50 mg	100 mg	
Nicotinamide (vitamin B3)	100 mg	200 mg	
Calcium pantothenate (equiv. pantothenic acid/vitamin B5)	50 mg (45 mg)	100 mg (90 mg)	
Pyridoxine hydrochloride (equiv. pyridoxine/vitamin B6)	50 mg (41.1 mg)	100 mg (82.2 mg)	
Folic acid (vitamin B9)	400 µg	800 µg	
Cyanocobalamin (vitamin B12)	400 µg	800 µg	
Calcium ascorbate dihydrate (equiv. ascorbic acid/vitamin C)	121 mg (100 mg)	242 mg (200 mg)	
Retinyl palmitate (equiv. vitamin A)	450 µgRE (1,500 IU)	900 µgRE (3,000 IU)	
Cholecalciferol (equiv. 250 IU vitamin D3)	6.25 µg (250 IU)	12.5 µg (500 IU)	
Biotin (vitamin H)	300 µg	600 µg	
Group 2: N-acetylcysteine group			
Capsule 1: N-acetylcysteine	500 mg	2,000 mg	4
Capsules 5, 6, 7: equivalent placebos of Capsules 2, 3, 4			6
Group 3: Placebo group			
Capsule 8: equivalent placebo of Capsule 1			4
Capsules 5, 6, 7: equivalent placebos of Capsules 2, 3, 4			6

µgRE = micrograms of retinol equivalent.
* Total dose of ubidecarenone is 200 mg and d-alpha-tocopherol is 40.32 mg (60 IU), combining capsules 4 and 5.

Exclusion criteria

The following people will be ineligible to take part in the study: individuals with a known or suspected active systemic medical disorder, recent gastrointestinal ulcers or renal stones, individuals who are pregnant or lactating; individuals who have a diagnosis of epilepsy; those who are currently taking > 250 mg of NAC, > 250 mg of ALC, > 25 mg of ubiquinone, or > 200 µg of selenium/day (a 1-month washout period will be required if participants currently taking these study preparations would like to take part); those currently enrolled in any other intervention study; individuals needing warfarin or phenytoin; individuals who are intolerant to or have had an anaphylactic reaction to any components of the preparation; and those with an inability to comply with either the requirements of informed consent or the treatment protocol. The exclusion of those on selenium is due to its antioxidant properties that are mechanistically similar to those of NAC, which provides cysteine to the glutathione pathway (http://www.genome.jp/kegg-bin/show_pathway?ec00450+2.9.1.1).

Withdrawal criteria

Withdrawal from the trial occurs if the participants cease taking their trial medication for 7 consecutive days or if a participant ceases effective contraception or becomes pregnant. Dose changes to existing medications (either increases or decreases in dose) or addition or removal of an agent are acceptable and participants are allowed to continue in the trial. Participants are withdrawn from the study if they withdraw consent or at the discretion of the investigator in case of adverse events or loss to follow-up.

Measurements

The participants are assessed at baseline using a structured clinical interview, the MINI-Plus. A set of validated outcome measures is completed at baseline and at weeks 4, 8, 12, 16, and 20. The measures include the Montgomery Asberg Depression Rating Scale (MADRS),[66] HAM-D,[67] Bipolar Depression Rating Scale (BDRS),[68] Young Mania Rating Scale (YMRS),[69] CGI's Improvement and Severity scales,[70] Patient Global Impression-Improvement subscale, Social and Occupational Functioning Assessment Scale (SOFAS),[71] Longitudinal Interval Follow-up Evaluation – Range of Impairment Functioning Tool (LIFE RIFT),[72] and Quality of life Enjoyment and Satisfaction Questionnaire (Q-Les-Q).[73] Information on substance use is also gathered. At week 2, a phone call is made to assess adherence to trial medication and also to assess any adverse events that may have occurred. In addition, a modified food frequency questionnaire, a physical activity questionnaire, and a Standard Assessment of Personality – Abbreviated Scale (SAPAS)[74] are administered at week 4. Demographic data are also collected, including, age, weight, height, sex, psychiatric history and duration of illness.

A purpose-designed qualitative written assessment will be undertaken to explore the effects of treatment on participants' physical and mental health. Clinician case notes from each visit will be used to explore differences over time between the CT, NAC, and placebo groups.

Adherence is monitored using pill counts of returned clinical trial material. Adverse events are recorded, addressed according to medical assessment, and monitored. For consenting participants, blood samples are collected at baseline and at week 16, to be stored and later analyzed for serum levels of pyruvate, lactate, and blood glucose as well as oxidative markers such as cysteine, glutathione, the lipid peroxidation products malondialdehyde and thiobarbituric acid reactive substances, oxidative enzymes (catalase, superoxide dismutase and glutathione peroxidase, GSH-Px), polymorphisms of the GSH-Px, γ-glutamylcysteine synthetase, and glutamate cysteine ligase subunit genes, and mtDNA polymorphisms. Change in mean MADRS total score is the primary outcome measure.

Data analysis

Baseline demographic and clinical characteristics will be examined. Measures of central tendency (mean, median) and variability (standard deviation, minimum and maximum) will be estimated for each continuous measure, and proportions estimated for categorical variables. Graphical displays (e.g., histograms and boxplots) will be produced. Transformations will be used when distributional assumptions are not fulfilled for inferential tests. Analysis of the two treatment arms will be compared to placebo and will first be conducted independently of the other. This was not designed as a comparator study to compare the CT and NAC treatments to each other, but rather to explore the benefits of both treatments independently. Comparisons between treatment groups will be made as well. The treatment groups will be compared on baseline demographic and clinical variables using t-tests or Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables. If significant baseline group imbalance is detected on a particular variable and that variable is correlated with outcome at $r > 0.30$, then it will be included as a covariate in the inferential analyses. Each of the statistical tests above will use a two-tailed alpha level of 0.05. Mixed-effects linear regression analyses will compare intervention groups on the repeated assessments of the MADRS over 16 weeks. The mixed-effects model will include a random intercept and slope over time and fixed effects for treatment and the stratification variable site. A treatment by time interaction will be used to determine if the change in MADRS score varies between the intervention group over the course of acute treatment. The treatment by time interaction will be evaluated using a likelihood ratio test. Analysis of the qualitative data will use NVivo, with the qualitative differences between CT and placebo and NAC and placebo being assessed by blinded researchers. We will use an operationalized methodology that we have developed and published.[75]

Training

All potential raters are required to view the overview training modules and complete reliability and inter-rater reliability assessments. All raters are required to conduct a set of observed interviews on a yearly basis. Any raters failing to meet stringent certification criteria on an ongoing basis will be required to undergo additional remedial training and observation.

Statistical power

Overall power to detect significant differences between the actual pattern of means will be determined. Assuming a correlation of post-treatment scores with baseline measurements of 0.7 and an effect of the dosage such that the experimental (usual treatment and adjunctive CT) group differs from controls (usual treatment and placebo) by 0.75 standard deviation, power will be maintained above 90% with 75 subjects in each group. Pairwise comparisons with 75 subjects per group in the three groups would enable effects smaller than 0.6 standard deviations to be detected with a power of 80%. These effect sizes are in the small to moderate range. The experiment would thus be capable of detecting differences between groups of clinical and scientific interest with a total sample size of 225.

Follow-up of adverse events

The research clinician will follow up any participant that has completed or withdrawn from the study and that has an ongoing adverse event 30 days after trial medication has ceased. This is based on recommendations from the Orygen Youth Health Research Centre Study Services Unit (internal communication) suggesting that adverse events should be followed up at a time period which is five times the half-life of the agent. Based on available research, cyanocobalamin (vitamin B12) has the longest half-life of any of the agents in the study (6 days). As such, any participants who have an ongoing adverse event at the end of their participation are asked to attend a final phone call or visit 30 days after project completion/withdrawal.

If a participant withdraws (or is withdrawn) from the study early, they are requested to attend a final face-to-face interview, scheduled to occur as soon as possible, to capture mood symptoms. If the participant is experiencing a current adverse event at that time, they will be asked to be phoned 30 days later to follow up that event. If the participant does not attend a final face-to-face visit upon withdrawal, verbal permission will be sought for a final monitoring phone call (30 days post-discontinuation).

Participants are instructed that, should they have an ongoing adverse event that is concerning them following the completion of the trial, they should contact their

treating physician rather than wait until the follow-up phone call 30 days later.

Results

Current data on oxidative stress and mitochondrial dysfunction in BD, together with the results of previous trials, present a compelling indication to examine the role of mitochondrial enhancing agents in BD. To date, we have randomized 81 participants with a view to complete the study in early 2015.

Discussion

Bipolar depression remains an unanswered clinical problem with a progressive and relapsing course. Recent advancements in the understanding of neurodegenerative process and disease progression of BD have focused on mitochondrial dysfunction, oxidative stress, neurotrophic factors, and inflammation[76] rather than on neuroreceptor pathways. Hence, novel treatments are aiming to modulate oxidative, inflammatory, and neurotrophic systems. In particular, attempts to modulate mitochondrial dysfunction seem promising. The current RCT follows a "systems biology theoretical approach" and, to our knowledge, will be the first to evaluate the efficacy of NAC administered alone or with additional nutraceuticals that target mitochondrial dysfunction in participants experiencing current bipolar depression. The advantages of the present study include its double-blind design, the use of face-to-face clinical interviews rather than self-reported questionnaires to generate quantitative and qualitative data, and the inclusion of blood sampling that would allow searching for correlates of biological factors.

The study will also contribute to the understanding of the pathophysiology of bipolar depression. The collection of blood samples at baseline and at the end of the treatment phase will allow investigation of relevant mitochondria-related molecules, as well as those involved in oxidative stress and inflammation. This will not only provide an indication of the mechanisms by which these novel treatments are effective, but might also provide new targets for development of future therapies.

Most importantly, in contrast to many RCTs, the present trial uses commercially-available products which require no additional regulatory approval. Consequently, participants can continue (or commence, if in the placebo group) taking active treatment following the completion of the study. This unique feature is extremely important, as clinicians could use these agents adjunctively to augment existing treatments. Moreover, once recruited into the trial, participants will not be withdrawn even if their medication (i.e., TAU) is altered, reflecting "real-life circumstances."

The consensus in the field of BD research is that the greatest unmet clinical need is bipolar depression. This study provides a novel therapy to target this phase of BD specifically. Given that the agents are safe, affordable, and tolerable, if positive, this study will provide information with a high impact both clinically and scientifically.

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