Brain, Behavior, and Immunity 61 (2017) 50–59

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

# Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects

John R. Kelly [a,b], Andrew P. Allen [a,b], Andriy Temko [c], William Hutch [d], Paul J. Kennedy [a], Niloufar Farid [b], Eileen Murphy [e], Geraldine Boylan [d], John Bienenstock [f], John F. Cryan [a,g], Gerard Clarke [a,b], Timothy G. Dinan [a,b,*]

[a] *APC Microbiome Institute, University College Cork, Ireland*
[b] *Department of Psychiatry and Neurobehavioral Science, University College Cork, Ireland*
[c] *Department of Electrical and Electronic Engineering, University College Cork, Ireland*
[d] *INFANT Research Centre and Department of Pediatrics & Child Health, University College Cork, Ireland*
[e] *Alimentary Health Ltd., Cork Airport Business Park, Cork, Ireland*
[f] *Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada*
[g] *Department of Anatomy and Neuroscience, University College Cork, Ireland*

ARTICLE INFO

*Article history:*
Received 8 September 2016
Received in revised form 28 October 2016
Accepted 16 November 2016
Available online 16 November 2016

*Keywords:*
Psychobiotic
Brain-gut axis
Stress
Cognition
Memory
EEG

ABSTRACT

*Background:* Preclinical studies have identified certain probiotics as psychobiotics – live microorganisms with a potential mental health benefit. *Lactobacillus rhamnosus* (JB-1) has been shown to reduce stress-related behaviour, corticosterone release and alter central expression of GABA receptors in an anxious mouse strain. However, it is unclear if this single putative psychobiotic strain has psychotropic activity in humans. Consequently, we aimed to examine if these promising preclinical findings could be translated to healthy human volunteers.
*Objectives:* To determine the impact of *L. rhamnosus* on stress-related behaviours, physiology, inflammatory response, cognitive performance and brain activity patterns in healthy male participants.
*Methods:* An 8 week, randomized, placebo-controlled, cross-over design was employed. Twenty-nine healthy male volunteers participated. Participants completed self-report stress measures, cognitive assessments and resting electroencephalography (EEG). Plasma IL10, IL1β, IL6, IL8 and TNFα levels and whole blood Toll-like 4 (TLR-4) agonist-induced cytokine release were determined by multiplex ELISA. Salivary cortisol was determined by ELISA and subjective stress measures were assessed before, during and after a socially evaluated cold pressor test (SECPT).
*Results:* There was no overall effect of probiotic treatment on measures of mood, anxiety, stress or sleep quality and no significant effect of probiotic over placebo on subjective stress measures, or the HPA response to the SECPT. Visuospatial memory performance, attention switching, rapid visual information processing, emotion recognition and associated EEG measures did not show improvement over placebo. No significant anti-inflammatory effects were seen as assessed by basal and stimulated cytokine levels.
*Conclusions: L. rhamnosus* was not superior to placebo in modifying stress-related measures, HPA response, inflammation or cognitive performance in healthy male participants. These findings highlight the challenges associated with moving promising preclinical studies, conducted in an anxious mouse strain, to healthy human participants. Future interventional studies investigating the effect of this psychobiotic in populations with stress-related disorders are required.



## 1. Introduction

An abundance of preclinical studies have shown that probiotics acting via the brain-gut-axis can affect brain development, function and behaviour (Bercik et al., 2011a; Buffington et al., 2016;

* Corresponding author at: Department of Psychiatry and Neurobehavioral Science, Biosciences Institute, University College Cork (UCC), Cork, Ireland.
*E-mail address:* t.dinan@ucc.ie (T.G. Dinan).

http://dx.doi.org/10.1016/j.bbi.2016.11.018
0889-1591/

Cryan and Dinan, 2015; Desbonnet et al., 2014, 2010; Hsiao et al., 2013). This has prompted a growing interest in the possibility of targeting the gut microbiota to beneficially impact human brain function and behaviour. Psychobiotics have been defined as bacteria that ingested in adequate amounts produce a positive mental health benefit (Dinan et al., 2013).

Considering the potential impact of putative psychobiotics upon central nervous system processes, especially stress, mood, anxiety and cognition (Cryan and Dinan, 2012; Dinan et al., 2015), the prospect of targeting the gut microbiota as a potential modifiable risk factor for stress-related disorders is appealing (Kelly et al., 2015). Preclinical research has indicated that chronic probiotic administration can reduce anxiety-like and depressive-like behaviour, and can normalise associated physiological outputs such as corticosterone, noradrenaline, brain-derived neurotrophic factor (BDNF) and immune function (Bercik et al., 2011b; Bravo et al., 2011; Desbonnet et al., 2010; Janik et al., 2016; Messaoudi et al., 2011). There is a growing appreciation of the need to translate this promising preclinical work to the clinic while at the same time recognising the challenges inherent in this process (Kelly et al., 2016b).

To date, there are indications from a number of sources that highlight the opportunities in this regard, for example, probiotic use in irritable bowel syndrome (IBS) (O'Mahony et al., 2005; Whorwell et al., 2006), a stress-related brain-gut axis disorder associated with high rates of psychopathology (Whitehead et al., 2002) as well as altered hypothalamic-pituitary adrenal (HPA) axis activity (Kennedy et al., 2014b) and cognition (Kennedy et al., 2015, 2014a). A number of proof-of-principle studies in healthy human volunteers have demonstrated that multi-strain probiotics, fermented drinks containing probiotics, or prebiotics, can alter resting brain activity, cognitive performance, baseline physiological stress outputs and self-reported psychological variables (Benton et al., 2007; Chung et al., 2014; Messaoudi et al., 2011; Mohammadi et al., 2015b; Schmidt et al., 2015; Steenbergen et al., 2015; Tillisch et al., 2013). More recently, *Bifidobacterium longum* 1714, selected based on pre-clinical evidence (Savignac et al., 2014, 2015), was shown to reduce stress levels and to produce a neurocognitive profile associated with enhanced memory in healthy volunteers (Allen et al., 2016).

By utilizing a well-validated preclinical screening platform, developed to inform efficient selection of prospective psychobiotic strains, we identified *L. rhamnosus* (JB-1). In these studies, which were carried out in the stress-sensitive BALB/c mice, ingestion of the JB-1 strain reduced anxiety in the elevated plus maze and despair-like behaviour in the forced swim test. Moreover, there was enhanced learning in a fear conditioning paradigm and reduced stress-induced corticosterone levels. At a brain level there were marked alterations in central GABAA and GABAB receptor levels (Bravo et al., 2011). Furthermore, a magnetic resonance spectroscopy study, also conducted in BALB/c mice showed that treatment with the JB-1 strain significantly elevated central GABA levels by 25% after four weeks of treatment (Janik et al., 2016). In addition, *L. rhamnosus* treatment modulates the immune system (Forsythe et al., 2012; Karimi et al., 2009; Kozakova et al., 2016; Ma et al., 2004), intestinal motility (Wang et al., 2010), gut barrier function (Patel et al., 2012; Wang et al., 2012) and the enteric nervous system (Kamiya et al., 2006; Ma et al., 2009). Taken together, these preclinical studies identify *L. rhamnosus* as a candidate psychobiotic with the one of the most comprehensive behavioural, physiological and neurobiological profiles.

We employed a randomized, placebo-controlled, cross-over, repeated measures design to examine the effects of the JB-1 strain compared to placebo on the psychobiological response to an acute, controlled stressor (Schwabe et al., 2008; Schwabe and Wolf, 2010) and assessed cognitive performance on tests assessing memory, sustained attention, social cognition and emotional processing. In addition, we measured the immune response to this candidate psychobiotic by measuring a panel of cytokines. Finally, to ascertain if the JB-1 strain effected brain activity patterns, we assessed brain activity in frontal, parietal and central regions using EEG following 4-week supplementation with the JB-1 strain in comparison to placebo, as these regions have been associated with memory and sustained attention (Coull et al., 1996; Hales et al., 2009) and are sensitive to anxiolytics (Fukami et al., 2010) and psychobiotics (Allen et al., 2016).

## 2. Methods

### 2.1. Subjects

Approval of the study protocol was granted by the Cork University Hospital ethics committee (Protocol Number: APC057) and conducted in accordance with the ICH Guidelines on Good Clinical Practice, and the Declaration of Helsinki. Written informed consent was obtained from all subjects before any study procedures were conducted.

Participants were aged between 20 and 33 years of age. Inclusion criteria were as follows: aged between 18 and 40 years, able to speak English, in good health as determined by the investigator. Male participants were selected to avoid the influence of the menstrual cycle, which can impact upon cortisol output and other readouts and all preclinical studies with this bacteria to date have focused on male animals (Bravo et al., 2011). Exclusion criteria were as follows: having a significant acute or chronic illness, following a diet or taking a medication that would interfere with the objectives of the study, pose a safety risk or confound the interpretation of the study results; to include, probiotics, antibiotics, antipsychotics, anxiolytics, laxatives, enemas, anti-coagulants and over-the counter non-steroidal anti-inflammatorys (NSAIDS), antidepressants or any other psychotropic medication. We also excluded people with evidence of immunodeficiency, bleeding disorder or coagulopathy, colour blindness, dyslexia or dyscalculia, or receiving any treatment involving experimental drugs.

### 2.2. Design

A repeated measures cross-over design was employed. Participants were screened at an initial visit for psychiatric disorder using the MINI International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) and demographic and baseline psychological information was collected. Following screening, participants completed neurocognitive visits and acute stress visits utilizing the socially evaluated cold pressor test (SECPT) at baseline, at 4 weeks and at 8 weeks. See Supplementary Fig. S1 for the study design. Participants were administered placebo capsules for four weeks or *L. rhamnosus* capsules for four weeks and they then switched to the alternative treatment. See Table 1 for detailed participant characteristics.

### 2.3. Materials

Both active and placebo capsules contained corn starch, magnesium stearate and silicon dioxide. The count for *L. rhamnosus* (JB-1) in the active capsules was $1x10^9$ colony-forming units (CFU). Participants were instructed to take one capsule each morning.

### 2.4. Tests from the CANTAB battery

Tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) were presented on a touch-screen monitor,