From Isoniazid to *Bifidobacterium*: The Gut Microbiome as a Novel Antidepressant Target

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Introduction

Many people do not know that the first antidepressant discovered was actually an antibiotic. Isoniazid was an antibacterial drug developed in the USA in the 1950's for treating tuberculosis. Unexpected side effects of euphoria, psychostimulation, increased appetite, and improved sleep prompted an interest in the medication as a potential antidepressant. Subsequent clinical trials confirmed antidepressant efficacy, which was attributed to isoniazid's ability to inhibit monoamine-oxidase enzymes and, therefore, increase levels of monoamines such as noradrenaline, serotonin and dopamine in the brain. The resultant 'monoamine hypothesis of depression' proved fruitful and heralded the development of further antidepressant medications, including the tricyclic antidepressants (TCAs) and serotonin-specific reuptake inhibitors (SSRIs), both of which act to increase central monoamine levels.

At the time it was not considered that the antimicrobial action of isoniazid might be responsible for, or at least contributing to, its antidepressant action. Over six decades later, in the era of a new understanding of the microbiome-gut-brain (MGB) axis, we can reasonably deliberate on such a possibility. The gut-brain axis is a bidirectional communication system involving neural, endocrine and immune pathways which allow the central nervous system (CNS) and gastrointestinal tract (GIT) to interact with, and respond to, each other rapidly and effectively. Thus, gut homeostasis and function have the ability to reciprocally impact emotional states and behaviour. It is becoming increasingly clear that a major player in this complex system is our gut bacteria. Trillions of bacteria reside in our GIT, vastly outnumbering our own human cells. We now appreciate that the immense collective genetic material of these gut bacteria, comprising the 'gut microbiome', has the ability to shape neurodevelopment and impact psychological functioning to a remarkable extent. While monoamine-oxidase inhibition undoubtedly contributed to the antidepressant action of isoniazid, it is highly plausible that its antimicrobial action also played a role in alleviating depressive symptoms.

Development of Psychobiotics: From the Laboratory to the Clinic

It is notoriously difficult to develop new psychotherapeutics. This is due, in part, to limited knowledge about the neurobiological basis for mental illness and the absence of targetable biomarkers. Many landmark psychotropic medications, such as lithium for mania and chlorpromazine as the first antipsychotic, were discovered serendipitously. The development of 'psychobiotics' has followed a more logical and step-wise course. While the term 'probiotic' refers to is a live organism that, when ingested in adequate amounts, exerts a health benefit, a 'psychobiotic' is one which is specifically beneficial for mental health. Most microbiome research over the last decade has focussed on understanding the mechanisms of MGB interaction through in-vitro and animal studies. While ongoing mechanistic studies are needed in the laboratory, a major effort is currently underway to progress the exciting preclinical findings to human studies and clinical trials with psychobiotics.

Psychobiotics in the Laboratory:

The psychobiotic narrative first gained traction in 2004 when a landmark study (Sudo et al., 2004) demonstrated that the gut microbiome could dramatically influence the development and function of the hypothalamic-pituitary-adrenal (HPA) axis, the body's primary stress response system which culminates in the production of cortisol, and has consistently been shown to be dysfunctional in

major depressive disorder (MDD). This Japanese research team demonstrated that germ-free (GF) mice (mice born and housed in sterile conditions, thus lacking a microbiome) exhibited an exaggerated stress response with greater cortisol release in comparison to their control counterparts. Strikingly, the abnormal response was partially reversed by colonisation with faeces from the control mice and completely reversed by feeding with a specific bacterial strain, *Bifidobacterium infantis*. However, the reversal only occurred if recolonization took place at an early stage, indicating a critical time period for normal, microbiome-dependent HPA-axis development. That the gut microbiome could influence the stress response was a seminal discovery which generated much speculation about the antidepressant potential of probiotic bacteria such as *Bifidobacterium*. However, it was early days and much remained to be elucidated about the mechanisms of MGB interaction. A dysfunctional HPA axis is only one component of the multifaceted aetiology of depression and questions remained about whether the microbiome could influence other pathways of interest in depression research such as immune regulation and serotonin metabolism.

The next decade saw great efforts undertaken to increase understanding of the relationship between the gut microbiome and immune system. It was well-recognised that depression was associated with a state of low-grade inflammation characterised by elevated pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-alpha) (O'Brien et al., 2004) but the source of these excess inflammatory markers was unknown. It now appears that the gut microbiome plays a role in generating this pro-inflammatory state. Under normal homeostatic conditions gut microbes are safely confined to the gut and prevented from extra-intestinal access by the tightly-adherent gut epithelial barrier. However, gut permeability can be increased by various factors, such as chronic stress, a well-established precipitant of depression. The resultant 'leaky gut' can lead to translocation of gut bacteria, and bacterial components such as lipopolysaccharides (LPS), into the bloodstream, thus stimulating a low-grade immune response such as that seen in depression (Maes et al., 2008). Such a theory has been supported by studies that demonstrate the ability of various probiotics to improve gut barrier function (Ait-Belgnaoui et al., 2012) and normalise the immune disturbance seen in animal models of depression (Desbonnet et al., 2010).

The potential of the gut microbiome as an antidepressant target was further reinforced by evidence that it could influence neurotransmitter pathways. In the first instance, gut bacteria can directly produce many common human neurotransmitters including gamma-aminobutyric acid (GABA), noradrenaline, serotonin, acetylcholine and dopamine (Roshchina, 2010). Serotonin, the most well-studied of neurotransmitters in relation to depressive illness, appears to be particularly susceptible to influence by the gut microbiome. A key study in 2009 revealed that the plasma serotonin levels of GF-mice were almost 3 times less than those of conventional mice (Wikoff et al., 2009). It was subsequently demonstrated that this differential serotonin level was secondary to the remarkable ability of gut microbes to directly promote the synthesis of serotonin from its amino acid precursor, tryptophan, in intestinal enterochromaffin cells (Yano et al., 2015). Furthermore, the gut microbiome was also shown to influence serotoninergic levels in the hippocampus, an area of the brain which plays an important role in stress, anxiety and depression (Clarke et al., 2013).

Another possible mechanism of action of psychobiotic bacteria may lie in their ability to produce short-chain-fatty acids (SCFAs) from the fermentation of non-digestible carbohydrates and proteins in the colon. The main SCFAs include propionate, acetate and butyrate and these bacterial metabolites appear to exert far-reaching effects in the body including a role in immune signalling and regulation of plasma lipid levels. Butyrate, in particular, is of major interest given its ability to

regulate gene transcription and it has been shown to demonstrate an antidepressant effect in mice (Schroeder et al., 2007).

Psychobiotics in the Clinic:

The major challenge in drug development lies in translating what seems promising in the laboratory into the clinical setting. The complexity of human disease means that only a very small fraction of new therapeutics progress through to the drug development process from preclinical evaluation to successful clinical trials. The challenge of psychobiotic identification and translation is no different. Despite promising preclinical findings, results in human studies have been modest. However, the evidence base is growing and clinical research on the antidepressant properties of psychobiotics is hopeful.

Most human studies investigating the potential of probiotics to improve mood have been conducted in healthy subjects and results have been variable (Table 1). A combination of *Lactobacillus helveticus* and *Bifidobacterium longum,* administered to 66 healthy adults resulted in slight improvements in mood (Messaoudi et al., 2011), as did a polybiotic combination of various *Actobacillus, Lactobacillus, Bifidobacterium* and *Streptococcus* strains (Mohammadi et al., 2016). *Lactobacillus casei*-Shirota improved mood in healthy adults with low baseline mood scores (Benton et al., 2007) and, although it did not improve mood in patients with chronic fatigue syndrome, it did reduce anxiety scores (Rao et al., 2009). Results in older adults have been less optimistic. Three studies (Chung et al., 2014, Shinkai et al., 2013, Ostlund-Lagerstrom et al., 2016), two of which had up to 300 participants, investigated single probiotics of various *Lactobacillus* strains in healthy adults over 65 years of age. They reported no benefits in terms of mood although there was an improvement in cognition noted with *Lactobacillus helveticus* IDCC3801 (Chung et al., 2014).

Several probiotic trials have been undertaken in patients with depression and two of three have reported positive findings (Table 2). An Iranian team described improvements in Beck Depression Inventory (BDI) scores in adults with a diagnosis of MDD following 8 weeks of consumption of a polybiotic containing *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum* (Akkasheh et al., 2016). Interestingly, patients also showed significant decreases in serum insulin and CRP concentrations along with increased plasma glutathione levels, thus demonstrating a beneficial probiotic effect on metabolic, immune and anti-oxidant parameters alongside the antidepressant action. A major limitation of the study was that no information was provided on the concomitant use of antidepressant medication and so it was unclear whether the probiotic was being used as an adjunctive or sole treatment. Another *Lactobacillus/Bifidobacterium* polybiotic containing alternative species of the genera (*Lactobacillus helveticus* and *Bifidobacterium longum*) found no benefit in terms of improving mood or moderating inflammatory or other biomarkers in patients with depression (Romijn et al., 2017), thereby highlighting the differential antidepressant potential of species within the same bacterial genus. A third clinical trial demonstrated an antidepressant effect of *Bifidobacterium longum* NCC3001 consumed over 10 weeks by patients with comorbid irritable bowel syndrome (IBS) (Pinto-Sanchez et al., 2017). However, the presence of IBS represented an obvious confounding factor and depressive symptoms were merely self-reported on screening questionnaires with no diagnostic interview performed to establish a clinical diagnosis of MDD.

When one takes this human data as a whole, it provides tentative but definite optimism for the future of psychobiotics in the treatment of depression, most likely as an adjunctive strategy alongside traditional pharmacological and psychological therapies. Most benefit seems to be derived by those with low mood and depressive symptoms at baseline, a conclusion confirmed by recent

meta-analysis (Ng et al., 2018). Effects in healthy subjects with normal baseline moods appear to be more limited although the use of psychobiotics as a preventative strategy for depression in those at higher risk must be considered.

Alternative Methods of Microbiome Manipulation

Of course, psychobiotics are only one means of altering the microbiome, and perhaps even, the least effective. While the term 'psychobiotics' originally referred to beneficial live organisms such as bacteria, the definition has been expanded in recent years to include 'prebiotics'. Prebiotics are non-digestible carbohydrates which are selectively fermented by bacteria in the large intestine, and can therefore be used to target beneficial host bacteria by specifically supporting and enhancing their growth (Bindels et al., 2015). Prebiotics include substances such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch and other soluble dietary fibres (though not all dietary fibres are prebiotic, i.e. not all modify selective gut microbiota). Natural sources of prebiotics include fruits and vegetables such as asparagus, leek, banana and chicory, as well as grains such as oats and wheat, foodstuffs which have become increasingly lacking in Western-style diets. Evidence for the potential of prebiotics to improve psychological health is accumulating. A FOS+GOS combination demonstrated significant antidepressant and anti-anxiety effects in mice exposed to chronic stress (Burokas et al., 2017) and in healthy human volunteers, GOS supplementation for 3 weeks resulted in suppression of the neuroendocrine stress response and an increase in the processing of positive versus negative attentional vigilance (Schmidt et al., 2015).

Another means of modifying the microbiome is through the use of faecal microbiota transplantation (FMT), a process which involves transferring the faecal matter from one individual to another, thereby colonising the recipient with the donor's microbiota (Khoruts and Sadowsky, 2016). It has been used to explore the potential transference of disease phenotypes to healthy animals by microbiome transplantation from specific human conditions or from animal models of disease. FMT from patients with MDD to microbiota-depleted rats resulted in the recipient rodents developing a depressive phenotype, both behaviourally and biochemically (Kelly et al., 2016). Similar findings were seen following depression-related FMT to GF-mice (Zheng et al., 2016). Such studies strongly support an aetiological role for the gut microbiome in depressive illness and have garnered interest in the role of FMT as a therapeutic intervention in psychiatric disorders.

Most evidence for the therapeutic use of FMT in humans has been in the treatment of refractory *Clostridium difficile* infection (CDI) but there is also evidence emerging that it may be beneficial in functional gastrointestinal disorders such as IBS, and in metabolic syndrome (Mullish et al., 2018). A recent Japanese study observed psychiatric symptoms in 17 patients who underwent FMT for the treatment of IBS, functional diarrhoea or functional constipation. Patients, with elevated depression scores at baseline, experienced a significant improvement in mood which correlated with an increase in microbiota diversity (Kurokawa et al., 2018). Although this was an open-label, uncontrolled, observational study, it does raise the possibility that FMT may be of benefit in depression. There has, to date, been only one FMT interventional study in a neuropsychiatric population. Kang et al (Kang et al., 2017) administered oral FMT from healthy donors to 18 children diagnosed with autism spectrum disorder (ASD) over a 10-week period. They reported an increase in overall bacterial diversity and significant improvements in both gastrointestinal and autistic behavioural symptoms, which were maintained at assessment 8 weeks after treatment had ended. The use of FMT for psychiatric illness is in its infancy but is no doubt a field which may offer exciting new therapeutic opportunities.

The final, and probably most important, method of introducing microbiota change is by targeting the diet. There is strong evidence that adherence to a Mediterranean diet, as well as lower intakes of food items such as processed meats and trans fats, confer protection against depression (Lassale et al., 2018) The mechanisms by which specific dietary factors promote resilience to depressive illness are not fully understood, but it is likely that the gut microbiome plays a significant role. It has been shown that a change in diet can dramatically and rapidly alter the microbiome composition (for review see Singh et al., 2017) and thus, alter levels of health-promoting bacteria and beneficial bacterial metabolites such as SCFAs. The potential for significantly reducing the incidence of depression and other neuropsychiatric diseases through large-scale dietary interventions is widely recognised and has led to the development of new initiatives such as The International Society for Nutritional Psychiatry Research to promote the growth of this field.

Conclusion:

Antidepressant therapy has come a long way from Isoniazid and the mono-amine hypothesis. However, perhaps the journey will ultimately prove to have been a circular, rather than linear, one. That the first antidepressant arose from an antibiotic is surely a notable coincidence in the current psychobiotic climate and forces one to reconsider and question widely-accepted concepts with fresh eyes. The traditional view of functional gastrointestinal disorders such as IBS is that these are anxiety- or stress-driven conditions, allowing for the concept of a top-down, brain-gut influence. New insights on the gut microbiome have turned these assumptions on their head and it may well be our gut that is driving the rapidly-escalating incidence rates of depression and anxiety.

Nonetheless, the subject of psychobiotics is still relatively new. Much work needs to be done to further delineate MGB interaction mechanisms and establish the extent of influence of this system on physiological and psychological processes. Further human studies are needed to improve characterisation of normal and ‘dysbiotic’, or unbalanced, microbiota configurations and to investigate patterns related to specific disease processes. Solid conclusions from probiotic studies are limited by the significant heterogeneity across trials, in particular in relation to the strain of probiotic, dosage levels and duration of treatment. Further studies are imperative to shed light on these variables and optimise psychobiotic treatment strategies as this exciting field moves forward.

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