ILSI 2021 Annual Symposium Session 2: The Microbiome beyond the Gut

Transcript of the presentation, Prebiotic and Probiotic Modulation of Cognition and Emotion, Philip Burnet, PhD, University of Oxford, United Kingdom

Thank you to the committee for inviting me at this excellent conference. Oh, I know time is moving on, so for the audience's sake I'll try and make this quick, because I know some of you will be approaching lunch lunchtime, some of you will be approaching dinner time, and some of you will be wanting to go to bed. So, I think I'll just start by saying: what I'd like to do is talk about how we used prebiotic and probiotic supplements as tools to test whether gut bacteria could actually modulate brain function. Now, this is not only a way of determining whether there are other peripheral mechanisms that modulate our brain, but also to develop a potentially new treatment for psychiatric disorders, especially those which are resistant to current therapies. I should say I am a neuroscientist, but I work closely with psychiatrists. But still, am quite sane.

So just my conflict of interest, some of the work is industrial funded. But a lot of it is from academic funding.

So why look at pre and probiotics? So first of all, I'll just mention very briefly why we were interested in looking at gut bacteria and brain modulation. And then I'll go into the prebiotic effects specifically of cognition, the higher function of the brain, in rats and humans. And then I'll go on to probiotic effects, which is more of the more recent studies we've done, particularly on emotional behavior, and I'll discuss that. And then go on to how we looked at probiotics and low mood in humans.

So why are we doing this? Why are we manipulating gut bacteria to look at the effect on the brain? Well, the influence of gut bacteria on host metabolism and immunity has been known for a long time, but it wasn't until 2004 when Sudo and his colleagues showed that germ-free mice had a higher response to stressors. Okay? So, there were highly stressed, anxious animals in the absence of gut bacteria, as opposed to their counterparts, control counterparts, which, when exposed to a stressor, showed a normal response. So, these were hyper responsive to stress. Now, we were interested in this in particular, because not only did they show their stress response we exaggerated in these mice, but also in the brain, the glutamate NMDA receptor subunits and obligatory subunits, the NR1, NR2A, were reduced in the brain.

Now, I'm particularly interested in the NMDA receptor because it's important for the maturation of the brain, particularly cognitive function. And its hypo function has been implicated in pathogenesis of schizophrenia. And so, my search mainly focuses around ways of augmenting this receptor. And so, we started looking at manipulating gut bacteria to achieve this based on Sudo's work showing that germ-free animals, a complete absence of gut bacteria, reduced these receptors in the brain.

So first of all, prebiotics. Why did we want to go into prebiotics? Well, at the time when we started this, there was already, as you saw after Sudo's work, literature on how probiotics might affect brain function. But we wanted to go for prebiotics because we wanted to augment the gut bacteria that were already in the gut, the indigenous populations, which there are more of. You can manipulate more gut bacteria with prebiotics than probiotics. Obviously, probiotics are a few strains or a multi-strain, but with prebiotics you hit quite a large percentage of the beneficial bacteria. And a lot of these things are found in things we probably don't like to eat, but nevertheless they're there. And the main one is the [inaudible 00:04:50] as a supplement for fructo-oligosaccharides, or FOS. And these are simple short chains of fructose, flanked by glucose, as I'm sure most of you know.

Now, there are also galacto-oligosaccharides where, instead of a fructose, there's galactose. Now these don't normally appear naturally in nature. These are more synthetic, especially long chains. Of course, you do get glucose galactose chains in these products, but they're not as long as the synthetic ones which are manufactured. Now this is interesting because we and others have shown that bifidobacteria are particularly fond of galacto-oligosaccharides. So, it is quite bifidogenic, the galacto-oligosaccharides. And we've shown that just feeding rats with galacto-oligosaccharides and look at their fecal bifidobacteria count, that there are more bifidobacteria in the gut than there are when you feed them with FOS. So, this wasn't new, this is something that others have shown as well. But what is interesting with prebiotics is you can get actually two hits, not only do you increase the population of beneficial bacteria, which may then impact the brain, either by modulating the immune system and/or the vagus nerve, but also the prebiotics themselves are broken down to potentially neuroactive substances, the short chain fatty acid metabolites, acetic acid, butyric acid, and propionic acid.

So here we thought prebiotics were good, because we amplify a larger population of good bacteria already residing in the gut. And also, there will be a supply of short chain fatty acids, which benefits the gut themselves. And I'll show you, discuss how they might go into circulation and affect the brain. So, we chose the galacto-oligosaccharides for the reasons that it's more bifidogenic.

And our first question was: do these prebiotics increase NMDA receptors? We've seen that in germ-free mice they decrease, so does increase in gut bacteria increase these? And we did actually find that feeding rats with actually both fructo-oligosaccharide and galacto-oligosaccharides increase NMDA receptors in specific brain regions important for attention, such as the frontal cortex, and the hippocampus for memory and spatial memory. So, these are a Western blot showing the changes in the subunit. We also found that giving the prebiotics increased one of the co-agonists of this receptor, and that's d-serine. So not only did it increase the receptor, but it increased the amount of the co-agonist that's needed for the receptor to function, as well as glutamate. So that was interesting. But of course, in animals these might be a homeostatic response. This doesn't necessarily indicate that these receptors were functional.

So, our next step was to investigate whether the increase in NMDA receptors in the brain were functional and caused ultimately a behavioral effect in the end. And in particular, as I mentioned, the NMDA receptors are important for optimal cognitive function, as well as neuro development. So, we use a cognitive test to see if particularly the B-GOS prebiotic improved cognition in rats. Now, but first to do that, we went further upstream and looked at the function of the NMDA receptors.

So, we did this by using in vivo iontophoresis. So basically, a cannula in the frontal cortex of the rat injects an NMDA receptor agonist, NMDA itself. And we measured the electrophysiological response of

cells in the brain to Microiontophoresis in NMDA. So micro injected NMDA. And we found ... this is a dose response. So, you see at different concentrations of NMDA there's an increase in response following prebiotic intake. So, this was a three-week prebiotic intake. Daily ingestion of this galacto-oligosaccharide. So, the increase in NMDA receptors actually correlated with an increase in their function.

But we also went further downstream, as I said, to see if this translated to an improvement in behavior. And lo and behold, we did actually find that the attention or cognitive flexibility of rats was increased. And I won't go into detail explaining this test, but basically cognitive flexibilities, as most of you will understand, is adapting to new changes in the environment. Okay? So, you're flexible about adapting to your surroundings. So, this is the important part. Animals who are on the control, they take so many trials to find a reward in a task. But those with the prebiotic didn't take very long to work out where the reward was. Obviously, there's obstacles and so on, they'd have to work out where the reward is by figuring out what's changed and what hasn't changed in their environment.

So prebiotic feeding, particularly the galacto-oligosaccharide, which increases bifidobacteria, increased NMDA receptors, which increased in function and increased cognitive flexibility. And this specific function of the brain is centered around the medial prefrontal cortex. So that's an important executive function that we all need, that makes us human, if you like.

So very briefly, what could the mechanisms be? Well, as I mentioned, that prebiotics are broken down by bifidobacteria into short chain fatty acids, acetate, butyrate, and propionate. Now, propionate and butyrate is taken up by the gut itself and uses an energy exhaust. But a lot of the acetate which is produced does actually go into the circulation. And several studies before us showed that acetate uptake, there's an interaction between NMDA receptors and acetate uptake in the brain. And indeed, pharmacological levels of acetate injecting into animals reverses blockade of NMDA receptors. If you give more acetate, NMDA receptors seem to function more. So, this might be a mechanism by which the prebiotics had their pro-cognitive effect in our animals. The galacto-oligosaccharides broke down, produced lots of acetate, that flooded the brain, increase NMDA receptors, and then led to the increase in their transcription function [inaudible 00:13:00].

And the mechanism, as I mentioned with transcription, acetate, short chain fatty acids are being shown to be histone deacetylase inhibitors. Histone deacetylase, yes, inhibitors. When you inhibit HDAC you increase transcription. So, this is the hypothesis we followed that the increase in acetate might increase transcription of the NMDA receptors. However, as I emphasized, these studies have been done at pharmacological levels of acetate. What we did, when we gave the probiotics for animals, we did see an increase in plasma acetate after prebiotic feeding. And we also showed an inhibition of HDAC activity, as has been seen with pharmacological levels of acetate. However, when we injected acetate to make plasma levels comparable to prebiotic levels, not pharmacological levels, so it's 10 times lower than pharmacological levels, we didn't see an inhibition of HDAC activity. So, the prebiotic reduction of HDAC, which we certainly saw, was actually acetate independent. Even though it does the same thing as acetate, and we hypothesized it must be the acetate, it did seem to be we couldn't replicate it at the levels produced by the prebiotic.

So, we're looking to other mechanisms, as I said. Looking at the vagal gut connections, and the role of the micro-biome itself. We didn't actually measure microbial levels as such.

So, as I said, a lot of this work is mainly because of our interest in improving treatment of schizophrenia, which is affects 1% of population. It's quite a severe mental illness, and there was very little treatment [inaudible 00:15:08]. There's psychosis, which can be treated in 70% of psychosis patients. 30% are treatment resistant. There's negative symptoms such as withdrawal and isolation and so on. But the thing that always occurs with schizophrenia or early psychosis is a cognitive impairment. And this is thought to be arising from a hypofunction of the NMDA receptor. So, this fits in with what we're doing. We found a way of increasing NMDA receptor function, so could this work in schizophrenia? And of course, more recently there has been linked with the microbiota associated with psychosis symptom severity.

So, I should say that the reason why we are interested in cognitive impairment is because this is the factor that really stops people with psychosis functioning. So even when they have medication and they're not psychotic, they still find it difficult to function in society and sustain a job, keep relationships because of their cognitive impairment, or even decreased cognitive flexibility as I mentioned. So, what we did was we wanted to see if our findings in the animals, in the rats, translated to human population. So, as you can imagine we recruited people with schizophrenia, first episode psychosis, and we measured their overall level of cognition. And we gave them a prebiotic, the B-GOS prebiotic, to take for 12 weeks, daily for 12 weeks. And then after that they would take a placebo.

So, this was an open label study. So, some started off with the B-GOS, and then we changed to the placebo. And this is, if you look on the right here, B, you'll see those who started in the B-GOS, there was increase in learning, in cognition. And then when they were swapped to placebo there seemed to be a decrease. Whereas the placebo group, when they were put on the prebiotics, seem to have sustained their learning. Obviously, there's an overall learning effect, but certainly even in the presence of a learning effect of the cognitive tasks you could see that the prebiotic improved cognition. And when you states collapse these data, [inaudible 00:17:42] comparison, you will see that the prebiotic overall improved cognition in schizophrenia. So that's quite exciting. And we are trying to replicate this with a larger number, and more measures of the actual microbiome.

The thing I should say is that it didn't affect any metabolic immune parameters. As I said, even acetate, it didn't seem to change. So, we're not sure what's going on there. We're going to look at the microbiome itself and see if there's any clues there. And as the gut bacteria and immune [inaudible 00:18:23] are inextricably linked, we obviously measured some plasma immune markers, but again we didn't see any [inaudible 00:18:28]. And I should say these of course were medicated, so whether that was a compounder I don't know. But in the presence of medication this seems to be an effective prebiotic. Perhaps the prebiotic augmented the effect of the medication. We don't know. We're looking into that at the moment.

So that's prebiotics, what about probiotics, live cultures, which were mentioned in the previous talk? Well, since the Sudo study there have been many articles looking at rodents' prebiotics and anxiety. Anxiety is quite easy to measure in rodents. A bit more difficult to measure depression really, which we'll discuss. And obviously impossible to measure psychosis. We don't know if the rats hear voices or squeaks or whatever. But so a lot of work has been done on this. So, we didn't really pick this up here until after our prebiotic studies. And because we wanted to make the translational, we were following a systematic review, which a recent systematic review concluded that the more convincing data on pre and probiotics are actually with multi-species probiotics for depression. So not so much mood in healthy volunteers, which a lot of studies have done, including ours, but actually when there's a preexisting

deficit. So clearly with a pre-existing deficit or a model we're more likely to see the effect of the probiotic.

So, what we did was we wanted to check the anxiolytic and antidepressant effect in both rats and humans. Now, a lot of the literature there's quite a few labs doing animal studies, quite few labs doing ... or fewer labs doing human studies. But we wanted to see if the same probiotic in the same lab had the same effect in rats and humans, or similar effect, as much as can be translated. So, what we did, we measure anxiety using the light/dark box, where anxious animals spend longer time in a dark area of this test box. And we measured so-called depression with the forced swim test where animals are put into to some water. They naturally swim. And the faster they stop swimming, the greater the indices of so-called depression, or in this instance maybe learned helplessness. So, it's a bit controversial. But it is a quick test to see and has been reported to be successful showing antidepressant effect.

But anyway, we gave a multi-species probiotic, a fifth of the dose that's usually reported for mouse studies, in a strain of mice that has been shown to be anxious, an anxious type of strain. We found that after three weeks of study the animals seemed to be less anxious, spent more time in the light, and their latency to go out into the night was reduced. So, they were less anxious after three weeks. After two weeks didn't see much effect, so maybe it needs time to have an effect.

But in the forced swim test we didn't really see an antidepressant effect. There was no change in latency to float, or time spent floating there. The greater the number of times floating, the more depressed, if you like, the animal is. Or conversely, the less time floating the less depressed the animal is. So, we found in the animals, this multi-species probiotic, which contained bifidobacteria-lactobacilli combination, I won't say the commercial product, but this seemed to have an anxiolytic but not antidepressant. Arguably we could argue that that's not a good test [inaudible 00:22:57]. But like I said, it's a test that has been used by antidepressant drugs. So, we were just following what's been used before.

Now, we also found [inaudible 00:23:10] consistent with our interest in NMDA receptors, we did look at the expression of NMDA receptors, and we actually found a decrease in NMDA receptors, which might be actually consistent with studies in humans showing that blocking NMDA receptors has a rapid antidepressant effect. But I'm not going to share those data. I think what's more interesting is that we looked into whether the viability of the probiotic, which is important. So, what we did was we heat killed the bacteria and compared it, as previously, with the live bacteria. And this is the light/dark box measuring anxiety again. And as you can see, animals receiving the probiotic daily, again this was for three weeks as previously, were less anxious than the control. But also, the heat killed bacteria, those receiving heat killed bacteria where less anxious sanctions. So that was interesting because it showed that viability wasn't important for the probiotic. And it also indicates that perhaps it's the bacterial cell wall that interacts directly with the host gut which initiate responses perhaps of the vagus nerve and perhaps of the immune system, which then feeds back to the brain.

And certainly, depression has been linked to an increase in inflammatory response. Obviously a mild ... So, we did look at the immune system, and we did indeed find that [inaudible 00:24:54] were decreased, perhaps more so with heat killed than with live bacteria. However, because this was also seen at two weeks, we don't think that the anxiolytic effect of the probiotic was mediated by the immune response. So again, we're looking into more how we can dissect the actual pathway, the path between the gut and the brain, which is mediating the anxiolytic effect.

So, what about humans? So [inaudible 00:25:33] now we also look to see if we could translate our findings of the mice into humans. And as I said, in the systematic [inaudible 00:25:47] meta-analysis, it was shown that the most promising result would probably be from studies of depression [inaudible 00:25:57] probiotics. So certainly, we decided to recruit people with low mood. According to the patient health questionnaire nine, this is something that primary healthcare uses. And we chose moderate depression because these people would, although they had quite high levels of depression, they weren't receiving medication. They weren't being treated or reluctant to seek any treatment. So, we could actually have a preexisting deficit without medication confounding any of the data.

So, we designed a double-blind, two-group placebo-controlled trial, 16 billion cfu per day or placebo for four weeks. We did subjective ratings of depression. So, we did a before and after score of depression after four weeks. And anxiety, the State-Trait Anxiety [inaudible 00:27:00], and pre and post. And we did psychological and cognitive measures on the last day. The reason why we did this is because when mood changes, the processes that underlie them are actually [inaudible 00:27:21] psychological processes. So, it's not the case of we see euphoria or short-lasting euphoria at the end of the study because everyone was glad the study was over, we actually could measure robust psychological changes in their perception of negative and positive stimuli, which in a way provides information of the psychological mechanisms that underlie mood. And we also measured blood immune markers as we did with the animals. And unfortunately, we couldn't obtain brains, but we did blood immune markers. And the waking cortisol response. So, this is the stress hormone that increases when we wake up. We measured that before and after too.

So, what we found is that the multi-species probiotic actually decreased significantly depression scores and people with low mood. This indicates significant [inaudible 00:28:30] interaction. There was a slight, as expected, a placebo effect, but this wasn't significant. This was more significant, the actual probiotic. So that fits in with the antidepressant profile. But interestingly, converse to the animal, it wasn't anxiolytic. So, these are the results of the State Trait Anxiety Inventory. And as you can see, pre and post there was no changes between before and after supplementation, or between placebo and prebiotics. So, it seemed to have an antidepressant effect, not an anxiolytic effect, which is converse to the animals.

And I've put this graph in on the right here just to show you what was interesting, is one of the questions in the patient health questionnaire was about attention, about cognition. And particularly concentration. So, people are always asked, "Is your concentration very poor? Can you not concentrate on watching TV or reading the newspaper?" And it seems their concentration increased after probiotic intake. So, there is some sort of change in cognitive scores there. But again, it did not affect circulating immune markers, and we didn't see a change in [inaudible 00:29:58] cortisol, the measure of stress response, which is the same as we saw with the prebiotics [inaudible 00:30:05].

And finally, what about the psychological tests? What are the psychological underlying mechanisms? So, we used a test [inaudible 00:30:15] of emotional tasks. And basically, what these do is measure what our biases ... Subjects are shown pictures, words, and so on. And when they score, we can determine what sort of emotion they focus on, if you like. The specific emotional stimuli they focused on. Now, people with depression tend to focus on negative stimuli, whereas happy people tend to, or even antidepressant treated people, focus on positive stimuli. So, as you can see in this graph on the left here, there wasn't much difference between probiotic and placebo. But what did happen was that probiotic people actually focused more neutral faces. So, there was no emotion expressed by faces they see on a computer-based task. Now as I said often, people with depression, you'll see a reduction in happy, and

an increase in the negative emotions such as anger, fear, sad especially. And then when they're treated with antidepressants you see an increase in happy.

But here we actually saw it was neutral. So, they seemed to be unbiased towards their choice of whether they picked a negative or positive emotion. And the same happened in this dot-probe task, which is a measure of emotional attention. This is the placebo, but a lot of patients who have been on antidepressants actually show this profile. They show an increased bias towards happy stimuli. But the probiotics completely annulled both emotions. So, they seem to be like they don't care about which emotion is shown. They seem to rather be neutral. So, it seemed to be numbed by any emotional stimuli. And interestingly, in a reward learning task, the probiotic again, they seemed to care less about receiving a reward. Now, this reward learning is informative for measuring anhedonia. So, lack of pleasure in depression. And so, people with depression will have lower win trial and probably higher loss trial because they get less pressure out of winning. But here they didn't seem to care whether they won or lost.

So overall it seemed to be opposite to traditional antidepressants by showing, as we call it, reduces reward salience, or becomes impartial to specific emotional stimuli. So emotionally blunted they seem to be, for want of a better term.

So that's it. So, to summarize. Pre-clinical studies showing benefits of gut bacteria appear to be translational. So even the higher functions that we found with the prebiotics, the higher executive function such as cognitive flexibility, seem to be translational and in severe mental illness. And we need to understand mechanisms may not be the same as pharmacotherapies as we saw in the last slide, or between species. We saw in the animals there's an anxiolytic effect, not antidepressant effect, vice versa in humans. And so, we have shown that the pre and probiotics may assist in treatment of cognitive and mood disorders. But I don't think they're likely to help mental illness on their own. I know we showed that with low mood it may be helpful, which is interesting. Because low mood people, low mood not being treated makes up about 50% of the total population, people with depression per se. So that will be a useful treatment to roll out.

But I think that perhaps with the gut bacteria improving metabolism of the host is probably more likely to increase the effect of drugs. And the maintenance of a normal health gut may reduce the onset of brain disorders. So, although, like all the other speakers have said, we don't know what a healthy microbiome is, certainly nurturing the ones we know are beneficial to the gut may help give us some resilience to psychiatric disorders.

So finally, I'd like to thank everyone in the audience for your attention, and to thank all my colleagues in Oxford and around the country who have put up with me these past few years. [inaudible 00:35:35]. Thank you very much.