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Intestinal microbiota and its host: harmony or discord?

Is there a therapeutic role for a chemical entity with a microbiota-specific intestinal target? The example of cardiovascular and metabolic diseases

Professor Rémy Burcelin, PhD (Toulouse)

Director of Research Inserm
I²MC, Institute of Metabolic and Cardiovascular Diseases, Inserm U 1048,
Jean Poulhès Street, 31400 Toulouse, France
Director of Team 2 "Intestinal Risk Factors, Diabetes Mellitus"

Springer Healthcare

Notes are linked to the references page.

INTRODUCTION

It is now clearly established that both the metabolic activity and the molecular structure of the gut microbiota control the development of metabolic and cardiovascular diseases.[1-6] All along the gastrointestinal tract, from the mouth to the anus, bacteria that colonize the corresponding epithelia metabolize not only nutrients but also food components that are not absorbable by humans, along with cell residues and byproducts. In addition, they synthesize new molecules from those produced by the host, such as secondary bile acids.

Therefore, molecular constituents such as lipopolysaccharides, peptidoglycans and flagellin, metabolic products, especially those generated by fermentation, such as short-chain fatty acids, nitrosylated and sulphonated molecules, derivatives of eukaryotic cells (deconjugated bile acid), and serotonin-derived intestinal hormones, constitute a means of communication between the host and the microbiota.

Can a chemical entity aimed at a microbiota-specific intestinal target serve as a therapeutic solution in cardiometabolic diseases?

TOWARDS A THERAPEUTIC SOLUTION VIA THE INTESTINAL MICROBIOTA IN CARDIOMETABOLIC DISEASES

Prerequisites

Before considering intestinal microbiota as a therapeutic solution for cardiometabolic diseases, some important questions need to be addressed:

1) Does the gut microbiota of patients with a specific disease such as diabetes or obesity differ from that of non-affected individuals?
2) Does dysbiosis in patients with diabetes differ from non-diabetic individuals?
3) Is microbiota dysbiosis causal? And:
4) Can we identify the molecular mechanisms of dysbiosis?

All of these issues are currently being debated.

Validation in type 2 diabetes

Dysbiosis of the intestinal microbiota in human type 2 diabetes

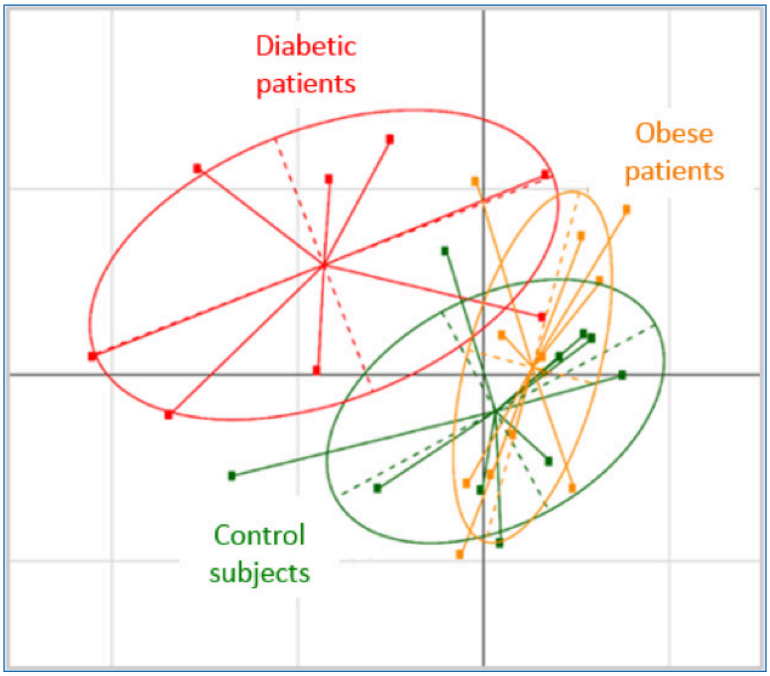
Various studies have shown the importance of gut microbiota dysbiosis in type 2 diabetes. Qin and colleagues[7] identified and validated approximately 60,000 markers associated with type 2 diabetes, opening the way to many avenues of research. The study proposed a list of bacteria positively and negatively associated with dysbiosis in this patient population.[7] However, these results raised arguments that the bacteria listed in the study were based on the fecal microbiota and may not be relevant to type 2 diabetes which, from a causal perspective, is more closely associated with dysbiosis of the ileal microbiota or the first half of the digestive tract.

Preliminary results of a study on the microbiota of the ileum mucosa conducted in our laboratory showed that sequencing of bacterial DNA distinguished obese diabetic and obese non-diabetic patients from individuals

in the control group (Figure 1: personal data). These results indicate that the microbiota and dysbiosis of the intestinal mucosa in diabetic obese and non-diabetic obese patients differ significantly from normal individuals in a potentially causal gut location. This causality has been seen in both humans and animals.

These observations validate prerequisite #1: it is a diseased microbiota that must be treated, and such certainty suggests new steps towards a therapeutic strategy targeting the microbiota in some metabolic diseases.

Figure 1. Sequencing of bacterial DNA from the ileal microbiota of healthy individuals, patients with type 2 diabetes, and non-diabetic obese patients: three different microbiota. *Personal data.*



Microbiota functions in type 2 diabetes

Qin and colleagues[7] analyzed specific bacterial genes from patients with type 2 diabetes and identified molecular pathways that revealed the existence of differential metabolism, including enrichment in membrane transport of branched-chain amino acids, sugars, sulfate reduction and oxidative stress. Thus, beyond identification of the types of bacteria present (names and taxa), functional analyses can identify metabolic pathways associated with phenotypes likely to generate a therapeutic substratum. These results suggest that it may be possible to treat type 2 diabetes by treating the microbiota and/or its targets.

Is dysbiosis of the microbiota causal?

Qin and colleagues[7] compared diabetic versus non-diabetic individuals in order to understand dysbiosis in type 2 diabetes and to answer this question; however, results of this study were of little relevance. A "before and after" comparison could shed some light on this issue, but this comparison may be unrealistic, if not impossible, owing to slow disease development in type 2 diabetes. Evaluating remission in already diabetic patients may be valuable, therefore. Another interesting aspect could be targeting the gut microbiota of patients with type 2 diabetes with specific drugs to control their blood glucose levels.

Metformin has been used over decades to control hyperglycemia in patients with type 2 diabetes. Today, we are still discovering its various mechanisms of action, one of them being the key fact that metformin alters intestinal microbiota.[8] Digestive difficulty after metformin intake has been reported in patients with type 2 diabetes.[8] It has now been established that these digestive disorders provide evidence of the therapeutic action of metformin in this patient population.

A recent study by Wu and colleagues[8] compared treatment-naive type 2 diabetes patients receiving a standardized dietary regimen for 4 months with metformin-treated patients. The study showed a perceptible dysbiosis, eventually evolving towards improvement. The OMICs approach[9] (metagenomic, transcriptomic, metabolomic analyses, etc.) also identified some bacterial functions as potential therapeutic targets, such as small molecules targeting the microbiota genes. These results suggest that particular elements of the microbiota may cause the dysbiosis seen in patients with type 2 diabetes.

In the study by Wu and colleagues[8], transfer of fecal samples from donors treated with metformin (obtained before treatment and 4 months after treatment) to germ-free mice showed improved glucose tolerance in mice receiving microbiota altered by metformin. These results indicate that it may be possible to improve type 2 diabetes using intestinal bacteria.

Molecular analyses were also performed in this study in order to identify correlations that could more accurately define this glycemic impact with metformin. Several positively associated candidate bacteria were identified, hardly "targetable" as a whole. Clustering made it possible to limit the number and identify three bacterial groups, suggesting the possibility of deriving three different molecules. The existence of interaction networks between candidate bacteria, as confirmed by metagenomic and microbiomic analysis, has led to the questioning of the potential existence of common bacterial functions. The authors conclude that metabolism of metalloproteases and metal transporters may be a preferential target for metformin on the microbiota.[8]

The effect of this action on the host remains to be determined.

Validation in obesity-induced hepatic steatosis

Hoyles and colleagues[10] used a phenomenic (hepatic transcriptomics, plasma and urinary metabolomics, and metagenomics) approach to study the development of hepatic steatosis in obese patients with and without insulin resistance, and reported the existence of correlations between steatosis and bacterial taxa. Taxonomic analysis based on clinical markers actually showed taxa associated with the disease. The authors reported a wide taxonomic diversity (394 species) and analyzed both metagenomic richness (comparison with 10 million genes) and microbial gene functions. Among the small number of metabolites released into the circulating blood or urine, potential candidates such as phenyl acetic acid or phenyl acetate were identified.[10] This molecule may be one of the vectors that express the microbial signature associated with the "hepatic steatosis" phenotype, and is likely to generate a small molecule which could be a potential drug. The metabolism of branched and/or aromatic amino acids could therefore become a target in hepatic steatosis, and the mechanism of the pathological effects of the intestinal bacteria could form the basis of a therapeutic solution based on small molecules.

The same study used transcriptomic analysis of peripheral blood to identify more than 2000 genes involved in steatosis from the earliest to the most advanced stages. Overall, 30 hypotheses were designed based on transcriptomic, microbiomic and metabolomic techniques. Among these, four groups of gene functions («core centers» or molecular centers) were identified: lipopolysaccharide (LPS)-centered inflammation, lipogenesis, amino acid metabolism, and immune defense reactions.[10] The study showed that, despite the fact that these are large biological systems, there is a hierarchy of molecular centers, which may lead to a potential therapeutic solution.

HOW TO APPROACH THIS THERAPEUTIC STRATEGY

Unbiased approach

The results of human studies suggest the possibility of developing a therapeutic strategy based on an unbiased approach. This approach consists of adding predictive algorithms to data compilation in order to obtain small but extremely refined targets such as:

1. A bacterium or a group of bacteria
2. A group of bacterial functions (metalloproteases, etc.)
3. A molecule produced by the bacteria that interacts with its ligand
4. Host–microbiota interaction

Approach based on a priori hypotheses

Among several hypotheses suggested to determine the crosstalk between the microbiota and its host, the most prevalent hypothesis is that the permeability of the intestine is responsible for metabolic inflammation. This hypothesis is based on translocation of dysbiotic microbiota into the organism as fragments (LPS, peptidoglycan, flagella, etc.) or as whole bacteria. This delivery to a functionally targeted tissue (such as the liver, which is located just behind the intestine) is supported by the immune system, thus inducing an inflammatory reaction.[11] Molecules such as LPS may be used as a starting point to suggest a molecular therapeutic solution. The action of anti-inflammatory LPS may be useful in antagonizing the deleterious effects of pro-inflammatory LPS, and blocking intestinal translocation with anti-inflammatory LPS may prevent the modification of tissue microbiota induced by intestinal dysbiosis.

Another preponderant hypothesis is that of altered intestinal immunity: in order to undergo translocation, bacteria must be «authorized». In metabolic diseases, an alteration in the intestinal immune system is associated with dysbiosis of the microbiota. Molecular mediators of this process have been identified. The bacterial antigen-presenting cell and its interaction with the adaptive immune system have undergone transcriptomic analysis, making it possible to restrict molecular hypotheses to immune co-activation with a number of key genes.[11] Once restored, immune co-activation is likely to induce natural defenses against the passage of bacteria that can generate inflammation. Therefore, targeting immune co-activation by small molecules to prevent gut permeability could be another option.

The tools

Blood and/or tissue biomarkers are necessary for a "who, why and how?" diagnosis. Indeed, bacterial DNA or "the tissue microbiota" may be a functional vector of inflammation in tissues such as adipose tissue, liver and pancreatic cells. In the blood, bacterial DNA is a diagnostic biomarker of therapeutic efficacy, category of patients, or prognosis. Thus, based on a simple blood test to sequence bacterial DNA biomarkers, it may be possible to diagnose certain diseases such as cancer or liver fibrosis, as reported recently,[12] and to propose a therapeutic target.

In obesity, sequencing of the vascular stroma (the cells surrounding adipocytes in humans) may show increased bacterial count. If these bacteria have a causal role in obesity, they are likely to serve as therapeutic targets. The bacterium Ralstonia, in particular, is a potential candidate. The functional vector for these bacteria is LPS, which may have anti-inflammatory activity for some and pro-inflammatory activity for others.

CONCLUSION ON "SMALL MOLECULE STRATEGIES"

Currently, it appears that systematic analysis of the intestinal microbiota makes it possible to identify the bacterial functions, and bacteria-derived molecules, associated with clinical phenotypes. Preclinical validation of these bacterial molecules will generate new targets for chemical therapeutic classes.

However, since the etiology of chronic metabolic and cardiovascular diseases seems to involve the gut microbiota, predictive diagnosis for classification of patients is crucial for better therapeutic solutions. Circulating bacterial DNA is a suitable biomarker for classifying etiology because its quantification is minimally invasive, and its diversity reflects indices of intestinal permeability and defects in the immune system, or even dysbiosis of the microbiota, all likely to cause development of cardiometabolic diseases. The same goes for liver diseases, or even autoimmune diseases such as type 1 diabetes. The identification of these molecules will make it possible to produce derivatives that will first topically affect the intestine, with low associated risk, and then activate, very early upstream, a series of physiological reactions. Such a process is likely to be useful in the management of numerous facets of human physiology.

The gut microbiota can interact with the host in three ways. Firstly, it can secrete molecules that act directly on target cells after absorption by the intestine. An example of this is LPS produced by bacterial cells, which interacts with adipose tissue stem cells to induce a local inflammatory response. Secondly, it can act on intestinal cells, which in turn transmit the bacterial signal to target tissues. Examples of this are the short-chain fatty acids such as butyrate that interact with insulin-secreting intestinal L cells to stimulate the secretion of insulin and control diabetes. Lastly, bacteria or even bacterial fragments can be translocated through the intestinal epithelium to the target tissues. For example, bacteria and bacterial DNA are found in the liver and adipose tissue, especially in the stromal vascular part of these tissues.

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