Higher Levels of Bifidobacteria and Tumor Necrosis Factor in Children With Drug-Resistant Epilepsy Are Associated With Anti-Seizure Response to the Ketogenic Diet

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Background: Recently, studies have suggested a role for the gut microbiota in epilepsy. Gut microbial changes during ketogenic diet (KD) treatment of drug-resistant epilepsy have been described. Inflammation is associated with certain types of epilepsy and specific inflammation markers decrease during KD. The gut microbiota plays an important role in the regulation of the immune system and inflammation. Methods: 28 children with drug-resistant epilepsy treated with the ketogenic diet were followed in this observational study. Fecal and serum samples were collected at baseline and three months after dietary intervention. Findings: We identified both gut microbial and inflammatory changes during treatment. KD had a general anti-inflammatory effect. Novel bioinformatics and machine learning approaches identified signatures of specific Bifidobacteria and TNF (tumor necrosis factor) associated with responders before starting KD. During KD, taxonomic and inflammatory profiles between responders and non-responders were more similar than at baseline. Interpretation: Our results suggest that children with drug-resistant epilepsy are more likely to benefit from KD treatment when specific Bifidobacteria and TNF are elevated. We here present a novel signature of interaction of the gut microbiota and the immune system associated with anti-epileptic response to KD treatment. This signature could be used as a prognostic biomarker to identify potential responders to KD before starting treatment. Our findings may also contribute to the development of new anti-seizure therapies by targeting specific components of the gut microbiota. Funding: This study was supported by the Swedish Brain Foundation, Margarethaemmet Society, Stiftelsen Sunnerdahls Handikappfond, Linnea & Josef Carlssons Foundation, and The McCormick Genomic & Proteomic Center.

Commentary

They are tiny but many: resident microorganisms in the human gut outnumber the total count of human cells and contribute approximately 200 g to the total body weight. The composition of our microbiota is highly versatile and is shaped by environmental factors, primarily our diet and the drugs we take. The microbiota in turn modulate many physiological functions of the human body, to the degree that some have suggested reclassifying them as a separate bodily organ. From their headquarters at the gut, they affect the rest of the body through metabolites they secrete and via immune, endocrine, and neural pathways, including the vagus nerve and the enteric nervous system. It is no wonder then that the profile of bacteria that inhabit the human intestines is being increasingly associated with diseases such as inflammatory bowel disease, cancer, and neurological disorders.

Preclinical works also demonstrated that the intestinal microbiome could be involved in epileptogenesis, and the microbiotal diversity differed between patients with epilepsy and healthy individuals. In small studies involving patients with epilepsy, both probiotics and antibiotics were associated with improvement in seizure frequency. In one patient, long-term seizure freedom was achieved after fecal microbiota transplantation for Crohn’s disease.

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The ketogenic diet is among the treatments most prone to interactions with the gut bacteria. In animal models, the anti-seizure effects of the ketogenic diet were convincingly shown to depend on the gut microbiota, and ketone bodies selectively inhibited bifidobacterial growth. A pilot study by Stafanie Prat-Nielsen from the Karolinska Institute and her colleagues confirmed in children with severe epilepsy the depletion of Bifidobacteria, in association with the ketogenic diet and identified dysbiosis of Bifidobacterium breve and B. longum. Now, the same group reports that the levels of B breve/longum and those of tumor necrosis factor (TNF) are predictors of response to the ketogenic diet among children with drug-resistant epilepsy.

To map the relationships between the gut microbes and treatment response, the researchers prospectively followed 28 patients aged 1.9 to 17.8 years. At the time of ketogenic diet initiation, all the children were receiving anti-seizure medications. Fecal and serum samples were collected at baseline and at 3 months on diet. Patients with at least a 50% reduction in seizures at 3 months were defined as responders. Machine learning was applied to datasets of taxonomic and functional profiles of the microbiota for identifying associations between the microbiome, the dietary intervention, and treatment response. In addition, 92 serum biomarkers of inflammation were analyzed simultaneously.

The dietary therapy was associated with a mean seizure reduction of 73.9% (range, 100%-51%) among the 16 responders. That of the 12 nonresponders was 4%, ranging from 40% reduction to a 19% increase in seizure frequency. The machine learning analysis identified distinct profiles of pretreatment gut microbiota which could differentiate between responders and nonresponders with an accuracy of 71.4%. Before the treatment, older responders (versus non-responders) had higher relative abundance of B breve/longum which are prominent members of the microbiota in infants, raising the question of why they linger in this group of children and adolescents. No discriminative features of the fecal microbiota could be identified during treatment. Responders also had higher pretreatment levels of TNF and other cytokines, but the diet eliminated those which are prominent in children with drug-resistant epilepsy.

The authors commented that their findings should be viewed as preliminary, given the small patient numbers and the absence of age-matched healthy controls. Another limitation was the lack of sensitivity analysis with regard to the 12 children who were fed by gastric tubes. Yet the findings demonstrated reproducibility of bifidobacterial dysbiosis and Bifidobacteria-TNF correlations. In addition, all efforts were made to minimize the impact of confounding factors. For instance, no changes were made in the pharmacological treatment over the 3-month study period, with the exception of valproate and topiramate dose reduction due to side effects (25%-37%, two patients, and 20%-25%, three patients, respectively).

Do these results imply that Bifidobacteria (or other microbes) and associated inflammatory factors contribute to epileptogenesis? Should patients with epilepsy avoid or consume Bifidobacteria-containing probiotics? What about antibiotics? It is hoped that future studies will address these questions. Such studies should ideally be multicentered to account for variation in nutrition and other environmental factors. Sufficient patient numbers will support the building of models for predicting the relative contribution of bacteria versus other factors to the variability in patient response to anti-seizure therapies. The featured work has provided data and insights which could support the design of such studies.

**Bugs and Drugs**

The gut bacteria are not only victims of dietary and pharmacological interventions, but they can also store drugs and even metabolize them. Sulfasalazine bioactivation by intestinal bacteria has been long established, but a recent study found that this phenomenon is more likely to be the rule than the exception—two-thirds of 271 orally delivered medications were metabolized by at least one bacterial strain. In that study, gut bacteria significantly reduced the levels of lamotrigine, oxcarbazepine, and phenytoin, with a drop of up to one-third in phenytoin in vitro levels over the 12-hour incubation. In another analysis, a model based on studies in mice predicted that the microbiome contributes 78% and 66% to the systemic levels of the 2 major murine metabolites of clonazepam. Intestinal microbes can therefore contribute to drug activation, inactivation, or formation of toxic metabolites. One might speculate that the need in dose reduction of valproate and topiramate in the children on ketogenic diet could have been related, at least in part, to depletion of bacterial species which metabolize those drugs.

**Bugs as Drugs**

Discovering the relationships between microbiota compositions and disease can open the door for the development of targeted therapies to support the proliferation of beneficial microbes. Consortia of bacteria have already shown success in the treatment of recurrent Clostridium difficile infection and bacteria-based therapies undergo clinical trials for their efficacy in cancer. Two studies which were published last year in Science found that fecal microbial transfer resulted in modest response among patients with melanoma who had previously failed to respond to immunotherapies. Potential future epilepsy therapies may contain more specific partial microbial fractures, target directly the culprit microbes (e.g., B breve and B longum), or provide an assortment of metabolites produced by beneficial bacteria. Compared to cancer, microbial therapies of epilepsy are still in their infancy. Success would largely depend on the relative contribution of bacteria to response among patients with epilepsy.

In conclusion, we are currently far from choosing therapies for epilepsy based on patients' gut, but the featured work is a first step in identifying novel biomarkers that could help us prescribe safe and effective treatments for individual patients. It is hoped that advances in this field will make precision medicine even more befitting to its title.
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