

Impact of Intestinal Microbiota Disparities on Sodium Valproate Concentrations in Pediatric Epileptic Patients

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Abstract: Pediatric epilepsy ranks among the most prevalent neurological disorders in children. The efficacy of sodium valproate in treating epilepsy is closely tied to its serum concentration, which exhibits substantial inter-individual variability. This study investigates the alterations in gut microbiota in pediatric epilepsy patients across various age groups following the administration of sodium valproate. The findings reveal a trend of decreased relative abundance of *Bacteroides* and *Blautia* in fecal samples with increasing age, coupled with an elevation in the abundance of *Ruminococcus*. Consequently, it is postulated that the primary factor initiating differences in sodium valproate concentration among pediatric epilepsy patients across different age groups is associated with changes in gut microbiota. Understanding the impact of gut microbiota on sodium valproate concentration is crucial for guiding the pharmacological treatment of epilepsy in affected children.

Keywords: Pediatric Epilepsy; Sodium Valproate Concentration; Gut Microbiota; Different Age Groups

1. Introduction

Pediatric epilepsy is a chronic brain disease caused by various factors, characterized by recurrent, paroxysmal, and transient central nervous system dysfunction due to excessive discharge of brain neurons. It is one of the most common neurological disorders in children^[1]. Valproic acid is the first-choice drug for treating pediatric epilepsy, with an efficacy rate of over 60%^[2]. The efficacy of sodium valproate in treating epilepsy is closely related to its serum concentration, with the expected therapeutic effect achieved within a blood drug concentration range of 50-100 µg/mL. However, there is significant inter-individual variability in sodium valproate blood concentrations, and the relationship between dose and blood concentration is unstable, especially in pediatric epilepsy patients^[3]. The exact mechanism of epilepsy onset remains unclear, and growing evidence suggests a close relationship between intestinal microbiota and neurological diseases^[4, 5]. The gut microbiota, consisting of an extremely vast, complex, and diverse microbial community, is acknowledged as the "hidden organ" of the body, playing an indispensable role in internal metabolism. In the context of epileptic states, differences in the gut microbiota among different age groups may be a crucial factor contributing to variations in the metabolism of valproic acid within the bodies of pediatric patients. Investigating the differences in the intestinal microbiota of pediatric epilepsy patients and their impact on sodium valproate concentrations is of great significance for guiding drug therapy in epileptic children.

2. Materials and methods

2.1 Data source

The observational group consisted of 155 pediatric epilepsy patients treated with valproic acid admitted to our hospital from July 2018 to July 2023. The control group comprised 148 healthy children admitted for physical examinations during the same period. There were no statistically significant differences in baseline data between the two groups ($P > 0.05$). This study was approved by the Medical Ethics Committee of our hospital.

Inclusion Criteria: Conforming to the seizure criteria formulated by the International League Against Epilepsy and confirmed as primary epilepsy through symptomatic and imaging examinations^[6]; age 1-14 years; patients regularly taking oral sodium valproate.

Exclusion Criteria: Secondary epilepsy; History of traumatic brain injury; history of central nervous system infections; concurrent neurodegenerative diseases; use of probiotics or antibiotics in the month prior to enrollment.

2.2 Methods

2.2.1 Sample analysis

Valproic acid sodium blood concentration measurement: Blood plasma samples from the pediatric patients were collected and analyzed using the AXSYM iSR1000 fully automated analyzer (Abbott Laboratories, USA) to determine the trough concentration of valproic acid.

Intestinal microbiota analysis: Collection of morning fecal samples from the pediatric patients, stored in tubes with preservative solution, and preserved at -80°C. Extraction of genomic DNA from fecal samples using a fecal genomic DNA extraction kit. Electrophoresis of the extracted DNA for band observation and identification. Submission of the collected DNA samples to Lianchuan Biotechnology Co., Ltd. for 16S rRNA sequencing.

2.2.2 Data Analysis

All statistical analyses were performed using MicrobiomeAnalyst and R 3.6.1 software. α -Diversity was assessed using various diversity and richness indices via USEARCH. ANOVA analysis and multiple t-tests were applied to compare α -diversity indices. PCoA analysis was conducted using Bray-Curtis distance. The "EdgeR" package was employed for identifying species with significant differences between groups.

3. Results

3.1 Monitoring results of valproic acid sodium blood concentrations in pediatric epileptic patients of different age groups

Considering the potential dosage variations in pediatric epileptic patients of different age groups, we conducted a statistical analysis of the ratio of valproic acid blood concentration to dosage (C0/D), representing the blood concentration per unit dose. The results are presented in Table 1.

Table 1: Valproic acid blood concentrations per unit dose in pediatric epileptic patients of different age groups

Age Group (years)	n	Mean Blood Concentration ($\bar{x} \pm s, (\mu\text{g/mL})/(\text{mg/kg})$)
≤ 3	29	3.44 ± 2.82
$3 < y \leq 6$	75	4.36 ± 2.33
$6 < y \leq 14$	51	6.89 ± 1.05

3.2 Characteristics of gut microbiota in children with epilepsy of different age groups after taking sodium valproate

Analysis of the gut microbiota in children of varying age groups with epilepsy, following the administration of sodium valproate, reveals significant differences in the abundance of specific bacteria, particularly focusing on Bacteroides, Blautia, and Ruminococcus. In comparison to normal children, those with epilepsy exhibit a notable decrease in the proportions of Lactobacillus, Bacteroides, and Actinomyces. As epileptic children age and undergo sodium valproate treatment, there is a correlated decrease in the abundance of Bacteroides and Blautia, and an increase in the abundance of Ruminococcus.

To assess the diversity of amplicon sequence variants (ASVs) between groups, the Chao1 index was utilized. Additionally, the Shannon index was employed to evaluate the evenness of the gut microbiota between groups. The results indicate that the gut microbiota in the normal group exhibits the highest diversity, while the diversity in epileptic children is lower than that in the normal group ($P=0.050$).

Interestingly, age does not appear to have a significant impact on the Chao1 index of the gut microbiota in children.

These findings suggest that epilepsy and sodium valproate treatment may influence the composition and diversity of gut microbiota, and these changes might be more pronounced in specific bacterial taxa. The observed alterations in gut microbiota could have implications for the overall health and treatment outcomes in children with epilepsy.

4. Discussion

4.1 Relationship between gut microbiota and epilepsy

The relationship between gut microbiota and epilepsy is an area of significant research interest. However, the scientific community is continuously delving deeper into the specific mechanisms and associations. Here are some current research findings and potential relationships:

① Immune System Regulation: Gut microbiota has the capacity to influence the host's immune system. Abnormal immune activity, linked to certain neurological diseases, including epilepsy, is a subject of investigation. Some studies propose that gut microbiota might impact the stability of the nervous system by modulating the immune system. There is a growing body of evidence suggesting that the immune mechanism plays a crucial role in the occurrence and development of epilepsy, especially autoimmune-related epilepsy. Epidemiological studies indicate a notable increase in epilepsy prevalence in individuals with autoimmune diseases. Autoimmune antibodies, such as N-methyl-D-aspartate receptor antibodies, glutamic acid decarboxylase 65 antibodies, thyroid-related antibodies, anti-phospholipid antibodies, antinuclear antibodies, anti-voltage-gated potassium channel antibodies, etc., have been detected in the serum or cerebrospinal fluid of some epilepsy patients. Therefore, the potential role of autoimmunity in epilepsy should be emphasized. ② Inflammation and Neurotransmitters: The imbalance in gut microbiota may lead to intestinal inflammation, and inflammatory factors may influence the central nervous system through various pathways. The imbalance of neurotransmitters may be associated with certain types of epilepsy. Gut microbiota can influence the synthesis and metabolism of neurotransmitters. Neurotransmitters generated by gut microbiota include gamma-aminobutyric acid (GABA), norepinephrine, glutamate, dopamine, serotonin, etc. ③ Short-Chain Fatty Acids (SCFAs): Metabolites such as short-chain fatty acids produced by gut microbiota during food digestion are believed to have an impact on the nervous system. Some SCFAs have been found to have antiepileptic effects. ④ Gut-Brain Axis: There is an interaction between gut microbiota and the central nervous system known as the gut-brain axis. This means that changes in gut microbiota may affect brain function through neural and immune pathways. The gut-brain axis refers to bidirectional communication between the central nervous system and gastrointestinal functions, involving the vagus nerve pathway, immune pathways, endocrine pathways, and metabolic pathways. Gut microbiota can regulate brain function and behavior through these potential pathways. The vagus nerve pathway, in particular, is the core of the gut-brain axis and has been extensively studied. Neurotransmitters secreted by gut microbiota and those generated by stimulating gastrointestinal cells, including serotonin, dopamine, and GABA, usually cannot pass through the blood-brain barrier. However, they may affect brain function through the vagus nerve, and experiments in animals have suggested that the vagus nerve may be a necessary condition for the action of gut microbiota on the brain.

Although there is a growing body of research suggesting a potential relationship between gut microbiota and epilepsy, this field is still evolving, and more research is needed to confirm these relationships and understand their specific molecular mechanisms.

4.2 Relationship between gut microbiota and sodium valproate metabolism

The relationship between gut microbiota and the metabolism of sodium valproate (valproic acid) is an area of interest. Sodium valproate is an antiepileptic drug commonly used in the treatment of epilepsy and bipolar disorder. Research has suggested that the gut microbiota may play a role in the metabolism of sodium valproate. Here are some points to consider:

Metabolism modulation: Gut microbiota has the ability to modulate drug metabolism, including the metabolism of sodium valproate. The metabolism of drugs in the gut can influence their bioavailability and efficacy. Microbial enzymes: Gut microbiota contains various enzymes that can metabolize drugs.

These microbial enzymes may contribute to the breakdown of sodium valproate into its metabolites. The specific enzymes involved and the extent of their impact on drug metabolism are subjects of ongoing research. Influence on drug levels: Changes in the composition and activity of gut microbiota may influence the levels of sodium valproate and its metabolites in the body. This could have implications for the therapeutic effects and potential side effects of the drug. Individual variability: The composition of gut microbiota can vary significantly among individuals. Therefore, the microbial metabolism of sodium valproate may vary from person to person, contributing to the observed variability in drug response. Clinical implications: Understanding the interplay between gut microbiota and sodium valproate metabolism may have clinical implications. It could potentially lead to personalized medicine approaches where drug therapies are tailored based on an individual's gut microbiota profile.

It's important to note that while there is emerging evidence suggesting a relationship between gut microbiota and drug metabolism, including sodium valproate, more research is needed to elucidate the specific mechanisms and clinical significance of these interactions. Additionally, factors such as diet, genetics, and overall health may also contribute to individual differences in drug metabolism.

4.3 Analysis of research results

Valproic acid is a broad-spectrum antiepileptic drug commonly used in clinical practice for the treatment of epilepsy syndromes, generalized seizures, various types of partial seizures, focal seizures, mixed-type epilepsy, and febrile seizures, particularly as a first-line treatment for pediatric epilepsy. The therapeutic concentration range of valproic acid is between 50-100 µg/mL, classifying it as a drug with a narrow therapeutic window. When the blood concentration is below 50 µg/mL, the efficacy of antiepileptic treatment is only 30-50%, and adverse reactions can occur with concentrations exceeding 100 µg/mL, reaching up to 50%. Achieving precision in the use of valproic acid is a critical issue in clinical applications. Valproic acid undergoes three main metabolic pathways in the body: the glucuronic acid metabolic pathway (50%), the β-oxidation pathway in mitochondria (40%), and the oxidation pathway mediated by cytochrome enzyme CYP450 (10%). The conversion of valproic acid to valproic acid glucuronide by UGT enzymes, leading to excretion in urine, is a significant factor influencing the blood concentration of valproic acid. The impact of transporters on valproic acid blood concentration is a recent research focus. It has been confirmed that the P-gp protein is primarily associated with the drug resistance mechanism of valproic acid, while the transporter affecting the blood concentration of valproic acid is mainly ABCB2.

Age, as a crucial physiological factor influencing pediatric epilepsy patients, has garnered significant attention. Numerous research findings indicate that when the blood concentration is below 50 µg/mL, the proportion of children is much higher than that of adults and the elderly. In the ranges of 50-100 µg/mL and greater than 100 µg/mL, the proportion of children is the lowest, and there is the greatest dispersion in blood concentration, showing significant differences from adults and the elderly. In monitoring data for pediatric epilepsy patients across different age groups, within the age range of children under 3 years old, the proportion below the minimum effective concentration is as high as 56.3%. With increasing age, the percentage of blood concentration within the effective concentration range substantially rises.

The gut microbiota constitutes a diverse and functionally complex community of microorganisms residing in the host's gastrointestinal tract. It has been confirmed that there are over hundreds of microbial species in the gut, with a count approximately 10 times that of the host's somatic cell number, forming a uniquely diverse microbial system. Changes in the types and quantities of bacteria play a role in regulating essential physiological processes such as host metabolism, energy balance, and immune regulation. Particularly, they play an indispensable role in drug metabolism. The gut microbiota primarily alters the internal processes of drugs through direct metabolism and the regulation of metabolic enzymes. The direct metabolic role of the gut microbiota on drugs is manifested through the secretion of glycosidases, nitroreductases, and azoreductases, which metabolize chemical components, thereby exerting pharmacological therapeutic effects. Additionally, the gut microbiota can modulate the expression of host metabolic enzymes and transporters, influencing the internal processes of drugs. Functional studies on the gut microbiota suggest that bacteria such as bacteroides, firmicutes, bifidobacterium, and ruminococcus have a clear role in regulating metabolic enzymes and transporters, with their primary mechanisms likely related to influencing bile acid metabolism processes. Significant changes in the gut microbiota can occur under the dual influence of age and disease, inevitably altering the internal processes of drugs.

Upon segmenting the age of the patients for analysis, it was observed that the blood drug concentration per unit dose showed the greatest dispersion in the 0-3 age group, with an overall lower concentration. With increasing age, the dispersion of blood drug concentration per unit dose gradually decreased, and the proportion of drug concentration within the effective range also increased accordingly. Simultaneously, for the same epileptic patient, the blood concentration of valproic acid exhibited a gradual upward trend with age. The results indicate significant differences in blood drug concentration among different age groups, and there is a positive correlation between the blood concentration of valproic acid and the age of pediatric epileptic patients. Furthermore, analyzing the gut microbiota of patients in different age groups revealed a relative decrease in the abundance of *Bacteroides* and *Blautia* in feces with increasing age, accompanied by an increase in the abundance of *Ruminococcus*. Therefore, it can be inferred that the primary initiating factor for the difference in valproic acid concentration among pediatric epileptic patients of different age groups lies in the gut microbiota. This suggests that the composition and function of gut microbiota change with age, leading to a reduction in the secretion of 7α -dehydrogenase dependent on gut microbiota^[7]. Consequently, this downregulates the expression of related proteins and mRNA^[8, 9], ultimately manifesting as metabolic differences in valproic acid among pediatric epileptic patients in different age groups.

5. Conclusion

The differences in valproic acid concentration among pediatric epileptic patients in different age groups may be primarily attributed to the gut microbiota. The composition and function of gut microbiota change with age, ultimately resulting in metabolic differences in valproic acid among pediatric epileptic patients of different age groups. This study aims to identify key factors influencing the intra-process differences of valproic acid in pediatric epileptic patients, providing new insights into the understanding of drug metabolism differences and offering a novel direction for achieving precision medicine.

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