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By addressing Gut dysbiosis and Endothelial dysfunction, Limosilactobacillus Reuteri reduces Preeclampsia in mice

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Abstract

Preeclampsia (PE) continues to be mysterious while being a major cause of maternal and newborn morbidity and mortality. We verified the link between PE and gut dysbiosis. It's unclear, though, if probiotics could enhance PE via controlling gut flora. It would be crucial to investigate specific probiotics that may reduce PE by modifying gut microbiota and clarifying intricate mechanisms.

Keywords: Preeclampsia; Limosilactobacillus reuteri ; Endothelial dysfunction

Introduction

Preeclampsia (PE), a prominent cause of maternal and newborn morbidity and mortality that is defined by maternal hypertension, proteinuria, and systemic endothelial dysfunction after 20 weeks of gestation, remains mysterious despite extensive research [1-2]. PE is associated with angiogenic dysregulation and endothelial dysfunction, and it is even thought to be an endothelial condition [3], despite the fact that the pathophysiology of PE is still completely unclear. It's interesting to note that recent research have found a connection between microbial communities and PE, suggesting that the microbiota may play a role in PE pathogenesis [4-5]. Growing evidence from our research and that of others has demonstrated that PE is an intestine dysbiosis-associated disease, where many of the disease's hallmark symptoms are caused by gut dysbiosis. Uncertainty persists on whether endothelial function and gut microbes interact directly in PE.

Methods

Making L. reuteri conditioned medium and cultivating L. reuteri (CM)

Our laboratory's L. reuteri CALM603 strain was isolated from fermented food and cultivated anaerobically at 37 °C in MRS broth medium using a Whitley A35 Anaerobic Workstation (Cat. 027312, Guangdong Huankai Microbial Sci. & Tech. Co., Ltd., China) (Don Whitley Scientific Limited, UK). When L. reuteri's OD600 nm in MRS broth medium reached one, the MRS broth was removed by centrifugation at 5500 g, 4 °C for 30 min, and bacterial growth continued in endothelial cell medium (ECM) anaerobically for 4 h. This was done in order to prepare L. reuteri CM [6-8]. The L.reuteri CM was then extracted from the ECM using a 0.22 m filter after centrifugation at 5500 g for 30 minutes at 4 °C.

The use of animals in experiments

We obtained C57BL/6 J mice from the Guangdong Medical Laboratory Animal Center (8 weeks old, 18-20 g). Individually housed in plastic cages with bedding made of broken up corncobs, the animals were kept in pathogen-free environments with temperatures between 20 and 25 °C and 12 h cycles of light and darkness. A typical lab food and unlimited amounts of water were given to the mice. The gestational day (GD) 0.5 was established by mating female mice with fertile male mice at a ratio of 2:1 during the course of an overnight period [9]. All animal experiments were carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Every research involving mice was The Southern Medical University's Zhujiang Hospital's Ethics Committee on Animal Experiments authorised all investigations involving mice.

Protocol 1 for experimentation

In order to eliminate the background microbiota, female mice were initially gavaged for five days with an antibiotic cocktail (vancomycin, 100 mg/kg; neomycin sulphate, metronidazole, and ampicillin, 200 mg/kg), then mated. The control group and the L-NAME group were then randomly assigned to groups of pregnant mice. L-NAME (40 mg/kg body weight, N5751, Sigma Aldrich, Allentown, PA, USA) was subcutaneously infused into pregnant mice every day from GD 9.5 to GD 17.5 to produce an experimental PE model. Control group pregnant mice received the same amount of PBS intravenously [10]. Tribromoethanol (1.25%, 0.2 mL/10 g) was used to completely anaesthetize mice on GD 17.5 before the foetus, placenta, and kidney were swiftly removed.

Experimental protocol 2

Prior to mating, female mice were initially gavaged with the aforementioned antibiotic combination for five days in a row. Then, pregnant mice were separated into three groups: control, L-NAME, and L-NAME+L.reuteri. Pregnant mice were subcutaneously injected with L-NAME (40 mg/kg body weight) every day from GD 9.5 to GD 17.5 in the L-NAME and L-NAME+L.reuteri groups, while controls received an equal amount of PBS. From gestation day (GD) 0.5 to gestation day (17.5), pregnant mice in the L-NAME+L.reuteri group received 200 L of PBS suspended L.reuteri (109 CFU/mL) by gavage, while pregnant mice in the control and L-NAME groups received equal amounts of PBS at the same time. Tribromoethanol (1.25%, 0.2 mL/10 g) was used to completely anaesthetize animals on GD 17.5 before the foetus, placenta.

RT-qPCR with RNA isolation

Using Trizol reagent, total RNA was extracted from placenta, colon, and HUVEC cells. The ultramicro UV spectrophotometer was used to measure the concentration and purity of total RNA (NanoDrop, Thermo Scientific, USA). The Evo M-MLV RT Premix (Accurate Biology, Hunan, China) was used to create cDNA in accordance with the manufacturer's recommendations. Using SYBR Green Master Mix, quantitative PCR analysis was performed on a ViiATM 7 Real-Time PCR System. Triplicate measurements of each sample were made (n = 3). The PCR conditions were 5 minutes at 95 °C, 40 cycles of 10 s at 95 °C and 30 s at 60 °C, 1 cycle of 15 s at 95 °C, 1 min at 60 °C, and 15 s at 95 °C, Using the 2CT approach, relative gene expression was calculated. Data for each sample were normalised to the internal control -Actin and expressed as a fold change in comparison to controls.

Discussion

This is the first study to demonstrate that the probiotic L. reuteri decreased with gut dysbiosis in the PE mouse model induced by NO blockade, and that L. reuteri treatment was effective in reducing hypertension and other PE-related symptoms in mice induced by NO inhibition by reducing gut dysbiosis and endothelial dysfunction. Our findings suggest that focusing on gut microbiota may represent novel therapeutic and preventive approaches for managing PE caused by endothelial dysfunction, and that L. reuteri may reduce PE by regulating gut dysbiosis and endothelial dysfunction.

Regarding the connections between NO synthesis, endothelial function, gut microbiota, and PE, this study made several novel discoveries. First and foremost, mice with PE caused by NO blockade showed gut dysbiosis linked to a decline in L. reuteri. In addition, L. reuteri could lessen the symptoms of PE and stop gut dysbiosis in mice with PE brought on by NO inhibition. Last but not least, L. reuteri may prevent PE growth by enhancing gut dysbiosis, NO production, and endothelial dysfunction. Because of this, our work is the first to identify gut dysbiosis as a crucial target in mediating PE caused by NO blockade and L. reuteri as a protective probiotic in preventing PE by modulating gut microbiota and endothelial function.

Conclusion

In conclusion, this is a novel finding that points to L. reuteri's significant contribution to PE improvement. In this paper, NO inhibition is described for the first time as a major promoting component in mediating gut dysbiosis in PE, and L. reuteri is described as a protective probiotic that inhibits PE via modifying gut microbiota and endothelium dysfunction. Our research provided fresh perspectives on PE by showing that L. reuteri might shield PE mice from gut dysbiosis and endothelial dysfunction by regulating eNOS/NO. Our findings imply that endothelium improvement, L. reuteri, or NO treatment may be possible PE prevention therapies. These findings also support the development of other potential therapeutic PE prevention techniques that target eNOS/NO and gut microbiota. Nonetheless, multi-center clinical trials ought to be carried out.

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