FULL TEXT GUIDE

1 Research Background

Studies have shown that drug- or antibiotics-induced diarrhea is associated with intestinal lactase dysfunction due to loss of activity. Thus, treatment with lactase supplements is a good option for most types of diarrhea due to the importance of lactase activity in the control of intestinal function. Various isoforms of the lactase gene have been identified and are widely expressed in the intestinal tract, with diversity enzyme activities. The expression, protein modification and isoforms can change in different micro-environments. AAD is not only associated with dysbacteriosis, but also intestinal lactase activity damage, leading to diarrhea. In the present study, we found that the activity of lactase in intestinal contents was significantly reduced in mice with antibiotics-induced diarrhea. In order to provide a basis for the mechanism of antibiotics-induced diarrhea, we investigated whether the alterations in lactase activity were caused by its expression. Therefore, we aimed to determine the mechanism of lactase activity from the viewpoint of genetic diversity and provide a basis for antibiotics-induced diarrhea.

2 Research motivation

The mechanism of antibiotics-induced diarrhea has been studied in a wide range of diverse microbes. However, less research has been less carried out on functional enzymes. In our preliminary study, we found that the activity of lactase in intestinal contents was significantly reduced in mice with antibiotics-induced diarrhea. We conducted the present study in order to determine the mechanism of lactase activity from the viewpoint of genetic diversity and provide a basis for antibiotics-induced diarrhea.

3 Research objectives

This study was carried out in order to provide a basis for the mechanism of antibiotics-induced diarrhea and to determine whether the alterations in activity were caused by its expression. We compared the diversity of bacterial lactase genes expressed in model mice with antibiotics-induced diarrhea and

in control mice.

4 Research methods

The mouse model of antibiotics-induced diarrhea was created by gastric perfusion with mixed antibiotics (26.67 mL·kg-1·d-1) composed of gentamicin sulfate and cephradine capsules administered for five days, and the control group received an equal amount of sterile water. The contents of the jejunum and the ileum were then collected, metagenomic DNA was extracted, followed by analysis of bacterial lactase genes using OTUs after amplification and sequencing. Qiime software was used to align the sequencing results, cluster analysis, PCA and ACE abundance indexing and Simpson diversity indexing analysis results. PCoA, NMDS, and Heatmap were analyzed in the R for diversity and similarity. SPSS21.0 software was used for statistical analysis and the results are expressed as means ± SE.

5 Research results

The results showed that there were significant differences in Chao1 and ACE indices between the two groups (P<0.05). As shown by PCA, PCoA and NMDS analysis, sample distribution in the control group was relatively intensive, and differences among individuals were small, while in the model group, they were dispersed and more diversified. The bacterial lactase genes in the intestinal contents from the control mice were related to Proteobacteria, Actinobacteria, Firmicutes and Unclassified bacteria. Of these, Proteobacteria showed the greatest abundance. In contrast, the bacterial population was less diversified and abundant in model mice, as the abundance of *Bradyrhizobium* sp. BTAi1, *Agrobacterium* sp. H13-3, *Acidovorax* sp. KKS102, *Azoarcus* sp. KH32C and *Aeromonas caviae* was lower than that in the control group. In addition, of the known species, the control group and model group had their own unique genera, respectively. For example, *Gordonia*, *Mycobacterium*, *Frankia*, *Microbacterium*, *Novosphingobium*, and *Aeromonas* were only seen in the control group. However, *Micromonospora*, *Paenibacillus*, and *Ensifer* were only found in the model group. To confirm our findings, the diversity and

abundance of bacterial species were clearly shown using Heatmap analysis. The lactase genes of *Gordonia*, *Frankia*, *Novosphingobium*, *Mycobacterium*, *Agrobacterium*, *Aeromonas*, and *Microbacterium* were highly present in the intestinal contents from the model group compared with the control group.

6 Research conclusions

There were significant differences between the control mice and model mice not only in types of lactase genes expressed, but also in their activities. Following the occurrence of antibiotics-induced diarrhea symptoms, the intestinal lactase genes changed, the number of strains was reduced and the abundance decreased, indicating changes in community structure and decreased diversity of lactase genes in antibiotics-induced diarrhea.