Suppression of intestinal putrefaction accumulation with sesame lignans

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Research objective

Indole, a putrefactive substance produced by gut bacteria, causes malodor in feces, and its metabolite indoxyl sulfate induces oxidative stress, cellular senescence, and fibrosis.[1)] Indole is derived from tryptophan in dietary foods, and is produced and accumulated by the gut bacterial enzyme tryptophan indole-lyase (TIL) (Figure 1).[2)] I hypothesized that the regulation of the enzymatic activity of TIL derived from gut bacteria would suppress the accumulation of indole in the gut, resulting in the prevention of the described health problems. A previous study showed that (+)-sesamin, sesaminol, and sesamol, which are sesame lignans and their derivatives found in sesame seeds, serve as potent inhibitors of gut bacterial TIL (Figure 1).[3)] These compounds would be applicable to dietary foods in daily diet to prevent health problems by reducing the accumulation of indole, as well as to therapeutic drugs. First, it is necessary to clarify the effects of sesame derivatives on TIL-expressing gut bacteria or host health, and their inhibitory activity on indole production.

In this study, to establish a novel strategy to prevent health problems caused by indole by inhibiting gut bacterial enzymes, I investigated the effects of sesame lignans and their derivatives on gut bacteria or hosts and their inhibitory activity on indole accumulation.

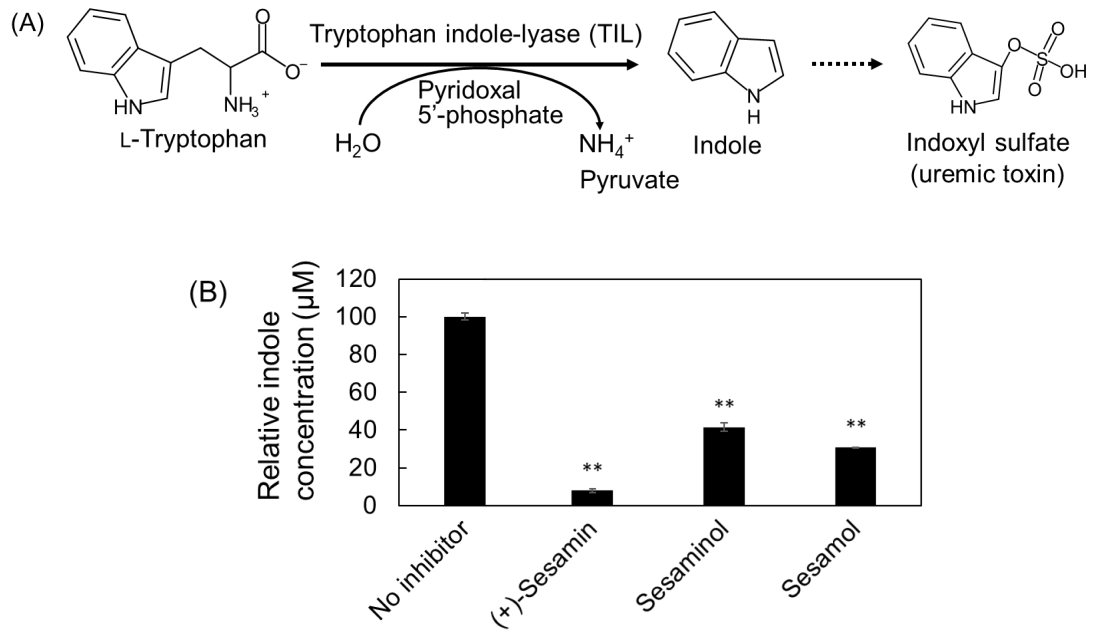

Fig. 1 Production of indoxyl sulfate and inhibition of tryptophan indole-lyase (TIL)

by sesame lignans

(A) Production of indole or indoxyl sulfate by gut bacterial TIL. (B) Screening for sesame lignans that inhibit TIL.

Methods

1. Inhibition of indole produced by gut bacteria

   First, bacterial species that encode homologs of *til* in the human gut were screened. In this study, type strains of the species purchased from the culture collection and strains isolated from human feces stored in our laboratory were used. The strains were incubated anaerobically in a reaction mixture containing tryptophan, a TIL substrate, and the indole level in the supernatant of the reaction mixture was monitored using HPLC. The inhibitory activity of sesame-derived compounds on bacterial indole production was assessed. Sesame lignans were added to a mixture of bacterial strains accumulating high concentrations of indole. After incubation, the concentrations of indole and sesame-derived compounds were measured by HPLC. The reaction mixture was applied to agar medium for the isolation of bacterial strains to quantify cell viability, and the effects of sesame derivatives on bacterial growth were assessed.

2. Supplementation of sesame derived-compounds to experimental mice

   Mice were divided into two groups. Mice in the control group were provided free access to AIN-76 and water; in contrast, mice in the sesamol group had free access to AIN-76 and sesamol-dissolved water. Mouse feces from each group were collected on various days during the experimental period. Genomic DNA was extracted from the feces, the V3-V4 region of the genes encoding 16S rDNA was amplified, and 16S amplicon sequence analysis was performed. Based on the obtained sequences, a diversity analysis or taxonomic study of the groups was performed.

Results

1. Inhibition of indole produced by gut bacteria

   Gut bacterial strains were incubated in a reaction mixture containing tryptophan, and indole in the supernatant was detected by HPLC. Indole production was confirmed in the samples with some strains belonging to the family *Bacteroidaceae* and some strains belonging to the families *Enterobacteriaceae* or *Morganellaceae* accumulated high concentrations of indole in the mixture. From these identified strains, *Bacteroides thetaiotaomicron*, *Citrobacter koseri*, and *Enterobacter* sp. were added to the mixture with or without sesame-derived compounds and incubated. Measurement of indole showed that sesamol addition resulted in a significant reduction in the accumulation of indole in the samples of each strain. In addition, results of HPLC showed that sesamol levels in the mixture were indistinguishable

before and after the reaction. The mixture was partially applied to agar medium, which was incubated anaerobically. Colonies formed on the agar were counted, and the number of viable cells was not significantly different between samples with and without sesamol. These results imply that sesamol supplementation suppresses indole accumulation by gut bacteria, is not metabolized by the bacteria, and does not affect cell viability.

2. Supplementation of sesame-derived compounds to experimental mice

   Total genomic DNA was extracted from mouse feces and amplicon sequencing based on 16S rDNA sequences was performed. Fecal microbiota of the control and sesamol groups were compared based on the obtained sequences. α-Diversity analysis revealed the absence of a significant difference in the number of amplicon sequence variants between groups. Principal coordinate analysis based on Bray–Curtis dissimilarity also showed low dissimilarity in microbial structure between the groups. The relative abundance of only a few bacterial species was significantly different, and a difference in the abundance of taxa encoding the *til*-homolog was not confirmed between the groups. These results indicated that sesamol supplementation did not have a critical effect on the gut microbiota of mice.

Conclusion

This study showed that sesamol supplementation did not affect the microbial structure of mouse feces and suppressed indole production by gut bacteria. Sesamol itself was not metabolized by the bacterial species used in this study and did not affect the cell viability of the strains in the range of the tested concentrations. Based on a previous report that sesamol inhibited bacterial TIL, it is possible that the suppression of indole production by gut bacteria in this study was partially due to the inhibition of bacterial TIL by sesamol supplementation.

References

1) Shimizu, H., *et al*. (2011) NF-κB plays an important role in indoxyl sulfate-induced cellular senescence, fibrotic gene expression, and inhibition of proliferation in proximal tubular cells. *Am. J. Physiol Cell Physiol.* **301:** C1201–C1212.
2) Phillips, R. S., Demidkina, T. V., Faleev, N. G. (2014) The role of substrate strain in the mechanism of the carbon–carbon lyases. *Bioorg. Chem*. **57:** 198–205.
3) Oikawa, D. *et al*. (2022) (+)-Sesamin, a sesame lignan, is a potent inhibitor of gut bacterial tryptophan indole-lyase that is a key enzyme in chronic kidney disease pathogenesis. *Biochem. Biophys. Res. Commun*. **590:**158–162.