学 位 論 文 の 要 旨

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学 位 論 文 名　Fenugreek Seeds Affect Intestinal Cholesterol Transporters in Caco-2 Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belonging to Fabaceae family has a long history of medical uses in Middle East, India, and China. The hypolipidemic properties of the fenugreek seeds have been demonstrated by basic and clinical studies. Some active transporters distributed in the gastrointestinal tract: Niemann-Pick C1-Like 1 (NPC1L1), ATP-binding cassette (ABC) transporter A1 (ABCA1), ABCG5, and ABCG8 mediate the intestinal cholesterol absorption. NPC1L1 is an influx transporter, whereas ABCG5 and G8 function together as an efflux pump excreting cholesterol out to the lumen. ABCA1 plays a role in intestinal cholesterol absorption on the basolateral membrane. These intestinal cholesterol transporters are regulated by transcription factors, such as a nuclear receptor: liver X receptor (LXR).

The purpose of this study is to clarify the mechanism for hypolipidemic effect of fenugreek in the intestinal absorption process. We used Caco-2 cell monolayers as an *in-vitro* model of the intestinal membrane. Effects of fenugreek seeds on the membrane transport activity for cholesterol were examined. Furthermore, we determined the mRNA expression of the major cholesterol transporters and the nuclear receptors in Caco-2 cells treated with or without

fenugreek.

MATERIALS AND METHODS

Fenugreek seeds were parched and comminuted into the coarse grain. The coarse-ground seeds were soaked and boiled in distilled water to collect the fenugreek seed water extract (fenugreek extract).

The Caco-2 cells were cultured on a polycarbonate filter set inside a 6-well plate to form confluent monolayers after cultivation for 16 to 21 days. The integrity of the monolayers was checked by the transepithelial electrical resistance. The viability of Caco-2 cells was tested with a homogeneous fluorescence assay.

The Caco-2 cell monolayer was pre-incubated with or without fenugreek extract for 24 or 48 h. After pre-incubation, cholesterol with [^{3}H]-cholesterol as a tracer was added to the apical chamber, and then the radioactivity in the basolateral chamber was measured periodically. The apical-to-basolateral permeability coefficient (P_{app}) of cholesterol was calculated as its flux rate. The effect of ezetimibe, an inhibitor of NPC1L1, on cholesterol transport was also examined in the intact cells. To evaluate the cholesterol accumulation into cells, the monolayer was lysed and the radioactivities in the lysate and medium were measured to calculate the cell/medium ratio.

Total RNA was isolated from the cells cultivated on a 6-well plate with or without fenugreek extract. Quantitative real-time PCR assay was utilized for determining mRNA expression levels of the genes involving in cholesterol transporters and nuclear receptors, and an internal reference, glyceraldehyde-3-phosphate dehydrogenase.

RESULTS AND DISCUSSION

The fenugreek extract did not change the cell viability within the concentration range of 0.01-1.0 mg/mL after the exposure for 48 h. Cholesterol was linearly translocated from the apical

to basolateral side across the cell monolayer up to 24 h. When ezetimibe was added in the apical side, the permeated amount of cholesterol was significantly decreased by about 35%, suggesting that NPC1L1 possibly functions in the Caco-2 cell monolayer. The exposure of the cells to fenugreek extract (1.0 mg/mL) significantly reduced the relative P_{app} of cholesterol in a time-dependent manner. Additionally, the cell/medium ratio of cholesterol was significantly decreased to about 70% by 48 h treatment with fenugreek extract. Net absorption from the apical to basolateral side depends on the balance of the three transporters: NPC1L1, ABCA1, and ABCG5/G8. Reduced net transport of cholesterol suggests that fenugreek extract inhibited the transport activity of the absorption transporters, NPC1L1 and ABCA1, more strongly than did the efflux transporter ABCG5/G8.

Fenugreek extract suppressed the mRNA expression levels of NPC1L1, ABCA1, ABCG5, and ABCG8. From these results, the decreased net transport of cholesterol may be due to inhibition of NPC1L1 and/or ABCA1 and not to stimulation of the efflux transporter ABCG5/G8. Considering that the accumulated amount of cholesterol in the cells was reduced, it is likely that fenugreek extract may influence the function of the influx transporter NPC1L1 rather than ABCA1. Fenugreek extract significantly decreased the LXRα gene expression, while farnesoid X receptor (FXR) was upregulated. Decreased expression of ABCA1, ABCG5, and G8 may be explained by LXRα-mediated downregulation caused by FXR activation.

CONCLUSION

The fenugreek seed water extract has inhibitory effects on mRNA expression and transport activity of the active transporters: NPC1L1, ABCA1, and ABCG5/G8, leading to the decreased net transport of cholesterol from the apical to basolateral side in Caco-2 cells. This may be one of the mechanisms for lipid-lowering effects of fenugreek seeds.