RESEARCH ARTICLE

Regulation of Cholesterol Transporter Protein by Black Pepper and Piperine

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Abstract

Black pepper extract *(Piper nigrum L.)* and piperine, the active compound of black pepper, were previously shown to block the uptake and absorption of cholesterol into Caco-2 cells. The present study was then aimed to determine the effect of black pepper extract and piperine on the expression of certain proteins functioning in the regulation of cholesterol transport in Caco-2 cells. The expression of proteins was determined by western blotting. The results showed that there was no change in the expression of ABCG5, ABCG8 and ACAT2 in cells treated with black pepper extract and piperine. The expression of cholesterol transporter, NPC1L1, in membrane fraction of Caco-2 cells was lower than that of control. This reduction was not observed in whole cell lysate. The disappearance of NPC1L1 in the membrane fraction may indicate that black pepper extract and piperine could regulate the translocation of NPC1L1 between cell membrane and cytoplasmic compartment.

Keywords Cholesterol, Black pepper, Piperine, NPC1L1.

Introduction

According to our previous study of twelve plant extracts, black pepper extract showed the best inhibition of cholesterol uptake in differentiated Caco-2 cells [1]. From our recent study, not only black pepper extract but also piperine effectively decreased cholesterol uptake and absorption in Caco-2 cells (unpublished data). To investigate their mechanism of action, we tested their effect on the expression of certain proteins functioning in cholesterol absorption. These proteins include Niemann-Pick C1-Like 1 (NPC1L1); a cholesterol transporter, acyl-coenzyme A cholesterol acyltransferase (ACAT2); a cholesterol esterification enzyme, ATP binding cassette proteins G5 (ABCG5) and G8 (ABCG8); cholesterol efflux proteins.

Method

Cell preparation

Caco-2 cells were obtained from the American Type Culture Collection (ATCC). Cells were grown in DMEM/F12 containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37 °C in CO_2 incubator containing 95% air and 5% CO_2. Cells were propagated in culture flasks and subsequently plated in 6-well plates. Caco-2 cells were cultured for 14- 21 days allowing cell differentiation.

Western blotting

Differentiated Caco-2 cells were treated with black pepper extract and piperine for 24 h. The cells were harvested in lysis buffer (50 mM Tris-HCl, pH 7.4, containing 0.5% SDS and 1% of protease inhibitor cocktail) and stored at -20°C before use. The protein concentrations were determined by BCA protein assay kit. Proteins in cell lysate were separated on SDS-polyacrylamide gel and transferred onto PVDF membrane. The membrane was blocked with PBS containing 5% skim milk at room temperature. Then, membrane was incubated with primary antibodies recognized NPC1L1, ABCG5, ABCG8 or ACAT2 overnight at 4°C. Proteins were detected using secondary antibodies IgG conjugated with horseradish peroxidase and then visualized by enhanced chemiluminescence.

Results

The result show that NPC1L1 level in membrane fraction was lower than that control, whereas in whole cell lysate was not altered after treating cells with black pepper extract and piperine for 24 h. (Fig. 1). The levels of ABCG5, ABCG8 and ACAT2 proteins did not change following such treatment (Fig. 2).

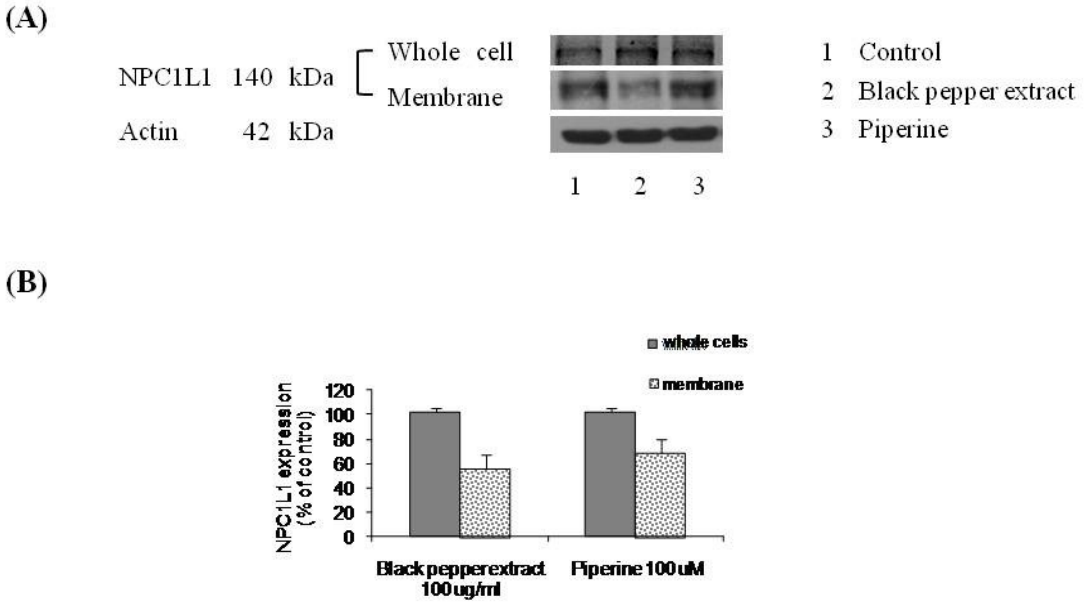

Figure 1 NPC1L1 protein expression in whole cell lysate and membrane fraction of differentiated Caco-2 cells incubated with black pepper extract and piperine for 24 h (A). Protein band densities were averaged from 3-4 experiments (B). Expression values ware normalized by actin. * $p<0.05$ compared to untreated cells (control).

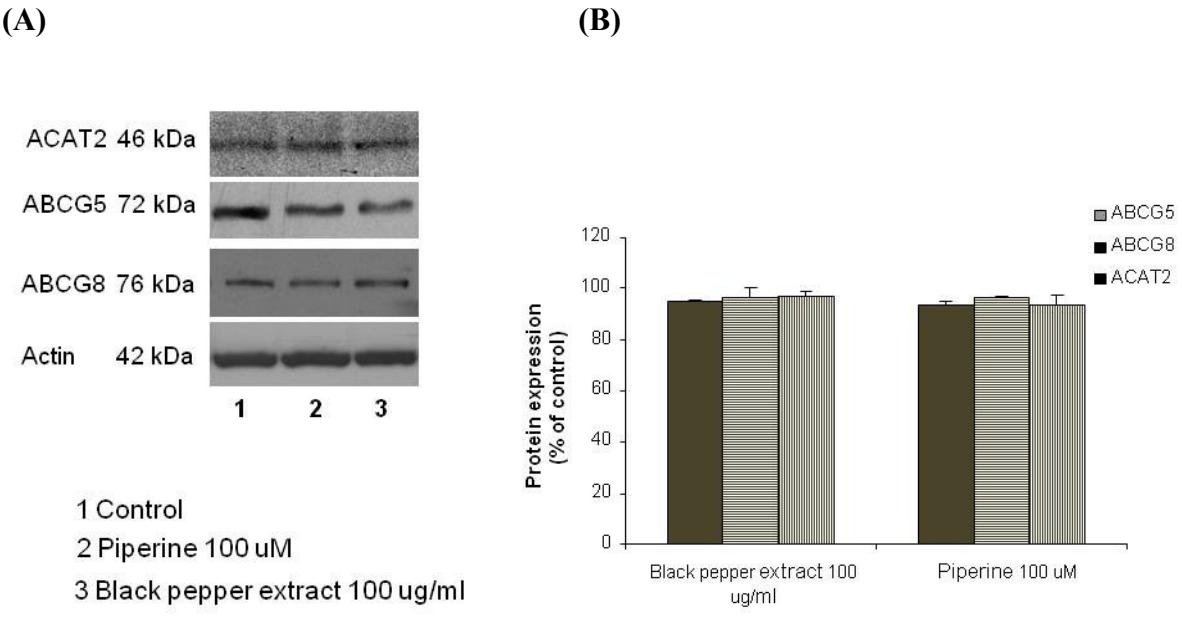

Figure 2 ABCG5, ABCG8 and ACAT2 protein expression in differentiated Caco-2 cells incubated with black pepper extract and piperine for 24 h (A). Protein band densities were averaged from 3 experiments (B). Expression values were normalized by actin.

Discussion

NPC1L1 is a cholesterol transporter protein found in both brush border membrane and the intracellular compartment of intestinal cells [2]. The present study showed that black pepper extract and piperine reduced NPC1L1 levels only in the membrane fraction. There was no change of NPC1L1 in whole cells lysate representing the total level of cellular NPC1L1 protein. This can be suggested that black pepper extract and piperine do not affect the overall expression of NPC1L1 but regulate the movement of NPC1L1 between cell membrane and cytoplasmic compartment. In this case, they promote the internalization of membrane NPC1L1 to the cytoplasm. There was evidence showing that cholesterol and ezetimibe (a cholesterol absorption inhibitor) can inhibit the internalization of NPC1L1 [3]. Although black pepper extract and piperine did not affect the expression of ACAT2 responsible for cholesterol esterification in this study, it was previously reported to inhibit the activity of ACAT1 and ACAT2 in mouse macrophages [4]. Possibly, black pepper extract or piperine alter only the activity but not the expression of ACAT2. In addition, no change of ABCG5 and ABCG8 expressions was observed in this study. Taken together with our previous study (unpublished data), black pepper extract and piperine do not have any effect on the cholesterol efflux.

Conclusion

One possible mechanism of the cholesterol absorption inhibitory activity of black pepper extract and piperine could be through the regulation of NPC1L1 translocation between cell membrane and cytoplasmic compartment. However, further study is required to verify this assumption.

Acknowledgements

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References

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