Functional Foods in Health and Disease **2013; 3(5):111-121**

Research Article Open Access

Benzotropolone moiety in theaflavins is responsible for inhibiting peptide-transport and activating AMP-activated protein kinase in Caco-2 cells

Ha-Young Park, Yuri Kunitake, and Toshiro Matsui*

Department of Bioscience and Biotechnology, Division of Bioresources and Biosciences, Faculty of Agriculture, Graduate School of Kyushu University, 6-10-1 Hakozaki, Fukuoka, 812-8581, Japan

***Corresponding Author:** Toshiro Matsui, PhD, Professor**,** Department of Bioscience and Biotechnology, Division of Bioresources and Biosciences, Faculty of Agriculture, Graduate School of Kyushu University, 6-10-1 Hakozaki, Fukuoka, 812-8581, Japan

Submission date: April 23, 2013; Acceptance date: May 23, 2013; Publication date: May 24, 2013

ABSTRACT

Objective: In the small intestine, peptide transporter 1 (PEPT1) plays a role in the transport of di- and tri-peptides. Recently, we found that theaflavins (TFs), dimeric catechins, inhibited the transport of di-peptides across Caco-2 monolayers by suppressing the expression of PEPT1 through AMP-activated protein kinase (AMPK) activation. In this study, we investigated the structural requirement of theaflavins for the effect, and the mechanism(s) underling theaflavin-induced AMPK activation.

Methods: Theaflavin-3'-*O*-gallate (TF3'G) was used for this study, since it possessed the most potent inhibition power for peptide-transport among theaflavins. Absorption ability was measured with Caco-2 cell monolayers treated with or without 20 μM sample (TF3'G or its related compounds) in an Ussing Chamber. The amount of Gly-Sar (a model of PEPT1-transporing peptide) transport at fixed time-points to 60 min was determined by fluorescent naphthalene-2,3-dicarboxaldehyde-derivatized assay (Ex/Em: 405 nm/460 nm). The apparent permeability coefficient (P_{app}) was used to evaluate the permeability. Expression of PEPT1 protein in Caco-2 cells treated with or without 20 μM TF3'G in the presence or absence of inhibitor (10 μM compound C as AMPK inhibitor or 25 μM STO-609 as CaMKK inhibitor) was evaluated by Western blot.

Results: The P_{app} value of Gly-Sar significantly ($P < 0.05$) decreased in 20 μM purprogallin-treated Caco-2 cells as well as in TF3'G-treated cells, together with the reduction of PEPT1 expression, while their monomeric catechins did not show any P_{app} reduction. In TF3'G-treated Caco-2 cells, the recovery of the reduced PEPT1 expression was found by 10 μM compound C, but not STO-609.

Conclusion: The study demonstrated that the benzotropolone moiety in theaflavins was a

crucial structural requirement for exerting the inhibition of intestinal peptide-transport, and the suppression of PEPT1 expression by theaflavins would be caused by activating LKB1/AMPK pathway, but not CaMKK/AMPK pathway.

Keywords: Theaflavin-3'-*O*-gallate, Peptide transport, PEPT1, Benzotropolone, AMP-activated protein kinase, Calmodulin-dependent protein kinase kinase