Kinetic Evaluation of Sustained Inhibitory Effect of Myricetin on Folate Transport by Proton-Coupled Folate Transporter

T. Yamashiro 1, K. Ohta 1, K. Inoue 2, M. Furumiya 3, Y. Hayashi 3, H. Yuasa 1
1 Nagoya City University, 2 Tokyo University of Pharmacy and Life Sciences, 3 Kinjo Gakuin University

**Purpose**
Myricetin is a flavonoid that has recently been suggested to induce sustained inhibition, which lasts after its removal, of proton-coupled folate transporter (PCFT/SLC46A1), which is responsible for the intestinal uptake of folate. The present study was conducted to characterize the inhibitory effect in more detail, focusing on kinetic aspects, to gain information that could be utilized to maintain the absorption of folate and antifolate drugs.

**Methods**
The uptake of [3H]folate was evaluated for a 2-min period at pH 5.5 and 37°C in MDCKII cells stably expressing human PCFT. The cells were pretreated with myricetin for 1 h to induce its inhibitory effect and myricetin was removed before the uptake assay. MDCKII cells stably expressing GFP-tagged PCFT was also prepared to assess its cellular localization and the effect of myricetin pretreatment on that.

**Results**
The extent of inhibition of PCFT-mediated transport of folate (5 μM) by myricetin (50 μM) was found to increase with an increase in pretreatment time, reaching about 80% inhibition at 60 min of pretreatment. The inhibition was found to last at the level up to 10 min after the removal of myricetin. Furthermore, the initial level of inhibition was dependent on myricetin concentration, and the inhibitory effect was kinetically attributable to a reduction in the maximum transport rate ($V_{max}$), whereas $V_{max}/K_m$ was unchanged due to a reduction in the Michaelis constant ($K_m$). On the other hand, the apical localization of GFP-tagged PCFT was found not to be altered by the pretreatment with myricetin (50 μM). Therefore, it is likely that PCFT may be functionally modulated by myricetin.

**Conclusion**
The sustained inhibition of PCFT by myricetin was kinetically attributable to a reduction in $V_{max}$, whereas the affinity of PCFT for folate was enhanced, as indicated by a reduction in $K_m$. This type of sustained PCFT inhibition could impose an even greater risk of the malabsorption of folate and antifolate drugs in addition to noncompetitive inhibition of PCFT by myricetin present in the medium during folate uptake, which has also been indicated recently.