Bioorganic & Medicinal Chemistry Letters 23 (2013) 183–187

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Polymethoxyflavones as agents that prevent formation of cataract: Nobiletin congeners show potent growth inhibitory effects in human lens epithelial cells

Yoshiki Miyata, Tetsuta Oshitari, Yuji Okuyama, Arata Shimada, Hideyo Takahashi, Hideaki Natsugari, Hiroshi Kosano *

Faculty of Pharma-Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

ARTICLE INFO

Article history:
Received 5 September 2012
Revised 16 October 2012
Accepted 29 October 2012
Available online 9 November 2012

Keywords:
Nobiletin
Polymethoxyflavone
Cataract
LEC proliferation
Matrix metalloproteinase-9

ABSTRACT

Posterior capsular opacification (PCO) is the most frequent complication and the primary reason for visual decrease after extracapsular cataract surgery. The proliferation and migration of leftover lens epithelial cells (LECs) after surgery may contribute to the development of PCO. To prevent PCO, a rational approach would be to inhibit both the proliferation and the migration of LECs using nontoxic xenobiotics. Nobiletin, one of the most abundant polymethoxyflavones (PMFs) in citrus peel, and its synthetic congeners displayed a potent inhibition of LEC proliferation. Structural features which enhance anti-proliferative activity have also been discussed.

Cataract is the most common cause of vision impairment in the world today. It is curable with highly effective surgery, which involves the extracapsular extraction of the natural opaque lens fibers and the implantation of an intraocular lens (IOL).[1] However, many patients gradually develop posterior capsular opacification (PCO), also known as after-cataract (Fig. 1).[2–7] PCO progresses through three stages, which can be roughly described as: (1) the improper proliferation of lens epithelial cells (LECs) left behind after-cataract surgery; (2) the migration of LECs onto the posterior capsule underlying the intraocular lens and into the light path; (3) an epithelial–mesenchymal transition (EMT) resulting in the formation of fibroblasts and spindle-like myofibroblasts.[8–13] The incidence of PCO within 2 months to 5 years after initial surgery is as high as 50% in adults and 100% in children.[14]

Flavonoids are a group of polyphenolic compounds ubiquitously distributed throughout the plant kingdom. Several studies report that some flavonoids have protective effects against lens opacification in both in vivo and in vitro models of cataract.[15–18] However, the detailed mechanism of action has not been addressed so far. Recently, we reported that nobiletin (**1**) (Fig. 2), one of the most abundant polymethoxyflavones (PMFs) in *Citrus* species, and its three metabolites inhibit the production of the pro-matrix metalloproteinase (proMMP)-9.[19] This enzyme plays an important role in the migration of LECs, which occurs during the second stage of PCO development. Therefore, these flavones could be potent lead compounds for developing chemotherapeutic agents against PCO. Moreover, the study of divergent nobiletin congeners has unveiled a relationship between their structures and their biological activities.[20]

On the other hand, the effects of flavones on LEC proliferation, occurring at the first stage of PCO development, have thus far been left almost unexplored. With the exception of studies on baicalein[21] and quercetin,[22] no systematic study of the growth inhibitory effects of flavones has been reported. Herein, we examined the effects of nobiletin (**1**) and 16 synthetic congeners on the proliferation of LECs, one of the major processes leading to PCO.

We first examined the effects of nobiletin (**1**) on the proliferation of the human lens epithelial cell line SRA01/04.[23,24] Cell growth curve assays were performed using Alamar Blue stain[25] to monitor the changes in growth over 4 days. As demonstrated in Figure 3A, the proliferation of SRA01/04 cells in the nobiletin-treated group was significantly suppressed in a dose-dependent manner when compared to the control group. This tendency became more apparent over time. On day 4, the inhibitory rates of **1** at doses of 32 and 64 μM were 47% and 80%, respectively. These findings strongly suggesting that nobiletin (**1**) inhibits the proliferation of LECs were substantiated by western blot analysis of expression of proliferating cell nuclear antigen (PCNA), a marker

Abbreviations: IOL, intraocular lens; PCO, posterior capsule opacification; LEC, lens epithelial cell; EMT, epithelial–mesenchymal transition; PMF, polymethoxyflavone; proMMP, pro-matrix metalloproteinase; PCNA, proliferating cell nuclear antigen; MAPK, mitogen-activated protein kinase.

* Corresponding author. Tel.: +81 3 3964 8191; fax: +81 3 3964 8195.
E-mail address: h-kosano@pharm.teikyo-u.ac.jp (H. Kosano).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.133

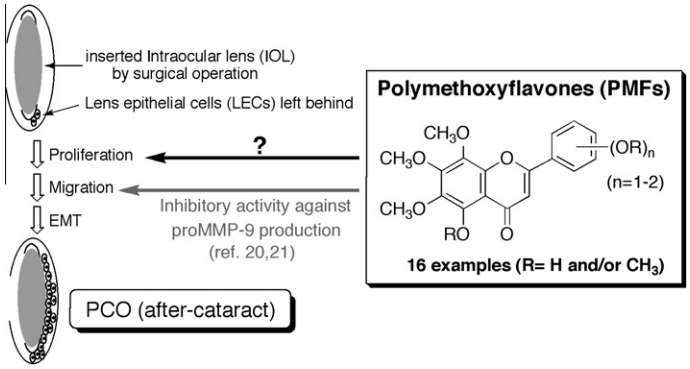

Figure 1. Pathogenesis of posterior capsule opacification (PCO) and proposed therapeutic roles of polymethoxyflavones (PMFs).

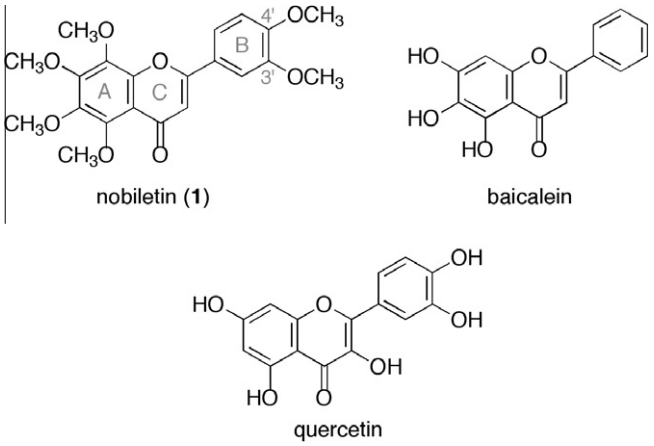

Figure 2. Structure of nobiletin, baicalein, and quercetin.

for cell proliferation.[26] As shown in Figure 3B, nobiletin (**1**) significantly decreased PCNA expression (p <0.05),[27] without significantly reducing β-actin expression.

Taking these observations into account, we next investigated the effects of other nobiletin congeners (Fig. 4) on the proliferation of SRA01/04 cells. In addition to the 14 PMFs reported previously,[19,20] compounds **7a**,[28] **7b**,[29] and **11**[30] were synthesized as per our previously reported protocol[19,20] as depicted in Scheme 1. Compound **7a** proved to be identical to the non-natural flavone synthesized previously.[31] Compound **11** was a flavone obtained from *Scutellaria baicalensis*.[32] The Alamar Blue assay[25] was performed using these compounds.[33,34] The results are shown as growth inhibitory rates compared to the control group on day 4. As demonstrated in Table 1, tangeretin (**6a**),[35] **7a**, **8a**, and **10**[35] were found to possess significantly stronger anti-proliferative activities, unlike that of nobiletin (**1**). Furthermore, in comparing the functional groups on the B ring, intriguing patterns emerged. Compounds **6b**, **7b**, and **8b**, which possess hydroxyl group(s) on the B ring, showed significantly lower inhibitory activities against LEC proliferation than compounds **6a** (p <0.001), **7a** (p <0.001), and **8a** (p <0.001), respectively. Similar results can be seen in comparisons between nobiletin (**1**) and **3** (p <0.01), between compounds **4a** and **4b** (p <0.001), and between compounds **5a** and **5b** (p <0.01) as well.[27] These observations suggest that a demethylation at the methoxy group(s) on the B-ring is associated with significant suppressive effects on the inhibition of LEC proliferation.

We also examined the effects of the 5-demethylated congeners **9**–**12** on the proliferation of SRA01/04 cells. Among these, compound **10** exhibited considerable inhibitory activity on cell growth. It is worth noting that compound **10** also exerted marked inhibitory effects (IC_{50}: 0.7 μM) on proMMP-9 production in PMA-treated cells.[20] Similarly, the growth inhibitory rates of compounds

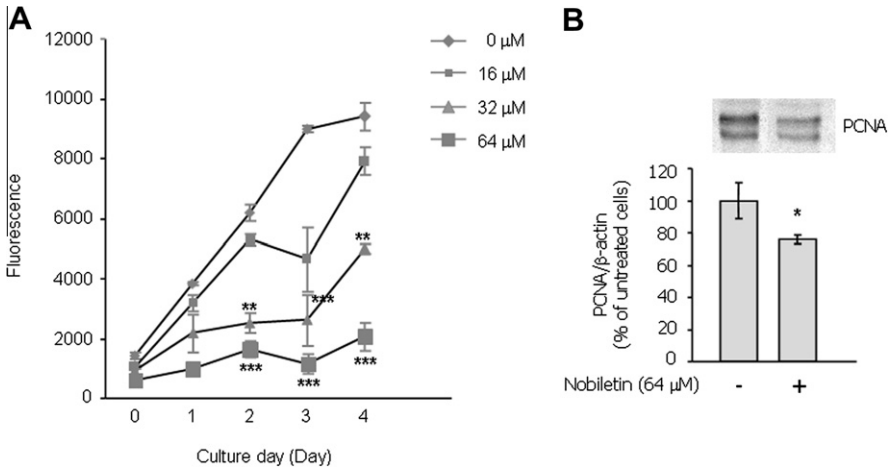

Figure 3. The antiproliferative effects of nobiletin on human lens epithelial cell line SRA01/04. (a) [A]: Cell growth curve assay by means of Alamar Blue stain. Data (n = 4) are shown as means ± SD. Asterisks indicate results that were significantly different from untreated control cells at each time (**: p <0.01; ***: p <0.001). [B]: Western blot analysis of proliferating cell nuclear antigen (PCNA) expression. Data (n = 4) are shown as means ± SD (*: p <0.05, significantly different from untreated group).