

## Polymethoxy Flavonoids: Possible Candidates for Overcoming Drug Resistance

Toshihiko Hirano\*

Department of Clinical Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

\*Corresponding author: Toshihiko Hirano, Department of Clinical Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan, Tel: +81-426-76-5794; Fax: +81-426-76-5798; E-mail: [hiranot@toyaku.ac.jp](mailto:hiranot@toyaku.ac.jp)

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### Introduction

The success of chemotherapy in cancer treatment is frequently limited by intrinsic or acquired multidrug resistance due to increased expression of a plasma membrane P-glycoprotein [1]. P-glycoprotein is an ATP-dependent transporter that effluxes many lipophilic anticancer agents out of cells, thereby reducing intracellular drug concentration and results in cancer cell survival. It has been reported that certain kinds of natural compounds and their derivatives [2-4] inhibit P-glycoprotein function, and overcome the multidrug resistance phenotype.

We have developed an multidrug resistant cell line namely MOLT-4/DNR from a T lymphoblastoid leukemia MOLT-4 cell line by exposing the parent cells to increasing concentrations stepwise of daunorubicin over three months [5]. This resistant subline MOLT-4/DNR has been revealed to overexpress functional P-glycoprotein and MDR1 mRNA [5]. The drug resistance in MOLT-4/DNR is closely related to the expression of P-glycoprotein and MDR1 mRNA [5], and therefore, this subline is a suitable model to investigate the agents which modify P-glycoprotein function and drug resistance.

Polymethoxyflavonoids tangeretin and nobiletin have 5-6 methoxy residues on the basic chemical structure of flavone [6]. These flavonoids were originated from citrus, and are also known to have several pharmacological activities including anticancer efficacies [6-8]. Then, we have evaluated the effects of polymethoxyflavonoids originated from citrus and other phenolic compounds on the drug resistant cell line MOLT-4/DNR [6].

### Polymethoxyflavonoid effects on growth of drug-resistant leukemia cells expressing functional P-glycoprotein

Growth-suppressive effects of naturally occurring polymethoxyflavonoids and other related polyphenolic compounds baicalein, wogonin, quercetin, and epigallocatechin gallate on a P-glycoprotein expressing multidrug resistant cell line MOLT-4/DNR have been examined [6]. All of these compounds inhibited cell growth with IC<sub>50</sub> values of 7.1-32.2 μmol/L, and the inhibitory effects were observed almost equally to the parent MOLT-4 and the daunorubicin-resistant cells. Tangeretin and nobiletin showed the strongest effects with IC<sub>50</sub> values of 7.1-14.0 μmol/L.

The growth of MOLT-4/DNR cells cultured in presence of daunorubicin combined with 5 μmol/L tangeretin or nobiletin was extensively inhibited, as compared to the growth in cells cultured with daunorubicin alone. The dose-response curves of daunorubicin

combined with tangeretin or nobiletin against the growth of MOLT-4/DNR cells shifted to almost the same level as those against growth of the parent MOLT-4 cells.

### Polymethoxyflavonoid effects on P-glycoprotein function, cell cycle and apoptosis of drug-resistant leukemia cells

We also investigated the action mechanisms of polymethoxyflavonoids by examining P-glycoprotein function, cell cycle, and apoptosis in these cells [6]. We have revealed that only 2.5% of MOLT-4 cells express P-glycoprotein, whereas 93.7% of MOLT-4/DNR cells express P-glycoprotein [9]. Polymethoxyflavonoids tangeretin and nobiletin inhibited P-glycoprotein function in MOLT-4/DNR cells, as estimated by R123 influx/efflux assay [6]. Nobiletin increased percentage of the G1 phase cells, whereas the flavonoid decreased percentage of the G2/M phase cells in both parental MOLT-4 and the drug-resistant MOLT-4/DNR cell line. Tangeretin and nobiletin did not efficiently induce apoptosis in cells of both MOLT-4 and MOLT-4/DNR cell line, even at high concentration (100 μmol/L), which can almost completely suppress the growth of these cells.

### Discussion

These observations suggest that naturally occurring polymethoxyflavonoids and related polyphenolic compounds inhibit growth of both T lymphoblastoid leukemia MOLT-4 cells and P-glycoprotein expressing daunorubicin-resistant MOLT-4 (MOLT-4/DNR) cells almost equally. The suppressive effects of the polymethoxyflavonoids tangeretin and nobiletin were the strongest among the compounds tested with the IC<sub>50</sub> values of 7.1-14.0 μmol/L, and that these flavonoids influence the cell cycle, but not apoptosis, of these cells.

The resistance of MOLT-4/DNR cells to daunorubicin has been reported to closely correlate with the expression of functional P-glycoprotein [5]. Multidrug resistance is recognized as one of the most common causes for failure of chemotherapy in treating cancer patients [10]. P-glycoprotein is an ABC transporter, which hydrolyses ATP and extrudes cytotoxic drugs from mammalian cells [1]. Since the polymethoxyflavonoids suppress the P-glycoprotein function in MOLT-4/DNR cells, the additive growth-inhibitory effects of these polymethoxyflavonoids in combination with daunorubicin are suggested to be mediated via suppression of P-glycoprotein function. Indeed, the additive effects were not observed against the growth of the parental MOLT-4 cells expressing less efflux activity [6]. These polymethoxyflavonoids were equally effective against the growth of parent MOLT-4 and the drug-resistant MOLT-4/DNR cells, suggesting that the compounds were not excluded by functional P-glycoprotein expressed on the MOLT-4/DNR cells.

Polymethoxyflavonoids were reported to influence cell cycle and induce G1 arrest in human cancer cells [11]. Daunorubicin prevents DNA replication and RNA synthesis, and therefore, the cell cycle modulating effects of the polymethoxyflavonoids are possibly additive or synergistic to the pharmacological action of daunorubicin against MOLT-4 and MOLT-4/DNR cell growth. The lack of apoptosis induction at concentrations that profound inhibitory effects on growth, and induce cytostatic effects (G1/S accumulation), suggests that cytostasis and not cytotoxicity is the most relevant biological effect of the polymethoxyflavonoids. The ability of these polymethoxyflavonoids to inhibit growth of MOLT-4 and MOLT-4/DNR cells without cell toxicity suggests that they interact selectively with mediators of cell cycle events in these cells. Other studies have also shown that polymethoxyflavonoids do not induce apoptosis in some human cell lines [11]. Tangeretin has been reported to protect cells against endoplasmic reticulum stress and neurotoxin [12]. Their selective interaction with mediators of cell cycle events and their consequent cytostatic effects may contribute to reduce the apoptosis events of these cells. Our observations suggest that such cytostatic effects of the polymethoxyflavonoids can be obtained in multidrug resistant cells despite that these cells highly expressed the functional P-glycoprotein molecules.

Toxic effects of polymethoxyflavonoids in mammals including human have little been reported, and they have rather been suggested to ameliorate dimethylbenz[a]anthracene induced kidney injury [13]. It has been reported that *Citrus reticulata* or *C. unshiu* peels contain nobiletin at  $0.83 \pm 0.13\%$  of the dry weight [14], suggesting that 100 g of these peels include  $830 \pm 130$  mg nobiletin. Pharmacokinetic profiles of polymethoxyflavonoids in human have not been examined so far. However, intake of sofalcone, a flavonoid drug generally used for treatment of gastric ulcer, at a dose of 100 mg is known to result in the maximum blood concentration of approximately 500 ng/mL. According to these observations, administration of 830 mg of nobiletin is suggested to give maximum blood concentration of approximately 10  $\mu\text{mol/L}$ , which would be around the  $\text{IC}_{50}$  values of this agent on the proliferation of MOLT-4 and the drug-resistant MOLT-4/DNR cell lines [6].

## Conclusive remarks

Naturally occurring polymethoxyflavonoids and related polyphenolic compounds exhibit suppressive efficacy on growth of both human T-lymphoblastoid leukemia MOLT-4 cells and the P-glycoprotein expressing daunorubicin-resistant MOLT-4/DNR cells. Among the polymethoxyflavonoids and related polyphenolic compounds examined, tangeretin and nobiletin showed the strongest effects with  $\text{IC}_{50}$  values less than 15  $\mu\text{mol/L}$ . These polymethoxyflavonoids influence cell cycle and inhibit growth of the parent and functional P-glycoprotein expressing cells almost equally. These observations raised the possibility that naturally occurring polymethoxyflavonoids could be a candidate for overcoming drug resistance in multidrug resistant leukemia cells expressing functional P-glycoprotein.

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