Review

Inhibitory Effects of Polyphenols on P-Glycoprotein-Mediated Transport

Shuji KITAGAWA

Niigata University of Pharmacy and Applied Life Sciences; 5–13–2 Kamishin'ei-cho, Niigata 950–2081, Japan.
Received October 21, 2005

Overexpression of P-glycoprotein (P-gp), a plasma membrane transporter which extrudes chemotherapeutic agents out of cells, has been associated with the multidrug resistance (MDR) of cancer cells. It has been revealed that flavonoids and other polyphenols inhibit P-gp activity. Due to their inhibitory activities of polyphenols on P-gp function and their physiological safety, they are possible candidates for modulators of MDR. To determine suitable candidates, it is important to clarify the structure–activity relationships of their inhibitory activities on P-gp function. Determining the structure–activity relationships is also meaningful because the intake of dietary polyphenols may also alter drug pharmacokinetics and pharmacodynamics *via* inhibition of P-gp-mediated drug efflux in tissues such as the intestinal epithelium, blood-brain barrier, hepatocytes and renal tubular cells. This is a review of our recent investigations using multidrug-resistant P-gp overexpressing KB-C2 cells.

Key words polyphenol; flavonoid; P-glycoprotein; KB-C2 cell; multidrug resistance (MDR)

Overexpression of the 170-kDa P-glycoprotein (P-gp), a plasma membrane transporter which extrudes chemotherapeutic agents out of cells, has been associated with the multidrug resistance (MDR) of cancer cells. Numerous studies suggest that the principal physiological role for P-gp is to protect the organism from toxic substances.[1] It is known that this efflux pump is also present in many normal tissues including the epithelium of the gastro-intestinal tract, the renal proximal tubule, the canalicular surface of the hepatocyte, and the endothelial cell surface comprising the blood brain barrier.[1] This ATP-dependent transporter extrudes a wide variety of structurally unrelated compounds, such as vinca alkaloids, etoposides, taxenes and anthracyclines.[2,3] Compounds such as verapamil, dihydropyridine analogs, quinidine and cyclosporin A reversed this P-gp-mediated multidrug resistance due to their inhibition of transporter activity.[2,3] In addition to these compounds, it has been revealed that flavonoids and other polyphenols modulate P-gp activity.[4—8]

The important antioxidant activities of polyphenols are well known. Various studies on the structure–activity relationships of their antioxidant activities have been reported.[9] On the other hand, polyphenols, especially flavonoids, are known to modify various physiological functions by interacting with celluar enzymes such as cytochrome P450 and membrane transporters such as glucose transporter.[10—12] In addition to these activities, it has recently been revealed that polyphenols inhibit the function of ABC transporters such as multidrug resistance associated proteins (MRPs) and breast cancer resistant protein (BCRP),[13,14] as well as P-gp mentioned above.

In this review we analyzed the effects of polyphenols on P-gp function from the standpoints of chemical structure, conformation and hydrophobicity by using multidrug-resistant human epidermal carcinoma cell line KB-C2 cells which overexpress P-gp.[15] Due to the inhibitory activities of polyphenols on P-gp function and their physiological safety, they are candidates for modulators of MDR. To find suitable candidates, it is important to clarify the structure–activity relationships of their inhibitory activities on P-gp function. To reveal the structure–activity relationships is also meaningful because the intake of dietary polyphenols may also alter drug pharmacokinetics and pharmacodynamics *via* inhibition of P-gp-mediated drug efflux in tissues such as the intestinal epithelium, blood-brain barrier, hepatocytes and renal tubular cells,[2] as well as *via* inhibition of cytochrome P450.[11]

INHIBITION OF SUBSTRATE EFFLUX BY P-GLYCOPROTEIN BY FLAVONOIDS

Flavonoids are a large group of polyphenolic compounds found commonly in fruits, vegetables and plant-derived beverages such as tea and red wine.[16] They are plant secondary metabolites and comprise more than 4000 known compounds. Flavonoids, which are water-soluble pigments responsible for the shades of yellow, orange, and red in flowering plants, are important factors for plant growth, development, and defense.[16] In addition to their well-known antioxidant activities, flavonoids have various biological activities such as anti-inflammatory, antiallergic, antiplatelet, and antitumoral activities.[17,18] They are found in various herbs and traditional Eastern medicines. Although bioavailability of dietary flavonoids is very limited, they have been believed to exert protective effects in particular cardiovascular disease and cancer.[19]

Flavonoids are composed of a common phenylchromanone structure, with one or more hydroxyl substituents. Variations in their heterocyclic ring give rise to flavonoid families such as flavanones, flavones, flavonols, flavanols, isoflavones, and anthocyanidins, as shown in Fig. 1. Although the structure–activity relationships of flavonoids on P-gp functions have been studied,[6,8,20,21] they are still not clear, especially concerning their effects in whole cells. We analyzed their effects on P-gp from the standpoints of chemical structure, conformation and hydrophobicity.[22,23]

We examined the effects of four tea catechins (flavanols), whose structures are shown in Fig. 2, quercetin (flavonol) and its glycosides, genistein (isoflavone) and cyanidin chloride (anthocyanidin) on the accumulation of P-gp substrate rhodamine 123, which has often been used for the study of various P-gp transport modulaotors, in KB-C2 cells. In Fig. 3 the effects of flavonoids are shown as relative accumulation amount of rhodamine 123 in KB-C2 cells in the presence of various flavonoids. Among four tea catechins, (−)epicate-

∗ To whom correspondence should be addressed. e-mail: kitagawa@niigata-pharm.ac.jp

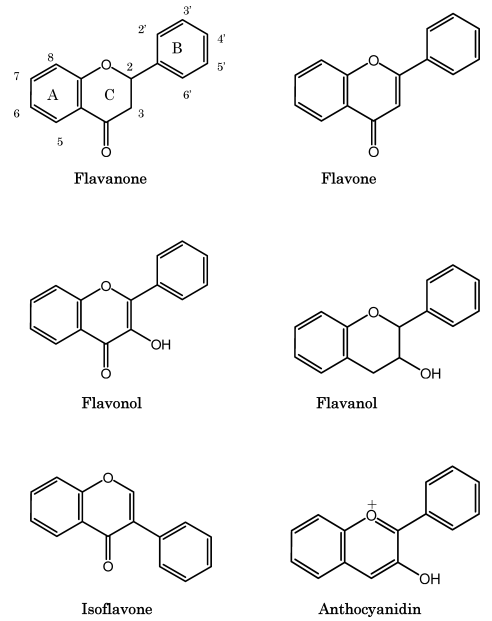

Fig. 1. Subclasses of Flavonoids Family

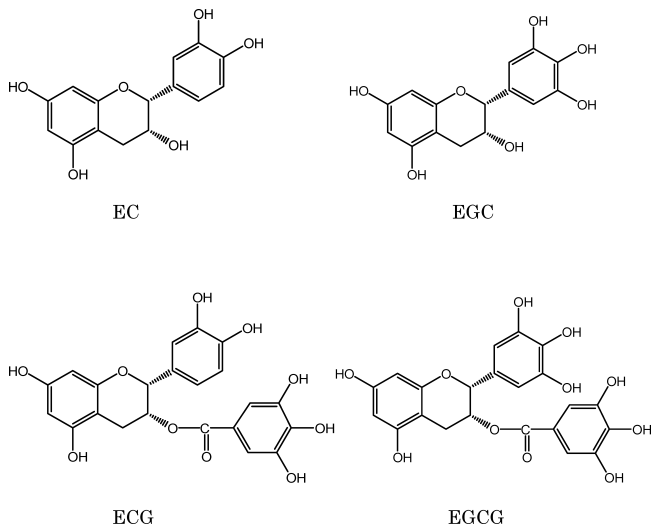

Fig. 2. Chemical Structures of Tea Catechins

EC, (−)epicatechin; EGC, (−)epigallocatechin; ECG, (−)epicatechingallate; EGCG, (−)epigallocatechingallate.

chin gallate (ECG) and (−)eigallocatechin gallate (EGCG), which are gallic acid esters and have galloyl moiety on the C-ring, increased cellular accumulation of the fluorescent substrate 2.1-fold and 3.7-fold at 100 μM, respectively.[22] Quercetin and cyanidin chloride also significantly increased cellular accumulation of the fluorescent substrate. The effect of EGCG was more significant than those of verapamil, which is often used as a P-gp inhibitor, and quercetin. On the other hand, isoflavone genistein had little effect. Two glycosides of quercetin, quercetin-3-*O*-glucoside and rutin, had no effect.[23] Increased accumulation of rhodamine 123 in individual cells in the presence of these catechins was confirmed by flow cytometry as shown for EGCG in Fig. 4.[22,23] The increase of cellular accumulation of rhodamine 123 in the presence of flavonoids is due to the inhibition of efflux as shown in Fig. 5 for the effect of EGCG.[22,23] The results obtained in these studies indicated that the effects of flavonoids on P-gp

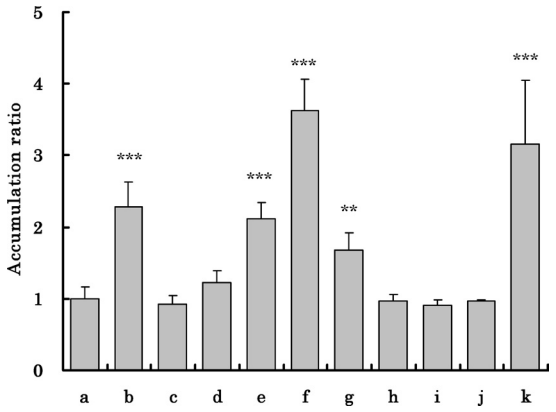

Fig. 3. Effects of 100 μM Flavonids on the Relative Accumulative Amounts of Rhodamine 123 in Multidrug-Resistant P-Glycoprotein Overexpressing KB-C2 Cells

Data are cited from refs. 22 and 23. a, control; b, verapamil; c, EC, d, EGC, e, ECG; f, EGCG; g, quercetin; h, quercetin-3-*O*-glucoside; i, rutin; j, genistein; k, cyanidin chloride. $^{**}p<0.01$, $^{***}p<0.001$ compared with the value in the absence of flavonoids. Data are means±S.D. of six experiments. The control value of accumulation was defined as 1.00.

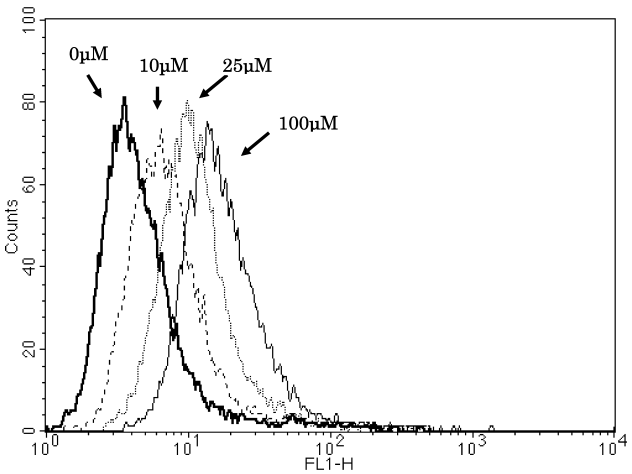

Fig. 4. Analysis by Flow Cytometry of the Effects of Kaempferol (0—100 μM) on the Intracellular Retention of Rhodamine 123 in the Accumulation Phase of Multidrug-Resistant P-Glycoprotein Overexpressing KB-C2 Cells[23]

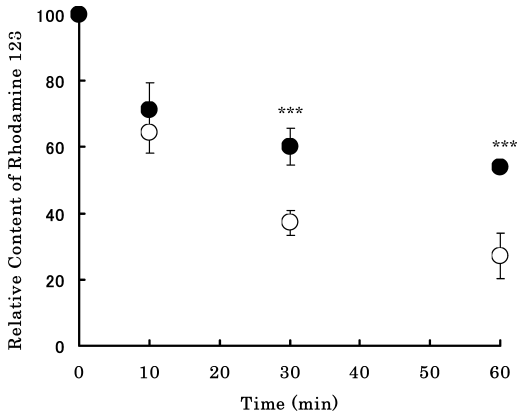

Fig. 5. The Efflux of Rhodamine 123 in the Presence (●) or Absence (○) of 100 μM EGCG[22]

Data are means±S.D. of six experiments. $^{***}p<0.001$ compared with the control value.

Table 1. Chemical Structures of Kaempferol, Quercetin, Baicalein, Myricetin, Fisetin, Morin and Naringenin

Compound	2,3-Double bond	3	5	6	7	2′	3′	4′	5′
Kaempferol	Yes	OH	OH	H	OH	H	H	OH	H
Quercetin	Yes	OH	OH	H	OH	H	OH	OH	H
Baicalein	Yes	H	OH	OH	OH	H	H	H	H
Myricetin	Yes	OH	OH	H	OH	H	OH	OH	OH
Fisetin	Yes	OH	H	H	OH	H	OH	OH	H
Morin	Yes	OH	OH	H	OH	OH	H	OH	H
Naringenin	None	H	OH	H	OH	H	H	OH	H

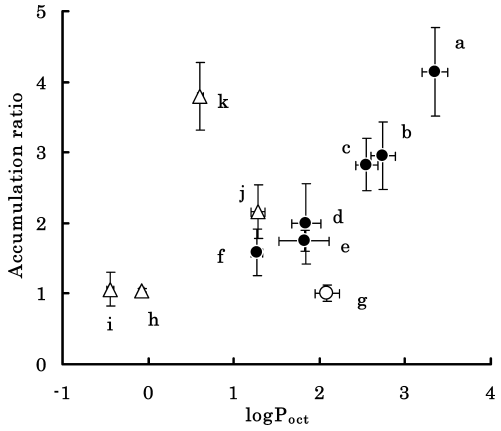

Fig. 6. Relationship between Logarithm Values of Partition Coefficients P_{oct} of Flavonoids and Relative Accumulative Amounts of Daunorubicin in the Presence of 100 μM Flavonoids

a (●), kaempferol; b (●), quercetin; c (●), baicalein; d (●) myricetin; e (●), fisetin; f (●), morin; g (○), naringenin; h (△), EC; i (△), EGC; j (△), ECG; k (△), EGCG. Data of daunorubicin accumulation are means±S.D. of six experiments.

function depend on their chemical structure.

RELATIONSHIP OF INHIBITORY EFFECTS OF FLAVONOIDS WITH THEIR CHEMICAL STRUCTURE AND HYDROPHOBICITY

To reveal the structure–activity relationship of the effects of flavonoids shown in Table 1 on P-gp, we examined their effects using daunorubicin as the P-gp substrate, because daunorubicin is more sensitive to inhibitory effects on P-gp-mediated efflux than rhodamine-123.[24] Therefore, the effects of flavonoids are expected to be more clearly detectable. Firstly we examined the effect of the double-bond between the 2- and 3-position in the C ring. As shown in Fig. 6, naringenin, which is flavanone and lacks the 2,3-double bond in the C ring like flavanols, had no effect on the accumulation of daunorubicin, although naringenin was more hydrophobic than myricetin, fisetin and morin.[23] These findings suggested the importance of the double bond in that position.

Since a hydrophobic interaction has been suggested for P-gp modulators,[6,21] we measured the partition coefficients P_{oct} of the flavonoids tested between *n*-octanol and PBS at 37 °C. We examined the relationship between their inhibitory activity on P-gp function and their partition coefficients. As shown in Fig. 6 for the effects at 100 μM, except the effects of tea catechins and naringenin (flavanones), the effects of kaempferol, quercetin, myricetin, morin, fisetin (flavonols) and baicalein (flavone), which commonly have 2,3-double bond in the C ring, corresponded well with the order of their P_{oct} values. Although these flavonols and flavone differ in the number and position of hydroxyl groups in flavonoid molecules, the difference seems to affect their activity toward P-gp *via* modifying their hydrophobicity.

From these findings, structure requirements were suggested for the inhibitory effects of flavonoids. Firstly, for flavonoids which do not have large substituents, the planar structure of flavonoids is important for their interaction with P-gp. For flavanones and flavanols, which lack the double-bond between the 2- and 3-position in the C ring, the stereoscopic relationship of the B ring with other A and C rings is different from that of flavones and flavonols which have the double-bond in that position. It has been reported that the torsion angles of the B ring in flavones and flavanols are smaller than those of flavanones.[14,25] Therefore, the double bond confers a special structure on flavone and flavonol molecules that are largely planar so that they may more readily intercalate between the hydrophobic amino acid residues of P-gp. The importance of the 2,3-double bond has also been suggested for the interaction of the flavonoids with MRP1 and MRP2.[14] It has also been revealed for the interaction of flavonoids with enzymes such as 15-lipoxygenases.[26]

Another point suggested from the study is that hydrophobicity is important for the inhibitory effects of the planar flavonoids, which do not have large substituents like the galloyl group, on substrate efflux by P-gp, regardless of the differences in the number and position of hydroxyl groups.

On the other hand, the effect of EGCG (flavanol), which does not have a planar structure, was much larger than expected from its hydrophobicity. The inhibitory effects of the tea catechins did not depend on their total hydrophobicity, but significantly depended on their chemical structure. The presence of galloyl moiety on the C-ring markedly increased the *n*-octanol/PBS partition coefficients of the catechins and their activity on P-gp. On the other hand, the presence of the trihydric pyrogallol group as the B-ring decreased the partition coefficients but increased the activity on P-gp, compared with the action on the corresponding catechins with a dihydric catechol B-ring.[22] Therefore, interaction of the non-planar tea catechins with P-gp seems to be different from that of the planar flavones and flavonols.

INHIBITION OF SUBSTRATE EFFLUX BY P-GLYCOPROTEIN BY OTHER POLYPHENOLS

Alkyl gallates (3,4,5-trihydroxybenzoic acid alkyl esters), propyl, octyl and dodecyl gallates, are widely used as food antioxidants in oils and butters.[27,28] They have also been shown to possess antibacterial activities.[29] In relation to the effects of tea catechins mentioned above, we tried to eluci-

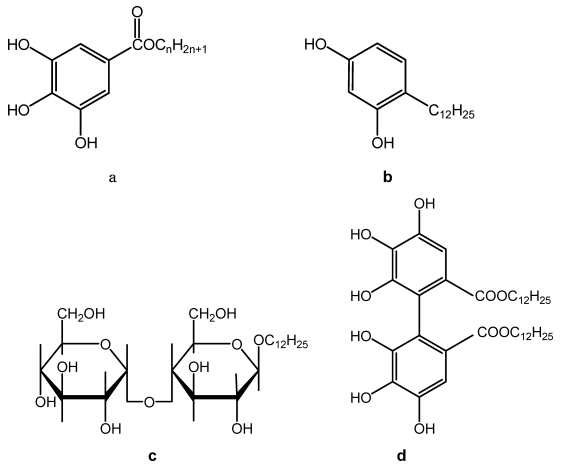

Fig. 7. Chemical Structures of Alkyl Gallates and Related Compounds Tested

(a) Alkyl (*n*-butyl (*n*=4), *n*-octyl (*n*=8), and *n*-dodecyl (*n*=12)) gallates; (b) *n*-dodecyl resorcinol; (c) *n*-dodecyl-*β*-D-maltoside; (d) *n*-dididecyl-(2,2′,3,3′,4,4′-hexahydroxy)-biphenyl-6,6′-dicarboxylate.

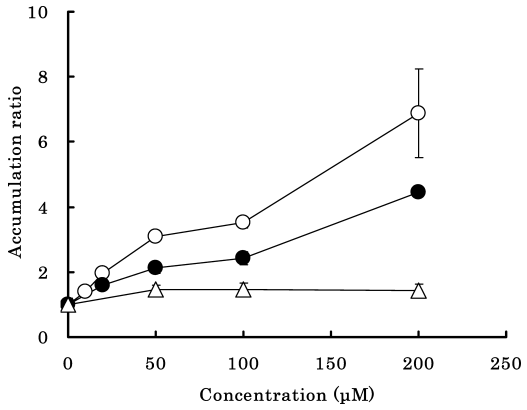

Fig. 8. Dose-Dependent Effects of *n*-Butyl (△), *n*-Octyl (●), and *n*-Dodecyl (○) Gallates on the Accumulation of Daunorubicin[30]

Data are means±S.D. of four experiments. The control value of accumulation was defined as 1.00.

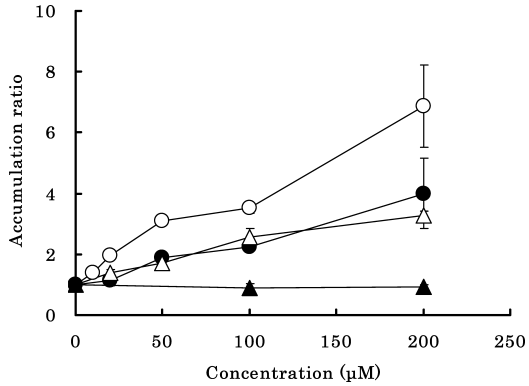

Fig. 9. Dose-Dependent Effects of *n*-Dodecylresorcinol (●), *n*-Dididecyl-(2,2′,3,3′,4,4′-hexahydroxy)-biphenyl-6,6′-dicarboxylate (△), and Lauric Acid (▲) on the Accumulation of Daunorubicin[30]

Data for *n*-dodecyl gallate (○) are also shown for comparison. Data are means±S.D. of four experiments. The control value of accumulation was defined as 1.00.

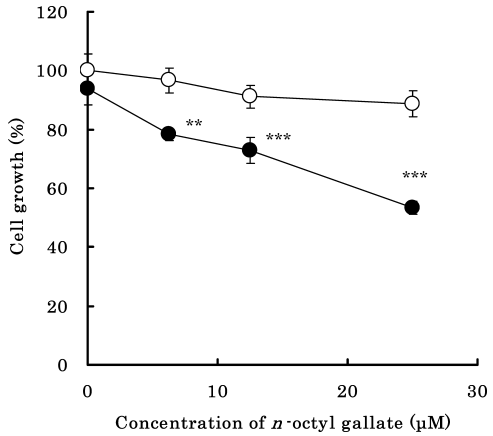

Fig. 10. Effect of *n*-Octyl Gallate Concentration on the Cytotoxicity of Daunorubicin in KB-C2 Cells[30]

○, control (in the absence of daunorubicin); ●, in the presence of 12.5 μM daunorubicin. Data are means±S.D. of four experiments. *** $p<0.001$.

date the role of both the hydrophobic moiety (alkyl chain) and gallic acid moiety on their modifying effects on P-gp function by clarifying the effects of three alkyl gallates and their related compounds (Fig. 7) on P-gp function.[30]

We examined the effects of *n*-butyl, *n*-octyl, and *n*-dodecyl gallates, whose numbers of carbon atoms in their alkyl chains are 4, 8, and 12 respectively, on the accumulation of daunorubicin in KB-C2 cells. As shown in Fig. 8 for the dose-dependent effects, *n*-butyl gallate had no significant effect, and marked increasing effects were observed for the other two alkyl gallates.[30] Maximum effects were observed for *n*-dodecyl gallate. The effect of *n*-dodecyl gallate was similar to that of EGCG. There was no effect of the nonionic detergent *n*-dodecyl-*β*-D-maltoside (Fig. 7), which has an *n*-dodecyl group but no phenol group.[30]

We furthermore examined the effect of *n*-dodecyl resorcinol (Fig. 7), which has an *n*-dodecyl group as its alkyl chain on the resorcinol ring. As shown in Fig. 9, *n*-dodecyl resorcinol increased the accumulation of daunorubicin. However, the effect was smaller than that of *n*-dodecyl gallate. The effect of *n*-dididecyl-(2,2′,3,3′,4,4′-hexahydroxy)-biphenyl-6,6′-dicarboxylate, which has an *n*-dodecyl gallate dimeric structure, was also smaller than that of *n*-dodecyl gallate. There was no effect of lauric acid, which has twelve carbon atoms but no phenol group. We confirmed that the enhanced accumulations of rhodamine 123 and daunorubicin in the presence of alkyl gallates was due to the inhibition of the P-gp-mediated efflux of the substrates by efflux experiment.[30]

To demonstrate the effect of alkyl gallates on the cytotoxicity of P-gp substrate anticancer drugs, we next examined the effect of *n*-octyl gallate on the cytotoxicity of daunorubicin. The addition of *n*-octyl gallate (6.25, 12.5, 25 μM) recovered the cytotoxicity of daunorubicin, as shown in Fig. 10. A similar effect on the recovery of daunorubicin cytotoxicity was also observed in the presence of 6.25 μM and 12.5 μM *n*-dodecyl gallte.[30] Although the effective concentrations of alkyl gallates were different from those in the accumulation study due to differences in cell numbers, the trend of daunorubicin cytotoxicity enhancement by alkyl gallates was consistent with the results described above, whereby alkyl gallate increased the cellular accumulation of daunorubicin due to their inhibition of P-gp-mediated drug efflux.

Other dietary polyphenols curcumin and resveratrol (Fig. 11), which are found in turmeric and grapes,[31,32] also

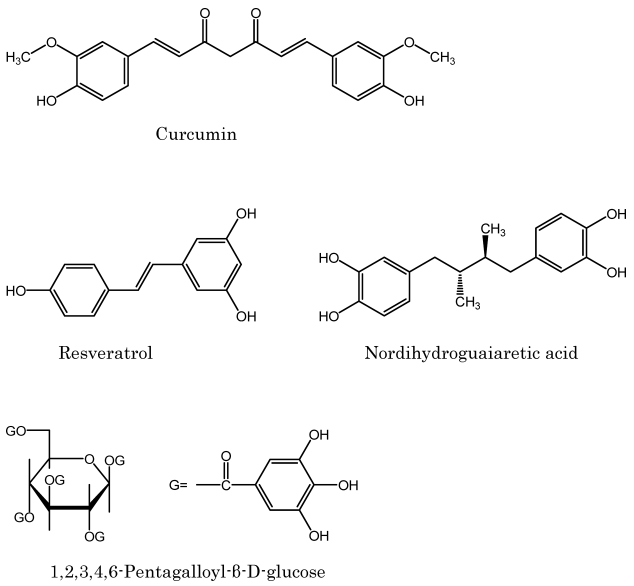

Fig. 11. Chemical Structures of Curcumin, Resveratrol, Nordihydroguaiaretic Acid, and 1,2,3,4,6-Pentagalloyl-β-D-glucose

inhibited substrate efflux by P-gp,[31,33] although the effect of the latter was small.[33] We recently found that polygalloylglucoses such as tannic acid and pentagalloylglucose (Fig. 11) had significant inhibitory effects on P-gp function. We also found inhibitory effects for nordihydroguaiaretic acid (Fig. 11). Therefore, there is a possibility that a large variety of polyphenols have inhibitory activities on P-gp function. As revealed for *n*-alkyl gallates and suggested by the effects of these polyphenols, a large hydrophobic region in addition to the phenolic hydroxyl groups seems to be commonly necessary for the interaction.

MECHANISM OF THE EFFECTS OF POLYPHENOLS

The inhibitory mechanisms of flavonoids and other polyphenols on P-gp function have been studied, and those shown in Table 2 have been suggested. Inhibition of substrate binding to P-gp and inhibition of ATPase activity have been reported. For example, flavonoids such as morin and silymarin have been reported to inhibit P-gp substrate azidopine binding to P-gp.[4,7] Inhibition of ATPase activity by EGCG has also been reported.[34] We also found significant inhibitory effects of polygalloylglucose on verapamil-stimulated P-gp ATPase activity. Flavonoids have been suggested to be modulators with bifunctional interactions at vicinal ATP-binding sites and steroid-interacting regions, which are expected to be in close proximity to the ATP-binding site, within a cytosolic domain of P-gp.[6,20] Flavonoids may induce their binding affinity towards NBD2 of P-gp through their ability to mimic the adenine moiety of ATP. Therefore, hydrophobic moiety of polyphenols may be important for interaction at the steroid-interacting hydrophobic sequence of P-gp.[6,20] Hydroxyl groups in polyphenols such as those in the gallic acid moieties of tea catechins and alkyl gallates may be important in polar interactions with P-gp,[35] possibly at the ATP-binding site. In addition to the direct interaction with P-gp, down regulation of MDR1 gene expression by polyphenols such as EGCG has also been reported.[34]

Table 2. Possible Mechanisms of Inhibitory Effects of Polyphenols on P-gp Function

Inhibition of substrate binding
Inhibition of ATPase activity
Down regulation of MDR1 gene expression

CONCLUSION

For the flavonoids which do not have large substituents such as the galloyl group, in addition to the presence of hydroxyl groups, the planar structure of the flavonoids and their hydrophobicity seem to be important, especially for their interaction with the hydrophobic region of P-gp. For non-planar flavonoids which have large substituents like the galloyl group and other polyphenols, the presence of a large hydrophobic region as well as the presence of neighboring hydrophilic hydroxyl groups seem to be important for the interaction with P-gp.

The bioavailability of polyphenols in food and food additives is limited Therefore, plasma concentration of each polyphenol seems to be low. However, synergistic effects of the various absorbed polyphenols on P-gp function should be considered.

Acknowledgments This study was supported by a grant from the Promotion and Mutual Aid Corporation for Private schools in Japan. I am grateful to Prof. Shin-ichi Akiyama of Kagoshima University for his kindness in providing KB-C2 cells.

REFERENCES

1) Bates S. E., "ABC Proteins," ed. by Holland I. B., Cole S. P. C., Kuchler K., Higgins C. F., Academic Press, London, 2003, pp. 359—391.
2) Bosch I., Croop J., *Biochim. Biophys. Acta*, **1288**, F37—F54 (1996).
3) Sharom F. J., *J. Membrane Biol.*, **160**, 161—175 (1997).
4) Castro A. F., Altenberg G. A., *Biochem. Pharmacol.*, **53**, 89—93 (1997).
5) Shapiro A. B., Ling V., *Biochem. Pharmacol.*, **53**, 587—596 (1997).
6) Conseil G., Baubichon-Cortay H., Dayan G., Jault J.-M., Barron D., Di Pietro A., *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 9831—9836 (1998).
7) Zhang S., Morris M. E., *J. Pharmacol. & Exp. Ther.*, **304**, 1258—1267 (2003).
8) de Wet H., McIntosh D. B., Conseil G., Baubichon-Cortay H., Krell T., Jault J.-M., Daskiewicz J.-B., Barron D., Di Pietro A., *Biochemistry*, **40**, 10382—10391 (2001).
9) Rice-Evans C. A., Miller N. J., "Flavonoids in Health and Disease," ed. by Rice-Evans C. A., Packer L., Marcel Dekker, New York, 1998, pp.199—219.
10) Barzilai A., Rahamimoff H., *Biochim. Biophys. Acta*, **730**, 245—254 (1983).
11) Hodek P., Trefil P., Stiborova M., *Chem.-Biol. Interact.*, **139**, 1—21 (2002).
12) Martin H.-J., Kornmann F., Fuhrmann G. F., *Chem.-Biol. Interact.*, **146**, 225—235 (2003).
13) Zhang S., Yang X., Morris M. E., *Mol. Pharmacol.*, **65**, 1208—1216 (2004).
14) van Zanden J. J., Wortelboer H. M., Bijlsma S., Punt A., Usta M., van Bladeren P. J., Rietjens I. M. C. M., Cnubben N. H. P., *Biochem. Pharmacol.*, **69**, 699—708 (2005).
15) Yoshimura A., Kuwazuru Y., Sumizawa T., Ikeda S., Ichikawa M., Usagawa T., Akiyama S., *Biochim. Biophys. Acta*, **992**, 307—314 (1989).
16) Pietta P., Gardana C., Pietta A., "Flavonoids in Health and Disease," 2nd ed., ed. by Rice Evans C. A., Packer L., Marcel Decker, New York, 2003, pp. 43—69.
17) Craig W. J., *Am. J. Clin. Nutr.*, **70**, 491S—499S (1999).

18) Prior R. L., Cao G., *Nutr. Clin. Care*, **3**, 279—288 (2000).
19) Walle T., *Free Radic. Biol. Med.*, **36**, 829—837 (2004).
20) Boumendjel A., Di Pietro A., Dumontet C., Barron D., *Medic. Res. Revs.*, **22**, 512—529 (2002).
21) Wang R. B., Kuo C. L., Lien L. L., Lien E. J., *J. Clin. Pharm. Ther.*, **28**, 203—228 (2003).
22) Kitagawa S., Nabekura T., Kamiyama S., *J. Pharm. Pharmacol.*, **56**, 1001—1005 (2004).
23) Kitagawa S., Nabekura T., Takahashi T., Nakamura Y., Sakamoto H., Tano H., Hirai M., Tsukahara G., *Biol. Pharm. Bull.*, **28**, 2274—2278, (2005).
24) Wang E.-J., Casciano C. N., Clement R. P., Johnson W. W., *Biochem. Biophys. Res. Commun.*, **289**, 580—585 (2001).
25) van Acker S. A. B. E., Bast A., van der Vijgh W. J. F., "Flavonoids in Health and Disease," ed. by Rice-Evance C. A., Packer L., Marcel Dekker, New York, 1997, pp. 221—251.
26) Sadik C. D., Sies H., Schewe T., *Biochem. Pharmacol.*, **65**, 773—781 (2003).
27) Aruoma O. I., Murica A., Butler J., Halliwell B., *J. Agric. Food Chem.* **41**, 1880—1885 (1993).
28) Leclercq C., Arcella D., Turrini A., *Food Chem. Toxicol.*, **38**, 1075—1084 (2000).
29) Kubo I., Fujita K., Nihei K., Nihei A., *J. Agric. Food Chem.*, **52**, 1072—1076 (2004).
30) Kitagawa S., Nabekura T., Kamiyama S., Takahashi T., Nakamura Y., Kashiwada Y., Ikeshiro Y., *Biochem. Pharmacol.*, **70**, 1262—1266 (2005).
31) Chearwae W., Anuchapreeda S., Nandigama K., Ambudkar S. V., Limtrakul P., *Biochem. Pharmacol.*, **68**, 2043—2052 (2004).
32) Martin A. R., Villegas I., La Casa C., de la Lastra C. A., *Biochem. Pharmacol.*, **67**, 1399—1410 (2004).
33) Nabekura T., Kamiyama S., Kitagawa S., *Biochem. Biophys. Res. Commun.*, **327**, 866—870 (2005).
34) Mei Y., Qian F., Wei D., Liu J., *J. Pharm. Pharmacol.*, **56**, 1307—1314 (2004).
35) Klopman G., Shi L. M., Ramu A., *Mol. Pharmacol.*, **52**, 323—334 (1997).