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Research Article

Plant Components Exhibit Pharmacological Activities and Drug Interactions by Acting on Lipid Membranes

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ABSTRACT: In light of a novel mode of action on lipid membranes, we studied pharmacological activities of representative medicinal plant components, flavonoids, and verified their possible interactions with membrane-acting drugs. Fluorescence polarization measurements with different probes revealed that 1–500 μM phytochemicals structure-dependently acted on biomimetic membranes prepared with phospholipids and cholesterol to change the membrane physicochemical property, fluidity, by preferentially acting on the deeper regions of lipid bilayers. In the structure and membrane activity relationship characterized, greater potencies to decrease membrane fluidity were associated with the polyphenol structures, flavonoids with hydroxyl groups at the 3-, 3′-, 4′-, 5-, 5′- and/or 7-position. Quercetin and (−)-epigallocatechin gallate, meeting the structural requirements, effectively inhibited at 1–10 μM the proliferation of tumor cells to show 74.3–75.5% inhibition after 48 h culture and the membrane lipid peroxidation induced by 10 μM peroxynitrite to show 96.8–100% inhibition. These antiproliferative and antioxidant flavonoids also changed the fluidity of cell membranes simultaneously with exhibiting pharmacological activities. The membrane action is, at least in part, mechanistically responsible for the disease preventive and therapeutic effects of medicinal plants containing flavonoids. The selected membrane-active phytochemicals, phloretin and capsaicin antagonistically or additively influenced at 25–500 μM the membrane-fluidizing effects of lidocaine and bupivacaine of clinically relevant concentrations. These results suggest that medicinal plant components may cause inhibitory or cooperative drug interactions with local anesthetics which act on lipid bilayers to modify the membrane environments for Na^+ and K^+ channels embedded in biomembranes.

KEYWORDS: antioxidant, antiproliferative, flavonoid, lipid bilayer, local anesthetic, membrane fluidity

INTRODUCTION

While one tends to believe that the majority of drugs are synthetic in origin, clinically important medications are linked to natural products, especially plants.[1] Plants still serve as a source for new drug discovery and development.[2] Medicinal potentials of plants are attributable to various classes of pharmacologically active components represented by alkaloids, flavonoids, terpenoids, steroids, coumarins, saponins, phenylpropanoids, lignans and stilbenoids.[3, 4] Such phytochemicals exert antitumor, anticarcinogenic, antimicrobial, anti-inflammatory, neuroprotective, anti-allergic, antithrombotic, anti-atherosclerosis, antihypertensive, apoptosis-inducing and lipid peroxidation-inhibitory activity. Of these various bioactivities, they have been best known for antiproliferative and antioxidant activity, both of which are closely associated with each other and provide medicinal plants with disease preventive and therapeutic utility. The antiproliferative and antioxidant mechanisms for plant components have been exclusively explained by their actions on key enzymes, receptors, ion channels and reactive oxygen species or their combinations.[5, 6] Most of these mechanistically relevant actions occur in the membrane environments within and through lipid bilayers. The requisite for accessing the enzymes, receptors and channels embedded in biomembranes and the reactive oxygen species present in biomembranes is the affinity for membrane lipids.

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The concomitant use and combination therapy of medicinal plants or herbs with conventional medicines have been increasing in popularity, potentially causing beneficial and adverse interactions between plant components and drugs. In contrast to the drug-drug interactions receiving enormous attention, the interactions of phytochemicals and drugs have not necessarily been extensively investigated despite the possibility of more frequent occurrence than anticipated.[7] As in the drug-drug interactions, pharmacokinetic and pharmacodynamic mechanisms are implicated in the antagonistic, additive and synergistic interactions with medicinal plants, that is, the induction and inhibition of drug-metabolizing enzymes (cytochrome P450s) and drug-transporters (P-glycoprotein) and the binding to receptors, enzymes and ion channels.[8] Medicinal plants could influence anticancer, cardiovascular, anticoagulant, anti-diabetic, antimicrobial, immunosuppressant, anti-anxiety, antidepressant, analgesic and anesthetic drugs.[9, 10] Perioperative risk has been suggested for patients consuming medicinal plants and the interactions with anesthetic agents have been presumed for several plant species.[11, 12] Phytochemical and drug interactions were previously studied at receptor, enzyme and channel levels, whereas only limited information is available at a membrane level,[13] although the drug-drug interactions are very likely to occur through the effects on lipid membranes.[14, 15]

Among bioactive phytochemicals, flavonoids constitute one of the most ubiquitous groups of components in medicinal plants as well as in nutraceutical foods and the herbal remedies containing flavonoids have been widely used in folk medicine.[16] Flavonoids possess the common skeleton of a chromane ring with aromatic rings attached at the 2- or 3-position and these rings are labeled with A, B and C (Figure 1). Based on different substitutions and the oxidation status of a heterocyclic C ring, they are divided into various subclasses, that is, flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanidins with an oxonium ion O^+ in the C ring, isoflavones with the C ring at the 3-position and chalcones lacking the C ring. The pharmacological activities of flavonoids as medicinal plant components cover a very broad spectrum. However, not only are their molecular mechanisms often unknown, but also their structure and activity relationship has been only partially understood.[17–19] Bioactive flavonoids have been suggested to act on lipid bilayer biomembranes to modify the membrane physicochemical properties,[20, 21] together with their drug interactions as well as in alkaloids and coumarins.[22, 23]

Apart from the conventional mechanisms, the investigations focused on a novel mode of action on lipid membranes might provide some insight into the beneficial effects of medicinal plants and into their interactions with prescribed drugs. Therefore, we comparatively studied the effects of structurally diverse phytochemicals including flavonoids and capsaicin (Figure 1, each individual structure is shown by the number in parentheses in the following descriptions and Figures) on biomimetic lipid membranes by measuring their induced changes in membrane fluidity, and then we addressed whether they exhibited antiproliferative and antioxidant activities associated with the comparative membrane effects. Based on the results of membrane fluidity experiments, we also verified the possible interactions of selected plant components with membrane-acting drugs, local anesthetics.

MATERIALS AND METHODS

Chemicals

Phytochemical flavonoids and capsaicin were purchased from Funakoshi (Tokyo, Japan), and local anesthetic lidocaine and bupivacaine from Sigma-Aldrich (St. Louis, MO, USA). Phospholipids: 1,2-dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-stearoyl-2-oleoylphosphatidylserine (SOPS) and porcine brain sphingomyelin (SM) and fluorescent probes: 2-(9-anthroyloxy)stearic acid (2-AS), 9-(9-anthroyloxy) stearic acid (9-AS), 12-(9-anthroyloxy)stearic acid (12-AS) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Avanti Polar Lipids (Alabaster, AL, USA) and Molecular Probes (Eugene, OR, USA), respectively. Cholesterol was purchased from Wako Pure Chemicals (Osaka, Japan), and diphenyl-1-pyrenylphosphine (DPPP) and peroxynitrite from Dojindo (Kumamoto, Japan). The concentration of peroxynitrite was spectrophotometrically determined according to manufacturer's directions, and then diluted to be 10 mM with 0.1 M NaOH. The prepared solutions were stored at −80°C and consumed within three weeks after preparation. Dimethyl sulfoxide (DMSO) of spectroscopic grade (Kishida, Osaka, Japan) and Dulbecco's phosphate buffered saline of pH 7.4 (PBS; Dainippon Pharmaceuticals, Osaka, Japan) were used for preparing reagent solutions. All other chemicals were of the highest grade available.

Biomimetic membrane preparation

Lipid bilayer membranes labeled with fluorescent probes were prepared as liposome suspensions according to the method of Tsuchiya et al.[24] In brief, an aliquot (250 μl) of the ethanol solutions of phospholipids and cholesterol

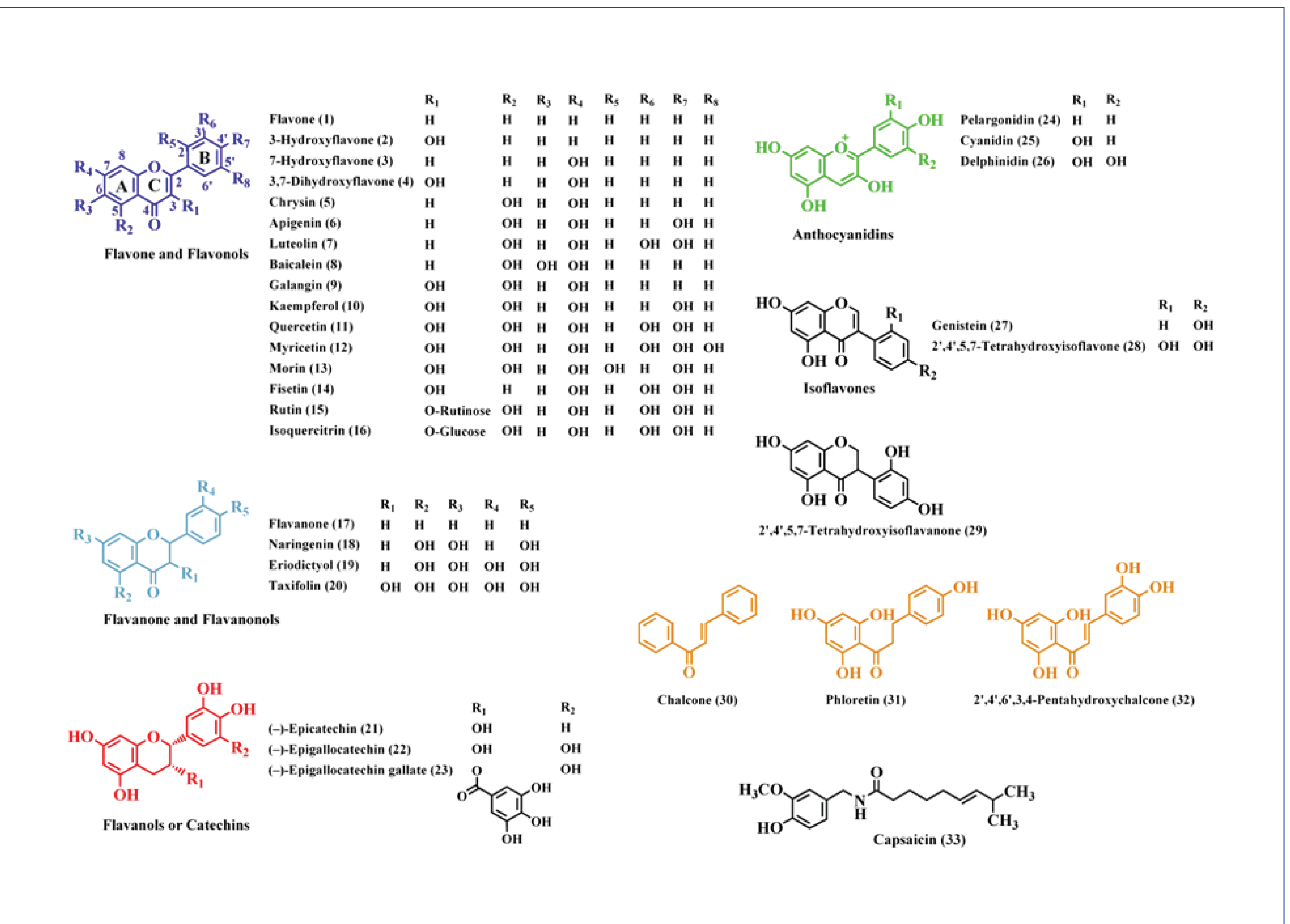

Figure 1: Plant component flavonoids and capsaicin used in this study.

(total lipids of 10 mM) and probes (2-AS, 9-AS, 12-AS or DPH, 50 μM for each) was injected four times into 199 ml of PBS under stirring above the phase transition temperatures of phospholipids. The compositions of lipids were 100 mol% DPPC for DPPC membranes, POPC, POPE, SOPS and cholesterol (48:24:8:20, mol%) for tumor cell-mimetic membranes,[25] and POPC, POPE, SOPS, SM and cholesterol (11:16.5:11:16.5:45, mol%) for peripheral nerve cell-mimetic membranes.[26]

Membrane effect comparison

The membrane effects of all the tested phytochemicals were compared by determining their potencies to change membrane fluidity. An aliquot of flavonoid and capsaicin solutions in DMSO was added to the membrane preparations to give a final concentration of 1–500 μM, followed by incubating at 37°C for 30 min. The DMSO concentration was adjusted to be less than 0.125% (v/v) of the total volume so as not to affect membrane fluidity. Thereafter, fluorescence polarization was measured at excitation and emission wavelengths of 367 nm and 443 nm for *n*-ASs, and 360 nm and 430 nm for DPH by an RF-540 spectrofluorometer (Shimadzu, Kyoto, Japan) equipped with a polarizer and a cuvette thermo-controlled at 37°C. Polarization values were calculated by the formula $(I_{VV} - GI_{VH})/(I_{VV} + GI_{VH})$ according to the method of Ushijima et al.,[27] in which I is the fluorescence intensity and the subscripts V and H refer to the vertical and horizontal orientation of the excitation and emission polarizer, respectively. The grating correction factor ($G = I_{HV}/I_{HH}$) is the ratio of the detection system sensitivity for vertically and horizontally polarized light, which was used to correct the polarizing effects of the monochromator.[28] Compared with controls, increasing and decreasing polarization value indicates a decrease (membrane rigidification) and an increase of membrane fluidity (membrane fluidization), respectively. Since the polarization values of control membranes differed by varying membrane lipid compositions and fluorescent probes, the polarization changes (%) relative to control values were used to compare between phytochemicals and between different membranes because percent changes are usable for the comparison regardless of the different polarization values of control membranes.[29] Fluorescent probes, *n*-ASs, selectively locate at a graded series of levels in lipid membranes. DPH localized in the hydrocarbon core of lipid bilayers reflect the fluidity changes in hydrophobic membrane regions.

Antiproliferative activity analysis

The effects of membrane-active flavonoids on tumor cell proliferation were analyzed as reported previously.[25] Briefly, mouse myeloma cells (Sp2/O-Ag14) were inoculated at 2.0 × 10^5 cells/ml in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum (ICN Biomedicals, Aurora, OH, USA). This cell line was chosen because the cells were successfully suspended in PBS and labeled with fluorescent probes in cell membrane fluidity experiments. An aliquot of quercetin (11) and (–)-epigallocatechin gallate (23) solutions in DMSO was added to the culture medium to be 10 μM for each. The final concentration of DMSO was 0.5% (v/v) so as not to affect cell proliferation. After culturing at 37°C for 24 and 48 h in a humidified 5% CO_2 atmosphere, the number of viable cells (trypan blue staining-negative cells) was counted by a hemocytometer, followed by comparing with controls (treated with DMSO vehicle alone) to determine the cell proliferation inhibition (%).

Determination of the effects on cultured cell membranes

Mouse myeloma cells (Sp2/O-Ag14) were cultured with or without quercetin (11) and (–)-epigallocatechin gallate (23) of 10 μM for each as described above. An aliquot of 2 ml of cell cultures was obtained after 24 h and 48 h culture, followed by centrifugation to collect cells. The collected cells were washed twice with and suspended in PBS, and then labeled with 2-AS and 12-AS according to the method of Furusawa et al.[30] The effects on cell membranes were determined by measuring fluorescence polarization as described above.

Antioxidant activity analysis

The effects of membrane-active flavonoids on membrane lipid peroxidation were analyzed as reported previously.[31] Briefly, DPPP-incorporated liposomal membranes with the molar ratio of DPPP to total lipids being 1:100 were suspended in PBS. The membrane lipid composition of POPC, POPE, SOPS, SM and cholesterol was the same as that of nerve cell-mimetic membranes described above. An aliquot (10 μl) of quercetin (11) and (–)-epigallocatechin gallate (23) solutions in DMSO was mixed with liposome suspensions (3.97 ml) to be a final concentration of 1 or 5 μM for each, followed by incubating at 37°C for 30 min. Membrane lipids were peroxidized by adding 20 μl of peroxynitrite solution in 0.1 M NaOH to be a final concentration of 10 or 50 μM, and then reacting at 37°C for 10 min. A corresponding volume of DMSO vehicle was added to controls. Membrane-incorporated DPPP quantitatively reacted with a lipid hydroperoxide to produce a fluorescent phosphine oxide in membrane lipid bilayers, which was fluorometrically measured at excitation and emission wavelength of 355 nm and 382 nm,

respectively. When peroxynitrite-induced increases in fluorescence intensity reached a plateau, the peroxidation of membrane lipids was defined as completed (100%). The antioxidant activity was determined by comparing the fluorescence intensity with controls.

Interactions of phytochemicals with drugs

Nerve cell-mimetic membranes were pretreated with 25 μM phloretin (31) or 50-500 μM capsaicin (33) at 37°C for 30 min. The pretreated and not-treated membranes were reacted with each 250μM lidocaine or bupivacaine at 37°C for 30 min, followed by measuring DPH fluorescence polarization as described above. Phytochemical and local anesthetic solutions in DMSO were added to the membrane preparations and the concentration of DMSO was adjusted to be less than 0.125% (v/v) of the total volume so as not to affect membrane fluidity. Control experiments were conducted with the addition of an equivalent volume of DMSO vehicle. The changes (%) of anesthetic membrane effects were obtained by comparing polarization changes induced by lidocaine and bupivacaine with those by phytochemical pretreatments.

Statistical analysis

All results are expressed as mean ± SE (n = 6 or 8 for membrane fluidity experiments and n = 5 for cell culture and antioxidant experiments). Data were statistically analyzed by ANOVA, followed by post hoc Fisher's PLSD test using StatView version 5.0 (SAS Institute, Cary, NC, USA). *P* values ≤ 0.05 were considered statistically significant.

RESULTS

Comparative effects on biomimetic membranes

Flavonoids differently increased *n*-AS fluorescence polarization of tumor cell-mimetic membranes at 10 μM, indicating that they structure-dependently acted on different regions of lipid bilayers to decrease membrane fluidity (Figures 2, 3 and 4). Delphinidin (26) exceptionally

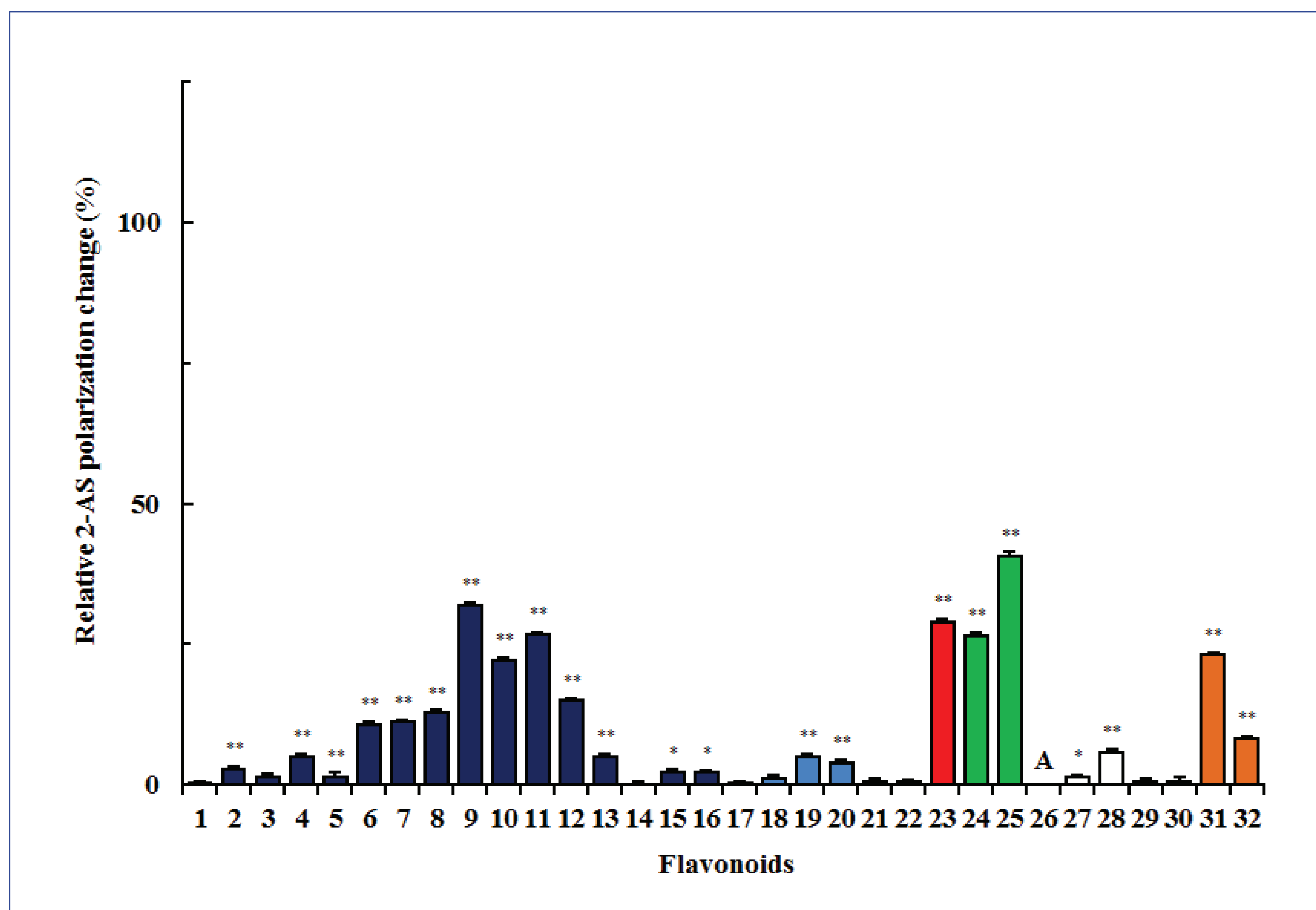

Figure 2: Comparative effects of flavonoids on biomimetic membranes shown by their induced 2-AS polarization changes. Flavonoids (10 μM for each) were reacted with tumor cell-mimetic membranes, followed by measuring 2-AS fluorescence polarization. Results are expressed as means ± SE (n = 8) of 2-AS polarization changes (%) relative to control polarization values. A: Aggregation induced. *p<0.05 and **p<0.01 *vs.* control.

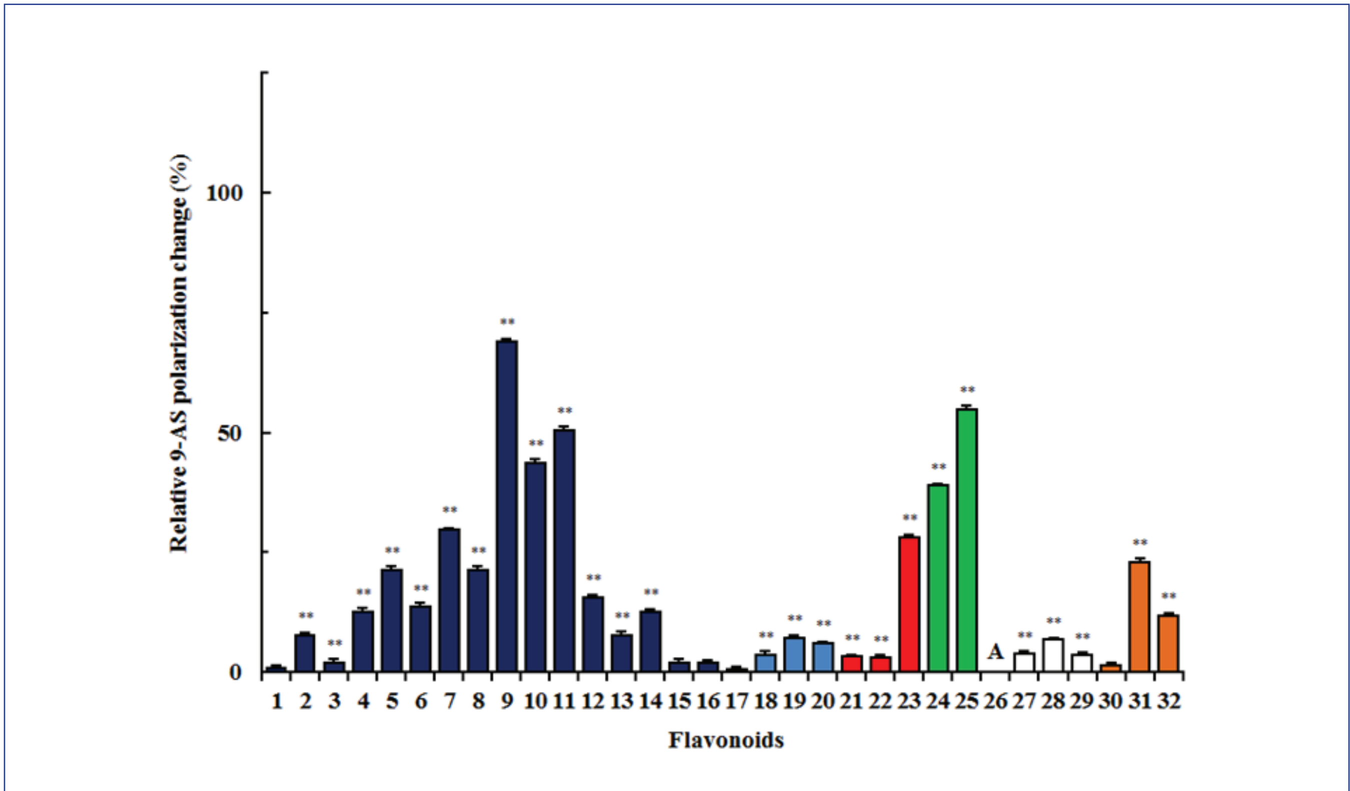

Figure 3: Comparative effects of flavonoids on biomimetic membranes shown by their induced 9-AS polarization changes. Flavonoids (10 μM for each) were reacted with tumor cell-mimetic membranes, followed by measuring 9-AS fluorescence polarization. Results are expressed as means ± SE (n = 8) of 9-AS polarization changes (%) relative to control polarization values. A: Aggregation induced. **p<0.01 *vs.* control.

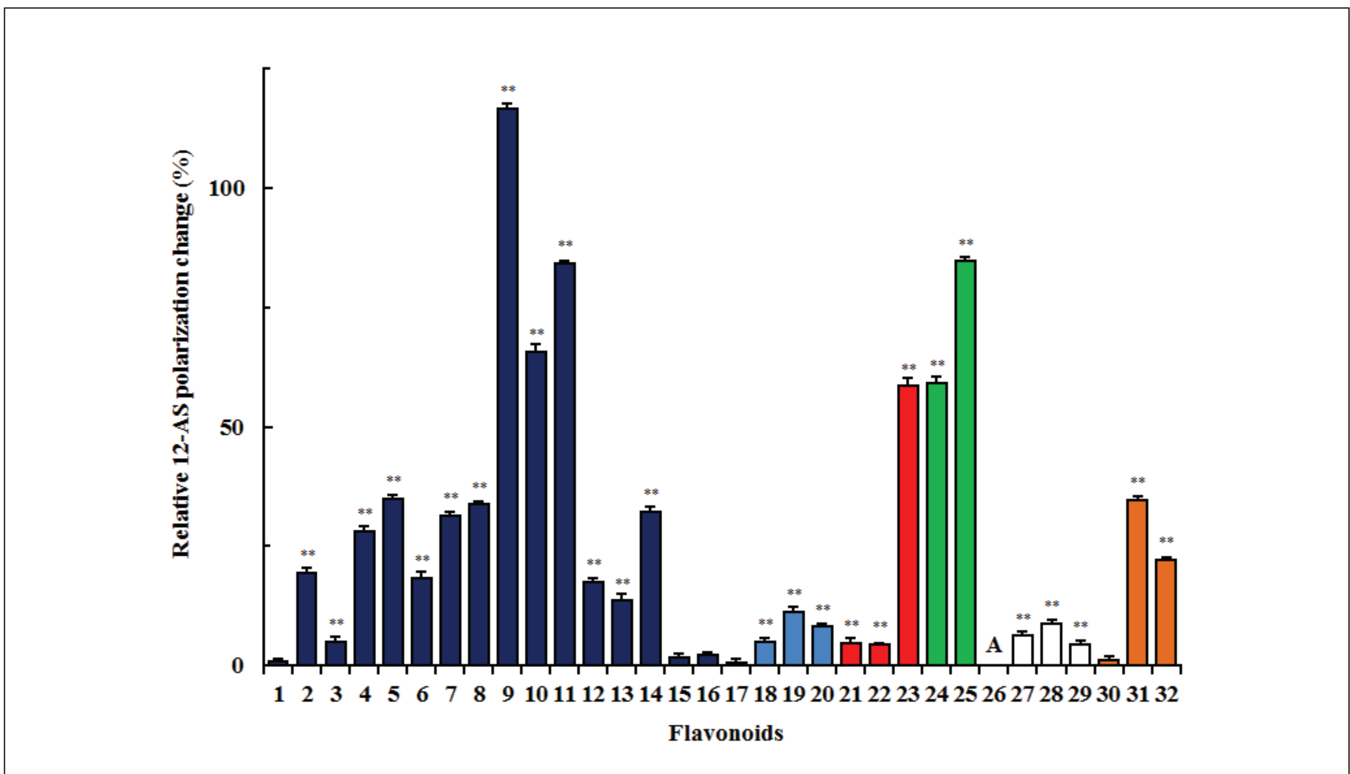

Figure 4: Comparative effects of flavonoids on biomimetic membranes shown by their induced 12-AS polarization changes. Flavonoids (10 μM for each) were reacted with tumor cell-mimetic membranes, followed by measuring 12-AS fluorescence polarization. Results are expressed as means ± SE (n = 8) of 12-AS polarization changes (%) relative to control polarization values. A: Aggregation induced. **p<0.01 *vs.* control.

induced the aggregation of liposomal membranes. In each subclass, relatively great membrane effects were shown by galangin (9), quercetin (11) and kaempferol (10) belonging to flavonols, (–)-epigallocatechin gallate (23) belonging to flavanols, cyanidin (25) and pelargonidin (24) belonging to anthocyanidins and phloretin (31) belonging to chalcones. These flavonoids significantly changed the membrane fluidity even at 1 μM. In the comparisons between structurally corresponding flavonoids (6, 7, 11, 28 and 18, 19, 20, 29, respectively), flavones and isoflavones were much more membrane-active than flavanones and isoflavanones, respectively. Flavonoids were more active compared with isoflavonoids as being apparent by comparing between kaempferal (10) and genistein (27). Glycosides rutin (15) and isoquercitrin (16) were much less effective in acting on the membranes than their aglycone-quercetin (11).

The relative polarization changes of 2-AS, 9-AS and 12-AS, which selectively locate from the surface to the inner regions of lipid bilayers with increasing *n* of *n*-ASs, indicated comparative effects on different depths of membranes. In the subclass of flavonols, myricetin (12), quercetin (11), kaempferol (10) and galangin (9) acted in increasing order of preference to the deeper regions of lipid membranes. Rutin (15) and isoquercitrin (16) comparatively preferred to act on the hydrophilic superficial regions. The greater potency to affect the hydrophobic regions was a characteristic common to membrane-active flavonoids (9), (11), (23), (25) and (31).

Antiproliferative and antioxidant activities

The selected membrane-active flavonoids, quercetin (11) and (–)-epigallocatechin gallate (23), were investigated as to whether they influenced the tumor cell viability and the membrane lipid peroxidation.

Quercetin (11) and (–)-epigallocatechin gallate (23) inhibited cell proliferation at 10 μM (Figure 5). Their antiproliferative activities were greater with increasing the culture time (24–48 h).

Both quercetin (11) and (–)-epigallocatechin gallate (23) were also effective at 1 and 5 μM in inhibiting membrane lipid peroxidation induced by different concentrations of peroxynitrite (Figure 6).

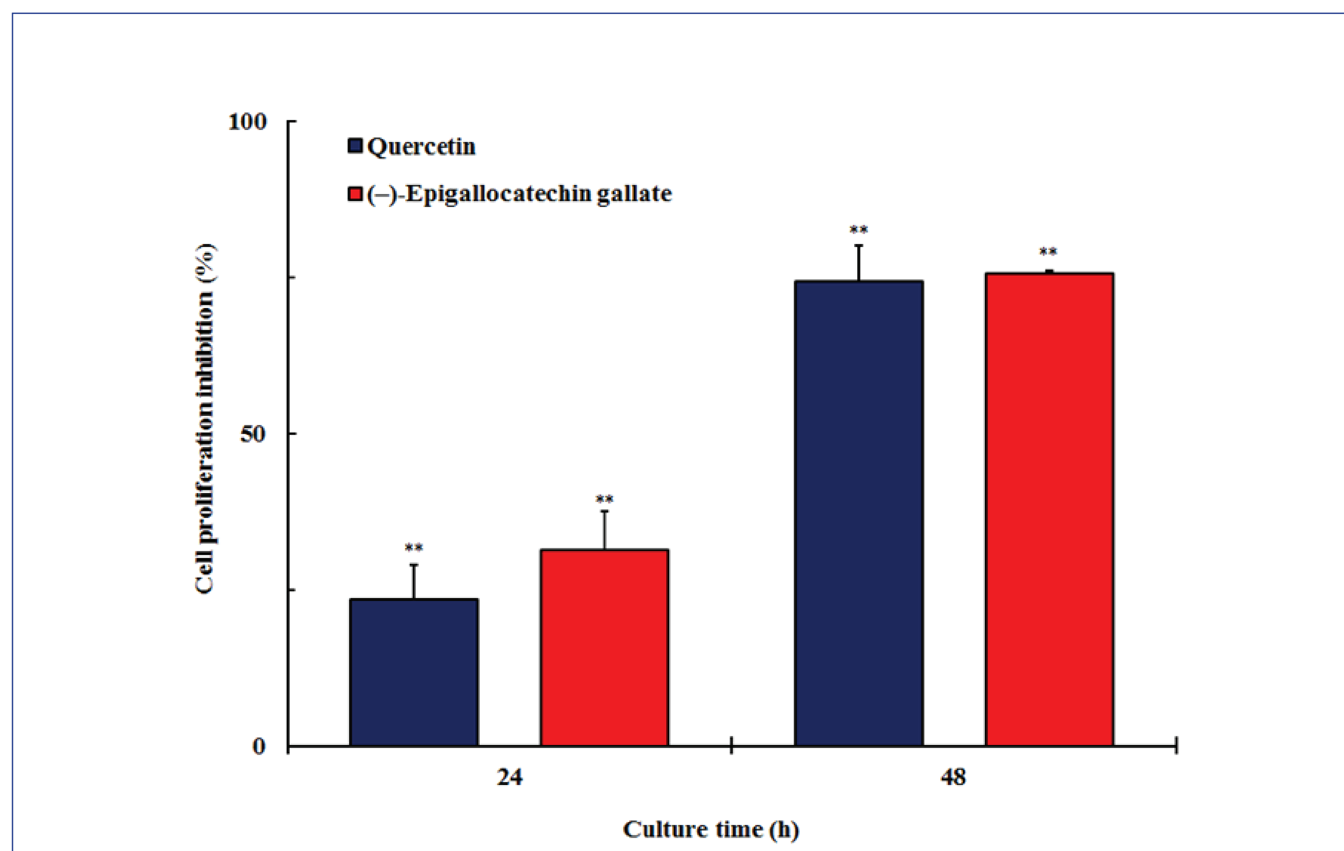

Figure 5: Antiproliferative activities of quercetin and (–)-epigallocatechin gallate on tumor cells. The inhibition of cell proliferation was determined after culturing tumor cells with quercetin and (–)-epigallocatechin gallate (10 μM for each) for 24 and 48 h. Results are expressed as means ± SE (n = 5). **p<0.01 *vs.* control.

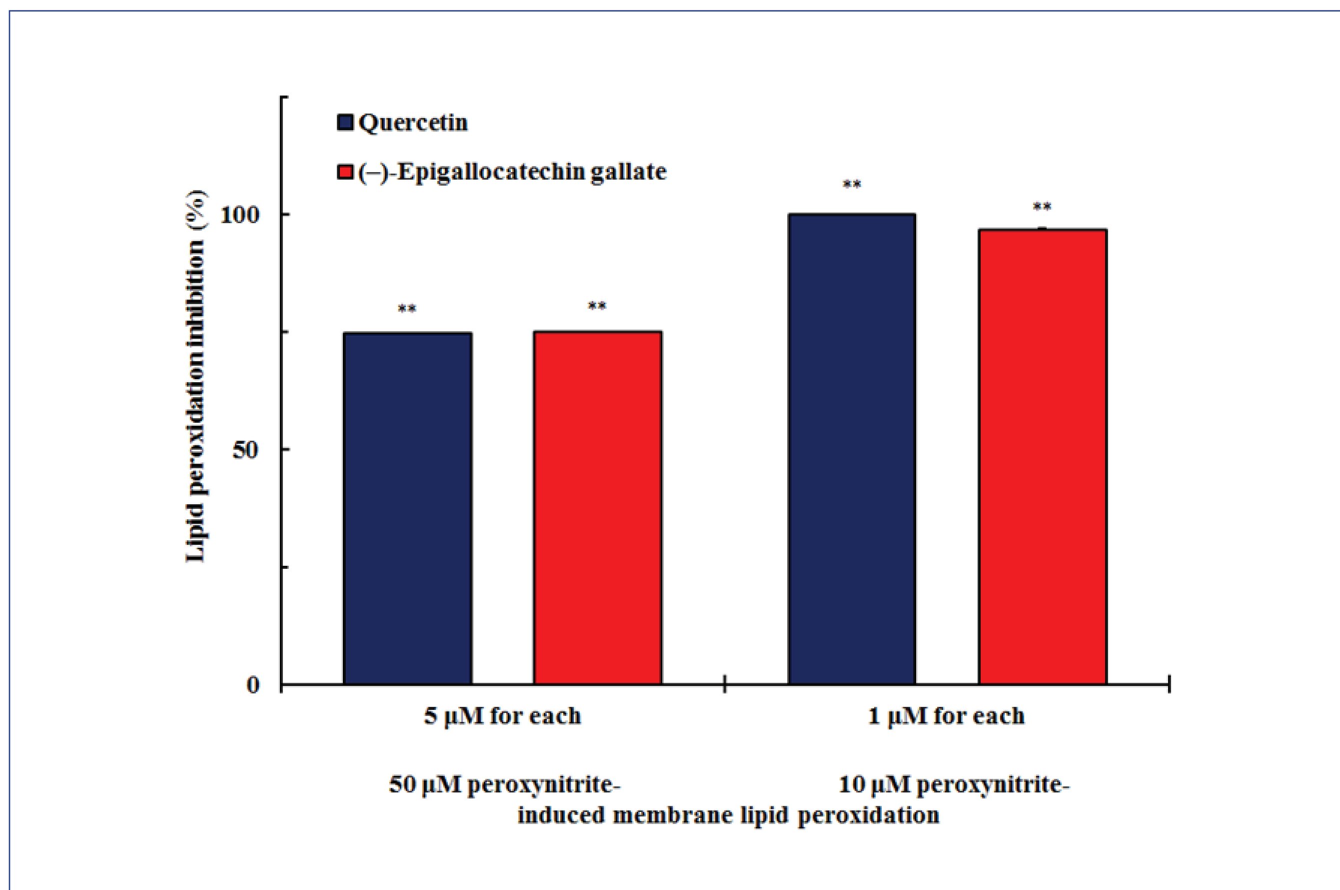

Figure 6: Antioxidant activities of quercetin and (–)-epigallocatechin gallate on membrane lipids. The inhibition of lipid peroxidation was determined by pretreating nerve-cell mimetic membranes with quercetin and (–)-epigallocatechin gallate (1 and 5 μM for each) and peroxidizing with 10 or 50 μM peroxynitrite. Results are expressed as means ± SE (n = 5). **p<0.01 *vs*. control.

Effects on cell membranes

Together with inhibiting tumor cell proliferation, quercetin (11) and (–)-epigallocatechin gallate (23) rigidified cell membranes at 10 μM as shown by 2-AS and 12-AS polarization increases (Figure 7A and B). Their membrane effects were more evident in the hydrophobic deeper regions of cell membranes.

Quercetin (11) and (–)-epigallocatechin gallate (23) also decreased at 1–10 μM the fluidity of nerve cell-mimetic membranes used for the antioxidant activity analysis.

Interactions of phytochemicals with local anesthetics

Phloretin (31) acted on nerve cell-mimetic membranes to decrease their fluidity at 10–50 μM in a concentration-dependent manner. On the other hand, capsaicin (33) showed biphasic effects on both DPPC membranes and nerve cell-mimetic membranes to increase the membrane fluidity at relatively low micromolar concentrations but decrease at relatively high concentrations as shown by different changes of DPH fluorescence polarization (Figure 8).

Lidocaine and bupivacaine acted on nerve cell-mimetic membranes at 250 μM to increase their fluidity as shown by DPH polarization decreases (Figure 9). Membrane-active phloretin (31) and capsaicin (33) inhibited the local anesthetics-induced membrane fluidization at 25 μM and 500 μM, respectively. At 50 μM, however, capsaicin (33) increased the potencies of both local anesthetics to fluidize membranes. Comparisons of the polarization decreasing degree between anesthetic alone and the combination indicated that the membrane interactions of capsaicin (33) with lidocaine and bupivacaine were additive rather than synergistic.

DISCUSSION

Firstly, we compared structural analogues which have a different number of hydroxyl groups in the A and B ring, a

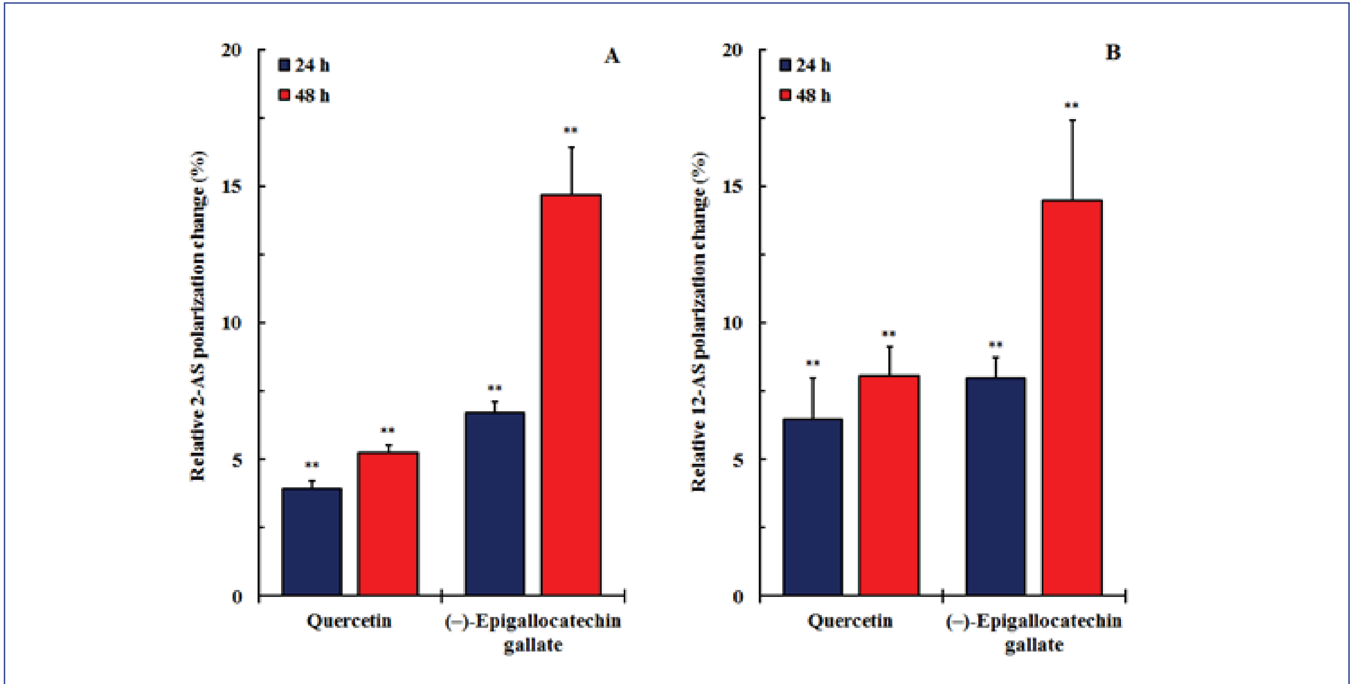

Figure 7: Effects of antiproliferative flavonoids on tumor cell membranes shown by 2-AS (7A) and 12-AS polarization changes (7B). Tumor cells were cultured with quercetin and (–)-epigallocatechin gallate (10 μM for each) for 24 and 48 h, followed by measuring 2-AS and 12-AS fluorescence polarization. Results are expressed as means ± SE (n = 5) of 2-AS or 12-AS polarization changes (%) relative to control polarization values. **p<0.01 *vs.* control.

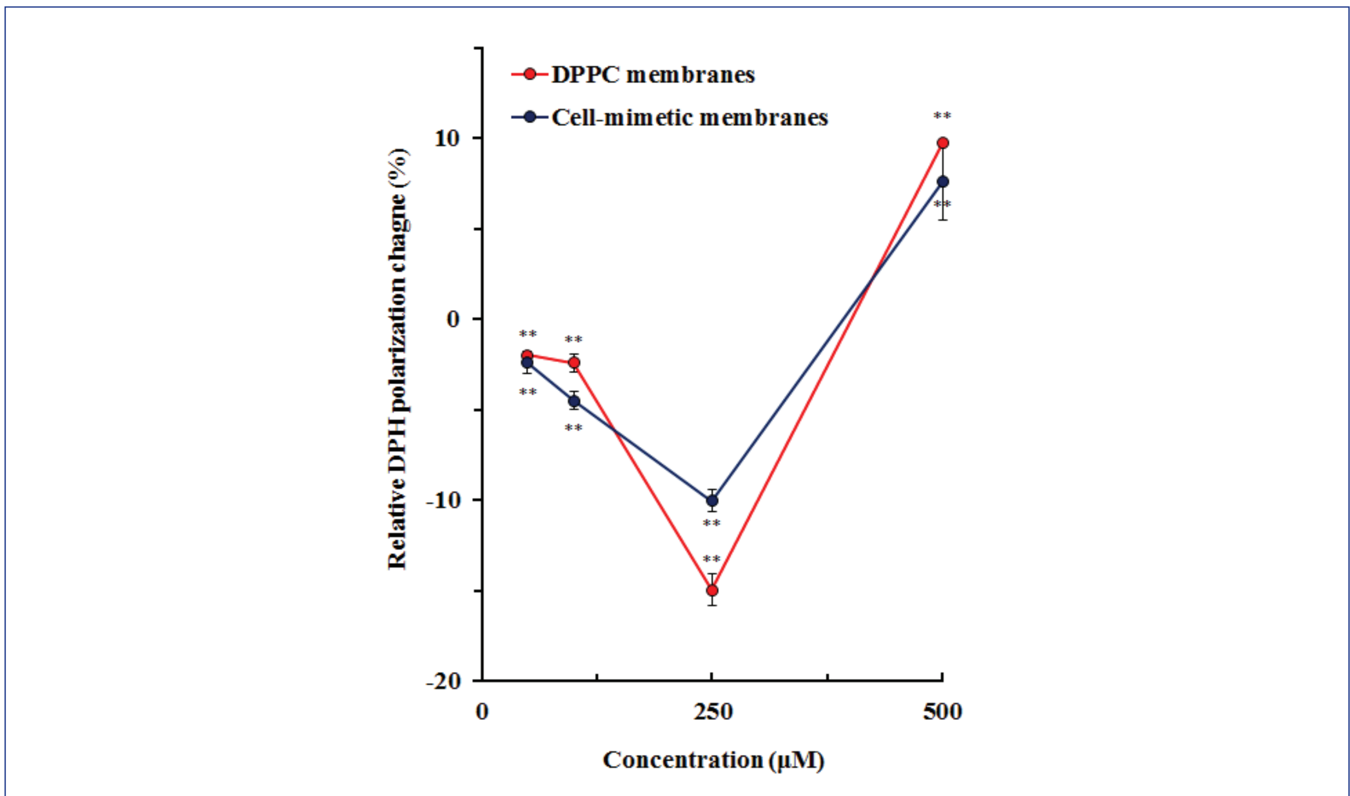

Figure 8: Effects of capsaicin on lipid membranes. Capsaicin of the indicated concentrations was reacted with DPPC membranes and nerve cell-mimetic membranes, followed by measuring DPH fluorescence polarization. Results are expressed as means ± SE (n = 6) of DPH polarization changes (%) relative to control polarization values. **p<0.01 *vs.* control.

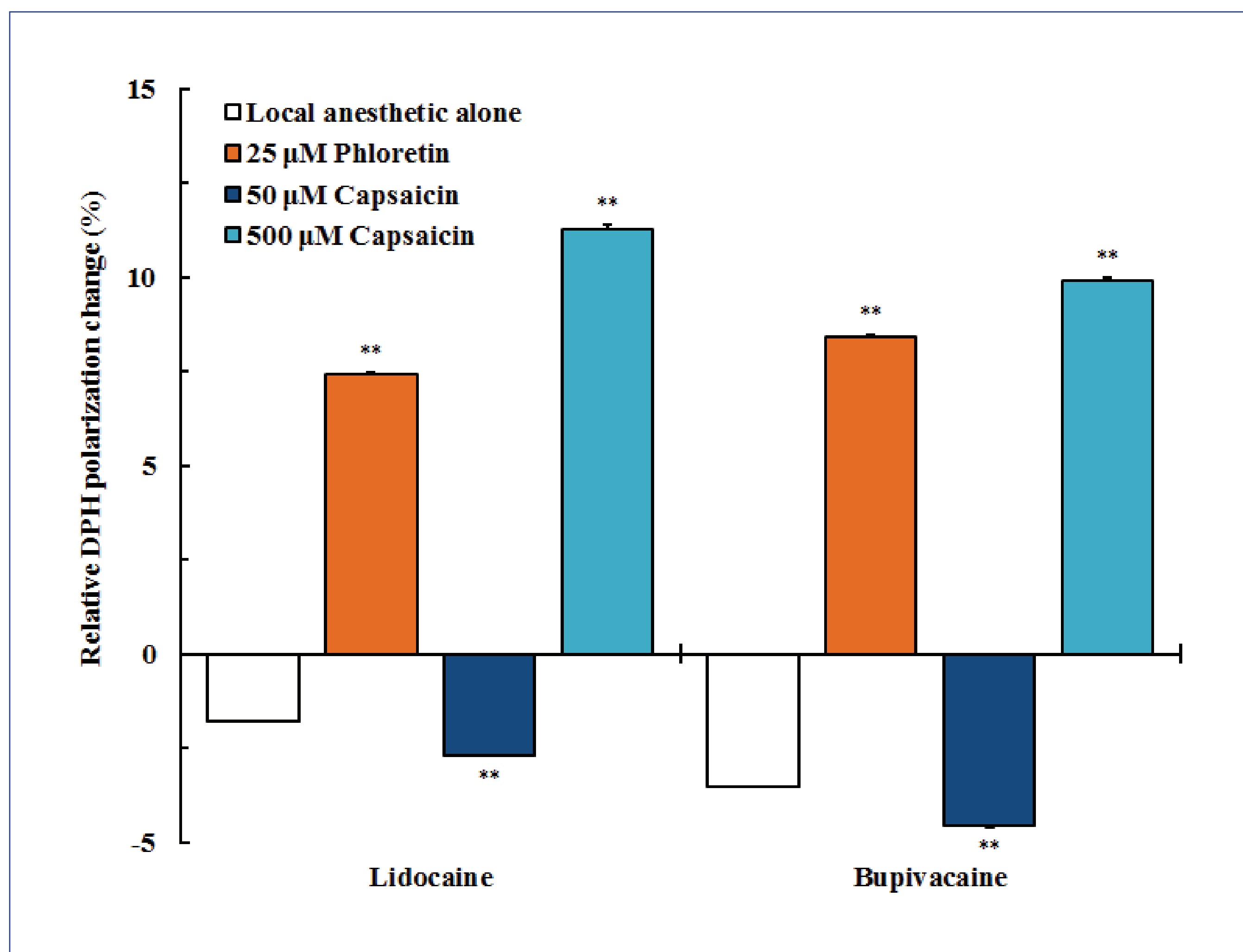

Figure 9: Membrane interactions of phytochemicals with local anesthetics. Nerve cell-mimetic membranes pretreated and not-treated with 25 μM phloretin or 50-500 μM capsaicin were reacted with lidocaine and bupivacaine (250 μM for each), followed by measuring DPH fluorescence polarization. Results are expressed as means ± SE (n = 8) of DPH polarization changes (%) relative to control polarization values. **p<0.01 *vs.* control.

different C ring and different substituents at the 3-position (Figure 1) to characterize the structure and membrane activity relationship. Flavonoids of low millimolar or sub-millimolar levels are known to quench the fluorescence of probes used for membrane fluidity experiments.[32] Since such quenching properties interfere with fluorescence polarization measurements, the comparative membrane effects were determined at 1–10 μM for each flavonoid. Consequently, the tested flavonoids have been revealed to act on biomimetic membranes and decrease their fluidity in a structure-dependent manner. The characterized relationship indicates that a 3-hydroxyl group and a 2,3 double bond in the C ring, 3',4'-dihydroxyl groups or the absence of any hydroxyl groups in the B ring and 5,7-dihydroxyl groups in the A ring provide the basic structure of flavonoid, not isoflavonoid, with greater membrane activities. In flavanols without a 2,3 double bond, a galloyl group is an alternative to a 3-hydroxyl group, which significantly potentiates the membrane action to provide the membrane-active structure of (–)-epigallocatechin gallate (23). The membrane effects of quercetin rutinoside (15) and glucoside (16) have been shown to be much less or negligible compared with the aglycone quercetin (11), while flavonoid glycosides-naturally present in plants under go the *in vivo* hydrolysis to the aglycones.[33, 34] The difference between aglycone and glycoside is attributable to an increase of hydrophilicity by glycosylation.

A set of *n*-AS fluorescence polarization have suggested that flavonoids preferentially act on the deeper regions of lipid bilayers by intercalating between the hydrocarbon chains of membrane lipids. One of determinants for the membrane-acting potency seems to be the hydrophobicity

to enhance the interaction with lipid bilayers,[21] as the membrane activity of flavonoids is well correlated to their relative lipophilicity or partition coefficient.[35] Steric configuration could additionally participate in the flavonoid and membrane lipid interactions.[36]

The structure and membrane activity relationship has suggested that the promising structures to exhibit pharmacological activities are galangin (9), quercetin (11), cyanidin (25), (–)-epigallocatechin gallate (23) and phloretin (31) in decreasing order of membrane-rigidifying intensity. Quercetin (11) is likely to be more bioactive than galangin (9) in the comparative studies reported previously.[37, 38] Anthocyanidins are easily decomposed under cell culture conditions and in the biophase,[39, 40] indicating the limited stability of cyanidin (25). Secondly, therefore, we addressed whether the selected membrane-active flavonoids, quercetin (11) and (–)-epigallocatechin gallate (23), exhibit pharmacological activities. Consequently, both flavonoids have been proved to inhibit both the proliferation of tumor cells and the peroxidation of membrane lipids at low micromolar concentrations, which almost agree with the concentrations to decrease the fluidity of biomimetic membranes. Quercetin (11) and (–)-epigallocatechin gallate (23) have been also revealed to exhibit the antiproliferative and antioxidant activities simultaneously with changing the fluidity of both the cultured tumor cell membranes and the biomimetic membranes used for antioxidant experiments.

Since the activation and suppression of cell proliferation occur in the lipid membrane environments and these events are governed by the physicochemical property of lipid bilayer biomembranes,[41] cell membranes and membranous organelles have been referred to as a novel target for antitumor agents.[42] The proliferating abilities of tumor cells are closely related to the altered properties of cell membranes as neoplastic and metastatic cells are known to have more fluid membranes than their normal counterparts because of decreasing cholesterol and increasing unsaturated phospholipids in membranes. The flavonoids-induced membrane rigidification would counteract the increased membrane fluidity of tumor cells. Flavonoids also affect tumor cell proliferation by inhibiting tumorigenesis-relating enzymes, inducing apoptosis, modulating proliferative signal transduction, arresting cell cycle progression and altering receptor functions or a combination of these mechanisms.[19] While cyclooxygenase, especially the inducible form cyclooxygenase-2, plays an important pathological role in tumorigenesis, the modification of membrane fluidity is associated with the inhibition of cyclooxygenase.[43] Changes in membrane fluidity should functionally influence tumorigenesis-relevant enzymes and receptors by disturbing the membrane environments optimal for the conformation of enzyme and receptor proteins embedded in biomembranes. Apoptosis is inducible through the alteration of tumor cell membrane fluidity.[44] In membrane dynamics, cell cycle is accompanied by membrane fluidity changes and the membranes of resting cells are more rigid than those of proliferating ones.[41] Quercetin (11) and (–)-epigallocatechin gallate (23) are considered to affect tumor cell proliferation by modifying the fluidity of cellular or plasma membranes.

The reaction between oxidants and membrane lipids is subject to the physicochemical property of lipid bilayers.[45] The effects of antioxidants are linked not only to scavenging reactive species such as peroxynitrite but also to reducing lipid peroxidation efficiency through the membrane fluidity changes.[46, 47] In more rigid or less fluid membranes, the diffusion of reactive species in lipid bilayers is hindered to decrease the kinetics of oxidation reactions, resulting in inhibition of the propagation of lipid peroxidation.[48] Quercetin (11) and (–)-epigallocatechin gallate (23) are speculated to influence membrane lipid peroxidation by rigidifying lipid bilayer membranes cooperatively with the scavenging effects on reactive oxygen and nitrogen species.

The membrane-acting mechanism to change membrane fluidity appears to underlie, at least in part, the inhibitory effects of plant component flavonoids on tumor cell proliferation and membrane lipid peroxidation.

Thirdly, in light of the mode of action on lipid membranes, we verified the possible membrane interactions between phytochemicals and drugs. Among membrane-acting drugs, anesthetics have been suggested to interact with plant or herbal medicines.[11, 12] Local anesthetics such as lidocaine and bupivacaine directly and indirectly block Na^+ and K^+ channels by binding to the membrane-embedded ion channels and changing the fluidity of nerve cell membranes.[49, 50] Amphiphilic local anesthetics act hydrophobically on phospholipid acyl chains and electrostatically on phospholipid polar head-groups, rearranging the intermolecular hydrogen-bonded network among phospholipid molecules and changing the orientation of the P-N dipole of phospholipid molecules, with a subsequent increase of membrane fluidity. Therefore, we selected phytochemical amphiphiles, flavonoid phloretin (31) with a long axis structure and alkaloid capsaicin (33) with a lipophilic hydrocarbon chain and a polar moiety, which might act on lipid bilayer membranes similarly to local anesthetics.

Consequently, phloretin (31) has been revealed to decrease the fluidity of nerve cell-mimetic membranes, whereas capsaicin (33) biphasically changes the fluidity depending on concentrations. The characteristic effects of capsaicin (33) are primarily due to its action on membrane phospholipids, not cholesterol, because it shows the biphasic feature common to DPPC membranes consisting of 100 mol% DPPC and cell-mimetic membranes consisting of phospholipids and cholesterol. By the concomitant uses, the membrane-fluidizing effects of lidocaine and bupivacaine of clinically relevant concentrations have been proved to be antagonistically inhibited by 25 μM phloretin (31) and 500 μM capsaicin (33), but additively increased by 50 μM capsaicin (33). A medicinal plant, *Glycyrrhiza uralensis*, was recently reported to show the pharmacokinetic interaction with lidocaine through the induction of cytochrome P-450s.[51] In addition, medicinal plant components may influence the effects of local anesthetics by modifying the membrane lipid environments for Na^+ and K^+ channels embedded in biomembranes.

While the clinical implication of the membrane interactions of phloretin (31) and capsaicin (33) with local anesthetics may be beyond the scope of this study, the speculative discussion is useful for the following researches. Phloretin (31) is assumed to act on lipid monolayers and bilayers to affect membrane structure and permeability.[52, 53] A polymeric mixture of polyesters of phloretin (31) and phosphoric acid was found to decrease the duration of infiltration anesthesia in guinea pigs by lidocaine, bupivacaine and prilocaine, and also to terminate their anesthetic effects.[54] Such an anti-local anesthetic effect was also proved in the infiltration anesthesia of human teeth.[55] The inhibitory drug interaction by phloretin (31) might be valuable to discontinue local anesthetic effects as soon as the dental treatment is finished in some cases of children and mentally retarded patients. Vanilloid receptor agonists, including capsaicin (33), are known to increase the effects of site 1 Na^+ channel blockers.[56] Capsaicin (33) was reported to potentiate the impulse block by lidocaine at 50 μM in rat sciatic nerves.[57, 58] The cooperative drug interaction by capsaicin (33) would be usable to increase and prolong local anesthetic effects.

CONCLUSIONS

This study describes one of possible mechanisms for the disease preventive and therapeutic effects of medicinal plants containing flavonoids. Flavonoids structure-dependently act on biomimetic and cellular membranes to modify the membrane fluidity by preferentially acting on the hydrophobic deeper regions of lipid bilayers. The most membrane-active quercetin and (–)-epigallocatechin gallate, meeting the structure and activity relationship, inhibit the proliferation of tumor cells and the lipid peroxidation together with affecting cell membranes. When concomitantly used with local anesthetics, phloretin and capsaicin decreasingly or increasingly change the membrane-fluidizing effects of lidocaine and bupivacaine which correlate to their anesthetic potencies. Medicinal plants may cause inhibitory or cooperative drug interactions with membrane-acting local anesthetics.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

ABBREVIATIONS

2-AS	2-(9-anthroyloxy)stearic acid
9-AS	9-(9-anthroyloxy)stearic acid
12-AS	12-(9-anthroyloxy)stearic acid
DMSO	dimethyl sulfoxide
DPH	1,6-diphenyl-1,3,5-hexatriene
DPPC	1,2-dipalmitoylphosphatidylcholine
DPPP	diphenyl-1-pyrenylphosphine
PBS	phosphate buffered saline of pH 7.4
POPC	1-palmitoyl-2-oleoylphosphatidylcholine
POPE	1-palmitoyl-2-oleoylphosphatidylethanolamine
SM	sphingomyelin
SOPS	1-stearoyl-2-oleoylphosphatidylserine

REFERENCES

1. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry 2001;56(3):237–43.
2. Lee KH. Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach. J Nat Prod 2010;73(3):500–16.
3. Kim YC. Neuroprotective phenolics in medicinal plants. Arch Pharm Res 2010;33(10):1611–32.
4. Afifi-Yazar FU, Kasabri V, Abu-Dahab R. Medicinal plants from Jordan in the treatment of cancer: traditional uses vs. *in vitro* and *in vivo* evaluations–Part 1. Planta Med 2011;77(11):1203–9.

5. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52(4):673–751.
6. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res 2008;7(3):1089–99.
7. Ioannides C. Drug-phytochemical interactions. Inflammopharmacology 2003;11(1):7–42.
8. Zhou SF, Zhou ZW, Li CG, Chen X, Yu X, Xue CC, Herington A. Identification of drugs that interact with herbs in drug development. Drug Discovery Today 2007;12(15/16):664–73.
9. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: an updated systematic review. Drugs 2009;69(13):1777–98.
10. Al-Omari IL, Afifi FU, Salhab AS. Therapeutic effect and possible herb drug interactions of ginger (*Zingiber officinale* Roscoe, Zingiberaceae) crude extract with glibenclamide and insulin. Pharmacog Commun 2012;2(1):12–20.
11. Frost EAM. Herbal medicines and interactions with anesthetic agents. Middle East J Anesthesiol 2006;18(5):851–78.
12. Muedra V, Moreno L. Knowledge of perioperative risk associated with use of medicinal plants. Rev Esp Anestesiol Reanim 2009;56(8):467–73.
13. Tsuchiya H. Inhibition of membrane effects of general anesthetic propofol by benzodiazepine inverse agonist tetrahydro-β-carboline. Int J Pharmacol 2012;8(6):542–2.
14. Tsuda K, Nishio I. A calcium channel blocker, benidipine, improves cell membrane fluidity in human subjects via a nitric oxide-dependent mechanism. An electron paramagnetic resonance investigation. Am J Hypertens 2004;17(12 Pt 1):1143–50.
15. Tsuda K, Nishio I. A selective estrogen receptor modulator, tamoxifen, and membrane fluidity of erythrocytes in normotensive and hypertensive postmenopausal woman: an electron paramagnetic resonance investigation. Am J Hypertens 2005;18(8):1067–76.
16. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci 1999;65(4): 337–53.
17. Kuntz S, Wenzel U, Daniel H. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. Eur J Nutr 1999;38(3):133–42.
18. López-Lázaro M. Flavonoids as anticancer agents: structure-activity relationship study. Curr Med Chem Anticancer Agents 2002;2(6): 691–714.
19. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. Med Res Rev 2003;23(4):519–34.
20. Ollila F, Halling K, Vuorela P, Vuorela H, Slotte JP. Characterization of flavonoid-biomembrane interactions. Arch Biochem Biophys 2002; 399(1):103–8.
21. Hendrich AB. Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. Acta Pharmacol Sin 2006;27(1):27–40.
22. Morris ME, Zhang S. Flavonoid-drug interactions: effects of flavonoids on ABC transporters. Life Sci 2006;78(18):2116–30.
23. Fasinu PS, Bouic PJ, Rosenkranz B. An overview of the evidence and mechanisms of herb-drug interactions. Frontiers Pharmacol 2012;3(69): 1–19.
24. Tsuchiya H, Ueno T, Mizogami M, Takakura K. Local anesthetics structure-dependently interact with anionic phospholipid membranes to modify the fluidity. Chem Biol Interact 2010;183(1):19–24.
25. Tsuchiya H, Nagayama M. Garlic allyl derivatives interact with membrane lipids to modify the membrane fluidity. J Biomed Sci 2008;15(5):653–60.
26. Svennerholm L, Boström K, Fredman P, Jungbjer B, Månsson JE, Rynmark BM. Membrane lipids of human peripheral nerve and spinal cord. Biochim Biophys Acta 1992;1128(1):1–7.
27. Ushijima H, Tanaka K, Takeda M, Katsu T, Mima S, Mizushima T. Geranylgeranylacetone protects membranes against nonsteroidal anti-inflammatory drugs. Mol Pharmacol 2005;68(4):1156–61.
28. Brittes J, Lúcio M, Nunes C, Lima JLFC, Reis S. Effects of resveratrol on membrane biophysical properties: relevance for its pharmacological effects. Chem Phys Lipids 2010;163(8):747–54.
29. Alfahel E, Korngreen A, Parola AH, Priel Z. Purinergically induced membrane fluidization in ciliary cells: characterization and control by calcium and membrane potential. Biophys J 1996;70(2):1045–53.
30. Furusawa M, Tsuchiya H, Nagayama M, Tanaka T, Oyama M, Ito T, Iinuma M, Takeuchi H. Cell growth inhibition by membrane-active components in brownish scale of onion. J Health Sci 2006;52(5):578–84.
31. Tsuchiya H, Ueno T, Tanaka T, Matsuura N, Mizogami M. Comparative study on determination of antioxidant and membrane activities of propofol and its related compounds. Eur J Pharm Sci 2010;39(1–3):97–102.
32. Schoefer L, Braune A, Blaut M. A fluorescence quenching test for the detection of flavonoid transformation. FEMS Microbiol Lett 2001;204(2):277–80.
33. Meiers S, Kemény M, Weyand U, Gastpar R, von Angerer E, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. J Agric Food Chem 2001;49(2): 958–62.
34. Montoro P, Braca A, Pizza C, De Tommasi N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chem 2005;92(2):349–55.
35. Uekusa Y, Takeshita Y, Ishii T, Nakayama T. Partition coefficients of polyphenols for phosphatidylcholine investigated by HPLC with an immobilized artificial membrane column. Biosci Biotechnol Biochem 2008;72(12): 3289–92.
36. Tammela P, Laitinen L, Galkin A, Wennberg T, Heczko R, Vuorela H, Slotte JP, Vuorela P. Permeability characteristics and membrane fluidity of flavonoids and alkyl gallates in Caco-2 cells and in phospholipid vesicles. Arch Biochem Biophys 2004;425(2):193–9.
37. Touil YS, Fellous A, Scherman D, Chabot GG. Flavonoid-induced morphological modifications of endothelial cells through microtubule stabilization. Nutr Cancer 2009;61(3):310–21.
38. Ahn MR, Kunimasa K, Kumazawa S, Nakayama T, Kaji K, Uto Y, Hori H, Nagasawa H, Ohta T. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. Mol Nutr Food Res 2009;53(5):643–51.
39. Kern M, Fridrich D, Reichert J, Skrbek S, Nussher A, Hofem S, Vatter S, Pahlke G, Rüfer C, Marko D. Limited stability in cell culture medium and hydrogen peroxide formation affect the growth inhibitory properties of delphinidin and its degradation product gallic acid. Mol Nutr Food Res 2007;51(9):1163–72.
40. Thomasset S, Teller N, Cai H, Marko D, Berry DP, Steward WP, Gescher AJ. Do anthocyanins and anthocyanidins, cancer chemopreventive pigments in the diet, merit development as potential drugs? Cancer Chemother Pharmacol 2009;64(1):201–11.
41. Muller CP, Krueger GR. Modulation of membrane proteins by vertical phase separation and membrane lipid fluidity. Basis for a new approach to tumor immunotherapy. Anticancer Res 1986;6(5):1181–93.
42. Daoud SS. Cell membranes as targets for anti-cancer drug action. Anticancer Drugs 1992;3(5):443–53.
43. Czapla K, Korchowiec B, Rogalska E. Differentiating oxicam nonsteroidal anti-inflammatory drugs in phosphoglyceride monolayers. Langmuir 2010;26(5):3485–92.
44. Baritaki S, Apostolakis S, Kanellou P, Dimanche-Boitrel MT, Spandidos DA, Bonavida B. Reversal of tumor resistance to apoptotic stimuli by alteration of membrane fluidity: therapeutic implications. Adv Cancer Res 2007;98:149–90.
45. Schnitzer E, Pinchuk I, Lichtenberg D. Peroxidation of liposomal lipids. Eur Biophys J 2007;36(4–5):499–515.
46. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 2008;585(2–3):325–37.
47. Dorta DJ, Pigoso AA, Mingatto FE, Rodrigues T, Pestana CR, Uyemura SA, Santos AC, Curti C. Antioxidant activity of flavonoids in isolated mitochondria. Phytother Res 2008;22(9):1213–8.
48. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 2000;373(1):102–9.
49. Yun I, Cho ES, Jang HO, Kim UK, Choi CH, Chung IK, Kim IS, Wood WG. Amphiphilic effects of local anesthetics on rotational mobility in neuronal and model membranes. Biochim Biophys Acta 2002;1564(1): 123–32.
50. Tsuchiya H, Mizogami M. Membrane interactivity of charged local anesthetic derivative and stereoselectivity in membrane interaction of local anesthetic enantiomers. Local Reg Anesth 2008;1:1–9.
51. Tang J, Song X, Zhu M, Zhang J. Study on the pharmacokinetics drug-drug interaction potential of *Glycyrrhiza uralensis*, a traditional Chinese medicine, with lidocaine in rats. Phytother Res 2009;23(5):603–7.
52. Cseh R, Benz R. Interaction of phloretin with lipid monolayers: relationship between structural changes and dipole potential change. Biophys J 1999;77(3):1477–88.

53. Auner BG, Valenta C. Influence of phloretin on the skin permeation of lidocaine from semisolid preparations. Eur J Pharm Biopharm 2004; 57(2):307–12.
54. Åberg G, Sydnes G. An inhibitory effect of polyphloretin phosphate (PPP) on local anaesthesia. Acta Anaesthesiol Scand 1975;19(5):377–83.
55. Nordenram A, Danielsson K. The effect of polyphloretin phosphate (PPP) on oral infiltration anaesthesia. An experimental investigation. Swed Dent J 1988;12(6):217–20.
56. Kohane DS, Kuang Y, Lu NT, Langer R, Strichartz GR, Berde CB. Vanilloid receptor agonists potentiate the *in vivo* local anesthetic activity of percutaneously injected site 1 sodium channel blockers. Anesthesiology 1999;90(2):524–34.
57. Shin HC, Park HJ, Raymond SA, Strichartz GR. Potentiation by capsaicin of lidocaine's tonic impulse block in isolated rat sciatic nerve. Neurosci Lett 1994;174(1):14–6.
58. Shin HC, Park HJ, Raymond SA. Potentiation by capsaicin of lidocaine's phasic impulse block in isolated rat sciatic nerve. Pharmacol Res 1994;30(1):73–9.