Biochemical Pharmacology, Vol. 58, pp. 1685–1693, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00212-9

COMMENTARY

Ginseng Pharmacology

MULTIPLE CONSTITUENTS AND MULTIPLE ACTIONS

*Anoja S. Attele, Ji An Wu and Chun-Su Yuan**

COMMITTEE ON CLINICAL PHARMACOLOGY, AND DEPARTMENT OF ANESTHESIA & CRITICAL CARE,
THE UNIVERSITY OF CHICAGO, CHICAGO, IL 60637, U.S.A.

ABSTRACT. Ginseng is a highly valued herb in the Far East and has gained popularity in the West during the last decade. There is extensive literature on the beneficial effects of ginseng and its constituents. The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins, which demonstrate the ability to target a myriad of tissues, producing an array of pharmacological responses. However, many mechanisms of ginsenoside activity still remain unknown. Since ginsenosides and other constituents of ginseng produce effects that are different from one another, and a single ginsenoside initiates multiple actions in the same tissue, the overall pharmacology of ginseng is complex. The ability of ginsenosides to independently target multireceptor systems at the plasma membrane, as well as to activate intracellular steroid receptors, may explain some pharmacological effects. This commentary aims to review selected effects of ginseng and ginsenosides and describe their possible modes of action. Structural variability of ginsenosides, structural and functional relationship to steroids, and potential targets of action are discussed. BIOCHEM PHARMACOL **58**;11:1685–1693, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. Panax ginseng; *Panax quinquefolius*; ginsenosides; steroidal saponins; pharmacological effects; herbal medicine

The ginseng root has been used for over 2000 years, in the belief that it is a panacea and promotes longevity. As described in Chinese traditional medicine textbooks, its effectiveness reaches mythical proportions [1, 2]. The efficacy of ginseng was known in the West by the 18th century, and the study of ginseng has a long history [2]. Recently there has been a renewed interest in investigating ginseng pharmacology using biochemical and molecular biological techniques. Pharmacological effects of ginseng have been demonstrated in the CNS and in cardiovascular, endocrine, and immune systems. In addition, ginseng and its constituents have been ascribed antineoplastic, antistress, and antioxidant activity. It is an herb with many active components, and there is evidence from numerous studies that ginseng does have beneficial effects [1, 3].

Seven major species of ginseng are distributed in East Asia, Central Asia, and North America [2]. Most studies of ginseng, including those cited in this commentary, have utilized constituents from three common species: *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng), and *Panax japonicus* (Japanese ginseng).

Active constituents found in most ginseng species include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids [1]. There is a wide variation (2–20%) in the ginsenoside content of different species of ginseng [2]. Moreover, pharmacological differences within a single species cultivated in two different locations have been reported. For example, the potency of extracts from *Panax quinquefolius*, cultivated in the U.S.A., for modulating neuronal activity is significantly higher than for the same species cultivated in China [4].

PHARMACOLOGICAL EFFECTS

Most pharmacological actions of ginseng are attributed to ginsenosides [2]. More than twenty ginsenosides have been isolated [3], and novel structures continue to be reported, particularly from *Panax quinquefolius and Panax japonicus* [5]. Figure 1 illustrates the structures of some ginsenosides. Since cardiovascular effects of ginseng have been well documented [3, 6], they will not be discussed here.

Effects on the CNS

Ginseng has both stimulatory and inhibitory effects on the CNS [7], and may modulate neurotransmission. Ginsenosides Rb_1 and Rg_1 play a major role in these effects [8, 9].

MEMORY, LEARNING, AND NEUROPROTECTION. Results of several animal studies show that Rb_1 [10], Rg_1 [11], and Re [12] prevent scopolamine-induced memory deficits. Central

* Corresponding author: Chun-Su Yuan, M.D., Ph.D., Department of Anesthesia & Critical Care, The University of Chicago, 5841 S. Maryland Avenue, MC 4028, Chicago, IL 60637. Tel. (773) 702-1916; FAX (773) 834-0601; E-mail: cyuan@midway.uchicago.edu

† *Abbreviations*: GABA, γ-aminobutyric acid; NK, natural killer; Cdk2, cyclin-dependent kinase-2; PKC, protein kinase C; GR, glucocorticoid receptor; and GRE, glucocorticoid response elements.

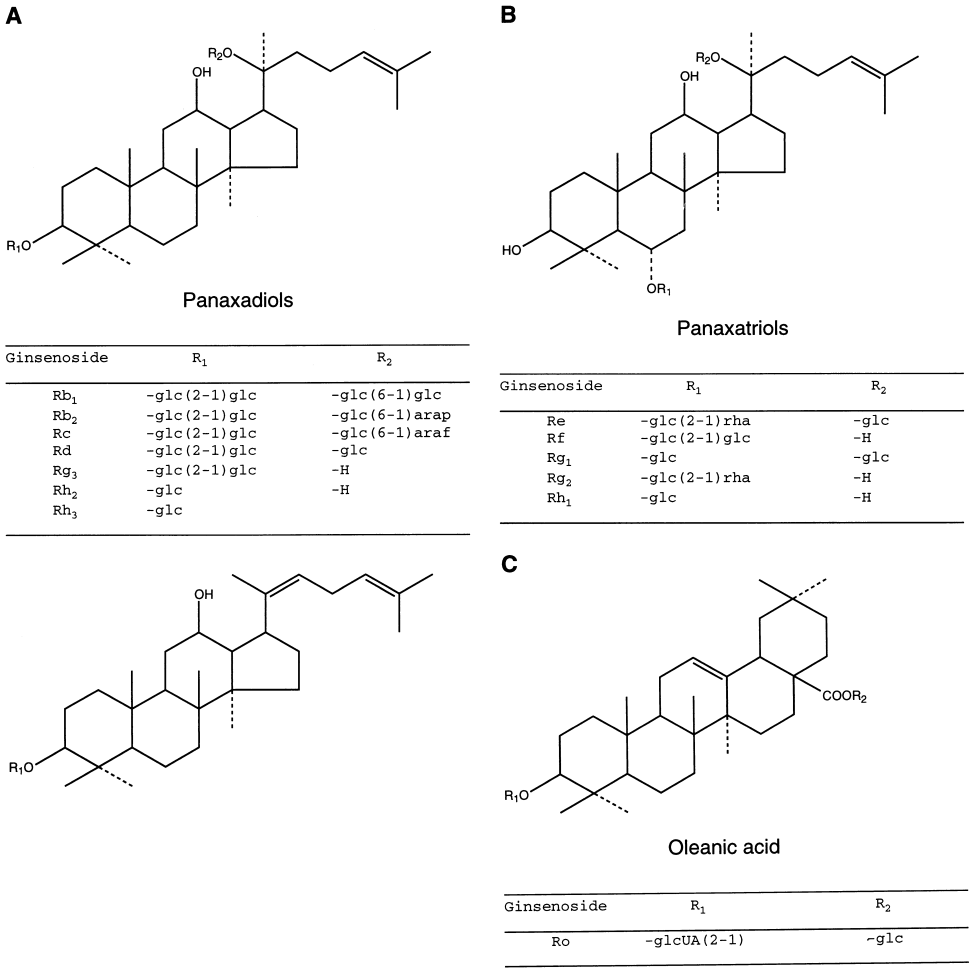

FIG. 1. Structures of ginsenosides discussed in the text. Based on chemical structure, there are two major groups: panaxadiols (A) and panaxatriols (B). Rh_3, as shown in the lower part of (A), differs from other panaxadiols at the side chain. Ginsenoside Ro, a nonsteroidal saponin, is shown in (C).

cholinergic systems have been implicated in mediating learning and memory processes [13]. Rb_1 was shown to increase the uptake of choline in central cholinergic nerve endings [9], and to facilitate the release of acetylcholine from hippocampal slices [10]. Both Rb_1 and Rg_1 appear to partially reverse scopolamine-induced amnesia by increasing cholinergic activity. Results from these investigations suggest that ginsenosides may facilitate learning and memory and are able to enhance nerve growth [14, 15].

Ginsenosides may also possess the ability to protect neurons from ischemic damage. Rb_1 was shown to rescue hippocampal neurons from lethal ischemic damage [16] and to delay neuronal death from transient forebrain ischemia [17] *in vitro*. In another study, Rg_1 was shown to increase membrane fluidity of cortical cells from 27-month-old rats [18]. Rb_1 increased the fluidity of synaptosomal membranes impaired by $FeSO_4$-cysteine [19]. Both Rb_1 and Rg_1 significantly decreased the hippocampal $[Ca^{2+}]_i$ level that was found to increase in aged rats [20].

NEUROTRANSMITTER MODULATION. Results of *in vitro* studies show that ginsenosides may modulate nerve transmission by decreasing the availability of neurotransmitters. Tsang *et al.* [8] demonstrated that ginseng extract concentration-dependently inhibits the uptake of GABA†, glutamate, dopamine, noradrenalin, and serotonin in rat brain synaptosomes. Ginsenosides compete with agonists for binding to $GABA_A$ and $GABA_B$ receptors [21]. Yuan *et al.* [22] demonstrated that *Panax quinquefolius* extracts interact with the ligand binding of $GABA_A$ receptors in brainstem neurons, which suggests that regulation of GABAergic neurotransmission may be an important action of ginseng.

OTHER CNS EFFECTS. An *in vivo* study that explored the effects of ginsenosides on drug-induced sleep showed that a mixture of Rb_1, Rb_2, and Rc prolonged hexobarbital sleeping time in mice, and decreased exploratory activity [23], suggesting a CNS-depressing effect. Other studies demonstrated that ginseng may ameliorate some adverse effects of morphine. Rats that were sensitized to morphine developed dopaminergic hyperfunction [24]. Kim *et al.* [25] showed that ginseng total saponin prevents the development of dopamine receptor supersensitivity induced by the chronic administration of morphine. Ginsenosides also may possess

antinociceptive properties. Ginseng total extract and Rf were shown to inhibit Ca^{2+} channels on primary sensory neurons to the same degree as opioids [26]. In addition, pretreatment of rats with ginsenosides inhibited substance P-induced pain behaviors [27].

Antineoplastic and Immunomodulatory Effects

Ginsenosides have been shown to exert anticarcinogenic effects *in vitro* through different mechanisms. Several ginsenosides show direct cytotoxic and growth inhibitory effects against tumor cells [28, 29]. Others have been shown to induce differentiation and inhibit metastasis [30, 31].

TUMOR CELL GROWTH INHIBITION AND APOPTOSIS. Ginsenoside Rh_2 inhibited growth and stimulated melanogenesis [28], and arrested cell cycle progression at the G_1 stage [32] in B16-BL6 melanoma cells. In association with G_1 arrest, there was a suppression of cyclin-dependent-kinase-2 activity. After oral administration, ginsenosides Rb_1, Rb_2, and Rc are metabolized by intestinal bacteria to a modified ginsenoside named M1 [33, 34]. Wakabayashi *et al.* [29] reported that M1 inhibited the proliferation of B16-BL6 mouse melanoma cells, and at a higher concentration induced cell death within 24 hr by regulating apoptosis-related proteins.

It has been reported that orally administered and subcutaneously injected Rh_2 inhibited growth of human ovarian cells transplanted into nude mice and significantly prolonged the survival times of the mice [35]. Intravenously or orally administered Rg_3 led to a decrease in lung metastasis of B16-BL6 melanoma cells [30]. Several studies that utilized medium-term and long-term anticarcinogenesis models in mice showed that ginseng extracts have a tumor inhibitory effect in mice exposed to chemical carcinogens [36, 37]. Results of a cohort study showed that ginseng consumers had a lower risk for gastric and lung cancer, suggesting that ginseng may have a non-organ-specific anticarcinogenic effect [38]. It seems that a large-scale, controlled clinical study is needed to validate this result.

ANTIMITOGENIC ACTIVITY. Sister chromatid exchange is regarded as a sensitive indicator of DNA damage [39] and significantly correlates with the mutagenic activities of many chemicals [40]. Rh_2 significantly suppressed both baseline and induced sister chromatid exchanges in human lymphocytes [41]. In addition, ginseng may enhance the proofreading activity of eukaryotic DNA polymerase. Cho *et al.* [42] showed that total ginseng extracts activated both polymerase and exonuclease activities of DNA polymerase δ.

DIFFERENTIATION AND INHIBITION OF METASTASIS. *In vitro* studies demonstrated that Rh_2 and Rh_3 induced differentiation of promyelocytic leukemia HL-60 cells into granulocytes, possibly by modulating PKC isoforms [31]. Total ginseng extract was shown to induce differentiation of cultured Morris hepatoma cells [43]. In addition, Mochizuki *et al.* [30] showed that Rg_3 significantly inhibited the adhesion and invasion of B16-BL6 cells into reconstituted basement membranes, and inhibited pulmonary metastasis.

IMMUNOMODULATORY EFFECTS. In general, immunomodulatory and anticarcinogenic activities of ginsenosides are discussed together. However, few investigations have viewed these two events as sequential steps. Yun *et al.* [44] followed the NK cell activity and the incidence of lung adenoma in mice treated with urethane or benzopyrenes. In mice administered ginseng, the NK activity was depressed for 4–24 weeks and then returned to control levels. Concurrently, in animals treated with ginseng, a lower incidence of lung adenoma was reported. Kim *et al.* [45] evaluated multiple immune system components in mice subchronically exposed to cyclophosphamide. This study also revealed that ginseng possesses some immunomodulatory properties, primarily associated with NK cell activity.

Ginsenoside Rg_1 was shown to increase both humoral and cell-mediated immune responses. Kenarova *et al.* [46] reported that spleen cells recovered from ginsenoside-treated mice injected with sheep red cells as an antigen showed significantly higher plaque-forming response and hemagglutinating antibody titers. In addition, Rg_1 increased the number of antigen-reactive T helper cells, T lymphocytes, and NK cells.

As described above, ginseng extracts and several ginsenosides have been shown to possess some anticarcinogenic and immunomodulatory effects. It will be interesting to see whether their efficacy can be observed in double-blind, randomized, placebo-controlled clinical studies.

WHY ARE THERE SO MANY DIVERSE EFFECTS?

Ginseng contains over twenty ginsenosides, and single ginsenosides have been shown to produce multiple effects in the same tissue [8, 47]. In addition, non-ginsenoside constituents of ginseng also exert pharmacological effects. Thus, it is not surprising that the overall activity of the herb is complex.

Ginsenosides and Steroids

Ginsenosides (except Ro) belong to a family of steroids named steroidal saponins [28, 31, 48]. They have been named ginsenoside saponins, triterpenoid saponins, or dammarane derivatives under previous classifications [49, 50]. Ginsenosides possess the four *trans*-ring rigid steroid skeleton, with a modified side chain at C-20 [51]. The classical steroid hormones have a truncated side chain (progesterone, cortisol, and aldosterone) or no side chain (estradiol and testosterone) [48, 52]. Many steroids have a β-OH group at C-3; ginsenosides (for example, Rb_1, Rb_2, Rc, and Rd) usually have a sugar residue attached to the same site [2, 51]. Sugar moieties are cleaved by acid hydrolysis during extraction, or by endogenous glycosidases to give the

aglycone [2, 48, 51]. Steroidal saponins, which share structural features with steroid hormones, have been used in the industrial synthesis of progesterone and pregnanolone [48].

Steroids possess numerous physiological activities, partly due to the nature of the steroid skeleton. The *trans*-ring junctions of the skeleton allow substituent groups, which interact with receptors, to be held in rigid stereochemically defined orientations [48]. In addition, the steroid skeleton endows the whole molecule with a favored structure to allow, for example, insertion into membranes [53]. Recent work showed that Rg_1 is a functional ligand of the nuclear glucocorticoid receptor [54, 55].

Structural Diversity of Ginsenosides

As illustrated in Fig. 1, ginsenosides exhibit considerable structural variation. They differ from one another by the type of sugar moieties, their number, and their site of attachment. Some sugar moieties present are glucose, maltose, fructose, and saccharose. They are attached to C-3, C-6, or C-20. The binding site of the sugar has been shown to influence biological activity. Rh_1 and Rh_2 are structurally similar, except for the binding site of the β-d-glucopyranosyl group. In Rh_1 the sugar is at C-6, and in Rh_2, at C-3. Ginsenoside Rh_2 decreased growth of B16-BL6 melanoma cells and stimulated melanogenesis and cell-to-cell adhesiveness. On the other hand, Rh_1 had no effect on cell growth and cell-to-cell adhesiveness, but stimulated melanogenesis [47]. Significantly, only Rh_2 was incorporated in the lipid fraction of the B16-BL6 melanoma cell membrane.

Ginsenosides also differ in their number and site of attachment of hydroxyl groups. Polar substituents interact with phospholipid head groups in the hydrophilic domain of the membrane. Consequently, the insertional orientation of ginsenosides into membranes would be influenced by the number and site of polar OH groups. Differences in the number of OH groups were shown to influence pharmacological activity. Ginsenoside Rh_2 and Rh_3 differ only by the presence of an OH group at C-20 in Rh_2. Although both Rh_2 and Rh_3 induced differentiation of promyelocytic leukemia HL-60 cells into morphological and functional granulocytes, the potency of Rh_2 was higher [31].

STEREOISOMERISM. Another factor that contributes to structural differences between ginsenosides is stereochemistry at C-20. Most ginsenosides that have been isolated are naturally present as enantiomeric mixtures [48, 56]. Since the modules with which they react in biological systems are also optically active, stereoisomers are considered to be functionally different chemical compounds [57]. Consequently, they often differ considerably in potency, pharmacological activity, and pharmacokinetic profile. Both 20(*S*) and 20(*R*) ginsenoside Rg_2 inhibit acetylcholine-evoked secretion of catecholamines from cultured bovine adrenal chromaffin cells [58]. However, the 20(*S*) isomer showed a greater inhibitory effect.

Ganodermic acid S compounds are steroidal saponins that share structural features with ginsenosides [59]. Twelve compounds of ganodermic acid S are either paired stereo- or positional isomers and show differential activation of human phospholipase C and A_2 by infiltrating into platelet membranes [60]. In this regard, the stereochemistry of the substituent was found to be the most important structural characteristic.

Structural alterations in the gut after oral administration also contribute to diversity. Certain ginsenosides, such as Rb_1 and Rg_1, are poorly absorbed after ingestion [61]. Rb_1 was hydrolyzed to compound K by intestinal flora [34]; compound K was shown to increase the cytotoxicity of antineoplastic drugs [62] and to induce apoptosis in B16-BL6 melanoma cells [29].

WHAT ARE THE UNDERLYING MECHANISMS OF ACTION?

Ginsenosides are amphiphilic in nature [48], and have the ability to intercalate into the plasma membrane. This leads to changes in membrane fluidity, and thus affects membrane function, eliciting a cellular response. There is evidence to suggest that ginsenosides interact directly with specific membrane proteins. Moreover, like steroid hormones, they are lipid-soluble signaling molecules, which can traverse the plasma membrane and initiate genomic effects. Figure 2 illustrates possible sites of action of ginsenosides, which are discussed below.

Ginsenosides and the Plasma Membrane

Cellular membranes may exist under conditions of curvature stress, being close to the hexagonal phase transition [63]. Consequently, the physicochemical properties of these membranes are sensitive to changes in membrane components and lipophilic agents, which may modulate curvature stress [64].

It has become increasingly evident that the lipid environment of membrane proteins, including ion channels, transporters, and receptors, plays an important role in their function [53]. In artificial and biological membranes, cholesterol, a major membrane lipid, is organized into structural and kinetic domains or pools [65]. Membrane proteins are thought to be localized selectively in cholesterol-rich domains (ACh receptor) or in cholesterol-poor domains (the sarcoplasmic Ca^{2+}-ATPase) [65]. Therefore, the biophysical properties of the different domains, rather than the bulk lipid, may selectively influence transmembrane protein function and mimic specificity at the effector level.

Ginsenosides may interact with the polar heads of membrane phospholipids and the β-OH of cholesterol through their OH groups. Moreover, their hydrophobic steroid backbone could intercalate into the hydrophobic interior of the bilayer. Both of these effects may contribute to altering the lipid environment around membrane proteins. Cholesterol is an intrinsic membrane lipid, which

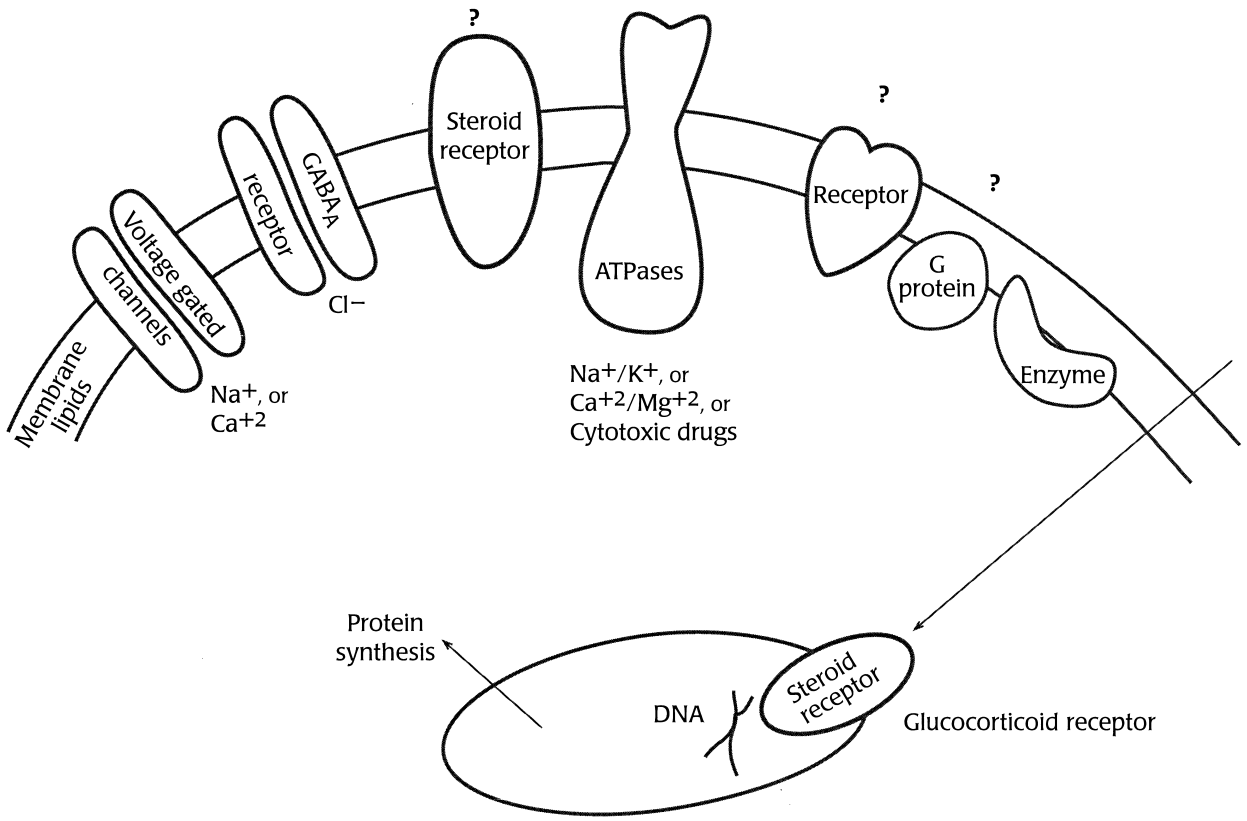

FIG. 2. Schematic drawing to illustrate potential sites of action of ginsenosides on plasma membrane and nuclear membrane. "?" indicates hypothetical sites.

shares the steroid backbone and amphipathic nature of ginsenosides. Cholesterol enrichment has an inhibitory effect on many membrane ATPases [53], and it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein [66]. In contrast, ginsenoside Rb_1 has been shown to increase Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity in neurons [19]. It is possible that some ginsenosides interact with membrane cholesterol and displace it from the immediate environment of ATPases. Since removal of cholesterol will lead to an increase in membrane fluidity [67], conformational changes that ATPases undergo during their transport cycle [68] may be facilitated.

Only recently has non-genomic action by steroids been widely recognized. Evidence for these rapid effects is now available for steroids of all classes [69]. In many of these cases, the steroid effect occurs at the membrane level and is not associated with entry into the cell. Several mechanisms for these effects have been proposed, including changes in membrane fluidity and activity of steroid hormones on plasma membrane receptors [70]. Ginsenosides also may modify membrane protein structure by changing membrane dynamics and modulating activity of ion channels, membrane-bound receptors, and enzymes. Consequently, a single ginsenoside may be capable of interacting with multi-receptor systems.

GINSENOSIDES AND MEMBRANE CHANNELS. Ginsenoside effects on membrane channels show similarities to steroid hormones including progesterone, estrogen, and vitamin D metabolites, which modulate rapid Ca^{2+} influx in several tissues [70]. Several ginsenosides inhibit Ca^{2+} influx through voltage-gated Ca^{2+} channels in adrenal chromaffin cells [31]. Of the five ginsenosides that were tested (Rb_1, Re, Rf, Rg_1, and Rc), the inhibitory potency was highest for Rc. Tachikawa *et al.* [71] showed that in bovine adrenal chromaffin cells, Rg_2 inhibited Na^+ influx through nicotinic receptor-gated cation channels, possibly by binding to the receptor-operated Na^+ channel. It is likely that the resulting decrease in catecholamine secretion may contribute to the anti-stress effects of *Panax ginseng*. Ginsenosides also can regulate Na^+ channels on nerve cells. Using standard patch clamp techniques, our group recently observed that extracts of *Panax quinquefolius* caused partial inhibition of neuronal Na^+ channels during activation and inactivation states (unpublished data).

Ginsenoside activity on membrane pumps is not limited to ion transporters. P-Glycoprotein is a membrane ATPase pump that actively exports cytotoxic compounds and contributes to anticancer multidrug resistance [72]. Several ginsenosides, including 20(S)Rh_2, inhibit the transport function of P-glycoprotein and increase sensitivity to cancer chemotherapeutics in resistant cells [62].

GINSENOSIDES AND GABA RECEPTORS. Several ginsenosides (Rb_1, Rb_2, Rc, Re, Rf, and Rg_1) [21] and total ginseng extracts [22] modulated the binding of the $GABA_A$ agonist muscimol. Ginseng extract and Rc decreased the affinity of binding of the $GABA_B$ agonist baclofen [21]. Like ginsenosides, steroidal compounds regulate GABAergic neurotransmission in the brain. Several endogenous steroids such as progesterone, androsterone, neurosteroids, and their metabolites stimulate $GABA_A$-mediated chloride ion flux [69].

GINSENOSIDES AND OTHER MEMBRANE PROTEINS. Agents that modify the physical properties of the phospholipid bilayer, such as its fluidity, can modulate the activity of membrane-bound G proteins in the absence of the receptors [73]. Some Ca^{2+} channels in sensory neurons are linked to G protein-coupled receptors [26]. Ginsenoside Rf was shown to produce antinociception by inhibiting Ca^{2+} channels on sensory neurons through a pertussis toxin-sensitive G protein [74]. However, whether Rf binds to the receptor or directly modulates G protein activity is not known.

One target molecule that may account for the anticancer effects of ginsenoside Rh_2 is Cdk2 [32], an intracellular cell cycle-regulating enzyme. It is not known whether Rh_2 directly inhibits Cdk2 activity. However, Cdk2 also can be suppressed indirectly via modulating signaling cascades originating at the cell membrane [75]. Rh_2 was shown to be incorporated in membranes to a level comparable to that of steroids [28]. That Rh_2 targets membrane components was also demonstrated by its ability to change membrane fluidity, adhesiveness, and cell surface sugar structures [28].

Some pharmacological effects of ginsenosides may be mediated by binding to steroid hormone receptors. Both neural and non-neural membrane steroid receptors have been reported, and in most cases steroids bind to the membrane receptors with specificity and modest affinity [70]. The differential effects of various ginsenosides on the lipid bilayer argue against a nonspecific activity. Rather, these effects suggest specific interaction between the ginsenoside and specific membrane proteins.

Membrane-associated enzymes that are sensitive to curvature strain such as PKC are highly responsive to perturbations of membrane structure [76]. Recent work has shown that the synergism exhibited by diacylglycerol and fatty acids in activating PKC is due to the synergistic effect of these molecules in inducing curvature strain in bilayers [64]. It was shown that ginsenosides Rh_2 and Rh_3 induced differentiation of human promyelocytic leukemia HL-60 cells into morphological and functional granulocytes by modulating PKC activity [31]. PKCs directly phosphorylate a number of intracellular proteins and regulate important cellular functions, including cell growth and cell differentiation [77]. Coincidentally, with the differentiation of HL-60 cells by ginsenoside Rh_2 there was an increase in PKC activity [31]. It is possible that ginsenoside Rh_2 and Rh_3 modulate PKC activity by altering curvature strain of the lipid bilayer. The ability of ginsenosides to independently target multiple plasma membrane-anchored proteins may account for the variety of responses that can be triggered.

Genomic Effects of Ginsenosides

As discussed earlier, ginsenosides belong to a family of steroids and share their structural characteristics [48]. Like steroids, they can traverse cell membranes freely. Moreover, their presence has been demonstrated within cells, particularly the nucleus [29]. According to the classical theory of steroid hormone action, steroids, which bind nuclear receptors, are thought to affect primarily the transcription of mRNA and subsequent protein synthesis [69]. Intracellular steroid binding proteins present possible attractive targets for ginsenosides.

A recent study showed the biological effects resulting from structural similarities between ginsenosides and steroids. Lee *et al.* [54] showed that Rg_1 is a functional ligand of the GR. In this regard, the binding of the synthetic glucocorticoid dexamethasone to the GR was competitively inhibited by Rg_1, although the affinity of Rg_1 for GR was lower than for dexamethasone. Ligand-occupied GR, when complexed with specific DNA sequences named GRE, regulated the transcription of target genes [78]. Subsequent to ligand binding, Rg_1 activated GRE containing reporter plasmids in a concentration-dependent manner. Moreover, the GR-mediated transactivation and growth inhibition of FTO2B cells by dexamethasone and Rg_1 were inhibited by the specific glucocorticoid antagonist RU486. Rg_1 exhibits many other features of a glucocorticoid, such as synergistic activation of gene transcription by cyclic AMP and the ability to down-regulate the GR content of cells [55].

After oral administration, ginsenosides Rb_1 and Rb_2 are metabolized by intestinal bacteria to compound K, also known as M1 [34], which induces apoptosis of tumor cells [29]. Compound K was shown to have a nucleosomal distribution [29]. This, together with the observation of up-regulation of the CDK inhibitor p27, and the down-regulation of c-Myc and cyclin D1, suggests that the modification of apoptosis-related proteins by compound K is induced by transcriptional regulation [29]. Another investigation showed the binding of Rb_2 to the transcription factor AP2 [79]. The subsequent genomic event was shown to be the induction of the SOD1 gene (Cu,Zn-superoxide dismutase), a key enzyme in the metabolism of oxygen free radicals.

SUMMARY AND FUTURE WORK

This commentary discusses ginsenoside effects that may be initiated at the cell membrane, as well as via intracellular protein binding. Consequently, ginsenosides may follow a dual model of action.

One pathway of ginsenoside activity involves binding to membrane receptors that trigger changes in electrolyte

transport systems, and activation of signaling pathways. In this regard, differences in lipophilicity between the ginsenosides and the cholesterol content of membrane domains may be important. Future studies should be carried out to demonstrate the partitioning of ginsenosides in membranes, and determine whether they induce changes in the structure of membrane proteins. Another possible mechanism by which ginsenosides produce pharmacological effects is by binding to plasma membrane steroid receptors. Research in this area is still in its infancy, although there is a growing interest in non-genomic signaling by steroids.

The second pathway of ginsenoside activity involves binding to intracellular steroid receptors, where the ligand/receptor complex acts as a transcription factor in the nucleus. The demonstration of ginsenoside Rg_1 as a functional ligand of the nuclear glucocorticoid receptor [54, 55] supports this view. More studies should be directed to showing that other ginsenosides may function as steroid receptor agonists, and to quantitating their binding. In this regard, computer-based imaging of functional groups on ginsenosides for the construction of pharmacophore models [80] would be useful. Future research also should focus on whether there is interaction between the two pathways of ginsenoside action, both of which may occur in the same cell. Therefore, the initial rapid response via membrane phenomena may be augmented by the delayed genomic response.

Two factors may contribute to the multiple pharmacological effects of ginseng. The first is the structural isomerism and stereoisomerism exhibited by ginsenosides, which increase their diversity. The second is the ability of ginsenosides to target membrane-anchored receptors and ion channels, as well as nuclear receptors. Certainly, the argument can be raised that evidence for most pharmacological effects of ginseng has been obtained from *in vitro* studies, many of which have not been confirmed *in vivo*. Nevertheless, the view that ginsenosides may initiate effects at the plasma membrane by interacting with multireceptor systems, and that they also freely traverse the membrane and produce genomic effects, complements the intriguing pharmacology of ginseng.

This work was supported, in part, by the Tang Family Foundation, and the Clinical Practice Enhancement & Anesthesia Research Foundation.

References

1. Lee FC, *Facts about Ginseng, the Elixir of Life*. Hollyn International Corp., Elizabeth, NJ, 1992.
2. Huang KC, *The Pharmacology of Chinese Herbs*. CRC Press, Boca Raton, FL, 1999.
3. Gillis CN, *Panax ginseng* pharmacology: A nitric oxide link? *Biochem Pharmacol* **54:** 1–8, 1997.
4. Yuan CS, Wu JA, Lowell T and Gu M, Gut and brain effects of American ginseng root on brainstem neuronal activities in rats. *Am J Chin Med* **26:** 47–55, 1998.
5. Yoshikawa M, Murakami T, Yashiro K, Yamahara J, Matsuda H, Sadoh R and Tanaka O, Bioactive saponins and glycosides. XI. Structures of new dammarane-type triterpene oligoglycosides, quinquenosides I, II, III, IV, and V, from American Ginseng, the roots of *Panax quinquefolium* L. *Chem Pharm Bull (Tokyo)* **46:** 647–654, 1998.
6. Chen X, Cardiovascular protection by ginsenosides and their nitric oxide releasing action. *Clin Exp Pharmacol Physiol* **23:** 728–732, 1996.
7. Saito H, Tsuchiya M, Naka S and Takagi K, Effect of Panax Ginseng root on conditioned avoidance response in rats. *Jpn J Pharmacol* **27**: 509–516, 1977.
8. Tsang D, Yeung HW, Tso WW and Peck H, Ginseng saponins: Influence on neurotransmitter uptake in rat brain synaptosomes. *Planta Med* **3**: 221–224, 1985.
9. Benishin CG, Actions of ginsenoside Rb_1 on choline uptake in central cholinergic nerve endings. *Neurochem Int* **21:** 1–5, 1992.
10. Benishin CG, Lee R, Wang LCH and Liu HJ, Effects of ginsenoside Rb_1 on central cholinergic metabolism. *Pharmacology* **42:** 223–229, 1991.
11. Yamaguchi Y, Haruta K and Kobayashi H, Effects of ginsenosides on impaired performance induced in the rat by scopolamine in a radial-arm maze. *Psychoneuroendocrinology* **20:** 645–653, 1995.
12. Yamaguchi Y, Higashi M and Kobayashi H, Effects of oral and intraventricular administration of ginsenoside Rg_1 on the performance impaired by scopolamine in rats. *Biomed Res* **17:** 487–490, 1996.
13. Perry EK, The cholinergic hypothesis—ten years on. *Br Med Bull* **42:** 63–69, 1986.
14. Takemoto Y, Ueyama T, Saito H, Horio S, Sanada S, Shoji J, Yahara S, Tanaka O and Shibata S, Potentiation of nerve growth factor-mediated nerve fiber production in organ cultures of chicken embryonic ganglia by ginseng saponins: Structure-activity relationship. *Chem Pharm Bull (Tokyo)* **32:** 3128–3133, 1984.
15. Salim KN, McEwen BS and Chao HM, Ginsenoside Rb1 regulates ChAT, NGF and trkA mRNA expression in the rat brain. *Brain Res Mol Brain Res* **47:** 177–182, 1997.
16. Lim JH, Wen TC, Matsuda S, Tanaka J, Maeda N, Peng H, Aburaya J, Ishihara K and Sakanaka M, Protection of ischemic hippocampal neurons by ginsenoside Rb_1, a main ingredient of ginseng root. *Neurosci Res* **28:** 191–200, 1997.
17. Wen TC, Yoshimura H, Matsuda S, Lim JH and Sakanaka M, Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia. *Acta Neuropathol* **91:** 15–22, 1996.
18. Li JQ and Zhang JT, Effects of age and ginsenoside Rg_1 on membrane fluidity of cortical cells in rats. *Acta Pharm Sin* **32:** 23–27, 1997.
19. Jiang XY, Zhang JT and Shi CZ, Mechanism of action of ginsenoside Rb_1 in decreasing intracellular Ca^{2+}. *Acta Pharm Sin* **31:** 321–326, 1996.
20. Min L and Juntian Z, Effects of ginsenoside Rb_1 and Rg_1 on synaptosomal free calcium level, ATPase, and calmodulin in rat hippocampus. *Chin Med J (Engl)* **108**: 544–547, 1995.
21. Kimura T, Saunders PA, Kim HS, Rheu HM, Oh KW and Ho IK, Interactions of ginsenosides with ligand-bindings of $GABA_A$ and $GABA_B$ receptors. *Gen Pharmacol* **25:** 193–199, 1994.
22. Yuan CS, Attele AS, Wu JA and Liu D, Modulation of American ginseng on brainstem GABAergic effects in the rat. *J Ethnopharmacol* **63:** 215–222, 1998.
23. Takagi K, Saito H and Tsuchiya M, Pharmacological studies of Panax Ginseng root: Pharmacological properties of a crude saponin fraction. *Jpn J Pharmacol* **22:** 339–346, 1972.
24. Bhargava HN, Cyclo (leucylglycine) inhibits the develop-

ment of morphine induced analgesic tolerance and dopamine receptor supersensitivity in rats. *Life Sci* **27**: 117–123, 1980.
25. Kim H-S, Kang J-G and Oh K-W, Inhibition by ginseng total saponin of the development of morphine reverse tolerance and dopamine receptor supersensitivity in mice. *Gen Pharmacol* **26:** 1071–1076, 1995.
26. Nah SY, Park NJ and McCleskey EW, A trace component of ginseng that inhibits Ca^{2+} channels through a pertussis toxin-sensitive G protein. *Proc Natl Acad Sci USA* **92:** 8739–8743, 1995.
27. Yoon S-R, Nah J-J, Shin Y-H, Kim S-K, Nam K-Y, Choi H-S and Nah S-Y, Ginsenosides induce differential antinociception and inhibit substance P induced-nociceptive response in mice. *Life Sci* **62:** PL319–PL325, 1998.
28. Ota T, Fujikawa-Yamamoto K, Zong ZP, Yamazaki M, Odashima S, Kitagawa I, Abe H and Arichi S, Plant-glycoside modulation of cell surface related to control of differentiation in cultured B16 melanoma cells. *Cancer Res* **47:** 3863–3867, 1987.
29. Wakabayashi C, Murakami K, Hasegawa H, Murata J and Saiki I, An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biochem Biophys Res Commun* **246:** 725–730, 1998.
30. Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tono-Oka S, Samukawa KI and Azuma I, Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb_2, 20(*R*)- and 20(*S*)-ginsenoside-Rg_3, of *Red ginseng*. *Biol Pharm Bull* **18:** 1197–1202, 1995.
31. Kim YS, Kim DS and Kim SI, Ginsenoside Rh_2 and Rh_3 induce differentiation of HL-60 cells into granulocytes: Modulation of protein kinase C isoforms during differentiation by ginsenoside Rh_2. *Int J Biochem Cell Biol* **30:** 327–338, 1998.
32. Ota T, Maeda M, Odashima S, Ninomiya-Tsuji J and Tatsuka M, G_1 phase-specific suppression of the Cdk2 activity by ginsenoside Rh_2 in cultured murine cells. *Life Sci* **60:** 39–44, 1997.
33. Hasegawa H, Sung J, Matsumiya S and Uchiyama M, Main ginseng saponin metabolites formed by intestinal bacteria. *Planta Med* **62:** 453–457, 1996.
34. Karikura M, Miyase T, Tanizawa H, Taniyama T and Takino Y, Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VII. Comparison of the decomposition modes of ginsenoside-Rb_1 and -Rb_2 in the digestive tract of rats. *Chem Pharm Bull (Tokyo)* **39:** 2357–2361, 1991.
35. Tode T, Kikuchi Y, Kita T, Hirata J, Imaizumi E and Nagata I, Inhibitory effects by oral administration of ginsenoside Rh_2 on the growth of human ovarian cancer cells in nude mice. *J Cancer Res Clin Oncol* **120:** 24–26, 1993.
36. Yun T-K, Kim S-H and Lee Y-S, Trial of a new medium-term model using benzo(a)pyrene induced lung tumor in newborn mice. *Anticancer Res* **15:** 839–845, 1995.
37. Yun TK, Experimental and epidemiological evidence of the cancer-preventive effects of *Panax ginseng* C.A. Meyer. *Nutr Rev* **54:** S71–S81, 1996.
38. Yun TK and Choi SY, Preventive effect of ginseng intake against various human cancers: A case study on 1987 pairs. *Cancer Epidemiol Biomarkers Prev* **4:** 401–408, 1995.
39. Perry P and Evans HJ, Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* **258:** 121–125, 1975.
40. Nishi Y, Hasegawa MM, Taketomi M, Ohkawa Y and Inui N, Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents. *Cancer Res* **44:** 3270–3279, 1984.
41. Zhu JH, Takeshita T, Kitagawa I and Morimoto K, Suppression of the formation of sister chromatid exchanges by low concentrations of ginsenoside Rh_2 in human blood lymphocytes. *Cancer Res* **55:** 1221–1223, 1995.
42. Cho SW, Cho EH and Choi SY, Ginsenosides activate DNA polymerase from bovine placenta. *Life Sci* **57:** 1359–1365, 1995.
43. Odashima S, Nakayabu Y, Honjo N, Abe H and Arichi S, Induction of phenotypic reverse transformation by ginsenosides in cultured Morris hepatoma cells. *Eur J Cancer* **15:** 885–892, 1979.
44. Yun YS, Moon HS, Oh YR, Jo SK, Kim YJ and Yun TK, Effect of red ginseng on natural killer cell activity in mice with lung adenoma induced by urethan and benzo(a)pyrene. *Cancer Detection Prev* Suppl **1:** 301–309, 1987.
45. Kim JY, Germolec DR and Luster MI, *Panax ginseng* as a poential immunomodulator: Studies in mice. *Immunopharmacol Immunotoxicol* **12**: 257–276, 1990.
46. Kenarova B, Neychev H, Hadjiivanova C and Petkov VD, Immunomodulating activity of ginsenoside Rg_1 from *Panax ginseng*. *Jpn J Pharmacol* **54:** 447–454, 1990.
47. Odashima S, Ota T, Kohno H, Matsuda T, Kitagawa I, Abe H and Arichi S, Control of phenotypic expression of cultured B16 melanoma cells by plant glycosides. *Cancer Res* **45:** 2781–2784, 1985.
48. Banthorpe DV, Terpenoids. In: *Natural Products* (Ed. Mann J), pp. 331–339. Longman Scientific and Technical, Essex, 1994.
49. Ourisson G, Crabbe P and Rodic OR, *Tetracyclic Triterpenes*. Holden-Day, Inc., San Francisco, 1964.
50. Boar RB, *Terpenoids and Steroids*. Bartholomew Press, Dorking, England, 1983.
51. Shibata S, Tanaka O, Shoji J and Saito H, Chemistry and pharmacology of Panax. In: *Economic and Medicinal Plant Research* (Eds. Wagner H, Hikino H and Farnsworth NR), Vol. 1, pp. 217–284. Academic Press, New York, 1985.
52. Heftmann E and Mosettig E, *Biochemistry of Steroids*. Reinhold Publishing Corp., London, 1960.
53. Bastiaanse EM, Höld KM and Van der Laarse A, The effect of membrane cholesterol content on ion transport processes in plasma membranes. *Cardiovasc Res* **33:** 272–283, 1997.
54. Lee YJ, Chung E, Lee KY, Lee YH, Huh B and Lee SK, Ginsenoside-Rg_1, one of the major active molecules from *Panax ginseng*, is a functional ligand of glucocorticoid receptor. *Mol Cell Endocrinol* **133:** 135–140, 1997.
55. Chung E, Lee KY, Lee YJ, Lee YH and Lee SK, Ginsenoside-Rg_1 down-regulates glucocorticoid receptor and displays synergistic effects with cAMP. *Steroids* **63:** 421–424, 1998.
56. Soldati F and Sticher O, HPLC separation and quantitative determination of ginsenosides from Panax ginseng, *Panax quinquefolium* and from ginseng drug preparations. *Planta Med* **39:** 348–357, 1980.
57. Islam MR, Mahdi JG and Bowen ID, Pharmacological importance of stereochemical resolution of enantiomeric drugs. *Drug Saf* **17:** 149–165, 1997.
58. Kudo K, Tachikawa E, Kashimoto T and Takahashi E, Properties of ginseng saponin inhibition of catecholamine secretion in bovine adrenal chromaffin cells. *Eur J Pharmacol* **341:** 139–144, 1998.
59. Shiao MS and Lin LJ, Two new triterpenes of the fungus Ganoderma lucidum. *J Nat Prod* **50:** 886–891, 1987.
60. Wang C-N, Chen J-S, Shiao M-S and Wang C-T, Activation of human platelet phospholipases C and A_2 by various oxygenated triterpenes. *Eur J Pharmacol* **267:** 33–42, 1994.
61. Odani T, Tanizawa H and Takino Y, Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. III. The absorption, distribution and excretion of ginsenoside Rb_1 in the rat. *Chem Pharm Bull (Tokyo)* **31:** 1059–1066, 1983.

62. Hasegawa H, Sung JH, Matsumiya S, Uchiyama M, Inouye Y, Kasai R and Yamasaki K, Reversal of daunomycin and vinblastine resistance in multidrug-resistant P388 leukemia *in vitro* through enhanced cytotoxicity by triterpenoids. *Planta Med* **61:** 409–413, 1995.
63. Rilfors L, Hauksson JB and Lindblom G, Regulation and phase equilibria of membrane lipids from *Bacillus megaterium* and *Acholeplasma laidlawii* strain A containing methyl-branched acyl chains. *Biochemistry* **33:** 6110–6120, 1994.
64. Goldberg EM and Zidovetzki R, Synergistic effects of diacylglycerols and fatty acids on membrane structure and protein kinase C activity. *Biochemistry* **37:** 5623–5632, 1998.
65. Schroeder F, Jefferson JR, Kier AB, Knittel J, Scallen TJ, Wood WG and Hapala I, Membrane cholesterol dynamics: Cholesterol domains and kinetic pools. *Proc Soc Exp Biol Med* **196:** 235–252, 1991.
66. Mas-Oliva J and Santiago-Garcia J, Cholesterol effect on thermostability of the (Ca^{2+}-Mg^{2+})-ATPase from cardiac muscle sarcolemma. *Biochem Int* **21:** 233–241, 1990.
67. Kirkwood A, Prichard JR, Schwartz SM, Meadow MS and Stemerman MB, Effects of low density lipoprotein on endothelial cell membrane fluidity and mononuclear cell attachment. *Am J Physiol* **260:** C43–C49, 1991.
68. Pederson PL and Carafoli E, Ion motive ATPases: Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* **12:** 146–150, 1987.
69. Wehling M, Specific, nongenomic actions of steroid hormones. *Annu Rev Physiol* **59:** 365–393, 1997.
70. Brann DW, Hendry LB and Mahesh VB, Emerging diversities in the mechanism of action of steroid hormones. *J Steroid Biochem Mol Biol* **52:** 113–133, 1995.
71. Tachikawa E, Kudo K, Kashimoto T and Takahashi E, Ginseng saponins reduce acetylcholine-evoked Na^+ influx and catecholamine secretion in bovine adrenal chromaffin cells. *J Pharmacol Exp Ther* **273:** 629–636, 1995.
72. Pastan I and Gottesman MM, Multidrug resistance. *Annu Rev Med* **42:** 277–286, 1991.
73. Gudi R, Nolan JP and Frangos JA, Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition. *Proc Natl Acad Sci USA* **95:** 2515–2519, 1998.
74. Mogil JS, Shin YH, McCleskey EW, Kim SC and Nah SY, Ginsenoside Rf, a trace component of ginseng root, produces antinociception in mice. *Brain Res* **792:** 218–228, 1998.
75. Sherr CJ, G_1 phase progression: Cycling on cue. *Cell* **79:** 551–555, 1994.
76. Zidovetzki R and Lester DS, The mechanism of activation of protein kinase C: A biophysical perspective. *Biochim Biophys Acta* **1134:** 261–272, 1992.
77. Nishizuka Y, The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* **308:** 693–698, 1984.
78. McEwan IJ, Almlöf T, Wikström A, Dahlman-Wright K, Wright AH and Gustafsson J, The glucocorticoid receptor functions at multiple steps during transcription initiation by RNA polymerase II. *J Biol Chem* **269:** 25629–25636, 1994.
79. Kim YH, Park KH and Rho HM, Transcriptional activation of the Cu,Zn-superoxide dismutase gene through the AP2 site by ginsenoside Rb_2 extracted from a medicinal plant, *Panax ginseng*. *J Biol Chem* **271:** 24539–24543, 1996.
80. Rohrer DC, 3D Molecular similarity methods: In search of a pharmacophore. In: *Structure-Based Drug Design* (Ed. Codding PW), pp. 65–76. Kluwer Academic Publishers, Norwell, MA, 1998.