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Effects of Neutral Dammarane Saponin from *Panax ginseng* on the *in vitro* Function of Polymorphonuclear Leukocytes

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Abstract

Although Saponin A from *Panax ginseng* has previously been shown to inhibit carageenin induced edema, a paucity of information exists on the effects of components from ginseng on the cellular inflammatory response, specifically polymorphonuclear leukocyte (PMNL) function. The purpose of this study was to determine the effects of isolated neutral dammarane saponins from ginseng (i.e., glycosidic derivatives of 20(S)-protopanaxadiol [ginsenoside Rb_1, Rb_2, and Rc] and 20(S)-protopanaxatriol [ginsenosides Re and Rg_1]) on *in vivo* PMNL function and to compare their effects with those produced by a steroidal anti-inflammatory agent (dexamethasone) and commercially available saponin. Dexamethasone, the ginsenosides and saponin were all shown to be potent inhibitors of PMNL chemotaxis using the ^{51}Cr assay with 5×10^{-8} M f-met-leu-phe [FMLP] as the chemoattractant. Inhibition of PMNL chemotaxis by dexamethasone, the ginsenosides and saponin were all shown to be both time-and dose-dependent and these agents did not affect cellular viability at the concentrations tested. Saponin and the ginsenosides were more potent inhibitors of chemotaxis than was dexamethasone. While oxidant generation (as measured by the luminol-enhanced chemiluminescence of PMNL using FMNL [10^{-6}] as the stimulus) was inhibited by dexamethasone, the ginsenosides (Rb_1, Rb_2, Rc, Re and Rg_1) and saponin at a concentration of 1 μM had no significant effect on PMNL chemiluminescence. Thus, the neutral dammarane saponins are potentially important modulators of PMNL function and their inhibitory effects may be differentiated from those of the steroidal anti-inflammatory agents.

Introduction

Ginseng is promoted for the prevention and treatment of a variety of unrelated ills including : aging, anemia, both hypertension and hypotension, diabetes, insomnia, muscle weakness and gastritis (1). Further, ginseng and its components have been reported to have wide range of physiological and pharmacological effects. Among the pharmacological effects of ginseng are : the control of phenotypic expression of cultured cancer cells (2), inhibition of adrenocorticotropin-induced (but not epinephrine-induced) lipolysis (3), hypoglycemic effects (4), and anti-inflammatory effects (5). The neutral dammarane saponins are in relatively high concentrations in ginseng (especially in the lateral roots and rhizomes of both wild and cultured ginseng) (6) and have displayed a wide range of pharmacological activities. The similarity in chemical structure of these triterpenoid plant glycosides to that of the glucocorticoids suggests a possible common pharmacological mechanism. For example, the inhibitory effects of ginsenoside Rh_2 on growth capacity and differentiation of cancer cells have been compared with those observed with the glucocorticoids (2).

Although these saponins have been suggested to be the major pharmacologically active substances of ginseng (6), components other than the saponins may account for the observed effects of ginseng. For example, the hypoglycemic effects of ginseng have been attributed to adenosine, a carboxylic acid, a peptide, glycans and, as yet, uncharacterized chemical constituents (4).

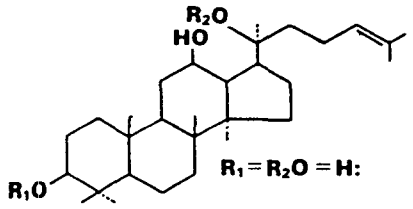
The action of ginseng saponins on cellular functions may be mediated, in part, by their interaction with membranes. For example, saponin Rc has been shown to cause agglutination of egg yolk phosphatidyl choline vesicles (7).

Ginseng may modulate the *in vivo* inflammatory response, since a previous study demonstrated Panax Saponin A (β, β'-20S-protopanaxatriol-diglucoside) to be an inhibitor of carrageenin-induced edema in the rat (5). The authors concluded that Panax Saponin A was anti-inflammatory agent but was not necessarily the only anti-inflammatory agent present in ginseng.

Polymorphonuclear leukocytes (PMNL) play a primary role in host defense against acute bacterial infections. Pathology can result from either an impairment of these leukocyte functions which frequently results in recurrent bacterial infections (8) or tissue damage as a result of uncontrolled recruitment of PMNL to the inflammatory site with subsequent release of proteases and oxidants (9).

In order for leukocytes to exert their antibacterial effects a specific series of events are necessary including : production of leukocytes in the bone marrow with their subsequent release into the vasculature, generation of chemotactic factors either by bacteria as a result of the inflammatory response, adherence of the leukocyte to the endothelial wall of the blood vessel, diapedesis, directed migration (chemotaxis) through extravascular space, and upon arrival at the site of infection-phagocytosis of the opsonized bacteria with subsequent bactericidal activity. The bactericidal activity of PMNL is in part a result of the generation of oxidants. Following opsonized particle ingestion or activation by suitable soluble stimuli, PMNL generate oxygen radicals (superoxide anion [O_2^-], hydrogen peroxide [H_2O_2], and hydroxyl radical [OH^-]). Associated with the generation of these oxidants is the emission of photons (i.e., chemiluminescence) which is enhanced by the addition of luminol (5-amino-2,3-dihydro-1, 4-phthalazine-dione).

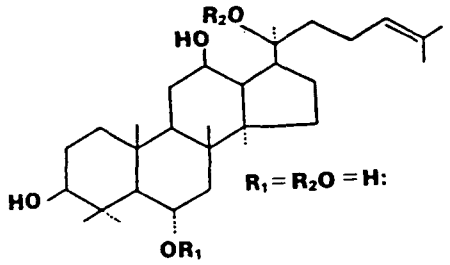
The present study sought to determine if the major neutral saponins of ginseng (i.e., the glycosidic derivatives



20(S)-protopanaxadiol

	R_1	R_2
GINSENOSIDE-Rb$_1$	-glc(2-1)glc	-glc(6-1)glc
GINSENOSIDE-Rb$_2$	-glc(2-1)glc	-glc(6-1)arap
GINSENOSIDE-Re	-glc(2-1)glc	-glc(6-1)araf

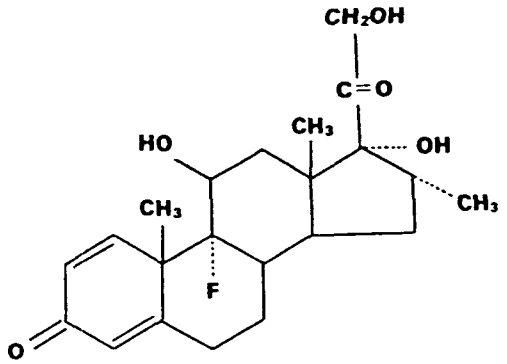
Figure 1. Structures of the ginsenosides Rb$_1$, Rb$_2$ and Rc.



20(S)-protopanaxatriol

	R_1	R_2
GINSENOSIDE-Re	-glc(2-1)glc	-glc
GINSENOSIDE-Rg$_1$	-glc	-glc

Figure 2. Structures of the ginsenosides Re and Rg$_1$.



Dexamethasone

Figure 3. Structures of dexamethasone.

of 20(S)-protopanaxadiol and 20(S)-protopanaxatriol) affected *in vitro* functions of human polymorphonuclear leukocytes (i.e., chemotaxis and chemiluminescence). The specific ginsenosides of 20(S)-protopanaxadiol tested included Rb$_1$, Rb$_2$ and Rc which differ only in the glycoside attached to the 20-hydroxyl group (Fig.1), while those of 20(S)-protopanaxatriol included Re and Rg$_1$ which differed in the glycosidic side chain in the 6-position (Fig.2). The effects of these neutral saponins from ginseng were compared with those of a steroidal anti-inflammatory agent, dexamethasone (Fig. 3), and commerically available saponin.

Methods

Isolation of neutral dammarane saponins :

Commerical saponin was obtained from Sigma Chemical Corp., St. Louis, MO. and a molecular weight of 610 was utilized to make up specific concentrations of this agent. Ginsenosides were prepared and purified from dry pulverized powder from *Panax ginseng* (C.A. Meyer) essentially by the methods of Sanada, *et al.* (10, 11). The preparation of these ginsenosides was as follows.

Extraction of Korean Ginseng :

Powdered Korean White Ginseng (10kg) was extracted three times by refluxing for 5 hours with methanol (MeOH, 24 liters). The MeOH extracts were concentrated to give 1.4 kg of solid extract.

Preparation of Total Saponin Fraction :

The concentrated MeOH extract was suspended in a small volume of water and extracted with 2.5 liters of ethyl ether three times to remove non-polar substances. The water layer was then extracted with n-butanol (2 liters) three times. The butanol layer was concentrated to give 280 gm of crude saponin fraction. The crude saponin fraction (140 gm) was dissolved in butanol (2 liters) and extracted with 2 liters of 5% NaOH (w/v) two times. The alkali-washed butanol layer contained 48 gm of ginsenoside mixture and was used for the starting material for the isolation of ginsenosides of protopanaxatriol series (*i.e.*, Rg$_1$ and Re). The alkaline layer was neutralized with d-HCL and extracted with butanol to give 75 gm of protopanaxadiol-rich ginsenoside mixture. This mixture was used as the starting material for the isolation of ginsenosides Rb$_1$, Rb$_2$ and Rc.

Isolation of Ginsenosides Rg$_1$ and Re :

The protopanaxatriol-rich ginsenoside mixture (30 gm) was chromatographed over a silica-gel column (5 cm, 500 gm) using $CHCl_3$: MeOH : H_2O (70 : 30 : 4) as the eluting solvent. The ginsenoside Rg$_1$-rich fraction eluted first from the column and was concentrated. The resultant amorphous powder was acetylated by treating with acetic anhydride ‖ pyridine mixture (1 : 1) for 24 hours at room temperature. The reaction mixture was digested with water resulting in crystalline substances which were recrystallized from MeOH to give ginsenoside-Rg$_1$ deca-acetate (mp. 252°C, yield 12.5 gm). The ginsenoside Rg$_1$ deca-acetate was saponified by refluxing for 5 hours in a 5% (w/v) NaOH ‖ 50% (v/v) MeOH solution to give pure ginsenoside-Rg$_1$. The saponified reaction mixture was concentrated to remove the MeOH and extracted with 100 ml butanol 3 times. The butanol layer was concentrated to give pure Rg$_1$ (yield 6.5 gm). The ginsenoside Re fraction was obtained by continued elution of the column with the same solvent. After removal of solvent the residue was dissolved in a

small volume of water. Ginsenoside-Re was crystallized by from the water solution by allowing it to stand overnight in the cold room (mp 196 °, yield 8.89 gm).

Isolation of Ginsenoside Rb_1, Rb_2 and Rc :

The protopanaxadiol-rich ginsenoside mixture (30 gm) was chromatographed on silica-gel column (5 cm, 500 gm) using $CHCl_3$: MeOH : H_2O (15 : 10 : 2.5) as eluent. The ginsenoside Rb_1-rich eluate was concentrated and the ginsenoside Rb_1 fraction was dissolved in a small volume of water. Ginsenoside Rb_1 was crystallized in the cold room and the crystals collected on a glass filter (3.5 gm, mp 205-7 °C). Further elution of the silica gel column gave the mixture of ginsenoside Rb_1 and Rc. This mixture was rechromatographed over a silica gel column using n-butanol : ethyl acetate : H_2O (42 : 12 : 10) as eluting solvent to obtain the pure ginsenoside-Rc fraction. Ginsenoside-Rc fraction was concentrated and crystallized by dissolving in a small volume of water (mp. 200°C, yield 0.7 gm). After the elution of ginsenoside Rb_1+Rc mixture, pure ginsenoside-Rb_2 was eluted by successive elution. Ginsenoside Rb_2 was also crystallized from water (mp 214-7°C, yield 1.2 gm). All ginsenosides were identified by comparing with authentic ginsenoside samples which were kindly supplied by Prof. S. Shibata using HPLC.

Isolation of human PMNL.

PMNL were isolated from peripheral venous blood of 21 to 35 year old, nonsmoking, male volunteers by dextran (average molecular weight 234,000) sedimentation of heparinized whole blood (12), separation of granulocytes from mononuclear cells by the Hypaque-Ficoll technique (13), and hypotonic saline lysis of residual erythrocytes (12). The PMNL suspensions were usually greater than 93% pure and greater than 95% viable as determined by trypan blue exclusion (14). Isolation procedures were done at room temperature and PMNL were exposed to agent and appropriate functional assays done immediately after cellular isolation.

Chemotaxis of ^{51}Cr-labeled PMNL.

The chemotaxis of ^{51}Cr-labeled PMNL (both untreated and treated was done essentially by the method of Gallin *et al.* (15). Briefly, the isolated PMNL were resupended in Gey balanced salt solution to give final concentration of 1.5 to 2.5 X 10^7 PMNL/ml. ^{51}Cr as sodium chromate in sterile isotonic saline was added to this celluar suspension (1μCi/(10^6 PMNL) and incubated at 37°C for 1 hour in an atmosphere of 5% CO_2 in air with agitation at 10 min. intervals. The incubation was stopped by adding cold Gey medium to give a total volume of 30 ml. The ^{51}Cr-labeled PMNL were collected by centrifugation at 5°C for 10 min at 400 Xg. The PMNL pellet was washed three times each with 30 ml of cold Gey medium and resuspended in Gey medium containing 2% (w/v) bovine serum albumin to give a final concentration of 3.0 X 10^6 PMNL/ml.

Test agents (the neutral dammarane saponins [Rb_1, Rb_2, Rc, Re, and Rg_1], dexamethansone, and commercial saponin) were dissolved and diluted in dimethylsufoxide (DMSO) so that the final concentration of DMSO added to ^{51}Cr-labeled PMNL was maintained at 1.0% (v/v) for all concentration of agent. ^{51}Cr-labeled PMNL were incubated with agents at 37°C for 3 hours (unless otherwise indicated) in an atmosphere of 5% CO_2 in air with agitation. This treatment of ^{51}Cr-labeled PMNL with DMSO or DMSO containing agent did not affect the release of ^{51}Cr from the labeled PMNL (data not shown).

Chemotaxis assays were done in quadruplicate blind well chambers with the upper and lower compartments being separated by two millipore (Sartorius Filters, Inc., Hayward, CA.) filters (top filter-5μ pore size, bottom filter-3μ pore size.) An optimal concentration of chemotactic agent [N-formyl methionyl leucyl phenylalanine (FMLP, 5 X 10^{-8}M)] was placed in the bottom chamber (0.22 ml) and 0.5 ml of ^{51}Cr-labeled PMNL (treated or untreated) were placed in the upper chamber. After incubation for 3 hours at 37°C in an atmosphere of 5% CO_2 in air, the contents of the upper compartment were aspirated and the filters removed. The filter on the attractant side was dip rinsed ten times in isotonic saline and its radioactivity determined using an automatic Beckman 4000 gamma-counter. The radioactivity of this bottom filter reflects the number of cells which have migrated through the top filter and embedded in the bottom filter in response to the chemotactic agent. Results of the chemotaxis assay were expressed as a mean percentage (± S.E.M.) of control.

Chemiluminescence of PMNL.

Luminol-enhanced PMNL chemiluminescence was done essentially by the method of Briheim *et. al.* (16) using a Packard Picolite Luminometer (Model No. A6500) at 35°C. Unlabeled-PMNL were treatment with DMSO or agent as similarly described for the chemotaxis assay. Chemiluminescence assays were performed in Hanks Balanced Salt Solution with calcium and magnesium but without phenol red. Chemiluminescence assays were started by addition of stimulus (FMLP) and the total volume of the assay mixture was 0.2 ml containing final concentrations of 1 X 10^{-6}M FMLP, 25 X 10^{-5}M luminol, and 5 X 10^6 PMNL/ml. Both the intensity and profile with time of the luminol-enhanced chemiluminescence (expressed as counts/second) were measured. The peak chemiluminescent response was observed for each of assays done in triplicate and expressed as a mean percent ± S.E.M of control (untreated)

Statistical analysis of data.

Comparison of treated with untreated PMNL responses was done by Students' T-test for unparied results.

Results

The effects of preincubation time on the inhibition of PMNL chemotaxis by the ginseng saponins and dexamethasone are shown in Figure 4. DMSO, used as a solvent for the saponins and dexamethasone, was shown not to affect chemotaxis. Inhibition of PMNL chemotaxis increased with increasing preincubation times (up to 3 hours) for dexamethasone, Rb_1, Rg_1 and saponin, all at 1 μM final concentrations. Although dexamethasone was not inhibitory to chemotaxis without preincubation, Rb_1, Rg_1 and commercially available saponin all inhibited chemotaxis in the absence of preincubation (i.e., at 0 time). Simillar effects of time of preincubation were also noted for Rb_2, Rc and Re (data no shown). Dexamethasone, saponin or the neutral dammarane saponins of ginseng did not affect cellular viability as indicated by the release of ^{51}Cr from the cells under these preincubation conditions (data not shown).

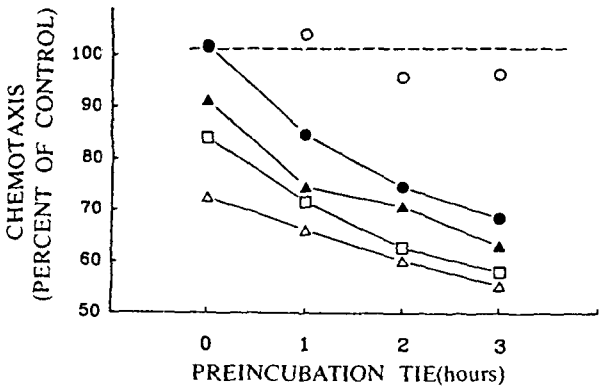


Figure 4. Effects of preincubation time on the inhibition of PMNL chemotaxis. Dashed line represents control chemotaxis (without additions) while the points represent the observed mean chemotaxis (4 replicates) with addition of : DMSO 1% v/v (○), dexamethasone, 1 μ, DXM (●), ginsenoside Rb_1, 1 μM (▲), ginsenoside Rg_1, 1 μM (□), and commercially available saponin, 1 μM (△).

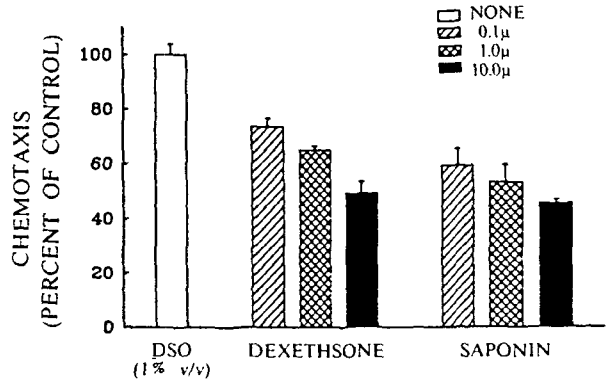


Figure 5. Dose-dependent inhibition of PMNL chemotaxis by dexamethasone and commercially available saponin. The results are expressed as mean percent of control (± S.E.M.) for five experiments Dexamethasone and commercially available saponin were significant ($p<0.01$) inhibitors at all concentrations tested as compared with the DMSO control.

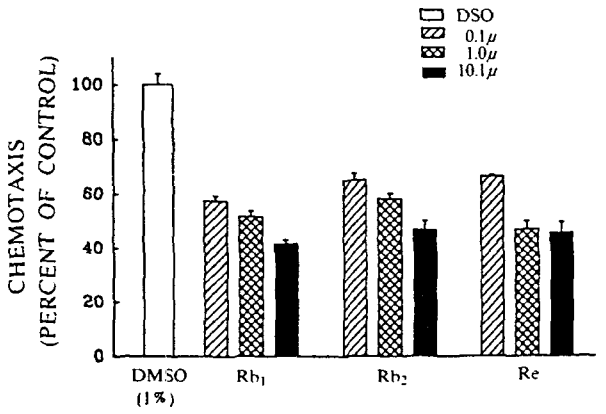


Figure 6. Dose-dependent inhibition of PMNL chemotaxis by the ginsenosides of the 20(S)-protopanaxadiol type (Rb_1, Rb_2 and Rc). The results are expressed as mean percent of control (± S.E.M.) for five experiments. All of the ginsenosides of the 20(S)-protopanaxadiol type at each of the concentrations tested were significant ($p<0.01$) inhibitors of PMNL chemotaxis.

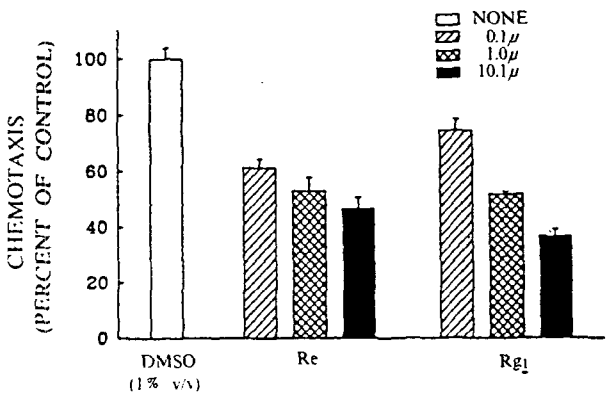


Figure 7. Dose-dependent inhibition of PMNL chemotaxis by the ginsenosides of the 20(S)-protopanaxatriol type (Re and Rg_1). The results are expressed as mean percent of control (± S.E.M.) for five experiments. All of the ginsenosides of the 20(S)-protopanaxatriol type at each of the concentrations tested were significant ($p<0.01$) inhibitors of PMNL chemotaxis.

Using three hours preincubation, dose response curves for dexamethasone, saponin and the neutral ginsenosides were generated using concentrations of 0.1, 1.0 and 10 μM (Figures 5-7). A dose-dependent inhibition of PMNL chemotaxis was observed with each of theses agents. Saponin was slightly more inhibitory than was dexamethasone (Figure 5). The glycosides of 20 (S)-protopanaxadiol (Rb_1, Rb_2 and Rc) were all inhibitory with possibly Rb- being the more potent inhibitor (Figure 6). The glycosides of 20(S)-protopanaxatriol (Re and Rg_1) were also inhibitors of chemotaxis, with Rg_1 being slightly more inhibitory than Re (Figure 7).

From these dose-response relationships, the concentration necessary to inhibit chemotaxis by 50% was estimated and compared with that obtained with dexamethasone (Table 1). The approximate effective dose of dexamethasone to achieve 50% inhibition of chemotaxis (11.5 μM) was arbitrarily assigned a relative inhibitory potency of 1. Saponin was approximately six times more inhibitory than dexamethansone, while Rb_1 and Rg_1 were approximately 8 to 9 times more inhibitory than dexamethasone. On the other hand, Rb_2, Rc and Re were only 2 to 4 times more inhibitory than dexamethasone.

The effects of these agents on FMNL-induced oxidative burst were then determined by measuring luminol-enhanced chemiluminescence. Typical time courses for the chemiluminescence of activated PMNL for control (untreated) cells and those treated with dexamethasone are shown in Figure 8A, while those for ginsenosides Rb_1 and Re are given in Figure 8B. Dexamethasone was a significant inhibitor of FMLP-induced PMNL chemiluminescence as compared to control. However, neither ginsenosides Rb_1 or Re significantly affected this chemiluminescence with similar results also being observed for DMSO, Rb_2, Rc, and Rg_1 treated PMNL (data not shown).

Similar results were obtained by comparing peak chemiluminescent responses with the summation of total counts over the entire 160 second observation period (Table 2). Mean (± S.E.M.) peak chemiluminescence for four experiments expressed as percent of control are

Table 1. Estimated Inhibitory Potencies of Dexamethasone Saponin, and the Neutral Saponins from Ginseng on Leukocyte Chemotaxis.

Addition	Estimated ED_{50} (μM)[a]	Relative Inhibitory Potency[b]
Dexamethasone	11.5	1.0
Saponin	2.0	5.8
Rb_1	1.3	8.8
Rb_2	5.0	2.3
Rc	0.7	1.6
Re	2.7	4.3
Rg_1	1.5	7.7

[a] Effective dose to achieve 50% inhibition of chemotaxis (ED_{50}) was estimated by semi-logarithmic plots of percent inhibition vs. dose.
[b] Relative inhibitory potencies are expressed as a ratio of the concentration of dexamethasone needed to achieve 50% inhibition of chemotaxis divided by that of the other agents.

Table 2. Comparison of Total Counts with Peak Counts in FMLP-induced, Luminol-enhanced Chemiluminescence[a]

Addition	Total Counts[c] (x 10^6)	Percent of Control	Peak Counts (x 10^6second)	Percent of Control
None	481.4	—	3.852	—
DMSO[b]	474.1	98.5	3.834	99.5
Dexamethasone	242.6	50.4	1.902	49.4
Rb_1	509.8	105.9	3.888	100.9
Rb_2	485.5	100.9	3.717	96.5
Rc	473.0	98.3	3.873	100.6
Re	489.2	101.6	3.790	98.4
Rg_1	487.8	101.3	3.668	95.2

[a] Results are expressed as mean counts for duplicate assays.
[b] Dimethyl sulfoxide (DMSO) was added at a concentration of 1% (v/v) while dexamethasone and the neutral dammarane saponins were added to give a final concentration of 1 μM in DMSO (1% v/v).
[c] Total counts represent the summation of counts over to 160-second assay after the addition of 1 μM FMLP.

shown in Figure 9. While 1 μM dexamethasone significantly ($p<0.01$) inhibited the peak chemiluminescent response, no significant inhibitory effect of 1% (v/v) DMSO, 1 μM ginsenosides or 1 μM saponin was observed.

Discussion

Panax Saponin A was previously shown to inhibit carrageenin-induced edema in the rat (5) suggesting that the neutral dammarane saponins of *Panax ginseng* might modulate inflammatory reactions. The purpose of the present study was to determine if these saponins might specifically modulate *in vitro* PMNL function (*ie.*, chemotaxis and the generation of oxygen radicals) and to determine if their effects on PMNL function mimicked those of a steroidal anti-inflammatory agent (dexamethasone).

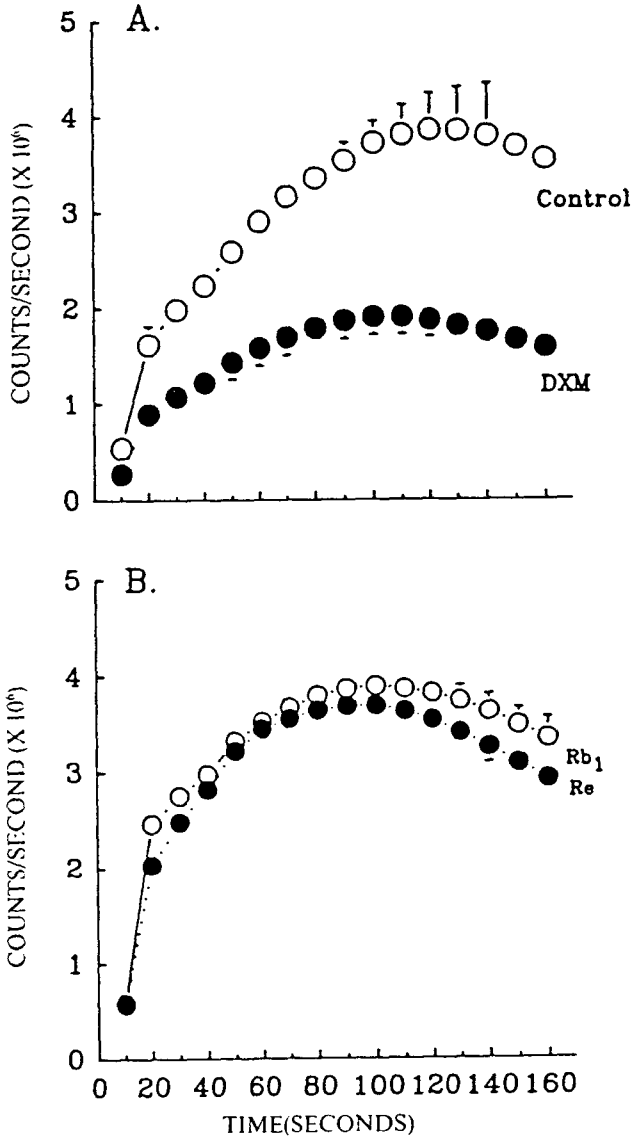


Figure 8. Time course for FMLP-induced chemiluminescence of PMNL. Results are expressed as mean (± S.E.M.) responses obtained for triplicate assays in a single experiment. Graph A denotes the time course for control (untreated) and dexamethasone (DXM, 1 μM) while graph B denotes the time course for ginseng saponin treated PMNL (1 μM). FMLP was addeed at time zero and the arrows indicate the peak response.

Dexamethasone has previously been shown to inhibit zymosan-induced production of chemotactic factor by rat PMNL (17) and in inflammatory sites (18,19), both apparently being mediated by leukotriene B_4. However, dexamethasone has also previously been shown to be directly inhibitory to *in vitro* chemotaxis of rat PMNL (20), chemotactic factor induced PMNL aggregation (21), bactericidal activity (22), and superoxide production and degranulation (23). Dexamethasone effects on neutrophil function may be mediated by its effects on sphingomyelin metabolism (24), membrane calcium release (23), or the production of a phospholipase A_2 inhibitory protein (25).

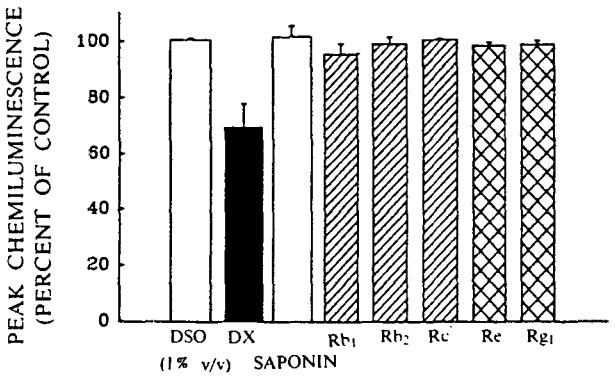


Figure 9. Effects of dexamethasone, saponin and the neutral demmarane saponins of ginseng on peak, FMLP-induced chemiluminescence of PMNL. Results are expressed as experiments. Dexamethasone (DXM, 1μM) significantly ($P<0.01$) inhibited the FMLP-induced chemiluminescence by PMNL, while dimethyl sulfoxide (DMSO), saponin (1 μM), and the neutral dammarane saponins (1 μM) han no significant ($p>0.05$) effect on this FMLP-induced chemiluminescence.

In the study, we have shown that like dexamethasone, the neutral dammarane saponins inhibit *in vitro* PMNL chemotaxis in a dose-dependent manner and that this inhibition of chemotaxis by preincubation for periods up to three hours. The ginseng saponins were more potent inhibitors of chemotaxis than was dexamethansone, with Rb_1 and Rg_1 being the more potent inhibitors. The structural basis for the relative inhibitory potency of each of the ginseng saponins is not apparent. While it seems likely that the dammarane skeleton (as an aglycone) is the inhibitory species, other studies have demonstrated that the type of glycosidically attached carbohydrate determines its interaction with phospholipid vesicles (7) and accounts for differences in its effects on the differentiation and growth of tumor cells (2). Differences inhibitory potencies between saponins within each group according to the aglycone skeleton [i.e., the saponins of the 20(S)-protopanaxadiol type (i.e., Rb_1, Rb_2 and Rc) and those of the 20(S)-protopanaxatriol type (*i.e.*, Re and Rg_1)] are probably not relevant to the effects of these saponins administered orally, since the glucosidic side chanins of these ginseng saponins are readily removed in the digestive tract.

Of fundamental significance in this study is the observation the neutral dammarane saponins, although more potent inhibitors of chemotaxis than was dexamethasone, did not inhibit PMNL chemiluminescence as did dexamethasone (all agents tested at a concentration of 1 μM). These data suggest that the mechanisms of inhibition of PMNL function by dexamethasone differ from those of the neutral dammarane saponins. The FMLP-induced chemiluminescence observed in this study is likely due to the extracellular reactions rather than intracellular reactions (16). The inhibitory effect of dexamethasone may be consistent with its inhibitory effect on degranulation (23). The lack of an inhibitory effect on these extracellular reactions by the ginseng saponins may indicate that these compounds do not similiarly affect degranulation.

Studies of the effects of the neutral dammarane saponins of ginseng on PMNL function have thus demonstrated that they are potent modulators of *in vitro* chemotaxis (but not FMLP-induced chemiluminescence) and at concentrations likely to be achieved in peripheral blood. Thus, the neutral dammarane saponins may be important mediators of inflammation. Their effects on chemotaxis may be beneficial in limiting the recruitment of PMNL to the inflammatory site and thus, limit the delivery of proteinases and oxidants which, if uncontrolled, produce pathology. However, the fact that only partial inhibitory was achieved even at relatively high concentrations of these saponins (*i.e.*, 10^{-5}M) suggests that their effects on chemotaxis would likely not prevent their response to an acute bacterial infection.

Although the neutral dammarane saponins probably constitute the major pharmacologically active substances of *Panax ginseng*, other ginseng constituents may also modulate *in vitro* PMNL function. In addition, yet to be determined is the effects of ginseng constitutents on *in vivo* PMNL function as well as cellular and humoral immunity. Our data provide preliminary evidence that ginseng or its constituents may modulate the inflammatory response and provide the empetus for these further studies.

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S.K.F. Chong : Your *in vivo* results are interesting, but I note that you have taken the opposite view of wanting to look at crude extract on neutrophil functions, but it is important to study the isolated purified fraction first.

Raymond B. Bridges : The effects of isolated compounds or fractions is obviously of primary interest. However, I am still concerned that the compounds of ginseng may have a synergistic or antagonistic effect so that the net effect of ginseng may be quite different from that observed with individual components.

S.K.F. Chong : Did you look at tumor necrosis factor (TNF) which would be generated by activated macrophages and may be responsible for the cytotoxic effects that you describe ?

S.C. Shim : The biological test has been conducted by prof. Woo Ik Whang of Medical College, Korea University. I will ask him to check it. Thank you for your suggestion.

Ara Der Marderosian : Are the polyacetylenes you isolated from ginseng toxic to normal cells ?

S.C. Shim : No, not at these low dose schedule we used.

인삼의 중성 Dammarane계 사포닌의 다형핵 백혈구 기능에 미치는 영향

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인삼에서 분리한 항염성분이 일찌기 파낙스 사포닌 (β,β'-20S-protopanaxatriol-diglucoside) 임이 Carrageenin edema test에 의하여 확인된 바 있다. 그러나 세포감염반응, 특히 다형핵 백혈구 (PNML) 기능에 관한 인삼성분의 효과에 대해서는 별로 알려진 바가 없다. 이 연구의 목적은 *in vivo* 시험을 통하여 PMNL 기능에 대한 인삼에서 분리한 중성 dammarane계 사포닌류 (Re와 Rg_1)의 효과를 알아보고자 하는데 있다. 사람의 말초혈에서 분리하였고 2.0% BSA 함유 RPMI 배지에 심어진 PMNL은 dimethylsulfoxide (DMSO)에 용해한 1 μM의 ginsenoside와 dexamethasone (DXM)으로 처리되었다. 37°C에서 3시간 배양후 PMNL을 세척하고 적당한 배지에 다시 심고 효능시험을 실시하였다. 화학주성(chemotaxis)은 주화성유인인자(chemoattractant) fMet-Leu-Phe(FMLP)를 5 X 10^{-8}M 사용하여 ^{51}Cr 분석으로 시행하였다. 처리된 PMNL의 luminole-nhanced chemiluminescence(luminol로 강화된 화학발광)도 역시 자극제로서 FMLP (10-6M)를 사용하였다. DMSO는 PMNL chemotaxis에 아무런 효과가 없었으나, Rb_2, Rb_3, Re와 Rg_1 (Rc는 아님)는 chemotaxis에 억제효과 (27.6-42.1%)를 나타내었느데 이는 스테로이드계 항염증제인 DXM으로 관찰한 것과 같거나 또는 더 강력하였다(29.6%). DXM이 PMNL 화학발광을 억제하는 반면에 중성 사포닌에서는 효과가 관찰되지않았다. 인삼뿌리에서 추출한 몇 종류의 중성 dammarane계 사포닌이 PMNL 기능을 조절하는 효과가 있었으며 그 효과는 항염증제인 dexamethasone과는 작용면에서는 다른 것으로 사료된다.