Panax Ginseng and Eleuthrococcus Senticosus Extracts— *in vitro* Studies on Binding to Steroid Receptors

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Abstract

We have examined the binding of 44% saponin from *Panax ginseng*, and extracts from *Eleuthrococcus senticosus* (Siberian ginseng) to classical steroid receptors *in vitro*. Both extracts had demonstrable affinity for progestin, mineralocorticoid and glucocorticoid receptors; the Siberian ginseng also bound to estrogen receptors. Highest affinity binding was to glucocorticoid receptors, with an approximate K_i of $8 \times 10^{-6} M$ for *Panax ginseng*. Such interactions may explain the reported glucocorticoid-like effects of ginseng *in vivo*.

Increasing interest in alternative therapy has led to an upsurge in the use of oriental medicinals in the West. *Panax ginseng* has been used for millenia in oriental society, and was recorded in 1596 in *Pent's'ao kang-mu* (Pharmaceutical Compendium) by Li Shih-Chen (Hu, 1977). Time-honoured and non-regulated use has led to a blurring of the distinction between possible pharmacological and placebo effects; recently, however, more scientific approaches have been adopted to determine the actions and potential benefits of ginseng. Most of this work has centered on *Panax ginseng* (PGS); Siberian ginseng (SGS) has also been the subject of an increasing number of reports, mostly from the USSR (Brekhman & Dardymov, 1969).

Consideration of the overall effects of PGS administration has prompted the suggestion that it may act via the hypothalamo-pituitary-adrenal axis (Fulder, 1977; Hiai *et al.*, 1979a). In addition, a study of re-

ported side effects in a number of patients led to the description of a syndrome not unlike that of glucocorticoid excess, termed the Ginseng Abuse Syndrome (Siegel, 1979). Experimental studies on rats have shown the ability of injected ginseng saponin to induce surprisingly rapid changes in ACTH and corticosterone (Hiai *et al.*, 1979a and b). Similarly, high oral doses of PGS saponin antagonised the effects of cortisone acetate in unilaterally adrenalectomized rats (Tanizawa *et al.*, 1981). Additional possible ginseng effects on steroid-modulated systems have been claimed by Punnonen & Lukola (1980), who reported that Rumanian (*Panax*) ginseng showed estrogenic effects on human uterus, and appeared to bind to estrogen and progestin receptors. In the present study we report the affinity of both *Panax ginseng* and Siberian ginseng (*Eleuthrococcus senticosus*) for steroid receptors for the five classically recognized types of adrenal and gonadal steroid—estrogen, progesterone, androgen, mineralocorticoid and glucocorticoid.

Materials and Methods

Estrogen receptor (ER) studies were performed on uterine cytosol from female Sprague-Dawley rats (body wt ~120 g); uteri from estrogen primed rats (0.4 μ g estradiol benzoate in oil at -48, -24 hrs) were used as a tissue source for progesterone receptors (PR); kidneys from adrenalectomized rats for mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) studies; and kidneys from 1-day castrate male Swiss mice for androgen receptor (AR) studies. Animals were supplied by Monash Central Animal House, Monash University Clayton, Victoria; all animals were maintained on Purina Lab chow, with 0.9% NaCl in place of tap water to drink post-adrenalectomy. Animals were killed by decapitation, and the tissues placed into ice cold buffer with all subsequent steps carried out at 0-4°C. For AR, ER and PR studies, tissues were homogenized in PTEDMG buffer (5 mM Na₂HPO₄, 10 mM Trizma base, 1.5 mM EDTA, 2 mM dithiothreitol (added fresh daily), 10 mM Na₂MoO₄, 10% glycerol v/v; pH 7.4 at 4°C). For MR and GR studies, tissues were homogenized in TSM buffer (100 mM Trizma base, 250 mM sucrose, 100 mM Na₂MoO₄; pH 7.4 at 4°C). Tissue was homogenized on ice with a Polytron PT10 (Kinematica GMBH Luzern, Switzerland), speed setting 3, for 2-3 three-second bursts. The homogenates were then centrifuged at 49,000 g in a Sorvall RC5 centrifuge for 1 hour, and the high speed supernatant (HSS) containing cytoplasmic steroid receptors used for competition studies. All tritiated steroids were from NEN, Boston, Mass; ligands used were estradiol (³HE₂, 115 Ci/m mole) for ER, promegestone (³HR 5020, 87 Ci/m mole) for PR, dihydrotestosterone (³HDHT, 45 Ci/m³ mole) for AR, aldosterone (³HA, 72 Ci/m mole) for MR, and dexamethasone (³HDM, 50 Ci/m mole) for GR. Non-radioactive E₂, DHT, A and DM were from Sigma, St. Louis, Mo; R 5020 was from NEN.

Competition experiments were carried out under conditions of time and temperature shown in preliminary studies adequate for equilibrium to be obtained. Incubations for ER (³HE₂ 2 nM), PR (³H-R 5020 5 nM) and AR (³HDHT 2 nM) were for 20 hrs at 4°C; for MR (³HA 2 nM) and GR (³HDM 10 nM) incubations were at 22°C for 40', followed by rapid cooling to 4°C (Grekin & Sider, 1980). Separation of bound and free steroid was by adsorption of receptor/ligand complex onto hydroxylapatite (HAP, Biorad Lab. Richmond CA). The pellet was washed twice, and bound counts eluted with ethanol as described previously (Pearce *et al.*, 1981). The ethanol was added to 10 ml of scintillation cocktail (PPO 10 g, POPOP 0.6 g, toluene 21), and counted in a Packard 460 c beta counter.

Non-specific binding of tracer, in the presence of

100-fold non-radioactive steroid, was subtracted from total binding to determine specific binding. This figure was taken as 100%, and specific binding in the presence of ginseng extracts expressed as a percentage thereof.

The Panax ginseng extracts were from the Office of Monopoly, Seoul, Korea, and were the gift of Dr. Shin Ho Kang of Dong A Pharmaceuticals, Korea, or purchased locally from David Wang Pty. Ltd., Melbourne. Such extracts were in the form of a thick paste containing 4% ginsenosides and used as such, or concentrated to 44% ginsenoside by extraction with water-saturated n-butanol (Sanada *et al.*, 1974). The extracts were pooled and a proportion of the thick black liquid treated by extraction with butanol in order to produce a ginsenoside concentrate (Sanada *et al.*, 1974). This was carried out by diluting with water (1:3 v/v) and applying 20 ml portions to each of a number of 25×75 mm prepacked columns of large-pore Kieselguhr ("Extrelut" Cat No. 11737, E. Merck, Darmstadt, West Germany). Each column was washed with 40 ml diethyl ether followed by 80 ml water-saturated n-butanol. The combined butanol extracts were evaporated to dryness *in vacuo* to give the ginsenoside concentrate, and assayed by the method of Honerlagen and Tretter (1979) using as a reference standard ginsenoside Rg₁ isolated by preparative thin-layer chromatography (Shibata *et al.*, 1966). The original extract and the concentrate were found to contain 3.96% and 44.0% (w/w) of ginsenoside Rg₁ equivalents, respectively.

As both 4% and 44% extract behaved similarly (see Figure 1) differing only in potency by a factor equivalent to the difference in ginsenoside concentration, detailed data for the purer material are shown. Since the Korean ginseng preparation examined was a mixture of ginsenosides, we estimated potency by taking the weight of total ginsenoside and dividing it by an approximate molecular weight of 1000 to give approximate molar values. The values thus obtained are therefore not absolute for a particular ginsenoside, but a relative figure for the mixture.

Siberian ginseng (*Eleutherococcus senticosus*) was obtained in liquid form (Medexport, USSR), dried (solids 7.7% w/v) and reconstituted to 10% of the original volume in PTEDMG or TSM buffer. Unlike the ginsenosides in preparations of panax ginseng, the eleutheroside concentration was unable to be determined, due to lack of a suitable standard. Results for Siberian ginseng accordingly were expressed as μ l of original liquid required to reduce specific binding of tracer to 50%, and were compared with concentrations of non-radioactive steroid required to achieve the same effect.

Results

As shown in Fig. 1 (a & b), *Panax ginseng* did not compete for tracer binding to either AR or ER, at concentrations up to 10^6 fold excess (2 mM). When two additional sources of ER were examined (ER +ve human breast tumor, freeze dried sheep uterus) *Panax ginseng* similarly showed no ability to compete for ER. This is in contrast with the findings using Rumanian *Panax ginseng* (Punnonen & Lukola, 1980). These authors report an increase in $^3\text{HE}_2$ binding with low ginseng concentrations; in our hands, this phenomenon appears to be a reflection of inadequate separation of bound and free tracer by charcoal, in the presence of ginseng extract.

In E_2 primed rat uterus, ^3H -R 5020 binds to progesterin receptors; in contrast with ER and AR, *Panax ginseng* competed for tracer binding to PgR (Fig. 1c). The relative potency, determined from the 50% competition concentrations, was $\sim 1:160,000$ that of R 5020 itself. The competition curve for *Panax ginseng* was parallel to that of R 5020, consistent with competitive inhibition of progesterin binding to PR.

Similarly, using the cell-free MR assay, we were able to demonstrate that *Panax ginseng* extract contained modest levels of mineralocorticoid receptor binding activity (Fig. 1d). On a molar basis, the relative potency compared with aldosterone was $\sim 1:10,000$.

Fig. 1. Competition by *Panax ginseng* (▲ original extract, 4% ginsenoside; △ concentrated extract, 44% ginsenoside) for classical steroid receptors. A: rat uterine estrogen receptors, $^3\text{HE}_2$ 2 nM, $n=4$, s.e.m. $\leq 4\%$; B: mouse kidney androgen receptors, $^3\text{HDHT}$ 2 nM, $n=6$, s.e.m. $\leq 6\%$; C: rat uterine progesterone receptors, $^3\text{HR5020}$ 5 nM, $n=4$, s.e.m. $\leq 5\%$; D: rat kidney mineralocorticoid receptors, ^3H Aldo 2 nM, $n=6$, s.e.m. $\leq 5\%$; E: rat kidney glucocorticoid receptors, ^3HDM 10 nM, $n=6$, s.e.m. $\leq 5\%$

Finally, *Panax ginseng* extract appeared to be most potent in displacing ^3HDM from GR (Fig. 1e), with a relative potency of 1:2000 compared with the highly active synthetic glucocorticoid, dexamethasone. Given this relatively high affinity, we have examined the displacement phenomenon in

Fig. 2. Lineweaver-Burke plot of ^3HDM binding alone ($\blacktriangle$), and in the presence of ginseng $2 \times 10^{-4}\text{M}$ ($\square$) or $4 \times 10^{-4}\text{M}$ ($\bullet$).

Fig. 3. Competition by Siberian ginseng (reconstituted extract of *Eleutherooccus senticosus*; $\square$) for classical steroid receptors. A: rat uterine estrogen receptors, $^3\text{HE}_2$ 2 nM, $n=3$, s.e.m. $\leq 6\%$; B: mouse kidney androgen receptors, $^3\text{HDHT}$ 2 nM, $n=4$, s.e.m. $\leq 7\%$; C: rat uterine progesterone receptors, $^3\text{HR5020}$ 5 nM, $n=4$, s.e.m. $\leq 5\%$; D: rat kidney mineralocorticoid receptors, $^3\text{H-Aldo}$ 2 nM, $n=4$, s.e.m. $\leq 9\%$; E: rat kidney glucocorticoid receptors, ^3HDM 10 nM, $n=4$, s.e.m. $\leq 7\%$. Added Siberian ginseng (2–200 μl) refers to volumes of original material.

a different manner. The results of studies with a range of ^3HDM concentrations, alone and in the presence of fixed concentrations of *Panax ginseng* extract, are shown in Figure 2 as a Lineweaver-Burke plot; the common intercept on the ordinate we interpret as evidence for competitive binding. The K_i thus determined was $8 \times 10^{-6}\text{M}$, and the approximate K_d for DM was 3 nM, a 2500 fold difference and of the same order as obtained in the competition experiments shown in Fig. 1e. Similar studies were not performed for the other steroid receptors, so that competitive inhibition of tracer binding by ginseng extracts remains probable, on the basis of parallel displacement curves, but not proven.

Eleuthrococcus senticosus: (SGS)

Like *Panax ginseng*, the extract of Siberian ginseng showed no affinity for AR; in contrast with the *Panax ginseng* extract, however, this extract showed modest affinity for ER (Figs. 3a, b). In terms of the original preparation, the content of ER-active material was equivalent to 2.1 ng E_2/ml , calculated from the doses necessary to produce a 50% inhibition of tracer binding. Siberian ginseng extract also competed for PR (Fig. 3c), with a content of PR-active material equivalent to 1.5 ng non-radioactive R 5020/ml original solution; activity in the mineralocorticoid receptor assay was more pronounced in Siberian ginseng (Fig. 3d), with levels of MR-active material equivalent to 77 ng aldosterone/ml; finally, activity in the glucocorticoid receptor assay was also high (Fig. 3e), with levels equivalent to 163 ng dexamethasone/ml of original material. Thus in Siberian ginseng extract, activity in terms of mineralocorticoid and glucocorticoid receptor binding seems most significant, as it does in *Panax ginseng*.

Discussion

The data presented here show that extracts of both *Panax ginseng* and *Eleuthrococcus senticosus* (Siberian ginseng) contain a substance or substances capable of interaction with certain classical cytoplasmic steroid receptors. That these substances are most likely the ginsenosides (and eleuthrosides) is likely given (a) the basic terpenoid structure of these compounds, and (b) the fact that extraction of material containing 4% ginsenoside, to give a 44% extract on the basis of ginsenoside content, showed a parallel increase in potency.

Though the ability of ginseng to prevent binding of tracer steroids to receptors is modest, the levels required are readily attainable in a person regularly taking ginseng, presuming near complete absorption after oral administration. Since binding to the appropriate receptor is an accepted preliminary step in steroid action, it appears reasonable to suppose that at least some of the *in vivo* effects ascribed to ginseng are a consequence of its ability to occupy steroid receptors.

There are, however, a number of relevant questions yet to be answered. For a steroid antagonist action, simple occupancy of cytoplasmic receptors by ginseng is an adequate mechanistic explanation; if, however, ginseng exerts glucocorticoid or mineralocorticoid agonist effects, the ginseng-receptor complex presumably binds to chromatin and affects DNA-directed, RNA-mediated protein synthesis.

A second question related to the possible *in vivo* effects of ginseng is the fate of ingested extract. Normally ginseng is taken orally; the *in vivo* experimental studies by Hiai *et al.* (1979a and b) involved intraperitoneal injection, a procedure in itself able to produce changes in plasma ACTH and corticosterone levels. After oral administration, various factors (digestion, ab-

sorption, hepatic or microbial metabolism) may decrease or increase the availability of the material in vivo, so that dose-equivalence in vitro may provide only an imprecise index of in vivo activity.

Third, in terms of the in vitro data on ginseng binding to GR detailed in this report, the relative potency of dexamethasone compared with corticosterone should be borne in mind. Injected into adrenalectomized rats, for example, DM is 1000 times as potent on a weight basis as corticosterone, measured by thymolysis or chronic ACTH suppression. Part of this enormous difference is due to the 3–10 fold lower affinity of the natural glucocorticoids for GR, compared with DM; the bulk of the difference, however, reflects substantial differences in plasma binding and metabolism of the steroids. Thus much more detailed information on plasma binding and metabolic fate of administered ginseng is required before any confident predications can be made of its potency in vivo relative to cortisol or corticosterone.

In addition to a potential in vivo interaction with GR, ginseng extracts show modest affinity for mineralocorticoid receptors. A mineralocorticoid agonist action would be consistent with the reports of hypertension and hypokalemia as stigmata of the ginseng abuse syndrome. One possible explanation for the mineralocorticoid-like action of ginseng preparations is the presence of added glycyrrhizins (licorice extracts). These compounds have been shown both to have hypertensive effects (Conn *et al.*, 1968) and to bind to mineralocorticoid receptors (Ulman *et al.*, 1975). Since the butanol extracts used in the present studies were free of glycyrrhizins, it appears that ginsenoside(s) may have mineralocorticoid-like activity, consistent with the traditional role ascribed to ginseng in modulating blood pressure levels.

Finally, a previous report (Punnonen & Lukola, 1980) implicated Rumanian (Panax)

ginseng as an estrogenic agent on human uterus, and claimed to show binding of extract to uterine ER and PgR. We have confirmed that Siberian ginseng extract does bind to these two receptors; but in our hands Panax ginseng differs in that it binds only to PgR, and very weakly at that.

Both Panax ginseng and Siberian ginseng therefore contain molecules capable of preventing the binding of tracer steroids to receptors in vitro. Further studies are currently being undertaken to distinguish and further examine this activity by radioreceptor assay.

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