PULMONARY VASCULAR EFFECTS OF GINSENOSIDES

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SUMMARY

We reported earlier (Br. J. Pharmac. 82, 485 - 491, 1984) that ginsenosides from Panax ginseng C.A. Meyer antagonized noradrenaline or prostaglandin $F_{2\alpha}$ - induced contractions of pulmonary and intrapulmonary arterial rings of rabbits. Because this effect resembled that of acetylcholine (ACh), we questioned whether these acitons were due to release of nitric oxide from vascular endothelium. We therefore determined whether ginsenosides could vasodilate preconstricted lungs and also protect against free radical injury, which normally eliminates the vasodilator response to ACh(J. Appl. Physiol. 71, 821 - 825, 1991). We found that ginsenoside Rg_1 or a mixture of saponins could a) vasodilate perfused, U_{46619} - preconstricted lungs, b) promote increased synthesis of nitric oxide by endothelial cells in culture and c) prevent the pulmonary edema often associated with free radical injury (Biochem. Biophys. Res. Comm. 189, 670 - 676, 1992). Thus, vasodilator and protective effects of ginsenosides against free radical injury may reflect enhanced synthesis and release of nitric oxide. These data suggest that ginsenosides may be useful in treatment of pulmonary and systemic hypertension. Aided by grants from the National Institutes of Health, Bethesda, MD.

INTRODUCTION

We reported earlier that GS (a mixture of saponins from Panax ginseng) reversed NE or $PGF_{2\alpha}$ - induced contraction of rabbit pulmonary artery and intrapulmonary arteries (1). This effect qualitatively resembled that of ACh in perfused, pre - constricted rabbit lungs in which the cholinergic agonist acts by generating NO from the vascular endothelium (2, 3). We therefore determined whether GS (and the individual ginsenosides Rb_1 and Rg_1) had similar actions in the perfused rabbit lung and in endothelial cells in culture.

Our experiments with a perfused rabbit lung model indicated that GS, like ACh, could dilate pre - constricted lungs. Prior studies in this laboratory (3) showed that the vasodilator response to ACh is converted to vasoconstriction following brief electrolysis of the inflowing perfusion medium. We proposed (3, 4) that such reversal of ACh action reflects oxygen free radical - mediated injury to the endothelium, which disrupts the normal release of NO by ACh. Consistent with this proposal, electrolytic injury decreased lung serotonin uptake, an established reflection of pulmonary endothelial inury (2). GS protect against myocar-

dial injury following ischemia/reperfusion (7), reportedly by reducing the damaging effects of free radicals and lipid peroxidation in the heart. For this reason and because several antioxidants prevent electrolysis - induced endothelial injury (4), we also determined whether the ginsenoside Rb_1, which is reported to have antioxidant properties (5, 6), might also protect the pulmonary vasculature against electrolysis - induced injury.

MATERIALS AND METHODS

Chemicals and Drugs : Drugs and chemicals were from the following sources : U_{46619} (9, 11 - dideoxy - 9, 11 - methyl - anoepoxy prostaglandin $F_{2\alpha}$), Cayman Chemicals (Ann Arbor, MI) : [14C] - L - arginine (57.8 mCi/mmol) and [14C] - L - citrulline (53.7 mCi/mmol), NEN/Dupont (Willimington, DE) : Dowex AG 50 WX - 8, Bio - Rad Laboratories (Richmond, CA) : N - ω - nitro - L - arginine (N - arg) and all other chemicals, Sigma Chemical Co. (St. Louis, MO). Ginsenosides (GS) were prepared from Korean red ginseng (Panax ginseng C.A. Meyer) as described by Shibata et al.(8). Ginsenosides Rb_1 and Rg_1 were purified from GS by the method of Ando et al. (6). Molecular weights of Rb_1 and Rg_1 are 1109 and 801 respectively.

Lung perfusion : Lungs were perfused as described earlier (2, 4). After an initial period of 30 minutes of non - recirculating (SP) perfusion to remove blood (Fig. 1), recirculation was started and vascular reactivity was determined by 1) addition of U_{46619}(5 - 30 nM) to the recirculating reservoir to cause an increase in perfusion pressure to 30 - 35 mmHg., and 2) cumulative addition of ACh (50 - 200 nM final concentration) to produce vasodilation. Drugs were then flushed from the vascular space by 10 min of SP perfusion.
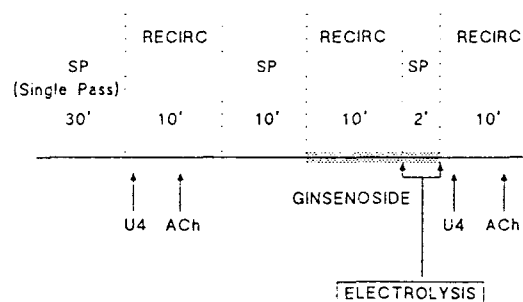

Figure 1. Proctocol used to evaluate the pulmonary vascular response to ACh before and after electrolysis and the effects of ginsenosides (GS). U_4 refers to infusion of U_{46619} (see "Methods and Materials" for details).

After this first "challenge", lungs were again constricted with U_{46619} and the direct vasodilator effect of GS, Rb_1 or Rg_1 on preconstricted lungs was assessed by adding each (50 - 200 μM) instead of ACh. Effects of ginsenosides pretreatment on ACh - vasodilatation in control experiments or after ES were studied by adding the saponins (20 - 200 μM) to the recirculation medium for 10 min (Fig. 1). Following this pretreatment, ES was applied (2, 4) by passing a 20 mA current for 2 minutes between platinum electrodes sealed through the perfusion tubing proximal to the lung. Next, recirculating perfusion was again started with fresh Krebs' medium and challenge with U_{46619} and ACh was repeated as above. In some experiments, N - arg (100 μM) was recirculated through the lungs for 5 min to block the release of NO and challenge with U_{46619} and ACh was repeated.

After each experiment, the lungs were weighed before and after drying to constant weight in an oven at 55°C. Wet to dry weight ratio is an index of edema production.

Endothelial cell culture : Bovine aortic endothelial cells (BAEC) were isolated from segments of bovine aortae (9) and bovine pulmonary artery endothelial cells (BPAE) was purchased from ATCC (American Type Culture Collection). Endothelial cells were grown in Dulbeco's modified Eagle's medium (DMEM) containing 0.48 mM L - arginine, 2 mM L - glutamine, 1 mM sodium pyruvate, 1 mM Hepes and 10% (vol/vol) fetal calf serum. Endothelial cells were identified as such by their cobblestone - like appearance when seeded onto 6 well plates and by positive immunostaining for angiotensin converting enzyme (ACE) and factor VIII.

Assay of NO synthase : NO synthase activity was measured by monitoring the conversion of [14C] - L - arginine to [14C] - L - citrulline (9) in confluent monolayer of endothelial cells which were arginine - deprived for 30 min before addition of [14C] - L - arginine (final concentration 850 nM, 0.05 μ C i / ml). 1 min later, GS, Rb_1 or Rg_i were added (final concentration 10 μM) and incubation continued for 15 min at 37°C. To inhibit the activity of nitric oxide synthase, N - ω - nitro - L - arginine (100 μM) was added to cells 20 min before addition of [14C] - L - arginine and ginsenosides. Cells were then washed with PBS, harvested in ice - cold Hepes/EDTA buffer (20 mM/ 2 mM, pH 6.0), sonicated (10 sec., three times) and centrifuged (10,000×g, for 15 min at 4°C) and the supernatant applied to a 1 - ml Dowex 50 cation exchange column. [14C] - L - citrulline in the effluent and a subsequent 1 ml water wash was quantified by liquid scintillation spectrometry. The sole radioactive component was verified as [14C] - L - citrulline by thin layer chromatography(data not shown). Percent conversion was calculated as dpm of [14C] - L - citrulline /dpm [14C] - L - arginine × 100.

Statistics : All values represent mean ± S.E.M. Differences among groups were determined by one - way ANOVA with Newman - Keuls test (11). Values were considered significantly different if P<0.05.

RESULTS

Effect of GS on basal vascular tone, U_{46619} - induced vasoconstriction and ACh vasodilatation : Recirculation of 20 μg/ml GS did not alter basal vascular tone (i.e. perfusion pressure), although we consistently noted that more U_{46619} was required to produce a 30 - 35 mmHg change in perfusion pressure (Fig. 2) thus confirming prior observations (1) which pointed to inhibition of smooth muscle contractility by GS.
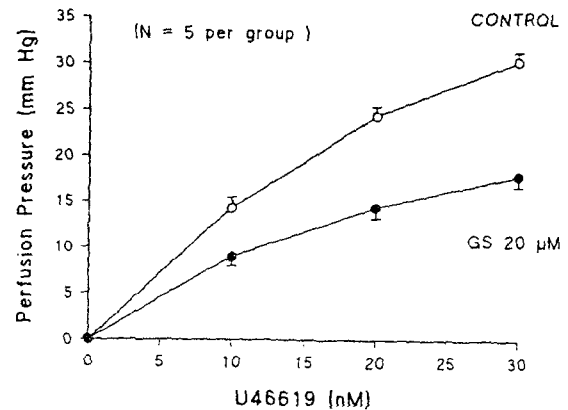

Figure 2. Effect of GS on U_{46619} - induced vasoconstriction. In presence of GS (20 μg/ml), vasoconstrictor response to 10 - 30 nM U_{46619} was significantly reduced (P <0.01).

Effect of GS on ACh - induced vasoconstriction after ES : We confirmed our earlier observations (4) that after ES, ACh consistently produced vasoconstriction (Fig. 3). Treatment of lungs with 50 μg/ml GS before and during ES prevented ACh - induced vasoconstriction, which normally followed the injury. Although 20 μg/ml GS had less protective effect than higher concentrations, it did eliminate ACh - induced vasoconstriction after ES (Fig. 3).
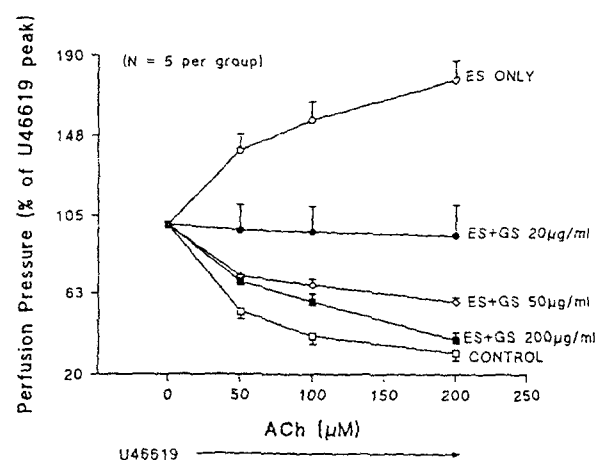

Figure 3. Preservation of the ACh vasodilator response by 50 μg/ml or 200 μg/ml GS. Notice that 20 μg/ml did not preserve the ACh vasodilatation.

Effect of GS on ES - induced pulmonary edema : Control wet/dry weight ratios for lungs in this series of experiments were 7.5±0.1 ; these values are somewhat higher than control data in other experiments carried out in this laboratory (2). However lungs exposed to ES had significantly higher ratios (11.0± 1.0 ; P<0.05), which fell to values similar to the experimental controls in the presence of 50 μg/ml (7.5± 0.3) or 200 μg/ml GS (7.6± 0.4) ; 20 μg/ml GS did not protect against edema (wet/dry weights were 8.7± 1.4). GS in the absence of ES did not cause pulmonary edema.

Vasodilator actions of ACh and GS before and after N - arg : GS, Rb_1 and Rg_1 significantly dilated lungs preconstricted by U_{46619} (P<0.05). 200 μg/ml of GS or 200 μM of either Rg_1 or Rb_1 caused 39%, 22% or 15% reduction, respectively, in the peak U_{46619} - induced pressure change (Fig. 4). After blocking the release of NO by N - arg (100 μM), a relatively lo-

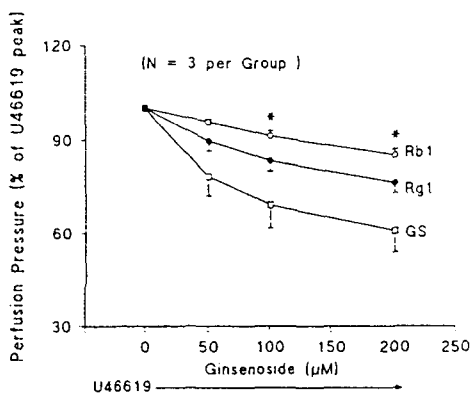Figure 4. Direct vasodilator actions of ginsenosides. Notice that GS (50 - 200 μg/ml) as well as ginsenosides Rg_1 and Rb_1 (50 - 200 μM), significantly vasodilated lungs preconstricted by U_{46619}. GS, Rg_1 or Rb_1 reduced peak perfusion pressure during U_{46619} infusion by 39%, 26% or 15% respectively (P<0.05).
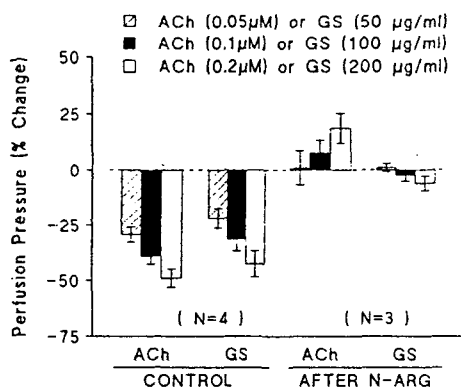

Figure 5. Changes in vasodilator actions of ACh and GS before and after N - arg. Vasodilator reponses to ACh (n=4) and GS (n=3) were abolished by 100 μM N - arg. Notice that after N - arg, ACh caused slight vasoconstriction.

wer concentration (10nM vs 30 nM) of U_{46619} was required to raise perfusion pressure to 30 - 35 mmHg. In addition, the vasodilator effects of both ACh and GS were completely eliminated (Fig. 5). Notice (Fig. 5) that 200 nM ACh caused slight vasoconstriction after N - arg, possibly because it acted directly on muscarinic receptors in the vascular smooth muscle (2, 4). N - arg also eliminated the vasodilator action of GS.

Effect of GS on arginine to citrulline conversion in BAEC & BPAE : As shown in Fig. 6, [14C] - L - citrulline production from [14C] - L - arginine, as an index of NO production, was significantly enhanced by 10 μg/ml GS or 10 μM Rg_1 (20.3% or 22% vs 14.9% in controls) but not by Rb_1 in BAEC. In addition, Rg_1 also significantly increased total [14C] - L - arginine transport into cells. This enhanced uptake and converison was also observed in the presence of 0.1 μM bradykinin (data not shown). With BPAE, a similar increase in arginine transport and citrulline conversion were observed after Rg_1 while Rb_1 had no effect(Fig.7). These effects of Rg_1 in BPAE were significantly reduced by addition of N - ω - nitro - L - arginine, a nitric oxide synthase inhibitor, suggesthing that Rg_1 might activate nitric oxide synthase in vascular endothelial cells.

DISCUSSION

The GS we used represents a complex mixture of individual ginsenosides, which produce a range of effects on the cardiovascular and central nervous systems (12). Each of the ginsenosides of *Panax ginseng* may have qualitatively and quantitatively different effects on the cardiovascular system producing a complicated range of actions (13), described as "adaptogenic" effects, which are reported to provide non - specific resistance to circulatory stress (12). The complex nature of the mixture probably explains the fact that, in our experiments, GS relaxed pulmonary vessels but constricted others, including renal vessels (1) and
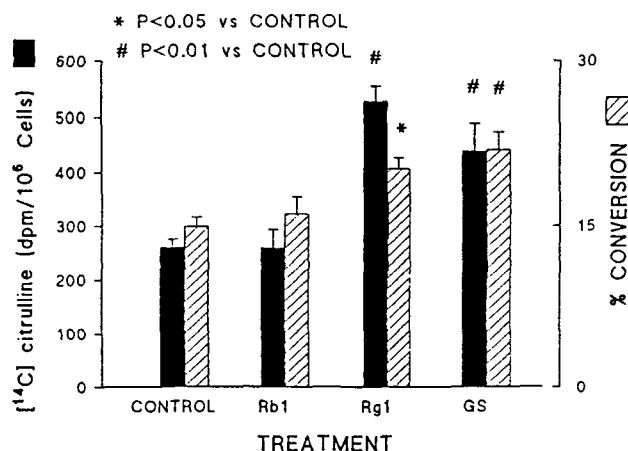

Figure 6. GS (10 μg/ml) and Rg_1 (10 μM) significantly (P <0.01) increased both uptake and [14C] - L - arginine and conversion to [14C] - L - citrulline in culture while Rb_1 lacked this effect in BAEC (n=12).

that Rg_1 and GS (but not Rb_1) in the present study significantly increased NO production as reflected by conversion of arginine to citrulline in BAEC (Fig. 6).

Our experiments establish that GS significantly diminished the vasoconstrictor activity of the thromboxane analog U_{46619}(Fig. 2), but did not directly alter vascular tone *per se*. This is qualitatively similar to effects on pulmonary arterial segments *in vitro* described previously (1). Also, GS and Rg_1 significantly vasodilated pre – constricted lungs. Both effects could be due to release of an endogenous vasodilator substance by GS. The fact that both actions were inhibited by N – arg (Fig. 5), an inhibitor of NO synthase (10), suggests that NO itself could be the substance involved. Experiments with BAEC and BPAE in culture support this possibility ; we found (Fig. 6 & 7) that GS and Rg_1 (but not Rb_1) significantly enhanced converison of arginine to citrulline, which is a measure of concomitant NO production. These observations are also consistent with the fact (Fig. 4) that Rb_1 had less inhibitory action on U_{46619} – vasoconstriction than either GS or Rg_1.
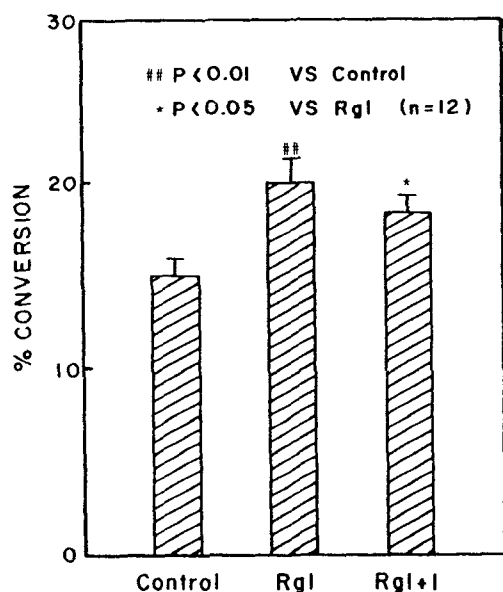

Figure 7. Stimulating effect of Rg_1 (10 μM) on [14C] – L – arginine uptake and [14C] – L – citrulline conversion. This effect is significantly reduced in BPAE by addition of N – arg (100 μM) (n=12).

If some ginsenosides enhance NO production, they might do so by direct action as cholinergic agonists. However, this seems unlikely because, after ES, ACh caused vasoconstriction, while ACh in the presence of 50 or 200 μg of GS per ml caused vasodilatation. We therefore propose that this protective effect is due to the production of endogenous NO at a site distal to cholinergic receptor. We reported earlier (3) that ES injury could be prevented by antioxidants including SOD, catalase or salicylate (a hydroxyl radical scavenger). It is thus of interest that NO acts as an antioxidant and also decrease lipid peroxida-

tion *in vitro* (14). Perhaps, therofore, NO released by GS as well as a direct antioxidant action of the ginsenosides (5), might contribute to protection against lung endothelial injury caused by electrolysis.

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