# *IN VITRO* EFFECT OF *PANAX GINSENG* ON PHYTOHAEMAGGLUTININ-INDUCED LYMPHOCYTE TRANSFORMATION AND ITS *IN VIVO* EFFECT

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## ABSTRACT

The effect of hydrocortisone or *Panax ginseng*, and/or a combination of hydrocortisone and Panax ginseng on phytohaemagglutinin (PHA-P)-induced transformation of peripheral blood lymphocytes were studied in 4 normal healthy adult volunteers. Increasing concentrations of *Panax ginseng* 0.16-1.60μg/ml caused a dose-related inhibition of PHA-P transformation of lymphocytes. A combination of 500μg/ml hydrocortisone and 0.80μg/ml *Panax ginseng* produced a greater suppression of PHA-P stimulation than either drug used alone. This suggests that *Panax ginseng* has a steroid-like effect *in vitro*, and may have a potentiating effect with hydrocortisone on T-cell mediated immunity. The *in vivo* effects of *Panax ginseng* were further studied in 6 healthy adult volunteers. PHA-P transformation studies were performed before and after *Panax ginseng* capsules were taken for 2 weeks and at a higher dosage at 4 weeks. Our results showed that in 3 subjects, there was significant inhibition of PHA-P transformation at 4 weeks.

## INTRODUCTION

Although the root of *Panax ginseng* has long been used in traditional medical practice in the Far East, the chemical, pharmacological and biochemical properties of *Panax ginseng* have only recently been scientifically studied.[1,2] Two of the saponin glycosides isolated from the methanol extract of Panax ginseng were found to have prolonged anti-inflammatory properties based on albumin stabilising activity, and carrageenin oedema testing in intact rats.[3] In this respect, Panax ginseng appears to share some properties of non-steroidal anti-inflammatory drugs. Further studies have shown that Panax ginseng could influence corticosterone secretion via the pituitary adenocortical axis.[4,5] We therefore compared the action of extracts from the ginseng root with hydrocortisone on the transformation of human lymphocytes by PHA-P.

## METHOD

Venous blood was obtained from four normal healthy adult subjects. The blood was separated over Ficoll-Hypaque[6], and the cell suspension washed twice in Hanks' balanced salt solution (HBSS, Gibco Europe Ltd). The lymphocytes were adjusted to a concentration of 1 x $10^6$ $ml^1$ in RPMI 1640 (Gibco Bio-Cult, Scotland) containing 10% fetal calf serum, antibiotics,

fungizone and 10mM HEPES (N-2 hydroxypiperzine N'-2-ethylenesulphonic acid buffer).

A dose-response curve was established using several blood samples, and a dose of 100$\mu$g ml$^{-1}$ of PHA-P (Difco Lab Ltd.) was shown consistently to produce maximum stimulation of lymphocytes. The experiment was performed with PHA-P alone, or PHA-P in the presence of different concentrations of the drug solutions of hydrocortisone and/or *Panax ginseng*. The concentration of *Panax ginseng* used was calculated as that plasma concentration obtained in vivo after taking a "usual" dose and assuming a distribution volume of 15% body weight. The peripheral blood lymphocytes were incubated at 37°C for 72 hours and 5$\mu$Ci of tritiated thymidine (21 Ci/mmol, Amersham, UK) was added to each culture tube 18 hours before termination. DNA was extracted and its activity estimated according to a standard method[7] and the results

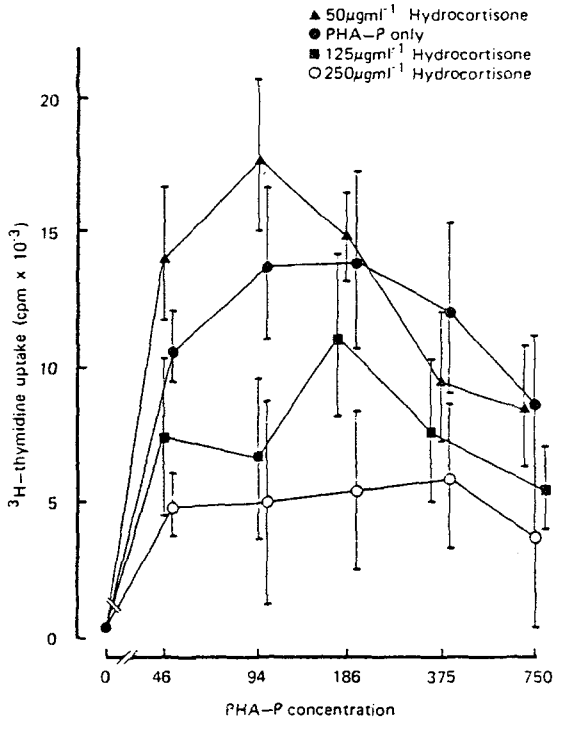


Fig. 1. Effect of hydrocortisone on $^3$H thymidine incorporation in PHA-P stimulated peripheral blood lymphocytes; (●) no drug; (▲) 50$\mu$g ml$^{-1}$ hydrocortisone; (■) 125$\mu$g ml$^{-1}$ hydrocortisone; (○) 250$\mu$g. ml$^{-1}$ hydrocortisone. Each point represents the mean ± SEM of four experiments carried out in triplicate.

expressed as counts per minute per tube of $^3$H thymidine uptake.

**Standard binding curve using $^3$H-triamcinalone ($^3$H-TC on lymphocytes from tonsils.**

Tonsil lymphocytes were separated on isopaque-ficoll, washed in RPMI and resuspended in RPMI containing 10% FCS. The cells were incubated in plastic culture flask at 37°C for 45 mins. to remove monocytes then counted.

The lymphocytes were washed in RPMI and resuspended at a concentration 2 x 10$^7$ cells/ 500$\mu$ of the cell suspension were added to 100ul aliquots of serial dilutions of $^3$H-TC, starting with 1nm of $^3$H-TC.

The cell were votexed gently before incubation at 37°C for 6 hrs. At the end of the incubation period, 2mls of PBS (4C) was added to each tube, which were then centrifuged for 10 min. at 1000g. The cells were washed twice with PBS then resuspended in 1ml of 95% ethanol plus 0.5ml PBS, before adding 10ml scintillant and counting on a counter. The optimal dose of $^3$H-TC was determined from this binding assay.

## INHIBITION OF $^3$H-TC BINDING

Various concentrations [up to 1mg/ml] of unlabelled TC, cortisone or fractions of ginseng were added in equal volumes to tubes containing 10$^7$ lymphocytes, just prior to the addition of the optimal dose of $^3$H-TC. Inhibition of labelled TC binding was determined by a decrease of lymphocyte bound counts, as compared with lymphocytes to which no "inhibitor" was added.

## RESULTS

The effects of hydrocortisone on PHA-P induced transformation of peripheral blood lymphocytes was initially studied. Lymphocytes were stimulated with serial dilutions of PHA-P (1:128 to 1:8). In the presence of increasing concentrations of hydrocortisone hemisuccinate (50-250$\mu$g ml$^{-1}$). the dose response curve was shifted to the right with a decreased maxima, as described previously by Heilman.[8] (Fig. 1).

However, further incremental doses of hydrocortisone did not further inhibit PHA-P transformation.

The experiment was repeated with *Panax ginseng* extract at concentration of 0.16 - 1.60μg $ml^{-1}$ (Fig. 2). A similar dose-related suppression of the PHA-P transformation of peripheral blood lymphocytes was obtained.

The effect of a combination hydrocortisone (500μg $ml^{-1}$) and *Panax ginseng* (0.80μg $ml^{-1}$) was compared with *Panax ginseng* or hydrocortisone alone on PHA-P stimulation of lymphocytes (Fig. 3). Hydrocortisone reduced the response of the lymphocytes to PHA-P by 44% at both PHA-P dilutions of 1:64 and 1:32, and ginseng reduced it by 72% and 64% at the same respective dilutions. The combination of hydrocortisone and *Panax ginseng* produced a greater suppression (90%) of PHA-P stimulation of lymphocytes.

We subsequently showed that the time course experiment at 48 and 96 hours showed inhibition of $^3H$ thymidine incorporation in the presence of *Panax ginseng* or hydrocortisone.

However, the viability of lymphocytes performed on two normal subjects at concentrations of 0.02μg and 200μg of *Panax ginseng* extract was 60% and 90% respectively. This may suggest that *Panax ginseng* at high concentrations may have an adverse effect on lymphocytes.

Binding of $^3H$-TC to $10^7$ lymphocytes was in theregion of 5% of the total counts added however addition of excess TC [100 fold] reduced the $^3H$-TC binding by 60%. Excess cortisone reduced $^3H$-TC binding by 67% and excess ginseng by 50%. On repeating the assay on tonsil derived lymphocytes from another patient, the degree of "competitive binding" was reduced using TC, cortisone and ginseng [Table 2].

A spectrum of fractions from ginseng was analysed. No one fraction showed appreciably different levels of "cpm. binding" with $^3H$-TC. The levels of apparent competitive binding of the ginseng fractions were increased dramatically by increasing the concentrations of the fraction. At 1mg/ml of ginseng fraction RE, RG, RF, the $^3H$-TC binding was decreased by

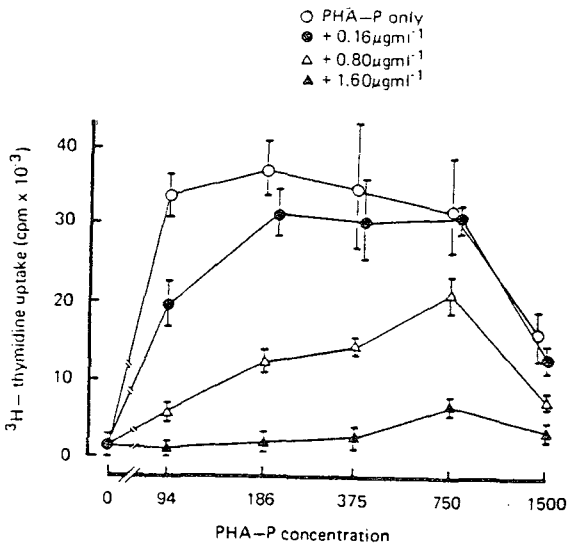


Fig. 2. Effect of *Panax ginseng* on $^3H$ thymidine incorproation in PHA-P-stimulated peripheral blood lymphocytes; (○) no durgs: (●) 0.16 μg. $ml^{-1}$ ginseng; (△) 0.80μg.$ml^{-1}$ ginseng; (▲) 1.60μg. $ml^{-1}$ ginseng. Each point represents the mean ± SEM of four experiments carried out in triplicate.

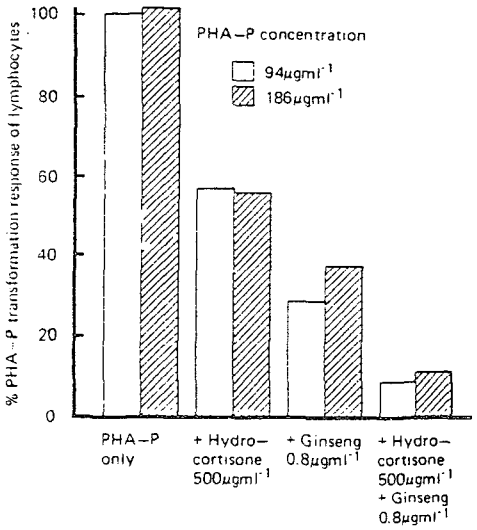


Fig. 3. Effect of a combination of hydrocortisone and Panax ginseng compared with hydrocortisone or Panax ginseng alone on the percent transformation of PHA-P-stimulated peripheral blood lymphocytes. 100% transformation equals 38x $10^3$ cpm.

72%. However, it must be stressed that the fall in $^3H$-TC was due to the ginseng lysing the lymphocytes.

TC at a concentration of 5μ/ml caused re-

Table 1. Inhibition of $^3$H thymidine incorporation into normal lymphocytes in the presence of Panax ginseng or hydrocortisone succinate after 48 h of incubation with PHA-P

| Healty donors No. | Lymphocyte thymidine uptake in presence of | | | Lymphocyte thymidine uptake in presence of | |
|---|---|---|---|---|---|
| | 10% FCS | 10% FCS + panax ginseng, 1.0 μg | index of inhibition | 10% FCS + hydrocortisone 125 μg | index of inhibition |
| 1 | 13,362 (458) | 1,127 (153) | 0.92 | 330 (88) | 0.97 |
| 2 | 14,412 (149) | 208 (158) | 0.98 | 255 (168) | 0.98 |
| 3 | 21,018 (255) | 390 (268) | 0.97 | 210 (162) | 0.99 |
| 4 | 27,009 (259) | 178 (178) | 1.0 | 135 (155) | 1.0 |

Figures in parentheses represent unstimulated values.

Table 2. Inhibition of $^3$H thymidine incorporation into normal lymphocytes in the presence of Panax ginseng and hydrocortisone succinate after 96 h of incubation with PHA-P

| Healty donors No. | Lymphocyte thymidine uptake in presence of | | | Lymphocyte thymidine uptake in presence of | |
|---|---|---|---|---|---|
| | 10% FCS | 10% FCS + panax ginseng, 1.0 μg | index of inhibition | 10% FCS + hydrocortisone 125 μg | index of inhibition |
| 1 | 24,618 (471) | 472 (244) | 0.98 | 137 (90) | 0.99 |
| 2 | 35,718 (696) | 425 (369) | 0.99 | 162 (97) | 0.99 |
| 3 | 26,119 (289) | 1547 (552) | 0.94 | 290 (144) | 0.99 |
| 4 | 44,057 (593) | 566 (371) | 0.99 | 1511 (313) | 0.97 |

Figures in parentheses represent unstimulated values.

duced viability whilst a 10 fold increase in this concentration was required to produce the same degree of cell death using ginseng i.e. 50μg/ml. At 250μg/ml, fraction RE, RG, RF caused a 95% fall in viability [Table I].

Commercial ginseng capsules caused equivalent reductions in TC binding.

## DISCUSSION

The *in vivo* effects of *Panax ginseng* capsules were assessed in five healthy volunteers who took 2 capsules daily for two weeks, and subsequently 2 capsules twice a day for a further two weeks. After the first two weeks, 2 of the 5 volunteers showed significant inhibition of PHA-P stimulated transformation at 35% and 42%. After 4 weeks, only 3 of the volunteers were taking the ginseng capsules. All 3 volunteers showed significant inhibition at 44%, 55% and 59%. The *in vivo* results, although performed in a few subjects do confirm our *in vitro* studies. However, a larger number of subjects should be studied.

The results of these experiments have displayed a marked competition of cortisone for the triamcinalone binding site, suggesting that there exists a common steroid receptor. Initial experiments showed ginseng to act as an apparant "competitive inhibitor" of steroid binding however the higher concentrations of ginseng required to increase the inhibition of steroid binding, have clearly been shown to cause lymphocyte mortality. It seemed most likely that the apparent competitive inhibition produced by ginseng is merely a reflection of cell viability. The extent of cell damage by these levels of ginseng is remarkable in the light that ginseng is generally considered as harmless.

**I.P. Lee:** You used 50μg deranging to 250μg/ml. In that ml, how many cells were present?

**Chong:** The cell concentration was 1-2 x $10^6$ /ml.

**I.P. Lee:** Physiologically I'm wondering whether the amount of ginseng we take actually reach that kind of concentration in our blood volume, because *in-vitro* study like this one and the one that I presented awhile ago shows citotoxicity at certain concentration beyond, say, 0.04gm per $10^6$ cells. That amount is a horrendous amount of ginseng. So that, essentially, you don't reach that kind of concentration at per ml basis.

**Chong:** Sure, but the point that should be stressed is that the concentration of ginseng which I used was based on a pharmacological dose which was suggested by the Office of Monopoly here and Pharmacon Company, Lugano. Now we worked out that the concentration of ginseng that I added was in fact a minuscule amount compared to what is actually swallowed. The concentrations of ginseng that were used were about 0.8 to 1.60μg/ml. That is a very tiny amount of ginseng and it showed inhibition on PHA-transformation. It is a crude test for lymphocyte but a pretty effective test used worldwide by immunologists. Now I agree with you that perhaps the number of peripheral lymphocytes that were extracted was small, but that is the usual amount of peripheral lymphocyte which most immunologists would actually accept as an acceptable range.

**I.P. Lee:** That is true. Though *in-vivo* situation, the lymphocyte concentration is not in the $10^6$ /ml level. It is rather scarce and there are a lot of other cells that can sink the binding of your fractions. So I think you can dilute out the concentration of ginseng. The second question is that you had thymidine incorporation studies to look at the lymphocyte transformation. I'm just wondering whether you have looked at the thymidine kinase activity or the free thymidine pool within the cell possibly altered by ginseng itself or by the hydrocortisone. Becauase that would in turn affect actual thymidine incorporation into the DNA. If this mechanism is either by the thymidine kinase alterationor because of the membrane or pool size is altered by the transport of neucleotides, that can also affect DNA synthesis. So I was wonderingn whether you have corrected that parameter to interprete correctly.

**Chong:** Yes, I think that's a point. That experiment which I described is a standard experiment which is performed to assess any immunosuppressive drug or any drug that we want to look at to see whether it is but immunological properties. As far as I know it is a non-specific test, but it does show that it has an effect *in-vitro* on peripheral lymphocyte. Just one point you made about whether that really applies *in-vivo*. I took the precaution of actually doing that same experiment *in-vivo*. We've got those 5 volunteers to swallow *Panax ginseng* over a period of a month. We found that it also causes an inhibition of peripheral blood lymphocyte *in-vivo* experiments. Although we had 5 volunteers, only 3 of them actually worked. So I don't think that is significant to make a major statement *in-vivo*, so I'm refraining from saying that it is cytotoxic *in-vivo*. I agree with you, but I think further experiment should be done *in-vivo*.

**F.J. Lee:** I agree with Dr. Lee who just mentioned about the cytotoxicity. You mentioned that at the 250μg/ml level of ginsenoside, there was cell death. I don't think that is a suprise at all, because 250μg/ml of any individual ginsenoside would be equivalent to a humongous amount of ginseng itself. So I think it is a kind of anticipated result.

**Chong:** Yes, I accept that because what we used were saponin fractions. Although we've only showed rather high concentrations, what I'm saying is that perhaps at lower concentrations, say, from about 125μg/ml of the extract going up to about 250μg to 1mg of the active components, there is a range of effect which I see ranging from immunosuppressive to cytotoxic. I don't really know what that means. I don't think anybody knows at the present point of time whether it is due to membrane

effect or cytotoxic effect or whether it actually is an effect on nucleus. I think one have to carry out further experiment on individual cell itself before he can actually say or reach a conclusion.

**F.J. Lee:** Just one more comment is that anything can be cytotoxic at an extremely high concentration.

**Chong:** Yes, I agree.

**Fulder:** I want to suggest that in doses about 0.8μg/ml, you got guite a severe reduction in lymphocyte transformation. You were already seeing an inhibition of cell multiplication which is actually nothing to do with PHA-inhibition but direct effect on the cells. Is that possible?

**Chong:** Yes, I think that is possible. It is extremely difficult to dissect out cytotoxicity from immunosuppression. It could just be that the effect, the so called steroid-like effect that I showed in my initial experiment were indeed due to the reduced cell viability. I accept that.

**Fulder:** There is a confusion in the published work done on this question of steroid. We found that, in fact, feeding ginseng saponin to rats produced increased sensitivity or binding of steroid to cell nuclei in brain cells. In this case you were getting an additive effect, you were getting more steroid rather than competitive effect. There is a paper in Australia indicating that you do get competition with ginseng and various steroids. We are dealing with saponin. We are dealing with surface active materials which can easily affect membrane fluidity, membrane structure. You could use small doses and just look at the steroid actually getting into cells, which were more meaningful perhaps than doing competitive studies with large doses of saponin.

**Chong:** Could I clarify just one thing? I did not do toxicity test on *Panax ginseng.* What I was interested in was the therapeutic potential of using ginseng in clinical medicine. That's what we're all interested in. I'm not saying that it is clinically toxic. No, that's not what I meant to say. What I'm tring to say or pointing out to everyone here is that there maybe a possibility that ginseng could have antimitotic effect. I mean, the point was raised very eloquently by Dr. Lee saying that it could actually be useful in treating skin tumors. That might just be possible. If one could show that it is antimitotic, it could perhaps possibility be used in cancer theraphy. I mean we ought to consider that as a possibility. But I'm not saying that it is *in-vivo* toxic. I haven't done any toxicity experiment and I don't claim to have done so.

**Fulder:** You made a conclusion or statement on the basis of data that ginseng may have immunosuppressive effect. This is in contrast to other reports which indicate immunostimulative effect. My understanding on lymphocyte transformation is rather changeable. If you take a cup of coffee or have a cold, it will change according to all sorts of conditions *in-vivo.* So, do you really feel this is meaningful as in an *in-vivo* study, actually draw the conclusion of immunosuppression from the *in-vivo* study you've done?

**Chong:** Yes, let me comment on that. I think you are absolutely right if you are talking about an *in-vivo* system. It is a very crude test. No, even *in-vitro,* many people would consider that it would be a crude test. But the important point to realize is that it consistantly showed an inhibition on stimulation test that we did on our system. It does'nt conclusively say that it is immunosuppressive but it shows that there is a trend to immunosuppression. That is all that we are trying to point out.

## 식물성 혈구 응집소(Phytohaemagglutinin)로 유도한 림파구 변형에 관한 인삼의 in vitro 및 in vivo 효과

S. K. F. Chong

Hydrocortisone과 ginseng 그리고 hydrocortisone과 ginseng의 혼합물이 phytohaemagglutinin (PHA-P)으로 유도한 모세혈관 림파구 변형에 미치는 영향을

검토하기 위하여 정상인 4명을 대상으로 연구하였다.

인삼의 농도를 0.16㎍/ml에서 1.60㎍/ml까지 증가시킴에 따라 PHA-P의 림파구 변형에 상관성이 있는 억제현상을 보였으며, hydrocortisone 500㎍/ml과 ginseng 0.8㎍/ml을 동시 투여하였을 경우 각각 단일 물질 투여시 보다 상당한 PHA-P 변형의 억제효과를 나타냈다.

이는 인삼이 in vitro상에서 steroid와 유사한 효과를 갖고 있음을 시사해 주며, 면역에 hydrocortisone과 더불어 상당한 효과를 가지고 있다는 것을 보여주고 있다.

in vivo상에서 6명의 정상인을 대상으로 PHA-P 변형에 대하여 인삼의 효과를 연구한 결과 4주에 이르러 상당한 억제효과가 관찰되었다.

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