Effects of Ginseng Saponins and its Fractions on Mouse Tryptophan Pyrrolase Activity *in vivo*

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생쥐의 *in vivo* 에서의 Tryptophan Pyrrolase 활성에 미치는 인삼사포닌과 그 분획물의 영향

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적 요

약 20 g 되는 생쥐에 인삼사포닌은 매일 체중 20 g당 0.02 mg을, 디올계 와 트리올계 사포닌은 0.01 mg을 피하주사하여 *in vivo* 에서의 Tryptophan Pyrrolase(TP) 활성의 변화를 측정하여 다음의 결과를 얻었다.

1. 인삼 사포닌을 주사한 3시간 후에 TP 활성은 약간 증가하였으나 통계 학적으로 유의성이 없었으며, 24시간 후에는 정상수준으로 복귀 하였다. 1 주간 처리군은 61.11%의 유의성 있는 증가를 나타내었다.

2. 디올계 사포닌 처리로 TP 활성은 3시간 후에 12.10% 증가하였으나 유 의성이 없었으며, 12시간에 64.96%의 유의성 있는 증가를 보였으며, 24시간 에 30.78%로 감소하였으나 유의성이 없었다. 1주간 처리군은 100.58%의 유의성있는 증가를 나타내었다.

3. 트리올계 사포닌 처리는 3시간에 59.17%의 유의성 있는 활성증가를 일 으켰으며, 24시간에 거의 대조군의 수준으로 복귀하였다. 1주간 처리군은 66.98%의 유의성 있는 증가를 나타내었다.

INTRODUCTION

Tryptophan pyrrolase (TP) is the haem-dependent liver cytosol enzyme which catalyses the conversion of L-tryptophan into formyl kynurenine. TP is an inducible enzyme, and adrenal corticosteroids and tryptophan itself are known to be inducing agent (Knox and Auerbach, 1955; Knox, 1951). In intact rat liver, the TP activity was increased by the administration of other compounds such as histidine, ephinephrine, and histamine, as well as tryptophan (Knox and Mehler,

1951; Knox, 1951). These non-substrate compounds appeared to act in a second way which was distinguished from the substrate-induced enzyme adaptation by the production of generally smaller increases in TP activity and by their action only in the presence of adrenal glands. Glucocorticoids cause a hormonal-induction involving the synthesis of new apoenzyme, whereas tryptophan produces a substrate-type enhancement consisting of decreased degradation of pre-existing apoenzyme in the presence of the normal rate of its synthesis. Rat liver TP is also regulated by its cofactor haem and by NAD(P)H (Badawy and Evans. 1975, 1976).

A few papers dealing with the effects of ginseng extracts on the rat liver TP activity were published recenlty. Brekhman *et al.* (1971) have reported that the ginseng extract does not influence either the activity or the hormonal induction of TP, and under stress conditions ginseng decreases the activity in normal rat and does not affect it in adrenalectomized animals. Oura *et al.*(1972) have reported that the induction of TP and tyrosine transaminase, which is observed with cortisone, did not occur by fraction 4 of ginseng. However, Jung *et al.* (1978) have reported that ginseng saponins and its fractions increased TP activity in one hour *in vitro.*

The present experiment was carried out to study the effects of ginseng total saponins, diol- and triol-saponin on the TP activity *in vivo,* and some aspects of the increase of TP activity by ginseng extracts are discussed.

MATERIALS AND METHODS

1. Materials

ICR mice weighing about 20 g were kept under controlled environmental conditions.

Four-year old Korean white ginseng roots (Keumsan, 50 pcs/300 g) were powdered and used for extraction and fractionation.

2. Methods

(1) Extraction and Fractionation of Ginseng Saponins

Total saponins were extracted according to the modification of Shibata *et al.* (1966) and Woo *et al.* (1973). The procedure are summarized in Jung *et al.*(1978). Fractionation of diol- and triol-saponin was carried out by the procedure proposed by Shibata(1967). The extracted substances were diluted with saline and then used.

(2) Determination of Enzyme Activity

The experimental animals were subcutaneously injected with 0.02 mg of total saponins, and/or 0.01 mg of diol- and/or triol-saponin per 10 g of body weight per

·day. The experimental animals were killed 3 hours, 12 hours, 24 hours or one week after injection. Animals for one week experiment were injected with the same amount of saponins as described above every day. The control animals were injected vehicle only.

The animals were killed by decapitation and the livers were removed and chilled by ice immediately. After operation 1 g of liver was homogenized with 7 ml of cold 0.1 M KCl solution containing 0.0025 N NaOH (pH: 7.0). Tryptophan pyrrolase activity was determined according to Knox and Auerbach (1955). Protein was estimated by the biuret method (Gonall et al., 1949).

RESULTS

The effects of ginseng total saponins and its fractions on mouse hepatic TP activity was determined in vivo 3 hours, 12 hours, 24 hours or one week after injection of the saponins.

As shown in Table 1, the total saponins of ginseng increased the TP activity by 26.12% after 3 hours, which was not significant statistically. The activity was returned to the control level after 24 hours. However, the TP activity was significantly increased by 61.11% ($p < 0.05$) in the liver of one week treatment.

Table 1. Effects of total saponins of Korean ginseng on tryptophan pyrrolase (TP) activity* in mice liver.

	Treatment	No. of mice	Average body wt.	TP Activity$\pm$S.E.	t-test
3 hrs	Saline (0.01 ml/10 g. B. wt.)	10	20.6	0.9375 ± 0.1380	
	Saponins (0.02 mg in 0.01 ml)	8	19.2	1.1824 ± 0.2051	not significant
	Change rate (%) (saponins/saline)	8/10		+26.12%	
24 hrs	Saline (0.01 ml/10 g. B. wt.)	7	18.5	0.8213 ± 0.1478	
	Saponins (0.02 mg in 0.01 ml)	8	18.9	0.8569 ± 0.0564	not significant
	Change rate (%) (saponins/saline)	8/7		+4.33%	
1 wk	Saline (0.01 ml/10 g. B.wt.)	8	17.4	0.7370 ± 0.0726	
	Saponins (0.02 mg in 0.01 ml)	7	17.4	1.1874 ± 0.1623	$p < 0.05$
	Change rate (%) (saponins/saline)	7/8		+61.11%	

* TP activity unit: moles kynurenine/g protein/hr. at 37°C

As shown in Table 2, the effects of diol-saponin on TP activity were similar to the case of the total saponins measured 3 hours after injection. However, TP activity was increased by as much as 64.98% (p<0.01) after 12 hours, but again decreased to 30.78% after 24 hours, which was not significant. One week treatment caused a significant value of 100.58% increase (p<0.01).

The effects of triol-saponin on the TP activity are shown in Table 3. The activity was increased by 59.17% (p<0.01) 3 hours after injection. But again the activity returned to its control level after 24 hours. It was significantly increased

Table 2. Effects of diol saponin on TP activity* in mice liver.

	Treatment	No. of mice	Average body wt.	TP Activity±S.E.	t-test
	Saline (0.01 ml/10 g. B.wt.)	10	20.6	0.9375±0.1380	
3 hrs	Diol (0.01 mg in 0.01 ml)	6	23.3	1.0500±0.1604	not significant
	Change rate(%) diol/saline	6/10		+12.1%	
	Saline (0.01 ml/10 g. B.wt.)	8	22.5	0.7465±0.0526	
12 hrs	Diol (0.01 mg in 0.01 ml)	7	22.4	1.2314±0.1265	p<0.01
	Change rate(%) diol/saline	7/8		+64.96%	
	Saline (0.01 ml/10 g. B.wt.)	7	18.5	0.8213±0.1478	
24 hrs	Diol (0.01 mg in 0.01 ml)	7	22.4	0.5685±0.0580	not significant
	Change rate(%) diol/saline	7/7		−30.78%	
	Saline (0.01 ml/10 g. B.wt.)	5	20.8	0.5201±0.0690	
1 wk.	Diol (0.01 mg in 0.01 ml)	7	21.1	1.0432±0.2165	p<0.01
	Change rate(%) diol/saline	7/5		+100.58%	

* See footnote to Table 1.

Table 3. Effects of triol saponin on TP activity* in mice liver.

	Treatment	No. of mice	Average body wt.	TP Activity±S.E.	t-test
	Saline (0.01 ml/10 g. B.wt.)	10	20.6	0.9375±0.1380	
3 hrs	Triol (0.01 mg in 0.01 ml)	8	21.9	1.4922±0.2258	p<0.01
	Change rate (%) triol/saline	8/10		+59.17%	
	Saline (0.01 ml/10 g. B.wt.)	7	18.5	0.8213±0.1478	
24 hrs	Triol (0.01 mg in 0.01 ml)	4	22.3	0.8211±0.0687	not significant
	Change rate(%) triol/saline	4/7		−0.02%	
	Saline (0.01 ml/10 g. B.wt.)	5	20.8	0.5201±0.05690	
1 wk	Triol (0.01 mg in 0.01 ml)	8	22.8	0.8685±0.0238	p<0.01
	Change rate(%) triol/saline	8/5		+66.98%	

* See footnote to Table 1.

by 66.98% (p<0.01) by one week treatment similar to the case of total saponins and diol-saponin.

DISCUSSION

The present results showed that the TP activities of experimental groups of 3 hour treatment were increased with some difference by the total saponins, diol- and triol-saponin. Only triol-saponin caused a significant 59.17% increase.

The TP activity was increased as many as 6 times within 4 to 10 hours after injecting tryptophan to rats and this phenomenon was called as substrate-induced enzyme adaptation (Knox and Mehler, 1951; Lee, 1956). The TP activity was increased in both normal and adrenalectomized rats by cortisone treatment and it was distinguished as hormone-induced enzyme adaptation (Knox and Auerbach, 1955). Then Brekhman *et al.* (1971) and Oura *et al.* (1972) could not observe hormone-induced TP adaptation by ginseng extract or fraction 4 of ginseng. Oura *et al.* observed some differences in effects between fraction 4 and cortisone, and suggested that the observed effects of ginseng extract would not be due to the elevated concentration of adrenocortical steroids.

Since a significant increasing effect did not appear in the present results by the total saponins or diol-saponin in the earlier period, it may not be able to interprete as an induced adaptive synthesis as well as substrate or cortisone induced adaptation. Moreover, because triol-saponin showed much less increasing rate than that of the above mentioned induced enzyme adaptation, it is suggested that the present result of slight increase may be caused by other mechanisms.

The increase of the TP activities by the treatment of total saponins and/or diol- and triol-saponin returned to its control level after 24 hours. A maximum increase of protein synthesis by Panax Saponin A (PSA) containing triol-saponin was shown 4 hours after injection (Han *et al.*, 1973). A maximum increase in a nuclear RNA was also shown 4 hours after administration of *Radix ginseng* extract. But the rate returned almost to its control level after 24 hours in the rats (Oura *et al.*, 1971). From the present results of 3 hour treatment of triol-saponin and of the returned activity to its control level after 24 hours it seemed that time-course effects of the above mentioned protein synthesis and increase in the TP activity by ginseng would have a certain relation. And also, the result that the increased rate of biosynthesis by prostisol containing diol-saponin reached a maximum value after about 8 to 12 hours (Oura *et al.*, 1972) agreed with the present result showing the increased activity after 12 hours by diol-saponin treatment. Therefore it is thought that the increased activity by ginseng may due to the increased biosynthesis of the TP *in vivo*.

For one week treatment animals the same doses of saponins as described in methods were repeatedly injected every day. In this case, the TP activities by total saponins, diol-and/or triol-saponin were significantly increased by about 60% to 100%. This increasing effect of one week treatment is a contrast to the Jung and Kim's report (1971) that TP activity decreased from 6th day after X-ray irradiation.

The reason for the increase of the TP activity in one week treatment should be discussed. Increased TP activity was observed under the presence of adrenal gland by epinephrine and histamine (Knox and Mehler, 1951), X-ray and stressing drugs (Thomson and Mikuta, 1954). These agents cause the release of adrenocortical hormone. Suh (1960) considered that the eosinopenic response of the mice which were administered with *Panax ginseng* for 3 weeks was due to the increasing tendency of basal metabolic rate by accelerated utilization of the corticoids and resulting fall of corticoids in the circulation which may stimulate the liberation of ACTH from the pituitary gland. Another possibility for the eosinopenic response might be that the symphatomimetic action of *Panax ginseng* stimulate the adrenal medulla which in turn cause the secretion of the adrenalin. Moreover Oura and Hiai (1974) have postulated that the active ginseng principle seemed to have an ACTH-inducing action. Accordingly, ginseng principle may induce the ACTH secretion or accelerate the secretion of adrenalin, thereupon may cause the increase of TP activity. Another aspects of the increases in the TP activity by three kinds of ginseng treatment were showed by the experiment *in vitro* in one hour (Jung *et al.*, 1968).

From the above mentioned studies and the review by Harper et al. (1977), it should be considered the possiblities that under the control of endocrine system the TP activities in ginseng treated animals may be increased not only by the increased biosynthesis of the enzyme but also by stabilizing the existing enzyme against degradation as well as by the action of tryptophan analogue (e.g, ascorbate) forming oxidized holoenzyme or by the complex of these actions. This subject would be clarified by further studies.

The pattern of acceleration in the TP activity by ginseng treatment shows the characteristics of gradual process by long period administration. In view of distinguishing this characteristics from prementioned induced-adaptation. it can be called ginseng-related enzyme activity increase.

SUMMARY

0.02 mg of Korean ginseng total saponins, and/or 0.01 mg of diol- and/or triol-saponin per 10 g of body weight per day were subcutaneously injected in mice wei-

ghing about 20 g. The changes of tryptophan pyrrolase (TP) activity *in vivo* were determined and the following results were obtained.

1. With total saponins treatment, TP activity increased slightly after 3 hours, which was not statistically significant. The activity returned to its control level after 24 hours. One week treatment caused a significant 61.11% increase.

2. With diol-saponin treatment, TP activity increased slightly after 3 hours, which was not significant. The activity increased 64.98% after 12 hours, and then again returned to its control level after 24 hours. One week treatment caused a significant 100.58% increase.

3. With triol-saponin treatment, TP activity increased 59.17% after 3 hours and it returned to its control level after 24 hours. One week treatment caused a significant 66.98% increase.

REFERENCES

Badawy, A.A.-B. and M. Evans, 1975. The regulation of rat liver tryptophan pyrrolase by its cofactor haem. Experiments with haematin and 5-aminolaevulinate and comparison with the substrate and hormonal mechanisms. *Biochem. J.* **150**(3) : 511—520.

Badawy, A.A.-B. and M. Evans, 1976. The regulation of rat liver tryptophan pyrrolase activity by reduced nicotinamide-adenine dinucleotide (phosphate). Experiments with glucose and nicotinamide. *Biochem. J.* **156**(2) : 381—390.

Brekhman, I.I., G.D. Bierdishev and V.G. Golotin, 1971. Influence of *Eleutherococcus* and ginseng extracts on the activity and the adaptive synthesis of tryptophan pyrrolase in rats. *Izv. Akad. Nauk. USSR, Ser. Biol.* (1) : 31—37. *CA* 74, 139198s.

Gonall, A.G., C.J. Bardawill and M.M. Bavid, 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**(2) : 751—766.

Han, B.H., C.H. Kim and Y.N. Han, 1973. Stimulating effect of Panax saponins on the C^{14}-leucine incorporation. *Korean Biochem. J.* **6** : 63—71.

Harper, H.A., V.W. Rodwell and P.A. Mayes, 1977. Review of Physiological Chemistry. Lange Medical Publications, California, pp. 352.

Jung, N.P. and D.J. Kim, 1971. Effect of body weight and dual effect of X-ray irradiation on tryptophan pyrrolase activity. *Korean J. Physiol.* **5**(1) : 19—22.

Jung, N.P., S.C. Kim and C.K. Yoo, 1978. Effects of ginseng saponins and its fractions on tryptophan pyrrolase activity *in vitro. J. of Nat. Sci. Res. Inst., Yonsei Univ.* **1** : 47—51.

Knox, W.E., 1951. Two mechanisms which increase *in vivo* liver tryptophan peroxidase ativity: Specific enzyme adaptation and stimulation of pituitary-adrenal system. *Brit. J. Exp. Path.* **32** : 462—469.

Knox, W.E. and A.H. Mehler, 1951. The adaptive increase of the tryptophan peroxidase-oxidase system of liver. *Science* **113** : 237—238.

Knox, W.E. and V.H. Auerbach, 1955. The hormonal control of tryptophan peroxidase in the rat. *J. Biol. Chem.* **214**(1) : 307—313.

Lee, N.D., 1956. The induced increase in the tryptophan peroxidase activity of rat liver: Time studies. *J. Biol. Chem.* **219**(1) : 211—220.

Oura, H. and S. Hiai, 1974. Biochemical action of *Panax ginseng* principle. Proc. International Ginseng Symp. The Central Research Institute, Office of Monopoly, Seoul, pp. 23—32.

Oura, H., S. Hiai and H. Seno, 1971. Synthesis and characterization of nuclear RNA induced by *Radix ginseng* extract in rat liver. *Chem. Pharm. Bull.* **19**(8) : 1593—1605.

Oura, H., S. Nakashima, K. Tsukada and Y. Ohta, 1972. Effect of *Radix ginseng* on serum protein synthesis. *Chem. Pharm. Bull.* **20**(5) : 980—986.

Shibata, S., 1967. Active principle of ginseng roots. *Tampaku-Shitsu, Kakusan, Kosa* **12**(1) : 32—38.

Shibata, S., O. Tanaka, T. Ando, M. Sado, S. Tsushima and T. Oshawa, 1966. Chemical studies on oriental plant drugs XIV. Protopanaxadiol, a genuine sapogenin of ginseng saponins. *Chem. Pharm. Bull.* **14**(16) : 595—600.

Suh, K.S., 1960. The effects of *Panax ginseng* of the eosinophils in mice. *The New Medical Journal* **3**(12) : 91—99.

Thomson, J.F. and E.T. Mikuta, 1954. Effect of total body X-irradiation on the tryptophan peroxidase activity of rat liver. *Proc. Soc. Exp. Biol. and Med.* **85** : 29—32.

Woo, L.K., B.H. Han, D.S. Park and W.L. Lah, 1973. Special difference of dammarane glycones of ginsengs. *Kor. J. Pharmacog* **4**(4) : 181—184.