THE ROLE OF *PANAX GINSENG* IN DETOXIFICATION OF XENOBIOTICS

F.C. Lee, J.K. Park, E.K. Kim, J.K. Ko, J.S. Lee and K.Y. Kim
Korea Ginseng &Tobacco Research Institute, Korea

ABSTRACT

The balance between metabolic activation of xenobiotics and detoxification of their active metabolites may play a vital role in controlling mutagenic and carcinogenic processes. To assess the possible role of *P. ginseng* C.A. Meyer in detoxification of xenobiotics, we studied the effects of ginseng on several parameters of the monooxygenasd system, including benzo(a) pyrene monooxygenase(AHH) and benzo(a) pyrene epoxide hydratase(EH) as well as effects of ginseng on the conjugation system. Test animals receiving ginseng saponin-fraction induced epoxide hydratase activity to over 150% (20mg/kg b.w.) of the control and increased glutathione transferase activity (GSH-T) up to 140% (20mg/kg b.w.) of the control, whereas no significant changes were observed in the benzopyrene monooxygenase activity (AHH).

Such a selective induction of the inactivation enzyme epoxide hydratase, combined with a marked elevation of the detoxifying enzyme glutathione transferase, without a concurrent induction of benzopyrene monooxygenase which is responsible for the formation of carcinogenic intermediates, demonstrates that ginseng has the potential to alter the metabolic course of carcinogenic polycyclic aromatic hydrocarbons, and thereby enhance detoxification. Thus, ginseng may play an important role in the prevention of tumors caused by carcinogens.

INTRODUCTION

Polycyclic aromatic hydrocarbons(PAH) constitute an important class of potentially hazardous chemicals which are ubiquitous in our environment. They are universal products of the combustion of organic matter, such as gasoline. Cigarette smoke also contains a significant amount of these hydrocarbons, mainly BP.

It is now generally accepted that the mutagenic and carcinogenic effects of polycyclic aromatic hydrocarbons are due to metabolic activation of these hydrocarbons by the cyt. P-450 mediated monooxygenase system.[1] BP monooxygenase, that is, the aromatic hydrocarbon hydroxylase is responsible for the formation of potentially mutagenic and carcinogenic metabolites, such as arene oxides and diol epoxides. These active metabolites are present in most mammaliam tissues and represent ulitimate carcinogenic metabolites, because they react with nucleophiles of macromolecules in tissues, such as protein and DNA.

However, the biological system has means of removing these carcinogens from the system. Epoxides are converted by epoxide hydratase to less reactive dihydrodiols and phenols and readily excreted by the conjugation system. That is, this epoxide hydratase renders potential carcinogens less reactive and less toxic. Therefore, the balance between metabolic activation of benzopyrene to epoxides by aromatic hydrocarbon

hydroxylase and detoxification of the epoxides either by epoxide hydratase or by direct conjugation may play a vital role in controlling mutagenic and carcinogenic processes.

To assess the role of *P. ginseng* in detoxification of polycyclic aromatic hydrocarbons and other xenobiotics, we studied the effects of ginseng saponin-fraction on several parameters of the monooxygenase system, such as cyt. P-450, BP monooxygenase (AHH), BP-epoxide hydratase (EH), aminopyrine demethylase and aniline hydroxylase activity, as well as the effects of ginseng on conjugation capacity.

MATERIALS AND METHODS

1. Chemicals

^{3}H-benzo(a)pyrene(BP) (S.A. 40 Ci/mmole), ^{3}H-benzo(a)pyrene-4, 5-oxide(BP 4,5-oxide (S.A. 287.5 mCi/mmole), 3-hydroxybenzo(a) pyrene (3-OH BP) and 4, 5-dihydroxy-4, 5-dihydrobenzo(a)pyrene (BP 4, 5-diol) were obtained from Midwest Research Institute, Kansas City, MO., USA.

2. Animal Treatment

Sprague-Dawley rats(Male, ~ 200g) were used. Saponin-fraction dissolved in water was orally administrated. For long-term experiment (1-4 wks), animals were fed ad libitum a normal diet containing saponin-fraction.

3. Fractionation of Liver Homogenates

Livers from Sprague-Dawley rats fasted overnight were homogenized in Potter-Elvejam homogenizer with 4 parts of 50mM Tris buffer (pH 7.4) containing 0.25M sucrose. The homogenates were centrifuged twice at 10,000 x g to yield postmitocondrial supernatant. Microsomal and cytosolic fractions were obtained by centrifugation of the 10,000 x g supernatant at 100,000 x g for 1 hr.

4. Preparation of Ginseng Saponin-fraction

Alcoholic ginseng extract (75% ethanol) was diluted with water and extracted with ether. The aqueous layer was then extracted 3 times with buthylalcohol, the extracts combined, concentrated and partially purified by column chromatography over active charcoal.

5. Enzume Assays

CYTOCHROME P-450. The microsomal cytochrome P-450 content was determined by the method of Omura and Sato[2] employing the reduced versus reduced CO-difference spectrum (extinction coefficient 91 $mM^{-1} cm^{-1}$).

ARYL HYDROCARBON HYDROXYLASE (AHH). The aryl hydrocarbon hydroxylase (BP monooxygenase) activity was quantitated using ^{3}H-benzopyrene.[3,4] Assay mixture consists of 50 mM Tris-HCl (pH 7.4) containing 5 mM $Mgcl_2$, 0.3 mM NADPH, 0.1 mM ^{3}H-BP and 50μg protein (10,000 x g fraction) in a final volume of 0.1 ml and incubated for 10 min at 37°C. The enzymatic reaction was stopped by addition of chilled acetone (50μl). The major reaction product, 3-OH BP was separated by TLC (Quantum Industries) in benzene and TLC scrappings of the product were counted in a Beckman Liquid Scintillation Spectrophotometer.

EPOXIDE HYDRATASE (EH). Assays were performed[4] at 37°C in 0.1M glycin buffer (pH 9.2) containing 50μM ^{3}H-BP 4, 5-oxide and 10μg protein (10,000 x g fraction) in a final volume of 0.1ml. The enzymatic reaction was stopped by addition of 50μl chilled aceton. The reaction product, BP 4,5-diol was separated by TLC in benzene/EtOH (95:5) and radioactivity of the product was determined by liquid scintillation spectrometry.

AMINOPYRINE DEMETHYLASE. The demethlase activity was determined by measurement of HCHO release using Nash reagent.[5] The incubation mixture contained 20 mM phosphate buffer (pH 7.0), 40μM NADPH, 20 mM Nicotinamide, 10mM aminopyrine and microsomal protein in a final volume of 2 ml.

ANILINE HYDROXYLASE. Anilin hydroxylase activity was quantitated by measurement of p-aminophenol formed (630nm). The assay

mixture consisted of 20mM phosphate buffer (pH 7.0), 20mM nicotinamide, 40μM NADPH, 6.7mM aniline and microsomal protein in a final volume of 2ml.[5]

GLUTATHIONE TRANSFERASE (GSH-TRANSFERASE). The trnsferase activity was determined by employing 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate.[6] The assays were performed in 0.1M phosphate buffer (pH 6.5) containing 1mM glutathione, 1mM CDNB and cytosolic protein in a final volume of 3ml. The conjugation product was then quantitated spectrophotometrically at 340nm.

RESULTS AND DISCUSSION

Potentially mutagenic and carcinogenic materials such as arene oxides and diol epoxides are produced by the microsomal monooxygenase system (aryl hydrocarbon hydroxylase (AHH). When the hydroxylase activity was assayed in the presence of 50μgs of 10,000 x g fraction of liver homogenates, as shown in Fig. 1, control animals gave about 28,000 dpm which represents about 40 nmoles. In test animals the rate of 3-OH

ARYL HYDROCARBON HYDROXYLASE ACTIVITY

SAPONIN-FRACTION (mg/kg b.w.)	(^{3}H) – BENZOPYRENE 3-OH DPM x 10^{-3} (10, 20, 30, 40)
0 (CONTROL)	
10	
20	
30	

Fig. 1. Incubations were performed in 50mM Tris-HCl buffer (pH 7.4) containing 5mM $MgCl_2$, 0.3mM NADPH, 0.1mM ^{3}H-BP (0.3μCi/nmole) and 50 μg protein (10,000 x g supernatant fraction of liver homogenates) in a final volume of 0.1ml. Values represent means ± SD (n=12).

formation was also about 28,000 dpm at a 10 and 20mg dose of saponinfraction/kg b.w. and 26,000 dpm at a dose of 30mg. So, there was no significant change in AHH activity between the control and the test group which received saponin-fraction.

On the other hand, a significant induction effect was observed with epoxide hydratase (Fig. 2), which is believed to be an inactivator of the carcinogenic intermediates. Epoxide hydratase

EFFECT OF SAPONIN-FRACTION ON BENZOPYRENE EPOXIDE HYDRATASE ACTIVITY

SAPONIN-FRACTION (mg/kg n.w.)	EH ACTIVITY n moles/mg protein/min (1.0, 2.0, 3.0, 4.0, 5.0, 6.0)	
O (CONTROL)		100
10		107.7
20		154.3
30		150.8

Fig. 2. Assay mixture contained 50μM ^{3}H-BP 4, 5-oxide, 10μg protein (10,000 x g supernatant fraction of liver homogenates) and 0.1 M glycine buffer(pH 9.2) in a final volume of 0.1ml. Animals were sacrificed 24 hr after a single oral dose of Saponin-Fraction. Each value is expressed in means ± SD (n=15).

activity in control animals was 3.5 nmoles/mg protein/min. In test animals at a single dose of 10mg saponin-fraction there was a slight increase in activity. The induction level was then elevated to over 150% of the control at higher doses, 20 and 30mg/kg b.w. These contrasting effects obtained with aromatic hydrocarbon hydroxylase and epoxide hydratase indicate that ginseng brings about a selective induction of monooxygenase increasing only the level of the inactivation system, which is very important in detoxification of active metabolites.

Cyt. P-450 content was determined by the method of Omura and Sato, employing the reduced versus reduced-CO difference spectrum.

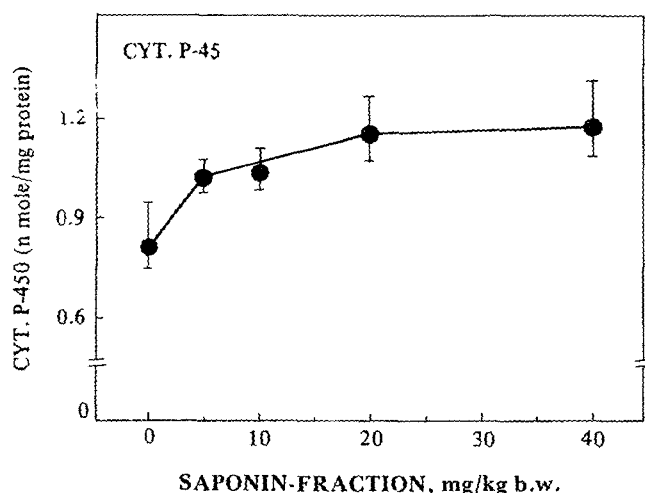

Fig. 3. Microsomal Cyt. P-450 content was determined utilizing the reduced versus reduced-CO difference spectrum. Values represent means ± SD (n=7).

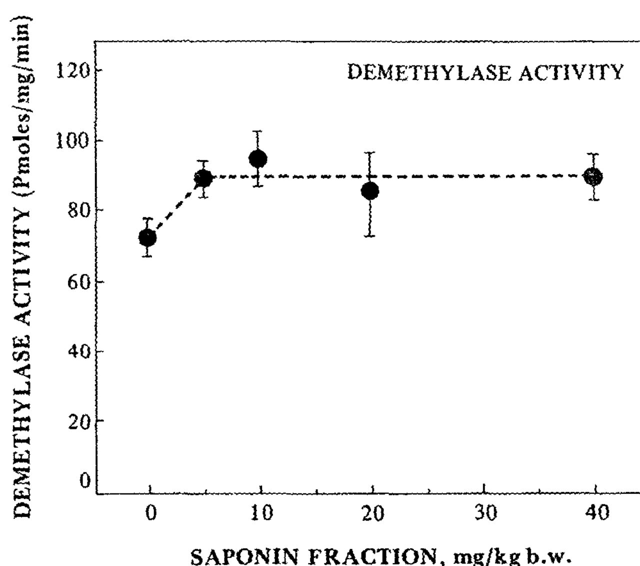

Fig. 4. Demethylase activity was determined by measurement of the amount of HCHO liberated (Nash reagent). Animals were sacrificed 24 hr. after a single oral dose of Saponin-Fraction. Results expressed are means ± SD (n=7).

Cyt. P-450 was increased, as shown in Fig. 3, in a dose dependent fashion to a maximum of 1.2 nmoles/mg protein, that is, 140% of the control level at a single dose of 20mg/kg b.w. and at 40mg about the same level of induction was maintained.

Aminopyrine demethylase activity (Fig. 4) determined by measurement of formaldehyde release exhibited an induction of 125% of the control at a single dose of 5mg saponin-fraction/kg b.w. and maintained the same level of induction even at 40mg dose. Quite a different effect was observed, however, with aniline hydroxylase. As you can see from the data shown in Table 1, after one week of treatment at a daily dose of 10 mg saponin-fraction/kg b.w., there was no significant change between the control and test group. Even after 4 weeks of treatment, control and test groups exhibited about the same activity—3.3 nmoles for the control group and 3.2 nmoles for the test.

The selective induction of monooxygenase parameters, such as cyt. P-450, epoxide hydratase and aminopyrine demethylase activity, without a concurrent induction of AHH and aniline hydroxylase activity, demonstrates that ginseng could be used as a valuable tool in the study of the role of EH in the overall biotransformation of PAH's leading to mutagenic and carcinogenic metabolites.

Table 1. Aniline Hydrxoylase Activity

Treatment (week)	Hydroxylase Activity (nmole/mg/min)	
	Control	Test
1	3.33 ± 0.01	3.20 ± 0.02
2	4.90 ± 0.01	5.10 ± 0.04
4	3.33 ± 0.03	3.50 ± 0.05

Hydroxylase activity was determined by measurement of p-aminophenol formed. Incubation mixture consisted of 20mM phosphate buffer (pH 7.0), 20mM nicotinamide, 40μM NADPH, 6.7mM aniline and microsomal protein in a final volume of 2ml. Test animals were fed a normal diet containing Saponin-Fraction. Results expressed are means ± SD (n=5).

Conjugation reaction is a true detoxifying reaction, bt which epoxides as well as other oxygenated metabolites are converted to water soluble conjugates and readily excreted. Therefore, the levels of conjugating enzymes may be key determinants of the course of BP metabolism. Effects of saponin-fraction of GSH transferase activity was determined by employing 1-chloro-2, 4-dinitrobenzene(CDNB) as substrate. In test

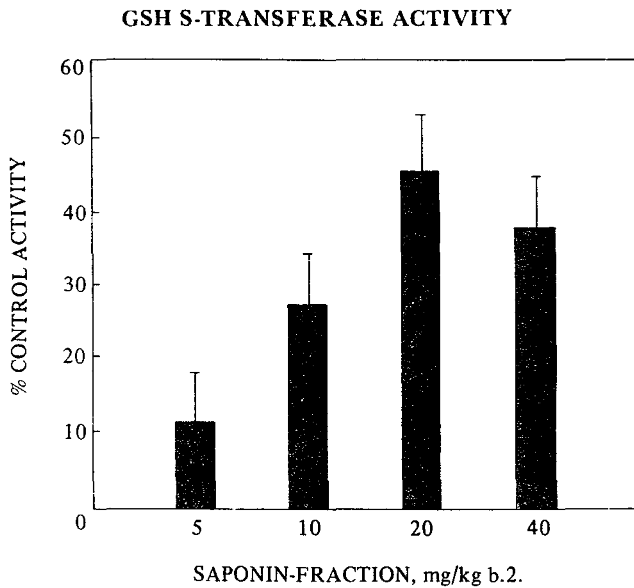

Fig. 5. GSH-T assays were performed in 0.1M phosphate buffer (pH 6.5) containing 1mM GSH, 1mM 1-chloro-2, 4-dinitrobenzene and cytosolic protein in a final volume of 3ml. The conjugation product was quantitated spectrophotometrically at 340nm. Results are expressed in means ± SD (n=9).

animals which received saponin-fraction, the transferase activity was increased in a dose dependent fashion, as shown in Fig. 5, up to 140% of the control activity at an optimal dose of 20mg/kg b.w. The activity was then slightly decreased at a higher dose of 40mg. In addition, we examined the effect of ginseng on glucose-6-phosphate dehydrogenase[7] which is an important enzyme in the control of the conjugation system. Preliminary experiments (data not shown) reveal that ginseng saponin-fraction also increased the G-6-P dehydrogenase activity.

CONCLUSION

Selective induction of benzopyrene epoxide hydratase activity and elevation of the conjugation capacity, brought about by ginseng saponinfraction suggests that ginseng has the potential to alter the metabolic course of carcinogenic polycyclic aromatic hydrocarbons, as well as other xenobiotics and thereby enhances detoxification.

Thus, ginseng may play an important role in the prevention of tumors, caused by potential carcinogenic compounds.

Cha: How did you assay the epoxide-hydratase activity? Was it by TLC scanning of the 4, 5-diol?

Lee: Yes, you are right.

Cha: Is there any evidence that ginseng has protective effect against chemical carcinogenesis? Do you know any evidence or is there any published literature on that?

Lee: Yes, there are numerous papers published. If you are interested in them, I will be able to give you some later.

Cha: These are *in-vitro* experiments. Do you have any plan to continue, for instance, with some organ level study?

Lee: Yes, of course, we'd like to proceed with an *in-vivo* experiment as long as funds and time permit.

독성물질 해독작용에 미치는 인삼의 효능

이재열, 박진규, 김은경, 고지훈, 이정숙, 김경영
한국인삼연초연구소, 한국

생체에 투입되는 이물질(xenobiotics)들이 돌연변이를 일으키고 나아가서는 암을 유발시키게 되는 것은 이물질들이 생체내에서 어떤 양상으로 대사되느냐, 즉 이물질이 독성화되는 과정과 해독되는 과정에 있어서의 활성도의 차이에 따라 좌우되는 것으로 알려져 있다.

따라서 인삼이 이물질 대사 및 해독작용에 어떤 영향을 미치는가를 밝혀보기 위하여 동물 실험을 통하여 발암물질로 알려진 Benzopyrene과 그 외 다른 유독한 화학약물들의 대사과정에 미치는 영향을 조사하여 보았다.

인삼투여군에 있어서 cyt. P-450와 관련된 Monooxygenase system이 선택적으로 유도되었고 또 해독작용에 필수적인 Conjugation도 현저히 상승되었다. 이와 같은 이물질 해독작용의 선택적 유도는 인삼이 이물질 대사과정에 영향을 미쳐 독성물질 해독 항진효능이 있음을 제시해 주는 것으로 해석되어진다.

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