Ginseng Prevents DNA-adduct Formation in Rat Hepatocytes *in vitro* Treated with DMBA

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ABSTRACT

It is an established fact that most of the carcinogens implicate bay-region diol epoxides as the ultimate carcinogenic metabolites. These electrophiles react with nucleophilic sites in the cells to form adducts. It is the formation of carcinogenic-DNA adducts that is thought to initiate carcinogenesis. In our previous study we have reported chemopreventive property of Ginseng on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice. In this study we have examined the effect on formation of DMBA-DNA adducts in rat hepatocytes pretreated with ginseng. Primary cultures of rat hepatocytes were used. The cells wete treated with ginseng for 24 hrs and then with DMBA (10μg) for 18 hrs. Cells were then harvested, their DNA was isolated and analyzed by P^{32} labelling. A significant reduction in the levels of DMBA - DNA adducts (adducts/10^8 nucleotides) was observed in all cultures pretreated with ginseng. The viability of cells was not affected by pre-treatment with ginseng. Our finding suggests that ginseng block or suppresses the events associated with chemical carcinogenesis by inhibiting metabolic activation of the carcinogens.

Introduction

It is an established fact that drug metabolizing and conjugative enzymes are critical determinants of the carcinogenecity of chemicals (Wattenberg,1985, Yang *et al.*, 1992, Morse and Stoner, 1993). Several plant products including those present in our diets have been shown to reduce the incidence and mortality of certain cancer in high-risk human populations (Coldin,1985, Prashar and Kumar, 1995, Prashar *et al.*, 1995 Banerjee *et al.*, 1996).

We have reported earlier the modulatory influence of ginseng on the activities of cytochrome P 450, cytochrome b_5 and arylhydrocarbon hydroxylase enzymes in the liver and gluathione-S-transferase and reduced glutathione level in the liver. These enzymes play an important role in the detoxification of carcinogens and mutagens. We have also reported the chemopreventive property of ginseng on 7,12-dimethylbenz(a)anthracene induced skin papillomagenesis in male Swiss albino mice. There was a significant reduction in the tumor formation (average number of tumors per mouse and cumulative number of papillomas) was observed in mice orally fed with ginseng (Kumar, 1993).

It is a well-established fact that most of the polycyclic hydrocarbons have either identified or implicated in the bay-region diol epoxides as the ultimate carcinogenic metabolites (Sims *et al.,* 1974, Jerina and Daly, 1976, Thakker *et al.,* 1979, Sims and Grover, 1981). These electrophiles react with nucleophilic sites in cells to form DNA adducts (Brooks and Lawley, 1964). It is the formations of carcinogen-DNA adduct formation that is thought to initiate carcinogenesis (Dipple *et al.,* 1993). In the present study an attempt has been made to observe the effect of rat hepatocytes pre-treated with ginseng on the DMBA-DNA adduct formation.

Materials and Method

Chemicals

7, 12-DMBA was purchased from Sigma Chemicals Co. USA. Ginseng extract was obtained from Amsar Co, Indore, India. Reagents and materials for P^{32} post labelling were done according to standard method.

Isolation and treatment of hepatocytes.

Liver was perfused *in situ* with solutions of collagenase and hepatocytes were isolated according to standard procedures (Berry *et al.,* 1991). Cell viability was tested by tryphan blue method. Cells were harvested in tissue culture flasks in modified culture medium containing 10% fetal calf serum. The flasks were kept in a humified incubator at 37℃ and 5% CO_2. 3-4 hrs later the unattached cells were removed and the medium was replaced by fresh medium without any fetal calf serum. The hepatocytes were treated with ginseng extract for 24 hrs and then the cultures were incubated for 18 hrs again after treatment with DMBA. 24hrs later the cells were harvested. DNA was isolated by a phenol/chloroform extraction procedure including RNAase digestion to remove RNA (Gupta, 1984). Cell pellets were suspended in 10 mM EDTA (0.5ml) containing 1% SDS and 0.5 mg proteinase K and incubated at 37℃ for 1 hr. The mixture was then extracted sequentially with equal volumes of phenol, phenol:chloroform:isoamyl alcohol(25:24:1), then chloroform:IAA (24:1, twice) 0.1 vol 5 M NaCl and 2 vol cold ethanol were then added. Precipitated DNA was redissovled in 1 mM SSC/EDTA (200ml) to which was added 50 mM Tris pH 7.4 (24ml), RNAase A (10mg/ml, 3 ml) and RNAase T1(5 U/ml, 3ml) and mixture incubated at 37℃ for 15 min. The solution was then extracted twice with chloroform: IAA and the DNA were precipitated by the addition of 5 M NaCl (20ml) and cold ethanol (2 vol).

^{32}P-Postlabelling analysis

^{32}P-postlabelling analysis, using the nuclease P_1 digestion method of sensitivity enhancement,was carried. Labeled adducts were resolved by polyethyleneimine cellulose TLC.

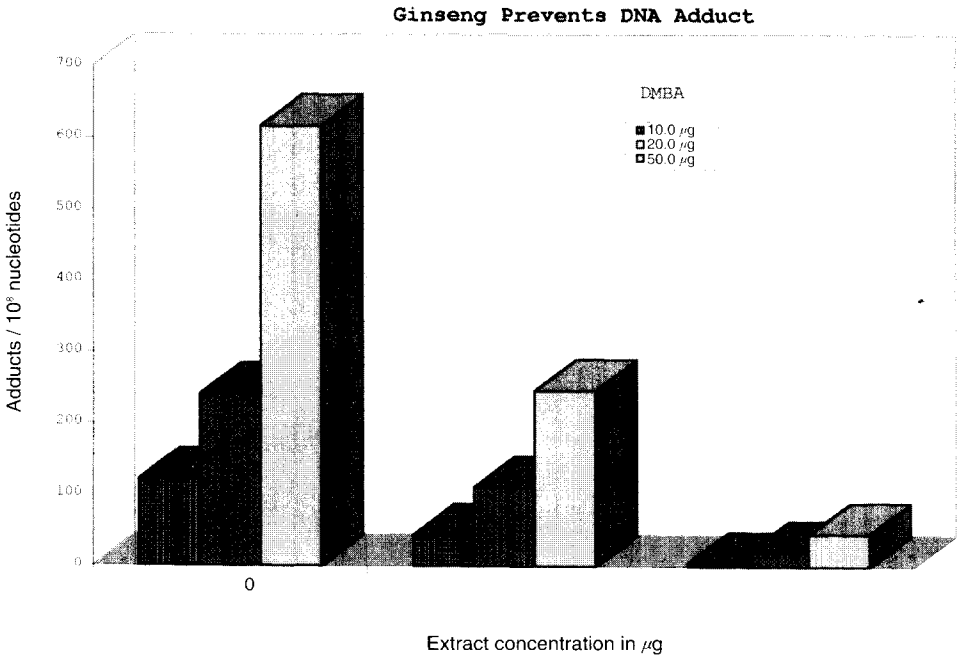
DNA Adduct

DNA adduct level was measured and quantified by comparing the amount of radioactivity detected in the chromatograms with the specific activity of the [γ-^{32} P] ATP used in the labeling reaction (Reddy and Randerath, 1986). The specific activity of the [γ-^{32}P] ATP was determined by measuring the T4 polynucleotided kinase-catalyzed incorporation of radioactivity into a known amount of 2' -deoxyadenosine 3' monophosphate(dAp).

The levels of adduct present in DNA samples were expressed as total numbers of adducts/10^8 nucleotides.

Results and Discussion

In the present study the hepatocytes treated with different concertration of ginseng did not show any change in their morphology as well as in their viability percentage. DNA adduct formation was determined by the measurement of radioactivity in the adduct spots observed on thin layer chromatogram. The radioactivity was measured due to ^{32}P- postlabelling. The DNA isolated from rat hepatocytes treated with DMBA showed the pattern of four spots. Similar pattern was observed in hepatocyte treated with different concentration of ginseng prior to DMBA treatment. However the intensities of the DNA adduct formation was less as compared to DMBA treated group alone. There were no radioactive adducts spots in the control cells, without any treatment of either DMBA or gin-



seng.

In cells pretreated with vehicle, the mean level of DNA adducts were 121.6, 240.7 and 616.4 adducts /10^8 nucleotides following treatment with 10, 20 and 50 μg DMBA respectively. However there was significant reduction in the adduct formation of the hepatocytes pretreated with 50μg of ginseng at all the doses of DMBA (43.8 adducts/10^8nucleotides at 10 μg, 110.7 adducts/10^8 nucleotide and 246.4 addusts/10^8 at 50μg. At higher doses of ginseng extract there was more significant decrease in the adduct formation (DMBA-DNA) at all the doses of DMBA. The adduct formation was reduced to 95%, 85% and 74% at 10, 20 and 50μg doses of DMBA.

In our previous report on chemopreventive action of ginseng on DMBA induced papillomagenesis in the skin of Swiss albino mice, we have already shown that there is a signigicant reduction in the tumor incidence, cumulative tumor frequency and tumor burden.

Several naturally occurring dietary or non-dietary consitituents, as well as parts of several species of edible plants having pharmacological activity, may influence the hepatic biotransformation enzyme profiles that are involved in activation and detoxicification of xenobiotic compounds, including chemical carcinogens (Bradford, 1976, Bradford and Bjeldanes, 1984 and Wattenberg, 1983). There is a reduction in the malanoaldehyde formation and cytochrome b_5 activity. There is no significant effect on arylhydrocarbon hydroxylase, DT-diaphorase and cytochrome P-450. However there is significant enhancement in the GSH level. In addition it was revealed that ginseng could augument the levels of soluble SH groups in the liver of mice (Kumar,1993). Ginseng has also been reported to have significant amount of phenolic compounds and vitamins. This GSH /GST enzyme system and the presence of phenols have been reported to be responsible for the conjugation of xenobiotics with glutathione and thus brings cellular detoxification. It is possible that ginseng by increasing the GSH contents and phenolic substance could potentially decrease the carcinogenic effect of DMBA (Meister and Anderson, 1983, Orrenius and Moldeus, 1984, Newmark, 1987, Ketterer, 1988 and Coles and Ketterer, 1990). 7,12-DMBA is a potent tumor initiator for mouse skin (Slaga *et al.*, 1979) and both it syn-and anti-bay region dihydrodiol epoxides are the main metabolites responsible for binding to DNA (Swaicki *et al.*, 1983, Dipple, *et al.*, 1983). The DNA adducts have been analysed by HPLC (Dipple *et al.*, 1983, Bigger *et al.*, 1983, Dipple *et al.*, 1984, Digiovanni *et al.*, 1985) and by ^{32}P-postlabelling(Randerath *et al.*, 1985). It has been established that the anti-diasteromer reacts mainly with adenine residues in DNA whereas the syn-diasteromer reacts mainly with guanine residues (Dipple *et al.*, 1983, DiGiovanni *et al.*, 1986). It is the formation of the carcinogen-DNA adducts that is thought to initiate carcinogenesis (Dipple *et al.*, 1993). The present study contribute information towards a dose dependent reduction in the DMBA-DNA adducts in mice hepatocytes pretreated with ginseng extract.

These results suggest that the inhibitory effect of ginseng on DMBA-DNA adduct formation have a chemopreventive effect.

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