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SHORT COMMUNICATION

In vitro chemopreventive effects of plant polysaccharides (*Aloe barbadensis Miller, Lentinus edodes, Ganoderma lucidum* and *Coriolus versicolor*)

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A plant polysaccharide, Aloe gel extract, was reported to have an inhibitory effect on benzo[*a*]pyrene (B[*a*]P)–DNA adduct formation *in vitro* and *in vivo*. Hence, chemopreventive effects of plant polysaccharides [*Aloe barbadensis Miller* (APS), *Lentinus edodes* (LPS), *Ganoderma lucidum* (GPS) and *Coriolus versicolor* (CPS)] were compared using *in vitro* short-term screening methods associated with both initiation and promotion processes in carcinogenesis. In B[*a*]P–DNA adduct formation, APS (180 μg/ml) was the most effective in inhibition of B[*a*]P binding to DNA in mouse liver cells. Oxidative DNA damage (by 8-hydroxydeoxyguanosine) was significantly decreased by APS (180 μg/ml) and CPS (180 μg/ml). In induction of glutathione *S*-transferase activity, GPS was found to be the most effective among plant polysaccharides. In screening anti-tumor promoting effects, APS (180 μg/ml) significantly inhibited phorbol myristic acetate (PMA)-induced ornithine decarboxylase activity in Balb/3T3 cells. In addition, APS significantly inhibited PMA-induced tyrosine kinase activity in human leukemic cells. APS and CPS significantly inhibited superoxide anion formation. These results suggest that some plant polysaccharides produced both anti-genotoxic and anti-tumor promoting activities in *in vitro* models and, therefore, might be considered as potential agents for cancer chemoprevention.

Cancer is one of the leading causes of death in the world despite newly developed tools for treatment and diagnosis (1). Chemoprevention is a preventive strategy used to reduce the incidence of human cancer either by inhibiting the initiation and spread of carcinogenesis or by preventing exposure to high levels of carcinogens (2,3). Much effort has thus been made in the search for cancer chemopreventive agents. Consequently, many agents have been shown to be effective for certain cancer chemoprevention, while other compounds are undergoing clinical trials (4–6). For the screening of potential chemopreventive agents, *in vitro* short-term tests can be applied because they are less time consuming, and also inexpensive and simple. Further *in vitro* tests can serve as biomarkers or endpoints of cancer to provide valuable insights into the mechanisms underlying carcinogenic processes and help screen for new chemopreventive agents (7,8).

Plant polysaccharides have traditionally been used around the world as a folk remedy for various diseases due to their multiple biological properties including anti-inflammation, wound healing, antihepatitis, anti-ulcer and anti-neoplastic effects (9–11). Aloe polysaccharide (APS) has recently been shown to exert an inhibitory effect on benzo[*a*]pyrene (B[*a*]P)–DNA adduct formation *in vitro* and *in vivo* (12). A number of other plant polysaccharides from *Basidiomycetes*, such as *Lentinus edodes* (LPS), *Ganoderma lucidum* (GPS), and *Coriolus versicolor* (CPS), have also been shown to produce anti-tumor effects, thus being potentially useful in cancer therapy (13–15). However, the mechanisms responsible for chemopreventive effects of plant polysaccharides are poorly understood.

In the present study, several structurally similar plant polysaccharides (APS, LPS, GPS and CPS) were screened for their chemopreventive effects using biomarkers involved in chemical carcinogenesis. Biomarkers used for initiation stage of cancer were: (i) DNA adduct formation (e.g. B[*a*]P–DNA adducts); (ii) 8-hydroxydeoxyguanosine (8-OH-dG), representing oxidative DNA damage; and (iii) induction of glutathione *S*-transferase (GST) activity. Biomarkers for promotion stage of cancer were (i) phorbol myristic acetate (PMA)-induced tyrosine kinase (TK) activity increase in human leukemia cells [HL-60; American Tissue Culture Collection (ATCC), Manassas, VA]; (ii) PMA-induced ornithine decarboxylase (ODC) activity elevation in Balb/3T3 cells (ATCC); and (iii) free radical formation in PMA-induced HL-60 cells (ATCC). LPS, GPS and CPS were prepared as described previously (15) and APS provided by ALOECORP (Harlingen, TX).

The total polysaccharide content was measured by the phenol–sulphuric acid method (16) and protein content was determined by the Bradford method (17). The sugar composition of crude polysaccharides was analyzed by HPLC (18). The purified polysaccharides were white to off-white fluffy powders with $<$16% protein and $>$67% carbohydrates (Table I). APS consists of ~0.5% protein and 85.1% polysaccharides (73% being mannose). Carbohydrate analysis indicated that glucose was the major sugar in GPS and CPS. However, mannose and arabinose were the major monosaccharides in LPS. A B[*a*]P–DNA binding assay was carried out with mouse normal liver NCTC clone-1469 cells (1×10^7) plated in culture flasks with NCTC-clone 115 medium (Gibco BRL, Grand Island, NY) supplemented with penicillin, streptomycin and 10% fetal bovine serum (FBS; Gibco BRL). The cells were maintained for 6 h, treated with ^{3}H-labeled B[*a*]P (4 nmol/ml, 52 Ci/nmol) (Amersham, Arlington Heights, IL) in the absence or presence of polysaccharides (6, 20, 60 or 180 μg/ml) and incubated for 12 h. After incubation, whole cells were harvested and DNA was isolated from the cells by addition of DNAzol (MRC, Cincinnati, OH). Concentration-dependent inhibition of

Abbreviations: 8-OH-dG, 8-hydroxydeoxyguanosine; APS, *Aloe barbadensis Miller* polysaccharide; CPS, *Coriolus versicolor* polysaccharide; GPS, *Ganoderma lucidum* polysaccharide; GST, glutathione *S*-transferase; LPS, *Lentinus edodes* polysaccharide; ODC, ornithine decarboxylase; PMA, phorbol myristic acetate; TK, tyrosine kinase.

Table I. Major components of plant polysaccharides and their sugar composition

Plant species	Polysaccharides (Glu, Man, Gal, Ara) (%)[a]	Protein (%)	Others (%)[b]
APS	85.1 ± 4.25 (7.2, 73.7, –, –)	0.5 ± 0.02	14.4
LPS	94.6 ± 1.72 (–, 64.0, –, 31.5)	0.4 ± 0.05	5.0
GPS	67.3 ± 3.68 (82.3, 1.6, –, –)	16.4 ± 2.49	16.3
CPS	89.5 ± 3.76 (62.5, 14.2, 2.7, –)	4.6 ± 0.25	5.9

Each value represents the mean ± SD of triplicate experiments.
[a]Percent sugar composition [glucose (Glu), mannose (Man), galactose(Gal) and arabinose (Ara)] in polysaccharide fraction.
[b]Other components include water, minerals and lipid.

Table II. A summary of *in vitro* chemopreventive effects of plant polysaccharides

Parameters	APS	LPS	GPS	CPS
Inhibition of B[*a*]P–DNA adduct formation	++	–	+/–	+/–
Inhibition of 8-OH-dG formation	+	+/–	+/–	+
Induction of GST	–	+/–	+	–
Inhibition of TK activity	+	+/–	+/–	+/–
Inhibition of ODC activity	+	–	+/–	+/–
Inhibition of superoxide anion formation	+	+/–	+/–	+

++, Strong positive effect in a dose-dependent manner ($P < 0.01$); +, positive effect in a dose-dependent manner ($P < 0.05$); +/–, moderate effect in a dose-dependent manner, but not significant; –, no effect.

^{3}H-B[*a*]P–DNA adduct formation was determined as described previously (19) and was observed only with APS treatment (Table II). APS at concentrations of 20–180 μg/ml significantly inhibited ^{3}H-B[*a*]P–DNA adduct formation by 20–50% of control (8.95 ± 2.13 pmol/mg DNA). Except for LPS, GPS and CPS at all concentrations produced moderate effects on DNA adduct formation. Using our experimental conditions, no cytotoxic effect on the 1469 cells was observed at 180 μg/ml concentration. Effects of APS, GPS, LPS and CPS on 8-OH-dG and superoxide anion formation are shown in Figure 1. The baseline levels of 8-OH-dG as measured by the method of Kasai *et al*. (20) in NCTC-clone 1469 cells were 2.47 ± 0.76 residues/10^5 dG (data not shown) whereas a significant increase in 8-OH-dG formation was observed in cells treated with B[*a*]P (4.47 ± 0.76 residues/10^5 dG). APS and CPS at concentrations of 180 μg/ml significantly inhibited 8-OH-dG formation, but LPS and CPS produced moderate effects on B[*a*]P-induced 8-OH-dG formation (Figure 1A). Effects of APS, LPS, GPS and CPS on free radical formation were investigated in PMA-induced HL-60 cells using a superoxide dismutase-sensitive cytochrome c reduction system as described by Pick and Mizel (21). In 96-well plates suspended in Hank's balanced salts solution (Gibco BRL), a time-dependent increase in cellular production of free radicals was observed in HL-60 cells (1×10^6 cells) treated with PMA (data not shown). As shown in Figure 1B, APS and CPS at 180 μg/ml concentration significantly inhibited the free radical formation in PMA-induced HL-60 cells. However, GPS and LPS produced moderate effects on PMA-induced inhibition of free radical formation. GST activity was measured in NCTC-clone cells (1×10^6 cells/dish, supplemented with 10% FBS) treated with or without plant polysaccharides as described previously (22). GPS significantly increased GST activities by 20–30%, but APS and CPS did not, and LPS moderately

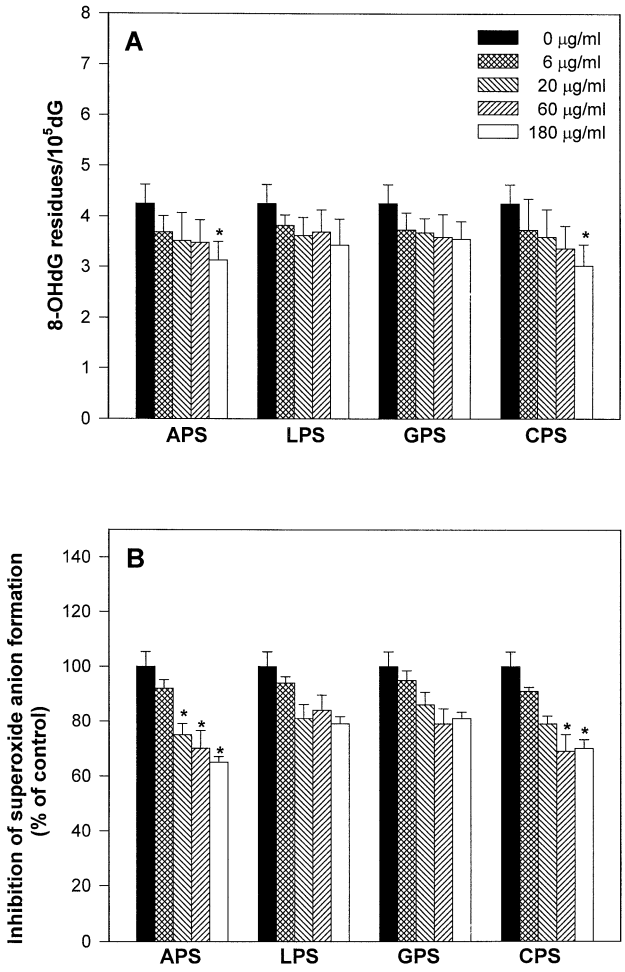

Fig. 1. Effects of polysaccharides on B[*a*]P-induced 8-OH-dG formation in NCTC-clone 1469 cells and on superoxide anion in HL-60 cells treated with PMA. (**A**) The cells were incubated for 24 h in medium containing B[*a*]P (1 μg/ml) in the absence or presence of polysaccharides. (**B**) The cells were treated with PMA (80 μM). Each value represents the mean ± SD of triplicate experiments. *, value is significantly different from control at $P < 0.05$. Quantitative differences between dose group values were analyzed statistically using ANOVA (analysis of variance) with a multiple comparison post-test by the Bonferroni method.

increased GST activities (Table II). PMA-induced TK activities were measured in HL-60 cells treated with APS, GPS, LPS and CPS (Table II). HL-60 cells were cultured in RPMI-1640 media (Gibco BRL) supplemented with 10% FBS and allowed to reach a maximum density of 1×10^6 cells/ml. The cells were then plated at a density of 5×10^5 cells in six-well plates, treated with PMA alone (0.1 μM in 0.001% dimethyl sulfoxide; Sigma, St Louis, MO) to induce TK activity, and immediately followed by the addition of genistein (10^{-3} M), as a positive control or with various concentrations of polysaccharides for 24 h at 37°C in an incubator containing 5% CO_2. TK activity was measured using poly(Glu–Tyr) (4.1:0.9; Sigma) as a substrate based on a previous method (23). In cells treated with PMA alone for 6 h, TK activity was significantly increased by 50% compared with control. Genistein, as a positive control, almost completely inhibited TK activity (data not shown). APS produced concentration-dependent inhibition of TK activity and was significantly inhibited by 30% at the highest concentration. In contrast, other polysaccharides induced moderate effects on TK activity. ODC activities were measured by the method

described previously (24). When Balb/3T3 cells were treated with PMA alone, ODC activity was significantly increased by 50% compared with control. Among plant polysaccharides tested, APS induced concentration-dependent inhibition of ODC activity and significantly decreased it by 20–30% of control at 180 μg/ml (Table II). However, LPS, GPS and CPS produced moderate effects on ODC activity.

Polysaccharides produced immunoenhancing and anti-tumor effects in several studies (9,10). Our previous study indicated that APS exerted anticarcinogenic effects on inhibition of B[*a*]P–DNA adduct formation by interfering with B[*a*]P absorption *in vivo* (12). The detection of DNA adducts in mammalian cells exposed to a particular carcinogen has been used as a biomarker for screening of anti-genotoxic chemopreventive agents. In the present study, the chemopreventive effects of polysaccharide extracts on B[*a*]P-induced DNA adduct formation in NCTC-clone cells were determined. A concentration-dependent inhibition of ^{3}H-B[*a*]P–DNA adduct formation was observed following APS treatment. It is possible that treatment with this polysaccharide inhibits the uptake of B[*a*]P and subsequently binding to cellular DNA.

It has been also suggested that the chemopreventive mechanisms of some compounds are related to the inhibition of carcinogen activation systems or to the induction of detoxification enzymes (25). In our study, GPS significantly increased GST activity by 20–30%. Although the induction of GST activity was low, it might be possible that plant polysaccharides could be effective in the induction of other detoxification enzymes. The mode of action of polysaccharides on GST activity is not clearly understood, but the induction of GST activity by GPS appears to be related to the amount of protein present in polysaccharide–protein complexes. Singh *et al*. (26) demonstrated that chlorophyllin also significantly elevated GST activity and glutathione levels in the liver and skin tissue.

Mechanisms of the inhibition of tumor promotion by polysaccharides toward different types of promoters remain unclear. However, tumor formation can be prevented by certain agents including retinoids, difluoromethylornithine (DFMO) and indomethacin, that block induction of ODC (27,28). Therefore, it is possible that ODC inhibition may be useful for the screening of chemopreventive agents. Moreover, it has been demonstrated that protein phosphorylation by TK is closely associated with cellular proliferation and differentiation. The inhibition of tyrosine TK may lead to suppression or reversal of carcinogenic processes (29). In our study, ODC and TK activities were significantly decreased by APS as compared with control in a concentration-dependent manner.

There are various naturally occurring products and synthetic compounds having both blocking and suppressing properties. The effect of certain fruits and vegetables containing high levels of natural antioxidants has been associated with scavenging of free radicals (30,31). Hence, the decrement in the rate of oxidative DNA damage induced by fruits and vegetables may serve as a basis for chemopreventive mechanisms. Liu *et al*. (32) suggested that mushroom polysaccharide extracts possessed superoxide and hydroxyl radical scavenging activities. However, the mechanism of free radical scavenging by polysaccharides is still not fully understood. It is possible that the protein content of polysaccharide extracts may be directly effective in free radical scavenging activity. It is known that phenolic compounds may react with superoxide radicals by a one-electron transfer mechanism (33). APS and CPS also significantly inhibited free radical formation in PMA-induced HL-60 cells, but it is not clear whether the mechanism of superoxide and hydroxyl radical scavenging by plant polysaccharides examined in this study is similar to that of phenolic compounds. In general, plant polysaccharides showed moderate or significant effects on the modulation of *in vitro* biomarkers associated with the carcinogenesis process. This effect might be related to the composition of sugars in polysaccharides as well as protein content.

In conclusion, the chemopreventive potential of polysaccharides was evaluated using biomarkers for carcinogenesis. Among polysaccharides tested, APS was found to be the most effective in anti-genotoxic and anti-promoting activities. GPS was also effective in GST activity induction and CPS was effective in inhibition of both 8-OH-dG formation and superoxide anion formation. LPS was the least effective and showed only moderate effects on some biomarkers such as 8-OH-dG, GST, TK and superoxide anion formation. These results suggest that some plant polysaccharides might be useful for chemoprevention application.

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