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Protective Effects of [6]-Shogaol on Cell Surface Glycoconjugates Abnormalities in 7,12-Dimethyldenz(a)anthracene (DMBA) Induced Hamsters Buccal Pouch Carcinogenesis

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KEYWORDS

[6]-shogaol, Glycoconjugates, 7,12-dimethyldenz(a)anthracene, Hamsters, Carcinogenesis

A B S T R A C T

Present study, we investigated that [6]-shogaol protect cell surface glycoconjugates abnormalities in 7,12-dimethyldenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral squamous cell carcinoma was induced by 0.5% of DMBA in hamster buccal pouch (HBP). The levels of glycoconjugates (Protein bound hexose, Hexosamine, Lipid bound sialic acid, Total sialic acid and Fucose) were analyzed by using specific colorimetric methods. The cell membrane glycoconjugates levels were significantly increased in DMBA induced hamsters. Our results shows, oral administration of [6]-shogaol (20 mg/kg b.wt) significantly decreased levels of protein bound hexose, hexosamine, total sialic acid, lipid-bound sialic acid and fucose in plasma and buccal tissue on tumor bearing hamsters. Overall findings concluded that phenolic compond of [6]-shogaol inhibit cell surface glycoconjugates abnormalities in DMBA induced HBP carcinogenesis.

Introduction

Glycoconjugates, a complex of proteins such as glycoproteins, glycolipids, glycopeptides, peptidoglycans, and lipopolysaccharides, are the essential components of the cell membrane. These cell surface elements are involved in cell differentiation and intercellular recognition and also play a crucial role during neoplastic transformation associated with abnormalities of cell surface molecules (Murray, 1996). Analysis of cell membrane glycoproteins levels in plasma and tissue could afford precious information for diagnosis and understanding tumor development, treatment monitoring and management of cancer (Manoharan *et al.*, 2004). Sialic acid is a carbohydrate sugar moiety play key role in the determination of cohesive and adhesive. Fucose plays a major

role in cancer and its spread to other neighbouring cells. Vasanti *et al.,*(1998) has been reported that fucose and mannose have important role in showing the development of cancer cells. Aberrant levels of glycoconjugates were reported in both human and experimental carcinogenesis (Manoharan *et al.,* 2004; Baskaran *et al.,* 2011).

Zingiber officinale Roscoe (Ginger) in the family of *Zingiberaceae*, it has long history and widely used for cooking and medicinal herb for various illness. Ginger has several constituent of pungent compounds and little quantity of [6]-shogaol are found in the fresh root of ginger, but much quantity in being the dried and cooking processed roots (Chrubasik *et al.,* 2005). [6]-shogaol is a phenolic nature and it has various chain lengths and α, β-unsaturated carbonyl moiety (Schwertner and Rios, 2007). [6]-shogaol is a natural dietary compound with well known pharmacological activities such as anti-inflammatory, neuroprotective, antipyretic, analgesic, and antitussive (Pan *et al.,* 2008a; Ha *et al.,* 2012; Tan *et al.,* 2013). However, no results available on [6]-shogaol protects the DMBA induced experimental carcinogenesis. This study evaluated the protective effect of [6]-shogaol on DMBA induced cell-surface abnormalities during DMBA induced hamster buccal pouch carcinogenesis model.

Materials and Methods

Chemicals

The chemical carcinogen of 7, 12-dimethylbenz (a) anthracene (DMBA), was purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India. And other chemicals (analytical grade) were obtained from Hi Media Laboratories, Mumbai, India. [6]-shogaol was purchased from Natural Remedies Pvt, Bangalore, India.

Animals

The golden Syrian hamsters, aged 8-10 weeks, weighing range between 80-120 g, were purchased from National Institute of Nutrition (NIN), Hyderabad, India. Animals were housed in polypropylene cages and provided certified standard pellet diet and water ad libitum at room temperature (27±2 °C) with relative humidity (55±5%) with a 12 h light- dark cycle in an experimental room. The experimental design was approved by IAEC, Annamalai University, (Register number 160/1999/CPCSEA/ 10801), Annamalainagar, India.

Experimental Design

A total number of 40 hamsters were divided into four groups. Group 1 animals served as untreated control. Groups 2 and 3 induced oral carcinogenesis by painting with 0.5% of 7,12-dimethybenz[a]anthracene (DMBA) in liquid paraffin three times per week for 16 weeks (Shklar, 1972). Group 2 received no other treatment. Group 3 orally administered with [6]-shogaol (20 mg/kg b.w) thrice a week on days alternate of the DMBA application. In group 4 animals were received [6]-shogaol alone at 40 mg/kg b.wt. At the end of the experimental period 16th week, animals were sacrificed after overnight fasting.

Biochemical Studies

The biochemical analyses were carried out in the plasma and buccal mucosa of experimental hamsters. The precipitate was obtained in plasma after treating with 95 % ethanol was used for the estimation of protein bound hexose and hexosamine. The defatted tissues obtained after treating buccal mucosa with methanol and chloroform was used for the estimation of glycoproteins. To the dry defatted tissues remaining after lipid extraction, 0.1N H_2SO_4

was added and hydrolyzed at 80°C for 1h. It was cooled and the aliquot was used for sialic acid estimation. To the remaining solution, 0.1N sodium hydroxide was added and kept in an ice bath for 1 h. From these aliquots, the protein bound hexose, hexosamine, total sialic acid and fucose were estimated by the methods methods of Niebes (1972), Wagner (1979), Warren (1959), and Dische and Shettles (1948) respectively

Statistical Analysis

Statistical results were expressed as mean ± SD. The significant differences between the groups were statistically assessed by DMRT using SPSS 11.0 software package. $P< 0.05$ was considered statistically significant.

Results and Discussion

Effect of [6]-shogaol on Plasma Glycoconjugates

The levels of cell surface glycoconjugates (protein bound hexose, hexosamine, total sialic acid, lipid-bound sialic acid and fucose) in the plasma of control and experimental hamsters in each group depicted in Table 1. The DMBA induced tumor bearing animals shows increased the levels of glycoconjugates in the plasma as compared to untreated control hamsters. Oral administration of [6]-shogaol (20 mg/kg b.wt) to DMBA induced hamsters significantly reversed the levels of glycoconjugates near to normal levels. However, [6]-shogaol alone treated experimental animals has no unpredictable changes in glycoconjugates level.

Effect of [6]-shogaol on Buccal Mucosa Glycoconjugates

Table.2 shows the levels of buccal mucosa glycoconjugates (protein bound hexose, hexosamine, and total sialic acid) in the control and experimental hamsters in each group. Over expression of glycoconjugates was observed in DMBA induced buccal mucosa as compared to control hamsters. Additionally, oral administration of [6]-shogaol (20 mg/kg b.wt) to DMBA induced hamsters significantly revert the levels of protein bound hexose, hexosamine, and total sialic acid to near normal level. Control and [6]-shogaol alone treated experimental animals has no changes in the levels of glycoconjugates.

Table.1 Plasma Glycoconjugates Levels in Control and Experimental Animals in each Group

Treatment group	Protein bound hexose (mg/dl)	Protein bound hexosamine (mg/dl)	Total sialic acid (mg/dl)	Lipid-bound sialic acid (mg/dl)	Fucose (mg/dl)
Control	92.17 ± 8.46^{a}	68.12 ± 5.83^{a}	47.18 ± 3.97^{a}	15.86 ± 1.57^{a}	8.34 ± 0.66^{a}
DMBA	161.10 ± 15.09^{b}	129.81 ± 8.73^{b}	77.5 ± 6.53^{b}	29.2 ± 2.06^{b}	21.57 ± 1.90^{b}
DMBA+ [6]-shogaol	93.67 ± 9.39^{ac}	70.32 ± 6.51^{ac}	47.94 ± 4.30^{a}	16.02 ± 1.08^{a}	8.81 ± 0.74^{a}
[6]-shogaol alone	91.98 ± 9.08^{a}	68.38 ± 7.25^{a}	47.01 ± 4.85^{a}	15.78 ± 1.22^{a}	8.89 ± 0.78^{a}

Values are expressed as mean ± SD (n=10). Values that are not sharing a common superscript in the same column differ significantly at $p < 0.05$

Table.2 Buccal Mucosa Glycoconjugates Levels in Control and Experimental Animals in each Group

Treatment group	Protein bound Hexose (μg/mg protein)	Protein bound hexosamine (μg/mg protein)	Total sialic Acid (μg/mg protein)
Control	83.79 ± 7.63[a]	18.56 ± 1.59[a]	16.01 ± 1.22[a]
DMBA	162.57 ± 15.38[b]	49.32 ± 4.30[b]	31.23 ± 2.36[b]
DMBA+ [6]-shogaol (20mg/kg b.w)	91.05 ± 8.29[a]	20.13 ± 1.64[a]	16.98 ± 1.42[a]
[6]-shogaol alone (20mg/kg b.w)	82.9 ± 7.08[a]	18.78 ± 1.61[a]	16.31 ± 1.15[a]

Values are expressed as mean ± SD (n=10). Values that are not sharing a common superscript in the same column differ significantly at $p < 0.05$

Cell surface glycoconjugates principal role in the regulation of epithelial cell proliferation and communicate cell signalling and diagnostic marker for carcinogenesis. In the present study, we analysed the cell surface glycoconjugate in DMBA induced HBP carcinogenesis. The functionally altered glycoproteins are over expressed, it affect cell to cell interaction and metastatic characteristics it take place in cancerous cells. Several studies reported that aberrant cell surface glycoconjucates are well documented in both human and experimental carcinogenesis model (Suresh *et al.,* 2007; Rajalingam *et al.,* 2008; Senthil *et al.,* 2007; Manoharan *et al.,* 2012).

In the present study, observed over expression of glycoconjucates in plasma and buccal mucosa on DMBA-induced tumor bearing animals. Similarly, Silvan et al (2011) has been reported that increased cell surface glycoproteins were due to abnormality glycosylation and elevated lysosomal hydrolases activities in cancer. Another report documented that over expression of glycoproteins were significantly increased in malignant tissue and plasma (Senthil *et al.,* 2007; Aranganathan *et al.,* 2005). Over expression of fucose is a terminal pentose sugar of glycoprotein chain content due to anomalous fucosylation in cancer. It has been predicted that up normal expression sialyl and glucosyl transferase activities which could be accountable for modification of cell surface glycoconjugates in cancerous condition. Malignant cells stimulate synthesis of glycoconjugates, and it subsequently shedding into the plasma as well as related tumor bearing buccal tissues (Vasanti *et al.,* 1998; Sebzda *et al.,* 2006).

Generally, phytotochemical has antioxidant properties that protect cell membrane abnormalities and inhibit tumorgenesis (Suresh *et al.,* 2007; Silvan *et al.,* 2011). In our results, oral administration of [6]-shogaol could inhibit the abnormalities of cell surface glycoconjugates in the plasma and buccal tissues in DMBA induced hamsters. Thereby, our results strongly suggested that [6]-shogaol protect cell surface abnormalities on malignant transformation. The phenolic agent of [6]-shogaol, having α, β-unsaturated carbonyl moiety. Thus structural specificity may inhibit oxidative damage of cell membranae and cellular proteins. Srinivasan *et al.*,(2008) showed that the phenolic nutrients possess inhibitory action against carcinogenesis. Thus the phenolic agent may significantly inhibit the action of glycosyltransferases thereby modulate glycoconjugates synthesis and protected the

cell surface abnormality, structural integrity indicating its potent anticancer property.

Conclusion

Our results suggest that [6]-shogaol has considerable potential to protect the cell surface glycoconjugates during DMBA-induced oral carcinogenesis. The protective effect of [6]-shogaol is probably due to its suppressive effect on glycoprotein synthesis by modulating the activities of the enzymes involved in the glycosylation. Further studies are therefore be warranted to assess the [6]-shogaol efficacy on the activities of enzymes involved in the process of glycosylation.

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Conflict of Interest

The authors have declared having no conflict of interest.

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