Original Research Paper

Volume-9 | Issue-8 | August - 2019 | PRINT ISSN No. 2249 - 555X

Zoology

SULFORAPHANE REDUCED GENOTOXICITY OF AFLATOXIN B1 IN HUMAN LYMPHOCYTE CULTURE.

Md. Sultan Ahmad* — Department of Zoology, S.N. (PG) College, Azamgarh, 276001 (U.P) India. *Corresponding Author

Afsar Ali — Department of Zoology, S.N. (PG) College, Azamgarh, 276001 (U.P) India.

ABSTRACT This work was carried out to study the antigenotoxicity of sulforaphane against Aflatoxin B_1, using Four concentrations viz; 40, 50, 60, and 80 µg /ml in the study. The data were collected at three durations of 24, 48, and 72 h for *in vitro* experiments. It was found that Sulforaphane significantly reduced chromosomal aberrations from 29.49, 52.47 and 68.65% at 24, 48, and 72h produced by Aflatoxin B_1 to 14.32, 28.00 and 41.88% level, similarly sister chromatid exchanges get reduced from 16.76 ± 1.50(SE) to 8.64 ± 0.75(SE) at 48 h of treatment and replication index was enhanced from 1.27 to 1.54 *in vitro*. In this study we have noticed the chromosomal aberrations (CAs), sister chromatid exchanges (SCEs) were reduced and replication index was enhanced in human lymphocyte cultures. The trend of reduction was dose-dependent.

KEYWORDS : Sulforaphane, Antigenotoxicity, Chromosomal aberrations, Sister chromatid exchanges, Replication index.

1. INTRODUCTION

It was found direct relationship between chromosome abnormalities, mutagenesis and carcinogenesis [1], *in vivo* and *in vitro* screening for chromosome aberrations is an established tool in the evaluation of potential hazards to the genetic material due to environmental agents. Aflatoxin B_1 is potent carcinogen [2]. It is consistently genotoxic, producing chromosomal anomalies *in vivo* and DNA damage, gene mutation and chromosomal anomalies *in vitro*. Sulforaphane (1-isothiocyanate-4-methyl sulfinyl butane) is a natural compound, found in cruciferous vegetables especially in young broccoli sprout and cabbage. It was found that sulforaphane is the most potent booster of phase-2-enzymes, acted as defense mechanism, triggering broad-spectrum antioxidant activity that neutralizes many free radicals, cycling over and over again before they can cause the cell damage that may cause mutations leading to cancer [3]. Sulforaphane blocked the formation of mammary tumors in rats treated with a potent carcinogen [4]. In our earlier study, we have shown the antigenotoxic effects of vitamins of C and E and some natural products in human lymphocytes culture [5, 6]. In the present study, we have evaluated the protective effects of sulforaphane against Aflatoxin B1 induced genotoxicity in human lymphocyte cultures. Sulforaphane reduced the chromosomal aberrations, sister chromatid exchanges and the replication indices were enhanced in the *in vitro* experiments.

2. MATERIALS AND METHODS

List of test chemicals and their doses

Chemicals	Doses (g /ml)	Source
1.Aflatoxin B1	50	Sigma, St. Louis, MO
2 Sulforaphane	40, 50, 60, 80	Sigma, St. Louis, MO

2.0. *In vitro* study

2.1. *Chromosomal aberration analysis*

Human lymphocyte cultures were set by adding 0.5 ml of whole blood (from two adult and healthy donors, occupationally not exposed to mutagens) to 4.5 ml of RPMI 1640 (Gibco, USA). Lymphocytes were stimulated to divide by adding 0.1 ml of phytohaemagglutinin– M (PHA– M, Gibco). The cultures were incubated at 37°C with 5% CO_2 for 72 hours in dark. Aflatoxin B1 at a final concentration of 50 g was added at 0 hour and kept for 24, 48 and 72 hours of duration, which served as positive control. Subsequently, sulforaphane was added along with Aflatoxin B1 and the cultures were kept for 24, 48 and 72 hours. Solutions of Aflatoxin B1 were prepared in DMSO and were treated along with S_9 mix (0.8 ml) in cultures. After 6 hours of incubation the cells were collected by centrifugation and the pellets were washed twice in pre warmed (37°C) medium to remove the drug and S_9 mix and re-incubated for 24 hours in fresh medium supplemented with antibiotic and fetal calf serum. Colchicines 0.20 g/ml, (Micro lab) were added to the cultures, 2.5 hours prior to harvesting. The cells were collected by centrifugation (10 min, 1200 rpm), the hypotonic treatment (0.075 KCl) was given for 10-12 min at 37°C and the recollected cells after centrifugation were fixed in methanol: acetic acid (3:1). Preparation of slides, staining and scanning was done under code. A total of 300 well - spread metaphases were analyzed per treatment per duration for all types of chromatid and chromosome type of aberrations and aberrations were scored [7].

2.2. *Sister chromatid exchange analyses*

Analysis of SCE was carried out following the fluorescent plus Giemsa technique [8]. The cells in the cultures were exposed to 5-bromo-2 – de oxyuridine (BrdU 2 g/ml; Sigma) after 24 hours of initiation of culture. After 90min. of this pulse treatment the cells were spun down and the supernatant discarded. The cells were washed twice to remove any traces of the drug, phyto-products and the liver metabolites. Finally the cell pellets were re-suspended in fresh medium supplemented with fetal calf serum, antibiotics and BrdU, and kept for another 24 hours in the dark at 37°C. One day old slides were stained in Hoechst 33258 stain (Sigma 0.5 g/ml), exposed to UV lamp (254 nm) for 30 min. and incubated in 2X SSC (0.3 M NaCl, 0.03M Sodium citrate; pH 7.0) at 60°C for 90 min and stained for sister chromatid. The slides were coded prior to scoring and 50 well- spread metaphase cells were scanned per concentration and the number of exchange scored .

2.3. *Cell cycle kinetics*

Cells undergoing 1st (M_1), 2nd (M_2) and 3rd (M_3) metaphase divisions were detected with BrdU – Harlequin technique for differential staining of metaphase chromosome by studying 200 metaphases for each combination and duration. The replication index (RI), an indirect measure of studying cell cycle progression, was calculated by applying the following formula [9].

$$RI = \frac{M_1 x_1 + M_2 x_2 + M_3 x_3}{100}$$

2.4. *Statistical analyses*

Student's two-tailed "t" test was used for calculating the statistical significance in CAs and SCEs, where as 2x3 chi- square test (χ^2) was used to analyze the cell cycle kinetics.

3. RESULTS

***In vitro* effect of Sulforaphane**:

The role of sulforaphane in reducing metaphase aberrations due to Aflatoxin B_1 is quite significant, being from (29.49, 52.47, and 68.65%) to (14.32, 28.00, and 41.88%) at the highest dose (E_4) and for the three different duration viz., 24, 48 and 72 h. There was no any change in basal clastogeny of the cell (2.00 and 2.75) by sulforaphane. The effects showed linear dose-response relationship. The effective maximum reductions in the clastogeny were 51.44, 46.63 and 39.0% percent at three different durations respectively as shown in Fig.1.

The effect on sister chromatid exchange counts were similarly reduced; however the experiments were conducted only for 48 h for all treatments including control. The 50 metaphases were scored for each treatment along with S_9 treatment as shown in Table 1. Aflatoxin B_1 produced 14.52 and 15.76 mean SCE per cell, in the absence as well as in the presence of S_9 mix. Those values were reduced to 08.38 and

08.64 SCE per cell due to highest concentration of sulforaphane.

The replication index calculated showed significant elevation of R.I in comparison to Aflatoxin B_1. Here the dose-effect relationships were almost linear as shown in Table 2.

4. DISCUSSION

In another work Zhang et al [4] in their earlier study had shown that the anticarcinogenic activities of sulforaphane and structurally related synthetic norbormyl isothiocyanate is related to phase-II enzyme induction that is associated with reduced susceptibility of animals and their cells to the toxic and neoplastic effects and block chemical carcinogens. In view of this result authors have studied the capacity of sulforaphane to inhibit the genotoxicity of Aflatoxin B_1. Sulforaphane helps to mobilize the human body's natural cancer fighting resources and reduced the risk of developing cancer. Within hours of being ingested, sulforaphane enters the blood stream where it circulates widely to trigger the immune system response to carcinogens. The phytochemical acts by inducing a series of proteins termed phase-2 detoxification enzymes, which act as scavengers for cancer causing molecules before they can damage DNA and promote cancer. Studies have shown that eating two pounds of broccoli a week can provide enough sulforaphane to lower colon cancer risk by half [10].

In a study conducted by Gamet-Payrastre et al [11] shows that sulforaphane induce cell cycle arrest and apoptosis in HT_{29} human colon cancer cells and aid in the fight of colon cancer. Frydoonfar et al [12] have studied the effect of Sulforaphane on cell proliferation of an HT_{29} colon cancer cells, was significantly reduced by sulforaphane.

The mechanisms by which L-Sulforaphane causes growth arrest and apoptosis induction have been studied extensively in human prostate cancer cells [13]. It has been demonstrated that the L-Sulforaphane - induced generation of reactive oxygen species, is caused by inhibition of mitochondrial respiratory chain enzymes [14]. Oral administration of sulforaphane inhibited prostate cancer progression and pulmonary metastasis in a transgenic mouse model of prostate cancer without causing weight loss or any other side effects [15]. The L-Sulforaphane mediated inhibition of prostate cancer development and metastasis in transgenic mice correlated with suppression of cellular proliferation and augmentation of natural killer cell lytic activity [16]. Thus the above discussion supported our finding, which shows that sulforaphane reducing the genotoxic effects of Aflatoxin B_1 *in vitro* system.

Acknowledgement

University Grant Commission [UGC F.No.42-500/2013(SR)], New Delhi for providing major research projects.

Table 1 Analysis of sister chromatid exchanges (SCE) after treatment with Aflatoxin B1 along with Sulforaphane *in vitro,* in the absence as well as presence of S_9 mix.

Treatment	Duration (h)	METAPHASE SCORED	Total SCE -S_9	Total SCE +S_9	Range	SCE /Cell ± SE -S_9 mix.	SCE /Cell ± SE +S_9 mix.
Aflatoxin B_1	48	50	726	788	3- 12	14.52± 1.00	15.76± 1.00
AF B_1+SF_1	48	50	662	615	3- 12	13.24± 1.00	12.30± 1.00
AF B_1+SF_2	48	50	610	552	2- 12	12.20± 1.00	11.04± 1.00
AF B_1+SF_3	48	50	526	490	1-11	10.52± 0.75	09.80± 0.75
AF B_1+SF_4	48	50	419	432	1.11	08.38± 0.70	08.64± 0.75
Normal	48	50	214	213	0-6	04.28± 0.40	04.26± 0.40
DMSO	48	50	219	217	0-6	04.38± 0.40	04.34± 0.40
DMSO+SF_3	48	50	215	215	0-6	04.30± 0.40	04.30± 0.40

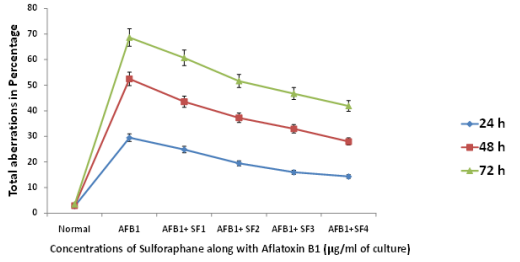
Note: SF; concentrations of Sulforaphane, AFB1 x/kg.bw; Aflatoxin B1 5 µg/ml/culture, SE; Standard error, DMSO; dimethyl sulphoxide. Calculations were significant at < 0.05 probability level

Table2 Analysis of cell cycle kinetics after treatment with Aflatoxin B1 along with Sulforaphane *in vitro,* in the absence as well as presence of S_9 mix.

Treatment	Cell scored	S9-mix	(%) cell in			Replication Index	2×3 chi square test
			M_1	M_2	M_3		
Aflatoxin B_1	200	-S_9	68	29	03	1.35	
		+S_9	75	23	02	1.27	
AF B_1+SF_1	200	-S_9	64	32	04	1.40	
		+S_9	72	25	03	1.31	
AF B_1+SF_2	200	-S_9	59	35	06	1.47	Significant
		+S_9	65	29	06	1.41	Significant
AF B_1+SF_3	200	-S_9	54	39	07	1.53	Significant
		+S_9	57	34	09	1.52	Significant
AF B_1+SF_4	200	-S_9	51	42	07	1.56	Significant
		+S_9	55	36	09	1.54	Significant
Normal	200	-S_9	38	53	09	1.71	
DMSO	200	+S_9	34	54	12	1.78	
DMSO +SF_3	200	+S_9	35	52	13	1.78	

Note: 2×3 Chi square (χ^2) test were conducted, SF concentrations Sulforaphane, AFB1 x/kg.bw; Aflatoxin B_1 5 µg/ml/ culture, DMSO; dimethyl sulphoxide. Calculations were made at < 0.05 probability level.

Fig. 1



REFERENCES

[1]. Snadberg AA. The chromosomes in human cancer and leukemia. Elsevier 1980, New York.
[2]. Bhattacharya RK, Francis AR, Shetty TK. 'Modifying role of dietary factors on the mutagenicity of Aflatoxin B1: In vitro effect of vitamins'. Mutation Res 1987; 188: 121-128.
[3]. Talalay P, Cho CG, Posner GH. "OPC Factor Sulforaphane." Johns Hopkins University (USA) 1992.
[4]. Zhang Y, Kensler T, Cho CG, Posner GH, Talalay P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothicyanates. Proc Nat Acad Sci (USA) 1994; 91: 3147-3150
[5]. Ahmad S, Hoda A, and, Afzal M. Additive action of vitamins of C and E against hydrocortisone-induced genotoxicity in human lymphocyte chromosomes. Int J Vit Nutr Res 2002, 72(4), 204 -209.
[6]. Ahmad MS, Sheeba, Afzal M. Amelioration of genotoxic damage by certain phytoproducts in human lymphocyte cultures. Chem Biol Interactions 2004; 149, 107-115.
[7]. Maron DM, Ames BN. Revised method for the Salmonella mutagenicity test. Mutat Res 1983; 113:173-215.
[8]. Latt SA, Wohllel JC. Optical studies of the induction of 33258 Hoechst with DNA, Chromatin and metaphase chromosomes. Chromosome 1975; 52: 297-316.
[9]. Tice R, Schneider EL, Rary JM. The utilization of BrdU incorporation into DNA for the analysis of cellular Kinetics. Exp Cell Res 1976; 102: 232-236.
[10]. Fahey JW, Zhang Y, Talalay P. Broccoli Sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens, Proc. Natt Acad Sci

(USA) 1997; 94(19): 10367-10372.
[11]. Gamet- Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, Chevolleau S et al. Sulforaphane a naturally occurring isothiocyanate induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer Res 2000; 60 (5): 1426-1433.
[12]. Frydoonfar DR, McGrath, Spigelman AD. Sulforaphane inhibits growth of a colon cancer cell line. Col Disease 2004; 6(1): pp.28.
[13]. Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E. Sulforaphane inhibits histone deacetylase activity in BPH-1, Ln-Ca-P, and PC-3 prostate epithelial cells. Carcinogenesis 2006; 27: pp.811–9.
[14]. Xiao D, Powolny AA, Antosiewicz J. Cellular responses to dietary cancer chemopreventive agent D, L-sulforaphane in human prostate cancer cells are initiated by mitochondrial reactive oxygen species. Pharmaceut Res 2009; 26: 1729–1738.
[15]. Singh SV, Herman-Antosiewicz A, Singh AV. Sulforaphane-induced G2/M phase cell cycle arrest involves checkpoint kinase 2 mediated phosphorylation of Cdc25C. J Biol Chem 2004; 279: pp.25813–22.
[16]. Burnstein KL. Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. J Cell Biochem 2005; 95: pp.657–69.