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The Effects of Aflatoxin B1 on Chromosome Aberrations and Sister Chromatid Exchanges in Human Lymphocytes

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ABSTRACT

Aflatoxin B1 (AFB1) is a ubiquitous mycotoxin produced by toxicogenic *Aspergillus* species. AFB1 has been reported to cause serious adverse health effects, such as cancers and abnormal development and reproduction, in animals and humans. The aim of this study was to investigate the ability of aflatoxin B1 (AFB1) to induce chromosome aberrations (CA) and sister chromatid exchanges (SCE) in human lymphocytes. AFB1 exposure significantly increased the number of CA and the frequency of SCE when compared with the control group. This significant increase in CAs as well as SCE may be attributed to the fact that AFB1 can induce genotoxicity through DNA damage. Thus, the present study indicates that AFB1 was genotoxic in human lymphocytes.

Introduction

Mycotoxins are toxic secondary metabolites produced by fungi. The most prominent mycotoxin, aflatoxin, belongs to a group of difuranocoumarins that is produced by *Aspergillus flavus* and *Aspergillus parasitic* (Busby and Wogan, 1984). These kinds of chemical substance can easily contaminate a variety of plants contented to human usage, as an example, corn, peanut, sorghum, wheat, nut, and rice (Cleveland et al., 2003). Aflatoxin production depends on many factors such as substrate, temperature, pH, relative humidity and the presence of other fungi. It has been identified 18 types of aflatoxins; the most frequent in foods are B1, B2, G1, G2, M1, and M2 (Bhatnagar et al., 2002). Aflatoxin B1 (AFB1) is actually one of the most important mycotoxin around the world as a result of its own wide-spread occasion, its own higher poisoning as well as its own financial effects (Mary et al., 2015). Aflatoxin B1 is the most potent hepatocarcinogen known and is classified by the International Agency for Research on Cancer in class 1B (carcinogenic to humans) (WHO, 1993). It is also the most prevalent aflatoxin usually found in cases of aflatoxicosis and is responsible for acute toxicity, chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (Creppy, 2002).

Several reports have shown the detrimental effects of AFB1 on the liver (Hassan et al., 2015), testis, epididymis (Agnes and Akbarsha, 2001; Hamzawy et al., 2012), Kidney (Abdel-Hamid and Firgany Ael, 2015; Arora et al., 1978), Heart (Abdulmajeed, 2011; Mohamed and Metwally, 2009), ovary (Ibeh et al., 2000), and the brain (Bahey et al., 2015). Aflatoxin B1 mutagenic effects have been well documented in a number of *in vitro* and *in vivo* models, where the presence of DNA adducts, DNA breaks, gene mutations, induction of DNA synthesis and inhibition of DNA repair have been determined, as well as increases in the rate of chromosomal aberrations, micronuclei and sister chromatid exchanges (SCE) (Madrigal-Santillán, et al., 2010; Witt et al., 2000; Woo et al., 2011)**.** In this study, the genotoxic effect of AFB1 was investigated by using chromosomal aberrations (CAs) and sister chromatid exchange assays which provide sensitive and rapid monitoring of induced genetic damage as primary DNA damage in human lymphocyte cell culture *in vitro*.

Materials and methods

Subjects

One healthy non-smoker and non-alcoholic adult males (26 year old) were recruited in the study to donate blood for lymphocyte cultures. About 20 ml blood was collected in heparinized collection tubes.

Chemicals

All the chemicals used, including AFB1, were purchased from Sigma Chemical Co., St. Louis, MO. Finally, Pb-Max Culture media was obtained from Thermofisher scientific (USA).

Cell cultures

Blood lymphocytes cultures were initiated by adding 1 mL of freshly withdrawn blood into tissue-culture flask containing 9 mL of complete lymphocyte Pb-Max media (RPMI 1640 medium supplemented with suitable amount of fetal bovine serum, glutamine, Penicillin-Streptomycin and Phytohaemagglutinin). AFB1 working solutions (100X) were prepared just prior to use by dissolving the compound in distilled water.

Chromosome Aberration Assay

Lymphocytes cultures were initiated by adding 1 mL of fresh heparinized whole blood to 9 mL of PB max complete lymphocyte Pb-Max media. Cultures were incubated in the dark at 37℃ for 72 h in a CO2 incubator with appropriate humidity. AFB1 were added to cultures in the last 24 h of incubation time. A negative control (untreated cultures) and a positive control (0.2 µg/mL mitomycin-C) were also used and was added in the last 24 h of incubation time. As a positive control, Cisplatin (1 µg /mL, final concentration) was used and was added in the last 24 h of incubation time. Colchicine (10 µg /mL) was added to cultures for 2 h prior to harvesting period. Cultures were then centrifuged at 1000 xg for 5 min, decanted and the cellular pellet was gently resuspended in 10 mL hypotonic solution (0.075 M KCl) at 37℃ for 20 min. The cellular suspension was centrifuged at 1000 xg for 5 min and the cellular pellet was fixed with three changes of ice-cold methanol: acetic acid (3:1).

The cellular suspension was then dropped on prechilled microscope slides to obtain metaphase spreads. The slides were stained with 5% giemsa stain (pH 6.8) for 15 min. The slides were analyzed blindly using medical microscope at 1000 magnification. About 200 well-spread metaphases were scored per each AFB1 concentration for the presence of chromosomal aberrations including (C-metaphase, chromosomal gap, chromosomal fragments, chromatin bridge and stickiness) (Alzoubi et al., 2012; Khabour et al., 2015).

Sister chromatid exchange assay

After lymphocyte cultures were established, a 5-bromodeoxyuridine solution was added to the culture media prior to incubation to achieve a final concentration of 20 µg /mL; this concentration of BrdUrd was maintained throughout the experiment.

Cultures were incubated at 37°C in CO_2 incubator for 72 h. AFB1 was added to cultures in the last 24 h of incubation time. Before harvesting of cultured lymphocytes, Colchicine (10 µg /mL) was added to cultures for 2 h. Cultures were then centrifuged at 1000 xg for 5 min, decanted and the cellular pellet was gently re-suspended in 10 mL hypotonic solution (0.075 M KCl) at 37°C for 20 min. The cellular suspension was centrifuged at 1000 xg for 5 min and the cellular pellet was fixed with three changes of ice-cold methanol: acetic acid (3:1). The cellular suspension was then dropped on pre-chilled microscope slides to obtain metaphase spreads. The slides were stained with the fluorescent-plus-Giemsa technique as described previously (Azab et al., 2009). The slides were analyzed blindly using medical microscope at 1000 magnification. About 200 M2 metaphase spreads were analyzed per each AFB1 concentration for presence of Sister-chromatid exchanges (Alzoubi et al., 2014; Khabour et al., 2016).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by two-tailed t-test when the ANOVA test yielded statistical differences ($p< 0.05$ or 0.01). A value of $p< 0.05$ was used as the criterion for statistical significance. All data were expressed as the mean ± SD.

Results

Chromosomal aberrations (CAs)

The data illustrated in Table 1 showed the chromosomal aberrations (CAs) including stickiness, chromosomal gap, chromosomal fragments, Robertosonian Centric Fusion (RCF) and polyploidy in lymphocytes after treatment with AFB1 (1, 2, 4, 8 and 16 mg/mL). AFB1 induced a statistically significant increase in chromosome aberrations in cultured human cells. The most frequent types of aberrations were Stickiness and gaps. The frequency of chromosomal aberrations also increased with increasing doses of AFB1. In control cultures, the mean frequency chromosomal aberrations per 200 cells was 4 ± 0.82. The mean percentages of the induced chromosomal aberrations were 5 ± 1.3%, 9 ± 1.3%, 18 ± 0.8%, 22 ± 1.4% and 33 ± 1.7% at AFB1 doses of 1, 2, 4, 8, and 16 mg/kg respectively.

Table 1. Percentage of chromosomal aberrations (CAs) in human lymphocytes culture after treatment with AFB1.

Dose (mg/kg)	No. of metaphase	% Chromosomal aberration (CAs)					Aberration % (mean ± S.D)
		Stickiness	Gap	Fragment	RCF*	Plyploid	
1	200	2	2	1	0	0	5.0 ± 1.3
2	200	4	3	1	1	0	9.0 ± 1.3 **
4	200	7	6	2	2	1	18 ± 0.8**
8	200	10	8	1	2	1	22 ± 1.4**
16	200	15	9	2	4	3	33 ± 1.7 **
N.C	200	2	1	1	0	0	4.0± 0.82

NC= Negative Control; *Robertosonian Centric Fusion (RCF); ∗∗Significant from the control $p<0.01$.

Sister chromatid exchange (SCE)

Table 2 shows the results of the SCE analysis performed in blood human lymphocytes treated with AFB1. In control cultures, the mean frequency SCE per 200 cells was 2.25 ± 0.96. In the treated cultures the mean value was 3.3 ± 0.95, 4.3 ± 1.6, 5.0 ± 0.8, 5.9 ± 1.2 and 6.8 ± 0.95 for 1, 2, 4, 8 and 16 mg/mL, respectively. According to the results, four acute doses (2, 4,8 and 16 mg/kg) of AFB1 tested in the present study, induced a significant increase in the frequency of SCEs ($p<0.05$ and $p<0.01$) in the cultured human lymphocytes.

Table 2. Average of in vitro induction of sister chromatid exchanges (SCEs) in human lymphocyte culture after treatment with AFB1.

Dose (mg/kg)	No. of metaphase	SCE/Cell (mean ± S.D)	Min-max SCE
1	200	3.3 ± 0.95	1-4
2	200	4.3 ± 1.6 *	2-5
4	200	5.0 ± 0.8 **	2-6
8	200	5.9 ± 1.2 **	2-7
16	200	6.8 ± 0.95 **	3-6
N.C	200	2.25 ± 0.96	1-4

N.C= Negative Control; ∗ Significant from the control $p<0.05$. ∗∗ Significant from the control $p<0.01$.

Discussion

Chromosomal aberrations (CAs) and sister chromatid exchange (SCE) analysis of human lymphocytes are used as the most rapid, sensitive and useful assays to detect the potential genotoxicity of chemicals (Rahman et al., 2002). They have been considered to be the markers of early biological effects of carcinogen exposure (Liou et al., 2002). In this study, genotoxic activity of AFB1 were examined using chromosomal aberrations and sister chromatid exchange test systems. The extensive use of chromosomal aberrations can be found mainly for evaluating the genotoxicity in human subjects (Kao-Shan et al., 1987). Moreover, there are numerous evidences reported in past studies in which researchers have shown a strong correlation between induction of CAs and the risk of cancer (Norppa et al., 2006; Ray et al., 2001). The results of these studies showed that AFB1 induced different type of CA such as stickiness, chromatid gap, fragment, Robertsonian Centric Fusion (RFC) and polyploidy. The lowest dose of (1mg/kg) of AFB1 could induce any significant increase in the frequency of CA. However, the doses 2, 4, 8, and 16 mg/kg of AFB1 induced a significant increase in the frequency of CA. The results are corresponding with the previous studies indicating rising frequency of CA succeeding AFB1 treatment *in vivo* (Ito el al., 1989; Adgigitov et al., 1984; Anwar et al., 1994; Krishnamurthy and Neelaram, 1986) and *in vitro* (Batt et al., 1980; Fadl-Allah et al., 2011; Werner et al., 1992). The results obtained from this study also indicate a significant increase in the ratios of the SCEs by AFB1 in lymphocytes, which is in accordance with the previous reports (Groopman and Kensler, 1999; Geyikoglu and Turkez 2005; Turkez and Geyikoglu, 2010). The SCEs are formed by toxic oxygen metabolites in cultured human leukocytes and other mammalian cells (Weitberg et al., 1983). From the present study, it can be concluded that AFB1 induce chromosomal alterations and DNA damage in human lymphocytes.

Conflict of interest statement

Author declares that there is no conflict of interest.

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