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Research Article

In vitro genotoxic effects of Acrylamide in human lymphocytes

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ABSTRACT

Acrylamide (AA) is an industrial chemical produced in foods during cooking at high heat. It is classified as neurotoxic and probably carcinogenic to humans. AA is known to be metabolized to glycidamide (GA) by cytochrome P450 2E1-mediated epoxidation which is responsible for AA's in vivo genotoxicity. The current study was carried out to evaluate the genotoxic aspects of the AA by chromosomal aberration assay and sister chromatid exchange assay on cultured human peripheral blood lymphocyte. After the treatment of the lymphocytes with four concentrations of AA (2,4,8,16 and 32 mg/mL) it was noticed that, AA did not significantly increase the chromosome aberrations (CA) frequency or sister chromatid exchanges (SCE) frequency compared to control with all concentrations. the results show that AA itself, without metabolic activation have a week direct clastogenic effect upon human chromosome and did not induce primary DNA damage. The results obtained in this study suggest that other tests should be performed to clarify whether AA has genotoxicity in chromosome aberrations.

Keywords: Acrylamide; Glycidamide; Genotoxicity; Mutagenicity; DNA damage; chromosome aberrations; sister chromatid exchanges.

1. INTRODUCTION

AA is a vinylic compound produced as result of mailled reaction between asparagine residues and glucose. It is found in carbohydrate rich foods that have been Baked and fried at excessive temperatures (Mottram et al. 2002;Stadler et al. 2002).The discovery of AA in foods raised concern about the potential health effects of everyday exposure through the diet (Tareke et al., 2002).

The International Agency for Research on Cancer (IARC) has classified AA as potentially carcinogenic substance to human (IARC 1994). The genotoxicity, neurotoxicity, carcinogenicity and reproductive toxicity of AA has been demonstrated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment in 2001(Keramat *et al.* 2011, Carere 2006). The genotoxicity of AA has been studied in a variety of assays. In vivo the genotoxic activity of AA revealed highly contradictory results. It has mutagenic activity, and has been shown to produce gene mutation, chromosomal aberration, micronuclei (MN) formation, single-strand breaks, aneuploidy and polyploidy (Taubert et al., 2006). In addition, LoPachin and Gavin (2012) reported that AA can cause pathological alterations in the brain and spinal cord in rats leading to neurobehavioral modifications.However, some in vitro studies indicated that AA is not genotoxic in bacterial assays (Dearfield et al., 1995). Moreover, AA did not cause genetic mutations in Salmonella, Escherichia coli (E. coli), or Klebsiella pneumoniae in the presence or the absence of an exogenous activation system (Besaratinia and Pfeifer, 2005). In the other hand, The genotoxic potential of AA has been demonstrated in mammalian cell tests for mutagenicity using the mouse lymphoma assay, chromosome aberration assay, and micronucleus assay (Mei *et al.*, 2008; Yang *et al.*, 2005). Cytogenetic assays, such a chromosome aberrations and sister chromatid

exchanges are detectable by cytogenetic analysis of chromosome in metaphase. These cytogenetic endpoint are widely used to assess the mutagenic and carcinogenic potential of chemical compounds (Albertini et al. 2000).In the present study, we investigated the ability of AA to induce chromosomal aberrations (CA) and sister chromatid exchange (SCE) in vitro using cultured human lymphocytes.

2. MATERIALS AND METHODS

2.1. 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.

2.2. chemicals

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

2.3 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). AA working solutions (100X) were prepared just prior to use by dissolving the compound in distilled water.

2.4. Chromosomal aberrations (CAs) 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 ◦C for 72 h in a CO2 incubator with appropriate humidity. AA 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 AA concentration for the presence of chromosomal aberrations including (C-metaphase, chromosomal gap, chromosomal fragments, chromatin bridge and stickiness) (Alzoubi et al., 2012 and Khabour et al., 2015).

2.5. 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 ℃ in CO2 incubator for 72 h. AA 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 ℃ 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 AA concentration for presence of Sister-chromatid exchanges (Alzoubi et al., 2014a; Khabour et al., 2016).

2.6. Statistical analysis

All of the results were expressed as mean ± standard error. Te differences among the groups were evaluated with Student's t-test. Te mean difference was considered significant at the 0.05 level.

3. RESULTS

3.1. Chromosomal aberration (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 AA (2,4,8,16 and 32 mg/mL). Total aberrant metaphase were found to be 5% and 31% for negative and positive control, respectively. AA did not show any clastogenic activity in human lymphocytes in vitro, since the frequency of total chromosome aberrations observed after treatment of cells with different concentrations of AA was not statistically different from that of the negative controls.

3.2. Sister Chromatid Exchange Assay (SCE)

Table 2 shows the results of the SCE analysis performed in blood human lymphocytes treated with AA. In control cultures, the mean frequency SCE per 200 cells was 2.3±0.3. In the treated cultures the mean value was 1.2±0.2 , 2.1±0.1, 2.1±0.1, 1.5±0.1, 1.6±0.2 and 2.2.6±0.1 for 2,4,8,16 and 32 mg/mL, respectively. According to the results, there was no significant increase in SCE frequencies in all concentrations compared with the control.

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

treatments		**Chromosomal aberrations (%)**					
Concentrations (mg/ml)	**Number of metaphases examined**	**Stickiness**	**Gap**	**Fragment**	**RCF***	**Polyploid**	**% of Aberrant cells**
2	200	2	0	1	0	0	3
4	200	4	3	0	0	0	7
8	200	6	0	2	1	1	10
16	200	5	1	1	0	1	8
32	200	4	2	1	0	0	7
N.C	200	2	1	2	0	0	5
P.C	200	12	4	6	3	6	31

NC= Negative Control PC=Positive Control
*Robertosonian Centric Fusion (RCF).

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

treatments		**sister chromatide exchanges**	
Concentrations (mg/ml)	**Number of metaphases examined**	**SCE/Cell ± S.E**	**Min-max SCE**
2	200	1.2 ± 0.2	0-2
4	200	2.1 ± 0.1	0-2
8	200	1.5 ± 0.1	0-3
16	200	1.6 ± 0.2	0-2
32	200	2.2 ± 0.1	0-3
N.C	200	2.3 ± 0.3	1-3

N.C= Negative Control

4. DISCUSSION

In vivo genotoxicity of AA has been showed by various rodent genotoxicity tests including micronucleus formation in peripheral blood [Cao et al., 1993; Abramsson-Zetterberg, 2003; Manjanatha et al., 2005], increase in abnormal sperm [Sakamoto et al., 1986] , transgenic gene mutation in liver [Manjanatha et al., 2005], and gene mutation and Comet assay in various organs [Manjanatha et al., 2006; Ghanayem et al., 2005; Wang et al., 2010]. AA has also shown mutagenic effects in germinal cell [Dearfield et al., 1995]. In F344 rats, oral administration of AA induced tumours in the central nervous system, the thyroid glands, peritesticular mesothelia and the mammary glands [Freitas et al., 1995; Tanaka et al., 1995]. It was reported that AA enhanced the frequency of dominant lethal mutations probably accompanying with chromosome aberrations leading to death of embryo [Shelby et al., 1987; Adler et al., 1994]. Recently , the results presented by Dobrovolsky et al. (2016) suggested that AA induced DNA damage in rat liver. In contrast, the in vitro genotoxicity of AA is still controversial. AA was negative in Ames assay [Zeiger et al., 1987; Knaap et al., 1988; Tsuda et al., 1993]. AA also induced Tk mutation in the MLA but did not induce Hprt mutation in V79 cells [Moore et al., 1987; Knaap et al., 1988; Tsuda et al., 1993; Baum et al., 2005; Mei et al., 2008]. In addition Baum et al., (2005) reported that AA produced negative results in the Comet assay with V79 cells and human. AA could induce DNA damage in the PC Cl3 and FRTL5 rat thyroid cell lines, as well as in human lymphoblastoid TK6 cells in the Comet assay (Koyama et al., 2006). Thus, AA is clearly genotoxic in vivo, although it's in vitro genotoxicity remains unclear because it is not metabolically activated in standard in vitro systems [Koyama et al., 2006; Koyama et al., 2011]. AA is metabolised to GA, presumably by cytochrome P450 2E1 (CYP2E1), which quickly reacts with cellular DNA and protein [Rice, 2005; Sumner et al., 1990; Ghanayem et al., 2005]. GA has been reported to be 100–1000 times more reactive with DNA than AA (Segerback et al., 1995). GA is known to be clastogenic and mutagenic in vitro and in vivo (Paulsson et al., 2003; da Costa et al., 2003). The present study, we investigated the genotoxic properties of AA with human lymphocytes in absence of metabolic activation. statistical analysis showed no obvious increase in the number of aberrant metaphases or sister chromatide exchange levels. These findings indicated that genotoxic effects of AA have not been observed under our conditions tested. The results of our studies support the concept of AA is not genotoxic by itself but becomes activated to its primary epoxoide genotoxic metabolite GA via epoxidation (Baum et al., 2008). Clearly, AA is not metabolically activated in human blood or lymphocytes (Baum et al., 2005). In conclusion, AA could not be metabolized to GA by in vitro metabolic activation system commonly used in genotoxicity tests. In vivo, on the other hand, GA is apparently responsible for AA-inducing genotoxicity. Although AA may exhibit genotoxicity in in vitro mammalian cells at high concentrations, its positive response is not relevant for its major genotoxicity.

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