

Protective Effect of Sodium Selenite against the Genotoxicity of Aflatoxin B₁ in Human Whole Blood Cultures

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ABSTRACT

This study was designed to investigate the effects of selenium and aflatoxin on human whole blood cultures (WBC) in relation to induction of sister-chromatid exchange (SCE). The results showed that the frequency of SCEs in peripheral lymphocytes was significantly increased by the direct-acting mutagen AFB₁ (at doses 5 and 10 µM except for 1µM) compared to controls. When sodium selenite (Na₂SeO₃) was added alone at a molar ratio of 5x10⁻⁷ and 1x10⁻⁶, cells did not show significant increase in SCE frequency. Whereas, SCE rates induced by the various AFB₁ concentrations could be significantly reduced by the presence of Na₂SeO₃ in a clear dose-related manner. These results indicated that selenite and AFB₁ mutually antagonized their ability to cause DNA damage leading to the formation of SCEs. However, selenium didn't completely inhibit induction of SCEs by AFB₁ compared to controls. This is first report describing, the protective ability of selenium against AFB₁ genotoxicity on human WBC.

Key words: Aflatoxin B₁, selenium, sister-chromatid exchanges, genotoxicity, whole blood cultures

INTRODUCTION

Dietary selenium is an essential trace element in human nutrition (Shi et al. 1995). Sodium selenite is an anticarcinogenic/ antimutagenic agent that exhibits carcinogenic/mutagenic properties in some short-term test systems used for the detection of DNA-damaging agents. One such test system is sister-chromatid exchange (SCE) induction (Ray, 1984). SCEs were significantly potentiated by the presence of Na₂SeO₃ (Lin and Tseng, 1992). From the viewpoint of genotoxicity, selenium has not been adequately studied (Cemeli et al. 2003). In contrast, AFB₁, human carcinogen and the most potent genotoxic agent, is mutagenic in many model systems and produces chromosomal aberrations, micronuclei, sister-chromatid exchange, unscheduled DNA synthesis, and

chromosomal strand breaks as well as forms adducts in rodent and human cells (Wang and Groopman, 1999). Selenium has been shown in animal studies to inhibit aflatoxin hepatocarcinogenesis (Shi et al. 1995). These inhibitory effects are supported by many diverse mechanisms, including inhibition of carcinogen formation, modulation of carcinogen metabolism, inhibition of mutagenesis and genotoxicity, inhibition of cell proliferation (Lu et al. 1996).

It is important to verify lack of toxicity of selenium on different systems and to investigate mechanisms of its action throughout the whole processes of mutagenesis. The mutagenicity of AFB₁ has been demonstrated using many model systems including HeLa cells, *Bacillus subtilis*, *Neurospora crossa*, *Salmonella typhimurium*, and Chinese hamster ovary (CHO) cells (Wang and

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Groopman, 1999). However, not enough studies have been carried out to evaluate the genotoxicity of selenium with AFB₁ on blood cultures. Therefore, the objective of this work, was to investigate sodium selenite and its interaction with AFB₁ in the SCE test using human whole-blood cultures.

MATERIALS AND METHODS

Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). Whole heparinized blood from four healthy non-smoking donors between age 25 and 28 with no history of exposure to any genotoxic agent were used in the experiments. Questionnaires were obtained for each blood donor to evaluate exposure history, and in addition, informed consent forms were signed by each donor. For all volunteers hematological and biochemical parameters were analysed and any pathologic finding has not been detected.

A total of 0.5 ml of heparinized blood was cultured in 5 ml of culture medium (Chromosome Medium B, Biochrom, Leonorenstr. 2-6.D-12247, Berlin) with 5µg/ml of phytohemagglutinin (Biochrom). AFB₁ (C₁₇H₁₂O₆, Sigma Chemical Co., St. Louis, MO. USA) (in concentrations of 1, 5 and 10µM) and sodium selenite (Na₂SeO₃, Sigma, St. Louis) (in concentrations of 5x10⁻⁷ and 1x10⁻⁶ M) added to the cultures just before incubation. In addition, to each individual, lymphocyte culture without AFB₁ and Na₂SeO₃ were studied as a control group. The experiments were performed on 12 groups as follows:

- Group 1: Control
- Group 2: Sodium selenite (5x10⁻⁷M) alone.
- Group 3: Sodium selenite (1x10⁻⁶M) alone.
- Group 4: AFB₁ (1µM) alone.
- Group 5: AFB₁ (5µM) alone.
- Group 6: AFB₁ (10µM) alone.
- Group 7: AFB₁ (1µM)+Na₂SeO₃ (5x10⁻⁷M).
- Group 8: AFB₁ (5µM)+Na₂SeO₃ (5x10⁻⁷M).
- Group 9: AFB₁ (10µM)+Na₂SeO₃ (5x10⁻⁷M).
- Group 10: AFB₁ (1µM)+Na₂SeO₃ (1x10⁻⁶M).
- Group 11: AFB₁ (5µM)+Na₂SeO₃ (1x10⁻⁶M).
- Group 12: AFB₁(10µM)+Na₂SeO₃(1x10⁻⁶M).

Groups of 7-12 represented simultaneous treatment with AFB₁ and Na₂SeO₃.

With the aim of providing successive visualization of SCEs, 5-bromo-2'-deoxyuridine (Sigma, St. Louis, final concentration 20µM) was added after culture initiation. The cultures were incubated in complete darkness for 72h at 37°C. Exactly 70h and 30 min after beginning of incubations, colcemid (Sigma, St. Louis) was added to the cultures to achieve a final concentration of 0.5 µg/L. After hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged, and then differentially stained for the inspection of SCE rate according to fluorescence plus Giemsa (FPG) procedure (Perry and Wolff, 1974). For each treatment condition, well-spread second division metaphases containing 42-46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell.

Statistical analyses

Experimental data were analyzed using one-way analysis of variance (ANOVA) to determine whether any treatment significantly differed from controls and/or each other. Significant differences between the controls and/or treated samples were confirmed by Fisher's least significant difference (LSD) test.

RESULTS

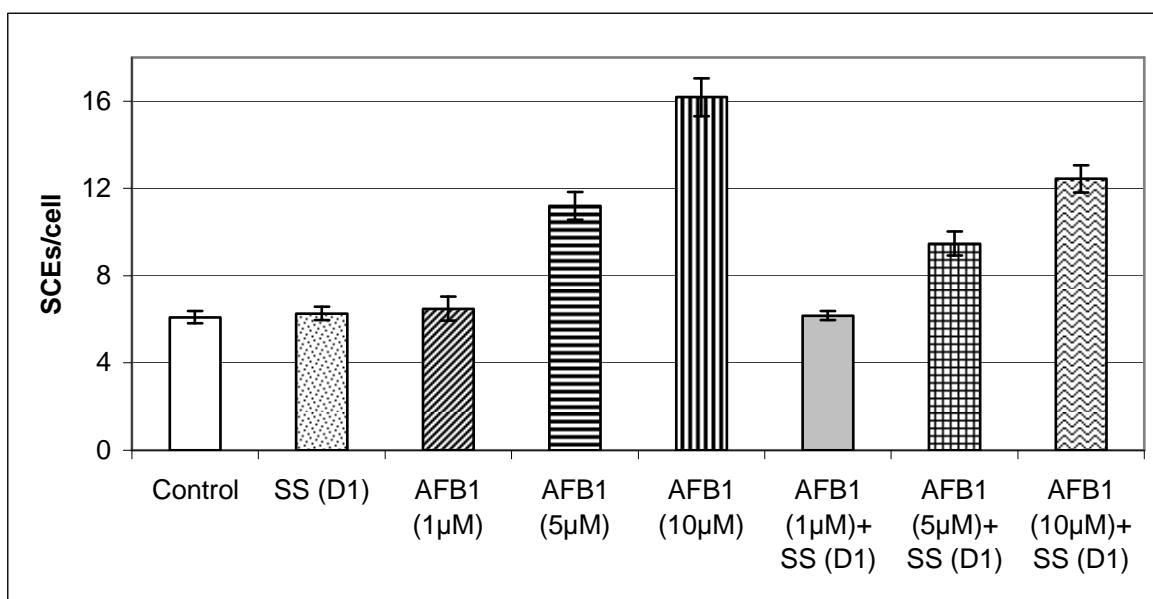
The effects on the number of SCEs of AFB₁ and Na₂SeO₃ in human WBC are shown in Table 1.

Sodium selenite alone, in concentrations of 5x10⁻⁷ and 1x10⁻⁶M did not significantly affect SCE rates in human lymphocytes. In contrast, the increasing concentrations of AFB₁ (5 and 10µM) elevated the frequencies of SCEs in these cells compared to controls. A significant increase in SCE frequency wasn't observed at the lowest AFB₁ dose (1µM). Na₂SeO₃ significantly reduced the number of AFB₁-induced SCEs. A dose-dependent decrease in SCEs was demonstrated, with inhibition observed at selenium concentrations of 5x10⁻⁷M or greater (Table 1, Figs. 1 and 2). However, the rates of SCEs following the applications of selenium and aflatoxin together were significantly high in comparison with control values.

Table 1 - The effects on the number of SCEs of AFB₁ and Na₂SeO₃ in human WBC.

Culture types	Number of samples	Range of SCEs	SCEs/cell
Control ⁺	4	0-8	6.10 ± 0.28 ^a
SS (D ₁)	4	2-9	6.27 ± 0.31 ^a
SS (D ₂)	4	2-10	6.35 ± 0.42 ^a
AFB ₁ (1μM)	4	3-11	6.49 ± 0.55 ^a
AFB ₁ (5μM)	4	4-19	11.20 ± 0.63 ^b
AFB ₁ (10μM)	4	3-28	16.18 ± 0.87 ^c
AFB ₁ (1μM)+SS (D ₁)	4	3-10	6.17 ± 0.21 ^a
AFB ₁ (5μM)+SS (D ₁)	4	3-17	9.47 ± 0.55 ^d
AFB ₁ (10μM)+SS (D ₁)	4	3-22	12.44 ± 0.62 ^e
AFB ₁ (1μM)+SS (D ₂)	4	1-9	6.38 ± 0.53 ^a
AFB ₁ (5μM)+SS (D ₂)	4	3-16	8.66 ± 0.45 ^f
AFB ₁ (10μM)+SS (D ₂)	4	4-21	11.46 ± 0.80 ^b

+ Without aflatoxin B₁ and sodium selenite. SS=sodium selenite. +SS (D₁)=sodium selenite treated culture with low concentration. +SS (D₂)= sodium selenite treated culture with high concentration. Values are mean ±SD. Means in the same column followed by the same letter are not significantly different at the p<0.05 level.

**Figure 1** - The effects of AFB₁ and Na₂SeO₃ (5x10⁻⁷ M) on the number of SCEs in human WBC.

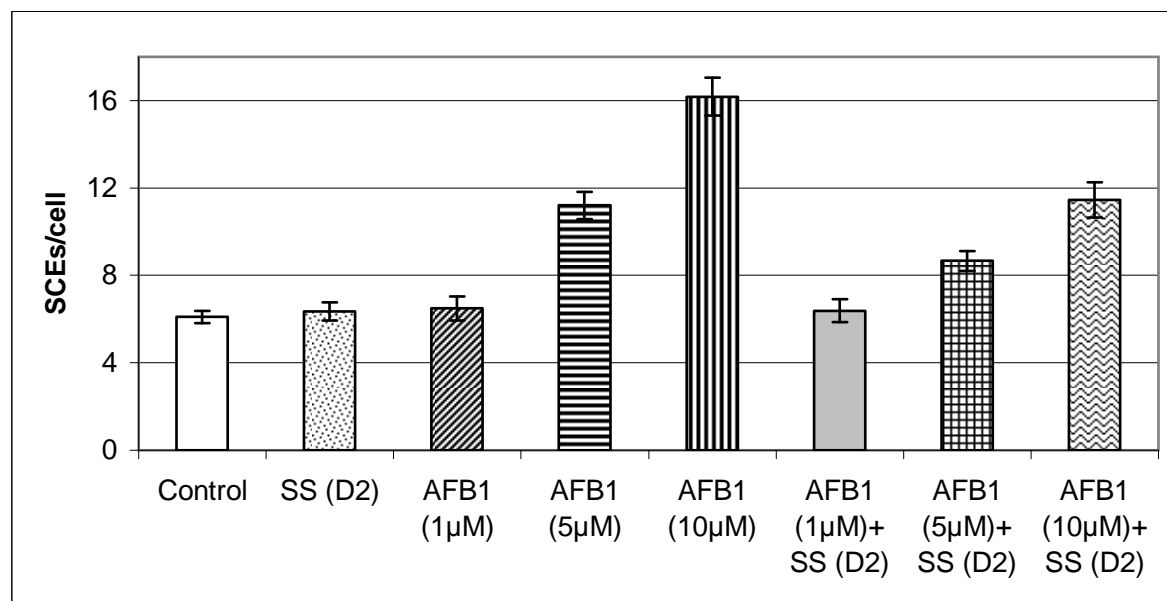


Figure 2 - The effects of AFB₁ and Na₂SeO₃ (1x10⁻⁶ M) on the number of SCEs in human WBC.

DISCUSSION

The normal human leukocytes stimulated to produce toxic oxygen metabolites cause sister chromatid exchanges in cultured mammalian cells (Weitzman and Stossel, 1981; Weitberg et al. 1983). Recent studies have shown that aflatoxin B₁ enhances reactive oxygen species formation and causes oxidative damage (Chan et al. 2003). It has also been reported that AFB₁ play a primary role in the generation of AFB₁-mediated genetic damage (Wang and Groopman, 1999). Thus, apparently aflatoxin B₁ (5 and 10µM) reacted with components of human WBC resulting in the formation of toxic intermediate compounds. Also, some of the oxygen products might cause SCE formation in peripheral lymphocytes. In a previous study, common oxidative damage, including formation of 8-oxodeoxyguanosine (8-oxodG) was observed in rat hepatic DNA following exposure to AFB₁ (Wang and Groopman, 1999). In the present study, SCEs were increased in cells treated with AFB₁ alone (except for 1µM) and this effect was greatly magnified with AFB₁ dosage. A time- and dose dependent increase in hepatic levels of 8-oxodG residues in liver DNA treated with AFB₁ has been reported (Shen et al., 1995; Yaborough et al., 1996).

The risk for AFB₁ hepatocarcinogenesis could be modified in animals by using a number of

chemoprotective agents (Wang and Groopman, 1999). A dramatic reduction of AFB₁-induced SCEs in peripheral lymphocytes by the increase in the amount of Na₂SeO₃ was demonstrated by our study. Apparently selenium might be affecting as an antioxidant. Because, selenium is a prosthetic group essential for the catalytic activity of glutathione peroxidase (GSHpx) (Chow, 1979). The selenium-dependent glutathione peroxidase can detoxify both hydrogen peroxide and lipid hydroperoxides (Leopold, 1976; Sandstrom and Marklund, 1990). AFB₁-induced reactive oxygen species formation and lipid peroxidation (LPO) might play a role in its cytotoxicity (Chan et al., 2003). AFB₁-induced LPO was also found in hepatocytes (Liu et al., 1999). In the present study, erythrocytes were present in the incubation medium. Glutathione peroxidase activities increased significantly in erythrocytes from mice supplemented with selenium dietary (Arai et al., 2002). Erythrocytes are known to have GSHpx and glutathione-S-transferase (GST) (Ozturk and Gumuslu, 2004). On the other hand, glutathione is a major component of RBCs (Ray, 1984) that plays a central role in the antioxidant defenses of cells (Meister, 1983). It is a cofactor of the enzyme glutathione peroxidase (Leopold, 1976). Again, glutathione conjugates with AFB₁ (Madle et al., 1986). Thus, it is possible that AFB₁-induced oxidative damage acts as an intermediate for the

genetic damage. However, a mechanism consisting of glutathione-Se-reactive oxygen species formation from Na₂SeO₃ and AFB₁ involving the participation of glutathione in RBCs might play a key role in this antagonism between AFB₁ and selenium. Also, it has been reported that the induction of detoxification enzymes (GSHpx and GST) following exposure to aflatoxin might contribute to the reduction in covalent binding of AFB₁ to macromolecules (Loury and Hsieh, 1984). Covalent binding of AFB₁ to adenosine (Andrea and Haseltine, 1978), cytosine (Yu et al., 1991) and guanine in DNA in vitro has also been reported (Wang and Groopman, 1999). In cultured CHO cells, selenium treatment did not affect AFB₁-DNA binding (Shi et al., 1995). Whereas, Chen et al. (1982) found that covalent binding of AFB₁ to liver DNA and RNA was greater in chicks fed the selenium-deficient diets than the chicks supplemented with selenium or vitamin E or both (Shamberger, 1985).

Despite the uncertainty about the specific role of selenium in human WBC, the SCE test showed that sodium selenite was non-genotoxic, while AFB₁ induced DNA damage. It was also shown that sodium selenite decreased the genotoxicity of AFB₁ when administered at the same time in a clear dose-related manner. This is first report describing the protective effects of Se against AFB₁ genotoxicity on human WBC. It could be possible that AFB₁-induced reactive oxygen species formation and oxidative damage could also contribute to its genotoxicity. By SCE test, it was proven that the protective effect was an important cytogenetic characteristic of sodium selenite. This study also showed that selenium did not completely inhibit induction of SCEs by AFB₁.

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