Combined Effect of Aflatoxin and Vitamin A on Clastogeny in Mice Chromosomes

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Mycotoxins are secondary metabolites of toxigenic strains of certain fungi. *Aspergillus flavus* is one such very common fungus that infests food and feed commodities (Ranjan 1986), and elaborates a toxic substance called aflatoxin. The most common aflatoxin is B₁ (AF-B₁), though B₂, M₁, M₂, G₁ and G₂ forms are also found. Dietary concentrations of AF-B₁ have been reported to be mutagenic (Sinha *et al.* 1987, Bilgrami and Sinha 1988), teratogenic and carcinogenic (Butler 1974, IARC 1976, Bilgrami *et al.* 1986a, b). Sincere efforts therefore, must be made to protect the domesticated animals, poultry birds and even human beings from its hazards. Vitamin C is one such bioantimutagen (Sinha and Bose 1989) that could minimise the genotoxicity and general toxicity (Bilgrami *et al.* 1989) of aflatoxin. Present experiments were, therefore, designed with an aim to find out similar effects, if any, of another vitamin, the vitamin A, which has also been shown to prevent, delay or retard carcinogenesis induced in laboratory animals by a variety of chemicals (Moon *et al.* 1983, Newberne and Rogers 1981, Sporn and Newton 1981).

Material and method

Four to five-week old albino Swiss mice (*Mus musculus*) of both sexes, obtained from an inbred laboratory stock, were used. The animals were separated into six groups, and were subjected to various treatments. The vitamin was administered either along with the toxin or as a post- or pre-treatment to toxin feeding. Three types of control were used: toxin only, vitamin only, neither toxin nor vitamin. The general plan of administration is given in Table 1. Two repeats of experiment were performed, each with six animals matched for their sex and age.

It is worth-remembering that the dose of toxin is almost the same which the population usually and inadvertently takes in with food and feed. The dose of vitamin A is proportionate to the dose recommended for human body (therapeutic dose). Vitamin A (as palmitate "Aquasol-A") used in this study was provided by U. S. Vitamin (India) Ltd. Animals were sacrificed after a week of the termination of the treatment, and chromosome preparations of bonemarrow cells were made following sodium citrate-aceto-alcohol-flame drying-Giemsa staining technique (Kilian *et al.* 1977). 200 well-spread metaphase plates from each group of animals were screened after random selection at the rate of 15–20 plates per animal.

Results and discussion

Both gross and individual types of abnormalities were found in all the variants. Their rate in toxin-treated animals was significantly higher than in the control variant, the rate being equal irrespective of 6 or 12 weeks of the treatment (Sinha and Bose 1989). The overall frequency of cells with chromosomal abnormalities was found to be higher in vitamin-treated

mice which may be due to hypervitaminosis (Elsas and McCormick 1986). Not only the pre- and post- administration of vitamin A, but also its concurrent administration were poorly efficacious in minimising the total frequency of aflatoxin-induced abnormalities.

Table 1. Summary of the experimental protocol

Experimental variants	Symbol	Description
1. Toxin only	T	0.05 µg of toxin/animal/kg. b. wt. daily with food for 6 and 12 weeks in 50±10 ppb concentration.
2. Vitamin only	V	66.06 I. U. of vitamin A/animal/kg. b. wt. daily for 12 weeks through intubation.
3. No treatment	C	No administration of toxin or vitamin.
4. Toxin and vitamin together	T+V	The toxin (dose as in 1) and vitamin (as in 2). administered together for 12 weeks.
5. Toxin feeding after vitamin feeding	VT	Vitamin treatment for 6 weeks followed by toxin treatment for another 6 weeks (doses as in 1 and 2).
6. Toxin feeding followed by vitamin feeding	TV	The toxin treatment (dose as in 1) for 6 weeks followed by vitamin treatment (dose as in 2) for remaining 6 weeks.

Table 2. Incidence of chromosomal abnormalities in bone marrow cells of mice treated with aflatoxin and vitamin A

	Total abnormal cell no. %±S. E.	Abnormality types			
		Individual type		Gross type	
		No. %±S. E.	Description of abnormality	No. %±S. E.	Description of abnormality
C	10 5.0±1.54	04 2.0±0.98	af=3, Ctb=1	06 3.0±1.20	Poly-2, C-mito-1, Clum-1, Hypo-2
V	34 17.0±2.65 ^{a,b}	12 6.0±1.67 ^{a,b}	af=8, Ctb=3, Ctg-1	22 11.0±2.21 ^a	Poly-1, C-mito-1, Clum-2, Hypo-18
T	64 32.0±3.29 ^{a,c}	35 17.5±2.68 ^{a,c}	af=21, Ctb-5, Ring-1, Ctg-3, Ictb-3, Mc-2	32 16.0±2.59 ^a	Poly-2, C-mito-5, Clum-6, Stick-2, Hypo-17
V+T	51 25.5±3.08 ^a	09 4.5±1.46 ^b	af=5, Mc-1, Ctb-2, Ctg-1	44 22.0±2.93 ^{a,c}	Poly-3, Hyp-35, Clum-2, Stick-1, C-mito-3
VT	58 29.0±3.20 ^a	14 7.0±1.80 ^{a,b}	af=7, Ctb-2, Ictb-1, Ctg-4	46 23.0±2.97 ^{a,c}	Poly-3, Hyp-33, Stick-2, Clum-2, C-mito-6
TV	47 23.3±2.99 ^a	19 9.5±2.07 ^a	af=8, Ctb-6, Ring-1, Mc-1, Ctb-3	30 15.0±2.52 ^a	Poly-2, C-mito-4, Stick-2, Hyp-22

^{a,b,c} indicate significant difference with corresponding value in the control, toxin and vitamin variants, respectively.

af=acentric fragment; Ictb=isochromatid break; Stick=stickiness; Ictg=isochromatid gap; Poly=polyploid; Ctb=chromatid break; Hypo=hypoploid; Ctg=chromatid gap; Clum=clumping; Mc=metacentric chromosome; C-mito=C-mitosis.

By considering the individual type abnormalities separately, the concurrent administration of aflatoxin with vitamin A, as well as the pre-treatment with vitamin A could significantly minimise the toxin-induced abnormality rate. The gross type abnormalities remained least affected. Thus, vitamin A (as palmitate) shows some protective effect against aflatoxin-induced chromosome/chromatid breaks. It can be therefore said that the vitamin A somehow repaired the toxin-induced breaks, but it failed to combat the damages induced in mitotic ap-

paratus, the latter being generally responsible for gross type abnormalities. The exact mechanism of the antimutagenic property of vitamin A is not known, but the spectrum of effects observed in the present study is in conformity with the observations made by earlier workers, who in *in vitro* kinetic studies found that retinol inhibits AF-B₁-DNA adduct formation by virtue of its ability to interact with microsomal enzyme component(s), thereby interfering competitively with the bioactivation of AF-B₁ (Firozi *et al.* 1987, Bhattacharya *et al.* 1989). Specific interaction of vitamin A with microsomal enzyme component (s) in relation to AF-B₁ metabolism, however, is still to be ascertained.

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Summary

Therapeutic dose (66.06 I. U.) of vitamin A, when administered concurrently with or as a pre-treatment to crude aflatoxin (0.05 µg) administration (per oral) for 12 weeks to albino Swiss mice, *Mus musculus*, could cause significant decrease in the incidence of toxin-induced individual type chromosome abnormalities (Control— $2.0 \pm 0.98\%$, Toxin— $17.5 \pm 2.68\%$, V→T $7.0 \pm 1.80\%$, V+T $4.5 \pm 1.46\%$); the incidence of gross type abnormalities remained unaffected. Post-treatment with vitamin was ineffective in minimising the clastogeny. The toxin alone could induce changes like chromatid break, chromatid gap, metacentric chromosome, acentric fragment, polyploidy, clumping, stickiness, etc. It is suggested that supplementation of food with vitamin A can be useful to combat the genotoxicity of aflatoxin.

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