Bull. Environ. Contam. Toxicol. (1991) 47:428–432

Bulletin of Environmental Contamination and Toxicology

Cytotoxicity of Aflatoxin on Red Blood Corpuscles

R. J. Verma and P. J. Raval

Department of Life Sciences, Bhavnagar University, Bhavnagar-364 002, India

Aflatoxins are a group of coumarin derivatives produced and released by toxigenic strains of Aspergillus flavus and Aspergillus parasiticus group of fungi. These toxins frequently contaminate food/feed stuffs of animals including human beings. Among all mycotoxins so far studied, aflatoxin B1 (AFB1) is found to be most potent hepatotoxic, hepatocarcinogenic and mutagenic (Stoloff, 1977; Busby and Wogan, 1984; Groopman et al., 1988).

Occurrence of anemia during aflatoxicosis has been reported in several animals such as rats (Panda et al., 1975; Ranjan, 1987; Verma et al., 1989), guinea pigs (Panda et al., 1975; Ranjan, 1987), CD-1 mice (Reddy et al., 1987) rabbits (Clark et al., 1980) and cattle (Patterson, 1983; Brucato et al., 1986). This clinical symptom could result from inhibition of hematopoiesis, defective hematopoiesis, increased destruction of RBC or a combination of all three. The exact mechanism of aflatoxin action is not clearly understood. In the present investigation, we report morphological aberrations and increased rate of hemolysis caused by aflatoxins in vitro.

MATERIALS AND METHODS

Inbred strains of rabbits (Oryctolagus ruficaudatus) used in the present investigation were provided with food and water ad libitum. Aflatoxin was produced by growing Aspergillus parasiticus (NRRL 3240) on SMKY liquid medium as described by Diener and Davis (1966). Culture filtrate was extracted with chloroform and aflatoxin content was determined spectrophotometrically by the method of Nabney and Nesbitt (1965).

Samples of blood were collected from ear-pinna of rabbits directly into EDTA bulbs. After dilution with saline, the samples were centrifuged at 1000 rpm for 10 min. Supernatants were discarded and the RBC pellet was further washed twice with saline by centrifugation. Final RBC suspension was prepared in saline to have 2×10^4 cells/ml. For examining the

---

Send reprint requests to (DR. R. J. VERMA) at the above address.

effect of aflatoxin on RBC, two sets of the tubes were prepared as follows : (a) Control tubes containing 1.0 ml RBC suspension, and (b) treated tubes containing 1.0 ml RBC suspension and aflatoxin (0.35 to 3.50 µg/ml). Aflatoxin solutions were prepared in saline. Total volume of each tube was made to 2.0 ml by adding saline. All the tubes were incubated at 37 C for 16 h. Morphological alterations in RBC was observed after staining RBC smear with Leishman's stain. Tubes were centrifuged at 1000 rpm for 10 min and colour density of supernatant was measured spectrophotometrically at 540 nm (Mukherjee, 1988). Percent hemolysis was calculated by the formula as :

$$\text{Percent hemolysis} = \frac{\text{Absorbance of individual tubes} \times 100}{\text{Absorbance with 100\% hemolysis}}$$

RESULTS AND DISCUSSION

Cells in the control tube remain settled in the bottom with almost clear ambient solution. Morphologically cells remained unaltered.

Effect of various concentrations of aflatoxin is shown in Fig. 1. At low concentrations (0.35 to 1.10 µg/ml) there was appearance of tinge red colour in the medium; most of the cells remain settled in the bottom. Morphological observation revealed concentration dependent swelling of RBC. Hemolysis occurred at 1.4 µg/ml and above it. Concentration dependent increase in hemolysis was noted between 1.4 to 3.1 µg/ml. The maximum (75%) hemolysis occurred at 3.1 µg/ml concentration of aflatoxin. The amount of pellet at the bottom of the tube decreased, accompanied by appearance of red colour in the ambient solution.

AFB1 is reported to induce cytotoxicity and transformation in culture cells (Schwartz and Perantoni, 1975). Kaden et al. (1987) noted mutations besides toxicity as a result of AFB exposure to TK6 and HrMl cells in culture. Cytotoxicity of aflatoxin on mouse hepatoma cell line HePa-1 was reported by Karenlampi (1987).

Exact mechanism of its action is not clearly understood. It is presumed with above observations that aflatoxin causes destabilization of plasma membrane with influx of water inside the cell. Lipid peroxidation is regarded as one of the primary key events in cellular damage (Plaa and Witschi, 1976; Mead, 1976) and the relationship between GSH levels, lipid peroxidation and cell lysis has been reported (Anundi et al., 1978; Younes and Seigers, 1984; Toskulkao and Glinsukon, 1988). Present investigation clearly indicates that higher concentration of aflatoxin in the blood may cause self destruction of erythrocytes (hemolysis) whereas, lesser concentration will result in morphological alterations and natural elimination of RBC by its destruction at reticuloendothelial tissues. It might be the

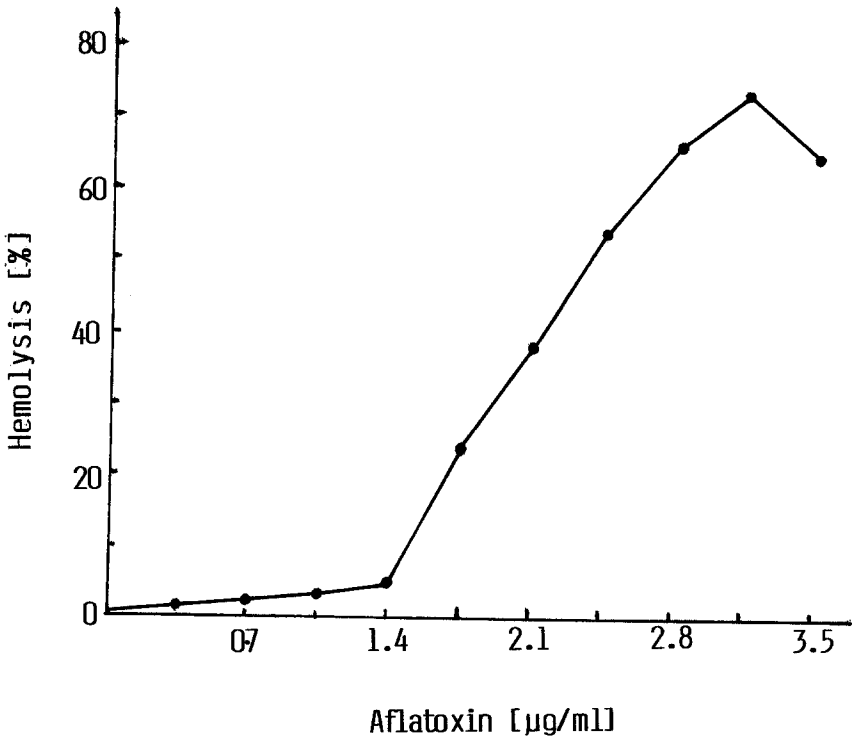

Figure 1 Showing effect of aflatoxin on red blood corpuscles

reason for occurrence of anemia during aflatoxicosis.

In summary, cytotoxicity of aflatoxin on RBC of rabbits was investigated in vitro. When saline suspension of RBC was treated with crude aflatoxin (obtained from culture filtrate of Aspergillus parasiticus (NRRL 3240) for 16 h at 37 C) the treated cells appeared swollen and spherical. The extent of swelling varied with the concentration of aflatoxin. Besides swelling, hemolysis too occurred. Hemolysis was more pronounced with higher concentration of aflatoxin (1.4 to 3.5 µg/ml) where ambient suspension became uniformly coloured due to released hemoglobin.

Acknowledgments. Authors are thankful to Prof. H.C. Dube for his constant encouragement. Financial assistance from Gujarat Council of Science and Technology is gratefully acknowledged.

REFERENCES

Anundi I, Kristoferson A, Hogberg J (1978) Xenobiotic induced toxicity in isolated hepatocytes. Excerpta Medica, Int Cong Series 440:270-280.

Brucato M, Sundlof SF, Bell JU, Edds GT (1986) Aflatoxin B1 toxicosis in dairy calves pretreated with selenium-vitamin E. Am J Vet Res 47 : 179-183.

Busby WF, Wogan GN (1984) Aflatoxins. In : Searle CE (ed) Chemical Carcinogens, ACS Monograph 182, American Chemical Society, Washington DC pp. 945-1136.

Clark JD, Jain AV, Hatch RC, Mahaffey EA (1980) Experimentally induced chronic aflatoxicosis in rabbits. Am J Vet Res 41: 1841-1845.

Diener UL, Davis ND (1966) Aflatoxin Production by isolates of Aspergillus flavus. Phytopath 56 : 1390-1393.

Groopman JD, Cain LG, Kensler TW (1988) Aflatoxin exposure in human populations : Measurements and relationship to cancer. CRC Critical Rev Toxicol 19 : 113-145.

Kaden DA, Call KM, Konives EA, Thilly WG (1987) Killing and mutation of human lymphoblast cells by aflatoxin B1. Evidence for an inducible repair response. Cancer Res 47: 1993-2001.

Karenlampi SO (1987) Mechanism of cytotoxicity of aflatoxin Bl. Biochem Biophys Res Commun 145:845-860.

Mead JF (1976) Free radical mechanism of lipid damage and consequences for cellular membranes. In:Pryor WA (ed) Free radicals in Biology, Academic Press, New York, pp 51-68.

Mukherjee KL (1988) Medical Laboratory Technology : A procedure Manual for Routine diagnostic tests. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Nabney J, Nesbitt BF (1965) A spectrophotometric method for determining the aflatoxins. Analyst 90:155-160.

Panda PC, Murti AS, Murthy VS, Murti IAS (1975) Effect of aflatoxin on the haematological picture of albino rats and guinea pigs. Indian J Exp Biol 13:569-570.

Patterson DSP (1983) Aflatoxicosis in farm animals. Vet Res Comm 7 : 135-140.

Plaa GL, Witschi H (1976) Chemicals, drugs and lipid peroxidation. Ann Rev Pharmacol Toxicol 16:125-141.

Ranjan KS (1987) Studies on effect of aflatoxin infested meal on some laboratory animals. Ph.D. thesis, Bhagalpur University, Bhagalpur, India.

Reddy RV, Taylor MJ, Sharma RP (1987) Studies on immune function of CD-1 mice exposed to aflatoxin B1. Toxicol 43:123-132.

Schwartz AG, Perantoni A (1975) Protective effect of dehydroepiandrosterone against aflatoxin B1 and 7,12-dimethylbenz (a) anthracene induced cytotoxicity and tranformation in cultured cell. Cancer Res 35:2482-2487.

Stoloff L (1977) Aflatoxin - an overview. In : Rodricks JV, Hasseltine CW, Mehlman MA (eds) Mycotoxins in Human and Animal Health, Pathotox Publishers, Park Forest South I, pp.16-28.

Toskulkao C, Glinsukon T (1988) Hepatic lipid peroxidation and intracellular calcium accumulation in ethanol potentiated aflatoxin B1 toxicity. J Pharmacobio Dyn 11 : 191-197.

Verma RJ, Raval PJ, Dube HC (1989) Effect of aflatoxin on red blood cells of human beings and certain animals. J. Toxicol Toxin Rev 8: Abstract.

Younes M and Seigers CP (1984) Interrelation between lipid peroxidation and other hepatotoxic event. Biochem Pharmacol 33 : 2001-2003.

Received October 15, 1990; accepted January 17, 1991.