Bull. Environ. Contam. Toxicol. (1992) 49:855-860

Environmental Contamination and Toxicology

Alterations in Biochemical Composition of Skeletal Muscle during Aflatoxicosis in Rabbits

P. J. Raval and R. J. Verma

Department of Life Sciences, Bhavnagar University, Bhavnagar-364002, India

The Aspergillus flavus and A. parasiticus groups of aspergilli are ubiquitous and well known for their aflatoxin-producing potentials. It is apparent from animal experiments and from clinical observations in man that short exposures to large doses of aflatoxin produces acute toxicity which may become lethal, while exposures to small doses over a protracted period of time is carcinogenic. Little is known about exposure to moderate amounts of dietary aflatoxins which appears to be of common occurrence in many tropical and subtropical countries. In parts of India, 100% of maize samples have been found contaminated with aflatoxin in the range of 6250-15600 μg/kg (Krishnamachari et al., 1975). Feeding aflatoxin-contaminated diet (15 mg aflatoxin/kg feed) to young rabbits over a period of 60 days caused significant reduction in body weight, but weights of organs like liver, kidney, spleen, adrenal, thyroid and heart remained almost unaltered. Reduced weight gain, respiratory troubles, lethargy and internal hemorrhage were also noted (Verma and Raval, 1991). Several other workers have also reported similar changes in various animal species (Allcroft and Carnaghan, 1963; Newberne et al., 1966; Maryamma and Sivadas, 1975; Edds, 1973; Clark et al., 1982; Jayasekara et al., 1989; Harvey et al., 1989; Pier et al., 1989; Verma et al., 1991).

Skeletal muscles account for approximately 40% of the body weight and another 5-10% is due to smooth muscles. A change in biochemical composition of muscles is expected to accompany the weight loss noted during aflatoxicosis. Inorganic components of muscles influence stimulus-response coupling, membrane permeability, as well as, muscular contractions and relaxations. In the present investigation we have examined the biochemical alterations in skeletal muscles of rabbit under toxaemia due to consumption of aflatoxin-contaminated feed.

MATERIALS AND METHODS

A toxigenic strain of A. parasiticus (NRRL 3240) was grown on SMKY liquid medium at 28±2°C for 10 days as described by Diener

Send reprint requests to Dr. R. J. Verma at the above address.

and Davis (1966). Obtained culture filtrates were extracted with chloroform and its aflatoxin content quantified by spectrophotometric method (Nabney and Nesbitt, 1965). The crude aflatoxin concentrate in chloroform was mixed with feed (15 mg aflatoxin/kg feed) and left overnight to allow for chloroform evaporation (Kadian et al., 1988). Random toxigenic samples were frequently analyzed for toxin content. Feed for 'control' rabbits was prepared in similar way but for addition of toxin.

Twenty young inbred NewZealand strain of rabbits weighing 200-225 gm (approximately 1 month old) were segregated in two groups. Group I rabbits were fed with aflatoxin contaminated feed (15 mg/kg) continuously for 60 days, whereas, Group II animals received nontoxic feed to serve as control. The animals were maintained under laboratory conditions and fed with ration and water ad-libitum.

The rabbits were sacrificed by cervical dislocation and thigh muscle samples were isolated and processed for biochemical analysis. Small pieces of muscle samples were weighed and analysed for their glycogen (Seifter et al., 1950) and protein (Lowry et al., 1951) contents. For water content, muscle pieces were quickly excised, dried in an anhydric air oven and weighed. Lipids were extracted from a known quantity of powdered dry tissues employing the method of Folch et al. (1957) using a mixture of chloroform and methanol (2:1 v/v). The lipid content was measured gravimetrically and expressed as mg% dry tissue.

For analysis of inorganic components, known weights of non-fat dry tissues (NFDT) were ashed in a muffle furnace at 600°C for overnight and % ash content measured gravimetrically. Ash samples were digested in 1 N HCl and analyzed for their sodium and potassium contents by flame photometry, while calcium and magnesium were estimated by titration. Phosphorus was estimated by photocolorimetric method and values were expressed as MEq/ 1000 gm NFDT (Jackson, 1973). Statistical analyses were done by standard student 't' test.

RESULTS AND DISCUSSION

Alterations in biochemical components of thigh muscle (Table 1) showed statistically significant decrease in glycogen, protein ($P < 0.02$), lipid ($P < 0.05$) and water contents ($P < 0.001$).

Table 2 shows that but for calcium, there was invariably a decrease in the contents of various minerals. The decrease was significant for sodium, magnesium and phosphorus. Concentration of calcium showed significant increase ($P < 0.01$). A distinct ionic imbalance is also recorded with regard to sodium/potassium and calcium/magnesium ions.

The data indicate that aflatoxin-contaminated feed caused significant reduction in concentrations of glycogen, protein, lipid

Table 1. Effect of feeding aflatoxin-contaminated diet on biochemical profile in the skeletal muscles of rabbits

Parameters	Control	Treated
Glycogen[1]	0.56 ±0.05	0.38[b] ±0.06
Protein[1]	15.03 ±0.69	12.94[b] ±0.70
Lipid[2]	25.24 ±1.09	22.05[c] ±1.05
Water[2]	79.15 ±0.14	78.46[a] ±0.15

N = 10; Values are mean ± SE.
Values for the same parameter in the same row with different superscripts significantly differ at the level: $^{a}P < 0.001$, $^{b}P < 0.02$, $^{c}P < 0.05$
Values expressed as: (1) mg % fresh weight, (2) mg % dry weight

and water in thigh muscle, revealing enhanced catabolic rate, muscle wasting and emaciation during aflatoxicosis in rabbits.

Aflatoxin B_1 is known to inhibit DNA and RNA synthesis (Clifford and Rees, 1967; Newberne and Butler, 1969; Edds, 1973). Clifford and Rees (1967) have also demonstrated inhibition of total liver protein synthesis *in vitro* when AFB_1 and [^{14}C] leucine were incubated in liver slice preparations. Decrease in K^+ can be correlated with reduced protein synthesis (Harper, 1971). Thus reduced protein concentration in muscle during aflatoxicosis may be due to inhibition of protein synthesis.

Reduction in glycogen concentration coincides with decreased K^+ and increased Ca^{2+} concentration; the latter is known to enhance glycogen phosphorylase activity in muscles (McGilvery, 1979). Raval (1991) has also observed increased glucose-6-phosphatase activity in the liver of rabbits during aflatoxicosis.

Lipid concentration of muscle depleted after aflatoxin ingestion which may be due to increased lipolysis to support fuel requirement of muscle during aflatoxicosis. Higher intracellular Ca^{+2} during aflatoxicosis leads to mitochondrial dysfunction, inhibition of enzymes and denaturation of structural protein (Toskulkao and Glinsukon, 1988). Swelling and structural alterations in mitochondria have been reported during aflatoxicosis (Roy, 1968; Bose et al., 1991). Thus, energy requirements of muscles may be fulfilled by anaerobic metabolism as apparent by lactic

Table 2. Effect of feeding aflatoxin-contaminated diet on certain inorganic components in the skeletal muscle of rabbits

Parameters	Control	Treated
Ash[1]	3.62 ±0.61	4.04 ±0.26
Sodium[2]	152.58 ±7.26	131.43[d] ±6.57
Potassium[2]	173.24 ±29.43	169.47 ±16.75
Sodium/Potassium	0.88	0.78
Calcium[2]	124.71 ±4.36	144.53[b] ±4.51
Magnesium[2]	1422.93 ±52.39	1256.41[c] ±32.32
Calcium/Magnesium	0.09	0.11
Inorganic Phosphorus	818.37 ±41.51	590.43[a] ±39.09

N = 10; Values are mean ± SE.
Values for the same parameter in the same row with different superscripts significantly differ at the level: $^{a}P < 0.001$, $^{b}P < 0.01$ $^{c}P < 0.02$, $^{d}P < 0.05$
Values expressed as: (1) mg % non-fat dry tissue, (2) MEq/1000 gm non-fat dry tissue.

acidosis (Raval, 1991) which requires more fuel than during aerobic conditions. Our data also indicate reduction in water content which correlates with reduction in Na^{+} concentration. Occurrence of dehydration is reported by Thurston et al. (1972) and Clark et al. (1980). The relative proportion of divalent cations are important for muscle contraction. Thus, increased Ca^{+2}/Mg^{+2} ratio altered aerobic metabolism causing lethargy which is well documented during aflatoxicosis. Decreased phosphorus concentration can be correlated with increased excretion of creatinine (Raval, 1991) which is a metabolically transformed form of phospho-creatine, an energy reservoir of muscles.

Acknowledgements 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

Allcroft R, Carnaghan RBA (1963) Groundnut toxicity; an examination for toxin in human food products from animals fed toxic groundnut meal. Vet Rec LXXV: 259-263

Bose S, Mathur M, Nayak NC (1991) A light & electron microscopic study on apoptotic bodies in acute aflatoxin B_1 induced injury in the rat liver. Indian J Med Res [B] 94: 134-138.

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

Clark JD, Jain AV, Hatch RC (1982) Effects of various treatments on induced subacute aflatoxicosis in rabbits. Amer J Vet Res 43: 106-110.

Clifford JI, Rees KR (1967) The action of aflatoxin B_1 on the rat liver. Biochem J 102: 64-75.

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

Edds GT (1973) Acute aflatoxicosis: A review. J. Amer Vet Med Assoc 162: 304-309.

Folch I, Lees M, Sloanestanly GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226: 497-509.

Harper HA (ed) (1971) Review of physiological chemistry, 13th edition, Maruzen Asian Edition, pp. 478-487.

Harvey RB, Kubena LF, Phillips TD, Huff WE, Corrier DE (1989) Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. Amer J Vet Res 50: 416-420.

Jackson ML (ed) (1973) Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Jayasekara S, Drown DB, Coulombe RA Jr, Sharma RP (1989) Alteration of biogenic amines in mouse brain regions by alkylating agents I. Effect of aflatoxin B_1 on brain monoamines concentrations and activities of metabolizing enzyme. Arch Environ Contam Toxicol 18: 396-403.

Kadian SK, Monga DP, Goel MC (1988) Effect of aflatoxin B_1 on the delayed type hypersensitivity and phagocytic activity of reticuloendothelial system in chickens. Mycopathologia 104: 33-36.

Krishnamachari KAVR, Bhat RV, Nagarajan V, Tilak TBG (1975) Investigations into an outbreak of hepatitis in parts of Western India. Indian J Med Res 63: 1036-1048.

Lowry DH, Rosenbrough NI, Farr A, Randall J (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275.

Maryamma KI, Sivadas CG (1975) Aflatoxicosis in goats (an experimental study). Indian Vet J 52: 385-392.

McGilvery RW (ed) (1979) Biochemistry. A functional approach 2nd edition. W.B.Saunders Company, Philadelphia.

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

Newberne PM, Butler WH (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals. A review. Cancer Res 29: 236-250.

Newberne PM, Russo R, Wogan GN (1966) Acute toxicity of aflatoxin B_1 in the dog. Pathol Vet 3: 331-340.

Pier AC, Belden EL, Ellias JA, Nelson EW, Maki LR (1989) Effects of cyclopiazonic acid and aflatoxin singly and in combination on selected clinical, pathological and immunological responses of guinea pigs. Mycopathologia 105: 135-142.

Raval PJ (1991) Histopathological and biochemical studies in rabbits under aflatoxin-induced toxaemia. Ph.D. Thesis. Bhavnagar University, Bhavnagar, India.

Roy AK (1968) Effect of aflatoxin on polysomal profiles and RNA synthesis in rat liver. Biochim Biophys Acta 169: 206-211.

Seifter S, Dayton S, Novic B, Muntwyler V (1950) The estimation of glycogen with anthrone reagent. Arch Biochem 25: 191-200.

Thurston JR, Richard JL, Cysewski SJ, Pier AC, Gram CK (1972) Effect of aflatoxin on complement activity in guinea pigs. Proc Soc Exp Biol Med 139: 300.

Toskulkao C, Glinsukon T (1988) Hepatic lipid peroxidation and intracellular calcium accumulation in ethanol potentiated aflatoxin B_1 toxicity. J Pharmacobio Dyn 11: 191-197.

Verma RJ, Raval PJ (1991) Morphological alterations in rabbits under aflatoxin induced toxaemia. J Anim Morphol Physiol (in press).

Verma RJ, Raval PJ, Dube HC (1991) Effect of aflatoxin on liver and blood cells of rats. Indian J Microbiol 31: 87-89.

Received February 28, 1992; accepted July 17, 1992.