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Effect of low protein diet on chronic aflatoxin B_1-induced liver injury in Rhesus monkeys

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The effect of dietary restriction of protein on monkeys fed 1.0 PPM of aflatoxin B_1 in their daily diet was investigated. The low and high protein diet, respectively, contained 5 and 20% casein.

By 38–40 wks, liver of monkeys in the low protein group exhibited large areas of hepatocyte necrosis, whereas those on high protein diet showed neoplastic nodules in the liver. These observations with 1.0 PPM of AFB_1 (high dose) in diet are different from earlier observations with 0.16 PPM (low dose) AFB_1 in diet. With the latter dose, while the low protein AFB_1 fed monkeys revealed preneoplastic lesions in the liver, no such alterations were recorded in the high protein, toxin fed animals.

These studies demonstrate that the dose of AFB_1 is important in determining the response of liver to a low protein diet in AFB_1 induced hepatocarcinogenesis.

Previously we communicated our observations on the interaction of protein calorie malnutrition and chronic low dose aflatoxin B_1 (AFB_1) induced injury in Rhesus monkeys [12]. The present study was undertaken to investigate the difference, if any, in the response of monkeys to a higher level of AFB_1 intoxication. In the earlier study with a dose of 0.16 PPM and 0.5 PPM in diet, no neoplastic leasions were observed at 120 wks in the animals fed a high protein diet. The animals that survived for 90 wks in the low protein group showed preneoplastic lesions [12].

Material and methods

Animals. Twelve Rhesus monkeys weighing between 1 to 1.2 kg were used for the experiment. Seven animals were reared on low protein diet (5% casein) while 5 animals were put on high protein diet (20% casein) to serve as controls.
Aflatoxin – AFB_1 was obtained from Makor Ltd. (Jerusalem). It was dissolved in propylene glycol, diluted in peanut oil and used as 1.0 PPM/day in the diet.
Feeding schedule – Animals were bottle fed a milk diet containing the required amount of aflatoxin. Details of the preparation of low and high protein milk diet and mode of administration of aflatoxin B_1 to monkeys has been described [12, 16].

Light microscopy, enzyme histochemistry and electron microscopy studies

At 16 wks, 3 animals each from both groups were subject to an open wedge biopsy of the liver. At

the time of the sacrifice of the animal, complete autopsy was performed.

Liver pieces were fixed in 10 percent formalin and paraffin sections cut at 4u thickness were stained with Haematoxylin and eosin and periodic acid Schiff's reagent for light microscopy. Fresh and cold formalin fixed cryostat sections of liver were used for histochemical staining; the former for glucose-6-phosphatase (G6Pase) and succinic dehydrogenase (SDH) and the latter for adenosine triphosphatase (AT-Pase), alkaline phosphatase (ALK Pase), and acid phosphatase (Acid Pase) according to the techniques previously described [15].

Results

General observations-until 25 wks, the animals in both the group appeared healthy and active. The monkeys in high protein group showed steady gain in weight where as those on low protein diet recorded no increase in weight. After 30 wks the monkeys in both the groups appeared listless, less active, and by 38 wks they stopped accepting feeds and a few; particularly in the low protein groups started passing high coloured urine. Two animals from low protein group died and the rest were sacrified between 38 to 40 wks.

Light microscopy

Low protein animals. Three animals subjected to open liver biopsy at 16 wks of AFB_1 administration showed mild alterations in the liver parenchyma. Normal lobular architecture of the liver was maintained. A significant degree of bile duct proliferation was seen in the portal tracts. The hepatocytes showed mild polyploidy and focal necrosis of hepatocytes.

Histopathology of liver pieces taken at postmortem examination (38–40 wks) revealed large areas of hepatocyte necrosis, chiefly periportal and was associated with extensive bile duct proliferation (Fig. 1). The surviving hepatocytes were confined to narrow islands. No regenerate activity

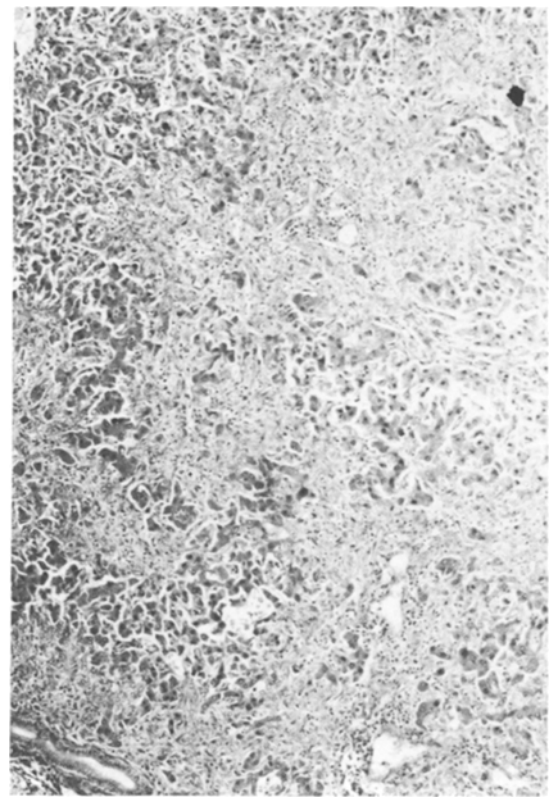

Fig. 1. Photomicrograph of liver from an AFB_1 fed monkey on low protein diet showing large areas of necrosis. H 2 E × 100.

occurred in these livers. A few foci showed cytoplasmic changes in the form of clear or acidophilic cytoplasm suggestive of preneoplastic lesions as described by Bannasch [3] in rats and observed in monkeys in our earlier experiments [12]. However, no neoplastic lesions were identified.

High protein animals. From biopsies taken at 16 wks, the livers of these animals showed changes that were milder though qualitatively similar to that observed in the low protein groups at this time interval.

Changes in the liver at the time of autopsy were prominent though different from those observed in the low protein animals at this tims interval. The architecture of the liver was obscured by nodules of regenerating hepatocytes. Many of the nodules showed preneoplastic cytoplasmic alterations. In addition, a few nodules causing com-

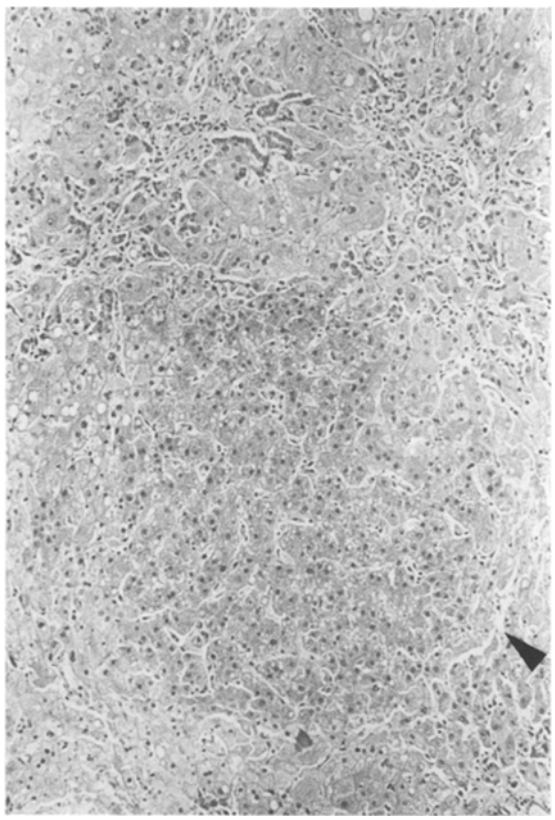

Fig. 2. Photomicrograph of liver from an AFB_1 fed monkey on high protein diet showing neoplastic nodule (arrow). H 2 E × 100.

pression of adjascent parenchyma were composed of small baseophilic cells, and concluded to be neoplastic foci (Fig. 2). Ductular proliferation was seen between the nodules.

Histochemical staining. A detailed histochemical study done on these livers showed a markedly heterogenous pattern of enzymes staining.

In the high protein group the majority of the nodules were negative for alk Pase, SDH, G6 Pase and ATPase, however, a few nodules revealed G6Pase, SDH and ATPase activity (Fig. 3). In the low protein group no nodules were identified but a ductular proliferation occurred. The cells constituting the ductules were positive for G6 Pase and SDH (Fig. 4).

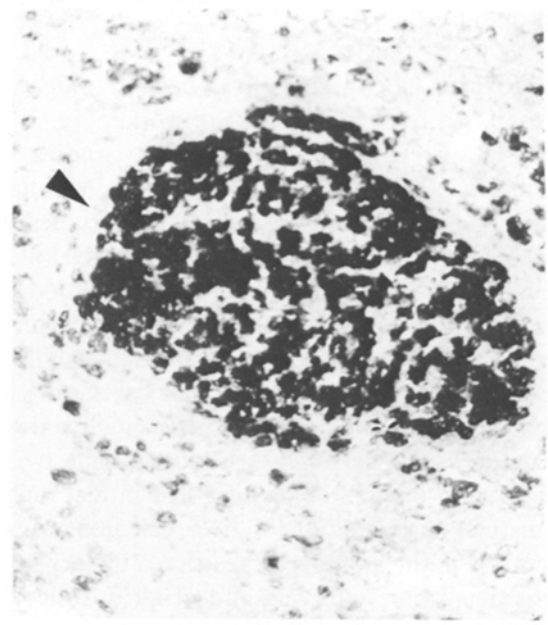

Fig. 3. Photomicrograph of liver from an AFB_1 fed monkey on high protein diet showing G 6 Pase positive nodule (arrow). G 6 Pase × 100.

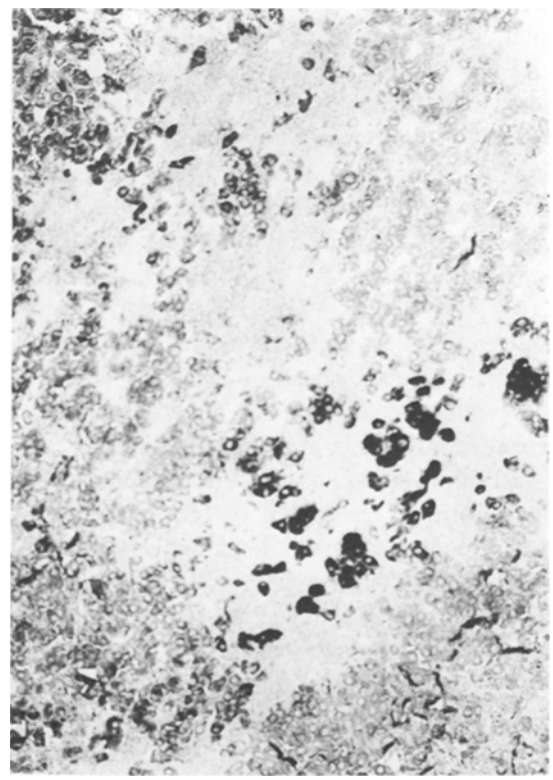

Fig. 4. Photomicrograph of liver from an AFB_1 fed monkey on low protein diet showing G 6 Pase positivity in ductular cells G 6 Pase × 100.

Discussion

Aflatoxin induced lesions have been investigated in monkeys by several groups of workers [1, 2, 6, 17]. However, there are very few studies in monkeys in which the role of protein calorie malnutrition has been investigated [7, 11]. Studies on the effect of malnutrition on AFB_1 induced liver injury are important as contamination of food by the mycotoxins and malnutrition are prevalent in certain areas of the world where incidence of liver disease, particularly hepatocellular carcinoma, is high [8, 13]. In our earlier studies using low dose of AFB_1 such as 50 μg/kg body weight twice weekly and 0.16 PPM or 0.5 PPM in diet daily, a distinct accentuation of liver cell injury was recorded in the animals on restricted dietary proteins at all time intervals and at all dose levels [12, 16].

In addition, an important feature of the earlier studies was the alterations observed by 18 wks, in the cell generation cycle of hepatocytes in the low protein fed monkeys given 50 μg/kg twice weekly of AFB_1. The cell generation cycle in this experiment was determined by cytoflourimetric analysis [16]. In another experiment [12] emergence of preneoplastic lesions at 90 wks was observed in low protein group of animals fed very low levels (0.16 PPM) of AFB_1 in diet. Monkeys given adequate protein diet and the same dose of toxin (0.16 PPM) in diet did not show any preneoplastic/neoplastic lesions on a follow up of 120 wks.

In contrast to the earlier experiments, in the present study, where a higher dose of toxin (1.0 PPM in diet) was used, the hepatic alterations appeared faster and pattern of injury was also different. By 40 wks the animals on high protein diet had neoplastic nodules in the liver whereas in animals on dietary restriction of proteins there were large areas of liver cells necrosis, a lack of hepatocytic regeneration and a few loci of preneoplastic alterations (but no neoplastic nodules) indicating that the pattern of response of liver to aflatoxin B_1 in monkeys is affected by the dose of toxin employed.

The appearance of neoplatic nodules in animals on adequate dietary protein and their absence from animals on protein deficient diet in the high dose experiment is similar to the observation of Madhaven *et al.* [10] in rats. As reported earlier, the observations from the low dose experiments in monkeys are quite different [12].

The mechanism for the difference in the response of protein deficient and control (high protein) animals to low and high dose AFB_1 intoxication is possibly related to the degree of necrosis produced by a particular dose of AFB_1. In the experiments using low dose of AFB_1 the appearance of preneoplastic lesions in the low protein group and their absence in high protein groups has been explained in a previous publication [12]. We proposed that the occurrence of significant liver cell necrosis in low protein animals with even a subnecrogenic dose of AFB_1 is responsible for the preneoplastic alteration in the low protein group. It appears that liver cell necrosis mediates its effect by inducing cell proliferation which is shown by a large number of investigators to have a profound influence on the emergence of neoplatic lesions [5, 14]. In monkeys on adequate protein diet, a subnecrogenic low dose of carcinogen may fail to induce any preneoplastic lesions as it does not provide the necessary stimulus for cell proliferation. However, in situations where dietary proteins are restricted, significant hepatocyte necrosis occuring even with a small dose of carcinogen is able to induce preneoplastic lesions due to cell proliferation and prolongation of the cell generation cycle as has been reported earlier [12].

The response of liver to AFB_1 is different when a high (necrogenic dose) dose-1.0 PPM of AFB_1 is used alongwith simultaneous restriction of proteins. There is a marked hepatocytic necrosis causing the deaths of animals. The necrosis produced in the high protein group with a dose of 1.0 PPM of AFB_1 (necrogenic dose) is not as extensive as in the low protein group. However the necrosis provides significant stimulus for cell replication resulting in the appearance of neoplatic nodules in this group.

A number of studies have been undertaken to investigate the histochemical alteration in liver

parenchyma during aflatoxin induced carcinogenesis in rats [3, 4, 9], however histochemical enzyme reactions have not been studied in monkeys during AFB_1 induced hepatocarcinogenesis. The histochemical features seen in this study show that the regenerative and neoplastic nodules are composed of heterogenous population of hepatocytes. The reaction observed in the neoplastic nodules in the livers of monkeys was similar to our earlier experiments and those of others on AFB_1 induced hepatocarcinogenesis in rats [3, 4, 9]. A significant degree of ductular cell proliferation was observed in the AFB_1 treated animals and was more marked in monkeys on low protein diet.

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References

1. Adamson PC, Corres P, Dalgand DW. Brief communication: Occurrences of primary liver carcinoma in rhesus monkey fed aflatoxin B_1. J Natl Can Instt 1973; 50: 549–51.
2. Alpert E, Serck-Hanssen A. Aflatoxin-induced hepatic injury in the Africa Monkey. Arch Envir Health 1970; 20: 723–8.
3. Bannash P. Sequential cellular changes during chemical carcinogenesis. J Can Res Clin Oncol 1984; 108: 11–22.
4. Butler WH, Hempsall V. Histochemical studies of hepatocellular carcinomas in the rats induced by aflatoxin. J Pathol 1989; 134: 157–70.
5. Craddock VM (1976) cell proliferation and experimental liver cancer In: Cameron HM, Linsell DA and Warwick eds liver cell cancer, Elsevier, Amsterdam, 1976; 152–201.
6. Cuthbertson WFJ, Laursen SC, Pratt DAM. Effect of groundnut meal containing aflatoxin on cynomolgus monkeys. Brit J Nut 1967; 21: 893–903.
7. Deo MG, Dayal Y, Ramalingswami V. Aflatoxin and liver injury in Rhesus Monkeys. J Pathol 1970; 101: 47–56.
8. Hendrickse RG. The influence of aflatoxins in child health in tropics with particular reference to Kwashiorkor, Tran Sec Trop Med 1984; 78: 427–35.
9. Kalengayi MMR, Desmet VJ. Sequential histological and histochemical study of the rat liver during aflatoxin B_1 induced carcinogenesis. Can Res 1975; 35: 2845.
10. Madhaven T, Gopalan C. The effect of dietary proteins on carcinogenesis of aflatoxin 1968; 85: 133–7.
11. Madhaven TV, Rao KS. Effect of dietary protein level on susceptibility of Monkeys of aflatoxin liver injury. Ind J Med Res 1965; 53: 984–9.
12. Mathur M, Nayak NC. Effect of low protein diet on low dose chronic aflatoxin B_1 induced hepatic injury in Rhesus monkeys Journal of Toxicology Toxin Reviews 1989; 8: 265–74.
13. Peers FG, Linsell CA. Dietary aflatoxins and liver cancer A population based study in Kenya. Brit J Can 1973; 27: 427–84.
14. Pound AW, Lawson TA. Effect of partial heptactomy on carcinogenecity, metabolism and binding to DNA of ethyl carbamate, J Natl Inst 1974; 53: 423–9.
15. Rizvi TA, Mathur M, Nayak NC. Effect of protein calorie malnutrition and cell replication on aflatoxin B_1 induced hepatocarcinogenesis in rats. J Natl Can Instt 1987; 79: 817–30.
16. Singal V, Mathur M, Ramalingaswami V, Deo MG. Aflatoxin B_1 induced injury in monkeys. Arch Pathol Lab Med 1985; 105: 105–8.
17. Svoboda D, Grady HJ, Higginson J. Aflatoxin B_1 injury in rat and monkey liver. Am J Pathol 1966; 49: 1023–38.

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