Effects of Aflatoxin B1 on Liver Nuclear DNA Biosynthesis in Growing and Day-old Chickens

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Summary

The inhibitory effects of aflatoxin B1 (AFB1) on hepatic DNA biosynthesis was compared in growing and day-old chickens. A single ip dose of AFB1 (0,25,50,100 or 200µg/kg BW) was administered to groups of hen and day-old female Hybro-broiler chickens pre-treated with [^{3}H]-thymidine. All the chickens were given labeled thymidine 22h prior to AFB1 administration and sacrificed 2h after AFB1 injection. Livers were removed and processed for DNA isolation and estimation of thymidine incorporation into nuclear DNA. A differential inhibitory effect of AFB1 on DNA biosynthesis in hen and chicks was noticed. The overall results showed that as compared to growing chickens, youngers are relatively more refractory to AFB1-induced inhibition in DNA biosynthesis. A single dose of AFB1 (25, 50, 100 or 200 µg/kg BW) administered to day-old chickens caused on average 23-28% inhibition in DNA biosynthesis. Whereas under similar conditions of dose and treatments to the growing group, a greater (36-47%) but dose-dependent inhibition in hen's liver DNA is recorded.

Key words: aflatoxin B1, age, chicken, DNA biosynthesis, liver

Introduction

Aflatoxins (B1, B2, G1 and G2) are a group of structurally related mycotoxins produced as secondary metabolites by toxigenic strains of *Aspergillus flavus* and *A.parasiticus.* Fungal growth and aflatoxin production on agricultural grains is

increased under favorable conditions of temperature and humidity (Allameh & Razzaghi 2001, Betina *et al* 1989). Aflatoxin B1 (AFB1) has been proved as a highly potent hepatocarcinogenic and hepatotoxic chemical in several animal species. AFB1 exerts its biological effects after metabolic activation by cytochrome P-450-dependent monooxygenase activity to its reactive form i.e. AFB1-8, 9-epoxide, leading to covalent binding to cellular macromolecules particularly DNA (Croy *et al* 1978, Swenson *et al* 1977). *In vitro* and *in vivo* studies showed that microsomal dependent cytochrome P-450 generated reactive AFB1-epoxide could be inactivated by cytosolic glutathione (GSH) S-transferases to prevent AFB1-DNA adduct formation (Degen & Neumann 1981, Lotlikar *et al* 1984).

Exposure of animals to aflatoxin-contaminated diet is associated with various histopathological and biochemical changes in the liver (Cullen & Newberne 1993, Newberne & Butler 1969). Inhibition of the biosynthesis of macromolecules (DNA, RNA and proteins) in liver is considered as the primary biochemical effects of AFB1 (Godoy & Neal 1976, Rogers & Newberne 1967, Yu 1981). Despite a bulk of information on the biological effects of aflatoxins on various animal species, very little has been documented on the effect of age on the metabolism of aflatoxins. In the recent years we have performed series of experiments to evaluate the efficiency of growing rats in metabolizing and eliminating aflatoxin metabolite. Our preliminary study *in vitro* revealed that microsomes drive from newborn rats are less capable of converting AFB1 to its reactive metabolite as judged by its adduct formation to DNA (Behroozikha *et al* 1992). Further studies carried out *in vivo* showed that delayed AFB1 epoxidation in growing rat liver is in consistent with the rate of AFB1 detoxification (Chelcheleh & Allameh 1995). More recently studies carried out on two selected age groups of rats showed a differential induction in the cytosolic glutathion S-transferase (GST) activity with concomitant increase in AFB1-GSH conjugation in tissues of weanling rats (Allameh *et al* 1999).

Mycotoxin contamination of feed has an enormous impact on the health of domestic animals for example, economic losses in the poultry industry from poor growth and efficiency of feed conversion have been associated with aflatoxins in feed (Jones *et al* 1982). These compounds cause a serious disease in trout, poultry,

livestock and other animals (Newberne & Butler 1969). Among the non-mammalian animals, chicken is relatively more resistant to the toxic and carcinogenic effect of aflatoxins (Allcraft 1969, Gawai *et al* 1992). With the development of broiler strains genetically selected for rapid growth a relative increase in the frequency of tumors in young chickens has been observed (Campbell & Appleby 1966, Hemsley 1966). In the present study an attempt has been made to investigate the inhibitory effects of different doses of AFB1 on the biosynthesis of DNA in livers of day-old and growing chickens.

Materials and Methods

Chemicals. Aflatoxin B1, dimethyl sulfoxide (DMSO) and calf thymus DNA were obtained from Sigma, U.S.A. [3H]-thymidine was the product of Amersham Inc. U.K. Other chemicals and reagents were from Merck, Germany.

Experimental procedures. Two different age groups of female Hybro-broiler chickens were taken for these experiments. Growing chickens were 45±5 day-old and divided into five subgroups each of 5. Likewise day-old chicks were divided into five subgroups. In order to measure the inhibitory effects of AFB1 on the incorporation of ^{3}H-thymidine in DNA, 22h prior to AFB1 a single dose of radioactive thymidine was injected intraperitoneally (ip) to all the chickens. The specific activity of stock solution of [^{3}H]-thymidine was 15.8Ci/mmol. Each young chicken was injected with thymidine prepared in normal saline solution (15µl of stock solution dissolved in 0.2ml solution for each young chicken). Likewise each of the growing chickens received 900µl of 3H-thymidine prepared in 2ml of normal saline solution. The inhibitory effect of AFB1 on thymidine uptake was compared in control and AFB1-treated chickens. Chickens in experimental groups were injected with a single i.p dose of AFB1, which was prepared in DMSO. Various doses of AFB1 viz. 0, 25, 50, 100 or 200µg/kg BW were prepared and administered to different experimental groups. Chickens in control groups were given DMSO alone.

All the chickens were sacrificed 2h after AFB1 treatment. Livers were removed and processed for DNA isolation and measurement of [^{3}H]-thymidine incorporation

into nuclear DNA. The results of [3H]-thymidine incorporation in tissue nuclear DNA was expressed as cpm/g liver. DNA content of the samples was measured by diphenylamine reagent, using calf thymus DNA as standard (Burton 1956).

Results

Administration of a single dose of aflatoxin B1 to groups of hen and day-old chickens pretreated with [3H]-thymidine, caused a differential inhibitory effects on DNA biosynthesis in adult and young chickens as judged by the rate of [3H]-thymidine incorporation into liver nuclear DNA (Figure 1). The rate of thymidine incorporation in DNA calculated for control groups (DMSO-treated) was considered as 100% and the rate of DNA biosynthesis inhibition due to AFB1 was calculated accordingly.
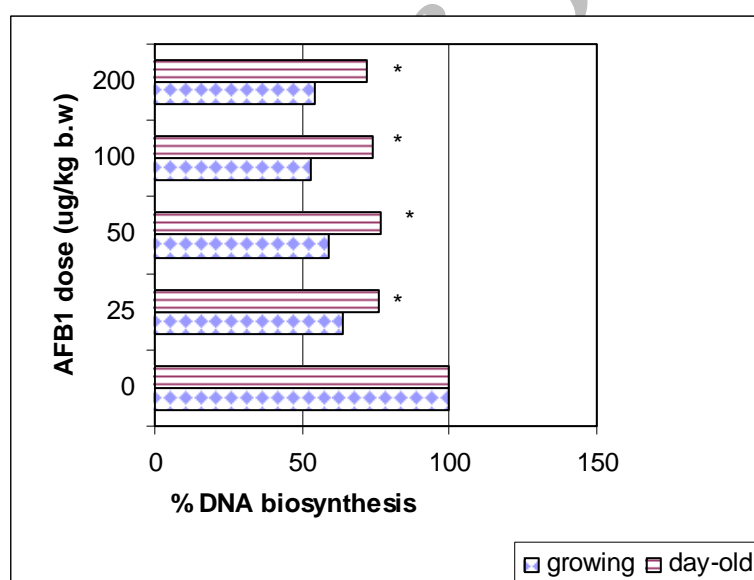

Figure 1. *Comparison of the effect of aflatoxin B1 on DNA biosynthesis in livers of day-old and growing chickens*

The rate of liver DNA biosynthesis was found to be inhibited by 36, 41 and 47% in chickens pretreated with 25, 50 or 100μg AFB1/Kg BW. Higher doses of AFB1 i.e. 200μg/kg given to growing chickens failed to further inhibit DNA biosynthesis. One day-old chicken showed relatively more refractory to AFB1 treatments. Doses of 25, 50, 100 and 200μg/kg of AFB1 resulted in 24, 23, 29 and 28% inhibition in liver DNA biosynthesis respectively. Results are presented as Mean±SEM of 5 analysis carried out in duplicate on 5 tissues from 5 separate chickens. The data from all the

AFB1-treated groups are significantly different with $p<0.05$ from their respective control groups.

Discussion

Inhibition of DNA biosynthesis has been accepted as a primary cellular alteration that is impacted by AFB1 administration and that represents an important initiation event through which toxin exerts its effects on the molecules and tissues. As a consequence of this the following biochemical processes may be affected: RNA and protein synthesis, lipid metabolism, mitochondrial function, lysosomal enzyme activities and membrane transport.

Inhibition in liver DNA biosynthesis is also linked to the metabolic activation and detoxification of AFB1 in the liver. The effect of age on AFB1 metabolism and biological effects was studied in rats. Our preliminary studies carried out to compare the activities of key enzymes involved in AFB1 metabolism in rats of various age groups revealed that in immature rat hepatic microsomal cytochrome P-450 together with GSH content and GST activity are significantly lower as compared to that in adults. These data were further confirmed *in vitro* in a reconstitution incubation system involving sub cellular fractions derived from livers of rats of different ages (Behroozikha *et al* 1992). When these findings were examined *in vivo* unexpected results were obtained. It has been observed that hour after administration of paracetamol, a hepatotoxic drug, to the young rats the activity of GST increases significantly. GST induction in response to the xenobiotics causes a surge in the rate of the xenobiotic-glutathione conjugate formation in the tissues. The inducibility of GST was confined to young rats and very little changes were observed in tissues of adult rats.

A single toxic dose of paracetamol administered to young and adult rats resulted in a 24-fold increase in drug-GSH conjugate formation (Allameh *et al* 1997). Such differences could be attributed to the differential induction in liver GST activity in adult and young rats (Allameh *et al* 1999). Studies on aflatoxin B1 metabolism in young and adult rats, revealed that due to lower levels of liver cytochrome P-450 in

young rats, the rate of AFB1 adduct formation to liver nuclear DNA is lower during early ages. It has been suggested that slowly generated epoxide is one of the protective mechanisms involved in modulation of deleterious effects of this toxic metabolite. Delayed formation of epoxide enables the detoxification enzyme system to efficiently eliminate the reactive metabolites (Chelcheleh & Allameh 1995). Further experiments carried out with aflatoxin B1 in rats show that administration of this carcinogen increases the GST activity in young tissues (liver and kidneys) more significantly than that in adults. This data suggest that probably immature rats are capable of eliminating aflatoxin metabolites more efficiently and relatively faster as compared to adults (Allameh *et al* 2000).

Very little has been documented on the molecular mechanisms of AFB1-induced liver damages in chicken. Differences observed in the inhibitory effects of AFB1 on liver DNA biosynthesis between hens and day-old chickens indicate that the differences in the drug metabolizing enzyme system in tissues of the two ages group. The percentage inhibition in liver DNA biosynthesis is greater in adults showing that the metabolic activation and formation of reactive metabolites in mature liver is relatively more efficient. In addition the rate of the detoxification of toxic metabolites via glutathione conjugation takes place with a normal rate, whereas the rate of cytochrome P-450 dependent epoxidation is relatively lower in young chickens as compared to that of growing. On the other hand, it seems that the development of phase II detoxification enzyme i.e. glutathione S-transferases precedes phase I xenobiotic metabolizing system. Furthermore there are evidences showing that the activity of cytosolic GSH S-transferase is induced more readily in younger animals in response to AFB1. The percentage inhibition of DNA biosynthesis due to different doses of AFB1 in hens was 36-47%. A single small dose of AFB1 caused about 36% inhibition in DNA biosynthesis. Higher doses of AFB1 increased the inhibition rate up to 47%.The process of DNA biosynthesis in young chickens showed a relatively more resistance to AFB1 treatments. A maximum 28% inhibition was recorded in livers of young chickens dosed with 200μg AFB1/kg. These data suggest that day-old chicken is more refractory to the primary biological effects of AFB1.

In conclusion, the differences between adult and young animals in drug metabolism are attributed to several factors as described briefly below:

-The delayed metabolic activation of AFB1 mediated by cytochrome P-450.

-More readily induction in AFB1 detoxification enzyme i.e. glutathione S-transferase in younger animals.

-Efficiency of liver in hydrolysis/repair of the AFB1-DNA adducts at early ages.

-Increased rate of aflatoxin conjugate formation and elimination through kidneys.

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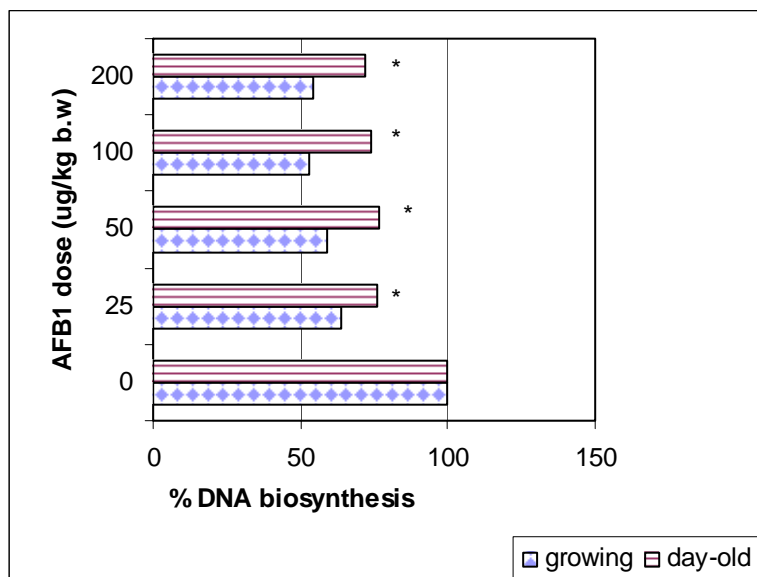
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