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COMPARISON OF THE PATTERN OF DISTRIBUTION OF AFLATOXIN B1 METABOLITES IN ADULT AND NEWBORN RATS

M. CHELCHELEH, A. ALLAMEH*, E. HAJIZADEH AND M.J. RASSAEE

From the Department of Biochemistry, Faculty of Medical Sciences, Tarbiat Modarress University, P.O.Box 14155-4838, Tehran, Islamic Republic of Iran.

ABSTRACT

Recently we have reported that newborn rats are deficient in the key enzymes involved in the biotransformation of aflatoxin B1 (AFB1), a known hepatocarcinogen.[1] Based on these data, *in vivo* experiments were carried out in order to investigate the bioavailability of this carcinogen in newborn rat tissues. Administration of a single dose (i.p.) of [^{3}H]AFB1 to groups of adult and neonatal rats resulted in the differential distribution of AFB1 metabolites in these animals. Uptake of aflatoxins by neonatal rats was about 50% of that in adults at all time intervals. In newborn liver, the level of aflatoxin reached its maximum 6 h after injection, and gradually decreased during the following 12 and 24 h. In adult liver the uptake was highest 2 h after AFB1 administration.

A decrease of radioactivity in liver relative to time was associated with a surge in aflatoxin levels in the sera of both age groups. Excretion of AFB1 metabolites was comparatively faster from newborn than from adult kidneys. Much lower radioactivity was measured in tissues such as stomach, intestine and lungs compared to liver. These observations indicate that the neonatal rat liver is less efficient in the bioactivation of AFB1, as a result of which free AFB1 (non-metabolized) may remain for a longer period of time in the organs of immature rats. *MJIRI, Vol. 9, No. 4, 347-350, 1996.*

INTRODUCTION

Aflatoxin B1 (AFB1), a known hepatotoxin and hepatocarcinogen, is a mycotoxin produced as a secondary metabolite by certain strains of *Aspergillus flavus*. *A. flavus* strains grow rapidly on a wide variety of foodstuffs under favourable conditions of moisture and temperature. Epidemiological data suggest that AFB1 contamination of human food may be partly responsible for the induction of human liver cancer in several parts of Africa and Asia.[2]

Human infants and animals may receive aflatoxin through the milk of mothers consuming a toxin-contaminated meal.[3] Powdered milk or formula can also be the source of aflatoxin contamination.

It is well established that AFB1 is a procarcinogen which requires bioactivation in order to generate the active metabolite AFB1-epoxide, which then interacts with cellular DNA to initiate carcinogenesis.[4] A cytochrome P-450-dependent oxidative reaction yields the active AFB1 metabolite. Glutathione (GSH) S-transferases that catalyse AFB1-GSH conjugation are thought to be a detoxification pathway for AFB1.[5,6]

The carcinogenicity of AFB1 in susceptible and

* To whom correspondence should be addressed.

resistant species parallels the ability of the liver to bioactivate the mycotoxin. AFB1-DNA binding and AFB1-GSH conjugate formation *in vitro* and *in vivo* are important factors in determining the susceptibility of animal species to the carcinogenicity of AFB1.[7] Therefore it is obvious that the ability of animal species to detoxify the carcinogen depends directly on the performance of this system.

In previous studies we have clearly shown that newborn rats are deficient in the hepatic key factors involved in the biotransformation of AFB1. Decreased levels of cytochrome P-450, cellular GSH and GSH S-transferases in newborn liver cause lower AFB1-DNA binding and AFB1-GSH conjugation *in vitro*.[1] The implication of these results in the pattern of distribution of AFB1 metabolites in various tissues of the newborn rat is interesting and is discussed below.

MATERIALS AND METHODS

Chemicals

[^{3}H]AFB1 (specific activity 18 Ci/mol) was purchased from the American Radiolabelled Chemical Co., St Louis, U.S.A. Unlabelled AFB1, dimethylsulfoxide (DMSO), 2,5, diphenyloxazole (PPO), p-bis-2(4-methyl)-5 phenyloxazole 2-ethylbenzene (POPOP) and ethylene diaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co., St. Louis. All of the solvents used in this study were of analytical grade from E. Merck, Germany.

Animals

Albino rats of Wistar strain were purchased from Razi Institute, Karaj, Iran. Animals were maintained on a commercial pellet available locally. Breeding was performed in the animal house of Tarbiat Modarress University. Male newborn rats were selected on the basis of their birth date. Age of the newborn rats used throughout this study was 17±2 days. Male adult rats used in this study were 3-5 months old.

Treatment

A group of newborn rats comprised of 9-12 male pups were divided into 3 subgroups. Animals were starved overnight prior to administration of a single i.p. dose of AFB1 (8 μCi [^{3}H]AFB1 containing 40 μg AFB1/100 g body weight dissolved in dimethyl sulfoxide (DMSO)). Similarly, 3 young adult rats were injected with AFB1. Animals were sacrificed 2 h after dosing. Other groups of rats were treated and killed 6, 12, and 24h after injection. Blood was collected for the measurement of radioactivity in the serum. The same protocol was followed for experiments at different time

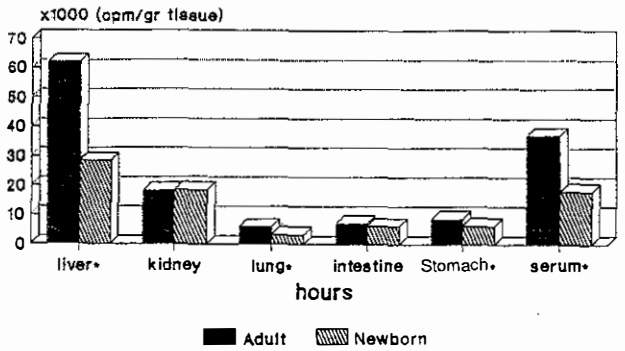

Fig. 1. Distribution of AFB1 metabolites in various tissues 2 hours after treatment. Results are mean ± S.E.M. of 3 samples in each group. Experimental details are as described under Materials and Methods. *P < 0.05; significantly different from other groups.

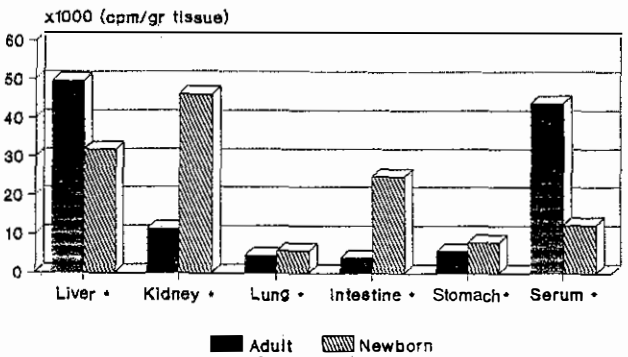

Fig. 2. Distribution of AFB1 metabolites in various tissues 6 hours after treatment. Results are mean ± S.E.M. of 3 samples in each group. Experimental details are described under Materials and Methods. *P < 0.05; significantly different from other groups.

periods of 2, 6, 12 and 24 hours after AFB1 treatment. Animals were sacrificed following the induction of light anesthesia by ether. Tissues including liver, kidney, intestine, stomach and lung were removed immediately and intestinal and stomach contents were evacuated. Due to the insufficient amounts of tissues obtained from neonatal rats, organs were pooled from 3-4 animals. The tissues were washed in a buffer containing 0.04% M EDTA, pH = 7.5. A weighed portion (0.5 g) of the tissues were homogenized in the same buffer to obtain a 10% homogenate. The homogenate was filtered through two layers of gauze. Then a measured amount of the filtrate was added to vials in duplicates, containing dioxane-based scintillation liquid, 0.6% PPO, 0.03% POPOP and 6% naphthalene. The radioactivity was measured using a B-counter. The results were expressed as cpm/g tissue.

Statistics

The statistical parameters were analysed by non-

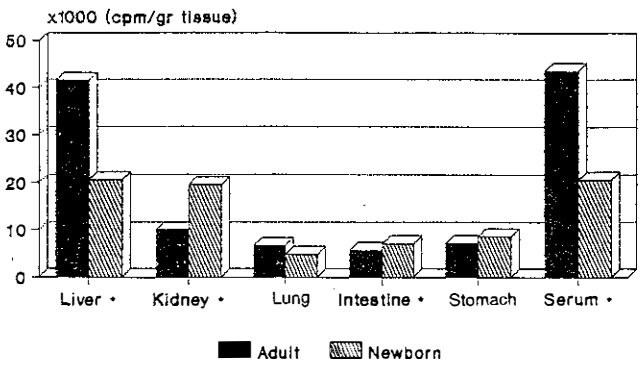

Fig. 3. Distribution of AFB1 metabolites in various tissues 12 hours after treatment. Results are mean ± S.E.M. of 3 samples in each group. Experimental details are as described under Materials and Methods. *P < 0.05; significantly different from other groups.

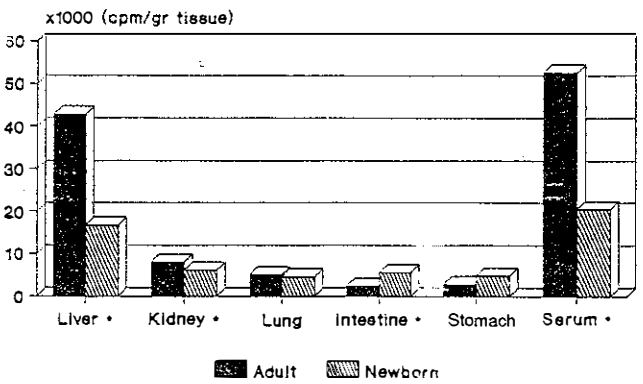

Fig. 4. Distribution of AFB1 metabolites in various tissues 24 hours after treatment. Results are mean ± S.E.M. of 3 samples in each group. Experimental details are as described under Materials and Methods. *P < 0.05; significantly different from other groups.

parametric Wilcoxion test using SPSS software.

RESULTS AND DISCUSSION

Newborn animals generally are more susceptible than adults of the same species to the lethal effects of drugs and environmental chemicals.[7] AFB1 readily reaches fetal and newborn tissues but pharmacokinetic studies on AFB1 in infant animals are very limited.

The results of the present study clearly show an overall difference in the uptake of aflatoxin metabolites into tissues of newborn and adult rats. The liver as the target organ for AFB1 is the major site of the deposition of aflatoxins in both age groups. Two hours after administration (Fig. 1) the amount of radioactivity in newborn liver is about 50% of that of adult tissue. The level of radioactivity in adult rat liver is associated with a gradual decrease at 2, 6, 12 (Fig. 3) and 24 h (Fig. 4) of AFB1 administration. The level of aflatoxin in newborn tissue reached its maximum 6 hours after injection (Fig. 2), after which it gradually decreased. This decrease is indicative of the translocation of aflatoxins from liver to serum. Serum levels of aflatoxin metabolites in pups were significantly higher than adults. The rate of renal excretion of aflatoxins was markedly higher in newborn rats compared to adults. This is probably due to the higher water content of the newborn body which facilitates the excretion of polar metabolites of the toxin.

Higher amounts of aflatoxins excreted via the kidneys during 6 h of treatment, although an important route of detoxification, could cause AFB1 adduct formation to renal cell DNA as has been observed in adult animals.[9]

Distribution of aflatoxin metabolites may be limited by their binding to plasma proteins, particularly albumin. AFB1 is known to bind strongly to albumin, a state in which it neither has no action nor can be metabolized, gain access to cellular sites or be eliminated.

Uptake of radioactivity by other tissues such as the lung, intestine and stomach in both adult and newborn animals was low. This clearly shows that the contribution of extrahepatic tissues is limited in the biotransformation of AFB1.

Low levels of AFB1 metabolites in newborn tissues could be related to the capacity of the newborn liver to handle AFB1. In this connection the level of cytochrome P-450 in hepatic tissue, which is responsible for the bioactivation of AFB1, is significantly lower than that of the mature organ. It is interesting to note that in newborn rats the phase II xenobiotic metabolizing factors, namely cellular GSH and cytosolic GSH S-transferases are also underdeveloped.[1] Therefore it is likely that AFB1 remains free (unmetabolized) in tissues and organs of the pups and may be a serious threat to cellular macromolecules for a longer period of time, as the biotransformation of AFB1 probably depends on the age-dependent development of these xenobiotic metabolizing factors.

The differential uptake of [^{3}H]AFB1 by adult and neonatal liver is directly related to the formation of two major metabolites. These metabolites are formed as a result of phase I (epoxidation) and phase II (GSH conjugation) activity of the xenobiotic metabolizing system.

The ability of adult and neonatal liver in formation of these metabolites *in vitro* and *in vivo* is the subject of our recent publications.[1,10] We have also observed that as a result of *in vitro* AFB1 biotransformation by subcellular fractions, the rate of microsome-mediated AFB1-DNA binding and AFB1-glutathione

conjugations are significantly lower in liver preparations obtained from neonates.[1] More recently[9] we have reported that AFB adduct formation to hepatic nuclear DNA *in vivo* is 3-4 times higher in adults as compared to neonates. In this study the role of GSH was also revealed when depletion of hepatic GSH was associated with a 2-3 fold increase in AFB1-DNA bindings.

In conclusion, the above mentioned evidence shows that lower levels of radioactivity in the organs of neonatal animals correspond to the rate of AFB1 biotransformation in the liver. It seems that formed polar metabolites are eliminated more rapidly in young rats. However, free AFB1 (non-metabolized) is deposited at a higher level in immature rats as a result of delayed metabolic activation of the carcinogen in neonatal liver. The fate of the deposited aflatoxin in organs at this age is not well known. However, it seems that in this situation cellular macromolecules remain exposed to the carcinogen for a longer period of time.

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