Biochemical Pharmacology, Vol. 31, No. 14, pp. 2327–2330, 1982.
Printed in Great Britain.

0006-2952/82/142327-04 $03.00/0

METABOLISM OF AFLATOXIN B_1 (AFB_1), AFLATOXIN G_1 (AFG_1) AND VITAMIN C INTAKE BY GUINEA PIG LIVER PREPARATION *IN VITRO*

F. DOMNGANG* and G. EMEROLE
Biochemistry Department, University of Ibadan, Ibadan, Nigeria

(*Received* 28 *October* 1981; *accepted* 1 *February* 1982)

Abstract—Male and female guinea pigs weighing 150–200 g were divided into three groups, with equal number of males and females in each group. They were fed an experimental diet which varied as follows: group I, 0 mg vitamin C/g of diet; group II, 1.08 mg/g and group III, 5.4 mg/g, for 28 days. Twenty-four hours after the last feeding, liver slices and 9000 *g* supernatant were prepared from each group, according to sex, and used for enzyme assays. For the demethylation assay, enzyme activity expressed as amount of formaldehyde produced from AFB_1 or AFG_1/hr/g fresh liver was seen to increase with the two levels of ascorbic acid intake in females. Males showed an enhancement of activity only in group II and remained with the same production of formaldehyde as above in group III. Although in each dietary group, the activity was higher in males than in females the variation in the amount of formaldehyde produced from one group to another was higher with females than with male guinea pigs. However with both sexes, the production of formaldehyde from AFG_1 was greater than from AFB_1. For the hydroxylation assay, enzyme activity was expressed as amount of metabolites (a) and (b) produced. Compared to group II, which offered a control level of ascorbic acid, group I fed without vitamin C showed a decreased production of metabolite (a) and (b) with males and females. Moreover, high intake of ascorbic acid in group III decreased the production of metabolite (a) and (b) in males, while in female guinea pigs the reduction was observed only with (b).

Biochemical studies have shown the importance of vitamin C in cancer [1]. With male guinea pigs, it has been shown that ascorbic acid was selectively concentrated in cancerous tissues and, as a result, normal tissues were depleted of this acid [2, 4]. Aflatoxins which are hepatocarcinogenic compounds were found to be metabolized by hepatic mixed function oxidase to a number of metabolites which are less toxic and carcinogenic than the parent compound [5]. The heart of that enzyme complex, cytochrome P-450 has been demonstrated in ascorbic acid deficient guinea pigs, to decrease activity to about 50 per cent of its normal value [6, 8]. With male and female guinea pigs, we therefore investigated the influence of various amounts of vitamin C intake on the *O*-demethylation and hydroxylation assays of aflatoxins B_1 and G_1 with liver slices and 9000 *g* hepatic supernatant, respectively.

MATERIALS AND METHODS

Male and female weanling guinea pigs (150–200 g) were utilized. The animals were fed normal diet for the first three days for acclimatization. They were then divided into three groups with equal number of males and females in each dietary group. Separate cages were used for each animal. All were fed experimental diet [9]. While group I was offered no vitamin C in the diet, group II received 1.08 mg vitamin C/g of diet and group III 5.4 mg/g. The animals were maintained on the basal diet and vitamin C doses for 28 days. Twenty-four hours after the last feeding, animals were killed by decapitation. The livers of animals in each group, according to the sex, were combined and divided into two portions. One of the portions was sliced in a cool medium (0–4°) as described by Dutton [10], and another homogenized and centrifuged at 9000 *g* by the method of Wagstaff and Street [11].

Enzyme assays were carried out with reagents obtained from Sigma Chemical Co. (St. Louis, MO). For the demethylation assay, an aliquot of the liver slices equivalent to 0.5 g of fresh liver, according to the sex, was added in an incubating medium consisting of 50 nmoles of AFB_1 (or AFG_1) in DMSO, and semicarbazide (50 μmoles) in McEwan's solution [12]. The total volume per flask was 5 ml. Incubation time was 1 hr at 37° in a Gallenkamp shaking bath. The reaction was stopped by addition of 2 ml saturated barium hydroxide followed by 2 ml of 35.6% (w/v) zinc sulphate. Formaldehyde produced from *O*-demethylation of aflatoxins, was estimated by the method previously described by Cochin and Axelrod [13] with Nash reagent [14]. Demethylation activity was expressed as nmoles of formaldehyde produced/hr/g fresh liver. For hydroxylation assay the method previously described by Dalezios *et al.* [15], Roebuck *et al.* [16] was slightly modified. An aliquot of 9000 *g* hepatic supernatant, equivalent to 0.5 g of fresh liver, was incubated with AFB_1 (50 nmoles in DMSO) in a medium consisting of potassium phosphate buffer (0.1 M, pH 7.4), $NADP^+$ (3 μmoles), glucose-6-phosphate (50 μmoles), nicotinamide (50 μmoles), magnesium

* Address for correspondence: Service de Biochimie, B.P. 812, Université de Yaoundé, Cameroun, West Africa.

chloride (25 μmoles). The total volume per flask was 5 ml. Incubation time was 1 hr at 37° in a Gallenkamp shaking incubator. The reaction was stopped by addition of 5 ml saturated NaCl. The mixture was stored no longer than 12 hr at 4–6° before being extracted 5 times with 10 ml of chloroform. The total extract was then concentrated to approximately 0.3 ml in the evaporator at 40° prior to spotting on an activated thin layer chromatographic plates coated with silica gel G (Hopkins and Williams). The developing solvent was ethyl acetate–chloroform 2:1. The estimation of metabolites produced from aflatoxin B_1 (AFB_1) was done by the methods previously described [17, 18]. Specific activity was expressed as nmoles of metabolites produced/hr/mg 9000 *g* supernatant protein. Protein in the supernatant was estimated by Biuret method [19] and vitamin C by the method of Roe and Kuether [20].

RESULTS AND DISCUSSION

Table 1 shows some basic parameters of experimental animals. Male and female animals in group II have the highest gain in body weight. Compared to the latter group the decrease in group III was 54 per cent and 57 per cent for the female and male guinea pigs, respectively. The effect of 28 days vitamin C intake on the concentration of that same vitamin in the liver is indicated. Results show that increased consumption of ascorbic acid leads to an increased concentration in the liver of both males and females. The animals of the latter sex in group I had 50 per cent less vitamin C in their liver than animals in group II and in group III, they have 324 per cent more vitamin C than those in group II. Although, males concentrated much more ascorbic acid in the liver than did females in the same dietary group, the percentage increases were almost the same. Males fed without vitamin C (group I) had 68 per cent less ascorbic acid in the liver than those fed 1.08 mg vitamin C/g of diet (group II), while animals fed 5.4 mg vitamin C/g (group III) had 230 per cent ascorbic in the liver than those fed 1.08 mg/g (group II).

Table 2 shows the results of demethylase activity. In female guinea pigs, increased intake of the two amounts of ascorbic acid was followed by an enhancement of *in vitro* *O*-demethylation of the two main aflatoxins: AFB_1 and AFG_1. The females in group I had 87 per cent less formaldehyde produced from AFB_1 than animals in group II, and in group III they had 150 per cent more formaldehyde also from AFB_1 than those in group II. With AFG_1, in females liver slices, the decreased activity in group I compared to group II was 10 per cent and the increased activity in group III was 118 per cent. Formaldehyde produced from the two aflatoxins, was higher in males than in females. For example in group III, males had 32 per cent more activity than females with AFB_1. However, the aflatoxin G_1 demethylation was greater in the same dietary group than the demethylation of aflatoxin B_1. With animals in group I the percentage increases of formaldehyde produced from AFG_1 compared to AFB_1 were 825 per cent and 407 per cent, respectively with females and males. From these percentage increases, it is

Table 1. Some basic parameters of experimental animals fed various levels of ascorbic acid for 28 days

Vitamin C levels in the groups (mg/g of diet)	Number of animals used (males, females)	Weight gain (g)		Liver weight/body weight (g/100 g)		Vitamin C level in the (μg/g)	
		Male	Female	Male	Female	Male	Female
Group I (0)	15–20	a	8.50 ± 5.40	4.49 ± 0.98	4.43 ± 0.43	*2.48 ± 0.83	1.65 ± 0.15
Group II (1.08)	12–16	39.85 ± 8.97	37.33 ± 3.21	2.50 ± 0.17	3.43 ± 0.96	7.78 ± 1.02	3.31 ± 0.52
Group III (5.4)	12–15	17.30 ± 3.58	17.23 ± 1.88	3.78 ± 0.41	4.20 ± 0.96	*25.65 ± 0.83	*14.06 ± 1.32

Values represent mean ± S.E. of 3 experiments with 4–5 animals per experiment subgroup.
* Shows values which are significantly different from group II at $P = 0.05$ (Student's *t*-test).
a. Animals which lost body weight and were not able to gain after 28 days of experimental feeding.

Table 2. Demethylation assay of aflatoxins B_1 (AFB_1) and G_1 (AFG_1) by liver slices of guinea pigs: nmoles of formaldehyde produced/hr/g fresh liver

Vitamin C levels in the groups (mg/g of diet)	AFB_1		AFG_1	
	Male	Female	Male	Female
Group I (0)	*1.073 ± 0.042	*0.536 ± 0.023	*5.445 ± 0.105	4.96 ± 0.63
Group II (1.08)	11.532 ± 0.803	4.24 ± 0.120	13.681 ± 0.350	5.54 ± 0.293
Group III (5.4)	14.021 ± 0.289	*10.586 ± 0.879	15.022 ± 0.440	*12.072 ± 0.580

Each value represents mean ± S.E. for three experiments with four or five animals per experimental subgroup according to the sex. Livers were pooled for each subgroup, according to the sex, and 5 replicates were made per assay.

* Used when value was significantly different from group II at P = 0.05 (Student's *t*-test).

observed that female guinea pigs demethylated AFG_1 9 times more than AFB_1 and male 5 times. Therefore, in group II and group III, there was some reduction of that discard both in males and females. For example with males the formaldehyde produced from AFG_1 in group II was 19 per cent higher than from AFB_1 and in group III 7 per cent. Moreover, the male guinea pigs ability to demethylate AFB_1 or AFG_1 was not as sensitive as that of the females to the increased intake of ascorbic acid from group II to group III. Thus, the activity demethylase using AFB_1 in group III animals, compared to those in group II was 22 per cent higher with males and 150 per cent with females. Similarly with AFG_1, the increases were 10 per cent and 118 per cent with males and females, respectively.

Hydroxylation activity is represented in Table 3. Two main unidentified metabolites with R_f values different from AFB_1 (R_f:0.61) and AFM_1 (R_f:0.41) were observed. Their R_f values were 0.46 and 0.32 for the metabolite (a) and the metabolite (b), respectively.

Metabolite (a)

Dietary vitamin C deficient guinea pigs reduced the production of the metabolite (a) both in females and in males when compared to the group of animals fed 1.08 mg/g (group II). With males and females the reduction was respectively, 21 per cent and 75 per cent compared to group II. Meanwhile, high intake of ascorbic acid in group III tended to increase the production of (a) with females, and almost no increase with males. But the activity hydroxylase in male animals was higher than in females in all groups.

Metabolite (b)

With male guinea pigs, an increased production of (b) was seen from group I to group III, and with females a decrease was revealed in group I and group III compared to group II. In males the enhancements were 220 per cent from group I to group II and 40 per cent from this latter group to group III. In females, the reductions were 25 and 75 per cent, respectively. As in the case of the metabolite (a) the production of metabolite (b) was higher with males than with females. For example in group II males production of (b) was almost three times greater than females, and in group III it was fifteen times.

According to those results, the general *O*-demethylation of AFB_1 and AFG_1 in both males and females, confirms the previous findings showing that the metabolism of many drugs *in vitro* decreased in vitamin C-deficient guinea pigs [21, 22], a species which does not synthesize vitamin C [23]. However, results of hydroxylase activity revealed some difference: the amount of metabolite (a) produced decreased in group I animals fed without vitamin C, compared to group II offered control levels of ascorbic acid both in females and males. Similarly, high intake of ascorbic acid, in group III, also reduced the production of the metabolite (a). With the metabolite (b), while female guinea pigs reduced its production either in group I or group III, males reduced only in group I and increased in group III. Thus, appeared a sex difference in the production of (b) by male and female guinea pigs added to the difference in the concentration of metabolites produced by hydroxylation, or the formaldehyde formed by *O*-demethylation of aflatoxins studied by male and female guinea pigs. These findings can be correlated to the results found by other workers [24, 25]. Therefore, the difference observed first between males and females in the same dietary group would be due to hormonal interaction, either with vitamin

Table 3. Hydroxylation assay of aflatoxin B_1 (AFB_1) by hepatic 9000 *g* supernatant of guinea pigs: nmoles metabolite produced/hr/mg protein

Vitamin C levels in the groups (mg/g of diet)	AFB_1 metabolized		Metabolite (a)		Metabolite (b)	
	Male	Female	Male	Female	Male	Female
Group I (0)	*0.274 ± 0.31	0.133 ± 0.040	0.077 ± 0.008	0.061 ± 0.011	*0.02 ± 0.003	0.018 ± 0.007
Group II (1.08)	0.789 ± 0.130	0.103 ± 0.030	0.098 ± 0.013	0.066 ± 0.015	0.064 ± 0.008	0.024 ± 0.003
Group III (5.4)	*0.382 ± 0.020	*0.050 ± 0.005	0.088 ± 0.014	*0.036 ± 0.005	*0.09 ± 0.016	*0.006 ± 0.001

Data represent mean ± S.E. of 3 experiments with 4–5 animals per experimental subgroup and per sex. Livers were pooled together for each dietary subgroup according to the sex and 5 replicates made per assay.

* Shows values which are significantly different from control at P = 0.05 (Student's *t*-test).

C itself, or the metabolism of aflatoxin affected by vitamin C. Secondly, the response of the animals to metabolize aflatoxin by hydroxylation pathway may be differently affected by vitamin C intake compared to *O*-demethylation pathway in the guinea pigs.

REFERENCES

1. E. Cameron, L. Pauling and B. Leibovitz, *Cancer Res.* **39**, 663 (1979).
2. E. Boyland, *Biochem. J.* **30**, 1221 (1936).
3. B. Sure, R. M. Theis and R. T. Harrelson, *Am. J. Cancer* **36**, 252 (1939).
4. A. F. Watson, *Br. J. exp. Path.* **17**, 124 (1936).
5. T. C. Campbell and J. R. Hayes, *Tox. appl. Pharmac.* **35**, 199 (1976).
6. E. Degkwitz, L. H. Kaufmann, D. Luft and H. Staudinger, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1033 (1972).
7. V. C. Zannoni, E. J. Flynn and M. Lynch, *Biochem. Pharmac.* **2**, 1377 (1972).
8. E. Deghwitz and K. S. Kim, *Hoppe-Seyler's Z. physiol. Chem.* **354**, 555 (1973).
9. Nutritional Biochemicals Corporation diet manual. Cleveland, Ohio. (1976).
10. G. J. Dutton, *Biochem. J.* **71**, 141 (1959).
11. D. J. Wagstaff and J. C. Street, *Tox. appl. Pharmac.* **19**, 10 (1971).
12. L. M. McEwan, *J. Physiol., Lond.* **131**, 678 (1956).
13. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
14. T. Nash, *Biochem. J.* **55**, 416 (1953).
15. J. I. Dalezios, R. I. Krieger and D. P. H. Hsieh, *Fedn Proc.* **31**, 733 (1972).
16. B. D. Roebuck, W. E. Siegel and G. N. Wogan, *Cancer Res.* **38**, 399 (1978).
17. J. Nabney and B. F. Nesbitt, *Analyst* **90**, 155 (1965).
18. J. V. Rodricks and L. Stoloff, *J. Ass. off. analyt. Chem.* **53**, 92 (1970).
19. A. G. Gornal, C. S. Bardwill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
20. J. H. Roe and C. A. Kuether, *J. biol. Chem.* **147**, 399 (1943).
21. P. H. Sato and V. C. Zannoni, *J. Pharmac. exp. Ther.* **198**, 295 (1973).
22. V. C. Zannoni and M. Lynch, *Drug. Metab. Rev.* **2**(193), 57 (1973).
23. J. J. Burns, *Nature, Lond.* **180**, 553 (1957).
24. H. J. Gurtoo, *Fedn Proc.* **35**, 330 (1976).
25. S. M. Pilch and G. F. Combs, *Fedn. Proc.* **38**, 302 (1979).