Afr. J. Med. med. Sci. (1976) 5, 55–62.

Species differences in the metabolism of aflatoxin B_1

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Summary

The metabolism of aflatoxin B_1 in a number of animals was investigated. Aflatoxin B_1 is metabolized relatively more slowly in liver slices of sheep than in the mouse, goat, guinea-pig, rabbit and golden hamster. The rate of metabolism of the toxin by the 10,000 *g* supernatant is faster than the metabolism by liver slices. This may be as a result of the substrate not penetrating the liver cells readily. Species differences exist in the *in vitro* metabolism of aflatoxin B_1 by hydroxylation and demethylation.

The sheep and White Rock cockerel demethylate aflatoxin B_1 poorly but the dog and duck do not demethylate the toxin at all. Of the animals studied, the duck, mouse and White Rock cockerel do not produce aflatoxin M_1 at all. The sheep and dog produce aflatoxin M_1 in comparatively large amounts, while the rat, goat and golden hamster produce aflatoxin M_1 in smaller quantities.

Résumé

On a étudié le métabolisme d'aflatoxine B_1 dans quelques animaux. L'aflatoxine B_1 est changée relativement plus lentement dans les tranches de foie du mouton que dans le souris, la chèvre, le cochon d'Inde, le lapin et le hamster d'oré. La vitesse du métabolisme de la toxine par le surnageant 10,000 *g* est plus vite que le métabolisme des tranches de foie. Cela peut être le résultat du substrat qui ne pénètre pas facilement cellules de foie. Ça existe les différences des espèces dans le métabolisme *in vitro* de l'aflatoxine B_1 en ajoutant un groupe d'hydroxine et en enlevant un groupe de methyl. Le mouton et le coq White Rock enlèvent l'aflatoxine B_1 médiocrement mais le chien et le canard n'enlèvent point la toxine. De tous les animaux étudiés, le canard, le souris et le coq White Rock ne rendrent pas du tout l'aflatoxine M_1. Le mouton et le chien rendrent relativement beaucoup de l'aflatoxine M_1, tandis que le rat, la chèvre et le hamster d'oré rendrent l'aflatoxine M_1 par petites quantités.

Introduction

The aflatoxins are a group of fluorescent mycotoxins produced by some strains of *Aspergillus flavus* Link (Sargeant *et al.*, 1961) and *A. parasiticus* (Codner, Sargeant & Yeo, 1963; Lie & Marth, 1967) growing on liquid culture media (Nesbitt *et al.*, 1962; Davis & Diener, 1968) and solid substrates such as cereal products, seeds and nuts (Allcroft *et al.*, 1961; Frank, 1966). Of all the aflatoxins, the metabolism of aflatoxin B_1 has been studied in some detail. The metabolism of aflatoxin B_1 involves the introduction of a hydroxyl group into the aflatoxin B_1 molecule. This may be done by the cleavage of the methoxy group (Shank & Wogan, 1965; Wogan, Edwards & Shank, 1967; Osiyemi, 1968) or by the direct introduction of a hydroxyl group into the molecule (Holzapfel, Steyn & Purchase, 1966; Allcroft *et al.*, 1966; Nabney *et al.*, 1967). Metabolism of aflatoxin B_1 by demethylation had been reported but the desmethyl aflatoxin B_1 has yet to be identified, while the direct hydroxylation of aflatoxin B_1 during metabolism results in the formation of aflatoxin M_1 (Butler & Clifford, 1965; Allcroft *et al.*, 1966; Holzapfel *et al.*, 1966; Nabney *et al.*, 1967). *In vitro*, aflatoxin B_1 has been shown to be metabolized in the rat by direct hydroxylation (Portman, Campbell & Plowman, 1968a; Portman, Plowman & Campbell, 1968b; Bassir & Emafo, 1970; Schabort & Steyn, 1969) and by o-demethyla-

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tion (Bassir & Emafo, 1970). The metabolism of aflatoxin B_1 by o-demethylation appears significant in species where the toxin is metabolized by o-demethylation (Bassir & Emafo, 1969).

Animals respond differently to the same foreign compound or drug for a number of reasons. Species and strain differences in the sleeping time of mammals after hexobarbitone administration is dependent on differences in the metabolism of the drug (Quinn, Axelrod & Brodie, 1954; Brodie, 1956; Backus & Cohn, 1966). Also, the duration of action of pethidine depends on its rate of metabolism. Dogs which metabolize pethidine rapidly are relatively immune to its toxic effect (Brodie, 1956). Although a number of species and strain differences in the response to a foreign compound are dependent on the duration of action of these compounds in the animal body (Brodie, 1956; Williams, 1967), other factors (Sims & Grover, 1968) such as inheritance (Jay, 1955; Quinn *et al.*, 1958) and differences in tissue sensitivity play some role. In this paper, species differences in the *in vitro* metabolism of aflatoxin B_1 were studied with a view to correlating the rate of metabolism of the toxin in different species with differences in their sensitivity to the toxin.

Materials and methods

Animals

Adult male mice (30–35 g), adult male rabbit (1·8–2·0 kg), adult male Wistar rats (140–150 g), duck of local strain (1·8–2·2 kg), White Rock cockerel (2·0–2·2 kg), adult male guinea-pigs (300–350 g) male dogs of local strain (5·4 kg), male goats (12·0–12·5 kg) and adult golden hamster (140–180 g) were used.

Preparation of liver slices and 10,000 *g supernatant*

Liver slices and the 10,000 *g* supernatant were prepared as previously described (Bassir & Emafo, 1970).

TABLE 1. Aflatoxin B_1 metabolism by liver slices of different species

Species	Percentage aflatoxin B_1 metabolized in 2 h by IG liver	Variance	Student's *t*	Probability
Duck	99·6±0·3	17·9		
			1·3	>0·05 Not significant
Rabbit	92·5±4·8	14·3		
Rat	83·8±5·4	26·8		
			1·9	>0·05 Not significant
Golden hamster	95·6±2·7	10·3		
Mouse	88·1±5·5	42·4		
			0·6	>0·05 Not significant
Dog	83·8±2·9	12·5		
Sheep	48·1±7·4	78·1		
			5·3	<0·001 Very significant
White Rock cockerel	96·3±1·9	5·4		
Guinea-pig	83·8±3·0	12·3		
			1·4	>0·05 Not significant
Goat	91·3±3·7	19·6		
Duck	99·6±0·3	17·9		
			1·1	>0·05 Not significant
White Rock cockerel	96·3±1·9	5·4		
Guinea-pig	83·8±3·0	12·3		
			4·5	<0·001 Very significant
Sheep	48·1±7·4	78·1		

TABLE 2. Aflatoxin B_1 metabolism by liver 10,000 *g* supernatant fraction of different species

Species	Percentage aflatoxin B_1 metabolized in 1 h by the equivalent of 1G of liver	Variance	Student's *t*	Probability
Duck	98·6±4·5	11·9		
			0·3	>0·05 Not significant
Rabbit	98·7±0·5	0·36		
Rat	88·8±0·3	5·6		
			1·7	>0·05 Not significant
Golden hamster	99·4±0·4	0·33		
Mouse	99·8±0·4	0·3		
			4·3	<0·001 Very significant
Dog	85·6±3·6	10·9		
Sheep	75·5±1·5	5·8		
			9·1	<0·001 Very significant
White Rock cockerel	99·4±0·4	0·43		
Guinea-pig	94·0±2·6	8·9		
			1·0	>0·05 Not significant
Goat	98·0±1·3	4·8		
Duck	98·6±4·5	11·9		
			1·4	>0·05 Not significant
White Rock cockerel	99·4±0·4	0·43		
Guinea-pig	94·4±2·6	8·9		
			3·0	<0·01 Very significant
Sheep	75·5±1·5	5·8		

Incubation mixtures for the isolation of metabolites and the study of demethylation of aflatoxin B_1

The metabolism of aflatoxin B_1 with liver slices and liver 10,000 *g* supernatant was studied as described by Bassir & Emafo (1969). The formaldehyde in the incubate was assayed colorimetrically with a spectrophotometer at 415 nm by the method of Cochin & Axelrod (1959) as modified by Stitzel *et al.*, (1966) using double strength Nash reagent (1953).

Results

Most of the aflatoxin B_1 in the incubation medium was metabolized *in vitro* by the liver slices or the liver 10,000 *g* supernatant within 2 h and 1 h respectively. The limiting factor in the metabolism of the toxin *in vitro*, is the diffusion of the substrate across the cell membrane because there is a greater metabolic rate of the toxin with the 10,000 *g* supernatant compared with the liver slices. The influence of diffusion of the substrate across the cell membrane is particularly significant in the metabolism of the toxin in the sheep, guinea-pig and mouse (Tables 1 and 2).

Species differences in the metabolic rate of aflatoxin B_1 were also observed. The goat, golden hamster, rabbit, duck, mouse, White Rock cockerel, and guinea-pig metabolize the toxin fairly rapidly while the sheep metabolizes it slowly. Even with the 10,000 *g* supernatant, the *in vitro* metabolic rate of aflatoxin B_1 in the sheep is of the order of 75·5 ± 1·5% compared with 94·4 ± 2·6% in the guinea-pig and 99·4 ± 0·4% in the White Rock cockerel during the same period.

On the biotransformation of aflatoxin B_1 into aflatoxin M_1, species differences were also observed. The duck, mouse and White Rock cockerel did not

TABLE 3. Influence of species differences on the biotransformation of aflatoxin B_1 to aflatoxin M_1 by liver slices

Species	Percentage aflatoxin B_1 converted to aflatoxin M_1 by IG liver in 2 h	Variance	Student's *t*	Probability
Duck	0	0		
			2·5	<0·025 Very significant
Rabbit	1·1±0·3	0·21		
Rat	1·6±0·6	0·15		
			2·5	<0·025 Very significant
Golden hamster	5·4±1·1	4·4		
Mouse	0	0		
			2·9	<0·01 Very significant
Dog	5·3±1·4	2·9		
Sheep	4·3±0·6	0·5		
			2·6	<0·01 Very significant
White Rock cockerel	0	0		
Guinea-pig	0·88±0·03	0·15		
			1·5	>0·05 Not significant
Goat	1·8±0·3	0·16		
Duck	0	0		
White Rock cockerel	0	0		
Guinea-pig	0·88±0·3	0·15		
			4·3	<0·001 Very significant
Sheep	4·3±0·6	0·5		

produce aflatoxin M_1 at all (Tables 3 and 4). Among the aflatoxin M_1 producers, the sheep and the dog were shown to produce this metabolite in amounts greater than that produced by any other species studied. While the dog, and sheep produced aflatoxin M_1 *in vitro* in the range of 4·3–5·4% the other animals were shown to produce aflatoxin M_1 in relatively moderate amounts.

In the demethylation studies of aflatoxin B_1, the results showed that all the animals studied except the duck and dog metabolize aflatoxin B_1 by demethylation. The golden hamster, rat, mouse and goat are good o-demethylators while the sheep and White Rock cockerel are poor o-demethylators of aflatoxin B_1 (Tables 5 and 6).

Discussion

In the *in vitro* metabolic studies of aflatoxin B_1 it is shown that the toxin is metabolized in varying amounts both by demethylation and hydroxylation in the rabbit, rat, golden hamster, sheep, guinea-pig and goat, by demethylation in the White Rock cockerel and by hydroxylation in the dog. The duck did not metabolize aflatoxin B_1 by either hydroxylation or by demethylation. Although the mouse metabolizes aflatoxin B_1 by demethylation and hydroxylation it does not produce aflatoxin M_1.

These species differences in the hydroxylation of aflatoxin B_1 call to mind previous reports in which species differences were observed in the hydroxylation of coumarin and biphenyl (Creaven, Park & Williams, 1965a, b). The ability of the rat liver slices and the 10,000 *g* supernatant to hydroxylate aflatoxin B_1 while the rat is unable to hydroxylate coumarin (Creaven *et al.*, 1965a) supports the hypothesis that a family of hydroxylases are available in the liver microsomes of the rat (Posner, Mitoma & Udenfriend, 1961). The metabolism of aflatoxin B_1 into aflatoxin M_1 *in vitro* in the rat, further supports

TABLE 4. Species differences on the biotransformation of aflatoxin B_1 to aflatoxin M_1 by liver 10,000 *g* supernatant fraction

Species	Percentage aflatoxin B_1 converted to aflatoxin M_1 in 1 h by the equivalent of 1G of liver	Variance	Student's *t*	Probability
Duck	0	0		
Rabbit	1·0±0·1	0·02		
Rat	1·7±0·1	0·03		
			1·11	>0·05 Not significant
Golden hamster	1·3±0·3	0·10		
Mouse	0	0		
Dog	5·4±1·2	3·4		
Sheep	4·8±1·0	2·6		
White Rock cockerel	0	0		
Guinea-pig	0·90±0·07	0·01		
			1·7	>0·05 Not significant
Goat	1·9±0·5	0·04		
Duck	0	0		
White Rock cockerel	0	0		
Sheep	4·8±1·0	2·6		
			2·4	<0·05 Significant
Guinea-pig	0·90±0·07	0·01		

earlier reports (Portman *et al.*, 1968a, b; Schabort & Steyn, 1969; Bassir & Emafo, 1970; Patterson & Roberts, 1971). Contrary to the report of Portman *et al.* (1968a) the mouse was not able to metabolize aflatoxin B_1 into aflatoxin M_1. This might be a case of strain differences in metabolism. Instead of aflatoxin M_1, the mouse used in our laboratory, metabolized aflatoxin B_1 into a substance with one or more hydroxyl groups based on its reaction with acetic anhydride in the presence of pyridine (Holzapfel *et al.*, 1966; Dutton & Heathcote, 1968). The metabolite has an R_F value of 0·20 as against R_F value of 0·25 of aflatoxin M_1.

Patterson & Roberts (1971) were unable to estimate demethylation of aflatoxin B_1 *in vitro* colourimetrically. In our studies, however, we observed demethylation of aflatoxin B_1 in a number of species. The golden hamster, rat, mouse and goat metabolized aflatoxin B_1 considerably by o-demethylation while the sheep and White Rock cockerel were poor aflatoxin B_1 o-demethylators. On the basis of our *in vitro* studies, in which zinc sulphate and barium hydroxide solutions were used as protein precipitants instead of tungistic acid, it is concluded that demethylation of aflatoxin B_1 is a significant method of detoxicification of the toxin in the golden hamster, mouse, goat, rabbit and guinea-pig since demethylation of aflatoxin B_1 has been reported to be important in the process of metabolism in the rat (Shank & Wogan, 1965; Wogan *et al.*, 1967). It is significant that the rapid demethylators of aflatoxin B_1 such as the rabbit, rat, golden hamster, and guinea-pig are more susceptible to aflatozin toxicity. With the exception of the duck, all the poor or non-demethylators of aflatoxin B_1 such as the mouse, sheep and White Rock cockerel are resistant to aflatoxin toxicity.

Since aflatoxin B_1 is as toxic as aflatoxin M_1 (Purchase, 1967) the production of large amounts of aflatoxin M_1 by the dog may be responsible for the tendency of this species to liver injury by aflatoxin B_1. Aflatoxin B_1 is metabolized slowly in the sheep

TABLE 5. Demethylation of aflatoxin B_1 by liver 10,000 *g* supernatant fraction of different species

Species	(m)μmol formaldehyde produced in 1 h by the equivalent of 1G of liver	Variance	Student's *t*	Probability
Duck	0	0		
Rabbit	56·3 ± 7·9	88·7		
Rat	75·1 ± 13·0	252	0·4	> 0·05 Not significant
Golden hamster	89·0 ± 9·1	117·4		
Mouse	68·3 ± 7·5	80·9		
Dog	0	0		
Sheep	43·3 ± 2·5	18·3	0·7	> 0·05 Not significant
White Rock cockerel	49·8 ± 8·9	95·6		
Guinea-pig	0	0		
Goat	61·1 ± 2·0	5·1		
Duck	0	0		
White Rock cockerel	49·8 ± 8·9	95·6		
Sheep	43·3 ± 2·5	18·3	1·9	> 0·05 Not significant
Rat	75·1 ± 13·0	252		
White Rock cockerel	49·8 ± 8·9	95·6	2·7	< 0·05 Significant
Golden hamster	89·0 ± 9·1	117·4		
Goat	61·6 ± 2·0	5·1	0·7	> 0·05 Not significant
Mouse	68·3 ± 7·5	80 9		

although aflatoxin M_1 is produced in relatively large quantities in this species. There appears to be no correlation of the resistance of sheep to aflatoxin toxicity and the production of aflatoxin M_1. The rapid metabolism of aflatoxin B_1 in the mouse and White Rock cockerel as well as their inability to metabolize aflatoxin B_1 into aflatoxin M_1 may be directly related to the resistance of the mouse and the cockerel to aflatoxin B_1-induced liver injury and hepatoma, provided the metabolite recovered in the incubated mouse liver is less toxic than aflatoxin M_1. The duck which metabolizes aflatoxin B_1 as rapidly as the White Rock cockerel is, however, very susceptible to aflatoxin toxicity. It is suggested, therefore, that factors other than the rate of metabolism of aflatoxin B_1 may be vital in determining the toxic and carcinogenic effects of aflatoxin B_1 in some animals. Differences in the binding of aflatoxin B_1 to DNA has been suggested as a probable cause for variations in species toxicity to aflatoxins (Rees, 1966). Other factors which had not previously been mentioned but which could be responsible for species differences in toxicity are the toxin receptor interaction and the differences in the binding of aflatoxin B_1 to liver proteins of the various species. It is known that the cardiotonic activity of cardiac glycosides is determined by differences in affinity between the drug and the receptor (Detweiler, 1967;

TABLE 6. Demethylation of aflatoxin B_1 by liver slices of different species

Species	(m) μmol formaldehyde produced by IG liver slices in 2 h	Variance	Student's *t*	Probability
Duck	0	0		
Rabbit	73·0 ± 7·1	174		
Rat	74·8 ± 3·1	33·7		< 0·05
			2·1	
				Significant
Golden hamster	59·4 ± 2·2	17·1		
Mouse	34·8 ± 2·1	15·8		
Dog	0	0		
Sheep	12·8 ± 1·7	4·4		> 0·05
			1·2	Not significant
White Rock cockerel	24·5 ± 2·4	89·7		
Guinea-pig	60·8 ± 3·9	51		> 0·05
			0·5	Not significant
Goat	72·8 ± 1·4	2·8		
Duck	0	0		
White Rock cockerel	24·5 ± 2·4	89·7		
Sheep	12·8 ± 1·7	4·4		< 0·001
			4·5	Very significant
Guinea-pig	60·8 ± 3·9	51		
Mouse	34·8 ± 2·1	15·8		< 0·005
			3·4	Very significant
Golden hamster	59·4 ± 2·2	17·1		

Okita, 1967). Also the differences in the binding of the carcinogen, N-hydroxy-N-2 fluorenyl acetamide to the rat liver accounts partly for sex differences in its toxicity (Weisburger, Grantham & Weisburger, 1964).

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(*Received* 14 *May* 1973)