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Short Communication

METABOLIC ACTIVATIONS OF DIMETHYLNITROSAMINE BY MALE AND FEMALE MICE AND RATS TO METABOLITES MUTAGENIC IN *Neurospora crassa*

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Differences in the ability to activate promutagens to mutagenic metabolites have been demonstrated between sexes of certain animal species [1]. Using preparations of mouse-kidney microsomes, Brusick and coworkers revealed that the mutagenicity of dimethylnitrosamine (DMN) in *Salmonella typhimurium* was detectable only when the preparation was derived from males [2,7].

Our early studies have shown that DMN, a potent procarcinogen, can be activated by different activation systems to metabolites mutagenic in *Neurospora crassa* [8]. Further studies were conducted to compare the activations of DMN to mutagenic metabolites by various organs from male and female mice and rats. Experiments were performed in both host-mediated assays and in vitro (microsome-mediated) activation systems. The adenine-3 (*ad-3*) test system of *N. crassa* was used for mutagenesis assays. The strains used and the test systems employed have been described elsewhere in detail [4]. In general, treatments were carried out as follows:

In the host-mediated assay with a forward-mutation system, conidia of tester strain H59 were injected intravenously into mice or rats. 10—12 week old CD mice and 5—7 week old CD rats were used. Immediately after injection of conidia, DMN (100 mg/kg) in distilled water was administered to the animals by intramuscular injection. In the control experiment, sterile distilled water was injected into animals. 16 h after DMN treatment conidia were recovered separately from liver, lung and kidney. The *ad-3* forward-mutation frequency of the recovered conidia was determined by the direct method [3]. Similar treatment conditions were also used for the reverse-mutation system with tester strain N23. The reversion frequency and survival of conidia recovered from different organs were determined by the methods previously described. [5].

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In the in vitro activation system, 9000 × *g* supernatants of organ homogenates (S9) were used. Preparation of S9 of liver, lung and kidney has been described previously [8]. Conidia (1.5×10^8) from testers (N23 for the reverse-mutation system and H59 for the forward-mutation system) were treated with or without DMN (100 mM) in the presence of S9, a NADPH-generating system (NADP, 2.34 mg/ml; G-6-P, 2.24 mg/ml; $MgCl_2 \cdot 6H_2O$, 1.59 mg/ml; KCl, 2.34 mg/ml) and O_2 at 37°C for 3 h. The survivals and mutation frequencies of DMN-treated and untreated conidia were determined by the methods as used in the in vivo activation system.

Results of the host-mediated assay using mice as hosts are summarized in Table 1. It appears that liver, lung and kidney of female and male mice can all activate DMN and that liver is the most effective organ for the conversion of DMN to metabolites mutagenic to *N. crassa*. The mutation frequencies are very similar between the conidia recovered from liver of male and female mice. The *ad-3* mutation frequency, however, dropped from 688.9 per 10^6 survivors among the conidia recovered from kidneys of male mice to 29.4 per 10^6 survivors among the conidia recovered from kidneys of female mice. It seems, therefore, that there is a striking sex difference in the activation of DMN to mutagenic metabolites by mouse kidney. In the *ad-3* reverse-mutation system with female mice as hosts, a very high reversion frequency in liver and a very low reversion frequency in kidney were found in the conidia recovered from DMN treated mice (Table 2). These results are comparable to the results obtained from the *ad-3* forward-mutation system.

Table 3 summarizes the results of the *ad-3* reverse-mutation assay using an in vitro activation system from mouse-organ homogenates. The *ad-3* reversion

TABLE 1

INDUCTION OF *ad-3* FORWARD MUTATIONS BY DMN IN H59 OF *N. crassa* VIA A HOST-MEDIATED ASSAY WITH CD_1 MICE AS HOSTS

Sex	Organ	Conc. of DMN (mg/kg)	Number of colonies ($\times 10^5$)	Percent survival	Number of *ad-3* mutants	*ad-3* mutants per 10^6 survivors
—	—	0	3.7	100	0	0
—	—	100 [a]	7.4	98.1	0	0
Male [b]	Liver [c] Lung Kidney	0	16.9	100	0	0
	Liver	100	5.4	13.6	444	822.2
	Lung	100	3.7	39.4	43	116.2
	Kidney	100	1.8	26.5	124	688.9
Female	Liver [c] Lung Kidney	0	15.8	100	1	0.6
	Liver	100	6.2	8.2	577	930.6
	Lung	100	10.7	40.9	35	32.7
	Kidney	100	1.7	37.4	5	29.4

[a] In vitro control, 100 μg per ml treatment solution.

[b] Data from Ref. 8.

[c] Conidia were pooled from three different organs. No difference in the mutation frequencies was found among the conidia recovered from different organs.

TABLE 2

INDUCTION OF REVERSE MUTATIONS BY DMN IN N23 OF *N. crassa* VIA A HOST-MEDIATED ASSAY WITH FEMALE MICE AS HOSTS

Conidia recovered from	Conc. of DMN (mg/kg)	Percent survival	Number of revertants	Revertants per 10^7 survivors
Liver [a]	0	100	0	0
Lung				
Kidney				
Liver	100	88.8	3984	2305.5
Lung	100	100	1952	471.0
Kidney	100	94.6	21	125.7

[a] Conidia were pooled from three different organs. No difference in the mutation frequencies was found among the conidia recovered from different organs.

TABLE 3

INDUCTION OF REVERSE MUTATIONS BY DMN IN N23 OF *N. crassa* WITH S9 OF ORGAN HOMOGENATES FROM CD_1 MICE

Sex	Organ	Conc. of DMN (mM)	Percent survival	Number of revertants	Revertants per 10^7 survivors
—	—	100	100	5	0.7
Male [a]	Liver	0	100	4	0.5
		25	100	21	4.2
		50	88.5	68	18.1
		100	94.1	119	26.1
	Lung	0	100	2	0.2
		25	97.0	154	29.4
		50	89.4	336	73.4
		100	96.6	1001	192.2
	Kidney	0	100	1	0.1
		25	99.2	76	15.2
		50	98.6	200	39.5
		100	94.6	411	85.7
Female	Liver	0	100	2	0.3
		25	99.5	60	10.5
		50	96.0	142	26.9
		100	100	319	52.9
	Lung	0	100	4	0.5
		25	100	174	32.1
		50	100	444	79.8
		100	101	1098	181.5
	Kidney	0	100	2	0.2
		25	92.8	131	24.3
		50	92.7	351	64.1
		100	91.7	684	126.9

[a] Data from Ref. 8.

TABLE 4

INDUCTION OF *ad-3* FORWARD MUTATIONS BY DMN IN H59 OF *N. crassa* WITH S9 OF ORGAN HOMOGENATES FROM FEMALE MICE

Organ	Conc. of DMN (mM)	Number of colonies ($\times 10^5$)	Percent survival	Number of *ad-3* mutants	*ad-3* mutants per 10^6 survivors
Liver [a] Lung Kidney	0	24.2	100	0	0
Liver	100	25.6	70.3	244	95.3
Lung	100	16.6	68.3	550	331.3
Kidney	100	17.7	72.9	277	156.5

[a] Conidia were pooled from three different organs.

frequency was the highest with S9 from lung followed by S9 from kidney, and the S9 from liver gave the lowest reversion frequency. Similar results were obtained in the *ad-3* forward-mutation system (Table 4). It is interesting to note that the relative efficiencies of the activation of DMN to metabolites mutagenic to Neurospora by liver, kidney and lung of mouse are in opposite order between in vivo and in vitro activation systems. Furthermore, it is surprising that the sex differences in the activation of DMN by mouse kidney observed in the in vivo activation system was not found in the in vitro activation system.

The results obtained from experiments with CD rats both in in vivo (Table 5) and in vitro (Table 6) activation systems were similar to those obtained with mice except that no sex difference in the in vivo activation of DMN was found with rat kidney.

TABLE 5

INDUCTION OF *ad-3* FORWARD MUTATIONS BY DMN IN H59 OF *N. crassa* VIA A HOST-MEDIATED ASSAY WITH CD RATS AS HOSTS

Sex	Organ	Conc. of DMN (mg/kg)	Number of colonies ($\times 10^5$)	Percent survival	Number of *ad-3* mutants	*ad-3* mutants per 10^6 survivors
Male [a]	Liver [b] Lung Kidney	0	14.6	100	14	9.6
	Liver	100	8.7	36.7	556	639.1
	Lung	100	7.8	59.5	136	174.4
	Kidney	100	1.3	57.0	48	369.2
Female	Liver [b] Lung Kidney	0	49.0	100	23	4.7
	Liver	100	18.7	58.4	1537	821.9
	Lung	100	13.1	80.8	207	158.0
	Kidney	100	4.6	70.7	208	452.1

[a] Data from Ref. 8.

[b] Conidia were pooled from three different organs. No difference in the mutation frequencies was found among the conidia recovered from different organs.

TABLE 6

INDUCTION OF REVERSE MUTATIONS BY DMN IN N23 OF *N. crassa* WITH S9 OF ORGAN HOMOGENATES FROM CD RATS

Sex	Organ	Conc. of DMN (mM)	Percent survival	Number of revertants	Revertants per 10^7 survivors
—	—	100	96.2	6	0.8
Male	Liver	0	100	1	0.1
		25	89.4	95	12.9
		50	92.8	202	26.5
		100	87.4	501	70.1
	Lung	0	100	1	0.1
		25	84.9	4179	592.3
		50	78.4	6908	1075.6
		100	82.5	8426	1255.8
	Kidney	0	100	2	0.2
		25	97	271	34.2
		50	99	766	93.6
		100	88.5	1540	227.2
Female	Liver	0	100	3	0.5
		25	98.9	100	21.7
		50	96.2	192	41.5
		100	100	404	80.6
	Lung	0	100	3	0.4
		25	80.6	1039	293.4
		50	84.9	2048	492.4
		100	80.9	4219	1144.5
	Kidney	0	100	1	0.1
		25	100	539	104.1
		50	100	1017	193.4
		100	100	1874	360.0

A sex difference in the activation of DMN to mutagenic metabolites by S9 of mouse kidney was found in the histidine reverse-mutation system of *S. typhimurium* [2,7]. However, in a forward mutation assay of *S. typhimurium* with an in vivo activation system, Pueyo et al. [6] found that there was no sex difference in the activation of DMN. The basis of the different results obtained from different studies is not known. However, it may be due to differences in the mouse strain used and/or differences in the mutagenesis assay systems employed. In the Neurospora assay system, the nature of the discrepancy between in vivo and in vitro activations of DMN by mouse kidney is not understood and requires further investigations.

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