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INDUCTION OF 6-THIOGUANINE RESISTANCE IN CHINESE HAMSTER LUNG CELLS TREATED WITH DIMETHYLNITROSAMINE' 2-AMINO-ANTHRACENE OR 7,12-DIMETHYLBENZ(A)ANTHRACENE IN THE PRESENCE OF RAT LIVER MICROSOMES

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SUMMARY

Chemically non-reactive carcinogens require metabolization in order to exert such biological effects as mutagenicity. The mutagenic activities of dimethylnitrosamine (DMN), 2-aminoanthracene (2-AA) and 7,12-dimethylbenz(a)anthracene (7,12-DMBA) have been studied at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster lung cells. These compounds induce 6-thioguanine (6TG) resistance in the presence of rat liver microsomes, but they do not induce 6TG resistance in the absence of rat microsomes. The frequencies of mutations induced by these compounds are dose-dependent.

INTRODUCTION

Recent advances in mutagenesis have focused attention on the need for activation of promutagens and procarcinogens [1, 3, 6, 10, 13—15, 17, 18]. The demonstration by Malling, that microsome activation provides an effective means for mutagenesis testing [14], has led a number of investigators to apply the same and/or modified techniques for the activation of environmental chemical agents [1, 3, 6, 10, 13, 15, 17, 18]. Currently, different

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Abbreviations: 2-AA, 2-aminoanthracene; 7,12-DMBA, 7,12-dimethylbenz(a)anthracene; DMN, dimethylnitrosamine; HGPRT, hypoxanthine guanine phosphoribosyl transferase; 6TH, 6-thioguanine.

tissue homogenate-mediated assays for mutation induction are widely utilized [1, 2, 13, 17, 18].

Most mutagenic and carcinogenic chemicals require metabolic activation [6, 15]. It is likely that mixed-function oxidase activity is responsible for the metabolic activation of most unreactive mutagenic and carcinogenic compounds into their chemically reactive metabolites [3]. It is now clear that bacterial cells [16] and many cultured mammalian cells lack mixed-function oxidase [12].

Most of the mutation research that has employed microsome activation has been carried out in prokaryotic cells. Little has been done, however, on the mutagenicity of chemical agents after microsome activation in cultured mammalian cells [13, 14, 17, 18]. In this paper we report our results from mutagenicity studies in which we used an in vitro rat microsome activation system and cultured Chinese hamster lung cells.

MATERIALS AND METHODS

Preparation of rat microsomes, cofactors, and mutagen mixtures

8 to 10 week-old male rats (Sprague–Dawley, Madison, Wis.) were used in all experiments. Each 10 g of rat livers was homogenized in 20 ml of ice-cold F10 [5] and then centrifuged for 20 min at 9000 × *g*. The collected supernatant fraction (S-9) was stored overnight at -20°C. 125 mg KCl, 120 mg glucose-6-phosphate, 61.25 mg NADP and 85 mg $MgCl_2 \cdot 6H_2O$ were dissolved in 5.3 ml of F10 medium. The treatment mixture was composed of 1 ml of this mixture, 4 ml of S-9 fraction, 0.1 ml of dimethylsulfoxide that contained mutagen, and 4.9 ml of F10 medium. In the control, F10 medium was used in the place of microsomes or mutagen.

Mutagenesis assays

Two million cells (Chinese hamster lung (CHL) cells, CCL39, obtained from the American Type Culture collection) in a 100 mm petri dish (P100) were treated with microsome-cofactor-mutagen mixture for 3 h in an incubator with 100% oxygen atmosphere. Then cells were inoculated into P100 dishes. Each dish contained 10^5 cells. A total of 7–16 dishes were used to determine mutant frequency for each data point. Selection with 6TG ($7.5 \cdot 10^{-5}$ M) for resistant cells was begun 48 h after inoculation and lasted for a period of 11 days. In order to estimate cell survival, 100 treated or untreated cells were inoculated into a 60 mm petri dish (P60) and were grown for 9 days. The dishes were then stained for colonies and the colony numbers were counted. This number was used to estimate cell survival in the calculation of mutation frequency. Methylene blue 2% in citrate buffer (pH 6) was used to stain the cells. Cells were rinsed with 0.9% saline, fixed with 95% ethanol and stained with methylene blue.

RESULTS AND DISCUSSION

An assay has been described for the measurement of mutation induction at the HGPRT locus in CHL cells (ATCC, CCL39) [7]. Resistance to 6TG has been used as the tool for this measurement. Optimal conditions for the recovery of 6TG-resistant cells have been defined [7] and a variety of chemical and environmental agents have been tested in this system [8, 9]. In this study we added microsomes to the cultured cells during the mutagen treatment.

Table I presents the mutation frequencies of different agents at different doses with a constant amount of microsomes. DMN, 2-AA or 7,12-DMBA are not mutagenic in CHL cells in the absence of microsomal activation. After microsomal activation, however, the mutagenic activity of these compounds becomes increasingly evident as the doses increased.

In order to provide convincing evidence for the nature of somatic mutation, one must demonstrate a clear dose–response relationship for induced mutation at a specific locus. The experiments of DMN, 2-AA, and 7,12-DMBA dose–response relationship to 6TG ($7.5 \cdot 10^{-5}$ M) resistance presented in Fig. 1 have been conducted using a dose range from 25 μg/ml to 200 μg/ml (of 2-AA or 7,12-DMBA) or 5 mM to 100 mM (of DMN). The data sets give estimated slopes of 0.85 ± 0.30, 1.43 ± 0.30 and 0.92 ± 0.06, respectively, for DMN, 2-AA, and 7,12-DMBA. All slopes are significantly different from zero at the 5% level.

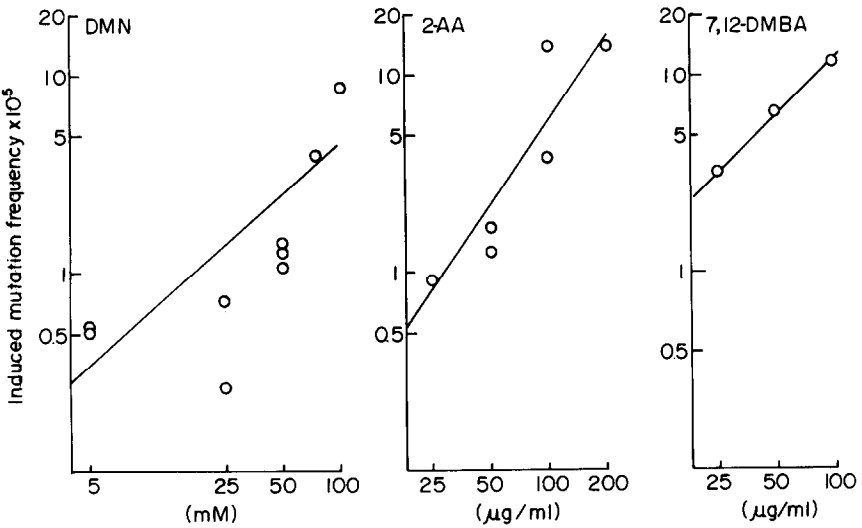

Fig.1. Induced mutation frequency as a function of chemical concentration. The straight-line fit for the averaged induced mutation frequencies over the doses tested are as follows: DMN: $\ln(Y) = -2.3863 + 0.8488 \ln(X)$; 2-AA: $\ln(Y) = -4.7905 + 1.4315 \ln(X)$; 7,12-DMBA: $\ln(Y) = -1.7759 + 0.9202 \ln(X)$; where Y is induced mutation frequency and X is concentration.

DMN, 2-AA, and 7,12-DMBA are known carcinogens [4]. We have confirmed the requirement of biotransformation by DMN and 7,12-DMBA for mutagenic action in cultured cells [10, 13, 17, 18]. We have also demonstrated that 2-AA requires biotransformation for mutagenic action in cultured cells as it does in bacterial systems [1]. Through the use of Chinese hamster cells and microsome activation, the mutagenic potential of carcinogens and other chemical agents can be detected and quantitated.

TABLE I

CALCULATION OF INDUCED MUTATION FREQUENCIES AFTER TREATMENT WITH DMN, 2-AA AND 7,12-DMBA

Mutation frequency was arrived at by taking the proportion of the number of mutants observed to the total number of survival cells. Induced mutation frequencies were obtained by subtracting the background mutation frequency observed in the presence of microsomes from the calculated mutation frequencies. Cells were incubated for 3 h in serum-free medium with a constant amount of microsome preparation (+) or without microsome preparation (-). The mutation frequencies obtained from dishes without microsomes and mutagen did not differ from those free of mutagen but containing microsomes nor from those without microsomes but containing mutagen. Mutation frequencies were increased with the increase of DMN, 2-AA and 7,12-DMBA in a constant amount of microsomes and were statistically different from that of the control (mutagen-free, microsome containing) at the 1% level or 5% level using binomial approximation [11]

Compound	Dose	S-9	Survival	Number of surviving cells $\cdot 10^{-5}$	Number of mutants observed	Mutation frequency $\cdot 10^{5}$	Induced mutation frequency $\cdot 10^{5}$
DMN	mM						
	0	-	61.1	9.17	19	2.07	
	0	+	50.8	7.62	17	2.23	
	50	-	64.0	9.60	20	2.08	
	50	+	36.8	5.52	18	3.26	1.03
	25	+	51.8	7.77	19	2.45	0.22
	5	+	52.8	7.92	22	2.78	0.55
	0	-	73.2	10.98	12	1.09	
	0	+	75.2	11.28	10	0.89	
	50	-	71.0	10.65	14	1.31	
	50	+	42.0	6.30	12	1.90	1.01
	25	+	46.5	6.65	11	1.65	0.76
	5	+	74.3	10.40	15	1.44	0.55
	0	-	98.0	14.70	63	4.29	
	0	+	100.0	16.00	60	3.75	
	100	-	90.0	13.50	66	4.89	
	100	+	33.6	5.04	64	12.70[a]	8.95
	75	+	37.0	5.92	48	8.11[a]	4.36
	50	+	51.0	8.16	46	5.64	1.89

TABLE I (continued)

Compound	Dose	S-9	Survival	Number of surviving cells · 10^{-5}	Number of mutants observed	Mutation frequency · 10^5	Induced mutation frequency · 10^5
2-AA	μg/ml						
	0	-	59.1	8.87	10	1.13	
	0	+	66.3	9.95	11	1.11	
	100	-	51.3	7.18	10	1.39	
	100	+	47.2	7.08	34	4.80[a]	3.69
	50	+	74.2	11.13	26	2.34	1.23
	25	+	52.1	7.82	16	2.05	0.94
	0	-	78.8	5.52	73	13.22	
	0	+	81.7	5.72	87	15.21	
	200	-	73.0	4.38	60	13.70	
	200	+	66.0	4.62	134	29.00[a]	13.79
	100	+	61.3	4.29	133	31.00[a]	15.79
	50	+	71.2	4.98	84	16.87	1.66
7,12-DMBA	μg/ml						
	0	-	59.7	4.19	11	2.63	
	0	+	62.5	4.38	11	2.51	
	100	-	42.8	3.00	8	2.67	
	100	+	40.9	2.86	40	13.99[a]	11.48
	50	+	44.1	3.09	20	9.39[a]	6.88
	25	+	45.2	3.16	18	5.70[b]	3.19

[a] 1% level.
[b] 5% level.

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