[CANCER RESEARCH 43, 1054-1058, March 1983]
0008-5472/83/0043-0000$02.00

Mutation of Chinese Hamster V79 Cells and Transformation and Mutation of Mouse Fibroblast C3H/10T½ Clone 8 Cells by Aflatoxin B_1 and Four Other Furocoumarins Isolated from Two Nigerian Medicinal Plants

Anthony O. Uwaifo,[1] Paul C. Billings,[2] and Charles Heidelberger[3]

University of Southern California Cancer Research Laboratories, Los Angeles, California 90033

ABSTRACT

Mutation by aflatoxin B_1 (AFB_1), imperatorin, marmesin, chalepin, and 8-methoxypsoralen (MOP), with and without black light (BL; long-wavelength ultraviolet light) activation, was determined at the hypoxanthine-guanine phosphoribosyltransferase locus (8-azaguanine resistance) in Chinese hamster V79 cells and at the ouabain locus in mouse C3H/10T½ cells. Transformation by these furocoumarins under the same activation conditions was also investigated in C3H/10T½ cells. In V79 cells, AFB_1 induced a 4-fold maximum mutation frequency over controls under BL activation at a concentration of 5 μg/ml; marmesin induced a 2-fold increased mutation frequency at 1.5 μg/ml; MOP induced a 19-fold increase at 10 μg/ml; chalepin induced a 3-fold increase at 5 μg/ml; and imperatorin induced a 20-fold increase at 10 μg/ml. Essentially no mutation was observed at the ouabain-resistant (*Ouaʳ*) locus in C3H/10T½ cells with any of these compounds. In the transformation assays, type II and type III foci were observed at a 1-μg/ml addition of AFB_1 with or without BL activation; while with MOP and imperatorin, these types of foci were observed only with BL activation. Marmesin, although relatively more cytotoxic than the other furocoumarins studied, with a 50% lethal dose of less than 0.5 μg/ml, was not as mutagenic or potentially carcinogenic as were AFB_1, imperatorin, or MOP with BL activation. These furocoumarins are considered to be involved in the etiology of the high incidence of skin cancer in Nigeria. Our experiments reinforce that concept and suggest that exposure to these furocoumarins may constitute a real carcinogenic hazard.

INTRODUCTION

The high incidence of primary liver cancer in Nigeria and other tropical countries (18) has been associated with a number of environmental factors (1, 19). Prominent among these is the mold metabolite AFB_1,[4] a furocoumarin that has been identified in the food and urine of normal and diseased Nigerians (4-6). Another important factor in the causation of such hepatomas is the hepatitis B virus (7). Apart from AFB_1, mutagens and carcinogens occurring naturally in plants taken as food or medicine are also suspect in this high incidence of hepatomas (6). In particular, chemicals such as furocoumarins that are structurally similar to AFB_1 and occur in plants ingested as food or medicine require further investigation. MAR, imperatorin, MOP, and chalepin are among such compounds (Chart 1). Imperatorin and chalepin are furocoumarins isolated from *Clausena anisata* (wild Rutaceae), a small tree found in the tropical forest of West African countries (1, 20). Concoctions of the roots of this plant are used for the treatment of hemorrhoids (8). Chalepin has also been isolated from *Ruta chalepensis* in Guatemala, where its concoction is used for the treatment of measles, scarlet fever, headaches, and heart diseases (8). Imperatorin, MAR, and MOP have been isolated from another medicinal plant, *Afraegle paniculata* (Rutaceae) (2). Preparations from this plant are ingested or applied topically for treatment of various disease conditions in Nigeria and other tropical countries (8). MOP has been reported to be mutagenic in mammalian and bacterial cells following irradiation with long-wave UV (9, 10). It has also been reported to induce transformation in mammalian cells (10) when thus irradiated. Although AFB_1 has been reported to bind to DNA following irradiation with near UV for about 1 to 4 hr (30), there are no reports on the mutagenicity and carcinogenicity of AFB_1 compared with those of other furocoumarins under UV activation. We report here such a study.

MATERIALS AND METHODS

Chemicals. AFB_1, 3-methylcholanthrene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and MOP were purchased from Sigma Chemical Co. (St. Louis, Mo.). Chalepin, imperatorin, and MAR were the generous gifts of Dr. Adesogan and Dr. Okorie, Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

Culture Techniques. V79 cells were grown in Dulbecco's medium supplemented with 8% dialyzed heat-inactivated calf serum and 2% dialyzed heat-inactivated fetal calf serum (25). C3H/10T½ clone 8 cells were grown in basal medium (Eagle's) containing 10% heat-inactivated fetal calf serum (26).

BL Treatment. All BL (long-wavelength UV) treatments were done in laminar-flow hoods on sterile elevated platforms. Cells were pretreated with test compounds for different lengths of time and rinsed with PBS containing test compounds before irradiation. Cells were irradiated with a GE BLB F40 fluorescent lamp with an emission range of 320 to 420 nm (maximum, 360 nm). This GE lamp has a built-in filter that absorbs 95% of the visible light and transmits a high percentage of near-UV energy, thus making additional filters unnecessary. BL exposure was measured with a J221 Black-ray UV meter (Ultra-Violet Products, Inc., South Pasadena, Calif.).

Cytotoxicity Assay. Since the establishment of equilibrium between the levels of a compound inside and outside of cells prior to irradiation is necessary for maximal effect (29), the minimal time for such an equilibrium to be established was determined for each compound. Two hundred cells were seeded per 60-mm dish, and after a 6-hr attachment period for V79 cells or a 24-hr attachment period for C3H/10T½ cells

[1] Fellow of the International Agency for Research on Cancer, Lyon, France. Present address: Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

[2] Supported by a Fellowship from Training Grant 1-T32-CA-09320 from the National Cancer Institute, NIH.

[3] To whom requests for reprints should be addressed.

[4] The abbreviations used are: AFB_1, aflatoxin B_1; MAR, marmesin; MOP, 8-methoxypsoralen; BL, black light; PBS, phosphate-buffered saline (0.9 mM $CaCl_2$; 27 mM KCl 1.5 mM KH_2PO_4; 0.5 mM $MgCl_2 \cdot 6H_2O$; 140 mM NaCl; and 8 mM $Na_2HPO_4 \cdot 7H_2O$, pH 7.4); *Ouaʳ*, ouabain-resistant.

Received July 2, 1982; accepted November 23, 1982.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/43/3/1054/2415318/cr0430031054.pdf by guest on 11 August 2024

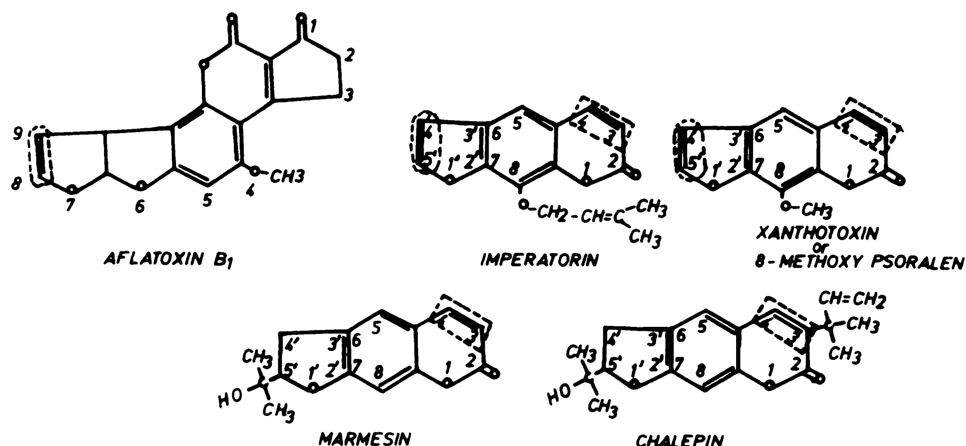

Chart 1. Chemical structures of AFB_1 and furocoumarins isolated from medicinal plants.

were treated with test compounds. After addition of test compound, the cells were incubated at 37° for various lengths of time prior to BL treatment. At each time point, the medium was removed, and the cells were washed with PBS. Subsequently, 5 ml of PBS containing the test compound were added to each dish, and the cells were irradiated in open dishes with BL. Controls were treated similarly but were not irradiated. Following BL irradiation, the PBS was removed and replaced with complete medium. The cells were grown for 7 days, fixed with methanol, and stained with Giemsa, and the surviving colonies were counted.

Mutation Assay. Except for the BL irradiation, as described above, the protocol followed in the mutation assays in V79 cells was as described by Peterson *et al.* (25) and in C3H/10T½ cells as described by Landolph and Heidelberger (15).

Transformation Assay. The protocol followed for all transformation assays was essentially that of Reznikoff *et al.* (26). However, cells were treated with furocoumarins for either 2 hr (AFB_1, imperatorin, MAR, and MOP) or 18 hr (chalepin) before irradiation. The medium was changed in all transformation experiments 48 hr after initial exposure of cells to the compounds. Transformed foci were classified as type II or type III, using the morphological criteria initially established by Reznikoff *et al.* (26).

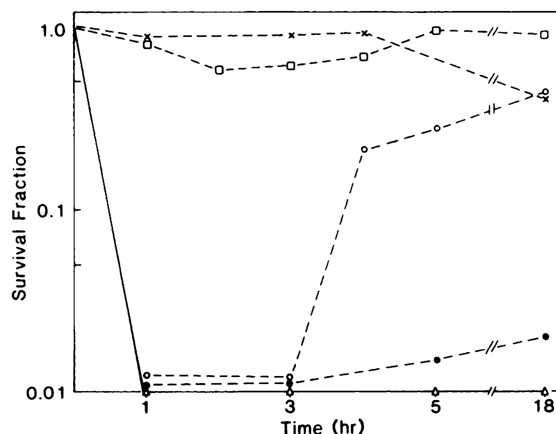

Chart 2. Effect of duration of incubation of V79 cells with furocoumarins prior to BL treatment on their cytotoxicity. No toxicity was observed with AFB_1, chalepin, imperatorin, or MOP in the absence of BL activation; therefore, these curves have been omitted. The toxicity of MAR is the same in the presence or absence of BL activation; hence, only the *solid line* is shown. □, AFB_1 (1 µg/ml); ×, chalepin (3 µg/ml); ○, imperatorin (10 µg/ml); △, MAR, (0.5 µg/ml); ●, MOP (10 µg/ml). Dose of BL irradiation, 4.25 J/sec/sq m for 10 min. - - - -, plus BL; ——, minus BL.

RESULTS

Cytotoxicity. One of the prime conditions governing the response of photobiologically active furocoumarins is the concentration of the compound at the biological site of action at the time of irradiation (3). The time interval required to attain the optimal effect was therefore determined for each furocoumarin in V79 cells (Chart 2). MAR required a minimum of a 1 hr incubation before irradiation for optimal effects; AFB_1, 2 hr; Imperatorin, 1 to 3 hr; MOP, 1 to 4 hr; and chalepin, 18 hr. The cytotoxicities of the furocoumarins in V79 and C3H/10T½ cells with and without BL activation are shown in Charts 3 and 4. The results obtained with both cell lines were qualitatively similar.

MAR is the most cytotoxic of the 5 furocoumarins, with or without BL activation. In V79 cells, the descending order of cytotoxicity is: without BL irradiation, MAR > chalepin > imperatorin > MOP > AFB_1; with BL irradiation, MAR > MOP > imperatorin > AFB_1 > chalepin. In C3H/10T½ cells, the order is: without BL, Mar > chalepin > AFB_1 > imperatorin > MOP; with BL, MAR > MOP > chalepin > imperatorin > AFB_1. Survival curves for the 5 furocoumarins at 1 µg/ml are shown in Chart 5 (V79 cells) and Chart 6 (C3H/10T½ cells) for

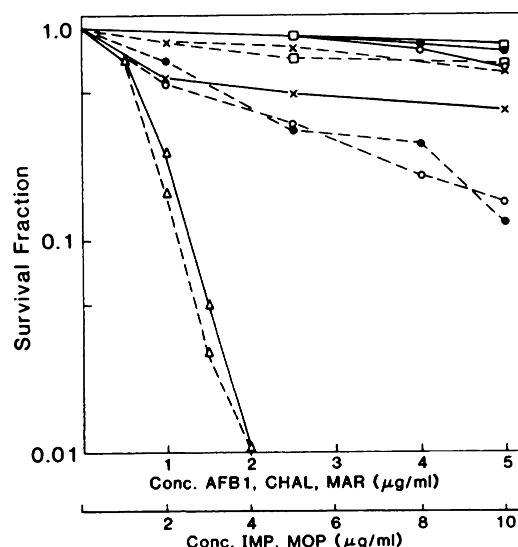

Chart 3. Cytotoxicities of furocoumarins in V79 cells at different concentrations with and without BL activation. Doses of irradiation: imperatorin (*IMP*) and MOP, 4.25 J/sec/sq m for 5 min; AFB_1, chalepin (*CHAL*), and MAR, 4.2J/sec/sq m for 10 min. □, AFB_1; ×, chalepin; ○, imperatorin; △, MAR; ●, MOP; - - - -, plus BL; ——, minus BL.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/43/3/1054/2415318/cr0430031054.pdf by guest on 11 August 2024

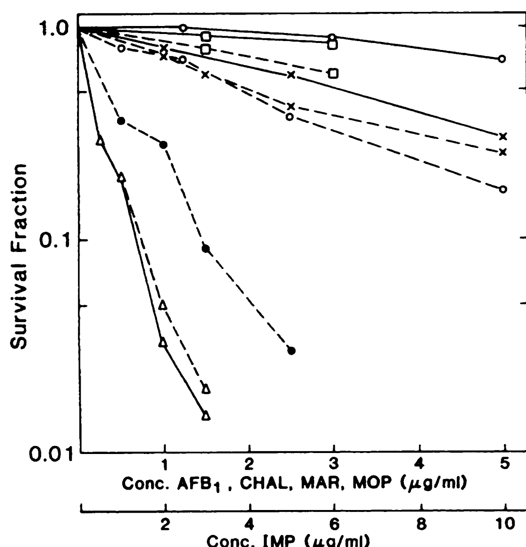

Chart 4. Cytotoxicities of AFB₁, chalepin (*CHAL*), imperatorin (*IMP*), MAR, and MOP in C3H/10T½ cells at different concentrations with and without BL activation. Dose of irradiation, 4.25 J/sec/sq m for 10 min. The curve for MOP without BL activation has been omitted from the chart due to the low level of toxicity observed. □, AFB₁; ×, chalepin; ○, imperatorin; △, MAR; ●, MOP; ----, plus BL; ——, minus BL.

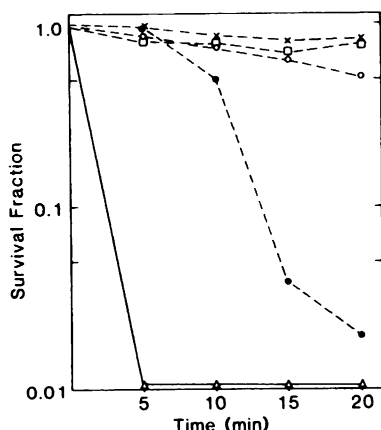

Chart 5. Relationship of duration of BL irradiation and cytotoxicity of furocoumarins at 1 μg/ml in V79 cells. The curves for AFB₁, chalepin, imperatorin, and MOP in the absence of BL activation have been omitted due to the low levels of toxicity observed. The toxicity of MAR was the same in the presence or absence of BL activation; therefore, only the *solid curve* for MAR is shown. Rate of irradiation, 4.2 J/sec/sq m. □, AFB₁; ×, chalepin; ○, imperatorin; △, MAR; ●, MOP; ----, plus BL; ——, minus BL.

different times of BL irradiation. The profile of the cytotoxicity curve for MAR in the 2 cell lines is that expected of one-hit survival curves (17, 32).

Mutation in V79 and C3H/10T½ Cells. The cytotoxicities and mutation frequencies produced by the furocoumarins in V79 cells are shown in Table 1. With the possible exception of imperatorin, essentially no mutation was obtained in the absence of BL. With BL irradiation, MOP and imperatorin produced a dose-dependent mutagenic response. At 10 μg/ml, imperatorin and MOP increased the mutation frequency about 20-fold above background. No mutation was detected at the *Oua^r* locus in C3H/10T½ cells with any of the furocoumarins (data not shown).

Transformation in C3H/10T½ Cells. The results of the transformation experiments are summarized in Table 2. AFB₁ induced type II and type III foci at 1 μg/ml, with and without BL activation. No foci were observed at the higher concentrations of 1.5 and 3 μg/ml. Under BL activation, MOP induced type II foci at concentrations of 1.0, 1.5, and 2.5 μg/ml. Imperatorin

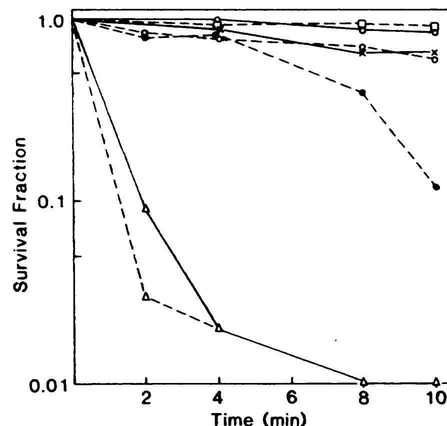

Chart 6. Relationship of duration of BL irradiation and cytotoxicity of furocoumarins at 1 μg/ml in C3H/10T½ cells. Essentially no toxicity was observed with AFB₁ and MOP in the absence of BL activation; therefore, these curves have been omitted. Equivalent toxicity was observed for chalepin in the presence or absence of BL irradiation. Consequently, only the *solid curve* for chalepin is shown. □, AFB₁; ×, chalepin; ○, imperatorin; △, MAR; ●, MOP; ----, plus BL; ——, minus BL.

Table 1
Mutagenicity of AFB₁, MAR, MOP, chalepin, and imperatorin with and without BL activation in V79 cells

Treatment	Survival[a]		*AzG^r*[b] mutants/10^5 survivors	
	−BL	+BL	−BL	+BL
AFB₁, 0.5 μg/ml	0.95	0.92	1.5 ± 0.4[c]	2.3 ± 0.5
AFB₁, 1.0 μg/ml	0.94	0.82	1.5 ± 0.3	2.6 ± 0.8
AFB₁, 1.5 μg/ml	0.92	0.74	1.4 ± 0.6	2.7 ± 0.5
AFB₁, 2.5 μg/ml	0.88	0.72	1.3 ± 0.7	2.9 ± 1.2
AFB₁, 5.0 μg/ml	0.85	0.70	2.4 ± 1.6	4.5 ± 1.3
DMSO, 0.5%	1.00	1.00	1.4 ± 0.6	1.4 ± 0.5
MAR, 0.25 μg/ml	0.98	0.98	1.6 ± 0.7	1.6 ± 0.3
MAR, 0.5 μg/ml	0.65	0.66	1.1 ± 1.0	1.7 ± 0.5
MAR, 1.0 μg/ml	0.27	0.17	1.1 ± 0.6	2.0 ± 0.5
MAR, 1.5 μg/ml	0.05	0.03	1.9 ± 0.8	2.4 ± 0.4
DMSO, 0.5%	1.00	1.00	1.2 ± 0.6	1.3 ± 0.5
MOP, 2.0 μg/ml	0.80	0.78	0.9 ± 0.3	1.9 ± 0.0
MOP, 5.0 μg/ml	0.80	0.32	1.1 ± 0.3	6.3 ± 0.4
MOP, 8.0 μg/ml	0.80	0.29	1.4 ± 0.4	8.8 ± 0.6
MOP, 10.0 μg/ml	0.77	0.12	2.9 ± 0.5	19.2 ± 0.9
DMSO, 0.5%	1.00	1.00	1.2 ± 0.6	1.3 ± 0.5
Chalepin, 1.0 μg/ml	0.58	0.94	0.5 ± 0.3	0.9 ± 0.3
Chalepin, 1.5 μg/ml	0.49	0.86	0.9 ± 0.3	0.9 ± 0.3
Chalepin, 2.5 μg/ml	0.47	0.85	0.7 ± 0.7	1.9 ± 0.6
Chalepin, 5.0 μg/ml	0.42	0.63	1.1 ± 0.8	3.8 ± 0.4
DMSO, 0.5%	1.00	1.00	1.5 ± 0.5	0.9 ± 0.4
Imperatorin, 2.0 μg/ml	0.82	0.55	2.3 ± 0.5	14.9 ± 0.9
Imperatorin, 5.0 μg/ml	0.81	0.35	4.3 ± 0.6	15.7 ± 0.9
Imperatorin, 8.0 μg/ml	0.80	0.20	4.5 ± 0.5	17.7 ± 0.8
Imperatorin, 10.0 μg/ml	0.78	0.15	7.8 ± 0.7	20.9 ± 1.0
DMSO, 0.5%	1.00	1.00	1.4 ± 0.4	1.5 ± 0.4
MNNG, 0.5 μg/ml[d]	0.32		5.7 ± 0.4[e]	

[a] Survival relative to DMSO controls. Absolute survival (plating efficiency) of V79 cells in DMSO controls was 0.41 ± 0.03.
[b] *AzG^r*, 8-azaguanine-resistant; DMSO, dimethyl sulfoxide; MNNG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine.
[c] Mean ± S.D. of 3 experiments.
[d] Cells were treated with MNNG as described previously (25).
[e] Mean ± S.D. of 15 experiments.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/43/3/1054/2415318/cr0430031054.pdf by guest on 11 August 2024

Table 2

Transformation of C3H/10T½ cells by AFB_1, MOP, imperatorin, MAR, and chalepin with and without BL activation

Compound	No. of experiments	Concentration (μg/ml)	Survival[a]		Transformation (no. of foci/no. of dishes scored)				No. of dishes with type II and type III foci	
					Type II		Type III			
			−BL	+BL	−BL	+BL	−BL	+BL	−BL	+BL
AFB_1	3	1.0	0.97	0.79	1/40	6/34	2/40	7/34	3	9
		1.5	0.88	0.75	0/39	0/39	0/39	0/39	0	0
		3.0	0.86	0.70	0/36	0/40	0/36	0/40	0	0
MOP	3	1.0	1.00	0.34	0/37	2/27	0/37	0/27	0	2
		1.5	1.10	0.10	0/40	3/50	0/40	0/50	0	3
		2.5	1.00	0.03	0/46	3/27	0/46	0/27	0	3
Imperatorin	3	1.0	1.00	0.78	0/36	0/38	0/36	0/38	0	0
		2.5	1.00	0.70	0/40	0/38	0/40	0/38	0	0
		5.0	0.93	0.56	0/41	0/41	0/41	14/41	0	12
MAR	3	0.25	0.30	0.32	0/58	0/60	0/58	0/60	0	0
		0.5	0.14	0.17	0/44	0/37	0/44	0/37	0	0
		1.0	0.03	0.05	0/42	0/36	0/42	0/36	0	0
Chalepin	3	1.0	0.88	0.82	0/27	0/50	0/27	0/50	0	0
		2.5	0.42	0.40	0/28	0/35	0/28	0/35	0	0
		5.0	0.30	0.27	0/30	0/30	0/30	0/30	0	0
MCA[b]	15	1.0	1.00		83/208		125/208		208	

[a] Survival relative to controls. Absolute survival (plating efficiency) of C3H/10T½ cells in controls was 0.29 ± 0.03.
[b] MCA, 3-methylcholanthrene. Cells were treated 24 hr after seeding with MCA for 48 hr (26).

induced typical type III foci at 10 μg/ml, while no type II or type III foci were observed at lower concentrations. MAR and chalepin did not induce any foci.

DISCUSSION

The concentration of furocoumarins at the biological site of action at the time of irradiation is a critical factor in their photobiological reactivity (3). In mammalian cells and in bacteria, the maximal response is usually attained after 1 to 2 hr (3). We found this to be true for MAR, AFB_1, MOP, and imperatorin but not for chalepin, which seems to require a much longer time to achieve the maximal response (Chart 2). For each compound, the time interval required for optimal photosensitization falls within a narrow time range, beyond which the photobiological response begins to fall. This situation might be due to a shielding effect arising from an excess of the compounds being concentrated within the cells and consequently reducing the dose of the impinging radiation (27). It is also possible that an excess of the furocoumarins at the biological site of action may have a quenching effect on the BL or may result in the photopolymerization of the compounds being sensitized (29).

The cytotoxicity data show that of the 5 furocoumarins examined, only MAR and chalepin have any significant cytotoxicity without BL activation in V79 and C3H/10T½ cells. The similarity of their cytotoxicity profiles seems to correlate with the similarity of their chemical structures (see Chart 1). These 2 compounds, in addition, are the only members of the group which have a saturated 4,5-bond, which is considered to be one of the 2 photochemically reactive sites of furocoumarins; the other photochemically reactive site is the 3,4-bond (27). Although the 3,4-double bond is present in chalepin and MAR, this site is not likely to be available for photobiological reactions in chalepin because of the large αα-dimethylallyl group on carbon 3 of the 3,4-double bond, which may exert steric hindrance on the double bond (Chart 1). The effect of the tertiary hydroxyl group situated on carbon 5 of the 4,5-double bond of the furan group on the direct reactions of MAR and chalepin may be linked to a destabilizing effect of this group on the valency electrons of the bond, which are usually stable (14). This stability is probably responsible for the chemical and biological inactivity of these compounds in the absence of irradiation (14). The relatively low cytotoxicity of BL-activated AFB_1 (Charts 3 and 4) can be explained on the basis of the presence of only one photobiologically reactive site, the 8,9-unsaturated furan bond, which is the equivalent of the 4,5-bond of the other furocoumarins. This restricts AFB_1 to the formation of monoadducts under BL activation, whereas imperatorin and MOP can form cross-linked adducts with the 3,4- (pyrone) and 4,5- (furan) double bonds.

BL activation increased the mutagenicity of MOP and imperatorin in V79 cells. Under these conditions, a good dose response was observed with these compounds. However, no mutation was observed at the *Oua*^{r} locus in C3H/10T½ cells with any of the furocoumarins in the absence or presence of BL activation. The *HGPRT* locus is capable of detecting both point and frame-shift mutations (16), while the *Oua*^{r} locus detects only point mutations (15, 16). Our results, therefore, suggest that BL irradiation activates these furocoumarins into forms which are capable of generating frame-shift mutations but do not generate point mutations.

We observed transformed type II and type III foci with AFB_1 at a concentration of 1 μg/ml with and without BL activation, while no focus formation was observed at the higher concentrations of 1.5 and 3 μg/ml. This may result from increased cytotoxicity at these higher AFB_1 concentrations, resulting in a decreased transformation response. It has also been demonstrated in liquid holding experiments that lesions induced in DNA by furocoumarins under BL activation are repaired (31). This may explain why we observed transformation with imperatorin only at 5 μg/ml, the highest concentration used in this study.

MAR was found to be the most cytotoxic furocoumarin that

Downloaded from http://aacrjournals.org/cancerres/article-pdf/43/3/1054/2415318/cr0430031054.pdf by guest on 11 August 2024

we examined. However, it was negative in the mutation and transformation assays. In contrast, MOP and imperatorin were positive in the mutation and transformation assays following BL activation but were less cytotoxic than was MAR. These results suggest that the mechanisms of furocoumarin-induced cytotoxicity may be different from the mechanisms of furocoumarin-induced mutation and transformation.

The photobiological effects of furocoumarins, such as MOP, in whole animals (12, 13, 21, 23, 24) and cell culture (11, 28) are widely reported. Our results with MOP are in basic agreement with those reports. It is, however, difficult to evaluate the significance of our cytotoxicity, mutation, and transformation results with respect to the carcinogenic risks that these compounds pose to those who ingest them or apply them topically. Nevertheless, the frequency of skin cancer, as a percentage of all cancers in Nigeria and other tropical countries, is second only to primary liver cancer (18, 22). Although people residing in Nigeria and other tropical countries are exposed to high-intensity sunlight, they also contain high amounts of melanin pigment in their skin. Since melanin is an effective absorber of UV radiation (29), the *in vivo* photoactivation of furocoumarins in these populations may be limited. In contrast, the topical application of these furocoumarins may represent a greater carcinogenic risk. Epidemiological studies of the incidence of skin cancer and other cancers in Nigeria and other areas of the world in which furocoumarins are used in folk medicine are necessary for meaningful risk extrapolation of the data presented here.

In this study, we have examined the potential of photochemically activated furocoumarins to kill, mutate, and morphologically transform mammalian cells. Enzymatic or metabolic activation by the liver or other organs may also play a critical role in the actions of these compounds *in vivo*. Further work is needed to clearly define the roles and relative importance of metabolic and photochemical activation in the conversion of furocoumarins into genotoxic metabolites.

ACKNOWLEDGMENTS

We thank Dr. Joseph Landolph for invaluable advice. We also thank Hazel Peterson and Diane Nakadate for expert technical assistance.

REFERENCES

1. Abe, M. O., and Taylor, D. A. H. A quinolone alkaloid from *Oricia suaveolens*. Phytochemistry (Oxf.), *10:* 1167–1169, 1971.
2. Adesogan, E. K. Coumarins and other components of *Afraegle paniculata*. Phytochemistry (Oxf.), *12:* 2310–2312, 1973.
3. Alderson, T., and Scott, B. R. The photosensitizing effect of 8-methoxypsoralen on the inactivation and mutation of *Aspergillus conida* by near ultraviolet light. Mutat. Res., *9:* 569–578, 1970.
4. Bababunmi, E. A. Excretion of aflatoxin in the urine of normal individuals and patients with liver disease in Ibadan (Nigeria). *In:* H. E. Nieburgs (ed.), Detection and Prevention of Cancer, pp. 1729–1736. New York: Marcel Dekker, Inc., 1976.
5. Bababunmi, E. A., Uwaifo, A. O., and Bassir, O. Hepatocarcinogens in Nigerian foodstuffs. World Rev. Nutr. Diet., *28:* 188–209, 1978.
6. Berman, C. Primary carcinoma of the liver. Adv. Cancer Res., *5:* 55–96, 1958.
7. Blumberg, B. S., Larouze, B., London, W. T., Werner, B., Hesser, J. E., Millman, I., Saimot, G., and Payet, M. The relation of infection with the hepatitis B agent to primary hepatic carcinoma. Am. J. Pathol., *81:* 669–682, 1975.
8. Dalziel, J. M. *In:* The Useful Plants of West Africa. London: Crown Agents, 1937.
9. Emerole, G., Thabrew, M. I., Anosa, V., and Okorie, D. A. Structure-activity relationship in the toxicity of some naturally occurring coumarins: chalepin, imperatorin, and oxypeucedanine. Toxicology, *20:* 71–80, 1981.
10. Evans, O. L., and Morrow, K. J. 8-Methoxypsoralen induced alterations of mammalian cells. J. Invest. Dermatol., *72:* 35–41, 1979.
11. Fowler, J. F. Differences in survival curve shapes for formal multi-target and multihit models. Phys. Med. Biol., *9:* 177–188, 1964.
12. Griffin, A. C., Hakim, R. E., and Knox, J. The wave length effect upon erythemal and carcinogenic response in psoralen treated mice. J. Invest. Dermatol., *31:* 289–295, 1958.
13. Hakim, R. E., Griffin, A. C., and Knox, J. M. Erythema and tumor formation in methoxsalen treated mice exposed to fluorescent light. Arch. Dermatol., *82:* 572–577, 1960.
14. Lamola, A. A. Photosensitization in biological systems and the mechanism of photoreactivation. Mol. Photochem., *4:* 107–133, 1972.
15. Landolph, J. R., and Heidelberger, C. Chemical carcinogens produce mutation to ouabain resistance in transformable C3H/10T½ Cl 8 mouse fibroblasts. Proc. Natl. Acad. Sci. U. S. A., *76:* 930–934, 1979.
16. Landolph, J. R., and Jones, P. A. Mutagenicity of 5-azacytidine and related nucleosides in C3H/10T½ clone 8 and V79 cells. Cancer Res., *42:* 817–823, 1982.
17. Meynell, G. G., and Meynell, E. Theory and Practice in Experimental Bacteriology, Ed. 2, pp. 196–201. Cambridge, England: Cambridge University Press, 1970.
18. Oettle, A. G. Cancer in Africa, especially in regions south of the Sahara. J. Natl. Cancer Inst., *33:* 383–439, 1964.
19. Oginsky, E. L., Green, G. S., Griffith, D. G., and Fowlks, W. L. Lethal photosensitization of bacteria with 8-methoxypsoralen to long wave length ultraviolet radiation. J. Bacteriol., *78:* 821–833, 1959.
20. Okorie, D. A. A new carbazole alkaloid and coumarins from roots of *Clausena anisata*. Phytochemistry (Oxf.), *14:* 2720–2721, 1975.
21. O'Neal, M. A., and Griffin, A. C. The effect of oxypsoralen upon ultraviolet carcinogenesis in albino mice. Cancer Res., *17:* 911–916, 1957.
22. Osunkoya, B. O. Cancer in the tropics. *In:* R. L.Smith, and E. A. Bababunmi (eds.), Toxicology in the Tropics, pp. 6–8. London: Taylor and Francis Ltd., 1980.
23. Pathak, M. A., Daniels, F., Hopkins, C. E., and Fitzpatrick, T. B. Ultra-violet carcinogenesis in albino and pigmented mice receiving furocoumarins: psoralen and 8-methoxypsoralen. Nature (Lond.), *183:* 728–730, 1959.
24. Pathak, M. A., and Kramer,D. M. Photosensitization of skin *in vivo* by furocoumarins (Psoralens). Biochim. Biophys. Acta, *195:* 197–206, 1969.
25. Peterson, A. R., Peterson, H., and Heidelberger, C. Oncogenesis, mutagenesis, DNA damage, and cytotoxicity in cultured mammalian cells treated with alkylating agents. Cancer Res., *39:* 131–138, 1979.
26. Reznikoff, C. A., Bertram, J. S., Brankow, D. W., and Heidelberger, C. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. Cancer Res., *33:* 3239–3249, 1973.
27. Rodighiero, G., Musajo, I., Dall'acqua, F., Marciani, S., Caporale, G., and Ciavatta, L. Mechanism of skin photosensitization by furocoumarins. Photoreactivity of various furocoumarins with native DNA and with ribosomal RNA. Biochim. Biophys. Acta, *217:* 40–49, 1970.
28. Sasaki, M. S., and Tonomura, A. A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. Cancer Res., *33:* 1829–1836, 1973.
29. Scott, B. R., Pathak, M. A., and Mohn, G. R. Molecular and genetic basis of furocoumarin reactions. Mutat. Res., *39:* 29–74, 1976.
30. Shieh, J. C., and Song, P. S. Photochemically induced binding of aflatoxins to DNA and its effects on template activity. Cancer Res., *40:* 689–695, 1980.
31. Simons, J. W. I. M. Development of a liquid-holding technique for the study of DNA repair in human diploid fibroblasts. Mutat. Res., *59:* 273–283, 1979.
32. Spicer, C. C. The estimation of the parameters of an exponentially declining population. J. Hyg., *54:* 304–310, 1956.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/43/3/1054/2415318/cr0430031054.pdf by guest on 11 August 2024