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Chemopreventive Effect of Difluoromethylornithine (DFMO) on Mouse Skin Squamous Cell Carcinomas Induced By Benzo(*a*)pyrene

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Abstract The effect of the chemopreventive agent D,L-α-difluoromethylornithine (DFMO) on the incidence of skin squamous cell carcinoma was studied in SENCAR mice treated weekly with topical applications of benzo(*a*)pyrene (B(*a*)P) (0.15 mmol, 2×/week) on the dorsal skin. Animals were randomized to receive either chow or chow supplemented with DFMO (1 g/1 kg) and studied at 10, 15, 20, 25, and 30 weeks of B(*a*)P treatment. Morphometric analyses at each timepoint evaluated the epidermal thickness (ET) and the number of epidermal nucleated layers (NL). The ET increased from 12–17 μm as early as 10 weeks after B(*a*)P treatment, reaching 22 μm at 20 weeks, and 27 μm at 25 weeks (130% increase). The NL also increased markedly. A relatively modest increase in ET was observed in animals treated with B(*a*)P and DFMO (16% at 15 weeks, 53% at 20 weeks, and 85% at 25 weeks) as compared to controls. The relative increase in NL showed a similar pattern. Although extensive epidermal hyperplasia was seen early, clear-cut focal premalignant lesions were not identifiable before week 20 of B(*a*)P treatment. At 20 weeks, the most frequently noted focal premalignant lesions in carcinogen-treated animals (without DFMO) were moderate dysplasias. At 25 and 30 weeks, a large increase was seen in the incidence of more advanced dysplastic lesions and invasive carcinomas. In the group treated with B(*a*)P and DFMO, a marked reduction in the number of carcinomas was observed at 25 and 30 weeks. At 25 weeks, DFMO reduced tumor yield from 5.8 to 3.2 carcinomas per mouse. At 30 weeks, the reduction was from 13.1 to 5.7 carcinomas per mouse (57% reduction). Collectively, these data emphasize the strong chemopreventive effect of DFMO against tumors in the mouse skin complete carcinogenesis model, as indicated by the reduction of overall skin tumor incidence and the decreased epidermal hyperplasia in DFMO-treated animals. Morphometrically defined increases in ET and NL can be used as early biomarkers of DFMO chemoprevention in mouse skin tumorigenesis. J. Cell. Biochem. Suppls. 28/29:81–89. © 1998 Wiley-Liss, Inc.

Key words: chemoprevention; benzo-(*a*)pyrene; squamous cell carcinoma; skin tumor markers; difluoromethylornithine

Squamous cell carcinomas of the murine skin produced by complete carcinogenesis protocols provide an excellent animal model for human squamous cell carcinoma (SCC) [1–4]. This tumor type is the most frequent solid neoplasm occurring in skin and several other sites, i.e., esophagus, oral mucosa, larynx, lung, cervix, bladder, etc. The advantage of this model over the more frequently used two-stage carcinogenesis protocol is the production of malignant tumors preceded by precancerous or precursor lesions that are not very different from human precancerous (in situ) lesions. Another similarity with human carcinogenesis is that the tissues are exposed repeatedly to carcinogenic stimuli.

Experimentally induced SCCs have been used as model systems to study the effect of chemopreventive agents on the growth of this neoplasm [5–13]. Among the several chemopreventive chemicals used, D,L-α-difluoromethylornithine (DFMO) is one of the most promising. DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC), an enzyme that regulates mammalian polyamine biosynthesis

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[14,15]. Although the exact function of polyamines in mammalian cells is still unclear, they are considered to play a role in cell cycle regulation, cell division, tissue growth, and differentiation [14,16]. ODC is present in very small amounts in quiescent cells, and its activity can be increased significantly within a few hours of exposure to topical stimuli, such as hormones or drugs that can induce tissue regeneration and tumor promotion [14,17,18]. DFMO has been reported to inhibit carcinogenesis of skin [19,20], tongue [21], colon [22–25], breast [26], urinary bladder [27], kidney [28], and liver [29] in experimental animals. DFMO is one of the most extensively investigated chemopreventive agents; several Phase I studies have already been done [30–33].

Although DFMO has successfully inhibited skin carcinogenesis using the two-stage carcinogenesis protocol, no information is available regarding the effectiveness of this agent in other models of skin carcinogenesis.

In the present study, the effect of DFMO treatment on skin tumor development has been evaluated using the complete carcinogenesis model of mouse skin. This model was selected because it closely resembles multiple exposures to carcinogen insults seen in humans, especially those exposed to tobacco smoke. Early histological and morphometric changes were investigated as potential biomarkers of the chemopreventive activity of DFMO. Since epidermal hyperplasia is one of the earliest tissue changes indicative of cellular proliferation induced by chemical carcinogens and promoters, we elected to examine the epidermis for changes that could serve as early markers of carcinogenesis and its chemoprevention.

MATERIALS AND METHODS

Animals

Female SENCAR mice 4–9 weeks of age were obtained from the Fox Chase Cancer Center Laboratory Animal Facility, Philadelphia, PA. Animals were housed in disposable cages, in a temperature-controlled room (23°C) regulated with 12-hour light/12-hour dark cycles, with food and water ad libitum. All of the mice were housed in facilities which are fully approved by the American Association for Accreditation of Laboratory Animal Care. Prior to the experiment, animals were matched for body weight and randomized into five groups, receiving 3,4-benzo(*a*)pyrene [B(*a*)P] and DFMO as indicated (Table I). All mice were visually inspected twice a week for gross abnormalities and tumor formation. Mice were euthanized by cervical dislocation at specified times. Animals were euthanized at 10, 15, 20, 25, 30, and 35 weeks (Table I). Plasma was collected from animals in Groups 1 and 4 (n = 5 at each timepoint) for DFMO quantitation.

Carcinogen Preparation and Treatment

Benzo(*a*)pyrene (3,4-benzopyrene) [B(*a*)P], reagent grade (Sigma Chemical, St. Louis, MO) was used. The same batch was kept in the freezer at −20°C in weighed aliquots and used weekly. B(*a*)P was dissolved in acetone (HPLC grade) before application. Carcinogen weighing and manipulation was done in a biohazard room, specially designed for work with toxic and carcinogenic substances. Acetone (200 ml) containing 0.15µ mol of B(*a*)P was applied to the dorsal skin twice weekly for 35 weeks. Acetone alone was applied as control vehicle for animal groups receiving no carcinogen treatment. The animals were shaved 48 hours prior to treatment and only those in the resting phase of the hair cycle were used to start the experiments.

Chemopreventive Agent

DFMO (100% Efflornithine HC1) was supplied by Marion Merrell Dow Research Institute (Cincinnati, OH) and mixed in Purina 5001 chow at 0.1% by Dyets, Inc. (Bethlehem, PA).

TABLE I. Animal Groups for Study of Premalignant Changes and Tumor Incidence in Skin of Mice

Groups	Number of animals studied at week						Weeks of DFMO treatment	B(a)P 0.15µ moles twice weekly
	10	15	20	25	30	35		
1	10	10	10	10	10	10	0–35	+
2	—	—	—	10	10	10	20–35	+
3	10	10	10	10	10	10	None	+
4[a]	5	5	5	5	5	5	0–35	−
5	5	—	—	—	—	30	None	−

[a]All animals in group 4 were topically treated with 0.2 ml acetone without carcinogen. Animals in group 5 provided the negative controls.

Control and experimental diets were prepared and stored at room temperature. The drug was determined to be stable in the diet for at least 5 weeks when stored at room temperature. Fresh diet was synthesized approximately every 5 weeks and each shipment was analyzed for content and homogeneity prior to use.

DFMO Quantitation

Blood samples for DFMO analysis were obtained from the retro-orbital plexus of mice at the time of sacrifice and placed in a heparinized tube. Plasma was isolated by centrifugation at 3,000 rpm (4°C) for 10 minutes.

A new, sensitive protocol for the measurement of DFMO in mouse plasma and rodent chow was established, employing the Waters Pico-Tag method (Waters, Milford, MA). Briefly, filtrates containing proteins <10 kd were prepared from plasma and food by spinning all samples through Ultrafree-MC filters (Millipore Corp., Bedford, MA). All samples were dried under vacuum in pyrolized tubes, incubated with (6 × 50 mm) ethanol:Triethylamine: dH_2O:phenylisothiocyanate (7:1:1:1) for 20 minutes at room temperature, again under high vacuum for approximately 50 minutes, and stored at −70°C. At the time of analyses, each sample was resuspended in 80 ml of 6 mM sodium phosphate buffer, pH 7.2. Analysis were performed on a Waters Pico-Tag C_{18} HPLC column at 37°C. Solvent A consisted of 118 mM sodium acetate (pH 5.8), while solvent B consisted of 84% acetonitrile in water. Separation of DFMO was accomplished with an isocratic gradient of 94% A/6% B for 15 minutes at 1 ml/min, followed by a wash cycle of 100% B for one, 5 minutes. The column was then equilibrated at 95% A/5% B for 4 minutes with a step to 94% A/6% B 2.2 minutes before the next sample injection. DFMO elutes at approximately 10.2 minutes. The lower limits of detection were 5–10 pmol DFMO.

Histology

In order to identify flat in situ lesions that develop before macroscopically detectable neoplasms, we examined the dorsal skin for premalignant lesions using histological procedures. Systematic histological evaluation was accomplished by studying approximately 2 cm^2 of dorsal skin. Tissues were fixed in neutral buffered formaldehyde and in 70% ethanol. Each animal yielded ten blocks, 1–2 mm thick and 10 mm long. The longer dimension was parallel to

TABLE II. Epithelial Thickness (ET) and Nucleated Layers (NL) in B(*a*)P and B(*a*)P + DFMO Treatment*

	10 Weeks		15 Weeks		20 Weeks		25 Weeks		30 Weeks	
	ET	NL	ET	NL	ET	NL	ET	NL	ET	NL
Control (non-treatment) (Group 5)	12.0 ± 0.40	1.36 ± 0.03	—	—	—	—	—	—	—	—
Group 1 (B(*a*)P + DFMO)	14.1 ± 0.48	1.71 ± 0.10	14.0 ± 0.45	1.82 ± 0.08	18.4 ± 0.08	2.40 ± 0.17	22.2 ± 1.22	2.76 ± 0.16	30.2 ± 1.71	3.6 ± 0.24
Group 3 (B(*a*)P alone)	14.4 ± 0.47	1.67 ± 0.06	17.0 ± 0.40	2.32 ± 0.06	22.0 ± 0.81	3.65 ± 0.14	27.5 ± 2.04	3.89 ± 0.26	28.5 ± 1.55	3.42 ± 0.13
Group 4 (DFMO alone)	11.0 ± 0.20	1.24 ± 0.05	—	—	—	—	—	—		

*ET = Expressed as microns and represents the average thickness from basal lamina to granular layer inclusive. Areas as ET. Total of ten mice per time point, ten blocks per mouse, and 60 measurements per mouse or 600 measurements expressed as mean ± standard deviation of the mean of each animal group.

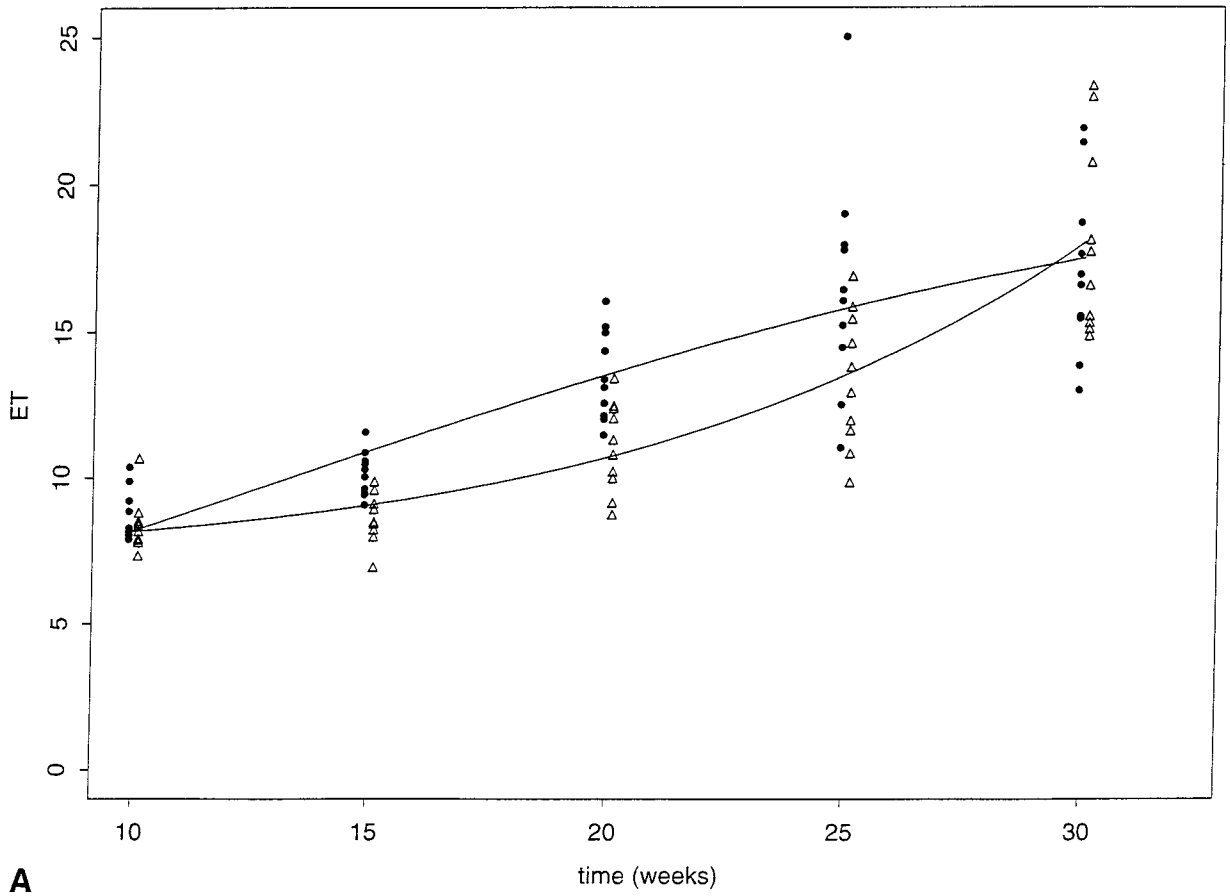

Fig. 1. **A:** Epidermal thickness (ET) and **(B)** number of nucleated layers (NL) in B(*a*)P + DFMO treated mice (Group 1) (triangles), or B(*a*)P alone (Group 2) (circles). Dots and triangles are shifted to avoid overlap. Smooth curves are least squares fits to a function of the form a + b*Phi[(t-c)/d], where Phi[.] is the cumulative normal distribution function. The span between curves indicates a delay of about 4 weeks for Group 1 in reaching an epidermal thickness equal to that of Group 2. A delay of >7 weeks is seen for Group 1 to reach the same average number of layers. Significant differences ($P < 0.05$) exist at 15, 20, and 25 weeks but not at 10 or 30 weeks for both measures.

the longitudinal cephalo-caudal axis of the animal and permitted a better visualization of the hair follicles. In order to obtain flat blocks, the entire piece of skin was placed on a piece of paper and prefixed for 1 hour to render the tissue harder and to avoid curling of the skin. After this prefixation period, the blocks were cut, labeled, and placed in separate vials and fixed overnight. Macroscopically detectable lesions ≥1 mm were separated from the skin and processed independently in separate blocks.

Morphometric Techniques

Lesions were classified according to previously published histopathological nomenclature. Morphometric parameters were evaluated in all animals at the selected timepoints. The epidermal thickness from the basement membrane to the granular layer (excluding the horny layer that is sloughed off irregularly in the different specimens) was measured in hematoxylin-eosin stained paraffin sections. Measurements were performed with a graduated reticule placed in the eye piece, and taken perpendicular to the basement membrane at each of the selected points. Ten measurements, a total of 60 points per animal, were taken for a total every 500 µm using six blocks per animal. Similarly, the number of nucleated layers was measured at the same time at each of these 60 points per animal. Values for each animal and group were expressed as the mean ± standard deviation of the mean; two-sample, two-sided T-tests (SAS proc TTEST), and Wilcoxon tests (SAS proc NPAR1WAY) were used to compare epidermal thicknesses between treatment

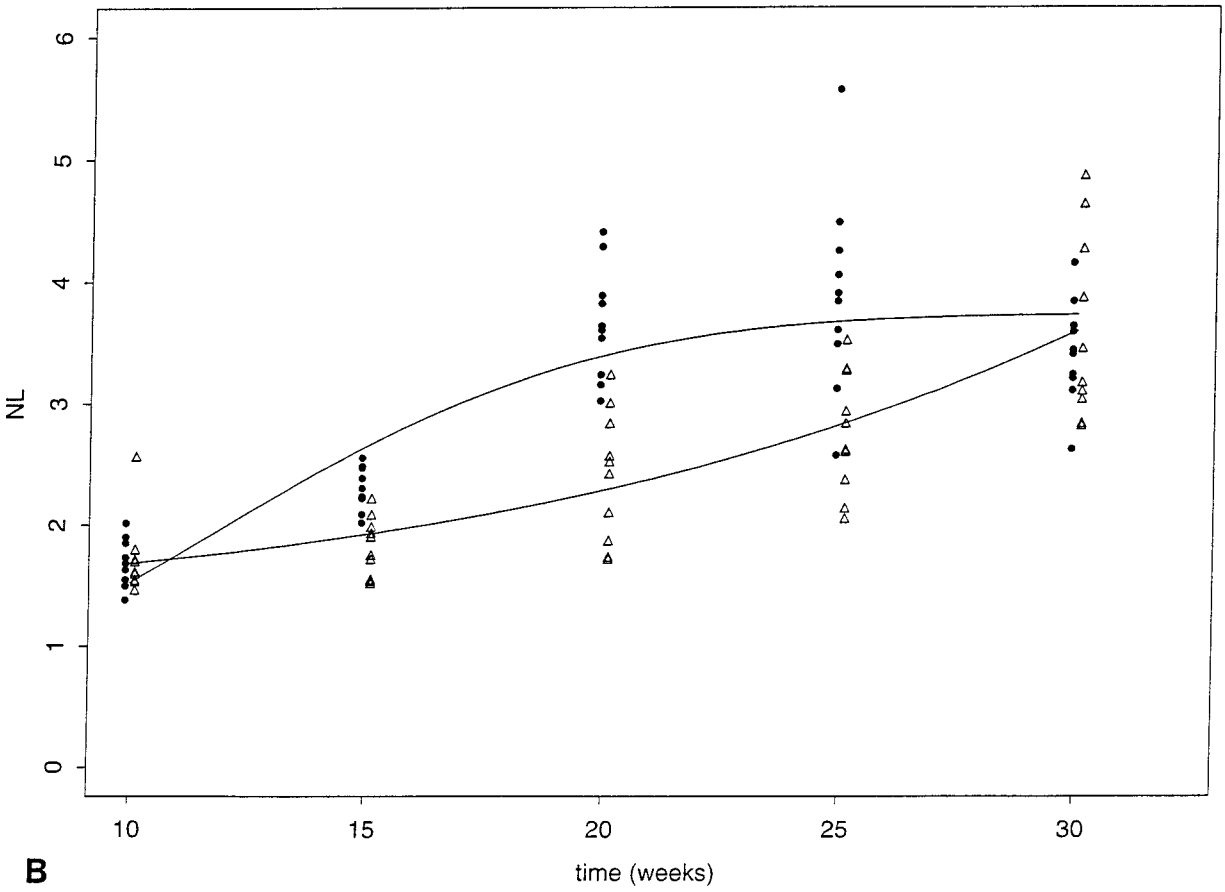

Figure 1. (Continued.)

groups or at different times within one group. All tests were conducted at the 5% level, though *P* values were usually much smaller.

RESULTS

Histopathology and Morphometry of Epidermal Hyperplasia

Histopathological evaluation of all animal groups, as well as morphometric analysis of Groups 1, 3, 4, and 5, was performed in order to determine the chemopreventive effect of DFMO on early changes produced by the carcinogen B(*a*)P, as well as to estimate the type and incidence of preneoplastic and neoplastic lesions.

In Group 3 treated with B(*a*)P alone, epidermal hyperplasia appeared in focal areas as early as 10 weeks after B(*a*)P treatment and became obvious after 15 weeks. At 20 and 25 weeks, extensive areas, no longer focal, showed marked hyperplasia. The main features of this hyperplasia were acanthosis (increased thickness of the spinous layer), hypergranulosis (increased thickness of the granular layer), and hyperkeratinization (increased thickness of the horny layer). The latter was usually orthokeratinized (no nuclei in the horny layer); occasionally, focal areas of parakeratinization (persistence of nuclei in the horny layer) were seen. This frequently coexisted with increased activity or hyperplasia of the basal layer and mild dysplasia of all cells in the underlying layers.

In addition to these changes in the interfollicular epidermis of carcinogen-treated animals, similar hyperplastic and mild dysplastic lesions were observed in the infundibular portion of the hair follicles. These changes were frequently contiguous to the equivalent layers of the adjoining interfollicular epidermis. The epidermal hyperplasia was characterized by an overall increase in epithelial thickness and in the number of nucleated layers, as evaluated using morphometric techniques. No change was

observed in the epidermis of animals in Groups 4 and 5 (DFMO alone and no treatment at all, respectively). Hyperplastic changes were also seen in animals treated with B(*a*)P and DFMO (Group 1). Nevertheless, the degree of the hyperplasia was markedly diminished, as demonstrated by histometric techniques. Table II and Figures 1A,B summarize the quantitative data.

In animals that did not receive DFMO (Group 3), the carcinogen treatment induced an increase in epidermal thickness (ET) as early as 15 weeks. At this timepoint, the ET had increased from 12 µm (Day 0) to 17 µm. At 20 weeks, it reached 22 µm (representing an 85% increase) and at 25 weeks, it measured 27 µm (representing a 130% increase). The number of nucleated layers (NL) in the epidermis also increased markedly from 1.36 in the control, to 2.32 at 15 weeks, 3.65 at 20 weeks, and 3.9 at 25 weeks, representing increases of 70, 168, and 186%, respectively, as compared to the control (Group 5). In another carcinogen-treated animal group fed ad libitum with a diet containing DFMO (1 g/kg) (Group 1), only a relatively modest increase in epidermal thickness (approximately 16%) was observed at 15 weeks, 53% at 20 weeks, and 85% at 25 weeks. The increase in the number of nucleated layers was not as marked when compared to the animals treated with carcinogen alone (32, 76, and 103% higher than the control at 15, 20, and 25 weeks, respectively). Table III shows the significance levels between Groups 1 and 2 (A) as well as within groups (B,C). Both Wilcoxon two-sample and T-tests were always in agreement for these comparisons.

TABLE III. Summary of Statistically Significant Differences in Groups 1 and 3*

	10 Week	15 Week	20 Week	25 Week
A. Comparison between Groups 1 and 3				
ET		X	X	X
NL		X	X	X
B. Comparison of ET over time within a group				
10				
15	X			
20	XY	XY		
25	XY	XY	XY	
30	XY	XY	XY	Y
C. Comparison of NL over time within a group				
10				
15	X			
20	XY	XY		
25	XY	XY	XY	
30	XY	XY	Y	Y

*X or Y indicate comparisons significant at 5% level, using both Wilcoxon and *t*-tests 2 sample tests. Numbers indicate weeks of comparison. Group 1 (X): B(*a*)P + DFMO. Group 2 (Y): B(*a*)P alone.

Histopathological Analysis of Premalignant and Malignant Lesions of the Skin

The histopathological evaluation of the skin lesions of animals treated with B(*a*)P alone (Group 3) was performed after 10, 15, 20, 25, 30, and 35 weeks. Numerous alterations, including regular epidermal hyperplasia, carcinoma in situ, papillomas, keratoacanthomas, and carcinomas, were identified. Focal lesions were not identified before week 20 of carcinogen treatment in any group. At 20 weeks, the most frequently noted focal premalignant lesions were classified as moderate dysplasias. A few severe dysplasias were also seen. Similarly, a few tumors could be detected at this timepoint. Most of these proved to be keratoacanthomas. At 25 and 30 weeks of treatment, an enormous increase was seen in the incidence of more advanced dysplastic lesions as well as invasive carcinomas (Table IV).

At 35 weeks, the last timepoint we investigated, malignant invasive lesions occupied most of the dorsal cutaneous surface. It was almost impossible to identify individual tumors since most of them had fused with each other. Thus, this latter timepoint was not considered in the present analysis.

In Group 1, we studied the effect of DFMO on B(*a*)P-induced skin carcinogenesis from the start of the experiment. A marked reduction in the number of carcinomas was observed at 25 and 30 weeks. As can be seen from Table III, DFMO reduced the tumor yield from 5.8 to 3.2 carcinomas per mouse at 25 weeks. At 30 weeks, the reduction was even more obvious from 13.1 to 5.7 carcinomas per mouse. Conversely, the incidence of pre-invasive lesions was higher in the DFMO-treated group than in the animal group that received B(*a*)P alone. This was especially true for moderate dysplasias at 25 and 30 weeks, which showed an approximate 40% increase in number.

The effect of coadministering DFMO in the diet starting at week 20 of the complete carcinogenesis protocol can be deduced from Table IV.

TABLE IV. Skin Tumors and Premalignant Lesions in B(*a*)P alone and B(*a*)P + DFMO-Treatment†

Weeks	Group	Treatment	MD	CIS + SD	PAP	KA	CA	CA + KA
15	3	B(*a*)P	0	0	0	2	0	2
	2	B(*a*)P + DFMO (20–30 weeks)	0	0	0	0	0	0
	1	B(*a*)P + DFMO (0–30 weeks)	0	0	0	1	0	1
20	3	B(*a*)P	82*	11*	1	8	7	15
	2	B(*a*)P + DFMO (20–30 weeks)	97	13	—	5	31	36**
	1	B(*a*)P + DFMO (0–30 weeks)	27*	3*	1	6	4	10
25	3	B(*a*)P	80	23	8	24	34	58*
	2	B(*a*)P + DFMO (20–30 weeks)	157**	32	9	19	18	37
	1	B(*a*)P + DFMO (0–30 weeks)	116	24	14	12	20	32*
30	3	B(*a*)P	64	41	25	34	97	131*
	2	B(*a*)P + DFMO (20–30 weeks)	77	59	1**	10	84	94**
	1	B(*a*)P + DFMO (0–30 weeks)	89	39	3	30	27	57*

†Ten animals per timepoint and group. B(*a*)P, topical application of 0.15 µmol twice weekly/DFMO 0.1% in the diet from week 1. The number of lesions represent a total for each group of ten animals. MD, moderate dysplasia; CIS + SD, carcinoma in situ + severe dysplasia; PAP, papilloma; KA, keratoacanthoma, CA, carcinoma (invasive).
**P* values 0.04 using the Wilcoxon two sample test comparing groups 1 and 3.
***P* values 0.003 using the Wilcoxon two sample test comparing groups 2 and 3.

DFMO decreased the number of tumors from 13.1 per mouse (Group 3) to 9.4 (Group 2) at 30 weeks. Similarly, the number of preneoplastic lesions was higher in the animals exposed to B(*a*)P plus chemopreventive agent (Group 1 and/or 2) as compared to B(*a*)P alone (Group 3).

Plasma Concentration of DFMO

Plasma levels of DFMO were monitored throughout the 35-week experiment. Plasma was obtained at the time of sacrifice from animals receiving DFMO alone (Group 4) or DFMO in combination with B(*a*)P (Group 1), and drug levels were quantified using HPLC methodology. The DFMO concentration of plasma from DFMO/B(*a*)P treated animals remained fairly constant over time, averaging between 4 and 11 nmol/ml of plasma. In contrast, plasma drug levels of mice receiving only DFMO increased 6-fold at 15 weeks of treatment, with drug concentrations varying significantly from animal to animal observed. Results from an independent experiment confirmed the observed peak in plasma DFMO levels at week 15.

At 20 weeks of treatment, plasma DFMO concentration correlated inversely with the number of pathologically confirmed lesions present in each animal. However, no relationship between drug concentration and tumor formation was observed in animals sacrificed at 30 and 35 weeks of treatment.

DISCUSSION

Using a protocol of complete carcinogenesis with B(*a*)P, we have observed an important chemopreventive effect of DFMO on tumor incidence after 30 weeks of treatment. This inhibition reached 57% and could be detected early using morphometric techniques, before tumors or even dysplastic lesions appeared. Measurement of two parameters (ET and NL) reflecting changes in epidermal volume were very informative.

The mean epidermal thickness had doubled at 20 weeks (as compared to Group 5 controls) and increased even further in later timepoints. The NL increased simultaneously with the ET and doubled at 20 weeks. At week 25, the value of NL was three times higher than that of the control epidermis.

Animals treated simultaneously with DFMO and B(*a*)P showed a much slower increase in ET values; i.e., at 20 weeks only a 50% increase, whereas treatment with B(*a*)P alone increased the epidermal thickness by almost 100%. Similar differences were maintained at 25 weeks. The NL also increased slowly at 20 and 25 weeks and was approximately 40% less in the DFMO-B(*a*)P-treated animals than in the group treated with B(*a*)P alone. It is clear from this morphometric analysis that both parameters are sensitive enough to be used as intermediate markers of the chemopreventive effect of DFMO in this model of chemoprevention of chemically induced skin squamous cell carcinoma.

DFMO treatment decreased the number of malignant tumors. Keratoacanthoma and papilloma incidence was also lower. Interestingly, the number of premalignant lesions was higher in this group than in the mice treated with

B(*a*)P alone. The probable mechanism of this elevation of in situ lesions after DFMO treatment could be an inhibition of tumor progression caused by the chemopreventive agent. It is probable that premalignant lesions take a longer time to progress to invasive carcinomas and consequently were found more frequently on the flat skin of the mice. Without DFMO treatment, these lesions progressed more rapidly into tumors, and, due to rapid and massive neoplastic growth, obliterated the adjacent epidermal surface that consequently cannot harbor premalignant lesions.

In animals of Group 2, DFMO, even when administered late, was able to decrease the number of tumors at 25 and 30 weeks (Table IV). Similar to Group 1, DFMO administration produced an accumulation of dysplastic lesions at 25 and 30 weeks. DFMO seems to inhibit tumor promotion and progression, inducing an early accumulation of preinvasive lesions and a final decrease of tumor incidence that is less marked if DFMO is administered late during carcinogenesis. It must be noted that in this model of skin carcinogenesis, B(*a*)P is applied throughout the experiment. A previous study by Albert et al. [2] has determined that this carcinogen has a strong promoting effect. It is probable that DFMO partially inhibits this effect in the present model and, in addition, has an inhibitory effect on the clonal evolution of progressively more malignant intraepidermal neoplastic cells [34]. It must be noted that there was no difference in body weight between the carcinogen-treated mice with and without DFMO. Nevertheless, these animals showed a decrease in body weight of approximately 25% (after 25 weeks) with respect to those mice that did not receive carcinogen treatment. Thus, it is unlikely that calorie intake played any role in the different incidence of premalignant and malignant lesions in the B(*a*)P-treated animals with and without chemopreventive agent.

Results from quantitating DFMO levels in rodent chow indicated that the differences between pellets and lots were not significant enough to account for the observed interindividual variability in drug levels. Variability in circulating drug concentrations is presumably due to differences in food consumption. Despite the limited sample size, the results from the present study suggest that the plasma concentration of DFMO present during the early stages of skin carcinogenesis may be an important determinant of chemopreventive activity.

In conclusion, these findings indicate the ability of DFMO to reduce carcinoma incidence in the mouse skin complete carcinogenesis model. Corresponding morphometric data suggests that ET and NL can be used as early biomarkers of the chemopreventive effect of DFMO. Measuring these epidermal parameters as early as 15 weeks can give an excellent predictive value of the chemopreventive effect of DFMO on skin tumorigenesis, since inhibition of tumorigenesis only becomes obvious after 10–15 weeks. The use of morphometric intermediate markers as endpoints of chemopreventive activity in this preclinical model provides a sensitive assay for evaluating the protective potential of other promising chemopreventive agents.

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