STUDIES ON THE MECHANISMS OF ACTION OF MODIFIERS OF SKIN CARCINOGENESIS[1]

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[3] Abbreviations used: BP, benzo(a)pyrene; DMBA, 7,12-dimethylbenzo(a)anthracene; TPA, 12-0-tetradecanoylphorbol-13-acetate; PAH, polycyclic aromatic hydrocarbons; MC, 3-methylcholanthrene; 2-0HBP, 2-hydroxybenzo(a)pyrene; 7 BrMe-12 Me BA, 7-bromomethyl-12-methylbenz(a)anthracene; BP-7,8-oxide, benzo(a)pyrene 7,8-oxide; DB(a,h)A, dibenz(a,h)anthracene; BA, benz(a)anthracene; DB(a,c)A, dibenz(a,c)anthracene; BP 4,5-oxide, benzo(a)pyrene 4,5-oxide; BP-7,8-diol-9,10-epoxide, (±)-7β, 8α-dihydroxy-9α-10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; BA-3,4-diol-1,2-epoxide, (±)-3α, 4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz(a)anthracene; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; PCB, polychlorobiphenyls; Poly I:C, polyinosinic: polycytidylic acid; ODC, ornithine decarboxylase; B(e)P, benzo(e)pyrene; 7,8-BF, 7,8-benzoflavone; DMSO, dimethylsulfoxide; BCG, Bacillus Calmette-Guerin; EPP, ethylphenylpropiolate; TLCK, toxyl lysine chloromethyl ketone; TAME, tosyl arginine methyl ester; TPCK, tosyl phenylalanine chloromethyl ketone.

ABSTRACT

Skin tumors can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation phase), followed by repetitive treatment with a weak or non-carcinogenic tumor promoter. There is a very good dose-response relationship between the induction of the number of papillomas per mouse at early times (10 to 20 weeks) by either tumor initiators and promoters and the final carcinoma incidence after a longer latency (20 to 50 weeks) in SENCAR mice. This system not only can be used to determine the tumor initiating and promoting activities of a compound but if the agent is given repeatedly by itself one can also determine if it is a complete carcinogen, i.e., if it has both tumor initiating and promoting activity. With the exception of a few pure tumor initiators there is in general a good qualitative and quantitative correlation between the ability of a polycyclic aromatic hydrocarbon (PAH) to act as a complete carcinogen and to act as a tumor initiator in mouse skin. In addition, if the agent is given concurrently with a known complete carcinogen or a tumor initiator one can also determine if the agent has co-carcinogenic or co-tumor initiating activity or even possibly anticarcinogenic activity. Likewise, if the agent is given concurrently with a known tumor promoter one can determine if the agent has co-promoting or anti-promoting activity.

There is a good correlation between the tumor-initiating activities of PAH and their abilities to bind covalently to DNA. In addition, various inhibitors of PAH tumor initiation show a strong correlation with their abilities to inhibit the binding of the PAH to DNA and their anti-tumor initiating activities. There is also a good correlation between the promoting abilities of phorbil esters to promote tumors and their abilities to

induce ornithine decarboxylase (ODC), cell proliferation and dark basal keratinocytes. However, when other nonpromoting hyperplastic agents are used only dark cell induction correlates. Certain polyamines and prostaglandins can enhance phorbol ester tumor promotion. Anti-inflammatory steroids, retinoids and protease inhibitors are potent inhibitors of tumor promotion. They inhibit tumor promotion by either inhibiting the 12-0-tetradecanoylphorbol-13-acetate (TPA) induced cell proliferation, ODC and/or dark basal keratinocytes. Certain weak promoters such as mezerein which mimics TPA in many biochemical and morphological effects are potent second step promoters in a two-stage promotion regimen.

INTRODUCTION

Current information suggests that chemical carcinogenesis is a multistep process with one of the best studied models in this regard being the two-stage carcinogenesis system using mouse skin. Table 1 summarizes some aspects of carcinogenesis in experimental animals and in man. T-1 Skin tumors can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation phase) followed by repetitive treatment with a noncarcinogenic tumor promoter. The initiation phase requires only a single application of either a direct or indirect carcinogen at a subthreshold dose and is essentially irreversible, while the promotion phase is brought about by repetitive treatments after initiation and is initially reversible, later becoming irreversible. This system not only can be used to determine the tumor initiating and promoting activities of a compound but if the agent is given repeatedly by itself one can also determine if it is a complete carcinogen, i.e., if it has both tumor initiating and promoting

activity. In addition, if the agent is given concurrently with a known complete carcinogen or a tumor initiator one can also determine if the agent has co-carcinogenic or co-tumor initiating activity or even possibly anti-carcinogenic activity. Likewise, if the agent is given concurrently with a known tumor promoter one can determine if the agent has co-promoting or anti-promoting activity. Furthermore, like most carcinogenesis systems, skin carcinogens may have additive or synergistic effects. This system has provided an important model for not only studying carcinogenesis and for bioassaying carcinogenic agents but also for the study of modifiers of carcinogenesis. Recently, the generality of the two-stage system or multistage carcinogenesis has been shown to exist in a number of systems besides the skin such as the liver, bladder, colon, esophagus, stomach, mammary, diaplacental as well as cells in culture (1).

Whenever a known skin carcinogen has been appropriately tested, it has shown skin tumor initiating activity (2-16). In a two-stage mouse skin system, initiation is the only stage that requires the presence of the carcinogen, and the measured carcinogenic potency of a chemical reflects its capacity for tumor initiation. The results in Table 2 show that there is both a good qualitative and quantitative correlation between T-2
the complete carcinogenic and tumor initiating activities of several chemical carcinogens in mouse skin. This is true when one considers the number of papillomas per mouse at early times (10 to 20 weeks) or the final carcinoma incidence after tumor initiation.

It is possible that a carcinogen lacking promoting ability would not be detected when tested as a complete carcinogen. In this regard, however, we have found a number of chemical compounds that have tumor initiating activity but either lack or

have very weak complete carcinogenic activity (4,9-11,14). These "pure" skin tumor initiators are listed in Table 3. T-3

There is a good dose-response relationship of many carcinogens used as tumor initiators in the two-stage carcinogenesis system using SENCAR mice. This is illustrated in Table 4. A good dose response relationship exists for 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BP) to initiate skin tumors in SENCAR mice. As can be seen a good correlation exists between the number of papillomas per mouse at 15 weeks and the final carcinoma incidence at 50 weeks. The percent of mice with papillomas has also a reasonable correlation but the dose response is very narrow. T-4

The dose-response ability of 12-0-tetradecanoylphorbol-13-acetate (TPA) to promote tumors after DMBA initiation is shown in Table 5. As was the case for tumor initiation, there is also a very good dose-response relationship for tumor promotion when considering either the number of papillomas per mouse at 15 weeks or the percent of mice with squamous cell carcinomas at 50 weeks. Similar results have been reported using Charles River CD-1 mice (5, 8) or ICR/Ha Swiss mice (17, 18). T-5

The SENCAR mouse was derived from crossing Charles River CD-1 mice with skin tumor sensitive mice (originally derived from Rockland mice) and selecting for sensitivity to DMBA-TPA two-state carcinogenesis for 8 generations starting with the F_1 cross (2). The mice developing the earliest and most papillomas after DMBA-TPA treatment were selected for each breeding. The SENCAR mice are between 10 and 20 times more sensitive to DMBA tumor initiation than the CD-1 mice, whereas the SENCAR mice are only between 3 and 5 times more sensitive to BP tumor initiation than the CD-1 mice (unpublished results).

Tumor Initiation

The tumor initiation phase appears to be an irreversible step which probably involves a somatic cell mutation as evidenced by a good correlation between the carcinogenicity of many chemical carcinogens and their mutagenic activities (19, 20). Most tumor initiating agents either generate or are metabolically converted to electrophilic reactants, which bind covalently to cellular DNA and other macromolecules (21). The Millers have proposed a significant general theory to explain the initial event in chemical carcinogenesis which states that all chemical carcinogens that are not electrophilic reactants must be converted metabolically into a chemically reactive electrophilic form which then reacts with some critical macromolecule to initiate the carcinogenic process (21).

Previous studies have demonstrated a good correlation between the carcinogenicity of several polycyclic aromatic hydrocarbons (PAHs) and their ability to bind covalently to DNA (22, 23). Table 6 summarizes our data which shows the strong correlation between the covalent binding of PAH to DNA and their tumor initiating activities. T-6

In order to help us better understand the mechanism of PAH carcinogenesis, we have been studying many compounds with the capacity to inhibit PAH tumor initiation. Table 7 summarizes various potent inhibitors of skin tumor initiation in mice. In most of our studies we have used PAH carcinogens which must be metabolized by the mixed-function oxidases to active form(s) before they are carcinogenic. Some of the flavones and antioxidants appear to inhibit carcinogenesis by inhibiting the metabolism of the carcinogen to its ultimate carcinogenic form (7, 24-27). 5, 6-Benzoflavone and T-7

quercetin have been found to be inhibitory to skin, lung and mammary carcinogenesis whereas 7,8-benzoflavone inhibits skin carcinogenesis by some polycyclic hydrocarbons and enhances carcinogenesis by others (7, 27, 28). The antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are widely used as food preservatives and have been shown to inhibit skin, lung, mammary, forestomach, colon and liver cancer in experimental animals induced by a wide range of chemicals (27). Similar inhibitory results have been noted for selenium and vitamins C and E (27). The noncarcinogenic polycyclic hydrocarbons and the environmental contaminants appear to inhibit skin carcinogenesis by inducing the metabolism of the carcinogen to detoxified products, thereby decreasing the binding of the PAH to DNA (29-33). This is epitomized by the environmental contaminants 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorobiphenyls (PCB) which are extremely potent inducers of polycyclic hydrocarbon carcinogen metabolism and potent inhibitors of their carcinogen effect (32-34). Although TCDD is one of the most toxic agents known, its inhibitory effect on polycyclic hydrocarbon carcinogenesis is at nontoxic dose levels.

Sulfur mustard inhibits tumor initiation by actually killing the initiated cells (35). The polyinosinic:polycytidylic acid (Poly I:C) and the anti-inflammatory steroids appear to inhibit tumor initiation by slowing down carcinogen metabolism by their anti-growth effect (36, 37). Some of the agents listed in Table 7 have been shown to inhibit carcinogenesis in a number of tissues and by a variety of chemical carcinogens indicating they may be useful agents in the chemoprevention of cancer in man (28). In general, the inhibitors of skin tumor initiation shown in Table 7 inhibit by either 1) alteration of the metabolism of the carcinogen (decreased activation and/or increased detoxification),

2) scavenging of active molecular species of carcinogens to prevent their reaching the critical target site(s) in the cells, or 3) competitive inhibition. In all cases this leads to a decrease in covalent binding to critical targets such as DNA. Table 8 reveals a good correlation between the ability of a number of compounds to inhibit tumorigenesis and their ability to inhibit the binding of the PAH to DNA. T-8

Tumor Promotion

In addition to causing inflammation and epidermal hyperplasia, the phorbol ester tumor promoters have been shown to have several other morphological and biochemical effects on the skin. These responses to phorbol ester tumor promoters are summarized in Table 9. Of all the observed phorbol ester related effects on the skin, the induction of epidermal cell proliferation, ornithine decarboxylase (ODC) and dark basak keratinocytes appear to correlate the best (38-41, 46). T-9

It is difficult to determine which of the many phorbol ester tumor promoter related responses are essential components of the promotion process. There is a good correlation between the promoting abilities of a series of phorbol esters and their ability to stimulate epidermal hyperplasia (38); however, the correlation fails if one looks at nonphorbol ester hyperplastic agents (54). Later O'Brien et al. (46) reported an excellent correlation between the tumor promoting ability of various compounds (phorbol esters as well as nonphorbol ester compounds) and their ability to induce ODC activity in mouse skin. However, mezerein a diterpene similar to TPA but with weak promoting activity was found to induce ODC comparable to that of TPA (55). Raick found that phorbol ester tumor promoters induced the appearance of "dark basal cells" in the epidermis, whereas ethylphenylpropiolate, a non-promoting epidermal hyperplasic agent, did not (39, 56). In addition, wounding induced a few dark cells but seemed to

correlate with its ability to be a weak promoter (40, 41, 56). In addition, a large number of these dark cells are found in papillomas and carcinomas (40, 41). Slaga et al. reported that TPA induced about 3 to 5 times the number of dark cells as mezerein which was the first major difference found between these compounds.

Various modifiers of the tumor promotion process have been very useful in our understanding of the mechanism(s) of tumor promotion. Table 10 summarizes the T-10
potent inhibitors of skin tumor promotion in mice by phorbol ester tumor promoters. The anti-inflammatory steroid, fluocinolone acetonide, was found to be an extremely potent inhibitor of phorbol ester tumor promotion in mouse skin (58). Repeated applications of as little as 0.01 ug almost completely counteract the skin tumorigenesis. Fluocinolone acetonide also effectively counteracts the tumor promoter induced cellular proliferation. Certain retinoids have also been found to be potent inhibitors of mouse skin tumor promotion (59). In addition, Sporn and coworkers have found that certain retinoids are potent inhibitors of lung, mammary, bladder and colon carcinogenesis (60). Verma and coworkers (59) have shown that certain retinoids are potent inhibitors of phorbol ester induced epidermal ODC activity. This plus their effect on epithelial differentiation appears to be related to their anti-carcinogenic effect. We have recently found that a combination of fluocinolone acetonide and retinoids produces an inhibitory effect on skin tumor promotion greater than that produced by each separately (61). Troll and Belman (62) have found that protease inhibitors, cyclic nucleotides, dimethylsulfoxide (DMSO) and butyrate also inhibit mouse skin tumor promotion by phorbol esters. Schinitsky and coworkers (63) reported the inhibitory effect of Bacillus Calmette-Guerin (BCG) vaccination on skin tumor promotion.

Multistage Promotion

Because of the many similarities in morphological and biochemical responses induced by TPA and mezerein, we felt that mezerein, although a weak promoter, would be a good candidate as a compound to be used in the second step of a two-step promotion protocol as originally reported by Boutwell (2). His results showed that promotion could be divided into two steps, conversion and propagation (2). After DMBA initiation, the conversion step was accomplished by a limited number of croton oil treatments which, with no further treatment, only produced a few tumors and the propagation step was accomplished by repeated treatment with turpentine, a non-promoting hyperplasic agent (2). The three step protocol (initiation-conversion-propagation) produced a significant tumor response but less than that observed when croton oil was given for the complete promotion step (2). However, recent results suggest that nonpromoting hyperplastic agents such as turpentine, ethylphenylpropiolate (EPP) and acetic acid when given repetitively after a few treatments with TPA were not able to complete the promotion process as reported by Boutwell (40, 54, 56). In fact, Raick reported that turpentine and EPP gave fewer tumors in a three-stage system than when DMBA was only followed by the limited TPA treatment (40, 56). Similar results were reported by Slaga et al. (54) using acetic acid as a second step promoter.

Our results on the use of mezerein as a second stage promoter are shown in Table 11. As illustrated TPA is about 50 times more active as a promoter than mezerein. When 2 ug of TPA are given twice weekly for only 2 weeks after DMBA initiation, very few tumors are induced compared to twice weekly treatments for 12 weeks. However, when mezerein is given at a dose of either 1, 2 or 4 ug twice weekly

T-11

after the limited TPA treatment, it induced a significant tumor response in a dose-dependent manner. The ability of mezerein to act as a potent second stage promoter was repeated in six separate experiments (57, 64). Also, shown in Table 11, is the ineffectiveness of EPP as a complete promoter and as a second stage promoter. As shown in Table 12, we have recently found that the protease inhibitor tosyl phenylalanine chloromethyl ketone (TPCK) can selectively inhibit step one of promotion, fluocinolone acetonide can effectively inhibit both step one and two of the promotion whereas retinoic acid specifically inhibits step two (64). T-12

Since the only major morphological or biochemical difference between the effects of TPA and mezerein on the skin is the ability of TPA to induce a large number of dark basal keratinocytes (40, 41), we were interested in determining the effects of various inhibitors of promotion on the appearance of these dark cells. We reasoned that if these dark cells are critical in the first stage of promotion and if FA and TPCK are potent inhibitors of stage I and RA of stage II, then FA and TPCK should counteract the appearance of these cells, whereas RA should not. This was, in fact, what we found (64). As hypothesized, FA and TPCK were found to effectively counteract the appearance of the dark cells induced by TPA, whereas RA had no effect.

Since TPCK was found to be a potent inhibitor of stage I of promotion but not of stage II and since TPCK counteracted the TPA-induced increase in the dark basal keratinocytes, we were interested in determining the effect of TPCK on TPA-induced ODC activity. Slaga and coworkers reported (64) that TPCK had very little effect on TPA- and mezerein-induced epidermal ODC activity; this was even evident at a relatively high TPCK dose level (20 ug).

The anti-inflammatory steroid, FA, not only counteracted the appearance of dark cells induced by TPA but also suppressed the hyperplasia induced by TPA. In fact, the skins from FA plus TPA treated mice appeared as untreated skin. This is in agreement with our previously reported observations on the inhibitory effect of FA on TPA induced inflammation, hyperplasia and DNA synthesis (58). TPCK appeared to have only a slight inhibitory effect on TPA induced inflammation and hyperplasia (64).

It is also of interest to point out that although RA inhibited Stage II of promotion, it had no inhibitory effect on the TPA or mezerein induced hyperplasia. However, certain retinoids have been found to be potent inhibitors of TPA and mezerein induced epidermal ODC activity (59). This data suggest that the induction of epidermal ODC activity followed by increased polyamines may be important in stage II of promotion. In this regard FA and TPCK have either no effect or only a slight inhibitory effect on TPA or mezerein induced ODC activity (64,65). FA does, however, significantly decrease the TPA induced spermidine levels in the epidermis (65). This plus FA's inhibitory effect on TPA induced hyperplasia may be responsible for its inhibitory effect on stage II promotion.

The data presented from this laboratory suggest that at least two stages are important in the promotion process, both of which can obviously be produced by repeated TPA treatment after tumor initiation. We believe that one of the important events in the first stage involves the induction of dark basal keratinocytes. The fact that mezerein is a potent second stage promoter, but only a weak complete promoter with much less ability to induce dark cells than TPA and that FA and TPCK inhibit stage I of promotion and inhibit the induction of dark cells by TPA whereas RA does

not, suggest that the dark cells are important in stage I of promotion (Figure 1). These dark basal keratinocytes are slightly increased by wounding which correlates with its weak promoting ability but not by EPP, which is a very weakly hyperplastic agent (39-41, 56). In addition, a large number of these dark cells are found in papillomas and carcinomas (40, 41). These dark cells may be primitive stem cells since we have found (unpublished results), as have others (40, 41), that they normally occur in large numbers in embryonic and newborn skin but are only present in very small numbers in adult skin. As stated earlier we feel that the induction of ODC activity followed by increased polyamines and increased cellular proliferation are important events in stage II of promotion. Figure 1 depicts the various stages, the important events in each stage and where the various inhibitors are effective. By seeking to divide the carcinogenic process into as many natural stages as possible and finding specific inhibitors of each stage, we will have a greater opportunity of understanding the important events in carcinogenesis as well as possibly securing a rational and effective basis for the prevention of cancer.

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TABLE 1

CARCINOGENESIS IN MAN AND EXPERIMENTAL ANIMALS

1. Complete Carcinogenesis
2. Cocarcinogenesis
3. Tumor Initiation
4. Tumor Promotion
5. Additive and Synergistic Effects of Carcinogens, Tumor Initiators and Tumor Promoters
6. Co-initiating and Co-promoting Agents
7. Anti-carcinogenesis
8. Anti-initiating and Anti-promoting Agents

TABLE 2

Comparison of complete carcinogenesis and tumor initiation in mouse skin[a]

Compound	Relative potency[b]	
	Complete Carcinogenesis (Carcinomas)	Tumor Initiation (Papillomas)
DMBA	100	100
MC	50	50
BP	30	30
2-OHBP	30	30
7BrMe-12MeBA	20	20
BP-7,8-oxide	20	20
DB(a,h)A	20	20
BA	5 ± 5	5
DB(a,c)A	0	3
pyrene	0	0
BP 4,5-oxide	0	0
Anthracene	0	0

[a]This is a summary of over 100 compounds which shows that an excellent qualitative and quantative correlation exists between complete carcinogenesis and tumor initiation in mouse skin.

[b]Relative potency was determined from dose-response data. DMBA was given a maximum value of 100.

TABLE 3

Agents that are possibly pure tumor initiators

Skin Tumor Initiators	Relative[a] Potency
BP-7,8-diol-9,10-epoxide	25
MNNG	15
BA-3,4-diol-1,2-dpoxide	2
BA	0.5
DB(a,c)A	0.2
Chrysene	0.1
Urethan	0.1

[a]Relative potency was determined from dose-response data. DMBA was given a maximum value of 100.

TABLE 4

DOSE RESPONSE STUDIES ON THE ABILITY OF DMBA AND BP TO INITIATE SKIN TUMORS IN SENCAR MICE [a]

The mice were treated one week after initiation with twice weekly applications of 5 ug of TPA

Initiator	Dose (nmoles)	# of papillomas per mouse at 15 weeks	% of mice with papillomas at 15 weeks	% of mice with carcinomas at 50 weeks
DMBA	100	22	100	100
DMBA	10	6.8	100	40
DMBA	1	3.2	93	22
DMBA	0.1	0.5	20	5
BP	200	7.5	100	55
BP	100	3.2	78	30
BP	50	1.4	60	18

[a] Data shown in this table is from T. J. Slaga, L. L. Triplett and S. Nesnow, "Comparison of complete carcinogenesis and tumor initiation in mouse skin". Submitted for publication.

Table 5

Dose-response studies on the ability of TPA to promote tumors after DMBA initiation

The mice were initiated with 10 nmoles of DMBA and promoted one week later with various dose levels of TPA

Promoter	dose (µg)	Time to first papilloma (wks)	# of papillomas per mouse at 15 weeks	with papillomas at 15 weeks	with carcinomas at 50 weeks
TPA	10	8	3.0	100	32
TPA	5	6	7.2	100	46
TPA	2	7	6.5	100	45
TPA	1	8	3.6	80	25
TPA	0.1	11	0.4	5	8

TABLE 6

Correlation of polycyclic aromatic hydrocarbons (PAHs) abilities to covalently bind to epidermal DNA with their tumor initiating activities[a]

PAHs	Relative ability to covalently bind to epidermal DNA[b]	Relative tumor initiating activity[c]
DMBA	10.0	10.0
MC	6.5	6.0
BP	3.3	2.0
DB(a,h)A	1.7	1.5
DB(a,c)A	0.8	0.2

[a]DMBA was given a value of 10 since it gave the maximum response in binding, ability to inhibit epidermal DNA synthesis and to initiate tumors in a two-stage system of tumorigenesis. All the other PAHs are expressed as values relative to DMBA's response.

[b]The relative abilities of various PAHs to covalently bind to epidermal DNA are based on dose-response binding studies. See references 7, 23 and 29 for details of actual binding levels.

[c]The relative tumor initiating activities are based on dose-response studies in Charles River CD-1 mice. See references 7, 8 and 29 for details.

TABLE 7

INHIBITORS OF TUMOR INITIATION

1. Antioxidants: butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and selenium
2. Flavones: 7,8-benzoflavone, 5,6-benzoflavone and quercetin
3. Vitamins: A, C and E
4. Certain noncarcinogenic polycyclic aromatic hydrocarbons: dibenz(a,c)anthracene, benz(a)anthracene, benzo(a)pyrene and pyrene
5. Environmental contaminants: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorobiphenyls (PCB)
6. Sulfur mustard
7. Polyriboinosinic-polyribocytidylic acid (Poly I;C)
8. Anti-inflammatory steroid

TABLE 8

Correlation of various compounds to inhibit tumor initiation by DMBA with their abilities to inhibit covalent binding of DMBA to epidermal DNA[a]

Inhibitors	Relative ability to inhibit DMBA tumor initiation by at least 50%	Relative ability to inhibit DMBA binding to by at least 50%
TCDD	100.0	100.0
DB(a,c)A	10.0	15.0
7,8-BF	5.0	8.0
B(e)P	5.0	3.0
BHA	0.2	0.1
BHT	0.1	0.1
Vitamin C	0.1	0.1

[a]TCDD was given a value of 100 since it gave the greatest inhibition of tumor initiation and DMBA binding to epidermal DNA. For example, TCDD at a 1 µg dose level almost completely inhibited DMBA tumorigenesis and DMBA binding to DNA. All the other compounds are expressed as values relative to TCDD's response. For example, BHA at a 1000 µg dose level inhibited DMBA tumor initiation and binding by at least 50%. See references 24-26, 29-34 for details.

TABLE 9

MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF MOUSE SKIN TO PHORBOL ESTER TUMOR PROMOTERS

Responses	References
Induction of inflammation and hyperplasia	2,5
Induction of dark cells	39-41
Induction of morphological changes in adult skin resembling papillomas and carcinoma cells	39-41
An initial increase in keratinization followed by a decrease	39
Increase in DNA, RNA and protein synthesis	42
Increase in phospholipid synthesis	43
Increase in histone synthesis and phosphorylation	44,45
Increase in ornithine decarboxylase activity followed by increase in polyamines	46
Decrease in histidase activity	47
Induction of embryonic proteins in adult skin	48
Increase in protease activity	49
Decrease in the isoproterenol stimulation of cAMP	50
Decrease response of G_1 chalone in adult skin	51
Increase in protein kinase activity[a]	
Increase in prostaglandin synthesis	52,53

[a]M. Mamrack, S. M. Fischer and T. J. Slaga (manuscript in preparation).

TABLE 10

INHIBITORS OF PHORBOL ESTER SKIN TUMOR PROMOTION

1. Anti-inflammatory steroids: cortisol, dexamethasone and fluocinolone acetonide
2. Vitamin A derivatives
3. Combination of retinoids and anti-inflammatory agents
4. Protease inhibitors: Tosyl lysine chloromethyl ketone, (TLCK); Tosyl arginine methyl ester, (TAME); Tosyl phenylalanine chloromethyl ketone, (TPCK); antipain and leupeptin
5. Cyclic nucleotides
6. Dimethylsulfoxide (DMSO)
7. Butyrate
8. Bacillus Calmette-Guerin (BCG)

TABLE 11

TWO-STAGE TUMOR PROMOTION AFTER DMBA INITIATION

The mice were initiated with 10 nmole of DMBA and followed one week later by twice weekly applications of 2 ug of TPA for 2 weeks. Starting on the third week of promotion the mice received either twice weekly applications of various dose levels of mezerein, EPP or only acetone.[a]

Exp. No.	Treatment Protocol (21 weeks)	Tumor Response	
		pap/mouse	% of mice with tumors
1.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }20\text{ wks}}$	8.2	100
2.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ acetone $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0	0
3.	DMBA $\xrightarrow{1\text{ wk}}$ mezerein 2 ug $\xrightarrow{2X/wk\text{ for }20\text{ wks}}$	0	0 0
4.	DMBA $\xrightarrow{1\text{ wk}}$ mezerein 4 ug $\xrightarrow{2X/wk\text{ for }20\text{ wks}}$	0.2	18
5.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein 1 ug $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	2.1	60
6.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein 2 ug $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	4.0	90
7.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein 4 ug $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	7.1	100
8.	DMBA $\xrightarrow{1\text{ wk}}$ acetone $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein 4 ug $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0.1	10
9.	DMBA $\xrightarrow{1\text{ wk}}$ EPP (14 mg) $\xrightarrow{2X/wk\text{ for }20\text{ wks}}$	0.1	10
10.	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ EPP (14 mg) $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0.2	12

[a] 96% or greater of the mice were alive at the end of the experimental period. The maximum percent standard deviation for the experiments was 16%.

TABLE 12

THE EFFECTS OF RA, FA AND TPCK ON TWO-STAGE PROMOTION AFTER DMBA INITIATION

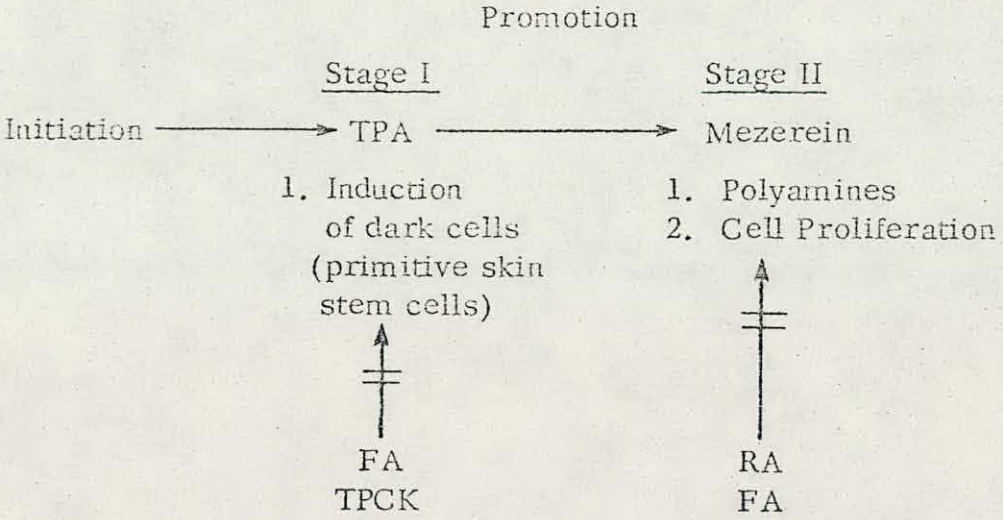
The mice were initiated with 10 nmoles of DMBA and followed one week later by twice weekly applications of 2 ug of TPA for 2 weeks (stage I). Starting on the third week of promotion the mice received twice weekly applications of 2 ug of mezerein (stage II). In some experiments FA, RA or TPCK were given simultaneously with either stage I or II.[a]

Exp. No.	Treatment Protocol 21 weeks	Tumor Response # of papillomas per mouse	Tumor Response % of mice w/tumors
1	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$	0	0
2	DMBA $\xrightarrow{1\text{ wk}}$ acetone $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0	0
3	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	4.2	92
4	DMBA $\xrightarrow{1\text{ wk}}$ TPA + FA(1 ug) $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0	0
5	DMBA $\xrightarrow{1\text{ wk}}$ TPA + FA (0.1 ug) $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0.4	26
6	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein + FA (1 ug) $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0.8	35
7	DMBA $\xrightarrow{1\text{ wk}}$ TPA + RA (10 ug) $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	4.0	88
8	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein + RA (10 ug) $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	0.8	34
9	DMBA $\xrightarrow{1\text{ wk}}$ TPA + TPCK (10 ug) $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	1.0	40
10	DMBA $\xrightarrow{1\text{ wk}}$ TPA $\xrightarrow{2X/wk\text{ for }2\text{ wks}}$ mezerein + TPCK (10 ug) $\xrightarrow{2X/wk\text{ for }18\text{ wks}}$	3.8	87

[a] 98% or greater of the mice were alive at the end of the experimental period. The maximum percent standard deviation for the experiments was 14%.

Figure 1 - A diagram of the various stages of skin carcinogenesis showing the important events in stage I and II of promotion and where FA, RA and TPCK inhibit promotion.

FIGURE 1


Promotion
Stage I
Stage II
Initiation
TPA
Mezerein
1. Induction of dark cells (primitive skin stem cells)
1. Polyamines
2. Cell Proliferation
FA
TPCK
RA
FA