ABSTRACT

The characteristics of the skin tumor promotion response with anthrone derivatives has been further examined in SENCAR mice. Chrysarobin, (1,8-dihydroxy-3-methyl-9-anthrone) was an effective skin tumor promoter when applied twice weekly with dose-dependent increases in both papillomas and squamous cell carcinomas between 25 and 100 nmol/mouse. A similar dose-response relationship for papilloma and carcinoma formation was observed when chrysarobin was applied once weekly. Interestingly, chrysarobin was approximately twice as active as a skin tumor promoter when applied once weekly versus twice weekly. Doses of 25, 100, and 220 nmol/mouse gave maximal papilloma responses of 2.90, 8.15, and 9.38 versus 0.73, 4.70, and 5.42 papillomas/mouse, respectively, in mice initiated with 25 nmol 7,12-dimethylbenz(a)anthracene. Thus, unlike 12-O-tetradecanoylphorbol-13-acetate (TPA), where a twice weekly application frequency is optimal, application of anthrone promoters such as chrysarobin once weekly is a more optimal frequency for papilloma development. Chrysarobin was also a much more effective skin tumor promoter when the start of promotion was delayed by an additional 10 weeks. Thus, groups of mice initiated with 10 nmol 7,12-dimethylbenz(a)anthracene and having promotion started in either the 3rd or the 13th week after initiation had maximal responses of 5.6 or 11.0 papillomas/mouse, respectively. In addition, the rate of papilloma development was faster in the delayed promotion group.

The progression of papillomas to carcinomas was examined in all chrysarobin-treated groups and compared with three groups of mice treated with 3.4 nmol TPA. After 60 weeks of promotion, the anthrone promoter-treated groups had carcinomapapilloma ratios 2.5 to 5.0 times higher than the TPA-treated groups. This was due primarily to the fact that similar carcinoma responses were observed in both anthrone- and TPA-treated mice at optimal promoting doses whereas the papilloma responses were significantly lower in the former groups. The data suggest that anthrone derivatives are very efficient tumor promoters. The results are further discussed in terms of mechanisms of skin tumor promotion.

INTRODUCTION

Skin tumor promotion and the phenomenon of tumor promotion in general have been studied extensively using the phorbol esters such as TPA1 (1, 2). The promotion response with TPA is characterized by the rapid production of a large number of papillomas followed by a relatively low incidence of squamous cell carcinomas on the backs of previously initiated mice (1, 3). The promoting action of the phorbol esters appears to be mediated in part by their interaction with protein kinase C (reviewed in Refs. 4 and 5). Many other classes of chemical agents are known to possess skin tumor-promoting activity (1, 2, 6). A number of these, such as the indole alkaloids (e.g., teleocidins) and polyacetates (e.g., aplysiatoxin), appear to work through the same or similar receptor mechanism as the phorbol esters (4–6), while others, such as anthralin, iodoacet acid, and benzoyl peroxide, do not (4). Few studies have fully characterized the promotion responses of these non-phorbol ester type tumor promoters.

Work in our laboratory has focused on a class of non-phorbol ester skin tumor promoters the basic structure of which is derived from anthrone (7, 8). These anthrone skin tumor promoters, of which anthralin and chrysarobin have been used as prototypes, are effective promoters of papilloma formation in previously initiated mouse skin (7–10). Several lines of evidence suggested that these compounds might be useful tools for studying the mechanism(s) of skin tumor promotion: (a) as noted above, anthralin (reviewed in Ref. 4) and chrysarobin (11, 12) do not compete for the phorbol ester receptor on mouse epidermal cells or in mouse epidermal particulate fractions; (b) at optimal promoting doses, the time course and magnitude of induction of epidermal ODC by chrysarobin is markedly different from that of TPA (8, 13); (c) we previously reported preliminary data showing that the carcinoma:papilloma ratio in mice promoted with chrysarobin was higher than in mice treated with optimal papilloma-promoting doses of TPA (8). Although fewer papillomas were present at optimal promoting doses of chrysarobin compared with TPA in the previous study (8), a similar number progressed to carcinomas in both groups, suggesting that this compound might be more efficient for selecting preneoplastic lesions.

In the present investigation we provide an in-depth analysis of the ability of chrysarobin to promote papilloma and carcinoma development in SENCAR mice. Through dose-response, altered treatment frequency, and delayed application experiments, we demonstrate that chrysarobin is an efficient skin tumor promoter in SENCAR mice especially when given once weekly or when the start of promotion is delayed for 10 weeks. Furthermore, while the carcinoma response was generally similar, the carcinomapapilloma ratio was 2.5 to 5.0 times higher in chrysarobin-treated mice than in TPA-treated mice. Possible mechanisms for the higher carcinoma:papilloma ratio and for the skin tumor-promoting actions of anthrones are discussed.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from Eastman Kodak Co. (Rochester, NY). Chrysarobin was obtained from ICN Pharmaceuticals, Inc. (K and K Laboratories Division, Plainview, NY), and purified by column chromatography as described previously (7). TPA was supplied by Chemicals for Cancer Research (Eden Prairie, MN).

Animals. Female SENCAR mice were obtained from the National Cancer Institute, Frederick, MD, or Research Biogenics, Inc., Bastrop, TX, and when 7 to 9 weeks of age were shaved on the dorsal side. Mice were allowed to stabilize for at least 2 days and only those mice in the resting phase of the hair growth cycle were subsequently used. All chemicals were applied topically to the shaved area in 0.2 ml acetone and control animals were treated with an equal volume of acetone. In the delayed promotion experiment, animals received twice weekly applications of acetone (0.2 ml) during Weeks 3 through 12 after initiation.

Tumor Induction Experiments. Each experimental group contained

1 Received 12/5/86; revised 3/25/87; accepted 4/2/87.

2 To whom requests for reprints should be addressed, at The University of Texas System Cancer Center, Science Park–Research Division, P. O. Box 389, Smithville, TX 78957.

3 The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz(a)anthracene; ODC, ornithine decarboxylase (EC 4.1.1.17).

3783
30 preshaved mice. Mice were initiated with either 10 or 25 nmol DMBA as indicated and beginning 2 weeks after initiation received topical applications of chrysarobin, TPA, or acetone. Thus, promotion in all groups except the delayed promotion group received the first promoter treatments at the beginning of the third week after initiation. The incidence of papillomas and/or carcinomas was observed and recorded weekly. Papillomas (Fig. 1A) were removed at random for histological verification. However, all carcinomas were verified histologically to be squamous cell carcinomas of varying degrees or grades of differentiation. The carcinoma incidence and the average number of carcinomas per mouse are expressed as a function of the mice at risk at the time of appearance of the first carcinoma (i.e., based on the effectual total). All tumor groups were run concurrently except those in Fig. 4 (mice obtained from Research Biogenics) which were run as a separate experiment. Statistical analyses of the differences between mean papilloma responses (i.e., papillomas per mouse) were performed using Student's t test whereas differences between carcinoma incidences were compared using the $\chi^2$ statistic. The level of significance was set in both cases at $P < 0.05$.

**Histological Evaluation.** Tissues for histological evaluations were prepared using conventional paraffin sections and hematoxylin-eosin staining. Squamous cell carcinomas were classified as Grade I, II, or III (G1, GII, GIII, respectively) according to the degree of differentiation as follows: G1 (Fig. 1B), a very well differentiated lesion with extensive areas of keratinization and horny pearls. The mitotic index in Grade I lesions was low and the cellular atypia moderate; GII (Fig. 1C), a lesion with more atypical cells and only small areas occupied by horny pearls; GIII (Fig. 1D), a very anaplastic lesion without signs of cornification and exhibiting a high mitotic index and also with marked nuclear and cellular pleomorphism.

**RESULTS**

Dose-Response Relationship for Tumor-promoting Activity of Chrysarobin Applied Twice Weekly. In previous dose-response experiments with chrysarobin, we were unable to demonstrate clearly a good dose-response relationship for promotion of papilloma formation (8). It was thought that this was due to a combination of factors: (a) the majority of doses used in our previous experiments were probably at or above optimal levels for promoting papillomas using a twice weekly application protocol; (b) with the lower dose utilized (i.e., 50 nmol), a longer duration of treatment may have been necessary to effect a significant papilloma response. Taken together, our previous experiments also suggested that any dose-response relationship with these anthrone derivatives would occur over a very narrow
TUMOR PROMOTION BY CHRYSAROBIN

Fig. 2. Dose-response relationship for skin tumor (papilloma) promotion with chrysarobin using a twice weekly application protocol. All mice were initiated with 25 nmol DMBA in 0.2 ml acetone followed 2 weeks later by twice weekly applications of various doses of chrysarobin. Thirty mice were used in each experimental group. Top, average number of papillomas per surviving mouse; bottom, percentage of mice with papillomas; •, 25 nmol; ○, 50 nmol; ■, 75 nmol; ◦, 100 nmol; △, 220 nmol; □, 440 nmol. Animals receiving the acetone vehicle (0.2 ml) at initiation followed by twice weekly applications of 220 nmol chrysarobin had 0.0 papilloma/mouse after 30 weeks of promotion. Animals initiated with 25 nmol DMBA followed by twice weekly applications of 0.2 ml acetone had no tumors at 30 weeks.

dose range similar to that observed with TPA (8). Fig. 2 shows the results of further dose-response experiments with chrysarobin for skin tumor promotion in SENCAR mice. In these experiments, mice were initiated with 25 nmol DMBA and promoted with twice weekly applications of chrysarobin at various dose levels. As shown, a good dose-response relationship was observed over the range of 25–100 nmol/mouse. At doses of 75 nmol/mouse and above, the average number of papillomas per mouse (Fig. 2, top) plateaued between 26 and 30 weeks of promotion except the 440-nmol/mouse group where the average number of papillomas per mouse dropped slightly after 22 weeks of promotion. The 50-nmol/mouse group plateaued after 36 weeks of promotion yielding 2.04 ± 0.73 (SD) papillomas/mouse (Table 1). The 25-nmol/mouse group had not plateaued by 30 weeks but continued to rise slowly over the 60-week observation period. At 60 weeks of promotion, the 25-nmol/mouse group had 0.73 ± 0.32 papillomas/mouse (see Table 1).

Effect of Application Frequency on Skin Tumor Promotion by Chrysarobin. At optimal promoting doses such as those used in the experiment shown in Fig. 2, chrysarobin is a very weak inducer of epidermal ODC (8, 13) compared to TPA. In addition, using a twice weekly application protocol, a 220-nmol application of chrysarobin leads to an ODC induction response of lower magnitude than that obtained after a single application (8, 13). These data suggested that twice weekly application might produce significant epidermal toxicity thus retarding the promotion response to chrysarobin. We recently found that application once weekly with chrysarobin induced epidermal ODC to a greater extent than twice weekly application (13), further supporting this hypothesis. In addition, a preliminary tumor experiment suggested that once weekly application of chrysarobin was more effective at promoting papillomas than a twice weekly application protocol (13).

Fig. 3 illustrates the promotion response in SENCAR mice receiving once weekly applications of 25, 100, 220, and 440 nmol chrysarobin. Interestingly, chrysarobin doses of 25, 100, and 220 nmol/mouse, in this once weekly protocol, gave maximal papilloma responses significantly greater ($P < 0.05$) than with the twice weekly protocol (2.90 ± 0.59, 8.15 ± 0.24, and 9.38 ± 1.11 versus 0.73 ± 0.32, 4.70 ± 1.37, and 5.42 ± 1.86, respectively). In addition, the rate of papilloma development was faster and the number of papillomas reached a plateau sooner in animals receiving treatments once weekly compared to animals receiving the twice weekly treatments (compare Figs. 2 and 3). Interestingly, the animals receiving once weekly applications of 440 nmol chrysarobin yielded a papilloma response lower than that observed with 220 nmol (significantly different, $P < 0.05$). This drop in papilloma response when 440 nmol chrysarobin was given once weekly (to ~5.0 papillomas/mouse) was similar to the maximum response obtained with doses of 100 nmol or greater using the twice weekly protocol. In a repeat experiment, animals initiated with 25 nmol DMBA followed 2 weeks later by once weekly applications of 220 nmol chrysarobin had 9.9 papillomas/mouse after 26 weeks of promotion. This value is essentially identical to that shown for the same dose in Fig. 3.

Fig. 3. Dose-response relationship for skin tumor (papilloma) promotion with chrysarobin using a once weekly application protocol. The conditions of the experiment were similar to those in Fig. 2 except that various doses of chrysarobin were applied once weekly. •, 25 nmol; ○, 100 nmol; ■, 220 nmol; △, 440 nmol. Animals receiving the acetone vehicle (0.2 ml) at initiation followed by once weekly applications of 220 nmol chrysarobin had 0.11 papilloma/mouse at 30 weeks of promotion.
Effect of Delayed Promotion on Skin Tumor Promotion by Chrysarobin. To further explore characteristics of the promoting action(s) of anthrone derivatives, a delayed promotion experiment was performed. Recently, Hennings and Yuspa (14) demonstrated that a 10-week delay in starting promotion with mezerein significantly altered the tumor response. Whereas twice weekly applications of mezerein started 2 weeks after initiation yielded a low tumor response, twice weekly applications started 10 weeks after initiation yielded a significantly greater tumor response. With the latter treatment protocol, tumors developed more rapidly and reached a number similar to that in the group of mice treated with TPA followed by mezerein (i.e., a two-stage promotion protocol) (14). Fig. 4 shows the results of a similar type of experiment with chrysarobin. In this experiment, groups of 30 mice each were initiated with 10 nmol DMBA and were followed 2 weeks later by twice weekly applications of TPA (3.4 nmol) or once weekly applications of chrysarobin (100 nmol). In a third group of mice, animals received acetone (0.2 ml) twice weekly starting in the third week after initiation and continuing through Week 12 after initiation. Chrysarobin treatments (100 nmol given once weekly) were then begun in Week 13 and continued for the duration of the experiment. Under these experimental conditions, the promoting action of chrysarobin was significantly enhanced. As shown in Fig. 4, both the rate of papilloma formation and the final papilloma yield were significantly \((P < 0.05)\) increased (~2-fold) compared to the group that received once weekly treatments beginning in the third week after initiation \((11.0 \pm 1.35\) versus \(5.6 \pm 0.21\) papillomas/mouse, respectively). The group of animals promoted with twice weekly applications of TPA developed an average of \(17.0 \pm 0.80\) papillomas/mouse after 22 weeks of promotion.

Comparison of Tumor Progression in Chrysarobin and TPA-treated Mice. To further analyze the characteristics of skin tumor promotion by anthrone derivatives, the various treatment groups in our dose-response experiments were continued for 60 weeks. Table 1 summarizes the dose-response relationships for carcinoma formation in SENCAR mice treated with chrysarobin. Also shown for comparative purposes are the papilloma and carcinoma responses in 3 groups of mice initiated with 25 nmol DMBA and followed 2 weeks later by twice weekly applications of TPA. This dose of TPA has previously been shown to be a maximal promoting dose for papilloma development in SENCAR mice (8). A good dose response for carcinoma formation was observed in the range of 25 to 75 nmol/mouse when chrysarobin was given as a twice weekly application protocol. Doses of 200 nmol/mouse and above were maximal for carcinoma formation.

To examine the progression of papillomas to carcinomas under the promotion influence of chrysarobin, we have calculated the carcinoma:papilloma ratio after 60 weeks of promotion with these anthrone derivatives. Examination of these values in Table 1 reveals that, in general, the carcinoma response followed closely the papilloma response for this class of tumor promoter. Thus, the carcinoma:papilloma ratio was similar in groups receiving the twice weekly or the once weekly applications of chrysarobin. In a separate experiment (data not shown), similar results were obtained with a group of mice initiated with 25 nmol DMBA and treated with 220 nmol anthralin once weekly for 60 weeks (carcinoma:papilloma ratio, 0.11). A notable difference was observed when comparing the chrysarobin (and anthralin) groups with the 3 groups of mice treated with TPA. In the TPA-treated mice, the carcinoma:papilloma ratio was considerably lower in all 3 groups \((2.5- to 5.0\)-fold depending on the anthrone treatment group used for comparison). One of the TPA-treated groups \((Group 10)\) gave a relatively low carcinoma response \((0.43\) carcinoma/mouse, \(33\%\) incidence) which was significantly different \((P < 0.05)\) than the other TPA-treated groups as well as the chrysarobin groups receiving optimal promoting doses. However, the papilloma response was also lower in this group \((Group 10)\). The other TPA groups gave papilloma and carcinoma responses expected based on previous work from our laboratories (8, 15) as well as others \((16, 17)\). Thus, our comparisons of papilloma
and carcinoma responses with the chrysarobin-treated groups are restricted to these latter TPA-treated groups (i.e., Groups 11 and 12). Regardless, the carcinoma:papilloma ratio was similar in all TPA-treated groups.

All squamous cell carcinomas reported in Table 1 were histologically verified and graded according to their degree of differentiation (Grades I–III with Grade III being the least differentiated as described in “Materials and Methods”). As shown in Table 2, the majority of the squamous cell carcinomas were relatively well differentiated and classified as Grade I. In addition, there were no significant differences between the anthrone and phorbol ester-treated groups with respect to the grade of carcinoma observed.

Incidence of Other Tumors in SENCAR Mice Promoted Topically with Anthrone Derivatives. It was also of interest to examine whether the incidence of certain tumors (spontaneous or otherwise) was affected with chronic topical exposure to anthrone tumor promoters. These data as well as the incidence of metastatic lung tumors are also given in Table 2. In the present study, we focused on tumors of the skin (including s.c. tumors), lung, hematopoietic system, and mammary gland since spontaneous tumors of these tissues have been shown to occur with a definite but low incidence in SENCAR as well as other stocks and strains of mice (18, 19). It should also be stressed that the summary in Table 2 is by no means an exhaustive examination of other tissues and organs. However, with respect to the tissues examined, we did not see any increase in incidence over that observed spontaneously in female SENCAR mice (18, 19) in groups treated with chrysarobin for the 60-week period. Thus, topical application of the anthrone derivative did not appear to provide any systemic promoting action under the conditions of our experimental protocol and for the tissues examined. In groups treated with TPA there also did not appear to be any significant increases in the incidence of other neoplasias for the tissues examined.

DISCUSSION

The present study was designed to further explore the characteristics of skin tumor promotion and progression by the anthrone class of skin tumor promoters. In previous experiments from our laboratory we examined the dose-response relationship for promotion of skin papillomas by anthralin and chrysarobin compared with TPA in SENCAR mice and a good dose-response relationship was not readily observed with the anthrone derivatives (8). In the present study, however, an excellent dose-response relationship was observed for the formation of both papillomas and squamous cell carcinomas when chrysarobin was given either once or twice weekly. Several factors may have contributed to the current results: (a) a higher initiating dose of DMBA was used in the present set of dose-response experiments (25 nmol versus 10 nmol); (b) application of chrysarobin once weekly proved to be a considerably more effective treatment schedule than treatments given twice weekly; (c) all treatment groups were carried for 60 weeks. It was important to extend the treatment time in the lower dose groups receiving twice weekly applications of chrysarobin (i.e., 25 and 50 nmol/mouse) to achieve a plateau and/or a significant tumor response.

As noted in our previous study, the time to reach a tumor response plateau (i.e., papillomas) with twice weekly applications of chrysarobin occurred between 25 and 30 weeks (see also Fig. 2 of the present study). With TPA, the papilloma response usually reaches a plateau on or before 15 weeks of promotion in SENCAR mice with a twice weekly application of optimal promoting doses (8, 15). In addition to this difference in the tumor response, the current data with the once and twice weekly applications of chrysarobin also show another notable difference compared with TPA. In this regard, it has been shown by several laboratories that some of the papillomas produced using optimal doses of TPA will regress or disappear before carcinomas develop even under continued exposure to the promoter (17, 20). This phenomenon is even more dramatic if TPA treatment is terminated at or about the time when the papilloma response plateaus in the promotion protocol (21). As shown in Fig. 2, when chrysarobin is applied using a twice-weekly regimen at doses of 220 nmol or less, little or no papilloma disappearance was observed. This was also true of the groups treated with chrysarobin in the once weekly application protocol (Fig. 3). Although the papillomas per mouse tended to drop after 30 weeks of promotion in the twice weekly treatment groups and after 24 weeks in the once weekly treatment groups, this was attributed primarily to progression of papillomas to carcinomas although some coalescence of papillomas was observed. In general, the first carcinomas appeared between Weeks 18 and 25 in the groups receiving the highest doses of chrysarobin and in the groups receiving TPA. The rate of carcinoma development (data not shown) and the overall grade of carcinoma (Table 2) was also similar in these groups. In other words, carcinomas appeared in anthrone- and TPA-treated groups at approximately the same time and developed at approximately the same rate despite the overall differences...
in the characteristics of the papilloma response. In addition to these observations, we also noted that many of the carcinomas appeared to arise from preexisting papillomas in the anthrone-treated mice, similar to that which has been observed with TPA (or croton oil)-treated mice (20, 21).

The existence of an optimum frequency of application for skin tumor promotion by phorbol esters has been known for some time (reviewed in Ref. 3). Much less is known for other classes of skin tumor promoters. As already noted there are differences in the magnitude of induction of epidermal ODC with different application frequencies of chrysarobin (8, 13). In this regard, once weekly applications led to a greater overall induction of epidermal ODC than with the twice weekly application protocol. In addition, we have recently found significant differences in the epidermal hyperplasias induced by twice weekly versus once weekly applications of chrysarobin.4 In this regard, once weekly applications of chrysarobin (220 nmol) led to an overall greater hyperplasia in terms of both epidermal thickness and number of nucleated interfollicular epidermal cells 48 h after the last of four applications. With both treatment protocols, however, abnormal looking basal cells were observed having both enlarged nuclei and cytoplasm. These latter observations were particularly striking in the skins of mice treated twice weekly. Other studies using cells in culture have documented the cytotoxic nature of anthrone derivatives, primarily anthralin (22–25). These data, which suggest that toxicity and epidermal regeneration may be important components of hyperplasia produced by anthrones, led to testing a once weekly application protocol. As shown in Fig. 3, chrysarobin was much more effective as a skin tumor promoter in SENCAR mice when given at a frequency of once weekly. A good dose-response was observed at doses of 25, 100, and 220 nmol/mouse. Interestingly, a dose of 440 nmol given once weekly yielded a papilloma response significantly lower than at the 220-nmol dose given once weekly but similar to the response with 220-nmol dose given twice weekly. These data, when taken together, allow us to hypothesize that toxicity may play an important role in modulating the promotion response with anthrones. The above data also suggest that there may be subclasses of initiated cells displaying differential growth properties and/or susceptibility to the cytotoxic effects of anthrone promoters. The existence of subclasses or subpopulations of initiated cells has been suggested by several investigators (17, 20, 21, 26). It is interesting to speculate that by the right choice of dose or treatment protocol, anthrone promoters may be capable of selecting for subclasses of initiated cells. We are currently exploring the above ideas in greater detail with both the anthrone and phorbol ester classes of promoters.

In light of the dramatic effect of changing the application frequency on the papilloma response, we examined the promoting action of chrysarobin after a 12-week (instead of the normal 2-week) interval between initiation with DMBA and the start of promotion. Interestingly, others have recently shown that delaying the start of promotion with mezerein by 10 weeks led to a significantly greater tumor response compared to a group of mice where mezerein treatment was begun 2 weeks after initiation (14). The data from our experiment shown in Fig. 4 demonstrate that a similar phenomenon occurred with chrysarobin.

It may be useful to point out that there are some very interesting similarities between chrysarobin and mezerein. In addition to the phenomenon of delayed promotion giving rise to an enhancement in the promotion response to both agents, neither agent functions as a Stage I promoting agent in the operational paradigm of two-stage promotion (8, 27). Furthermore, both agents are considered to produce significant epidermal toxicity which plays a critical role in modulating their promoting actions (12, 28, 29). Although the mechanism for the delayed promotion effect is unknown at present there are several possibilities. In this regard, TPA may be capable of causing the rapid expansion of most if not all classes of initiated cells after only several treatments (Stage 1 of promotion) whereas this process may occur more slowly in the absence of any treatment (i.e., spontaneous expansion of initiated cells into miniclonies as a result of delayed promotion) (14). Mezerein and chrysarobin when applied alone and shortly after initiation (1–2 weeks), may allow the expansion and expression only of certain subclasses of initiated cells which are more resistant to the toxic actions of these compounds. However, limited TPA

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
<th>Fibro-</th>
<th>Adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
<th>Fibro-</th>
<th>Adenocarcinoma</th>
<th>Metastases</th>
<th>Hematopoietic</th>
<th>Mammary gland adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>C*</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C+</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>22</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>20</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>23</td>
<td>7</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>24</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>18</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>38</td>
<td>34</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data represent total incidence after 60 weeks of promotion. Treatment group numbers correspond to those in Table 1.

* Animals received 25 nmol DMBA at initiation, followed by twice weekly applications of acetone (0.2 ml).

* Animals received 0.2 ml acetone at initiation, followed by twice weekly applications of 220 nmol chrysarobin.

* Animals received 0.2 ml acetone at initiation, followed by once weekly applications of 220 nmol chrysarobin.

treatments or delayed promotion might alter the further responsiveness of subclasses of initiated cells such that a larger proportion can now respond to a compound such as mezerein or chrysarobin. While the hypothesis that Stage I of promotion may occur spontaneously (14) is an interesting one it is not consistent with the data demonstrating the reversibility of Stage I of promotion in both SENCAR (30) and NMRI (31) mice. An alternate hypothesis, which we are currently exploring, is that alterations in the skin as a result of a 10-week delay (such as hair regrowth, etc.) reduce the toxicity to compounds such as mezerein and chrysarobin.

We previously tested chrysarobin as a second stage promoter but did not observe an enhancement in its promoting actions when 2 weeks of TPA treatment preceded the start of twice weekly applications with the anthrone (8). These data are also inconsistent with the first hypothesis above; however, only one promoting dose (220 nmol) and only one application frequency (twice weekly) was used in our previous work. The present work used a lower dose (100 nmol) and a once weekly application frequency (Fig. 4) which may have been more optimal for observing the enhancement. In any event, we believe that the above ideas should be explored in much greater detail not only with the anthrone derivatives but also with a variety of promoting agents other than the phorbol esters to determine the generality of this phenomenon. Regardless of the exact mechanism, it appears that delaying the start of promotion can enhance the promoting response to a compound (chrysarobin) which is a reasonably good complete promoter.

Another important finding in our present study is related to the carcinozma-papilloma ratio produced by promotion with anthrone derivatives. In this regard, animals receiving once or twice weekly treatments with the anthrones had 2.5- to 5.0-fold higher ratios than corresponding mice treated with optimal promoting doses of TPA (i.e., 3.4 nmol). This was due primarily to the fact that similar carcinoma responses were observed in both chrysarobin (and anthralin) and TPA-treated mice at optimal promoting doses while the papilloma responses (i.e., papillomas per mouse) were significantly lower in the former groups. The only exception was Group 10 (Table 1) where the carcinoma incidence was significantly lower than the other TPA and chrysarobin groups receiving optimal promoting doses (P < 0.05). In addition, although the papilloma response in Group 10 (12.2 ± 1.98) was not significantly different than in Group 8 (P < 0.05) it was significantly different (P < 0.05) than in all other chrysarobin-treated groups. It should be stressed that in our experience the tumor response in Group 10 (treated with TPA) was abnormally low; therefore, we have placed considerably less emphasis on the above noted exceptions. Furthermore, our previous work (8) clearly demonstrated significant differences in papilloma responses between mice treated with TPA versus chrysarobin, and in our present study the other TPA-treated groups in Table 1 had papilloma responses significantly different (P < 0.05) than all chrysarobin-treated groups. In the animals receiving once weekly applications of chrysarobin at optimal promoting doses (i.e., Groups 7 and 8 in Table 1) there was a higher number of carcinomas per mouse; thus, in these two groups both the lower number of papillomas per mouse and a higher number of carcinomas per mouse contributed to the increased carcinozma-papilloma ratio compared with the TPA groups. The values for conversion of papillomas to carcinomas in Table 1 are similar to those reported by other laboratories using maximal promoting doses of TPA (or croton oil) (17, 20, 21, 32). There are several possible explanations for these observations: (a) anthrone promoters may promote the expression of a subclass of papillomas with a high probability of progressing to carcinomas. This mechanism would be similar to that recently suggested for the reported similarity in carcinoma response in mice promoted with either TPA or mezerein alone following DMBA initiation (17); (b) optimal promoting doses of TPA, in terms of papilloma development, may not be optimal for progression of papillomas to carcinomas. Verma and Boutwell (33) showed that in female CD-1 mice, doses of TPA giving similar papilloma responses (i.e., 10 and 20 nmol/mouse) gave markedly different carcinoma incidences. This is most probably due to a combination of both local as well as systemic toxicity at the higher dose levels. Klein-Szanto et al. (34) have very recently demonstrated that chronic treatment of SENCAR mice with TPA alone markedly decreased their longevity. Thus, systemic effects of high doses of TPA may play a role in limiting conversion of papillomas to carcinomas. We are currently exploring both of the above possibilities in more detail at the present time.

In summary, anthrone derivatives are effective skin tumor promoters in SENCAR mice especially when administered using a once weekly application protocol or when the start of promotion is delayed for an additional 10 weeks. The biochemical and molecular mechanisms(s) by which this class of tumor promoters works remains unknown; however, as noted in the “Introduction,” they do not interact directly with the phorbol ester receptor in epidermal particulate or whole cell preparations. The further study of this class of compounds may help to better define critical events in the process of tumor promotion in general as well as a more detailed understanding of the biochemical and molecular events associated with anthrone skin tumor promotion.

ACKNOWLEDGMENTS

The authors wish to thank Joyce Mayhugh for her excellent secretarial assistance in preparing this manuscript and Judy Chesner for her excellent skills in the processing and preparation of tissues for histological evaluation. The authors also thank Judy Ing and John Riley for the photography and artwork, respectively, in the figures used in this paper and Beth Crysup for valuable technical assistance in conducting the tumor experiments.

REFERENCES

10. Van Duuren, B. L., Segal, A., Tseng, S.-S., Rusch, G. M., Loewengart, G.,
TUMOR PROMOTION BY CHRYSAROBIN


Characterization of Skin Tumor Promotion and Progression by Chrysarobin in SENCAR Mice

Francis H. Kruszewski, Claudio J. Conti and John DiGiovanni


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/14/3783

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.