Strain-specific Tumorigenesis in Mouse Skin Induced by the Carcinogen, 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one, and Its Relation to DNA Adduct Formation and Persistence

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ABSTRACT

The incidence of skin tumors has been studied in three strains of mice, namely, TO, C57BL, and DBA/2, after treatment with the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one. After either a single dose followed by croton oil promotion or a continual dose of the carcinogen, tumors were observed in the TO and C57BL strains, with the TO mice having the shorter mean latent period. The DBA/2 mice, however, appeared to be resistant to tumor formation by either treatment. To understand the mechanism of resistance, several criteria have been investigated. Metabolism of the carcinogen was assessed in terms of the total DNA adduct formation and the pattern of individual adducts after separation by high-pressure liquid chromatography, and no major differences between the three strains was found. Similarly, the rates of disappearance of the individual adducts when measured over 14 days posttreatment were not strain specific. Persistent binding of the carcinogen after 2 months was found in all three strains and could be reduced markedly if croton oil was administered throughout this period.

The ability of the phorbol esters to cause biochemical changes in both sensitive and resistant strains was indicated by the induction of ornithine decarboxylase in each of the three strains after treatment with either croton oil or its active component, 12-O-tetradecanoylphorbol-13-acetate.

INTRODUCTION

The 2-stage theory of carcinogenesis, comprising the 2 distinct phases, initiation and promotion, represents a move toward defining the individual steps in tumor development. This theory, although largely based on experiments involving the induction of tumors on the skin of mice, has been found applicable to a wide range of tissues in which chemically induced tumors arise (17). The specificity of chemical carcinogens in relation to both tissue and species has been related to events at the initiation stage. Both the metabolism of carcinogens to active electrophilic species and the presence of DNA repair enzymes capable of removing promutagenic lesions have been found to be both tissue and species specific. This specificity has further helped to define the steps involved in tumor initiation. Strain variation in the development of skin tumors, on the other hand, has been noted by a number of researchers (4, 14, 21, 22, 26), but little understanding of the basis of this variation has been achieved. The question of whether the genetic differences arise at the initiation or promotion phase is not yet fully understood. Since strain differences in response to a promotor could provide a tool for the analysis of the mechanism of promotion, this study was undertaken to determine the basis for this specificity.

The carcinogen, 11-methyl ketone (Chart 1, Structure II) has been extensively studied in this laboratory as the most potent member (9) of the series of monomethylated derivatives of the parent compound, 15,16-dihydrocyclopenta[a]phenanthren-17-one (Chart 1, Structure I). Here it has been used to study tumor induction in the mouse strains TO, C57BL, and DBA/2 with and without croton oil promotion. The TO mice have been shown previously to be sensitive to this carcinogen (11) while the C57BL and DBA/2 are reported to have low sensitivity to polycyclic hydrocarbon-induced tumor formation (22). This study compares the skin tumor incidence in these strains in relation to the formation and disappearance of DNA adducts in the skin.

MATERIALS AND METHODS

Radiochemicals and Enzymes. The carcinogen 11-methyl ketone was generally tritium labeled at the Radiochemical Centre, Amersham, U. K., and purified in these laboratories by column chromatography (8). The final specific activity was 17 Ci/mmol. DL-[1-14C]Omithine was purchased from the Radiochemical Centre. Pancreatic DNase I (type I), snake venom phosphodiesterase (type II), Escherichia coli alkaline phosphatase (type II), and pancreatic RNase (type III-A) were obtained from Sigma Chemical Co., London, U. K. Proteinase K was obtained from BDH, Poole U. K.

Tumor Incidence. The 3 strains of mice to be compared for skin tumor incidence, namely, TO (Thielers’ Original), C57BL, and DBA/2, were obtained from the Animal Breeding Unit of the ICRF, Mill Hill, London, U. K. Male mice (8 to 10 weeks old) were used in groups of 20 for all experiments. The dorsal skin was shaved with electric clippers (approximate area, 2.5 x 2.5 cm) 1 day before the initial treatment and at various times throughout the experiment. The carcinogen and/or promoting agent was applied to the shaved area of each mouse. To compare the strains by the 2-stage carcinogenesis system, one group from each of the 3 strains was given a single dose of 400 μg of unlabeled 11-methyl ketone in 40 μl of toluene followed by twice weekly applications of 10 μl of 1% (w/w) croton oil (Sigma) in toluene beginning 8 days later. For each strain, the control group received only croton oil. To compare the tumor incidence after continual carcinogen treatment, one group from each of the 3 strains received twice weekly applications of a low dose of 11-methyl ketone (50 μg in 10 μl toluene). The control groups received twice weekly applications of toluene alone. The incidence of papillomas was recorded twice weekly.

Analysis of DNA Binding. The analysis of DNA binding of 11-methyl ketone in mouse skin has been described previously (1, 12). Briefly, the labeled carcinogen (400 μg in 40 μl toluene) was applied to the shaved skin area of each mouse. At various times, mice in groups of 10 to 15
radiation. However, the cell populations which survive treatment with Adriamycin or with radiation are related but are probably not identical (13). AZQ is currently undergoing Phase II clinical trials, and although results of many of these trials are not impressive, the drug has some clinical activity (1). Results presented in this paper should encourage study of tumor response and toxicity for AZQ used with Adriamycin to treat the (uncommon) experimental tumors that are sensitive to Adriamycin. If such studies confirm the present findings, they might lead to Phase II trials of this drug combination used to treat human tumors that are sensitive to Adriamycin.

REFERENCES


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respectively (1, 10). Similarly, Peak VI which is unknown and Peak VIII which is derived from deoxyadenosine and the 15-hydroxy-3,4-diol (1) elute in a position similar to the corresponding peaks derived from TO mice (1). No major differences in the metabolic pathways leading to DNA adducts, therefore, exist among the 3 strains of mice.

The possibility of differences in the rate of loss of adducts between the 3 strains was investigated by measuring the binding ratio at various times up to 14 days. Chart 5 compares the rate of loss of adducts in each of the 3 strains. The half-life for disappearance of the total adducts is 6 to 7 days in each case. As found previously (2), this corresponds to the rate of turnover of the tissue (measured only in TO mice). Probably, little or no active removal of minor adducts was investigated by separation of the total adducts by HPLC at various time points. The results for DBA/2 mice are shown in Chart 6. Peak VIII is lost at the same rate as the major adduct (Peak VII), while Peak VI is lost rapidly with a half-life of 2 days. Whether Peak VI is actively removed from the DNA or is an unstable adduct has not yet been determined. The results were similar for C57BL mice and are identical to those described previously for TO mice (2). No differences, therefore, could be found in the loss of total or individual adducts in the 3 strains which could account for their differences in tumor susceptibility.

Chart 3. Induction of dorsal skin tumors in the 3 mouse strains by continual carcinogen treatment. Mice were treated with 50 μg of 11-methyl ketone twice weekly.

Table 1
Binding of 11-[G-3H]methyl ketone to skin DNA of the 3 mouse strains after 3 days

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total DNA binding* (nmol adduct/mol DNA-phosphorus)</th>
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<tbody>
<tr>
<td>TO</td>
<td>670</td>
</tr>
<tr>
<td>C57BL</td>
<td>994</td>
</tr>
<tr>
<td>DBA</td>
<td>919</td>
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*Total DNA binding is defined as the total amount of radioactive products eluting from the Sephadex LH-20 column after the normal nucleosides, in relation to the deoxyadenosine peak.

Chart 4. HPLC profile of radiolabeled 11-methyl ketone-DNA adducts derived from the skin of DBA/2 mice at 5 days (A) and C57BL mice at 3 days (B). Separation was on a reverse-phase Partisil 10 ODS column. The peak numbers have been assigned on the basis of cochromatography with both adducts from skin DNA of TO mice and also from in vitro metabolism (1, 2).

Chart 5. Loss of radiolabeled 11-methyl ketone-DNA adducts from DNA of skin of each of the 3 strains of mice. Total amount of adducts was calculated from the total radioactivity eluting after the normal deoxynucleosides on the Sephadex LH-20 column. The rate of loss of adducts shown is not adjusted for the rate of DNA turnover of the tissues.
Persistence of Adducts. Following a previous observation that carcinogen DNA adducts of 11-methyl ketone persisted in the DNA 1 month after treatment at a higher-than-expected level (12), persistence of adducts has been investigated in these 3 mouse strains. Table 2 shows the level of binding in each of the 3 strains 2 months after treatment with the carcinogen. Binding was higher than expected in all strains and represented approximately 5 to 10% of the binding ratio at the time of maximal binding (2 to 3 days). Treatment of the mice with croton oil throughout this period reduced the level of total binding considerably, but persistent binding was still clearly measurable. The reduction in binding caused by the croton oil may be due to its hyperplastic effect on the basal cells (3, 5). Neither persistent adduct formation nor croton oil-induced hyperplasia, however, appeared to be strain specific.

Measurement of ODC. The induction of ODC represents an early event in mouse epidermal cells following treatment with tumor-promoting agents (25). Although this event may or may not be related to the mechanism of tumor promotion (13), the induction is dependent on interaction of the tumor promoter with the epidermal cells and may indicate the presence of cell surface receptors. ODC induction has been measured in each of the 3 strains at 4.5 hr after treatment with either 1 mg of croton oil or 20 nmol of TPA, and the results are shown in Table 3. Although there is considerable variation, it is apparent that ODC induction does occur in each of the strains following both croton oil and TPA treatment.

DISCUSSION

The carcinogen, 11-methyl ketone, has been shown in these experiments to be active in TO and C57BL mice both as an initiator for skin tumorigenesis and as a complete carcinogen when applied continually in a low dose. With both protocols, no tumors could be found in the DBA/2 mice over the same time period. These results provided the basis for further analysis of these strains in terms of the capacity of their skin epidermal cells to metabolize 11-methyl ketone and to repair any DNA adducts which might be formed following carcinogen treatment and also for comparison of their response to promoting agents in order to detect any differences which might account for this strain specificity.

The metabolism of the carcinogen, 11-methyl ketone, has previously been studied in vitro using rat liver microsomes as a source of activating enzymes (10) and the major pathways of metabolism elucidated (1, 10). Likewise, the DNA adducts have been largely characterized (1), and the in vivo adducts in the DNA of the skin of TO mice have been found to be identical to those formed in vitro (1). Metabolism in the skin of 3 strains was compared by measuring the total DNA binding and separating the adducts by HPLC. High binding in all 3 strains suggested active metabolism of the carcinogen to the reactive diol-epoxide. This was confirmed by HPLC separation of the adducts since the pattern of adducts was similar for all 3 strains. No gross differences among the 3 strains in the metabolism of the carcinogen to reactive intermediates capable of covalent reaction with DNA could be found.

The possibility of a difference in the rate of repair of adducts contributing to strain differences was considered. This is a more difficult parameter to measure, especially in skin, since the basal cell population is proliferating relatively rapidly while the rest of the cell population is undergoing terminal differentiation. The overall loss of adducts from the DNA has been measured over a 14-day period following treatment. This measurement assumes that the repair capacity of the regenerating basal cells is the same as that of the fully differentiated keratinocytes. The method of Clausen et al. (7) for the separation of these 2 cell types may be useful in overcoming this problem in the future. The measurement of disappearance of adducts, however, revealed no differences among the 3 strains. As observed previously for TO mice (2), the major 11-methyl ketone-DNA adduct is probably not actively removed from the DNA of the skin of C57BL or DBA/2 mice but is simply lost due to normal cell proliferation (Chart 6). One minor adduct (Peak VI) whose identity is unknown (1) is lost relatively rapidly in all 3 strains. The question of whether repair...
enzymes have a role in averting tumor formation by polycyclic compounds remains largely unanswered, but it is clear that the DNA adducts of polycyclic hydrocarbons can be repaired (2, 15, 16, 18, 24, 27); there is also evidence that this repair, at least in the case of benzo(a)pyrene adducts in human fibroblasts, leads to a decrease in mutation frequency (33). There are few cases, however, where adduct repair may be correlated to a decrease in tumor yield. Eastman and Bresnick (16) have suggested that the strain specificity of mouse lung tumors induced by polycyclic hydrocarbons is related to persistence of adducts in the DNA. While this may be the case in mouse lung, our results with mouse skin and those of Phillips et al. (26) provide no evidence that persistence of adducts occurs in the mouse strains which eventually develop skin tumors. On the other hand, the DNA adducts of 11-methyl ketone were shown previously (2) to be rapidly removed in mouse liver, a fact which may be related to the resistance of this organ to 11-methyl ketone-induced tumorigenesis. So although tissues may differ in their repair capacity, there is no evidence that this is also strain specific.

The presence of residual DNA adducts after an extended time period may be related to the repair capacity of the epidermal basal cells. Persistent DNA adducts were found in the skin of all 3 strains 2 months after carcinogen treatment. Of the epidermal cells, only the basal cells could retain the adducts for this length of time. Treatment of the mouse skin of each strain throughout this period with croton oil produced very significant decrease in the adduct binding ratio, which is consistent with the pronounced hyperplastic effect of croton oil on mouse skin epidermis (3, 5). This is in contrast with the results of Burki et al. (6), who have suggested that TPA-induced skin hyperplasia may be strain specific. HPLC analysis of the adducts 2 months after treatment revealed little change in the pattern of adducts to that observed at 14 days (results not shown). Persistence of adducts, therefore, seemed to be associated with a general lack of repair rather than with the inability to remove any specific adduct. No differences could be detected between the sensitive and resistant strains.

Since carcinogenesis in skin can be divided into 2 stages, initiation and promotion, genetic differences conferring strain specificity could manifest themselves at either of these levels. Because no true assay for initiated cells exists, other criteria such as the presence of carcinogen-DNA adducts during a phase of cell proliferation must serve to indicate that initiated cells probably exist in the tissue. By the criteria of adduct formation and persistence, then, the skin epidermal cells of DBA/2 mice have been initiated. Resistance to tumor formation, therefore, may reside in the promotional phase of carcinogenesis. Tumor promoters have been shown to cause numerous cellular changes (13, 32), including the induction of a number of new enzyme pathways such as ornithine decarboxylase (25). Following skin treatment with either croton oil or its active component, TPA, induction of ODC was observed in all 3 strains of mice. This probably indicates the presence of TPA receptor sites on the epidermal cells of each strain. The investigation of other promotion-linked cellular changes in relation to strain differences may be more fruitful.

In conclusion, the results suggest that the genetic factors conferring strain specificity are not related to metabolism of the carcinogen, to the disappearance of DNA adducts, or to their persistence in the DNA. More likely, the differences in strain specificity are related to steps in the promotion stage of carcinogenesis although probably not to the initial cell surface binding of TPA. Recent reports that promotion may be divided into a 2-stage process (19, 28) might be used to investigate this possibility. The use of epidermal cell cultures (20, 34) from sensitive and resistant strains may help to analyze these questions in the future.

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