Effects of 7, 12-Dimethylbenz(a)anthracene and Estrogen on the Transplantation and Growth of a Rat Pituitary Tumor

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SUMMARY

7, 12-Dimethylbenz(a)anthracene, a carcinogenic polycyclic aromatic hydrocarbon compound, significantly enhanced the transplantability and stimulated the growth of a pituitary tumor in rats. Estrogen was less effective than 7, 12-dimethylbenz(a)anthracene. The combination of 7, 12-dimethylbenz(a)anthracene and estrogen showed a synergistic effect. Hormone production by the tumor was not impaired by these treatments.

INTRODUCTION

DMBA\textsuperscript{1} induces a variety of tumors in animals (14, 15, 18, 25). Mammary gland explants from normal rats treated with DMBA in short-term culture undergo anaplastic changes, giving rise to adenocarcinomas after transplantation to the isologous host (8, 9). Although the exact mode of action to this carcinogen is unclear, a direct effect on the genetic structure of susceptible cells seems very likely, as there are evidences for binding of DMBA to DNA, resulting in inhibition of DNA and rRNA syntheses (1, 2, 11, 12, 24) or stimulation of DNA synthesis (19).

Estrogens also induce a variety of tumors (5, 29, 34). Many tumors are hormone dependent, and sex hormones may cause their growth or regression, depending upon the nature of the neoplastic tissue (16, 17, 20, 27, 29). In cell culture, steroid hormones may modulate the function of tumor cells (3). Available evidences also point to a direct action of steroids on the genetic expression of the affected cells.

Although literature of the carcinogenic action of DMBA and of estrogen is abundant, little is known about the growth-promoting effect of these compounds on the preexisting tumors and on their function. We report here our observations on the stimulatory effect of DMBA and estrogen on the transplantability, growth, and hormone production of a rat pituitary tumor.

MATERIALS AND METHODS

DMBA was purchased from Sigma Chemical Company, St. Louis, Mo., and was used without further purification.

\textsuperscript{1}The abbreviations used are: DMBA, 7, 12-dimethylbenz(a)anthracene; ACTH, adrenocorticotropic hormone.

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A solution consisting of 20 mg of DMBA per ml oil was prepared. The sesame oil, USP, was purchased from City Chemical Corporation, New York, N. Y. A 2nd preparation of 15% fat emulsion with DMBA, 5 mg/g, was a gift from Dr. Paul E. Schurr, the Upjohn Company, Kalamazoo, Mich.

Estradiol benzoate in oil (Pregynon), 3.33 mg/ml, was purchased from Schering Corporation, Bloomfield, N. J. Prior to use, the solution was further diluted with sesame oil to a concentration of 1 mg/ml.

Rat anterior pituitary tumor originating from a clonal strain of cells, GH\textsubscript{1}, (36), was carried in inbred Wistar/Furth female rats for 4 and 5 generations before these experiments. This strain of cells produced growth hormone and prolactin (35), but no other hypophyseal hormones were detected during growth either in vivo or in vitro.

Seven-week-old Wistar/Furth female rats were purchased from ARS/Sprague-Dawley Company, Madison, Wis. The animals were fed Purina rat chow and tap water ad libitum. After a 2-week period of quarantine and acclimatization, the animals were deprived of food and water overnight prior to treatment. The rats were divided into 4 groups. In the 1st group, each rat received a single dose of 1 ml sesame oil by gavage; in the 2nd group, each rat received the same treatment of sesame oil and then weekly s.c. injections of estradiol benzoate, 0.2 mg, in 0.2 ml oil, near the hind legs; in the 3rd group, each rat received a single dose of 1 ml DMBA solution by gavage; and finally, in the 4th group, each rat received the same treatment of DMBA solution and then weekly s.c. injections of estradiol benzoate like the 2nd group. On the day following treatment, some of the rats from each group received transplants of tumor and others were kept without transplants as controls. GH\textsubscript{1} tumor tissue from a single donor was removed by sterile techniques and minced in Grand Island Biological Co. Medium 199. A volume of 0.5 ml suspension containing at least 2 million viable cells was injected s.c. in the posterior cervical area. During the experimental period, the body weight of each rat was recorded weekly. The earliest evidence for graft take was detected by palpation. Ten weeks after transplantation, all rats including the control animals were sacrificed by exsanguination through cardiac puncture. The liver, kidneys, spleen, and adrenal glands, as well as the tumor, were immediately dissected and weighed. Serum levels of growth hormone, prolactin, and corticosteroids were measured by radioligand competitive methods (4, 28, 30).

Due to the acute toxicity of DMBA administered p.o. and
the remarkable effect of estrogen and DMBA on observed
tumor growth, a subsequent experiment was designed to test
the efficacy of a smaller dose of DMBA in emulsion and also
to confirm the earlier observation. The rats were 8
weeks old when the tumor was transplanted. Each rat in 1
group received i.p. 10 mg of DMBA in emulsion, while the
other groups of rats received, as previously, 20 mg of
DMBA in 1 ml oil solution by gavage, or sesame oil alone.
Tumor transplantation was carried out the day following
DMBA or oil treatment. Estrogen injections were given at
10-day intervals. Detection of palpable tumor at the trans-
plantation site was recorded at weekly intervals. All rats
were sacrificed 10 weeks after transplantation.

RESULTS

The effects of estrogen, DMBA, and a combination of
estrogen and DMBA on tumor growth, 10 weeks after
transplantation, are summarized in Table 1. Estrogen alone
had some stimulatory effect on tumor growth, increasing
the tumor weight an average of 6-fold in comparison with
the tumors of untreated animals. DMBA consistently stim-
ulated tumor growth and in the dose given was more potent
than estrogen. The combination of the 2 compounds showed
a synergistic effect resulting in tumors that were, on an
average, 17-fold (Experiment 1) or 55-fold (Experiment 2)
heavier than those in the nontreated animals.

The potentiating influence of estrogen and DMBA on
tumor growth rate was also reflected by the transplantabil-
yity of the tumor. The cumulative number of rats with
palpable tumors at various intervals after transplantation is
summarized in Table 2. In both experiments, tumors could
be detected earlier in a larger proportion of rats treated with
estrogen and/or DMBA than of nontreated animals.

Within the 1st 2 days following treatment, approximately
40% of the rats receiving 20 mg of DMBA in oil by mouth
died. Indication for the acute toxicity of DMBA was also
observed by the weight loss of surviving animals during the
1st 2 weeks. The toxic reaction also occurred when the
gavage dose of DMBA solution was reduced by one-half.
When the animals were treated with an i.p. injection of 10
mg of DMBA in emulsion, no fatality was observed. This
level and route of administration of DMBA emulsion was
equally effective in stimulating tumor growth, but tumors
were detected at a later period after transplantation (Tables
1 and 2).

Data in Table 3 show the effects of the tumor on body as
well as viscera weights. The average animal and organ
weights, while little affected by the treatment in nontrans-
planted controls, were increased by the presence of tumor
and by the treatments that stimulated tumor growth. Since
the increased body weight was far in excess of the tumor
weight, the striking body-weight gain was attributed to the
general growth-promoting effect of growth hormone pro-
duced by the tumor. This view was confirmed by the direct
measurement of serum levels of rat growth hormone in the
various groups of rats (Table 4). Thus, tumors growing
under the influence of estrogen and DMBA maintain their
organ-specific function of growth hormone production. The

prolactin assay data shown in Table 4 indicate that prolactin
production by the tumor was also preserved. Especially
noteworthy are the increased serum concentrations of
prolactin and growth hormone in estrogen-treated tumor-
bearing as well as nontransplanted control animals. The
effects of the tumor on the adrenal gland were determined
by measurement of the gland weights (Table 3) and serum
levels of corticosteroids (Table 4). While a significant
increase in adrenal weight was observed in tumor-bearing
animals treated with the combination of DMBA and
estrogen, compared with that of nontransplanted controls
receiving the same treatment (Table 3), the treated tumor-
bearing rats had low serum concentrations of corticoste-
roids (Table 4).

DISCUSSION

The GH tumor studied in this report originated from a
normal rat pituitary gland after X-ray irradiation (37). The
success of transplantation and growth of this tumor was, at
the beginning, dependent on estrogen but later became
autonomous (13). The tumor was designated MtT/W, and
was carried in inbred Wistar/Furth female rats until a
clonal strain of tumor cells was established by cell culture
and designated GH (36). The tumor cells in culture or in
host animals exhibited the organ-specific function by pro-
ducing high levels of rat growth hormone and prolactin
(35). In the original strain, no other pituitary glycoprotein
or polypeptide hormones including ACTH were detected.
Since certain closely related types of pituitary tumors do
secrete ACTH and induce adrenal hyperplasia (7) and
since our data show increased adrenal weight in animals
bearing the largest tumors, a possibility that GH tumor
might dedifferentiate and start secreting ACTH during its
growing stage was suspected. However, this possibility
seems unlikely, as the same group of rats failed to have
elevated serum levels of corticosteroids. The cause of adre-
nal hyperplasia remains unclear.

It is interesting to note that estrogen was less effective
than DMBA in promoting tumor growth but greatly
stimulated prolactin and growth hormone secretion from
both the pituitary gland in control animals and by the
lumor. Estrogen stimulates hypophyseal production and
release of prolactin (22, 26, 33), and a similar effect on other
pituitary tumors has been reported (27). Estrogen also
stimulates release of growth hormone from the rat anterior
pituitary gland (10). Our results show that GH tumor, like
the normal gland, responds to estrogen by secreting more
growth hormone. Estrogen seems to act on the tumor like
other target organs by facilitating the genetic expressions
(31).

DMBA, on the other hand, accelerates tumor growth
with associated increases in serum growth hormone and
prolactin. DMBA seems to stimulate cellular division,
possibly by direct action in the nucleus on the genetic
activity which may not involve specifically hormonal pro-
duction. Since Dao et al. (8, 9), Koyama et al. (21), and
Sinha and Dao (34) have demonstrated that DMBA could
alter normal tissue in culture to induce neoplastic transfor-
DMBA and Estrogen Effects on Rat GH\textsubscript{T}umor

Table 1

Effects of E\textsubscript{2} and DMBA on tumor growth

GH\textsubscript{T}umor graft was transplanted to groups of rats receiving different treatments. The weights of their tumors were determined at 10 weeks after transplantation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
<td>Tumor wt\textsuperscript{(g)}</td>
</tr>
<tr>
<td>None</td>
<td>4</td>
<td>1.18 ± 0.77</td>
</tr>
<tr>
<td>E\textsubscript{2} alone</td>
<td>5</td>
<td>6.83 ± 10.60</td>
</tr>
<tr>
<td>DMBA alone</td>
<td>8</td>
<td>9.04 ± 5.51</td>
</tr>
<tr>
<td>DMBA + E\textsubscript{2}</td>
<td>9</td>
<td>20.33 ± 5.17</td>
</tr>
<tr>
<td>DMBA emulsion + E\textsubscript{2}</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} E\textsubscript{2}, estrogen (estradiol benzoate).

\textsuperscript{b} Rats without apparent tumor at sacrifice time were excluded.

\textsuperscript{c} Mean ± S.D.

\textsuperscript{d} \textit{p} < 0.05, as compared to nontreated group by Student’s \textit{t} test.

\textsuperscript{e} \textit{p} < 0.05, as compared to group treated with E\textsubscript{2} or DMBA alone.

Table 2

Effects of E\textsubscript{2} and DMBA on tumor transplantability

GH\textsubscript{T}umor graft was transplanted to groups of rats receiving different treatments. The data show the time of detecting palpable tumor in weeks after transplantation and the cumulative number of tumor-bearing rats/total number of rats receiving grafts in each group.

<table>
<thead>
<tr>
<th>Time after transplantation</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 4</td>
<td>Wk 5</td>
</tr>
<tr>
<td>None</td>
<td>1/5</td>
<td>4/5</td>
</tr>
<tr>
<td>E\textsubscript{2} alone</td>
<td>2/5</td>
<td>4/5</td>
</tr>
<tr>
<td>DMBA alone</td>
<td>5/9</td>
<td>9/9</td>
</tr>
<tr>
<td>DMBA + E\textsubscript{2}</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>DMBA emulsion + E\textsubscript{2}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} E\textsubscript{2}, estrogen (estradiol benzoate).

Table 3

Effects of E\textsubscript{2} and DMBA on body and viscera weights of control and GH\textsubscript{T}umor-bearing rats

The control groups were rats treated with E\textsubscript{2} and/or DMBA or not treated, as listed, but they did not receive GH\textsubscript{T}umor grafts whereas the GH\textsubscript{T} groups did. All rats were sacrificed 10 weeks after transplantation time.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Weight (g)\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>202.0 ± 5.6</td>
</tr>
<tr>
<td>No treatment</td>
<td>3</td>
<td>209.1 ± 8.2</td>
</tr>
<tr>
<td>E\textsubscript{2}</td>
<td>3</td>
<td>197.7 ± 7.0</td>
</tr>
<tr>
<td>DMBA</td>
<td>3</td>
<td>191.8 ± 17.6</td>
</tr>
<tr>
<td>DMBA + E\textsubscript{2}</td>
<td>4</td>
<td>217.0 ± 6.4</td>
</tr>
<tr>
<td>E\textsubscript{2}</td>
<td>5</td>
<td>239.8 ± 36.8</td>
</tr>
<tr>
<td>DMBA</td>
<td>8</td>
<td>232.9 ± 23.4</td>
</tr>
<tr>
<td>DMBA + E\textsubscript{2}</td>
<td>9</td>
<td>285.4 ± 13.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a} E\textsubscript{2}, estrogen (estradiol benzoate).

\textsuperscript{b} Mean ± S.D.

\textsuperscript{c} Significant difference (\textit{p} < 0.05) between control and GH\textsubscript{T} groups by Student’s \textit{t} test.

\textsuperscript{d} One rat had an exceptionally large tumor with proportionately increased body and viscera weights.
mation after transplantation to the host animals, and since there is evidence showing that DMBA binds to DNA (6, 23, 32, 38), it is very likely that the observed effect of DMBA on tumor growth is a direct stimulatory action on the affected cells rather than on the host animals.

The synergistic effect of estrogen and DMBA on tumor growth can be explained by their actions at different loci. It seems that the observed effects of DMBA and estrogen are mediated through different mechanisms, DMBA causing further neoplastic changes and estrogen stimulating hormonal secretion.

Since GH, tumor can be propagated in vivo and in vitro and maintains function by producing at least 2 specific hormones that can be measured quantitatively, it constitutes a useful model for the study of the mode of action of carcinogens. Nevertheless, since the overall tumor size varied considerably in our 2 experiments and may have been due to differences in the donor and recipients or other unknown factors, technical improvement to standardize such a model is necessary.

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