Photodestruction of Mouse Epithelial Tumors after Oral Acridine Orange and Argon Laser

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SUMMARY

The photosensitizing dye acridine orange selectively localized to mouse epithelial tumors after p.o. administration. Subsequent irradiation with a continuous-wave argon laser, at energy levels that did not cause heat damage, could produce partial or complete necrosis of these tumors.

INTRODUCTION

We report the use of the photosensitizing dye AO and a continuous wave argon laser to produce necrosis in mouse epithelial tumors.

AO, a diaminoacridine, combines with nucleic acids, both DNA and RNA (16); its well-known metachromatic nature is due to the formation of dye aggregates (16). The cytoplasm of living cells stains faintly (4, 16) at low dye concentrations, although there is evidence that AO is also concentrated in lysosomes (1, 18).

The photodynamic effect of acridine compounds was first reported by Oscar Raab in 1900 (13) and was studied in detail early this century by Tappeiner and Jodalbauer (15). In tissue culture cell death seemed to be dependent both on dye concentration and on the light energy to which the cells were exposed (8, 17).

Lewis and Goland (11) and Lewis et al. (12) reported in 1948 and 1946, respectively, that certain tumors in mice, including sarcomata and spontaneous mammary gland adenocarcinomata, were selectively stained after the mice were fed, p.o., various dyes including AO. They reported, further, that the administration of such aminoacridines could result in retardation of tumor growth (11, 12). More recently, the selective staining of malignant cells has resulted in the use of AO and fluorescence microscopy in exfoliative cytological diagnosis of cervical and vaginal cancer (3).

AO has an absorption peak at 492 nm in 1 μM aqueous solution, and forms at least 2 complexes in solution with both DNA and RNA, with absorption maxima at 465 and 502 nm (14). This behavior is probably modified somewhat in the more complex intracellular environment. The argon laser emits at 9 spectral lines at wavelengths ranging from 514.5 to 454.5 nm. This laser spectrum encompasses the maximum reported to produce chromosomal lesions in salamander lung tissue explants exposed to AO (2). Approximately 60% of the incident nonreflected light of these wavelengths is thought to penetrate the epidermis (6) and should therefore reach dermal tissues in sufficient intensity to activate a phototoxic dye present in a tumor. The maximum permissible skin irradiance level for human exposures to argon laser for up to 8 hr is 200 milliwatts/sq cm over areas of up to 25 cm (American National Standards Institute, Z-136-1, 1973.) At these irradiance levels, one need not be concerned with the pressure effects and nonlinear effects that may occur with the use of high energy-pulsed laser in cancer therapy (7, 9).

There have been reports of the use of photosensitizing agents and conventional light sources in experimental tumor destruction. Jesionek and Tappeiner (10) in 1903 claimed some success in the treatment of skin cancer using the photodynamic action of eosin, but then apparently abandoned the treatment. Recently, Diamond et al. (5) have used hematoporphyrin, which, like AO, is selectively taken up by tumor cells, with high-intensity light bulbs to induce incomplete destruction of gliomata implanted s.c. in the flanks of rats. Treatment with either light or hematoporphyrin alone had no effect.

We wanted to determine whether complete tumor necrosis without damage to surrounding tissues would occur if mice were fed AO, followed by argon laser exposure.

MATERIALS AND METHODS

To ensure that the combination of AO and argon laser irradiation was phototoxic to tumor cells, Ehrlich ascites cells were first studied. A suspension of 2 X 10⁶ washed cells per ml was incubated for 2 hr in the dark with AO in lactated Ringer’s solution. After incubation, the cells were washed twice before being resuspended in lactated Ringer’s solution. Following irradiation, the number of dead cells was assessed by staining with the nonvital dye, trypan blue.

Epithelial tumors were induced in inbred male C3H mice weighing between 25 and 30 g by the twice-weekly application of 100 μl of 0.66% benzo(a)pyrene in toluene. Six to 8 weeks after the 1st application, acanthomas began to appear, many of which subsequently developed into invasive ulcerating carcinomas. Benign epithelial tumors showing hyperkeratosis, acanthosis, and papillomatosis were defined as acanthomas; malignant epithelial tumors of squamous cell type, with

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3 The abbreviation used is: AO, acridine orange.

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invasion of the dermis and cytological features typical of cancer, were designated as carcinomas. The carcinogen was discontinued after a tumor appeared.

Tumor-bearing mice force-fed 6 mg/day for 3 days with AO, Color Index 46005, in corn oil showed localization of the dye in their tumors which stained yellow-orange. Normal skin was not stained, and a sharp margin was visible between the stained tumor and normal skin. Control animals that were not fed AO showed no tumor staining. Fluorescence microscopy confirmed the presence of dye in the tumors. A bright fluorescence was noted in the tumor tissue and little in normal skin. Necrotic areas of tumors were noticeably unstained. Dye fluorescence could also be observed in salivary glands, intestines, seminal vesicles, bladder, and prostate.

A group of 6 tumor-bearing mice were fed 15 mg AO daily for 4 days and kept in the dark. The tumor growth was compared over this period with that in a control group matched for tumor size. The mean increase in tumor size in the AO-fed group (34% of original size) was not significantly different from that in the control group (20%), as assayed by a pooled t test.

Twenty tumor-bearing mice were divided into 2 groups, closely matched for tumor size. One group was force-fed 5 to 25 mg daily of AO in corn oil for 3 to 5 days prior to irradiation. The mice in the other group served as unfed controls and were exposed to the laser alone. Unanesthetized mice were immobilized in a plastic cylinder with a central opening to allow exposure of the tumor and surrounding normal skin during irradiation. A Coherent Radiation Model 53 argon ion laser was used. This laser produced a beam in 8 spectral lines between 457.9 and 514.5 nm. The horizontal 1.5-mm diameter beam was expanded by a diverging lens (focal length, 250 mm) and then deflected by a mirror into an 8X beam-expander telescope. Apertures were used to select the central-most uniform part of the beam. Apertures were used so that, in each case, the field irradiated included the entire tumor and a margin of normal skin 3 mm in width. The irradiance was controlled by changing the distance of the diverging lens from the expanding telescope. The irradiance was measured with an Eppler three-eighth inch in diameter thermopile and was found to be uniform across the irradiated field and stable in time to within 10%. The surface temperatures of tumors and adjacent skin were measured with a BLH Electronics, Inc., HT, Type TCC-IS-200 microminiature thermocouple.

Mice were sacrificed 48 hr after irradiation, and tissues were processed by routine histological methods to determine both the degree of tumor necrosis histologically, and the state of adjacent noncancerous skin. Where tumor destruction was incomplete, we took a section through the center of the tumor and estimated histologically the depth of destruction, which was then expressed as a percentage of the total vertical dimension of the tumor.

RESULTS

Irradiation of Ehrlich ascites cells for 5 min at irradiances up to 480 milliwatts/sq cm or incubation with AO in the dark did not affect the viability of cells with respect to controls over the next 21 hr. Cells incubated with 38 μm AO and subsequently exposed 10 milliwatts/sq cm argon laser for 5 min were noted to be 95% viable with respect to controls 0.5 hr after exposure, and 53% were viable at 21 hr. Laser irradiances of 20, 100, and 250 milliwatts/sq cm progressively produced more rapid cell death and a higher percentage of dead cells at 21 hr. Lower AO concentrations required a higher laser exposure to produce the same percentage of cell death (Table 1).

The results of tumor irradiation at 3 different laser irradiances in the 20 tumor-bearing mice are shown in Table 2. Seven mice with 7 tumors, i.e., 2 acanthomas and 5 carcinomas, showed partial to complete tumor necrosis after p.o. administration of AO and laser irradiances (65 and 165 milliwatts/sq cm) that did not cause heat necrosis of normal surrounding tissue or necrosis of tumor in control mice not fed AO. Irradiation at 65 milliwatts/sq cm for 1 hr produced no histological change in 3 tumors on mice not fed AO or in 2 tumors on mice fed a total of 15 mg AO over the preceding 3 days, whereas feeding a total dose of either 30 or 75 mg AO over a 3-day period resulted in necrosis of part of the tumor or complete tumor necrosis after irradiation, with sparing of surrounding tissue (Figs. 1 and 2).

Hour-long irradiation at 165 milliwatts/sq cm also produced no histological evidence of necrosis in tumors on 3 mice that had not received AO. Partial to complete tumor necrosis was observed in mice fed 75 mg AO over 5 days prior to irradiation. Hour-long irradiation at 280 milliwatts/sq cm usually produced complete necrosis of tumors, whether or not the animal was fed AO.

The resting surface temperature on the tumors and adjacent skin was 32—34°C. Irradiation at 65 milliwatts/sq cm resulted in a surface temperature rise to 36—37°C, 165 milliwatts/sq cm resulted in a rise to 42—44°C, and 280 milliwatts/sq cm resulted in a rise to 54—56°C.

The normal skin of 3 pairs of nontumorous mice was

<table>
<thead>
<tr>
<th>AO concentration (μM)</th>
<th>% viability with laser irradiance (milliwatts/sq cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.38</td>
<td>100 (100) 100 (100) 100 (100)</td>
</tr>
<tr>
<td>3.8</td>
<td>100 (100) 100 (90)</td>
</tr>
<tr>
<td>11.5</td>
<td>100 (83) 100 (57)</td>
</tr>
<tr>
<td>38.0</td>
<td>100 (53) 100 (50)</td>
</tr>
</tbody>
</table>

Table 1

Percentage viability at 0.5 hr (and 21 hr) after irradiation of Ehrlich ascites cells incubated with AO and exposed to the argon laser for 5 min at different irradiances

In each case, viability is compared with control cells incubated with AO but not irradiated. Values between 95 and 105 are recorded as 100; this is consistent with the statistical error of the counting procedure.
Table 2

<table>
<thead>
<tr>
<th>Laser dose (milliwatts/sq cm for 1 hr)</th>
<th>AO dose (mg)</th>
<th>Histological type of tumor</th>
<th>Dimensions of tumors before irradiation (length x width x height in mm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>None</td>
<td>Acanthoma</td>
<td>2 x 2 x 2.5</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>Acanthoma</td>
<td>7 x 9 x 4</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>Carcinoma</td>
<td>8 x 6 x 2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5 x 3 (15)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Acanthoma</td>
<td>2 x 1.5 x 2</td>
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<td>None</td>
</tr>
<tr>
<td>5 x 3 (15)</td>
<td>Acanthoma</td>
<td>2.5 x 3 x 2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10 x 3 (30)</td>
<td>Acanthoma</td>
<td>3 x 1.5 x 1.5</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>10 x 3 (30)</td>
<td>Acanthoma</td>
<td>4 x 4 x 2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>25 x 3 (75)</td>
<td>Carcinoma</td>
<td>10 x 11 x 4</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>None</td>
<td>Acanthoma</td>
<td>4 x 4 x 3</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>Acanthoma</td>
<td>6 x 4.5 x 2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>Carcinoma</td>
<td>6 x 6.5 x 3</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>15 x 5 (75)</td>
<td>Carcinoma</td>
<td>4 x 7 x 2.5</td>
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<td></td>
</tr>
<tr>
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<td>Carcinoma</td>
<td>9 x 8 x 3</td>
<td>95</td>
<td></td>
</tr>
<tr>
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<td>Carcinoma</td>
<td>10 x 10 x 4</td>
<td>75</td>
<td></td>
</tr>
<tr>
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<td>8 x 9 x 4</td>
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<td></td>
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<tr>
<td>280</td>
<td>None</td>
<td>Carcinoma</td>
<td>5 x 5.5 x 2</td>
<td>Complete</td>
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<td>Carcinoma</td>
<td>8 x 7 x 6.5</td>
<td>Complete</td>
<td></td>
</tr>
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<td>12 x 12 x 5.5</td>
<td>75</td>
<td></td>
</tr>
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<td>Carcinoma</td>
<td>5 x 4 x 1.5</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>15 x 5 (75)</td>
<td>Carcinoma</td>
<td>9 x 9 x 2</td>
<td>Complete</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, number of days.

Fig. 1. Radiation, 65 milliwatts/sq cm; 75 mg AO. Squamous cell carcinoma with complete necrosis. The base of the tumor was invading muscle tissue. H & E, X 25.

irradiated at 65, 165, and 280 milliwatts/sq cm, respectively, for 1 hr. One mouse of each pair was fed 75 mg AO (15 mg for 5 days). No gross or microscopic changes were seen at 48 hr in the mice irradiated at 65 milliwatts/sq cm. Irradiation at 165 milliwatts/sq cm caused slight inflammatory changes in the dermis and s.c. fat. Irradiation at 280 milliwatts/sq cm caused moderate coagulation necrosis of epidermis, dermis, and s.c. fat, the changes being slightly less in the mouse not fed AO.

DISCUSSION

In this preliminary study, we have shown that the
photosensitizing dye, AO, selectively localizes in mouse epithelial tumors. Mouse tumors, including invasive squamous cell carcinoma, can be destroyed in animals fed AO p.o. in conjunction with argon laser irradiance (65 milliwatts/sq cm), which causes neither significant temperature elevation nor histological damage to normal skin or to the tissues surrounding the tumor. Within a limited dose range and at lower irradiance, tumor destruction appears to be dependent on dye dosage. As the laser irradiance was increased to 165 and 280 milliwatts/sq cm, histological changes were apparent in normal skin, presumably at least partly due to thermal effects.

This phototoxic system may eventually prove useful in cancer therapy both because of the ability to selectively destroy skin tumors and the present ability to deliver the argon laser beam via fiber optic systems to internal tumors.

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REFERENCES

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