Effect of Dihydroxyanthraquinone (NSC 279836) and Thoracic Irradiation on Long-Term Survival of Rats

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ABSTRACT

Dihydroxyanthraquinone (DHAQ; NSC 279836) is a recently synthesized compound that is structurally similar to Adriamycin and produces greater antitumor effects in murine model systems. We compared DHAQ to Adriamycin in rats, with and without irradiation of the chest at various intervals after drug treatment. A single injection of Adriamycin (1 mg/kg i.p.) had little effect on animal survival, even if combined with radiation (12 Gy 25 MV X-rays), >90% being alive at 1 year. A single injection of DHAQ (3 mg/kg i.p.) was equally ineffective up to 200 days after treatment (survival, >90%). However, between 200 and 370 days after treatment, all animals died, producing a median survival time of 280 days. Further, when DHAQ was combined with radiation, there was an increase in animal deaths between Days 30 and 200. For animals irradiated on Days 0, 43, and 93 after DHAQ treatment, only 50, 75, and 80%, respectively, survived to Day 200. All animals that survived past Day 200 subsequently died by 1 year, displaying the same kinetics of lethality as those animals that had received DHAQ only. A repeat experiment using DHAQ at 1 mg/kg produced similar results. Based on these findings, we conclude that DHAQ produces a long-term (>200 days) toxicity in rats that is not detectable by short-duration toxicity screening. In addition, radiation enhances short-term (<200 days) lethality, with the degree of enhancement decreasing as the interval between drug and radiation is increased.

INTRODUCTION

DHAQ3 (NSC 279836) is a newly synthesized compound (18) that has been shown to be an effective antitumor agent in a number of murine tumor model systems (2, 14, 18). Presently, the drug (in the form of the dihydrochloride salt; mitoxantrone; NSC 301739) is entering Phase II clinical investigations (13). Since DHAQ is structurally related to but not an analog of the DNA-intercalating antibiotics ADR and actinomycin D, care must be taken to avoid a repetition of the severe complications that developed from the early use of these chemotherapeutic agents. DHAQ has been shown in a rat model to cause less cumulative chronic cardiotoxicity than ADR (2). Although the combination of DHAQ and radiation therapy is effective against the L1210 murine leukemia system (6) and the Walker 256 fibrosarcoma (12), the long-term effects of this combination on normal tissues have yet to be resolved (9). Since it is possible that DHAQ may one day be used in conjunction with radiation therapy in the clinic, it seems prudent to establish a set of guidelines by which to estimate the potential for interaction of these 2 modalities for the production of normal tissue toxicity. In this study, we have investigated the effects on rat normal tissues of DHAQ, alone and in combination with ionizing radiation, and have compared the effects of these treatments to similar treatments with ADR.

MATERIALS AND METHODS

Male Sprague-Dawley rats were used to investigate the long-term effects of treatment with DHAQ, ADR, or radiation. Rats (175 to 200 g) were obtained from Sasco Industries, Omaha, Neb., quarantined for at least 2 weeks to assure freedom from disease, and then housed in sterile filtered cage racks for the duration of the experiment. DHAQ was either obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, or was synthesized in the Drug Development Laboratory of the Mid-American Cancer Center. The latter was used to assure that there had been no loss of activity during storage and shipment. ADR was obtained as commercially available from Adria Laboratories, Wilmington, Del. Drugs were dissolved in HBSS such that a volume of 1 ml (in the first experiment) or 5 ml (second experiment) was injected i.p. to deliver a dose of 3.0 or 1.0 mg DHAQ per kg body weight, respectively, or 1.0 mg ADR per kg. Control animals were given injections of HBSS only.

For X-irradiation of the thoracic cavity, 25 MV X-rays from a 40 MeV Sagittaire linear accelerator were used. Rats were lightly anesthetized with sodium pentobarbitol (Nembutal, i.p.) to insure immobility during the irradiation. The rats were positioned 5 at a time so that only the thoracic cavity was irradiated (a 4-cm-wide strip). The entire thoracic cavity was irradiated to assure that the heart and lungs were within the radiation field. The remainder of the rat was shielded by the collimator midline dose. Since a dose rate of 4 Gy/min and a total dose of 12 Gy was utilized, animals were not anesthetized for more than 10 min, thus minimizing any potential effects of the anesthesia itself on the development of long-term toxicity. Animals were periodically examined for any gross physical changes in size, hair coloration and texture, skin ulcerations, etc. Chest radiographs of representative animals were taken prior to sacrifice of animals for histological examination. Survival of animals was monitored daily and deaths were recorded. Median survival times were calculated and compared for statistical significance using a Gehan-Wilcoxon test for singly censored samples (4) and a modified t test (15). Animals that died (or were sacrificed because they appeared to be in the terminal stages) were also autopsied. Critical organs (heart, lungs, liver, esophagus, trachea) were grossly examined at autopsy, and any external abnormalities were noted and included in the specimens processed for histological examination.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DHAQ, dihydroxyanthraquinone; ADR, Adriamycin; HBSS, Hanks’ balanced salt solution.

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1 OneGy = 100 rad.
RESULTS

Prior to the investigation of the combined effects of DHAQ, ADR, and/or radiation, it was necessary to determine the responses of critical tissues to the single agents and to determine the treatment levels that could be tolerated by the animals. For determination of the optimum ADR dose, rats were given single i.p. injections of ADR at either 1.0 or 10 mg/kg. The higher concentration of ADR produced acute toxicity, with only 20% of the animals surviving past 65 days. During this same period, 90% of the animals receiving the lower dosage survived. Based on this result, the dose of 1.0 mg/kg was chosen for subsequent experimentation. Similarly, animals were given single i.p. injections of DHAQ (1, 3, 10, and 100 mg/kg). At 30 days after drug treatment, all animals in the 2 lower dosage groups were alive while all animals in the 2 higher dosage groups were dead. Based on this result, a standard dose of 3.0 mg/kg was chosen for future studies with DHAQ. Finally, the thoracic cavities of rats (including both the heart and the lungs) were irradiated with various doses, ranging from 10 to 30 Gy. A dose of 12 Gy was chosen as a dose that would produce a minimum of acute lethality while allowing for the development of long-term normal tissue damage.

Having determined permissible single doses of radiation, ADR, and DHAQ, we evaluated the toxicity produced by a combination of the 2 treatment modalities. On Day 0, animals received either HBSS, ADR (1.0 mg/kg i.p.), or DHAQ (3.0 mg/kg i.p.). Animals either were not treated further or else received 12 Gy X-rays to the thoracic cavity on Days 0, 43, 93, or 199 after drug treatment. Each treatment group originally consisted of 12 animals. However, all animals receiving a particular drug were considered as being drug treated only until they had received their subsequent irradiation. At 14 months after initiation of treatment, the following observations were made. No acute toxicity, i.e., lethality, was observed in the rats that received ADR and/or radiation, demonstrating that the ADR and radiation dose levels chosen were indeed tolerable in a large series of animals. In the group that received ADR alone, the first death was not recorded until Day 302 after drug administration; only one other death has occurred subsequently (Day 380). Irradiation alone produced a survival pattern similar to that of ADR alone, regardless of whether the irradiation was performed on Day 0 or at 1.5, 3, or 6 months after the initiation of the experiment. An average of 2 of 12 animals/treatment group died over the course (450 days) of the experiment. An additional trend was observed in that deaths occurred sooner after irradiation when animals were older at the time of irradiation. For example, deaths after irradiation at the initiation of the experiment (animals 3 months old) occurred on Days 250 and 335 after irradiation, whereas for those animals receiving irradiation 6 months after the start of the experiment (9 months old at irradiation) deaths occurred on Days 283 and 333 of the experiment (84 and 134 days after irradiation). When these 2 modalities were combined (ADR on Day 0 and X-irradiation at 0, 1.5, 3, or 6 months after drug administration), no additional lethality beyond that produced by the single agents was observed.

The results obtained with DHAQ were in striking contrast to the above results with ADR or radiation. A single injection of DHAQ was equally nontoxic (survival, >90%) as ADR up to 200 days after drug treatment. However, between 200 and 370 days after treatment, all animals died, producing a median survival time of 280 days (Chart 1). Further, when DHAQ was combined with radiation, there was an increase in the number of animal deaths between Days 30 and 200. For animals irradiated on Days 0, 43, and 93 after DHAQ treatment, only 50, 75, and 80%, respectively, survived to Day 200. All animals that survived past Day 200 subsequently died by 1 year, displaying the same kinetics of lethality as those animals that had received DHAQ only. For animals irradiated on Day 199 after DHAQ treatment, there was appreciable immediate toxicity associated with the anesthesia and/or the irradiation; 5 of 11 animals did not regain consciousness after the procedure. By comparison, 2 of 12 and 3 of 12 animals irradiated 199 days after treatment with HBSS or ADR, respectively, were also lost. The number of animals lost per group was not statistically different (p > 0.25).

An analysis of difference between median survival times was performed using a Gehan-Wilcoxon test (4). As shown in Table 1, median survival was decreased following DHAQ plus irradiation at Day 0 or 43, compared to that following treatment with DHAQ alone or DHAQ plus irradiation at Day 93 or 199. There were statistically significant differences (p < 0.05) of the median survival times between the controls and all animals that received DHAQ, between animals irradiated at specific times and animals that had received DHAQ and then been irradiated at the same times, and between DHAQ-treated animals irradiated either at Day 0 or 43 compared to those irradiated at Day 93 or 199. Since ADR was basically without effect on survival, the same results were obtained if ADR-treated animals replaced HBSS-treated animals in the comparison to DHAQ-treated animals.

Because of the severe lethality produced by DHAQ in the above experiment, we repeated the DHAQ ± radiation arm of the experiment using a lower dose of DHAQ (1.0 mg/kg) so as to obtain a direct comparison to ADR. A similar response to that shown in Chart 1 was obtained but with slightly different

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**Chart 1.** The survival of rats as a function of time after treatment with DHAQ and/or radiation. On Day 0, animals received a single i.p. injection of DHAQ (3.0 mg/kg). At times thereafter, animals received 12 Gy X-ray to the thoracic cavity. The vertical lines represent a decrease in the percentage of survival because of death of an animal; the diagonal lines to a point represent an apparent decrease in survival due to the sacrifice or other censor of an animal at that time.
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Analysis of median survival times: Experiment 1

| Treatment groups          | Median survival time (days) | Statistical differences (p) between treatment groups 
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&gt;400</td>
<td></td>
</tr>
<tr>
<td>X-ray (at Days 0, 43, 93, or 199)</td>
<td>&gt;400</td>
<td></td>
</tr>
<tr>
<td>DHAQ (3 mg/kg)</td>
<td>279</td>
<td></td>
</tr>
<tr>
<td>DHAQ + X-ray at Day 0</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>DHAQ + X-ray at Day 43</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>DHAQ + X-ray at Day 93</td>
<td>343</td>
<td></td>
</tr>
<tr>
<td>DHAQ + X-ray at Day 199</td>
<td>301</td>
<td></td>
</tr>
<tr>
<td>Control vs. DHAQ</td>
<td></td>
<td>&lt;0.0025</td>
</tr>
<tr>
<td>Control vs. DHAQ + X-ray at Day 0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Control vs. DHAQ + X-ray at Day 43</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Control vs. DHAQ + X-ray at Day 93</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>X-ray at Day 0 vs. DHAQ + X-ray at Day 0</td>
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<td></td>
</tr>
<tr>
<td>X-ray at Day 43 vs. DHAQ + X-ray at Day 43</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>X-ray at Day 93 vs. DHAQ + X-ray at Day 199</td>
<td>&lt;0.025</td>
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</tr>
<tr>
<td>DHAQ + X-ray at Day 0 vs. DHAQ + X-ray at Day 93</td>
<td>&lt;0.05</td>
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</tr>
<tr>
<td>DHAQ + X-ray at Day 0 vs. DHAQ + X-ray at Day 199</td>
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<td></td>
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<tr>
<td>DHAQ + X-ray at Day 43 vs. DHAQ + X-ray at Day 199</td>
<td>&lt;0.05</td>
<td></td>
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</tbody>
</table>

* Determined by Gehan-Wilcoxon test for singly censored samples.

Not significantly different because of excessive loss of animals during anesthesia for X-irradiation, resulting in small sample size. However, if the comparison is expanded to include 2 other groups that were irradiated simultaneously with the 2 in question, then the DHAQ-treated animals differ from all other animals irradiated at Day 199 (p < 0.025).

chronic cardiotoxicity in rats but at an accumulated dose greater than that required for ADR (2). Combined with a greater antineoplastic activity, this improved therapeutic benefit prompted continued investigation, both clinically and in the laboratory. The overall similarity of DHAQ to DNA-intercalating antibiotics such as ADR, actinomycin D, and lucanthone should aid in the design of protocols to test whether DHAQ will ultimately provide an adequate replacement for ADR in the treatment of cancer. DHAQ also has the advantage that it is relatively inexpensive and easy to synthesize and separate in comparison to the laborious isolation and purification steps required for antibiotics of microbiological origin. The greater cytotoxic and antitumor activity (as a function of concentration) of DHAQ compared to ADR may yet be an advantage despite any associated greater effect on critical normal tissues. Recently, the dihydrochloride salt of DHAQ (mitoxantrone) has passed clinical Phase I testing (13) and is now entering Phase II studies. It is also likely that clinical trials will eventually be conducted utilizing DHAQ in combination with radiation therapy. To date, only a few isolated incidences of patient cardiotoxicity have been reported, and these after full courses of ADR and other cancer chemotherapy agents. However, the possibility of long-term cardiotoxicity should not be dismissed. A relative lack of early acute cardiotoxicity is no assurance that long-term potentially fatal cardiomyopathy will not develop.

Based on our results, we conclude that DHAQ produces a long-term (>200 days) toxicity in rats that is not detectable by short-duration toxicity screening (1, 10, 16, 17). In addition, radiation enhances short-term lethality, with the degree of enhancement decreasing as the interval between drug and radiation is increased. At present, the critical tissue for this DHAQ-induced lethality has not been identified, but further investigations are underway to elucidate the mechanism of both the short-term and the long-term toxicity associated with the combination of DHAQ and radiation. While these in vivo investigations into the interaction of DHAQ and ionizing radiation on rat normal tissues cannot be extrapolated directly to a prediction of response in patients, it is nevertheless evident that DHAQ possesses the potential disadvantages of ADR and other DNA-intercalating agents. For this reason, appropriate precautions should be considered in the design of clinical trials to test the efficacy of DHAQ or other new anthracyclines proposed as anticancer agents, particularly in patients that have previously received known cardiotoxic drugs and/or thoracic irradiation.

ACKNOWLEDGMENTS

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REFERENCES

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