The Enhanced Response of the Ridgway Osteogenic Sarcoma to Roentgen Radiation Combined with Actinomycin D

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SUMMARY

A series of experiments is reported in which X-radiation, actinomycin D, and their combination were used in the treatment of mice bearing the Ridgway osteogenic sarcoma. This tumor responds well to treatment with either of the 2 modalities; "therapeutic synergism," as judged by tumor inhibition and increase in survival time, is obtained with combination therapy. The augmentation of therapeutic response was found to be independent of the sex of the tumor-bearing animal, the order in which the 2 treatments were given, the time interval up to 24 hr which separated the 2 treatments and the parenteral route used for the administration of actinomycin D. Retreatment with the combination during a period of tumor regression occasioned by a first course led to a high percentage of "cures" in animals thus treated.

Roentgen ray reactions in normal human skin and mucous membrane are enhanced in some individuals by actinomycin D (7, 40). This agent sometimes produces an early erythema after skin doses of 500 r or less, whereas, usually, doses of 3—4 times this amount of X-radiation alone are required to produce a similar response. Augmentat.ion of effects also has been demonstrated, both grossly and microscopically, in normal mouse and rat tissues (5, 16, 24, 26). Bases (1), Chan and Liebner (4) and Elkind et al. (8), employing tissue culture methods, have shown that there is an increased response to the combination of these agents. The effect of combined therapy on transplantable human and animal tumors in the hamster has been reported by Handler (18).

The response of malignant neoplasms in mice to combined treatment with actinomycin D and X-radiation was investigated in the series of experiments here recorded, which employed the Ridgway osteogenic sarcoma. Parts of this study have been reported in preliminary form in prior communications (5—7, 25—27).

MATERIALS AND METHODS

General.—Exploratory studies were made with the Cloudman malignant melanoma (S91) and the DBBRB mammary adenocarcinoma, both in the DBA/1 mouse; the P1534 lymphatic leukemia in the DBA/2 strain; and the Ridgway osteogenic sarcoma in the AKR inbred or AKR/J x DBA/2J F1 (AKD2F1) hybrid mouse. The ROS was found to be the most suitable for the purposes of this study, and all the results reported herein are based on experiments employing this tumor.

Transplant technic.—The tumors were maintained by serial passage in stock AKR mice. With aseptic technics, the neoplasm was harvested at the end of 3—4 weeks and passed through a tumor press. It was then diluted with sterile saline until the consistency allowed easy aspiration into a 1-ml tuberculin syringe. The site of injection was painted with tincture of iodine and alcohol before and after the introduction of 0.05—0.1 ml through a No. 18 gauge needle into the lateral aspect of the muscles of the right hind leg.

Animal data.—Animals of one sex, averaging 18—20 gm in weight, were used in any one experiment. They were housed in groups of not more than 10 to a cage. Approximately 21 days after implant, the animals were sorted so that the average tumor size was the same in all experimental groups. Tumor area (caliper measurements) was used as the index for tumor size. All animals were fed Ralston Purina mouse chow, with water, ad libitum. They were dipped, initially, in Aramite-15W (United States Rubber Co., Naugatuck, Conn.), (4 teaspoons/gallon of water), and were given a 5% solution of dy-piperazine (David Yellen Co., Canton, Mass.) for 12 hr in the drinking water. These procedures were routine on receipt of the animals and were repeated if parasite infestation occurred. The animal quarters were air-conditioned.

The following abbreviations are used: ROS, Ridgway osteogenic sarcoma; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.
and temperature and humidity were rigidly controlled at 74°F and 55%, respectively.

Actinomycin D preparation and administration.—Crystalline actinomycin D (Lederle) was dissolved in 95% alcohol to give a stock solution of 2 mg/ml. This was then diluted with normal saline to a final concentration of 15 μg/ml. The individual animals were then weighed, and the material was administered by a parenteral route on a dose-per-weight basis, either at 75 or 150 μg/kg, depending upon the individual experiment.

Irradiation technic.—Roentgen radiation was given with a Philips machine and with the use of the following factors: 250 kv (constant potential), 1 mm of Al added filter (HVL = 0.63 mm Cu), 15 ma, 40 cm target-to-skin distance, output 228 r/min. In some experiments, the entire rear half of the mouse was treated; in others, only the right hind leg was included in the beam. All animals were protected against back scatter by 2-mm lead shields topped with 0.5 cm of Masonite. Some of the experiments were conducted with the animals under light anesthesia induced with veterinarian Nembutal at a dose of 0.06 mg/gm (weight).

Statistical method.—Mean survival for each group was computed in the usual way: long term survivors ("cures") were cut off as of the date of termination of the experiment. Tumor growth rate was calculated for each animal (difference between initial tumor size and final tumor size divided by the survival time). All groups were tested against each other with Student's t test (38) for both survival and tumor growth rate.

EXPERIMENTAL PROCEDURES AND RESULTS

EXPERIMENT 1

This experiment was designed to test the effect of 2 courses of treatment given to animals, starting 21 and 44 days after implantation, as compared to that produced by a single course on the 21st day.

Procedure.—The study employed 7 groups of 8 animals each and was divided into 2 parts. One group received no treatment and served as control for both parts, which were designed as follows: In Part A, Group A1 was treated with actinomycin D (75 μg/kg i.p. on each of 3 successive days); Group A2 was treated with X-ray (400 r in air on each of 3 successive days); and Group A3 was treated with X-ray plus actinomycin D (doses and schedule as in Groups 1 and 2). In Part B, Group B1 was treated as A1, except that a 2nd course of actinomycin D was started on the 44th day; Group B2 was treated as A2, except for a 2nd course of X-ray therapy starting on the 44th day; and Group B3 was treated as A3, except for a 2nd course of combined therapy starting on the 44th day. The tumors were measured 3 times a week, and the animals were followed for survival.

Results.—Part A.—Both X-ray therapy and actinomycin D produced increased survival and regression of tumors in the animals so treated (Chart 1). The mice receiving the combination showed the most prolonged survival and the greatest suppression of tumor growth, as compared to those receiving either modality alone. Survival data were clouded by deaths caused by an intercurrent perianal...
infection—present in animals of all groups—that caused the deaths of some of the Group 4 animals that were free of tumor at the time.

Part B.—Actinomycin D retreatment led to no major improvement in survival rate or tumor regression (Chart 2). X-ray retreatment led to a 2nd reduction in tumor size and extended survival. In the combination Group B3, the tumors disappeared after initial treatment, as they did in the X-ray group. No recurrence was noted in any of the animals before the 2nd dose was given, and none became manifest thereafter. Four animals survived and were free of tumor on the 122nd day, when the experiment was terminated. All deaths in Group B3 were attributed to the perianal infection previously mentioned.

Actinomycin D was given about 6 hr after X-ray treatment in the studies described above. Another set of experiments was then performed to assess the effect of reversing the sequence of the 2 treatments and varying the time interval between them. In the 2nd part of this experiment, the influence of sex factors on response of the tumor to treatment also was investigated.

Experiment 2

Procedure.—On the 21st day after implantation, 54 AKD2F1 mice were divided into 6 groups of 9 animals each. Group 1 received no treatment. Group 2 was treated with actinomycin D (150 µg/kg i.v., once). Group 3 was treated with X-ray (1000 r in air, once). Group 4 was treated with actinomycin D 6 hr after radiotherapy. Group 5 was treated with the combination “simultaneously”; i.e., actinomycin D followed radiotherapy by not more than 10 min. Group 6 was treated with X-ray 6 hr after actinomycin D. The doses given to Groups 4, 5, and 6 were the same as those for Groups 2 and 3.

Results.—It was again shown that combined treatment produced significantly better results than X-ray therapy or chemotherapy alone, but no significant difference in tumor growth or survival could be detected between any of the combined treatment groups.

Experiment 3

Procedure.—Ten male and 10 female AKD2F1 mice were used in each of 8 groups, as follows: Group 1 received no treatment. Group 2 was treated with the combination “simultaneously”; i.e., X-ray (775 r in air, once) preceded actinomycin D (150 µg/kg i.p., once) by not more than 10 min. Group 3 was treated with actinomycin D 6 hr before radiotherapy. Group 4 was treated with actinomycin D 6 hr after radiotherapy. Group 5 was treated with actinomycin D 12 hr before radiotherapy. Group 6 was treated with actinomycin D 12 hr after radiotherapy. Group 7 was treated with actinomycin D 24 hr before radiotherapy. Group 8 was treated with actinomycin D 24 hr after radiotherapy.

The doses given to Groups 3–8 were identical with those for Group 2. In Group 7, actinomycin D was given i.v. rather than i.p. It is believed that the difference in parenteral route did not introduce a significant variable, in view of the 24 hr which elapsed between treatments.

Results (Charts 3, 4).—Tumor growth rate.—The female mice receiving radiotherapy 6 hr before actinomycin D (Group 4) showed a significantly different tumor growth rate. Tumors in these animals grew more slowly than those of female mice treated with X-ray 12 hr before actinomycin D (Group 6) (P < 0.05) and those irradiated 24 hr after actinomycin D (Group 7) (P < 0.01). No other significant differences were encountered within or among any of the treatment groups. Tumor growth rate of female controls (Group 1) was slightly faster than that of the males (P < 0.05).

Survival.—There were large variances in the survival times encountered in some of the groups, especially the males of Groups 3, 6, and 7 and the females of Group 4. A statistically significant difference (P < 0.01) was en-
CHART 3.—Experiment 3. The results of variation in time interval between administration of actinomycin D and X-ray, reversal in sequence of treatment, and influence of sex. Agents used were actinomycin D at 150 μg/kg, i.p., once, and X-ray at 775 r (air) once. The ROS was implanted in AKD2F1 mice. The use of a time interval (6 hr) is compared with "simultaneous" administration. Mean tumor sizes, in sq cm, are represented by bars, number of surviving mice by broken lines. There were 10 mice per group.

CHART 4.—Experiment 3. Results of 12- and 24-hr time interval studies.
encountered only between males and females of Group 7. Within and among all other groups, no differences could be detected statistically.

Comment.—There were several early deaths because of diarrhea among the female mice of Group 4, the only treated animals with a different tumor growth rate. Five of the 10 animals died during the period of complete tumor regression, and normal variations within the small remaining sample could account for the unusual pattern of tumor growth.

Conclusion.—The effect of combined therapy appears to be largely, if not completely, independent of: (a) sex; (b) the order in which the 2 treatment modalities are administered; (c) the time interval—ranging from 10 min to 24 hr—which separates the 2; and (d) the parenteral route by which actinomycin D is administered.

DISCUSSION

The actinomycins, initially isolated in Streptomyces cultures by Waksman and Woodruff (42) are presently a vital part of the armamentarium of the experimental biologist as well as the cancer chemotherapist. The carcinolytic activity against mouse tumors, demonstrated by Hackmann (17) and by Farber and associates (9, 10, 12) has been documented in various laboratory animals (18, 39) and in man. Many of the early trials in humans were conducted in European clinics, where actinomycin C was used. This experience was reviewed by Begemann in reporting his own clinical studies with this agent (2). Actinomycin D has also been found to be effective in the management of certain human cancers (10-13, 29, 30, 36, 40, 43).

The mechanism of action of actinomycin D has been investigated by numerous workers (14, 19, 21-23, 32, 37). Several discussions and reviews of this subject have appeared (3, 20, 28, 32, 34). It is now well established that actinomycin D binds with DNA and blocks DNA-dependent RNA synthesis.

It has been shown in several experimental systems that use of combined actinomycin D and X-radiation produces an enhanced effect (1, 4, 7, 16, 18). Elkind and colleagues (8), using tissue culture techniques, have found that actinomycin D interferes with recovery processes in X-irradiated mammalian cells.

It is known that higher doses of either X-radiation (G. J. D’Angio and C. L. Maddock, unpublished data) or actinomycin D (39) are effective in complete control of the ROS.

Whether use of the combination at lower doses results in mere summation of effects (“addition”) or something more than this (“potentiation”) is extremely difficult to assess. The statistical complexities inherent in analyses of this kind are many, as are the pharmacologic difficulties (15, 35, 41).

Dose-response curves for each of the two modalities and for their combination are necessary to establish, or to exclude, “potentiation.” Appropriate studies have been in progress in these laboratories for several years. Until this question becomes clearer, it seems best to apply the term “therapeutic synergism” (15, 41) to the combined effect here reported. By this is meant a better therapeutic result obtained by 2 or more agents in combination than by any one alone, without specifying whether the better result is one of “addition” or “potentiation.” Accepting this definition, “therapeutic synergism” has also been found by Ranner and Griem’s observations (31) on the lack of response to actinomycin D of rats bearing the Walker 256 carcinoaoma, a response that was in no way improved when 2500 r (air) was given 1 to 4 hr after the i.p. administration of 150 µg/kg of this agent.

In all the experiments reported here, the doses of actinomycin D and of X-radiation were lower than those required for total control of the ROS by either agent alone. At these lower dosage levels, better results in survival and tumor regression were obtained after combined therapy. In fact, “cures” were achieved not infrequently, especially among animals receiving a 2nd course of combined therapy during a period of tumor suppression.

With these laboratory data in mind, it is perhaps no idle hope that a continuing search for optimum treatment regimens might succeed in pointing the way to a clinical approach to similar problems in the human.

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