Disposition of [3H]Actinomycin D in Tumor-bearing Mice

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SUMMARY

A single, nonlethal dose of actinomycin D will cause total regression and cure of Ridgway osteogenic sarcoma in mice. A cure is not obtained with a single dose daily for 7 days, a dose regimen which kills 10% of normal C57BL/6 × DBA/2 mice. This suggests that actinomycin D is more effective on a single high-dose schedule than on chronic daily therapy. Analysis of drug exposure in Ridgway osteogenic sarcoma and normal mouse tissues following the single and multiple-dose regimens suggests the difference in therapeutic response is due to drug exposure at a higher concentration in Ridgway osteogenic sarcoma after the single high dose than after the multiple-dose regimen. This may be related to the higher drug concentration attained in blood following the single-dose regimen than is attained with the multidose regimen.

INTRODUCTION

Following a single, nonlethal dose, actinomycin D will cure ROS in mice (14-16). However, actinomycin D failed to produce a cure when administered each day on a 1- to 7-day schedule up to the LD10 dose (11). The total doses in both cases are similar (500 and 560 µg/kg).

Toxicity data for mice and rats indicate that the total LD10 dose for actinomycin D remains fairly constant over a wide range of dose schedules, provided that the intervals between doses do not allow time for host recovery from drug damage to normal tissues (4, 11). Toxicity studies in dogs indicate that fractionation of the dose reduces drug toxicity (6, 7).

This study was undertaken in order to evaluate drug exposure in tumor and normal mouse tissues following the single and multiple drug-dosing regimens used by Schabel (14) and to correlate tissue drug exposure with drug toxicity in tumorous and normal tissues.

MATERIALS AND METHODS

Male AKR × DBA/2 F1 (hereafter called AKD2F1) mice, 20 to 30 g, received s.c. implants of ROS and were divided into 2 groups. Treatment was begun in both groups on Day 12 after tumor implant, when median tumor size was 850 mg (13). One group received a single i.p. dose of 500 µg [3H]actinomycin D per kg. The other group received 80 µg [3H]actinomycin D per kg, i.p., each day for 7 days.

Four mice from each group were sacrificed at indicated times following drug administration. Tissues were pooled, minced, and thoroughly mixed. The total radioactivity in the pooled tissues was then used to determine actinomycin D concentration. The method of preparation of the injection solution, drug source, and determination of tissue radioactivity content have been previously reported (3). The radiochemical purity of the [3H]actinomycin D used in this study, which was determined by chromatography (3), was 96%.

Regression analysis of the data for the single 500-µg/kg i.p. dose was performed. The regression lines were then used to estimate tissue drug depletion half-life. The regression lines were transposed from semilog plots to arithmetic plots. On both plots, the Y axis represented µg of drug per g of tissue and the X axis represented time in hr. The area under the regression line was integrated between zero time and the time at which drug concentration in the tissues reached 0.005 µg/g. This value was chosen because an in vitro actinomycin D concentration of 0.005 µg/ml has been reported to inhibit synthesis of rRNA by mammalian cells (8). The integrated area (C × T value) was used as an estimate of tissue drug exposure (3).

For the dose regimen of 80 µg/kg for 7 days, the experimentally determined data points for the various tissues were joined by straight lines (Charts 1 to 3). The time at which the drug level in tissues reached 0.005 µg/g was estimated, assuming the drug depletion from the concentration experimentally determined at 216 hr is the average half-life value calculated for all the tissues following the single 500-µg/kg dose. The area under the joined data points was then integrated on an arithmetic plot with 0.005 µg/g as a limit to the area. Similarly, the integrated area under the joined data points is used as an estimate of the tissue drug exposure with the dosage regimen of 80 µg/kg for 7 days.

RESULTS AND DISCUSSION

Charts 1 to 3 illustrate the experimental data of selected tissues and tumor for both dose regimens used. A pattern
Actinomycin D in Tumor-bearing Mice

Chart 1. Small intestine levels of \(^{3}H\)actinomycin D in male ROS-bearing AKD2F, mice. est., estimated.

Chart 2. Kidney levels of \(^{3}H\)actinomycin D in male ROS-bearing AKD2F, mice. est., estimated.

Chart 3. ROS levels of \(^{3}H\)actinomycin D in male AKD2F, mice. est., estimated.

The results of this investigation are consistent with our previous report (3) that the depletion of actinomycin D from tissues of various mammalian species may be described by a 1st-order process.

A comparison of the estimates of tissue drug exposure (Table 1) following the 2 dose regimens suggests greater drug exposure to most normal tissues and tumor following the single-dose regimen. Although the evaluation of tissue drug exposure \((C \times T)\) is a useful tool for pharmacodynamic evaluation of drug dosing regimens, the concept (2, 9) must be applied with caution when evaluating cytotoxic agents in vivo. Cellular damage (morphological change) in ROS and some normal tissues following exposure to actinomycin D has been reported previously (15). Since this cellular damage may influence \(C \times T\) values, and because a vividly conspicuous difference in estimates of total drug exposure in the tissues for the 2 dose regimens is not evident, an explanation for the difference in therapeutic response has not been interpreted from a comparison of \(C \times T\) values.

Total urinary and fecal excretion of \(^{3}H\)actinomycin D following single i.p. doses of 80 and 500 \(\mu g/kg\) was 30.5 and 31.5%, respectively, of the administered dose in the 1st 24 hr.

Toxicity. It has been reported that actinomycin D toxicity is cumulative over a wide range of dose schedules (4). This conclusion is largely attributed to results of studies in the mouse and rat, with lethality as the end point. Both drug regimens in this study have similar total doses which approach the highest nonlethal dose in the mouse.

For both dose schedules, drug tissue concentrations attained were compatible with in vitro cytotoxic levels reported by Wilkoff et al. (18). Philips et al. (10) suggested that the lethal action of actinomycin D was due to damage of the intestinal mucosa, resulting in penetration of enteric toxins. This leads to the conclusion that in the mouse the toxicity of actinomycin D is cumulative over a wide range of dose schedules because the various schedules used all result in attainment and maintenance of drug levels sufficient to result in the necessary damage to intestinal mucosa to cause death. The long drug-depletion half-life and slow rate

similar to that shown for kidney and small intestine was found for other tissues evaluated (Table 1).
of recovery of the small intestine from actinomycin D damage (10, 15) support this hypothesis.

**Drug Concentrations in Blood.** Serum concentrations of [\textsuperscript{3}H\textsubscript{]}actinomycin D following a single i.p. dose of 500 \(\mu\)g/kg were 0.066 at 3 hr and 0.012 \(\mu\)g/ml at 24 hr. Following the initial i.p. dose of 80 \(\mu\)g/kg, serum concentrations of drug were 0.006 and 0.003 \(\mu\)g/ml, respectively, at 3 and 24 hr. Serum concentrations of [\textsuperscript{3}H\textsubscript{]}actinomycin D were measured at 3 and 24 hr following subsequent i.p. doses of 80 \(\mu\)g/kg. The highest drug concentration in serum from mice on the multidose schedule was one-eleventh of the concentration following a single high dose, the tumor drug concentration was greater than 7 times the maximum concentration achieved following the single-dose regimen which is not attained following a multiple-dose regimen.

The passage of drug through the cell membrane and the amount of actinomycin D bound to DNA are 2 factors that play major roles in the sensitivity of cells to actinomycin D (12). Data support the concept that drug transport across the cell membrane is rate limiting to intracellular binding of actinomycin D to DNA and thus to resulting cytotoxicity (1). Also, the cellular mode of actinomycin D toxicity is believed to be dose dependent and is probably different for high and low doses (8, 19).

It has been previously demonstrated in the rat, dog, monkey, and man that blood levels of actinomycin D are rapidly depleted due to uptake into the tissues and drug excretion (3, 17). The influx of actinomycin D into cells is a non-energy-dependent process which is 1st order over a wide range of drug concentrations (1, 12). Thus, increasing the actinomycin D concentration to which cells are exposed proportionally increases the intracellular drug concentration (5). Also, it has been demonstrated in vitro that tumor cells in a population are not homogeneous in their ability to absorb actinomycin D (5). This lack of tumor cell population uniformity may be due to a difference in drug transport across the cell membrane by individual cells. Cells that absorb low drug concentrations have a greater chance of survival and may give rise to a tumor cell line with a low drug absorption.

In this study, a higher blood level of the drug is attained following the single-dose regimen than is attained at any time with the multiple-dose regimen. Thus, higher tumor extracellular drug levels (both proximal to and distal from vascular bed) are attained following the single-dose regimen than following the multidose regimen. Following the single high dose, the tumor drug concentration was greater than 7 times the maximum concentration achieved following the multiple-dose schedule. This suggests that a similar higher drug concentration was achieved in all tumor cells, including cells that may exhibit altered drug cell membrane transport characteristics.

In progress in this laboratory are studies in the beagle dog comparing blood concentrations of [\textsuperscript{3}H\textsubscript{]}actinomycin D with time following a single high dose and a multidose regimen, with a similar total dose. Preliminary results demonstrate that the peak drug concentration in blood is related to the size of the dose and rate of administration. A comparative pattern similar to that reported for mice has been observed in dogs.

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