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Uptake *in vivo* and *in vitro* of actinomycin D by mouse leukemias as factors in survival

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THE ANTIBIOTIC, actinomycin D, has been used in the treatment of animal and human neoplasms.[1] The drug was shown to bind with guanine residues in DNA, leading to inhibition of DNA-dependent RNA polymerase;[1–3] the structure of the DNA-drug complex has been partly elucidated.[4–6] In addition to inhibition of RNA synthesis, the antibiotic has been shown to interfere with other metabolic processes in cells.[7–9] The disposition of actinomycin D in animals has been studied.[10–11] The drug was mainly found in kidney, liver, and spleen; in liver, the drug was concentrated in the nuclear fraction. Resistance to actinomycin D in bacterial [12–15] and mammalian[16–17] cells was found to be related to barriers to drug uptake.

We examined uptake of actinomycin D by six mouse leukemias, which varied in response to the drug from almost complete resistance to "cures". Sources of these tumors and methods of cell isolation have been described.[18, 19]

Data on the effect of actinomycin D on survival of tumor-bearing animals were obtained as follows. Animals were treated with actinomycin D (50 μg/kg) by i.p. injection from day 1 to day 10 after

inoculation with 10^6 tumor cells. Survival increase = 100 (T - C)/C. T = mean surival time in days of treated animals, and C = mean survival time of untreated control animals. Corresponding survival increase of these lines produced by therapeutic treatment with actinomycin D were: P388/57155, >190 per cent; P388, > 175 per cent; L1210, 60 per cent; P388/VCR, 40 per cent; P815/VLB, 13 per cent; P388/38280, 20 per cent.

For observations on drug uptake *in vitro*, cells were removed from tumor-bearing animals and resuspended in a buffered-salts medium. This consisted of 8 mM $CaCl_2$, 15 mM KCl, 65 mM NaCl, and 62 mM TES buffer, pH 7·2. TES, *N*-Tris (hydroxymethyl) methyl-2-aminoethansulfonic acid, was obtained from Calbiochem Corp. Aliquots of 150 μl of suspensions, containing 7–8 mg of cells (wet wt.), were incubated with 0·1 μg/ml of ^{3}H-actinomycin D* at 15–37° to obtain data shown in Fig. 1. Similar data were obtained with other lines discussed here. Incubations were ended by chilling

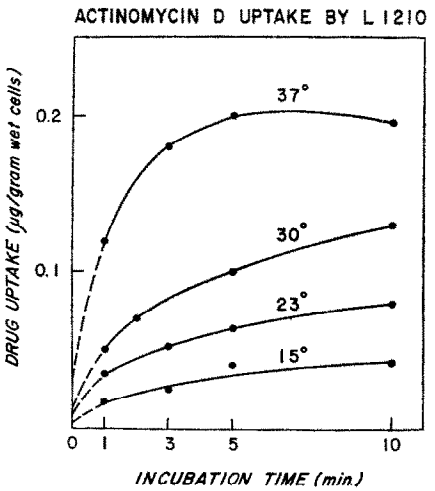

FIG. 1. Uptake of ^{3}H-actinomycin D by L1210 cells *in vitro*.

the tubes, and the cells were collected by centrifugation for 30 sec at 150 *g*. The cells were washed once by resuspension in ice-cold buffer and again collected by centrifugation. Siliconized glassware was used throughout, since binding of labeled actinomycin D to untreated glassware has been found. No evidence could be found for drug metabolism when perchloric acid extracts[20] of cells loaded with actinomycin D were chromatographed in any of several solvents[21–23]. Actinomycin D could not be washed from cells by 0·9% NaCl or by 0·25 M sucrose. The drug could be removed by chloroform, acidified ethyl acetate, or by heating cell suspensions to 100°. To measure cellular levels of labeled drug, cell pellets were uniformly suspended in 250 μl of 0·9% NaCl, and a 200-μl aliquot was removed, mixed with 10 ml of scintillator solution,† and radioactivity was measured with a Nuclear-Chicago Mark 1 liquid scintillation counter.

Uptake of actinomycin D *in vitro* was similar in all six cell lines tested, regardless of external drug level used. Data shown in Fig. 1 were obtained from L1210 cells, and similar results (± 15%) were obtained from any of five other cell lines: P388, P388/57155, P388/VCR, P388/38280, and P815/VLB. The final cell/medium drug distribution ratio of 2, obtained in L1210 cells after a 5-to 10-min incubation at 37°, could also be achieved at lower temperatures if incubation times were sufficiently prolonged.

* The labeled drug (4·2 c/m-mole, randomly labeled) was purchased from Schwarz Bio-Research Inc., and was also supplied by the National Cancer Institute.

† This consisted of 600 ml toluene, 400 ml methyl cellosolve, 60 g naphthalene, and 4 g BBOT, 2,5-*bis*-(2-(5-*tert*-butylbenzoxazolyl))-thiophene (packard Instrument Co.).

The extracellular drug level used for studies *in vitro* was 0·1 μg/ml. The drug distribution ratios achieved and the temperature sensitivity of drug uptake did not change when the external drug level was varied from 0·01 to 10 μg/ml.

Studies on cellular accumulation of the drug *in vivo* were carried out on mice, containing about 3 ml of ascitic fluid, at about 36 hr before death of the animals was expected. A therapeutic dose of the labeled drug (50 μg/kg) was injected in 0·4 ml of 0·9% NaCl; this usually amounted to 1 μg of drug (400,000 cpm) per mouse. At measured intervals, 0·2 ml samples of ascitic fluid were removed with a syringe and a No. 26 needle, and collected in weighed tubes. The cells were collected by centrifugation at 150 *g* for 30 sec and an aliquot of the supernatant fluid was withdrawn. This was mixed with an equal volume of 30% H_2O_2 for decolorization, the volume was brought to 0·2 ml with water, and radio activity was determined as outlined above. The cell pellet was freed from erythrocytes, if necessary, by brief resuspension in distilled water. The cells were collected by centrifugation and washed once by resuspension in 0·9% NaCl.[14] The cells were finally collected by centrifugation. The pellet was blotted dry, weighed, resuspended in 250 μl of 0·9% NaCl, and a 200-μl aliquot was removed for determination of radioactivity. All data were reported in terms of micrograms of drug taken up per gram of cells (wet wt.).

Data shown in Fig. 2 were obtained on single mice, but repeated experiments yielded results that did not differ from those shown by more than ± 10 per cent. Drug levels in the ascitic fluid reached 0·1 μg/ml just after injections and fell to 0·005 μg/ml within 4 hr after injections.

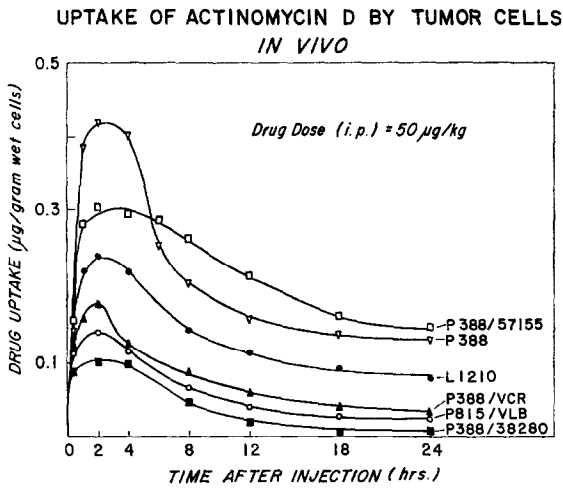

FIG. 2. Time course of ^{3}H-actinomycin D uptake and retention by animal leukemia cells *in vivo*.

The uptake and retention of the drug by tumor cells during the first few hours after drug injections appear related to the inherent sensitivity of these tumor lines to the drug as measured by drug-promoted survival time of tumor-bearing mice. The gradual loss of drug from cells after the initial uptake has been attributed by Harbers *et al.*[23] to detachment of drug from DNA during subsequent DNA synthesis. The reasons for the different behavior of these cell lines, with respect to drug uptake *in vivo* and *in vitro*, are unknown. The latter studies suggest that initial rates of drug uptake are similar in all cell types tested here.

Electrophoretically, actinomycin D showed no net charge over the pH range 3–11, and was quantitatively extracted from water solution, or from cells exposed to the drug, by chloroform. The drug was not extracted from cells by treatment with 0·01 N acetic acid at 60°, although in other experiments we found that this procedure extracted most low molecular weight compounds from the cells, including nucleotides, purines, pyrimidines, and amino acids. The nature of the permeability barriers to the uptake or retention of this highly lipid-soluble and uncharged drug molecule is unknown.

One actinomycin D-resistant cell line examined here, P388/38280, was originally selected for resistance to a terephthalanalide.[24, 25] Investigations showed that resistance to the latter drug was related to accelerated cellular excretion of most of the terephthalanilide that had initially been accumulated. Cross-resistance between terephthalanilides and vinca alkaloids has previously been noted.[26, 27] The present data suggest the possibility of a common mode of resistance to terephthalanilides, vinca alkaloids, and actinomycin D in some animal leukemias.

In animal leukemias, correlations have been found between drug response and rates of drug uptake with methotrexate[19], and between response and rates of drug phosphorylation to nucleotides with 5-fluorouracil[20] and cytosine arabinoside[28]. Actinomycin D represents a class of drugs in which the ability of cells to accumulate and retain the drug is an important determinant of response.

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