of Anatomy, for electron microscopic observations.
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Transport and Phosphorylation as Factors in the Antitumor Action of Cytosine Arabinoside

Abstract. *Survival of mice bearing different transplantable leukemias and treated with cytosine arabinoside was compared with uptake and subsequent phosphorylation of the drug in vitro. Capacity for nucleotide formation was correlated with response and is apparently an important determinant of drug sensitivity. Drug uptake, although apparently mediated, was similar in all cell lines.*

We have reported that variation in uptake of the nonmetabolized drug methotrexate (*1*) and variations in capacity for phosphorylation of the freely diffusible drug 5-fluorouracil (*2*) were determining factors of drug response in leukemias in mice. We now find that cytosine arabinoside is not freely diffusible, but that the rate of intracellular phosphorylation—not uptake of the drug—is correlated with drug response (Fig. 1).

Cytosine arabinoside (1-β-D-arabinofuranosyl-cytosine) inhibits growth of certain animal (*3*) and human (*4*) neoplasms, presumably by competitively inhibiting reduction of cytidine ribotides to deoxyribotides (*5*). Another possible mode of the drug's action is its own incorporation into the DNA and RNA of mammalian cells (*6*). The ability of cytosine arabinoside to interrupt DNA synthesis was not considered to be related to incorporation of the drug into terminal positions of cell DNA (*7*). Phosphorylation of the drug to nucleotides was required for its antitumor action, and impaired drug phosphorylation was associated with development of drug resistance in cultured P5178Y leukemia cells of mice (*8*).

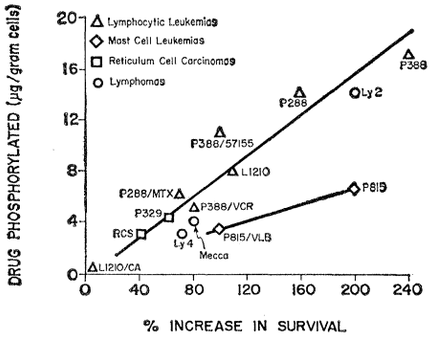

Fig. 1. Correlation between cytosine arabinoside phosphorylation in vitro and drug-promoted survival of tumor-bearing animals. Phosphorylation was determined by measurement of drug conversion to nucleotides. Results are shown in terms of micrograms of drug phosphorylated per gram of cells (wet weight) per 15-minute incubation at 37°C. Survival data were given by Wodinsky and Kensler (*9*). Each point represents cells from the tumor cell line indicated.

We examined 14 transplantable mouse leukemias which varied widely (*9*) in responsiveness to the drug (*1, 10*). Ascitic cells were removed from animals and suspended in buffer (*11*). Samples of 150 μl containing 7 to 8 mg of cells (wet weight) were incubated with 10 μg per milliliter of radioactive cytosine arabinoside or other nucleosides (*12*). Incubations were ended by chilling the tubes for 30 seconds. The cells were collected by centrifugation for 30 seconds at 150*g*, washed by suspension in ice-cold buffer, and collected by centrifugation. Uptake of drug and conversion to nucleotides were measured at this point by determining the total radioactivity in the cells. To determine the concentrations of labeled nucleotides in the cells, the pellets were again suspended in 250 μl of buffer at 37°C for 5 minutes and collected by centrifugation. All unmetabolized nucleoside was washed from the cells by this procedure; loss of labeled nucleotides was negligible. Cell radioactivity was measured by extracting intracellular components with 250 μl of 0.1*N* acetic acid at 60°C for 10 minutes. Cell debris, which contained negligible radioactivity, was removed by centrifugation, and a 200-μl fraction of the supernatant was used for radioactivity measurements (*13*).

Radioactive components of cell extracts were identified by paper chromatography. Drug nucleotides and unmetabolized drugs were extracted with perchloric acid, and the extracts were neutralized and concentrated (*2*). Diffusible and nondiffusible labeled cell components were identified with appropriate compounds as markers on chromatograms (*14*). Drug detoxification results from deamination of cytosine arabinoside to uracil arabinoside, an inactive product (*15*). Deaminase activity could not be found in L1210 or L1210/CA cells (*16*), nor did we obtain any chromatographic evidence of deamination of cytosine arabinoside by any cell line that we tested.

Of the four types of mouse leukemias tested, only the mast-cell leukemias showed significant sensitivity to cytosine arabinoside in the absence of a correspondingly high capacity for drug phosphorylation (Fig. 1). This may be related to our observation that rate of phosphorylation of other nucleosides by mast cells was relatively low (*17*).

Nucleotide formation in drug-resistant leukemias might be limited by impaired drug uptake. Therefore, it was important to show that cytosine arabinoside was not excluded from drug-resistant cell lines. We first studied uptake of the drug in L1210/CA, a line derived from L1210 by selection for drug resistance (*18*). Uptake of cytosine arabinoside by L1210/CA cells was essentially temperature-insensitive; a ratio of about 0.2 for intracellular to extracellular distribution of the drug was achieved within 3 minutes after the start of incubations (Fig. 2). This ratio was not increased by prolonging incubations. Accumulation of cytosine arabinoside by L1210 cells was highly temperature-sensitive and apparently concentrative (Fig. 2). But chromatographic examination of cell extracts showed that the labeled intracellular material accumulated at 37°C con-

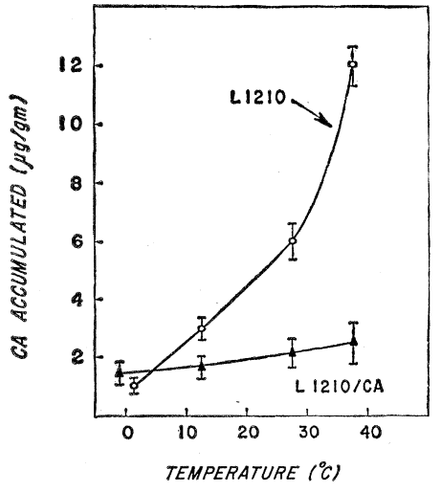

Fig. 2. Accumulation of cytosine arabinoside plus nucleotides of the drug by tumor cells as a function of temperature of incubation. Cells were incubated in medium containing 10 μg per milliliter of labeled drug for 15 minutes; the resulting intracellular accumulation is reported in terms of micrograms of drug per gram of cells (wet weight). After incubations at 37°C, $>$ 95 percent of the cell radioactivity in L1210/CA represented the unchanged drug; 70 percent of the radioactivity in L1210 represented drug nucleotides (*14*).

sisted mainly of drug nucleotides. Near 0°C, phosphorylation was inhibited, and drug accumulation was similar in both cell lines. In other studies, we found that uptake of cytosine arabinoside, during the initial 30 seconds of incubations, was similar in all 14 cell lines examined. Under these conditions, less than 10 percent of the cellular radioactivity accumulated at 37°C represented drug nucleotides.

The accumulation of cytosine arabinoside nucleotides by cell lines examined was increased by raising the drug concentration in the medium, thereby increasing the rate of drug uptake. This suggests that variations in the rate of cytosine arabinoside uptake, although apparently not a factor here, could affect responsiveness to the drug in other cell lines.

Uptake of cytosine arabinoside can be stimulated by pyrimidine and certain purine nucleosides (*19*). This structural specificity suggests interaction between transport systems (*20*). Our attempts to show an energy requirement for transport of cytosine arabinoside in L1210 or L1210/CA were unsuccessful. Jacquez (*21*) found evidence to suggest mediated transport of three pyrimidine nucleosides—thymidine, uridine, and fluorouridine—by Ehrlich ascites tumor cells.

We conclude that cytosine arabinoside does not diffuse freely into leukemia cells of mice. There is evidence to suggest that uptake of the drug is mediated; however, uptake was not impaired in drug-resistant cell lines. Variation in cellular capacity for phosphorylation of the intracellular drug provides the basis for responsiveness to cytosine arabinoside in the cell lines that we examined. Similar correlations between capacity for drug phosphorylation in vitro and drug response in vivo were found in human leukemias (*22*).

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11. TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] was obtained from Calbiochem Corp. This buffer contained 62 m*M* TES, *p*H 7.2; 65 m*M* NaCl; 15 m*M* KCl; and 8 m*M* $CaCl_2$.
12. Tritiated cytosine arabinoside (2 c/mmole) was purchased from Schwarz BioResearch Corporation. Other labeled nucleosides were purchased from New England Nuclear Corporation.
13. The scintillator solution was 10 ml per vial of 600 ml of toluene, 400 ml of methyl cellosolve, 60 g of naphthalene, and 4 g of 2,5-bis-[2(5-*tert*-butylbenzoxazolyl)] thiophene (BBOT, Packard Instrument Co.). A Nuclear-Chicago Mark I liquid scintillation counter was used.
14. Whatman No. 1 paper was used for descending chromatography. Solvent systems used were: 94.6 ml of 85 percent *n*-butanol plus 5.4 ml of concentrated ammonium hydroxide (*8*), and 100 ml of isobutyric acid plus 60 ml of 1*M* ammonium hydroxide adjusted to *p*H 6 [H. A. Krebs and R. Hems, *Biochim. Biophys. Acta* **12**, 172 (1952)]. In cell extracts, triphosphates of nucleosides predominated; lesser amounts of monophosphates and traces of diphosphates were also found.
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17. Conversion of labeled cytosine arabinoside, deoxycytidine, cytidine, adenosine, and uridine to nucleotides by P815 cells proceeded at 10 to 20 percent of the corresponding rates observed in L1210 cells. These studies were performed by incubating cells in medium containing the labeled nucleosides (0.1 m*M*) and then by determining accumulation of nondiffusible nucleotides. The identity of the latter compounds was verified by paper chromatography of cell extracts.
18. I. Wodinsky and C. J. Kensler, *Cancer Chemotherap. Rep.* **43**, 1 (1964). L1210/CA cells showed a specific enzymatic impairment; cytosine arabinoside and deoxycytidine were phosphorylated (*17*) at <1 percent and 10 percent, respectively, of the rates found in L1210. Phosphorylation of uridine, adenosine, deoxyadenosine, and cytidine proceeded at similar rates in L1210 and L1210/CA.
19. These included deoxycytidine, thymidine, thymine riboside, uridine, cytidine, deoxyadenosine, and 5-fluorouridine, but not guanosine, adenosine, cytosine, uracil, or arabinose. Addition of a 50-fold molar excess of any of the first group of compounds increased the ratio of distribution of cytosine arabinoside in L1210/CA cells from 0.2 to 0.4 (Fig. 2).
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Cell Division: Direct Measurement of Maximum Tension Exerted by Furrow of Echinoderm Eggs

Abstract. *Tensions exerted by cleavage furrows in isometric contraction were measured by means of flexible glass needles whose characteristics of bending had already been determined. The tension of* Astriclypeus manni *furrows in second division is* $3.04 \times 10^{-3} \pm 0.95 \times 10^{-3}$ *dyne; that for* Pseudocentrotus depressus *eggs in first division is* $2.00 \times 10^{-3} \pm 0.43 \times 10^{-3}$ *dyne. The tension required for cleavage probably does not exceed* 1.5×10^{-3} *dyne. According to existing morphological evidence, these values can be accounted for by a substance whose capacity for exerting tension does not exceed that of an actomyosin thread.*

Since echinoderm eggs divide despite removal, replacement, or drastic rearrangement of the endoplasm and subsurface cytoplasm (*1*), the mechanism of division must lie in or very close to the cell surface. The observation that the surface of the base of the furrow can bend a glass needle placed in its path at right angles to the cleavage plane (*2*) indicated that direct measurement of the maximum tension generated by the furrow should be possible.

Thin glass needles were drawn with an incandescent filament and micromanipulators; they were calibrated with a reference needle (*3*). Immediately after the furrow appeared, the calibrated needle was inserted through one polar surface, and a stouter holding needle was thrust in the opposite direction through the other polar surface. Both needles were positioned so that they passed through the cleavage plane and lay in approximately the same plane of focus (Fig. 1). Opera-

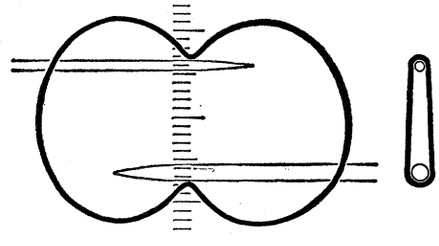

Fig. 1. (Left) Arrangement of cell and needles for determination. Upper, calibrated needle is deflected downward during cleavage. Lower, holding needle does not move. (Right) Schematic section through and parallel to the cleavage plane during isometric contraction. Diameter of the needles is exaggerated.