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EFFECTS OF PERSANTIN ON DEOXYCYTIDINE TRANSPORT BY MURINE LEUKEMIA CELLS

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SUMMARY

Effects of persantin on nucleoside transport were studied using a sub-line of the L1210 murine leukemia unable to metabolize deoxycytidine. Both uptake and exodus of deoxycytidine were inhibited by persantin; the inhibition was rapid, and could not be overcome by increasing the deoxycytidine concentration. Persantin accumulation was a temperature-insensitive and apparently non-saturable process.

INTRODUCTION

The drug persantin (dipyridimole, 2,6-bis(diethylamino)-4,8-dipiperidino-pyrimido-(5,4-*d*)pyrimidine) inhibits transport of several compounds by mammalian cells: adenosine[1–3], inorganic phosphate[4] and certain sugars[5] by erythrocytes and nucleosides by chick fibroblasts[6]. Persantin is used clinically to treat cardiac insufficiency. The drug is believed[7] to act by preventing loss of the vasodilator adenosine[8] from plasma *via* erythrocytes.

Previous studies on inhibition of nucleoside uptake by persantin employed a cell type which rapidly metabolized nucleosides. Effects of persantin on nucleoside exodus could therefore not be measured. This communication describes our studies on effects of persantin on uptake and exit of deoxycytidine using L1210/CA. This is a sub-line of the L1210 mouse leukemia which is unable to further metabolize deoxycytidine[9]. Accumulation of persantin by L1210/CA was also examined

MATERIALS AND METHODS

Chemicals

[2-^{14}C]Deoxycytidine (200 mC/mole) and [*carboxy*-^{14}C]cycloleucine (30 mC per mmole) were purchased from New England Nuclear Corp. Persantin was provided by Geigy Pharmaceuticals.

Tumor cells

L1210/CA, a cytosine arabinoside-resistant sub-line[10] of the L1210 leukemia was propagated by transplant into CDF_1 mice. Tumor cells were removed from the animals 7 days after inoculation with 10^6 cells. The line was periodically tested for

deoxycytidine kinase activity[11]. If measurable activity was found, the line was discarded, and a fresh inoculum was obtained from frozen stock*.

Measurement of nucleoside transport

Procedures are described in ref. 9. Aliquots (200 μl) of 10 % cell suspensions were incubated at 0 or 37° with labeled deoxycytidine in isotonic buffered-salts solution. Incubations were terminated by collection of cells by centrifugation for 30 sec at 500 × *g*, the fluid was removed and the radioactivity associated with the cell pellets was determined by liquid-scintillation counting. A correction was made for trapped extracellular fluid as described below. Water-permeable cell space was measured, and nucleoside uptake calculated as the distribution ratio between extracellular and cellular water. In other experiments, persantin was added before, with, or after the labeled deoxycytidine. Volumes of deoxycytidine and persantin were in the 2–5-μl range.

When we found that persantin inhibited exodus of deoxycytidine, we carried out some experiments in which the [^{14}C]deoxycytidine-loaded cells were washed in medium containing 0.2 mM persantin. This procedure eliminated the need for correction for trapped extracellular radioactivity.

Efflux of [^{14}C]deoxycytidine was measured from cells loaded by a 3-min incubation, at 37° in medium containing 0.1 mM of the compound. The cells were collected by centrifugation, then dispersed in fresh medium, at 0 or 37°. At timed intervals, cells were collected, and intracellular radioactivity measured. Alternatively, pre-loaded cells were suspended in fresh medium, and persantin (final level = 0.2 mM) was added at a measured interval later. The cells were then collected by centrifugation, and cellular radioactivity determined. The first method was appropriate for intervals of 0 5 min or more. The second was useful in measuring nucleoside exit during intervals of 0.05 min.

Determination of persantin accumulation

Cells were incubated in medium[9] containing 0.01–10 mM persantin for 1–10 min; then collected by centrifugation. In some experiments, the cell pellets were then resuspended in fresh medium for timed intervals, and then collected as before. Persantin was extracted from the cells with 2 times 200 μl of formamide and the extract was clarified by centrifugation. This procedure removed 99 % of the drug from the cell pellets. A 30-μl aliquot of the formamide extract was diluted with 3 ml of water, and the persantin concentration was determined by fluorimetry[12]. (Excitation wavelength = 305 mμ, fluorescence determined at 495 mμ.) Suitable blanks were obtained by formamide extraction of untreated cells.

Correction for trapped extracellular fluid

When cells were collected from media containing a labeled substrate, a correction for trapped extracellular radiactivity was necessary. The procedure used is outlined in ref. 9, and involved the use of labeled sulfate, to which L1210/CA cells are

* Presence of deoxycytidine kinase would lead to accumulation of intracellular radioactivity in the form of non-diffusible deoxycytidine nucleotides during incubations.

The L1210/CA cells are unable to form nucleotides from either cytosine arabinoside[13] or deoxycytidine[14].

impermeable. If the cell pellets were thoroughly washed, or efflux of labeled deoxycytidine into fresh medium were measured, the extracellular radioactivity was sufficiently diluted so that such a correction was not significant.

RESULTS

We have shown[9] uptake of deoxycytidine by L1210/CA to be a rapid process, with the nucleoside equilibrating between extracellular fluid and cell water within 1 min at 37°. Saturation kinetics and structural specificity of nucleoside transport were demonstrable only near 0°. In the present study, we found that persantin lowered the cell–medium distribution ratio of deoxycytidine (Table I), indicating that the drug had inhibited net accumulation of the nucleoside. (Persantin was added 0.5 min before deoxycytidine.) The results were not altered when the deoxycytidine level was varied between 0.1 and 10 mM, *i.e.*, the inhibition of deoxycytidine uptake by persantin was not competitive. The persantin concentration required to cause a 90% inhibition of deoxycytidine uptake was 0.1 mM at 37°, but only 0.01 mM at 0°. This was not related to the temperature-sensitivity of persantin uptake; the same amount of the drug was taken up by L1210/CA cells at 0 or 37° (Table II). The results shown in Table II were not altered by longer incubations, nor by addition of 10 mM deoxycytidine to the medium.

To measure the time required for persantin inhibition of labeled deoxycytidine uptake, we added the drug (final level = 0.1 mM) to an L1210/CA suspension at a measured interval after addition of [^{14}C]deoxycytidine (final level = 0.1 mM). At 37°,

TABLE I

INHIBITION OF DEOXYCYTIDINE UPTAKE BY PERSANTIN

Cells were incubated for 5 min at 37° in medium containing 0.1 mM [^{14}C]deoxycytidine and specified levels of persantin.

Persantin level (μM)	*Cell–medium distribution ratio of nucleoside*	
	Incubation temp.: 0°	37°
0	0.92	0.97
10	0.08	0.85
100	0.03	0.12
200	0.02	0.05
1000	0.01	0.01

TABLE II

PERSANTIN UPTAKE DURING 0.5-min INCUBATIONS

Persantin level (μM)	*Uptake (nmoles/g cells)*	
	Incubation temp.: 0°	37°
10	3.6	3.5
100	33	35
1000	320	365

equilibration between extracellular and intracellular deoxycytidine pools was complete within 0.2 min, leading to a distribution ratio of about 1, and addition of persantin after 0.2 min did not alter this distribution ratio. But addition of the drug at 0.05 or 0.1 min after deoxycytidine interrupted the equilibrium, resulting in a distribution ratio of less than one (Fig. 1). We interpret this to mean that persantin acts rapidly (within 0.05 min) to stop further uptake to nucleoside. When this experiment was carried out at 0°, persantin addition interrupted equilibration of external and internal deoxycytidine pools unless about 3 min had elapsed between addition of deoxycytidine and addition of persantin.

Persantin also inhibited exodus of accumulated deoxycytidine. Cells were pre-loaded with the nucleoside (see MATERIALS AND METHODS), then suspended in fresh medium at 0 or 37°. In the absence of persantin, deoxycytidine was rapidly lost (Fig. 2). When pre-loaded cells were suspended in medium containing 0.1 mM persantin, loss of deoxycytidine was inhibited (Fig. 2). The rate of deoxycytidine loss could be measured by collection of cells at intervals, following resuspension in fresh medium, and measuring intracellular radioactivity levels. The time required for loss of 50 % of initial

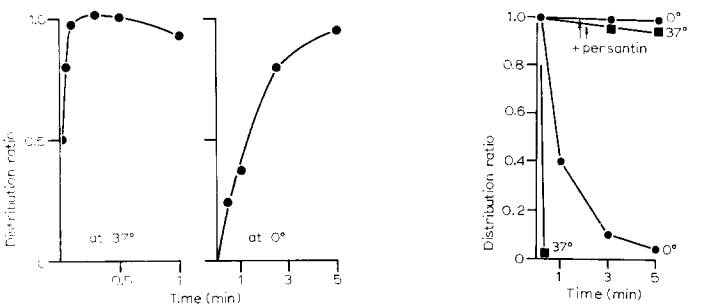

Fig. 1. Distribution ratio of labeled deoxycytidine at 0° and 37°. The intervals shown indicate the time of exposure of cells to deoxycytidine before addition of persantin (final level = 0.2 mM). The deoxycytidine level employed was 0.1 mM.

Fig. 2. Effect of 0.1 mM persantin on exodus of labeled deoxycytidine from cells at 0° or at 37°. The initial intracellular nucleoside level was 0.1 mM.

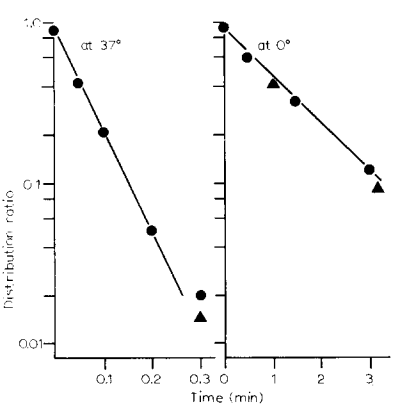

Fig. 3. Exodus of deoxycytidine from pre-loaded cells. ▲, cells were collected at indicated intervals after their resuspension in fresh medium; ●, pre-loaded cells were suspended in fresh medium, and persantin (0.1 mM) was added at indicated intervals following resuspension.

pools was found to be about 0.05 min at 37° and 1 min at 0° (Fig. 3)*. When the pre-loaded cells were suspended in fresh medium, and persantin added at intervals thereafter, the same rates of nucleoside loss were obtained. In this case, the time of incubation was measured from the moment of resuspension until the persantin was added. In other experiments, we found that 0.01 mM persantin was sufficient to prevent loss of deoxycytidine at 0°, but a level of 0.1 mM of the drug was required at 37° to completely inhibit loss of deoxycytidine.

Persantin, at 0.1–1 mM did not alter permeability of L1210/CA cells to tritiated water, nor their impermeability to labeled sulfate. But accumulation of the non-metabolized amino acid, cycloleucine, was apparently increased by 0.1 mM persantin

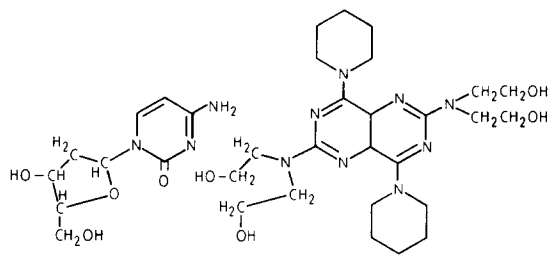

Fig. 4. Structures of persantin and deoxycytidine.

TABLE III

EFFECT OF PERSANTIN ON CYCLOLEUCINE TRANSPORT

Cells were incubated with 0.1 mM [^{14}C]cycloleucine for 10 min at 37°, then, if specified, washed in fresh medium for 3 min at 37°. Persantin was present at 0.1 mM as indicated.

Conditions	*Cell–medium distribution ratio of cycloleucine*
No persantin, cells not washed	3.0
Persantin present during incubation, cells not washed	7.5
No persantin, cells washed	1.4
Persantin present during wash	2.8

TABLE IV

PERSANTIN LOSS DURING WASHING

Data are expressed in terms of the fraction of persantin remaining after incubation of pre-loaded cells in fresh medium at 37°. Cells were loaded with the drug by a 3-min incubation at 37° in 0.1 mM persantin.

Wash time (min)	*Fraction of persantin remaining (%)*	
	Wash at 0°	*Wash at 37°*
3	66	50
10	60	45
30	50	42

* The initial distribution ratio represents the distribution of deoxycytidine between cell water and medium in the pre-loaded L1210/CA cells.

at 37° (Table III). When cells were pre-loaded with 0.1 mM cycloleucine for 3 min at 37°, subsequent loss of the amino acid into fresh medium was impaired by persantin.

If cells were pre-loaded with persantin by incubation in 0.1 mM drug for 3 min at 37° about half of the accumulated material was removed by a 3 min wash at 37° (Table IV). These results were not changed by shortening the initial incubation to 0.5 min, or by carrying out the initial incubation at 0°. When L1210/CA cells were exposed to 0.1 mM persantin for 3 min at 37°, subsequent accumulation of 0.1 mM [^{14}C]deoxycytidine was inhibited, as previously discussed here. Even when the persantin-treated cells were washed in fresh medium for 10 min before addition of the labeled deoxycytidine, uptake of the latter was still prevented by the remaining persantin.

DISCUSSION

Persantin blocks both uptake and exodus of deoxycytidine from a mammalian cell line (L1210/CA) unable to metabolize this nucleoside because of the absence of deoxycytidine kinase and deoxycytidine deaminase[9]. Persantin levels needed for this inhibition were lowered 10-fold when incubation temperatures were lowered from 37 to 0°. Accumulation of persantin was not altered by lowering the incubation temperature, nor was the fraction of accumulated persantin 'bound' altered. Washing of persantin-treated cells removed a fraction of the drug, but the 'bound' remainder was sufficient to inhibit deoxycytidine transport. Deoxycytidine did not inhibit accumulation of persantin, nor was the persantin-induced inhibition of deoxycytidine transport reversed at high deoxycytidine levels. This latter finding contrasts with other data[4,6] indicating that persantin inhibition of uptake of some substrates was competitive. SCHOLTISSEK[6] found evidence to suggest an interaction between nucleoside transport and subsequent nucleoside phosphorylation by kinases. Only other substrates for each nucleoside kinase could reverse the inhibition by persantin of uptake of one such substrate. In the L1210/CA line, deoxycytidine kinase is absent[13]. Therefore this mode of reversal of a persantin effect on deoxycytidine uptake is unavailable.

Levels of persantin used by SCHOLTISSEK[6] to inhibit nucleoside uptake by chick fibroblasts ranged between 0.1 and 1 % of levels used here. In a recent report, PLAGEMANN AND ROTH[15] found that 10 μM persantin inhibited by 40 % the incorporation of uridine into acid-soluble material by Nivokoff rat hepatome cells. This was judged to represent interference, by persantin, with uridine uptake. The 10 μM level is comparable to the persantin concentration needed to inhibit deoxycytidine uptake by L1210/CA cells; the lower magnitude of drug needed to inhibit nucleoside uptake fibroblasts might reflect in basic metabolic differences between such cells adhering to glass and animal tumor cells growing in ascitic fluid *in vivo*.

We were previously unable[9] to demonstrate clearly a temperature-dependence of deoxycytidine influx when low levels of the nucleoside were employed, since the shortest feasable incubation times were too long to permit accurate measurements of this rapid process. Deoxycytidine exodus could only be measured over intervals of 0.3 min or more. In the present study, data has been obtained to indicate that the time of onset of persantin-induced inhibition of inward and outward deoxycytidine transport is short, compared with rates of deoxycytidine transport. The drug was useful

delineating kinetics of deoxycytidine movement over shorter intervals (0.05–0.3 min).

Persantin accumulation was studied sufficiently to show that a temperature-insensitive and apparently non-saturable process is involved. The nature of the binding of the drug to L1210/C cells is unknown.

The mechanism of the inhibition of deoxycytidine uptake and exodus by persantin remains obscure. Some structural similarities between the two compounds are evident (Fig. 4). This similarity does not account for the ability of the drug to inhibit cycloleucine exodus, as described here, nor the persantin-induced inhibition of phosphate[4] and sugar[5] transport. Perhaps all of these effects are related to a common mode of drug action on membrane components involved in trans-membrane movement of many different substances.

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