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EFFECTS OF PERSANTIN ON SEVERAL TRANSPORT SYSTEMS OF MURINE LEUKEMIAS

DAVID KESSEL AND DAVID C. DODD

Department of Pharmacology and Toxicology and Division of Oncology, University of Rochester School of Medicine, Rochester, N. Y. 14642, and School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pa. 19348 (U.S.A.)

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SUMMARY

The effect of persantin on several transport systems was studied using murine leukemia cells. Low concentrations of persantin inhibited inward transport of phosphate, fucose, deoxycytidine and adenosine. At higher concentrations, inhibition of exodus of these substances from pre-loaded cells could be shown. In contrast, persantin preferentially inhibited efflux of *O*-methylglucose, uridine, uracil, and cycloleucine at levels which did not significantly slow influx. As the persantin level was elevated, entry of the latter group of compounds was also inhibited. These data have implications concerning the multiplicity of transport sites on the cell membrane.

INTRODUCTION

The drug persantin, chemically: 2,6-bis(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-*d*)pyrimidine, has been shown to inhibit many mammalian transport processes, although the spectrum of effects of this drug in a single cell type was previously not examined. Persantin inhibits inward and outward movement of adenosine through myocardial cell membranes[1-4], inward and outward movement of phosphate through erythrocyte membranes[5], uptake of sugars by erythrocytes[6], uptake of nucleosides by chick fibroblasts[7] and by rat hepatoma cells[8], and inward and outward movement of deoxycytidine by murine leukemia cells[9]. Persantin appears to lower levels of ADP in erythrocytes, possibly by inhibition of a membrane ATPase[10], and also slows mitochondrial potassium transport[11] and NADH-linked respiration[12].

This multiplicity of effects is difficult to interpret since measurements on many different cell types were involved under varying conditions. We have previously noted[9] that persantin could be considered a structural analog of nucleosides, and might competitively inhibit association of certain nucleosides with transport sites on this basis. Before constructing models to explain the ability of persantin to inhibit transport of other substances, we studied effects of the drug on outward and inward transport of several biochemicals by a readily accessible mammalian cell type, the transplantable mouse leukemia.

MATERIALS AND METHODS

Procedures for maintaining murine leukemia cell lines and for isolating cells have been described[9,13]. The cell lines employed were L1210; L1210/CA, which cannot metabolize deoxycytidine[13]; P388/38280, which poorly metabolizes uracil[14]; and P388/FUR, a line unable to metabolize uridine[15]. In the latter three cell sub-lines, the compounds named above are non-metabolizable substrates for study of transport phenomena uncomplicated by intracellular biotransformations.

Radioactive substrates were obtained from New England Nuclear Corp. The concentrations were adjusted to 10 mM with a specific activity of about 10^7 cpm/ml, as determined by liquid scintillation counting. 2-μl portions of stock solutions were mixed with 200-μl portions of cell suspensions containing 5–10 mg of cells, wet weight, for standard incubations. In some cases, the amount of carrier was adjusted to permit variation in substrate concentration.

Persantin (Geigy) stock solutions (1–50 mM) were made up in distilled water. Since persantin has a limited solubility in buffered solutions at a pH of 6.5 or above, some incubations were carried out in 150 mM NaCl. Otherwise, a buffered salts solution, previously described[16], was used. There was no difficulty in preparing solutions of 1–50 mM persantin in unbuffered solutions, *e.g.* distilled water or 0.9 % NaCl.

Although our procedures for measuring uptake and exit of labeled materials by murine leukemia cells have been described before[9,13], a brief summary of the method follows. To measure accumulation of substrate, incubations were carried out at 37 °C for 5 min, and the distribution ratio of label between extracellular and intracellular water was measured*. Incubations were terminated by centrifugation for 30 s at 500 × *g* in 10 mm × 30 mm glass tubes. Pellets were blotted dry, dispersed in 0.9 % NaCl, and radioactivity was measured by liquid scintillation counting. Control studies were carried out with tritiated water, to measure intracellular + extracellular space, and with labeled inulin, mannitol, or sulfate, to estimate extracellular space. If a given substrate (*e.g.* uridine) is accumulated in a non-diffusible form, *i.e.* uridine nucleotides, the cell pellets may be washed with 0.9 % NaCl at 0 °C after termination of incubation. The correction for trapped extracellular radioactivity could then be omitted. If persantin substantially inhibits 'wash-out' of a substrate, the post-incubation washing can be carried out in 0.9 % NaCl containing 10 mM persantin[9]. This permits a direct measurement of accumulation of certain substrates after washing cell pellets.

Cells may be pre-loaded with a radioactive substrate, and drug effects on exodus of that substrate measured, if the substrate is not extensively metabolized. Often, mutant cell types can be used here which lack kinases, phosphoribosyltransferases, phosphorylases, or similar enzymes involved in catabolic and anabolic biotransformations. For such studies cells are first incubated with labeled substrate, collected by centrifugation, then suspended in a relatively large volume of fresh medium. At intervals, portions of the suspension are centrifuged, the cells collected and intracellular radioactivity determined.

In specific experiments, the effect of persantin on uptake of labeled compounds, and on efflux from preloaded cells was measured. Details are given with each experi-

* Distribution ratio = cpm/μl in cell water ÷ cpm/μl in extracellular water.

ment described. The substrates employed here are fucose, a poorly metabolized monosaccharide; glucose, a rapidly metabolized sugar; glycerol, a slowly metabolized carbohydrate; 3-*O*-methylglucose, a non-metabolized monosaccharide; adenine; uracil; the nucleosides deoxycytidine, uridine, cytidine and adenosine; cycloleucine, a non-metabolized amino acid; and phosphate ($^{33}PO_4^{2-}$), a rapidly metabolized anion.

RESULTS

Suspensions of L1210 cells were incubated with the labeled substrates listed in Table I, together with various levels of persantin. Persantin strongly inhibited accumulation of radioactive fucose, deoxycytidine and adenosine. Accumulation of *O*-methylglucose, uridine, cycloleucine and uracil was enhanced by addition of the drug. Persantin barely altered accumulation of adenine and glycerol. Since deoxycytidine, uridine and uracil are all metabolized to nucleotides in L1210 cells, a portion of the observed accumulation represents unidirectional flux into non-diffusible pools. We therefore repeated the studies shown in Table I using cell lines unable to metabo-

TABLE I

INHIBITION OF ACCUMULATION OF LABELED SUBSTRATES BY PERSANTIN

Cells were incubated with 0.1 mM labeled substrate + specified levels of persantin for 5 min at 37 °C, the cells were collected by centrifugation, and levels of radioactivity in cell water and extracellular water measured.

Substrate	*Distribution ratio*						
Persantin level (μM):	*0*	*10*	*25*	*100*	*250*	*500*	*1250*
Glucose	2.5 *	—	1.4	0.62	0.3	—	—
3-*O*-Methylglucose	1.05	—	1.30	1.76	0.9	0.30	0.25
L-Fucose	0.4	0.36	0.28	0.20	—	—	—
Glycerol	1.46	—	1.4	1.42	1.3	—	—
Cycloleucine	3.0	—	5.8	7.5	—	3.6	—
Adenine	1.6	—	1.7	1.7	1.5	—	—
Uracil **	1.1	—	—	1.8	—	—	—
Uridine ***	2.28 *	4.2	6.3	2.18	1.1	—	—
Adenosine	4.7 *	—	3.1	2.2	—	—	—
Deoxycytidine	2.0 *	—	—	0.2	—	—	—
Phosphate	0.2 *	0.23	0.20	0.12	—	0.07	—

* Substantial conversion to non-diffusible metabolites.
** Similar values were obtained using fluorouracil.
*** Similar values were obtained using cytidine.

lize these substances. The data in Table II show that persantin inhibited influx of deoxycytidine but promoted accumulation of uracil and uridine in the sublines employed.

Although fucose, *O*-methylglucose and cycloleucine are essentially non-metabolized in L1210, glucose and phosphate are rapidly metabolized, and the data shown in Table I can only be taken to represent unidirectional influx of the latter compounds. We have no cell lines unable to metabolize these two substances.

L1210 cells were pre-loaded with labeled cycloleucine, fucose or *O*-methylglucose, and the effect of persantin on efflux was measured. The drug inhibited exodus

TABLE II

EFFECT OF PERSANTIN ON ACCUMULATION OF NON-METABOLIZED SUBSTRATES

Cells were incubated for 5 min at 37 °C with 10 mM labeled substrates + specified levels of persantin.

Substrate	*Cell line*	*Distribution ratio*	
	Persantin level (μM):	*0*	*100*
Uracil*	P388/38280	0.98	1.65
Uridine	P388/FUR	1.1	2.7
Deoxycytidine	L1210/CA	1.05	0.03

* Similar values were obtained using fluorouracil.

TABLE III

EFFECT OF PERSANTIN ON EXODUS OF SUBSTRATES FROM PRE-LOADED CELLS

Cells were incubated with labeled compounds (0.1 mM) for 5 min at 37 °C, then collected and placed in fresh medium for 5 min at 37 °C. Persantin (100 μM) was present during the latter incubation as specified.

Substrate	*Cell line*	*Distribution ratio*		
		Initially	*After washing*	*After washing**
3-*O*-Methylglucose	L1210	1.10	0.08	0.75
L-Fucose	L1210	0.36	0.12	0.15
Cycloleucine	L1210	3.2	0.73	2.91
Deoxycytidine	L1210/CA	0.96	0.05	0.93
Uracil	P388/38280	0.92	0.18	0.72
Uridine	P388/FUR	1.09	0.13	0.92

* In the presence of 0.1 mM persantin.

of cycloleucine and *O*-methylglucose. Using the L1210/CA, P388/FUR and P388/38280 lines, the effect of persantin on efflux of uracil, uridine and deoxycytidine was measured. All were inhibited by the drug (Table III).

DISCUSSION

From the data presented above, we conclude the following:

(1) Persantin, at 100 μM, enhanced net accumulation of the non-metabolized sugar *O*-methylglucose. Furthermore, exodus of this sugar from pre-loaded cells was inhibited. At high levels, persantin inhibited net accumulation of *O*-methylglucose. Persantin appears to preferentially inhibit outward transport of this sugar. We cannot tell whether glucose is a member of the same class, since rapid conversion of this sugar to non-diffusible metabolites precludes studies of glucose efflux.

(2) Persantin inhibits accumulation of fucose, although slight inhibition of the exit process could be demonstrated. The drug therefore preferentially inhibits inward transport of the class of sugars represented by fucose.

(3) Persantin enhances accumulation of cycloleucine by inhibition of outward transport. This phenomenon has been described before[9].

(4) Persantin is known to inhibit uptake of nucleosides; evidence is presented here to show that the drug preferentially inhibits outward flux of uridine and cytidine. In contrast, inward transport of adenosine and deoxycytidine is predominantly inhibited at low persantin levels.

(5) Persantin inhibits uptake of phosphate, but not as markedly as in erythrocytes[5]. Because of rapid metabolism of phosphate, no studies on drug effects on phosphate efflux were done.

(6) Persantin did not alter accumulation of adenine, guanine or glycerol.

The multiplicity of effects of persantin on transport processes can only mean that the drug interacts with a series of different inward and outward transport processes to varying extents. It seems unlikely that such phenomena could be explained on the basis of interference with energy requirements of transport processes. The drug molecule[9] does bear structural similarities to a variety of naturally occurring molecules, *e.g.* pyrimidines, purines, nucleosides and simple sugars. Persantin could therefore bind to a variety of transport sites on either side of the cell membrane and thereby alter the flux of substrates through membranes.

Although a common transport system for nucleosides has been suggested[17], we had previously reported evidence suggesting considerable complexity; *e.g.* adenosine competed with deoxycytidine for uptake, but not for exodus from L1210 cells[13]. The present data show preferential inhibition by persantin of *O*-methylglucose exodus and fucose uptake; of deoxycytidine uptake and of uridine exodus; of uracil exodus while accumulation of adenine remains unaffected. Studies of the effect of persantin on movement of various substances through mammalian cell membranes therefore appears to be a new and useful method for providing further delineation of various transport processes.

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REFERENCES

1 W. Kübler, P. G. Spieckermann and H. J. Bretschneider, *J. Mol. Cell. Cardiol.*, 1 (1970) 23.
2 F. W. Koss, G. Beisenherz and R. Maerkisch, *Arzneim. Forsch.*, 12 (1962) 1130.
3 W. Kübler and H. J. Bretschneider, *Arch. Ges. Physiol.*, 280 (1964) 141.
4 R. A. Bunag, C. R. Douglas, S. Imai and R. M. Berne, *Circ. Res.*, 15 (1964) 83.
5 E. Gerlach, B. Deuticke and J. Duhm, *Pflügers Arch.*, 280 (1964) 243.
6 B. Deuticke, J. Duhm and E. Gerlach, *Pflügers Arch.*, 280 (1964) 275.
7 C. Scholtissek, *Biochim. Biophys. Acta*, 158 (1968) 435.
8 P. G. W. Plagemann, *Biochim. Biophys. Acta*, 233 (1971) 688.
9 D. Kessel and T. C. Hall, *Biochim. Biophys. Acta*, 211 (1970) 88.
10 J. Duhm, B. Deuticke and E. Gerlach, *Biochim. Biophys. Acta*, 170 (1968) 452.
11 D. R. Sanadi and A. L. Fluharty, *Biochemistry*, 2 (1963) 523.
12 L. A. Sordahl, J. C. Allen, A. Schwartz and R. L. Pharo, *Arch. Biochem. Biophys.*, 138 (1970) 44.
13 D. Kessel and S. B. Shurin, *Biochim. Biophys. Acta*, 163 (1968) 179.
14 D. Kessel, P. Reyes and T. C. Hall, *Mol. Pharmacol.*, 5 (1969) 481.
15 D. Kessel, R. Bruns, and T. C. Hall, *Mol. Pharmacol.*, 7 (1971) 117.
16 D. Kessel and H. B. Bosmann, *Cancer Res.*, 30 (1970) 7695.
17 T. S. Lieu, R. A. Hudson, R .K. Brown and B. C. White, *Biochim. Biophys. Acta*, 241 (1971) 884.