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The effect of dimethyl sulfoxide on nucleoside transport in L-cells

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SUMMARY

Low concentrations of dimethyl sulfoxide were found to sharply inhibit the uptake of radioactive nucleosides into L-cells. The inhibition appeared to be at the level of nucleoside transport and the extent of inhibition varied widely with the nucleoside being transported; 5% dimethyl sulfoxide inhibited the uptake of cytidine and uridine by approximately 90% but had little effect on the uptake of adenosine or thymidine. The inhibition was reversible and was not caused by leakage of material from the cell.

Two inhibitors of nucleoside uptake in mammalian cells, persantin and phenethyl alcohol, although chemically dissimilar have analogous effects[1-3]. Both agents decrease the initial rate of nucleoside uptake[2,3]. This rate is thought to represent a rate-limiting step in nucleoside transport across the cell membrane[2]. Neither of the agents seem to interfere with nucleoside phosphorylation as shown by their failure to competitively inhibit nucleoside kinase *in vitro* and by their failure to alter the relative amounts of nucleoside and its phosphorylated derivatives in the intracellular pool[1-3]. These results suggest that both chemicals interfere with nucleoside transport rather than a subsequent step in uptake[2,3].

We have found that the effect of dimethyl sulfoxide matches these properties exactly, but also presents a new one; at low concentrations dimethyl sulfoxide is selective in its inhibition of the uptake of nucleosides. Fig. 1 shows the effect of increasing concentrations of dimethyl sulfoxide on the uptake of ribonucleosides into the trichloroacetic acid-soluble L-cell pool. It can be seen that adenosine uptake is almost unaffected at levels of dimethyl sulfoxide that sharply inhibit the uptake of cytidine and uridine. The uptake of guanosine appears intermediate in its sensitivity to dimethyl sulfoxide.

To make certain these results reflect a real effect on uptake and not the failure to accumulate nucleosides because of cell damage, the experiment described in Table I

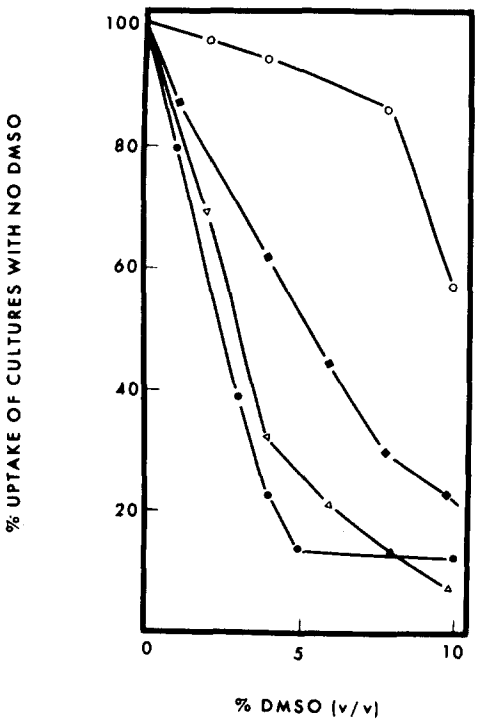

Fig. 1. The uptake of ^{3}H-labeled nucleosides into trichloroacetic acid-soluble material as a function of dimethyl sulfoxide (DMSO) concentration. L-cells (929 from Flow Laboratories, Rockville, Maryland), were grown to near confluency on 60-mm plastic Petri dishes containing 5 ml Eagle's medium with 10% calf serum. Monolayers were exposed to 1–10% dimethyl sulfoxide in growth medium for 2 h. One of the four ^{3}H-labeled nucleosides was added for an additional 45 min; the amount of nucleoside added per dish was as follows: 5 μC uridine (28.4 C/mmole); 5 μC adenosine (7.1 C/mmole); 2.5 μC cytidine (26.4 C/mmole); 0.5 μC guanosine (5.3 C/mmole). The cells were washed twice in phosphate buffered saline at 4° and extracted overnight in 5% trichloroacetic acid at 4°. Aliquots of the extracts were placed directly in Bray's scintillator and counted at ambient temperature. o–o, adenosine; ■–■, guanosine; Δ–Δ, cytidine; ●–●, uridine.

was performed. The results show that the cell pools retain approximately 70% of their original radioactivity after a 2-h incubation in nonradioactive medium and that 5% dimethyl sulfoxide causes no increased leakage of labeled cell material above the controls.

We measured the effect of dimethyl sulfoxide on the initial kinetics of adenosine and uridine uptake, as shown in Fig. 2. The results show that 5% dimethyl sulfoxide reduces the slope of the rate of uridine uptake *vs.* uridine concentration line by 92%, while the same concentration of dimethyl sulfoxide has no effect on adenosine. When this experiment was repeated using cytidine and thymidine, 5% dimethyl sulfoxide resulted in an 85% and 14% decrease in slopes, respectively. This substantiates the conclusion of the selectivity of dimethyl sulfoxide indicated in Fig. 1 and suggests that 5% dimethyl sulfoxide interferes with whatever rate-limiting step is responsible for the linear kinetics of pyrimidine ribonucleoside uptake.

It is possible that dimethyl sulfoxide interferes with uptake by decreasing nucleoside phosphorylation. To check this we prepared a cell sonicate capable of phosphorylating

TABLE I

THE EFFECT OF DIMETHYL SULFOXIDE ON NUCLEOTIDE LEAKAGE FROM PRE-LABELED CELLS

L-cell monolayers were exposed to [^{3}H] uridine or [^{3}H] adenosine for 150 min, then washed free of label with growth medium at 37°. One monolayer was extracted with 5% trichloroacetic acid to measure the intracellular pool counts/min at the time of transfer. The remaining monolayers were incubated for 30 or 120 min in growth medium with or without 5% dimethyl sulfoxide. Then the medium was removed and an aliquot counted to measure radioactivity lost from the cell. After further washing, the monolayers were extracted with 5% trichloroacetic acid to determine pool counts/min.

Nucleoside label	*% dimethyl sulfoxide after transfer*	*Time after transfer (min)*	*Counts/min in acid-soluble pool*	*Counts/min in medium overlay*	*% of original pool counts/min in medium*
Uridine	–	0	99 980	–	
	0	120	70 020	13 660	14
	5	30	–	7 120	7
	5	120	73 850	11 790	12
Adenosine	–	0	182 700	–	
	0	120	141 140	14 060	8
	5	30	–	7 710	4
	5	120	154 480	13 740	8

uridine *in vitro* to UTP and the intermediate phosphates, following the procedure of Plagemann[4]. The addition of up to 10% dimethyl sulfoxide was found to have no effect on the activity of this uridine kinase preparation.

A measure of the phosphorylation rate *in vivo* is the nucleotide levels of the acid-soluble cell pool. Inhibiting phosphorylation would be expected to increase free nucleosides and monophosphates at the expense of triphosphates. The effect of dimethyl sulfoxide on the uridine nucleotide levels was accordingly measured and the results are presented in Table II. There is no increase of uridine, nor any change in the proportions of nucleotides due to dimethyl sulfoxide.

TABLE II

THE EFFECT OF DIMETHYL SULFOXIDE ON THE COMPOSITION OF THE URIDINE NUCLEOTIDE POOL

L-cell monolayers in large Petri dishes were incubated for 90 min with or without dimethyl sulfoxide, then labeled for 2 h in the presence of [^{3}H] uridine. The cells were washed in cold phosphate buffered saline and extracted for 30 min with 0.5 M $HClO_4$ at 0°. An aliquot was counted to give the total label taken into the pool. A second aliquot was chromatographed and counted according to the procedure of Plagemann[4], along with reference nucleotides located by ultraviolet light.

% dimethyl sulfoxide	*Counts/min in extract*	*%*	*% as UTP*	*% as UDP and UDP-X*	*% as UMP*	*% as uridine*
0	73 844	100	42	32	26	<1
2	45 340	61	46	30	25	<1
4	19 790	27	46	30	24	<1

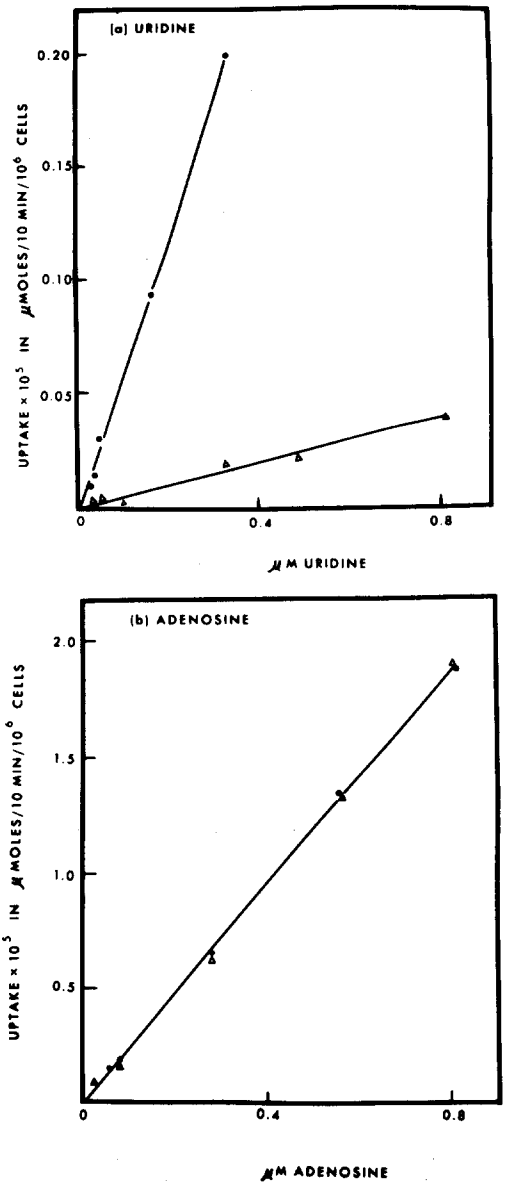

Fig. 2. The initial rate of nucleoside uptake as a function of external nucleoside concentration (μmoles/l) and dimethyl sulfoxide (DMSO). L-cell monolayers were incubated with or without 5% dimethyl sulfoxide for 90 min, then the desired concentration of ^{3}H-labeled nucleoside was added for 10 min. After washing with cold phosphate buffered saline the cells were dissolved overnight in 1 M NaOH at 37°. Aliquots were counted by liquid scintillation and from the known efficiency of counting the rates of uptake were calculated. ●–●, without dimethyl sulfoxide; Δ–Δ, with 5% dimethyl sulfoxide.

Further experiments showed that the dimethyl sulfoxide effect is readily reversible. When cell monolayers were washed free of dimethyl sulfoxide, they regained over 90% of their original capacity for uridine uptake within 15 min. In measurements of incorporation taken at 15-min intervals the inhibition of uridine uptake by dimethyl sulfoxide was fully established by 30 min.

It appears then that the most probable effect of up to 5% dimethyl sulfoxide is on nucleoside transport across the cell membrane. The dramatic inhibition of uptake of both cytidine and uridine by 5% dimethyl sulfoxide suggests that these nucleosides share a common transport system. Other indications about the specificity of nucleoside transport in L-cells are that adenosine and guanosine are distinguished and that the ribo- and deoxyribo-pyrimidine nucleosides also have separate transport systems, since thymidine and the pyrimidine ribonucleosides are so differently affected by 5% dimethyl sulfoxide.

Because of its selective effect on nucleoside transport, any conclusions about the effect of dimethyl sulfoxide on RNA synthesis when measured by the uptake of radioactive precursor into acid-insoluble material must be made cautiously. Fig. 3 shows

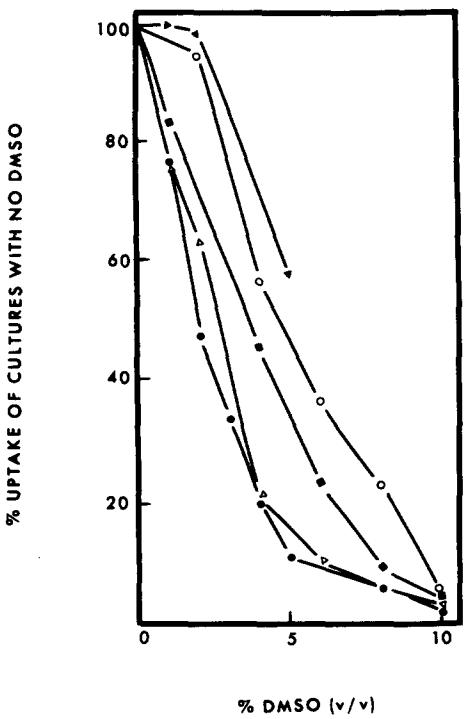

Fig. 3. The uptake of radioactive nucleosides and $^{32}P_i$ into trichloroacetic acid-insoluble material as a function of dimethyl sulfoxide (DMSO) concentration. Except for $^{32}P_i$, the experimental procedure was the same as that given in Fig. 1. The acid-insoluble material was dissolved in Nuclear Chicago Solubilizer for liquid scintillation counting. In the case of $^{32}P_i$ the cells were labeled as before (2 μC/ml) but RNA was purified by the procedure of Schmidt and Thannhauser[5]. Aliquots of the final NaOH digest of RNA were counted by liquid scintillation. ▲–▲, $^{32}P_i$; o–o, adenosine; ■–■, guanosine; Δ–Δ, cytidine; ●–●, uridine.

that the effect of dimethyl sulfoxide on apparent RNA synthesis is much smaller for $^{32}P_i$ and adenosine than for cytidine and uridine. The inhibition of incorporation of labeled cytidine and uridine into RNA by dimethyl sulfoxide is accounted for by the failure of transport of these compounds into the cell. However, Fig. 3 indicates that dimethyl sulfoxide also has a secondary effect on some step in RNA synthesis other than transport. For example, 5% dimethyl sulfoxide does not inhibit adenosine transport or pool accumulation (Figs. 1 and 2b), but still has a significant effect on its uptake into the acid-insoluble RNA fraction.

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