Bis(benzyl)polyamine analogues are substrates for a mammalian cell-transport system which is distinct from the polyamine-transport system

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Bis(benzyl)polyamine analogues (e.g. *NN'*-bis{3-[(phenylmethyl)amino]propyl}-1,8-diamino-octane [$C_6H_5CH_2NH(CH_2)_3NH(CH_2)_8NH(CH_2)_3NHCH_2C_6H_5$]) have previously been shown to regulate polyamine biosynthesis and growth of rat hepatoma (HTC) cells. Saturable uptake of the analogues, the ability of other bis(benzyl)polyamine analogues to compete for this uptake and the *trans*-acceleration of this uptake in pre-loaded cells indicate that these novel compounds are accumulated through the action of a transport system in HTC cells. A mutant Chinese-hamster-ovary (CHO) cell line, CHOMG, which lacks a functional polyamine-transport system, exhibited saturable bis(benzyl)polyamine uptake identical with that observed in the parental CHO cells, which have normal polyamine transport. The uptake of the analogue by both CHOMG and CHO cells was competitively inhibited by other bis(benzyl)polyamine analogues, but was insensitive to excess spermine. Treatment with α -difluoromethylornithine, an inhibitor of polyamine biosynthesis, resulted in the enhancement of spermine uptake in CHO cells but did not alter the uptake of a bis(benzyl)polyamine analogue by either CHO or CHOMG cells. Thus it appears that bis(benzyl)polyamine analogues are substrates for a mammalian-cell-transport system distinct from the polyamine-transport system.

INTRODUCTION

Spermidine, spermine and their biosynthetic precursor putrescine are known to be substrates for a plasma-membrane carrier-mediated transport system referred to as the 'polyamine-transport system' (Pegg & McCann, 1982; Jänne *et al.*, 1983; Tabor & Tabor, 1984; Pegg 1986, 1988; Porter & Jänne, 1987). A number of polyamine analogues and structurally related compounds have been shown to enter mammalian cells via the polyamine-transport system. These alternative substrates include methylglyoxal bis(guanylhydrazone) (MGBG) (Mandel & Flintoff, 1978; Alhonen-Hongisto *et al.*, 1980; Seppänen *et al.*, 1981; Jänne *et al.*, 1983; Rodrigues *et al.*, 1987), paraquat (Smith & Wyatt, 1981; Gordonsmith *et al.*, 1985; Byers *et al.*, 1987) and various alkylated spermidine and spermine derivatives (Porter *et al.*, 1984, 1987; Byers & Pegg, 1989). Since all of these compounds are toxic to cells in which they accumulate, the activity of the polyamine-transport system is crucial to the sensitivity of mammalian cells towards these agents. Resistance to these polyamine analogues in mutant cell lines which lack polyamine transport demonstrates the important permissive role the polyamine-transport system plays in the toxicity of these compounds (Mandel & Flintoff, 1978; Byers *et al.*, 1987; Rodrigues *et al.*, 1987; Persson *et al.*, 1988; Heaton & Flintoff, 1988). It has been suggested that potential chemotherapeutic compounds be designed so that the polyamine-transport system may be used to deliver agents into the cell (Porter *et al.*, 1984, 1985).

Bis(benzyl)polyamine analogues (e.g. *NN'*-bis{3-[(phenylmethyl)amino]propyl}-1,8-diamino-octane [$C_6H_5CH_2NH(CH_2)_3NH(CH_2)_8NH(CH_2)_3NHCH_2C_6H_5$]) inhibit the growth of the malaria parasites *Plasmodium falciparum* *in vitro* and *P. berghei* *in vivo* (Bitonti *et al.*, 1989b). Because accumulation of antimalarial

drugs (e.g. chloroquine) in infected erythrocytes often determines the drug's efficacy against the parasites (Fitch, 1970), the accumulation of bis(benzyl)polyamine analogues was examined in cultured human erythrocytes, the host cells of *P. falciparum*. This accumulation was found to have a saturable and apparently carrier-mediated component (Bitonti *et al.*, 1989c). The uptake system responsible for the accumulation of the bis(benzyl)polyamine analogue was also found to be apparently distinct from the transport system which facilitates the uptake of the natural polyamines and other polyamine analogues (Bitonti *et al.*, 1989c).

Exposure of cultured rat hepatoma cells (HTC) to a bis(benzyl)polyamine resulted in a down-regulation of polyamine biosynthesis and inhibited cell growth (Bitonti *et al.*, 1989a). It was of interest to know whether a transport system similar to that found in erythrocytes was present in other mammalian cells and to determine if the carriers responsible for this uptake were distinct from those which transport polyamines. In the present work, uptake of the bis(benzyl)polyamines was examined in HTC cells and in two Chinese hamster ovary (CHO)-cell lines, a parental CHO line and a MGBG-resistance mutant CHO-cell line (CHOMG) which lacks polyamine-transport activity (Mandel & Flintoff, 1978; Byers *et al.*, 1987). Uptake of the bis(benzyl)polyamines in these cells did appear to occur via a carrier-mediated transport system. Whereas previous studies with erythrocytes are suggestive of distinct transport systems for the natural polyamines and the bis(benzyl)polyamine analogues (Bitonti *et al.*, 1989c), the present study unambiguously distinguishes between the two uptake systems. By using the CHOMG-cell line it was shown that bis(benzyl)polyamines are substrates for a plasma-membrane transport system which is distinct from the polyamine-transport system.

Abbreviations used: CHO, Chinese-hamster ovary; DFMO, α -difluoromethylornithine; FBS, fetal-bovine serum; ODC, ornithine decarboxylase; MGBG, methylglyoxal bis(guanylhydrazone); MEM, minimal essential medium; PBS, phosphate-buffered saline (composition and pH are given in the text).

MATERIALS AND METHODS

Chemicals

[1,4- 14 C]Spermine tetrahydrochloride (111 μ Ci/ μ mol) was purchased from Amersham International. Other reagents were purchased from Sigma. All polyamine analogues (including *NN'*-bis{3-[(phenylmethyl)amino]propyl}-1,8-diamino[14 C]octane (48 μ Ci/ μ mol) and α -difluoromethylornithine (eflornithine; DFMO) were synthesized at the Merrell Dow Research Institute. Eflornithine and bis(benzyl)polyamine analogues are available upon request.

Cell culture

HTC cells were cultured in Swim's 77 medium supplemented with 10% (v/v) newborn-calf serum and were maintained at 37 °C in air/CO₂ (19:1) and 93% humidity. The MGBG-resistant CHOMG cells, and the parental CHO cells from which the CHOMG cells were derived, were generously provided by Dr. W. Flintoff, Department of Microbiology, University of Western Ontario, London, Ont., Canada. These cells were grown as monolayers at 37 °C in air/CO₂ (19:1) and 93% humidity in α -MEM (Gibco) with 10% fetal-bovine serum (FBS), penicillin (12 μ g/ml) and streptomycin (12 μ g/ml).

Uptake measurements

The uptake procedures used were based on those previously described for HTC cells (Bayer *et al.*, 1980) and for CHO cells (Byers *et al.*, 1987). The cells were plated in 24-well plates. After the indicated times, the growth medium was aspirated and uptake medium, containing the appropriate 14 C-labelled substrate, was added. Uptake into HTC cells was determined in Swim's medium supplemented with 10% (v/v) horse serum, rather than FBS, to avoid the oxidative formation of toxic metabolites from polyamines and polyamine analogues by bovine serum oxidases. α -MEM without FBS was used for the uptake medium in the CHO-cell experiments. The cells were incubated for 30 min under the same conditions as for growth, after which the cells were washed by repeated aspiration and addition of fresh medium, and finally washed with phosphate-buffered saline [PBS; 137 mM-NaCl/3.0 mM-KCl/16 mM-Na₂HPO₄/2.0 mM-KH₂PO₄/CaCl₂ (0.2 mg/l)/MgSO₄·7H₂O (0.2 mg/l)]. Uptake of bis(benzyl)polyamine analogues was linear for at least 30 min. HTC cells were then lysed by the addition of 0.2 ml of distilled water per well, and liquid-scintillation counting was performed on the supernatant. HTC cells in separate wells, without added radioactive substrates, were used for determination of cell number by counting on a haemocytometer. CHO cells were dissolved by sonication in 0.5 ml of 0.1 M-NaOH per well, and aliquots of this solution were used for protein quantification (Bradford, 1976) and for determination of radioactivity. Uptake data were analysed by Lineweaver-Burk (1934) plots to obtain apparent K_m and K_i values.

Detection of bis(benzyl)polyamine metabolism by CHO and CHOMG cells

CHO and CHOMG cells incubated with 2 μ M-MDL 27695 for various times were washed three times with 10 ml of PBS (4 °C) and then harvested in 10% (w/v) trichloroacetic acid by scraping the surface of the dish with a rubber policeman. The polyamine analogue MDL 27695 and its monobenzyl (MDL 28560) and free amine (MDL 26752) metabolites were derivatized with dansyl chloride, separated by h.p.l.c., and quantified on the basis of fluorescence detection (Bitonti *et al.* 1989a).

RESULTS

The uptake of [14 C]MDL 27391 (structures for MDL

Compound	<i>n</i>
MDL 27695	7
MDL 27391	8
MDL 27701	9

Fig. 1. Structures of bis(benzyl)polyamine analogues

compounds are given in Fig. 1) into HTC cells was saturable with an apparent K_m of approx. 1 μ M (Fig. 2). For comparison, the K_m for spermidine uptake by HTC cells was determined to be approx. 7 μ M. These kinetics suggested carrier-mediated uptake. MDL 27701, a structural analogue of MDL 27391 containing an additional carbon atom in the central methylene chain, competitively inhibited the uptake of [14 C]MDL 27391 with an apparent K_i of 4 μ M. Treatment of HTC cells with compounds which interfere with energy-dependent processes did not effect the uptake of [14 C]MDL 27391 (Table 1). When HTC cells were exposed to 10, 100, 500 or 1000 μ M unlabelled MDL 27391 for 60 min, washed free of remaining exogenous drug, and then exposed to 2.0 μ M-[14 C]MDL 27391 for 30 min, the uptake of [14 C]MDL 27391 was increased compared with that in cells

Fig. 2. Lineweaver-Burk plot of the uptake of MDL 27391 into HTC cells and its competitive inhibition by a structural analogue

HTC cells were plated in 24-well plates so that they were confluent within 4 or 5 days. After the cells were confluent, the growth media was replaced with Swim's medium supplemented with 10% horse serum containing 0.2–5 μ M-[14 C]MDL 27391 and 100 μ M-MDL 27701 where indicated. After a 30 min incubation at 37 °C the cells were washed three times with Swim's medium without serum, and the cells were lysed by the addition of 0.2 ml of distilled water. Scintillation assays were used to determine uptake. The cell number of representative cultures was determined by exposure to trypsin and cell counting using a haemocytometer.

Table 1. Uptake of the bis(benzyl)polyamine analogue MDL 27391 by HTC cells does not appear to be energy-dependent

HTC cells cultured in 24-well plates were preincubated with the indicated compounds for 15 min at 37 °C. [14 C]MDL 27391 was added to 2.0 μ M and the cells were incubated for an additional 30 min. Cells treated with iodoacetic acid and/or KCN were exposed to these compounds only for the duration of the uptake measurements. The mean control value for three experiments was 10.4 ± 1.6 pmol/30 min per 10^6 cells.

Inhibitor	Uptake (% of control)
1 mM-Dinitrophenol	90
50 mM-2-Deoxyglucose	130
1 mM-Dinitrophenol + 50 mM-2-deoxyglucose	107
1 mM- <i>N</i> -Ethylmaleimide	87
1 mM-Ouabain	111
5 mM- NaN_3	105
1 mM-Iodoacetic acid	86
1 mM-KCN	90
1 mM-Iodoacetic acid + 1 mM-KCN	138

which were not preloaded with unlabelled compound (Fig. 3; a concn. of 0 mM on the x-axis represents cells not preloaded with unlabelled compound). The extent to which uptake was increased correlated directly with the concentration of unlabelled MDL 27391 in the first incubation.

The uptake of [14 C]MDL 27391 was also examined in CHO and CHOMG cells to determine unambiguously the role of the polyamine-transport system in the accumulation of bis(benzyl)polyamines. Uptake of [14 C]MDL 27391 by both CHO and CHOMG cells was saturable (Fig. 4), with identical apparent-

Fig. 3. Transacceleration of MDL 27391 uptake of HTC cells preloaded with unlabelled MDL 27391

HTC cells were plated in 24-well plates so that they were confluent within 4 or 5 days. After the cells were confluent, the growth media was replaced with Swim's medium supplemented with 10% horse serum and 0, 10, 100, 500 or 1000 μ M unlabelled MDL 27391 and the cells incubated at 37 °C for 30 min. After this incubation the cells were washed three times with medium, and the uptake of [14 C]MDL 27391 was determined as described in the legend for Fig. 2.

Fig. 4. Saturable uptake of MDL 27391 by CHO (●) and CHOMG (○) cells

CHOMG and CHO cells were plated at 3×10^4 cells/ml per well in 24-well plates. At 72 h after plating the growth media was removed and the cells were washed with 1 ml of PBS (37 °C). The cells were exposed to α -MEM without FBS containing [14 C]MDL 27391 (0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 2.0, 2.4 and 2.75 μ M). At 0 and 30 min after the addition of [14 C]MDL 27391 the wells were washed twice with α -MEM without FBS (4 °C) and twice with PBS (4 °C). The cells were then dissolved in 0.1 M-NaOH, and scintillation and protein assays were performed to determine nmol/mg of protein values. Results are shown as means $\pm$ S.D. for three observations.

K_m values of approx. 1 μ M. The uptake of [14 C]MDL 27391 by both CHOMG and CHO cells was competitively inhibited (apparent K_i of approx. 2 μ M) by MDL 27695, a bis(benzyl) polyamine analogue shorter in chain length than MDL 27391 by one carbon atom, but was relatively insensitive to spermine (Fig. 5). Finally, treatment of CHO cells with 5 mM-DFMO, known to inhibit ornithine decarboxylase (ODC) and deplete intracellular putrescine and spermidine, enhanced the uptake of [14 C]spermine but not the uptake of [14 C]MDL 27391 (Table 2).

Although both CHO and CHOMG cells metabolized MDL 27695, this metabolism was not detectable during the first 30 min of exposure to a 2.0 μ M concentration of this analogue (Table 3). In fact, measurable quantities of the two known metabolites, the monobenzyl (MDL 28560) and the free amine (MDL 26752) derivatives, were not found until 1–2 h of incubation in CHO and CHOMG cells. This time course was comparable with that reported previously for HTC cells (Bitonti *et al.*, 1989a).

DISCUSSION

Uptake of bis(benzyl)polyamine analogues by a transport system different from that which serves to transport the natural polyamines, putrescine, spermidine and spermine, appears to be a common feature of a number of mammalian cell types, including human erythrocytes, rat hepatoma cells and CHO cells. Although initial studies in human erythrocytes were highly suggestive of this carrier-mediated transport system, the results of the present study are more conclusive, because of the use of 'wild-type' cell lines (HTC and CHO), which transport polyamines, and a mutant cell line which completely lacks the ability to transport polyamines (CHOMG). The availability of mammalian cells which provide negative and positive controls for polyamine transport have been used previously to examine the role of the polyamine-transport system in the toxicity of polyamine analogues and other compounds with structural similarities. The CHO/CHOMG model has been used to demonstrate that the

Fig. 5. Competitive inhibition of bis(benzyl)polyamine uptake into (a) CHO and (b) CHOMG cells by a structural analogue, but not by spermine

CHO and CHOMG cells were plated at 2.75×10^4 cells/ml per well in 24-well plates. At 72 h after plating, the growth media was removed and the cells were washed with 1 ml of PBS at 37 °C. The cells were exposed to α -MEM without FBS containing [14 C]MDL 27391 (0.5, 0.8, 1.1, 1.4 μ M) in the presence of 60 μ M-MDL 27695 ($\square$) or spermine ($\circ$) ($\bullet$, control). At 0 and 30 min after addition of [14 C]MDL 27391 the wells were washed twice with α -MEM without FBS (4 °C) and twice with PBS (4 °C). The cells were then dissolved in 0.1 M-NaOH. Scintillation and protein assays were performed to determine nmol/mg of protein values. Results shown are means for three observations.

Table 2. DFMO treatment increases the activity of the polyamine transport system without altering the uptake of the bis(benzyl)polyamine analogue MDL 27391

CHOMG and CHO cells were plated at 3×10^4 cells/ml per well as controls or 1×10^5 cells/ml per well with 5.0 mM-DFMO. At 72 h after plating, the growth media was removed and the cells were washed with 1 ml of PBS (37 °C). The cells were exposed to α -MEM without FBS containing 2.25 μ M-[14 C]MDL 27391 (48.3 μ Ci/ μ mol) or 2.25 μ M-[14 C]spermine (10.6 μ Ci/ μ mol). At 0 and 30 min after the addition of [14 C]MDL 27391 the wells were washed twice with α -MEM without FBS (4 °C) and twice with PBS (4 °C). The cells were then dissolved in 0.1 M-NaOH. Scintillation and protein assays were performed to determine nmol/mg of protein values. Results are shown as means $\pm$ S.D. for six observations. The difference in spermine uptake by control and DFMO-treated CHO cells was significant as determined by *t* test ($P < 0.01$). The differences for MDL 27391 uptake in DFMO-treated and control cells (CHO or CHOMG) were not significant by the same criteria.

Group	Uptake (nmol/h per mg of protein)
Spermine	
CHO/control	3.5 ± 0.36
CHO/DFMO	5.7 ± 0.8
MDL 27391	
CHO/control	0.30 ± 0.041
CHO/DFMO	0.30 ± 0.036
CHOMG/control	0.32 ± 0.045
CHOMG/DFMO	0.25 ± 0.045

activity of the polyamine-transport system is important in the cytotoxicity of MGBG (structurally analogous to spermidine), paraquat (structurally analogous to putrescine), and bis(ethyl)polyamine analogues (Mandel & Flintoff, 1978; Byers *et al.*, 1987; Byers & Pegg, 1989). On the basis of data obtained with CHO and CHOMG cells, it is obvious that the transport system responsible for the uptake of the bis(benzyl)polyamine analogues is distinct from the polyamine-transport system. The identical uptake of these compounds by cells with (CHO) and without (CHOMG) a functional polyamine-transport system provides conclusive and unambiguous evidence to this effect. The inability of spermine to inhibit this uptake and its lack of

Table 3. Time course of MDL 27695 metabolism by CHO and CHOMG cells

CHO and CHOMG cells were plated at 8×10^5 cells/10 ml per 100 mm dish in α -MEM with 10% FBS. After 72 h, the bis(benzyl)polyamine analogue MDL 27695 was added to 2 μ M. The cultures were incubated under growth conditions for the indicated length of time, washed three times with 10 ml of PBS (4 °C), and harvested in 10% trichloroacetic acid. These samples were dansylated and analysed by h.p.l.c. The results are means $\pm$ S.D. for three or four cultures and represent the levels of MDL 27695 and its two known metabolites, the monobenzyl MDL 28560 and the free amine MDL 26752. The level of detection is given where metabolites could not be detected.

Cells	Incubation period	Metabolite level (nmol/mg of protein)			
		MDL 27695 bis(benzyl)	MDL 28560 monobenzyl	MDL 26752 free amine	Total measured
CHO	30 min	0.69 ± 0.09	< 0.15	< 0.15	0.69
	1 h	1.07 ± 0.06	< 0.15	< 0.15	1.07
	2 h	1.18 ± 0.09	0.34 ± 0.03	0.34 ± 0.03	1.86
	4 h	1.46 ± 0.09	0.33 ± 0.03	0.29 ± 0.08	2.08
	8 h	0.81 ± 0.04	0.42 ± 0.04	0.36 ± 0.14	1.59
CHOMG	30 min	0.95 ± 0.14	< 0.15	< 0.15	0.95
	1 h	1.11 ± 0.13	0.32 ± 0.08	0.22 ± 0.06	1.65
	2 h	1.31 ± 0.12	0.25 ± 0.06	0.34 ± 0.05	1.90
	4 h	1.72 ± 0.11	0.26 ± 0.02	0.26 ± 0.07	2.24
	8 h	1.24 ± 0.19	0.22 ± 0.04	0.32 ± 0.06	1.78

induction by DFMO support these findings. The natural substrates of the carrier responsible for the uptake of the bis(benzyl)polyamine analogues have not been determined.

The saturable uptake of the MDL 27391 indicates that, at the concentrations used in these experiments, bis(benzyl)polyamines do not readily diffuse across the plasma membrane of HTC, CHO or CHOMG cells. Diffusion would not be expected, given the fact that the amines of these compounds are positively charged under physiological conditions. At higher concentrations, however, diffusion of these compounds into cells has been demonstrated (results not shown). This is in contrast with the uptake of these analogues by human erythrocytes, where diffusion was a major component of the uptake of bis(benzyl)polyamines, and a saturable component could only be demonstrated by manipulation of the temperature and pH of the uptake medium (Bitonti *et al.*, 1989c). The ability of analogous compounds to inhibit competitively the uptake of MDL 27391 supports the interaction of bis(benzyl)polyamines with a specific carrier/transporter on the mammalian cell membranes. Further evidence of carrier-mediated uptake is provided by the elevated [14 C]MDL 27391 uptake by HTC cells preloaded with unlabelled MDL 27391, referred to as 'transacceleration' (Willbrandt & Rosenberg, 1961; Bednarczyk & Medzihradsky, 1977; Medzihradsky, 1982). Increased transport in cells preloaded with substrate is believed to be due to the counterflow of the intracellular substrate, down a concentration gradient, via the transport protein, thus 'accelerating' the rate at which the binding site becomes available to the extracellular environment. The apparent lack of energy-dependence for the uptake of the bis(benzyl)polyamines by HTC cells suggests that this uptake is facilitated transport. In contrast, polyamine uptake has been reported to be active transport which requires metabolic energy (Seppänen, 1981; Porter *et al.*, 1982; Rinehart & Chen, 1984; Feige & Chambaz, 1985; Kumagai & Johnson, 1988).

The bis(benzyl)polyamine analogues are known to be substrates for mammalian-cell polyamine oxidase (Bitonti *et al.*, 1989a, 1990). The metabolic products of MDL 27695 were not detectable in either CHO or CHOMG cells during the first 30 min of exposure, indicating that metabolism did not play a role in the bis(benzyl)polyamine uptake. HTC cells also did not metabolize the bis(benzyl)polyamine analogues during the 30 min time used to measure uptake (Bitonti *et al.*, 1989a). These data are important, because it is possible that metabolism of a substrate could maintain a gradient which would drive uptake of that substrate via diffusion, and that saturation of the enzyme responsible for this metabolism would result in uptake which mimics saturable carrier-mediated uptake. In such a case other compounds which are substrates for that enzyme would inhibit the uptake by preventing the formation of the gradient. The lack of bis(benzyl)polyamine metabolism during the course of our uptake experiments supports the hypothesis that the uptake described for CHO and CHOMG cells is indeed a transport phenomenon. This is also indirectly supported by a linear and apparently non-saturable component of [14 C]MDL 27391 uptake observed at higher concentrations (results not shown). Since this second component is probably due to diffusion, it is unlikely that the saturable uptake which occurs at lower substrate concentrations is due to diffusion limited by a metabolism-generated gradient. Additionally, the saturable uptake of the bis(benzyl)polyamines by human erythrocytes does not require metabolism, since human erythrocytes lack polyamine oxidase activity (A. J. Bitonti, unpublished work).

Although the polyamine-transport system contributes significantly to the sensitivity of mammalian cells towards a number of polyamine analogues and related compounds which have potential value as cancer chemotherapeutic agents, the use

of this transport system to deliver drugs into cells may be limited by a regulatory decrease in polyamine-transport activity which occurs when intracellular substrate levels are increased (Byers, 1988). This regulation results in the maintenance of physiological levels of intracellular polyamines in cells exposed to an exogenous polyamine source and limits the accumulation of polyamine analogues. A means to by-pass the polyamine-transport system in the delivery of polyamines and polyamine analogues would be desirable. It is possible that polyamines and free-amine polyamine analogues could be delivered into cells as bis(benzyl)polyamines, which would in turn be metabolized to give the bioactive free amines. While the polyamine oxidase content of the target cell could be a limiting factor in this scheme, this enzyme is considered ubiquitous and is found constitutively at relatively high levels (Seiler *et al.*, 1980; Seiler, 1987) and therefore would probably not be limiting.

The finding that bis(benzyl)polyamines are substrates for a transport system distinct from the polyamine-transport system is significant for several reasons. First, it is evident that the uptake of the bis(benzyl)polyamines is not regulated by changes in intracellular polyamine pools, which suggests that DFMO pretreatment would not decrease and normal intracellular polyamine levels, would not prevent the accumulation of these compounds within mammalian cells. Secondly, an alternative transport system has been identified which may be targeted as a means to deliver drugs into mammalian cells. And thirdly, the uptake of the bis(benzyl)polyamines and their subsequent metabolism by polyamine oxidase provides an alternative to the highly regulated polyamine-transport system for the delivery of polyamines and polyamine analogues. Further investigation into the role of the uptake of bis(benzyl)polyamines and their effects on polyamine metabolism may have potential in both clinical and basic research.

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