Spermine uptake is necessary to induce haemoglobin synthesis in murine erythroleukaemia cells

Sophie CLÉMENT,* Jean-Guy DELCROS*§ and Burt G. FEUERSTEIN†‡||

* Laboratoire d'Immunochimie INSERM C.J.F. 89-05, Faculté de Médecine Lyon Sud, Oullins 69921, France, † Brain Tumor Research Center of the Department of Neurological Surgery and ‡ Division of Molecular Cytometry, Department of Laboratory Medicine, School of Medicine, University of California, San Francisco, CA 94143, U.S.A.

To determine whether intracellular uptake of spermine is necessary to induce haemoglobin synthesis in murine erythroleukaemia (MEL) DS 19 cells, we used single-step selection for resistance to N^1,N^{12} -bis(ethyl)spermine (BESM), a cytotoxic spermine analogue, to isolate clones deficient in polyamine transport. The cells were approximately 500-fold more resistant to BESM than parental cells and were unable to accumulate BESM, putrescine, spermidine or spermine. Addition of spermine to the polyamine-transport-deficient cells failed to induce haemoglobin synthesis. Hexamethylene-1,6-bisacetamide, a well-known differentiating

agent, induced haemoglobin synthesis in both parental and resistant cells. Polyamine-transport-deficient cells transfected with DNA purified from the parental cell line were further selected for their ability to grow in the presence of α -difluoromethylornithine and putrescine. The transfectants had an active transport system for polyamines, and spermine added to their culture medium accumulated inside the cells and induced haemoglobin production. These findings indicate that intracellular spermine uptake is required to induce haemoglobin production in MEL cells.

INTRODUCTION

Polyamines are biological polycations that play a major role in cell growth and differentiation [1,2]. In murine erythroleukaemia (MEL) cells, spermine and synthetic polyamine analogues induce haemoglobin synthesis [3,4]. In a previous study, we related the ability of polyamine analogues to induce haemoglobin synthesis in this cell line to the number of cationic charges they possess [4]. We therefore hypothesized that haemoglobin synthesis is mediated by binding of polyamine analogues to polyanionic cellular components. Compounds that induce terminal erythroid differentiation in MEL cells [5] can act at various cellular sites, including the plasma membrane and chromatin [6–8]. Thus active polyamine binding might occur at the outer surface of the plasma membrane [9–12]. However, because spermine and polyamine analogues accumulate in MEL cells, intracellular sites might also be important [4]. Spermine and polyamine analogues, for example, bind to DNA and chromatin, and alter their structure both *in vitro* and *in vivo* [13–15]. To determine whether the induction of haemoglobin synthesis by polyamines and polyamine analogues in MEL cells is mediated by events that take place at the outer surface of the plasma membrane or by direct interaction with intracellular components, we studied the ability of spermine to induce haemoglobin synthesis in MEL cells unable to accumulate polyamines and polyamine analogues.

EXPERIMENTAL

Chemicals

Minimal essential medium (MEM) and fetal calf serum (FCS) were obtained from Gibco (Eragny, France). Proteinase K was purchased from Boehringer-Mannheim (Meylan, France), and [1,4- 14 C]putrescine (110 mCi/mmol), [14 C]spermidine (110 mCi/

mmol) and [14 C]spermine (122 mCi/mmol) were from Amersham International (Amersham, Bucks, U.K.). All plastic material for cell culture was from Becton Dickinson (Lincoln Park, NJ, U.S.A.) and Costar (Cambridge, MA, U.S.A.). N^1,N^{12} -bis(ethyl)spermine (BESM) and α -difluoromethylornithine (DFMO) were kindly supplied by Dr. Raymond J. Bergeron (University of Florida, Gainesville, FL, U.S.A.) and Marion Merrell Dow (Cincinnati, OH, U.S.A.) respectively. All other reagents were purchased from Sigma (St. Louis, MO, U.S.A.).

Cell culture and terminology

MEL-DS 19 cells [3] were grown in suspension in MEM with 10% FCS at 37 °C in 5% CO₂. Cell growth was checked daily by counting cells with a haemocytometer. Resistant subclones are identified by BS (BESM resistance). Transfected cells are identified by T.

Mutagenesis and selection of resistant lines

Exponentially growing MEL-DS 19 cells were pretreated with ethylmethanesulphonate (400 µg/ml) for 24 h at 37 °C [16], centrifuged and resuspended in fresh medium. After 48 h, 1–100 µM BESM was added for 120 h. Surviving cells were cloned in semisolid medium with 1.44% (w/v) methylcellulose [4 Pa·s (4000 cP)] in MEM/10% FCS with 10 µM BESM. Cultures were examined immediately after inoculation to ensure that they were distributed as single cells. Surviving colonies were picked, cultured in suspension and retested for BESM resistance. BESM-resistant subclones (MEL-BS) were continually grown in 1 µM BESM, except for the week before measurement of polyamine uptake, when they were grown in the absence of BESM.

§ Present address: Groupe de Recherche en Thérapeutique Anticancéreuse, URA CNRS 1529-DRED 1266, Laboratoire de Biologie Cellulaire, 2 Avenue du Professeur Léon Bernard, F-35043, Rennes Cedex, France.

|| To whom correspondence should be addressed at Division of Molecular Cytometry.

Abbreviations used: MEL, murine erythroleukaemia; MEM, minimal essential medium; FCS, fetal calf serum; BESM, N^1,N^{12} -bis(ethyl)spermine; DFMO, α -difluoromethylornithine; TE, Tris/EDTA buffer (1 mM EDTA/1 mM Tris, pH 8); HBS, Hepes-buffered saline (0.14 M NaCl, 5 mM KCl, 10 mM sodium phosphate, 5.5 mM dextrose; 20 mM Hepes); MGBG, methylglyoxal bis(guanylhydrazone); HMBA, hexamethylenebisacetamide.

DNA isolation and transfection

High-molecular mass DNA from MEL-DS 19 cells was purified and transfected into MEL-BS 251 cells [17]. DS 19 cells were washed in Tris-buffered saline (0.14 M NaCl, 2.7 mM KCl, 25 mM Tris, pH 7.4) and resuspended in Tris/EDTA buffer (1 mM EDTA and 1 mM Tris, pH 8). They were then treated with 0.5% SDS and 20 µg/ml RNase in 10 mM Tris, pH 8, containing 0.1 M EDTA for 1 h at 37 °C and then with proteinase K (20 mg/ml) for 3 h at 50 °C. The cellular DNA was extracted with phenol, precipitated with ethanol in the presence of ammonium acetate, and resuspended in Tris/EDTA buffer.

Exponentially growing MEL-BS 251 cells (1×10^7) were washed in PBS. The DNA (40 µg of DS 19 DNA) was coprecipitated in an equal volume of double-strength Hepes-buffered saline (HBS) (0.14 M NaCl, 5 mM KCl, 10 mM sodium phosphate, 5.5 mM dextrose, 20 mM Hepes) by adding 2 M CaCl_2 dropwise to achieve a final concentration of 0.12 M. The cells were resuspended in 1 ml of the calcium phosphate/DNA suspension and after 20 min were diluted into serum-containing medium. After 6 h, the cells were washed with PBS, resuspended in 15% glycerol in HBS, incubated for 1 min at room temperature, and then diluted and washed with PBS. They were then resuspended in serum-containing medium, grown for 48 h, and transferred to medium containing 5 mM DFMO, 2 mM aminoguanidine and 50 µM putrescine for 2 days. Cloning in semisolid medium containing DFMO, aminoguanidine and putrescine was then carried out as described above. The cells were maintained under these selective conditions for approx. 2 weeks; colonies (MEL-T) were then selected and cultured in liquid medium.

Effect of drugs on cell growth

Exponentially growing cells were seeded at 5×10^4 cells/ml in the presence of drugs as indicated. When cells were grown in putrescine, spermidine or spermine, 2 mM aminoguanidine was added to prevent oxidation by serum amine oxidases [18]. To measure the effect of BESM (10 nM to 1 mM) or methylglyoxal bis(guanyldrazone) (MGBG) (10 nM to 0.1 mM) on cell growth, we defined the IC_{50} as the concentration of compound necessary to reduce cell growth to 50% of control growth after 3 days of culture. The effects of exogenous polyamines on DFMO-induced cytostasis were expressed as cell numbers after 3 days of culture in 5 mM DFMO with or without added polyamines. Cultures treated with 5 mM hexamethylene-bisacetamide (HMB) or 100 µM spermine were counted daily, and samples were collected for haemoglobin determination as described below.

Intracellular polyamine and BESM levels

Cell (1×10^6) were washed in PBS and extracted with 8% sulphosalicylic acid. The extracts were dansylated, and derivatized polyamines were separated by reverse-phase HPLC and quantified fluorimetrically [19].

Polyamine uptake

Aliquots of cell [$(0.5-3) \times 10^6$] were washed and resuspended in prewarmed MEM without FCS. Radioactive polyamine diluted in MEM was added (0.5 µCi; final concentration 0.5–30 µM). The cells were incubated at 37 °C for 10–30 min, and labelled uptake was blocked with ice-cold MEM (without FCS) containing a 1000-fold excess of unlabelled polyamine. The medium was removed by centrifugation and cells were washed with ice-cold MEM. The cell pellets were resuspended in 0.1 M NaOH, disrupted by sonication (Sonicator Annemasse S.A., Annemasse,

France; 70 V, two 10 s bursts) and aliquots of lysate were used to measure radioactivity (Scintillator Minaxi β Tricarb Packard-Pico Fluor 30; Packard Instrument S.A., Rungis, France) and protein [20]. Lineweaver–Burk plots were constructed, and K_m and V_{max} were determined by linear regression.

Haemoglobin production

Cells were scored for haemoglobin production by cytochemical staining with 3,3',5,5'-tetramethylbenzidine [21]. PBS-washed cells were stained for 2 min in 12% acetic acid containing 0.1% tetramethylbenzidine and 2% H_2O_2 . At least 100 cells were scored.

RESULTS

Overall strategy

Unlike other groups [16,22,23], we were not able to select cells deficient in polyamine transport with MGBG. Instead, we used BESM, a spermine analogue cytotoxic for MEL cells [4] and taken up by the polyamine-transport system [24]. Some 18 clones of BESM-resistant cells (MEL-BS) were isolated by exposing mutagenized MEL-DS 19 cells to BESM, and the resistance was accompanied by a deficiency in polyamine uptake.

One MEL-BS clone was then transfected with DNA purified from parental MEL-DS 19 cells, and 11 clones of transfectants (MEL-T) were selected for their ability to grow in the presence of both an inhibitor of ornithine decarboxylase (DFMO) and the product of its activity (putrescine). Cells that grew in the presence of DFMO were therefore required to take up extracellular putrescine. Each MEL-T clone had an active polyamine-transport system. The ability of spermine to induce haemoglobin production in both MEL-BS and MEL-T cells was then investigated.

Polyamine transport is reduced in MEL-BS cells

Mutagenized MEL-BS clones were unable to take up spermine and putrescine (Table 1). In MEL-BS lines, uptake of spermine and putrescine was 0.5–3% and 2–5.5% of controls respectively. Spermidine uptake was similarly affected (results not shown). The uptake of spermine and putrescine in MEL-T was similar to that in parental MEL-DS 19 cells.

Uptake of polyamines by MEL cells was dependent on time, temperature and concentration and was saturable (results not shown). V_{max} and K_m were similar for spermine and spermidine,

Table 1 Uptake of spermine and putrescine by BESM-resistant mutant (BS), transfectant (T) and parental MEL-DS 19 cells

The uptake of [^{14}C]polyamines (20 µM; 0.5 µCi) was measured after incubation in FCS-free MEM for 10 min (MEL-DS 19 and MEL-T cells) or 30 min (MEL-BS cells). The results for each BS and T clone are the average of two determinations that agreed within 20%; n is the number of clones tested.

Clone	Polyamine uptake (nmol/h per mg of protein)	
	[^{14}S]Spermine	[^{14}C]Putrescine
DS 19	5.29 $\pm$ 0.53*	4.60 $\pm$ 0.45*
BS	0.07 (0.02–0.15)† (n = 17)	0.17 (0.08–0.25)† (n = 17)
T	5.47 (5.08–5.68)† (n = 6)	4.63 (4.59–4.67)† (n = 2)

* Mean $\pm$ S.D.

† Median (range).

Table 2 Kinetic constants for polyamine uptake by MEL-DS 19 cells

MEL cells were seeded at 5×10^4 cells/ml and cultured for 3 days with or without 5 mM DFMO. The uptake of polyamines was measured at 10 min with [14 C]polyamines (0.5–30 μ M; 0.5 μ Ci). Values are means $\pm$ S.D. of three experiments.

Treatment	Polyamine	K_m (μ M)	V_{max} (nmol/h per mg of protein)
None	Putrescine	41.99 ± 7.80	13.88 ± 2.04
None	Spermidine	1.05 ± 0.31	4.66 ± 0.60
None	Spermine	0.81 ± 0.22	4.49 ± 0.43
5 mM DFMO	Spermine	0.16 ± 0.01	6.60 ± 0.15

Figure 1 Kinetics of spermine uptake by MEL-DS 19, MEL-BE and MEL-T cells

Cells were incubated for 10–30 min at 37 °C in the presence of various concentrations of [14 C]spermine diluted in MEM (without FCS) and the amount of radioactivity inside the cells was determined. Results are shown for MEL-BE 251 (○), MEL-BE 541 (●), MEL-T 151 (Δ), MEL-T 223 (▲) and MEL-DS 19 cells (□) as the mean $\pm$ S.D. of three determinations that agreed within 5%. Lineweaver–Burk plots are shown in the inset. K_m is expressed as μ M and V_{max} as nmol/h per mg of protein.

Table 3 Polyamine levels in exponentially growing MEL-DS 19, MEL-BE and MEL-T cells

The cells were harvested from suspension culture with a cell density of 5×10^5 to 1×10^6 cells/ml, and polyamine levels were determined. Each value is the mean $\pm$ S.D. of three determinations. The median and range are given for the BS and T clones tested.

Clone	Polyamine content (nmol/ 10^6 cells)		
	Putrescine	Spermidine	Spermine
DS 19	$0.43 \pm 0.04^*$	$2.57 \pm 0.27^*$	$2.11 \pm 0.18^*$
BS (6 clones)	0.61 (0.28–1.02)†	2.31 (2.06–2.44)†	1.75 (1.37–2.66)†
T (4 clones)	0.29 (0.26–0.44)†	2.17 (1.9–2.57)†	1.63 (1.33–2.07)†

* Mean $\pm$ S.D.

† Median (range).

but were significantly higher for putrescine (Table 2). Treatment of MEL cells with 5 mM DFMO induced an approx. 50% increase in V_{max} and an approx. 7-fold decrease in K_m for

Figure 2 Sensitivity of MEL-DS 19 and MEL-BE cells to BESM

MEL-DS 19 (Δ), MEL-BE 251 (●), MEL-BE 541 (○), MEL-T 151 (■) and MEL-T 223 (□) cells were cultured in the presence of BESM, and cells were counted 72 h after the addition of the drug. The number of cells in the flask containing BESM was compared with controls. Results are means $\pm$ S.D. for three flasks.

spermine. Figure 1 shows the kinetic analysis of spermine uptake in clones of MEL cells; poor spermine uptake in MEL-BE clones prevented accurate determination of V_{max} and K_m . K_m was lower in MEL-T than in MEL-DS 19 cells, but V_{max} was unchanged.

Polyamine levels and growth of MEL-BE and MEL-T cells

Polyamine levels in MEL-BE and MEL-T cells were 50–150% of control (Table 3). The growth of transport-defective or transport-competent MEL cells was comparable with or slightly slower than that of parental MEL-DS 19 cells (results not shown).

Decreased BESM and MGBG sensitivity in MEL-BE cells

Dose–response curves for the growth of BS clones in BESM are presented in Figure 2. The IC_{50} for BESM in MEL-BE cells was greater than 100 μ M ($\sim 200 \mu$ M for MEL-BE 251 and slightly higher than 100 μ M for MEL-BE 541), but was only $\sim 0.25 \mu$ M for MEL-DS 19 parental cells. The IC_{50} values for BESM in MEL-T 151 and MEL-T 223 were $\sim 0.08 \mu$ M and $\sim 0.10 \mu$ M respectively. BS clones were unable to accumulate BESM to levels comparable with those of parental MEL-DS 19 cells, even at a BESM concentration of 100 μ M (Figure 3).

Figure 3 BESM levels in BESM-treated MEL cells

MEL-DS 19 (Δ), MEL-BE 251 (●) and MEL-BE 541 cells (□) were seeded at 5×10^4 cells/ml in 100 μ M BESM, and the intracellular level of BESM was determined in 1×10^6 cells. Results are means $\pm$ S.D. for three determinations.

Figure 4 Sensitivity of MEL-DS 19 and MEL-BS cells to MGBG

MEL-DS 19 (□), MEL-BS 251 (●), MEL-BS 541 (○), MEL-T 151 (▲) and MEL-T 223 (△) cells were cultured in the presence of MGBG, and cells were counted 72 h after addition of the drug. The number of cells was compared with controls. Results are means $\pm$ S.D. for three flasks.

MEL-BS cells were also less sensitive to MGBG than parental cells (Figure 4). The IC_{50} was $\sim 4 \mu M$ and slightly less than $3 \mu M$ for MEL-BS 251 and MEL-BS 541 cells respectively, but it was $\sim 0.7 \mu M$ for MEL-DS 19. MEL-T cells were also more sensitive to MGBG than parental clones. In MEL-T 151 and MEL-T 223 cells, the IC_{50} values for MGBG were $\sim 0.15 \mu M$ and $\sim 0.25 \mu M$ respectively.

Exogenous polyamines do not prevent DFMO-induced cytostatis in MEL-BS cells

The growth of parental MEL-DS 19, mutant BS and transfectant T cells cultured for 3 days in the presence of 5 mM DFMO was inhibited by 80–90% (Table 4). When 50 μM putrescine and 5 mM DFMO were given simultaneously, growth inhibition decreased to 50% in MEL-DS 19 cells and was prevented in MEL-T cells. However, 50 μM putrescine did not lessen the cytostatic effect of DFMO in MEL-BS cells. When the putrescine concentration was increased to 500 μM , the growth of MEL-BS cells was still 50% inhibited. Furthermore, neither 50 μM spermidine nor 50 μM spermine lessened DFMO-induced growth inhibition of MEL-BS cells, but they did totally or partially prevent the cytostatic effect of DFMO in MEL-T cells (Table 4).

Spermine does not induce haemoglobin production in MEL-BS cells

Table 5 shows the percentage of cells producing haemoglobin in

Table 5 Effect of HMBA and spermine on haemoglobin production in MEL-DS 19, MEL-BS and MEL-T cells

Cells were cultured with or without 5 mM HMBA or 100 μM spermine (plus 2 mM aminoguanidine). After 72 h (for HMBA-treated cells) or 120 h (for spermine-treated cells), the cells were stained for benzidine positivity as described in the Experimental section. Values are the average of two determinations that varied by less than 20%. The median and range for 14 BS and 11 T clones and the primary data from the parental and two BS clones are presented. ND, not determined.

Clone	Cells producing haemoglobin (% of benzidine-positive cells)		
	No addition	HMBA	Spermine
DS 19	2	96	40
BS (14 clones)	1 (0–6)*	93 (70–98)*	3 (1–8)*
BS 151	1	39	2
BS 152	2	9	6
T (11 clones)	2 (0–7)*	ND	44 (28–54)*

* Median (range).

cultures of MEL-DS 19, MEL-BS and MEL-T cells treated for 3 days with 5 mM HMBA or for 5 days with 100 μM spermine. In all but two HMBA-treated MEL-BS clones, the percentage of MEL-BS cells producing haemoglobin after HMBA treatment was comparable with that of MEL-DS 19 cells (70–98%); a lower percentage of MEL-BS 151 and MEL-BS 152 cells produced haemoglobin in response to HMBA than parental DS 19 cells. After 5 days of treatment with spermine, haemoglobin was present in 40% of MEL-DS 19 cells and 30–50% of MEL-T cells; none of the MEL-BS clones contained haemoglobin. Only 2–7% of MEL-BS cells grown with spermine were benzidine-positive.

Spermine accumulated in MEL-DS 19 and MEL-T cells treated with 100 μM spermine (Figure 5a), but those cells were quickly depleted of spermidine (Figure 5b). Spermine at a concentration of 100 μM also inhibited the growth of MEL-DS 19 and MEL-T cells by 25–45% (results not shown). Spermine had no effect on spermine or spermidine levels (Figure 5) or on growth in MEL-BS cells (results not shown).

DISCUSSION

This study shows that intracellular spermine-binding sites govern spermine-induced haemoglobin production in MEL-DS 19 cells. Eighteen mutant MEL clones deficient in polyamine transport were unable to produce haemoglobin when treated with spermine.

Table 4 Ability of exogenous polyamines to prevent DFMO-induced cytostasis in MEL-DS 19, MEL-BS and MEL-T cells

Cells were cultured in 5 mM DFMO, 2 mM aminoguanidine and the polyamine at the concentration indicated. At 72 h, cells were counted and expressed as a percentage of the number of cells exposed solely to aminoguanidine. Values are means $\pm$ S.D. of three determinations. ND, Not determined.

Addition	Cell growth (% of control)				
	DS 19	BS 251	BS 541	MT 151	MT 223
None	11.9 $\pm$ 2.8	15.1 $\pm$ 2.1	13.8 $\pm$ 0.6	21.9 $\pm$ 0.2	14.3 $\pm$ 2.6
Putrescine (50 μM)	54.7 $\pm$ 4.3	13.6 $\pm$ 2.1	13.6 $\pm$ 1.5	110.8 $\pm$ 12.5	90.5 $\pm$ 4.3
Putrescine (500 μM)	99.0 $\pm$ 3.3	55.1 $\pm$ 6.3	54.5 $\pm$ 3.0	ND	ND
Spermidine (50 μM)	70.1 $\pm$ 4.2	19.6 $\pm$ 0.4	23.4 $\pm$ 1.5	106.1 $\pm$ 2.2	84.0 $\pm$ 13.1
Spermine (50 μM)	45.6 $\pm$ 3.7	18.9 $\pm$ 0.6	11.7 $\pm$ 2.4	56.9 $\pm$ 8.0	41.2 $\pm$ 3.1

Figure 5 Relative spermine and spermidine levels in MEL cells treated with 100 μ M spermine

MEL-DS 19 ($\circ$), MEL-T 151 ($\blacksquare$), MEL-T 223 ($\triangle$), MEL-BS 541 ($\bullet$) and MEL-BS 251 ($\square$) were cultured in the presence or absence of 100 μ M spermine (with 2 mM aminoguanidine). Cells were collected and intracellular polyamine levels were determined. Results are expressed as (a) the ratio of spermine levels in spermine-treated cells to those in control cells, and (b) the ratio of spermidine levels in control cells to those in spermine-treated cells.

When a polyamine-transport system was expressed in a polyamine-uptake mutant, the ability of spermine to induce haemoglobin production was restored. This close relationship between the presence of an active polyamine-transport system and the ability of spermine to induce haemoglobin production implies that intracellular uptake of spermine is necessary to induce haemoglobin production in MEL-DS 19 cells. Our observation that no revertant colonies were isolated when DFMO/putrescine selection was applied to untransfected MEL-BS cells (results not shown) confirms this relationship. Furthermore, since polyamine transport in itself did not greatly affect intracellular polyamine levels or growth, the absence of response to spermine was not related to modifications in polyamine metabolism or cell growth.

The well-known differentiating agent HMBA [25] induced normal production of haemoglobin in all but two polyamine-transport-deficient clones. This finding suggests that HMBA does not generally rely on polyamine transport to induce haemoglobin production.

The existence of a unique transporter or of several transporters specific for one or several polyamines has been described in mammalian cells [26]. Our data demonstrate that MEL cells transport putrescine, spermidine and spermine. The kinetic parameters were similar for spermine and spermidine uptake, but the K_m for putrescine was ~ 40 -fold higher. The uptake of all three polyamines was affected in MEL-BS cells selected for their deficiency in transporting an analogue of spermine (Table 1). Similarly, MEL-T cells selected for their ability to transport putrescine had a very active transport system for all three

polyamines. These results suggest that MEL cells express a transporter with different affinities for individual polyamines.

The loss of polyamine transport in MEL-BS cells could result from mutation in either the transporter itself or in another protein that regulates it. Although our data do not assess the gene encoding this loss, the stability of the resistant phenotype supports a genetic origin.

The apparently higher affinity for spermine exhibited in MEL-T than in parental cells may explain why 50 μ M putrescine completely prevented DFMO-induced cytostasis in MEL-T cells, but only partially prevented it in MEL-DS 19 cells (Table 4). The K_m for the uptake of polyamines in MEL-T cells cultured in the absence of DFMO was close to that in parental cells treated with DFMO (results not shown). It is possible that the MEL-T cells selected after extended contact with DFMO lack the normal mechanism responsible for feedback control of active polyamine uptake.

Mutant cells deficient in polyamine uptake were selected for their resistance to the toxic action of BESM, a potent anti-proliferative drug [27–31] taken up by a polyamine-transport system [24]. MEL-DS 19 cells are very sensitive to BESM (IC_{50} 0.3 μ M), whereas MEL-BS cells are resistant. The decreased capacity of MEL-BS cells to concentrate BESM [as evidenced by the low intracellular levels of BESM in treated cells (Figure 4) is a likely cause for their resistance to the cytotoxic drug.

MGBG is another cytotoxic drug that can be taken up through the polyamine-transport system [32,33]. Although resistance to MGBG has been used to select cells deficient in polyamine transport [16,22,23], MGBG-resistant cells with active polyamine transport have been isolated [34]. MEL-BS cells are much less resistant to MGBG than to BESM. This difference in sensitivity of MEL cells may explain why we could not isolate polyamine-uptake mutants by MGBG selection (results not shown), and indicates that MGBG may have additional mechanisms for uptake in MEL cells.

In summary our results demonstrate that MEL cells selected for resistance to BESM do not express haemoglobin when treated with spermine whereas parental and transfected cells with competent polyamine transport do. Induction of haemoglobin synthesis in MEL cells is related to the ability of the cells to accumulate spermine. This suggests that induction of haemoglobin production by spermine is mediated by interactions that take place within the cell.

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