J. Braz. Chem. Soc., Vol. 10, No. 4, 334-340, 1999.
Printed in Brazil.

0103 – 5053 $6.00 + 0.00

Article

Regioselective Binding of Spermine, N^1,N^{12}-Bismethylspermine, and N^1,N^{12}-Bisethylspermine to $tRNA^{Phe}$ as Revealed by 750 MHz ^{1}H-NMR and its Possible Correlation with Cell Cycling and Cytotoxicity

***Benjamin Frydman*[a], *William M. Westler*[b], *Aldonia Valasinas*[a], *Debora L. Kramer*[c], *and Carl W. Porter*[c]**

[a]*SLIL Biomedical Corp., 535 Science Drive, Suite C, Madison, WI 53711-1066 USA*

[b]*National Magnetic Resonance Facility, University of Wisconsin-Madison, Madison, WI 53706, USA*

[c]*Grace Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA*

Neste trabalho foi feito o estudo de ligação de espermina (SPM), N^1, N^{12}- bismetilespermina (BMS) e N^1, N^{12}-bisetilespermina (BES) ao $tRNA^{fen}$ usando RMN de ^{1}H a 750 MHz. As poliaminas foram enriquecidas com ^{13}C nos resíduos 5-CH_2 e 8-CH_2, sendo obtidos os picos cruzados por efeito de Overhauser nuclear entre as ressonâncias dos hidrogênios dos metilenos marcados com ^{13}C e vários hidrogênios de grupos imino de pares de bases do $tRNA^{fen}$ através de espectros 1D filtrados por ^{13}C. Foi encontrado que, enquanto SPM e BMS se ligam ao N(3)-H dos pares de bases T54-m^{1}A58, U50-A64 e U52-A62, BES liga-se só nos pares T54-m^{1}A58 e U50-A64. Esta regiosseletividade de ligação das três poliaminas ao tRNA foi correlacionada com seus efeitos biológicos no crescimento celular. Usando células de câncer de melanoma humano (MALME-3M), foi encontrado que SPM e BMS não tem efeito e é citostático, respectivamente, enquanto que BES é claramente citotóxica. Esta última poliamina também afeta o ciclo celular e, contrário ao comportamento de SPM e BMS, acarreta a uma clara parada do ciclo celular G_1 /S.

The binding of spermine (SPM), N^1,N^{12}-bismethylspermine (BMS) and N^1,N^{12}-bisethylspermine (BES) to $tRNA^{Phe}$ was studied using ^{1}H-NMR at 750 MHz. The polyamines were enriched in ^{13}C at the 5-CH_2 and 8-CH_2 residues and the nuclear Overhauser enhancement (NOE) cross peaks connecting the ^{1}H-NMR resonances of the ^{13}C-methylenes and several base paired imino protons of $tRNA^{Phe}$ were obtained using 1D ^{13}C-half filtered spectra. It was found that while SPM and BMS bind to the N(3)-H of base pairs T54-m^{1}A58, U50-A64 and U52-A62, BES binds only to T54-m^{1}A58 and U50-A64. This regioselectivity in the binding of the three polyamines to tRNA was correlated with their biological effects on cell growth. Using human melanoma cancer cells (MALME-3M), we found that SPM and BMS were without effect and cytostatic, respectively, while BES was distinctly cytotoxic. The latter also affected cell cycling and, at variance with SPM and BMS, lead to a distinct G_1/S cell cycle arrest.

Keywords: *^{1}H-NMR of polyamines, $tRNA^{Phe}$, melanoma, spermine*

Introduction

Spermine **1** (SPM) (Fig. 1) and spermidine (SPD) are polyamines widely distributed in biological systems where they are absolutely required for cell proliferation and also to support carcinogenesis[1-3]. Of the many hypotheses advanced to explain their biological effects[4], the most solid one concerns their interaction with nucleic acids[5-7]. Polyamines bind to ribosomal subunits, to DNA, and to

[a]Phone: 608-231-3854; Fax: 608-231-3892.

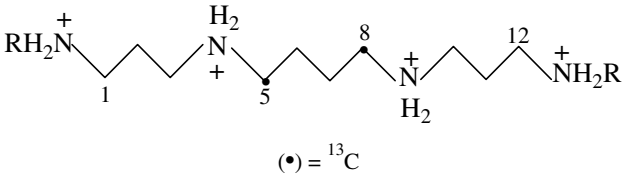

Figure 1. Structures of [$^{13}C_2$]-enriched spermine **1** (R=H), N^1,N^{12}-bismethylspermine **2** (R=CH_3), and N^1, N^{12}-bisethylspermine **3** (R=C_2H_5).

tRNA[1]. When binding to DNA they can cause DNA to condense and aggregate and can induce both B-to-Z and B-to-A transitions in certain DNA sequences[8-10]. The interaction of both SPM and SPD with tRNA has been extensively studied using solution ^{1}H-NMR[11a], ^{13}C-NMR[7], and ^{15}N-NMR[11b,11c] techniques. The polyamines are strong bases protonated at physiological pH and the aforementioned NMR studies showed that they bind to the nucleotides preferentially through hydrogen bonds generated by the protonated $–NH_2^+–$ residues (N^4 and N^9) internally present in the polyamine backbone (Fig. 1). The primary $–NH_3^+$ groups bind to tRNA much more weakly than the secondary $–NH_2^+–$ groups, thus casting doubt on the simplistic notion that polyamines are just "organic cations" analogous to Mg^{2+} or K^+. The ^{15}N-NMR experiments suggest that the specificity with which nitrogens in polyamines bind to tRNA is a consequence of the different hydrogen bonding modes that can be established between both types of molecules. More recently[11a] we have shown, using high field ^{1}H-NMR analysis, that SPM binds in solution to the TΨC loop of $tRNA^{Phe}$, at the corner of the L-shaped tRNA molecule where the TΨC and D loops meet. The importance of this part of the tRNA molecule for its correct *in vivo* function is known from random mutagenesis experiments[12].

Synthetic analogs of polyamines have been shown to be promising anticancer agents. They have the potential of killing cells and inhibiting cell growth both *in vivo* and *in vitro*[3,13]. A decade ago[13a] Bergeron and coworkers showed that N^1,N^{12}-bisethylspermine (BES) **3** (Fig. 1) was a powerful antineoplastic agent; these results led to the synthesis of a large number of highly cytotoxic N^α,N^ω-bisethyl polyamine analogs[3,13b,13c], two of which reached Phase I clinical trials. The former disclosure[13a] also reported that N^1,N^{12}-bismethylspermine (BMS) **2** (Fig. 1) was a much weaker antineoplastic agent than BES. This divergence between the inhibitory effects on cell growth of BES and BMS remained unexplored until very recently when their effect was evaluated *in vitro* in MALME-3M human melanoma cells. It was found that while BES is cytotoxic, BMS is cytostatic[14]. These results suggest that the antineoplastic effects of the N^α,N^ω-bisalkyl derivatives of spermine could be due, at least in part, to their effects on the protein synthesis process of the cell cycle rather than on the DNA replication process. The latter appears to be more affected by the N^α,N^ω-bisethyl derivatives of the higher homologs of spermine[15]. When the interaction with $tRNA^{Phe}$ of the N^α,N^ω-bisethyl derivatives of spermine and its homologs was examined using ^{15}N-NMR techniques[11b,11c] it was found that internal $–NH_2^+–$ groups (N^4 and N^9) formed the strongest hydrogen bonds with the nucleotides (as is the case with SPM or SPD), while the terminal $–NH_2^+C_2H_5$ groups (N^1 and N^{12}) formed the weaker bonds. The same was true for the N^α,N^ω-bismethyl derivatives; the strongest hydrogen bonds between the polyamine and tRNA were those formed by the central nitrogens (N^4 and N^9), independent of the alkyl substituent (methyl or ethyl) present at the terminal nitrogen groups. Binding of polyamines to tRNA has been shown to be relevant to the fidelity of the translation process[16-19], as well as to codon-anticodon recognition and ribosomal binding[20-21]. Polyamines also stimulate aminoacylation in general and their effects on the structure of tRNA compelled attention to the possible role of these compounds for protein synthesis[22]. We therefore decided to examine the binding regiospecificity of spermine (SPM) **1**, bisethylspermine (BES) **3** and bismethylspermine (BMS) **2** to tRNA using high field ^{1}H-NMR, and to attempt to correlate these results with their effect on the essential cell cycle processes and on the cell growth inhibition of a tumor cell line.

Results and Discussion

Regioselective binding to $tRNA^{Phe}$ of spermine (SPM) **1**, *bismethylspermine (BMS)* **2**, *and bisethylspermine (BES)* **3**

To identify the binding domains of **2** and **3** with tRNA we pursued the chemical and spectroscopic approach we devised to establish the binding of spermine **1** with $tRNA^{Phe}$.[11a] The strategy is based on the use of ^{1}H-^{1}H nuclear Overhauser enhancement (NOE) spectra where the spatial proximity between the polyamine analog and the tRNA protons could be determined by the appearance of peaks resulting from the ^{1}H-NMR resonances. In order to use this approach it is necessary to have a set of clearly resolved ^{1}H-NMR resonances of the tRNA assigned to well-defined regions within the structure. A valuable link between the structure of tRNA and its ^{1}H-NMR spectrum can be found in the 9.5-15.0 ppm region, where only the hydrogen bonded imino protons of the paired nucleotide bases resonate; these imino resonances have been assigned to individual bases in *E. coli*[23] and yeast $tRNA^{Phe}$ [24] (Fig. 2). We used these assignments to monitor the sites of binding, by measuring NOE cross peaks between the ^{1}H-imino resonances from the macromolecule and the polyamine analog protons. The upfield region of the tRNA spectrum (1.0-3.0 ppm) also allows the identification of the resonances of the methyl groups present in the $tRNA^{Phe}$ bases and was used for measuring NOE cross peaks with

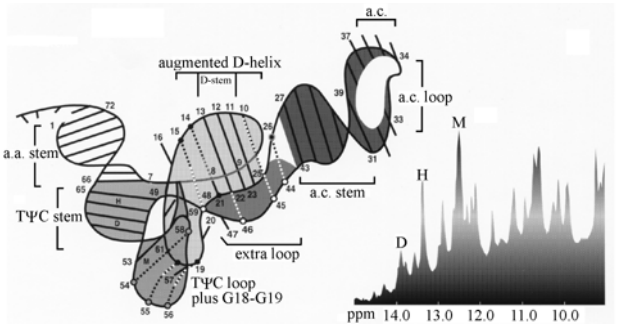

Figure 2. tRNA^{Phe} and the ^{1}H-NMR of its imino region recorded at 750 MHz.

the spermine protons.[11a] The aromatic region of the tRNA ^{1}H-NMR spectrum (6.0-9.0 ppm) is less useful for these determinations since the non-exchangeable aromatic protons have not been unequivocally assigned.

Ideally, labeling the $-NH_2^+-$, $-NH_2^+R$, and $-NH_3^+$ proton resonances of N^1,N^{12}-bisalkylspermines **2** and **3** or spermine **1** would be necessary, as these groups are directly involved in the binding process. Fast exchange with the aqueous solvent, however, precludes the observation of these resonances. This leaves the CH_2-protons of the methylenes α to the amino groups as the only practical choice for implementing this study. These protons resonate between 2.8-3.1 ppm; for the relative polyamine/tRNA concentrations that we use they will therefore be only marginally visible, due to the severe overlap with the tRNA sugar resonances starting at ca. 3.1 that integrate for hundreds of sites. In order to alleviate this problem we used polyamines enriched with ^{13}C in the carbon bonded to the $-NH_2^+-$ groups (Fig. 1). This allowed the acquisition of ^{13}C-half-filtered ^{1}H-NMR spectra where only protons bound to ^{13}C-heteroatoms appear along one of the frequency dimensions. The outline of the pulse sequences on which our tRNA/polyamine binding studies were based can be seen in Fig. 3. The sequence used in the 1D, ^{13}C-half-filtered, proton-proton NOE spectra is summarized in sequence (1):

$$D1 - 90^{H} - \Delta \pm 90^{C} - 180^{H} - 90^{C} - \Delta - 90^{H} - \\ - T_{mix} - 90^{H} - \delta - (-)\,90^{H} - \pm \text{acquisition} \quad (1)$$

The imino proton region of tRNA^{Phe} (9.0-15.0 ppm) shown in Fig. 4A was recorded in cacodylate buffer in the absence of Mg^{2+}. It is known[24,25] that the structure of tRNA^{Phe} in the absence of Mg^{2+} is basically the same as in the presence of Mg^{2+}, and that the ^{1}H-NMR spectrum in the region of 9.0-15.0 ppm is essentially independent of its presence. When the 1D ^{13}C half-filtered NOE spectrum of the imino region of the 1:8 tRNA^{Phe}:[5,8-$^{13}C_2$]-spermine complex was recorded, a strong NOE signal at 12.48 ppm could be measured (Fig. 4C). We had already shown [11a] that this interaction corresponds to the NOE cross peak of the

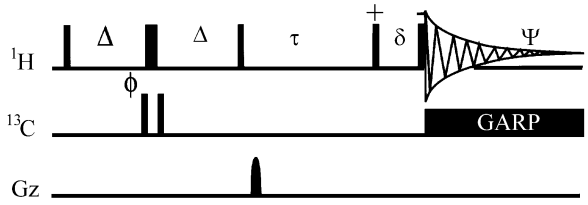

Figure 3. Pulse sequence used to obtain one-dimensional ^{13}C half-filtered proton-proton NOE spectra. The narrow and wide lines represent 90° and 180° pulses, respectively. G_z represents a pulse field gradient. Broadband decoupling of ^{13}C during the acquisition period was achieved by GARP modulation. Details of the timing, phases, and frequencies are given in Experimental.

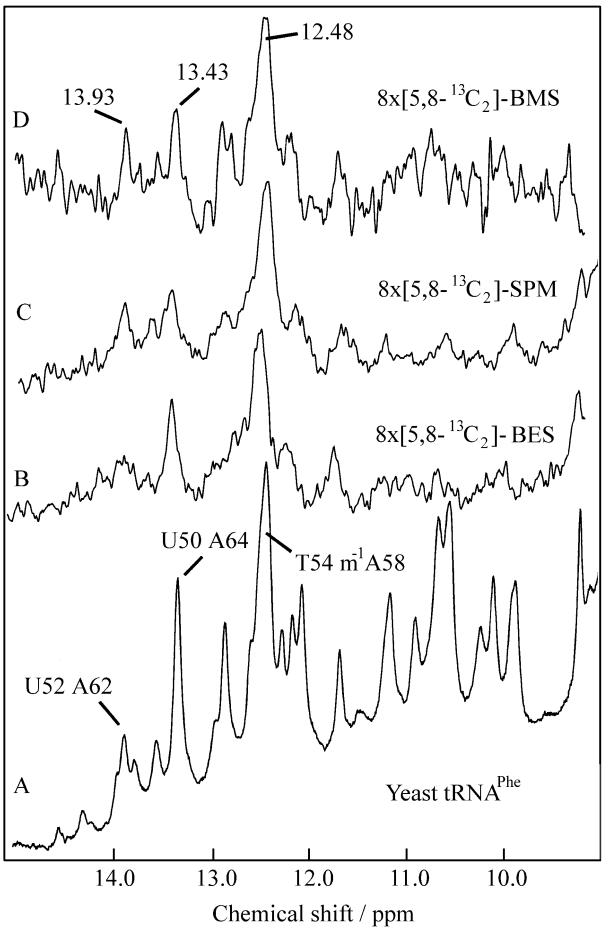

Figure 4. 750 MHz 1D ^{13}C half-filtered NOE spectra of the imino region of 8:1 complexes of [5,8-$^{13}C_2$]-enriched polyamines with tRNA^{Phe}. (A) Imino region of tRNA^{Phe}; 1D spectrum. (B) Bisethylspermine **3** added. (C) Spermine **1** added. (D) Bismethylspermine **2** added. The peaks of B-D represent NOE interactions between the spermine protons of the labeled carbons and the hydrogen bonded base-pair protons of tRNA^{Phe}. The NOE peaks are consistent with the binding of **1-3** near the TΨC loop of tRNA^{Phe}.

[5,8-$^{13}CH_2$] methylenes of spermine and the N(3)-H of the T54-m^{1}A58 base pair (Fig. 5). Base T54 on the TΨC loop is hydrogen bonded through its N(3)H to m^{1}A58 and this

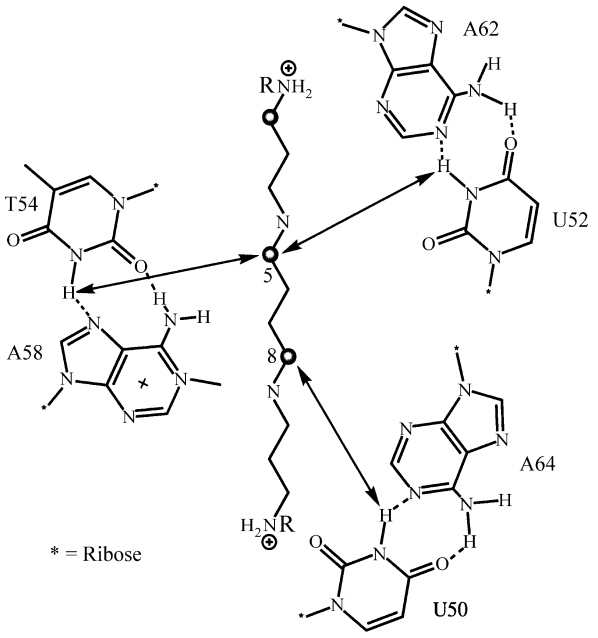

Figure 5. Outline of the ^{1}H-^{1}H cross peaks of ^{13}C-half-filtered NOE spectra of [^{13}C]-enriched spermines **1-3** with TΨC base pairs in tRNA^{Phe}.

base stacks on top of the three bases G57, G18, and G19 (the latter two of loop D) to form a stack of four purines. This interaction is critical for the stabilization of the TΨC loop at the juncture with the D loop (Fig. 2). Two weaker NOE cross peaks were also observed in the interaction of [5,8-$^{13}C_2$]-spermine and the imino protons of other tRNA^{Phe} base pairs. The NOE cross peak at 13.43 ppm (Fig. 4C) corresponds to the imino proton linking U50 to A64 on the T stem (Fig. 5). We confirmed Hilber's assignment of this resonance (resonance H)[25] to this base pair using 2D-NOE correlations. The second cross peak at 13.93 ppm corresponds to the interaction of spermine with an imino proton of a second UA base pair; namely U52 A62 (resonance D in Hilber's assignment[25]). Both U50 A64 and U52 A62 are base pairs of the T-stem and help locate the spermine molecule at the juncture of the TΨC loop and the D loop (Fig. 2). The importance of this part of the tRNA molecule for its correct *in vivo* function is well known[26]. When the interaction of [5,8-$^{13}C_2$]-N^{1},N^{12}-bismethylspermine (BMS) **2** with tRNA^{Phe} was examined using an analogous procedure to that used with [5,8,$^{13}C_2$]-spermine, the same pattern of NOE cross-peaks was obtained (Fig. 4D). Thus, the binding domain of BMS to tRNA^{Phe} is similar to that found for spermine. A different NOE cross peak pattern was obtained when the binding of [5,8-$^{13}C_2$]-N^{1},N^{12}-bisethylspermine (BES) **3** with tRNA^{Phe} was examined. While strong NOE signals were obtained with the imino protons of the T54 A58 and U50 A64 base pairs, the interaction with the U52 A62 imino proton could not be detected (Fig. 4B). It is therefore very likely that BES docks into the TΨC loop of tRNA^{Phe} with a different regioselectivity to that found for spermine and BMS. Since NOE effects are observed below 5 Å, the BES binding site will be further removed from the N(3)-H of base pair U52 A62 while SPM and BMS will bind in its close vicinity. It is also conceivable that the binding mode of the polyamines to tRNA^{Phe} at the juncture of the TΨC and D loops will affect the kink of the tRNA^{Phe} and therefore the specificity of aminoacyl-tRNA^{Phe} formation[22]. A linkage of this hypothesis with cell growth was sought using human melanoma cancer cells (MALME-3M).

Effects of spermine (SPM) **1**, *bismethylspermine (BMS)* **2**, *and bisethylspermine (BES)* **3** *on MALME-3M cell growth, cell cycle and polyamine pools.*

MALME-3M cells treated with 10 µM SPM grew similarly to untreated cells for 120 h (Fig. 6). In the presence of BMS the cells grew for 48 h and then completely ceased growing.. There was significant loss in cell number during BMS treatment to suggest a cytotoxic response. Compared to BMS, BES inhibited growth by 60% afer 48 h which was followed by a cytotoxic response as indicated by a steady loss in cell number to below the initial seeding density. Also with BES treatment, there was a significant number of detached cells previously characterized to be apoptotic[14]. Samples taken for cell cycle analysis at 24 h intervals up to 96 h treatment (Fig. 7) showed that SPM and BMS treat-

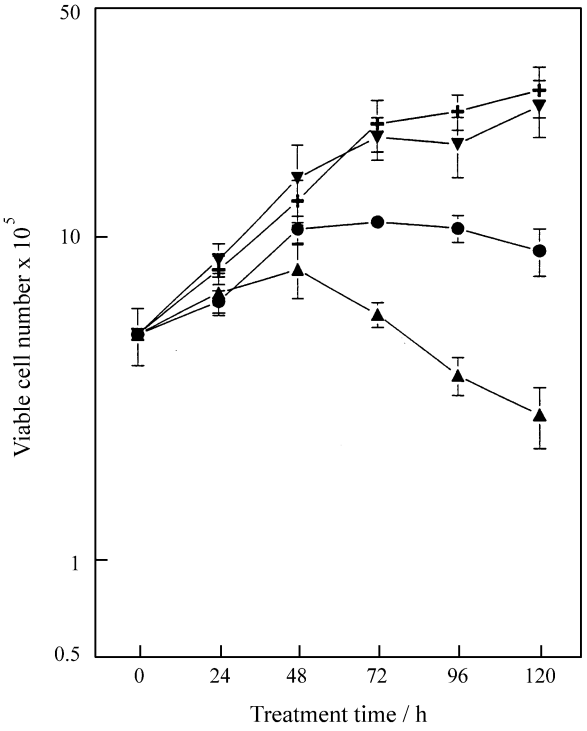

Figure 6. Growth of MALME-3M cells in the presence and absence of 10 µM SPM (▼) BMS (•) or BES (▲). Number of attached cells were determined at 24 intervals up to 120 h. Controls (+).

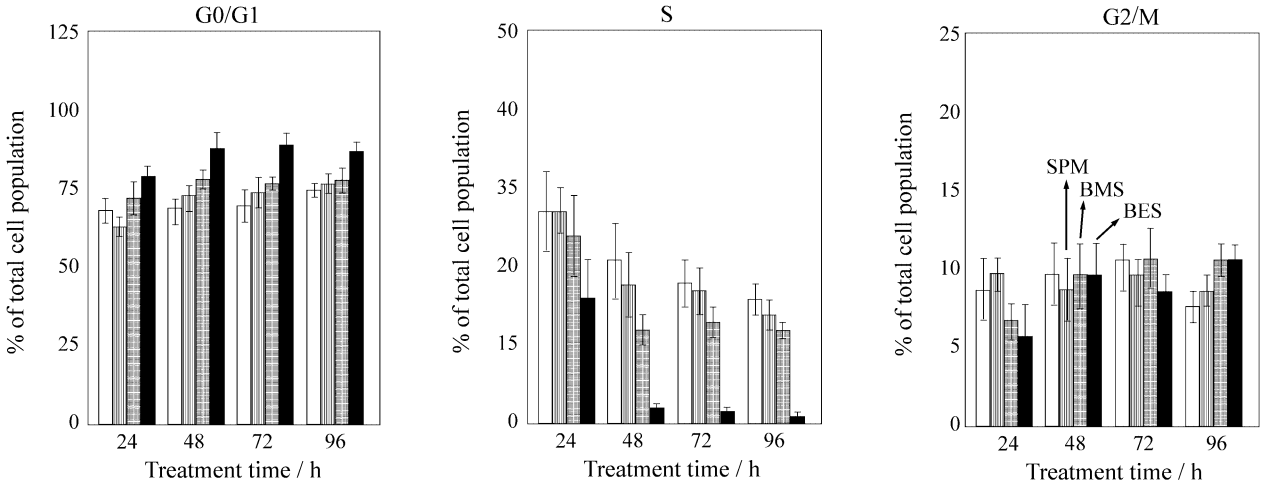

Figure 7. Cell cycle distribution of logarithmically growing MALME-3M cells following treatment with 10 μM SPM; BMS; and BES Controls at 24, 48, 72, and 96 h.

ments only marginally altered cell cycling and the cell population profile remained similar to that of untreated cells, with ~65% of cells in G_0/G_1, ~27% in S, and ~11% in G_2/M phase. In contrast, BES-treated cells showed a 50% decrease in S phase cells accompanied by an increase in G_1 cells only at 24 h. By 48 h, only 2% of the cells remained in S phase indicating a full G_1/S arrest. No significant change was observed for G_2/M phase cells, and the fact that 10% of the cells remained in this phase suggests the possibility of an additional block here.

The melanoma cells were also analyzed for polyamine and polyamine analog pools (Table 1). Decreases occurred in all three of the intracellular polyamines by 48 h and these natural pools were replaced with comparable amounts of BMS and BES. Thus, the distinct differences observed in effects on growth and cell cycle between these two analogs are independent of their abilities to accumulate intracellularly or to deplete polyamine pools. The results basically show three separate effects of SPM, BMS and BES on MALME-3M cell growth; namely, no effect by SPM, a cytostatic effect by BMS, and a cytotoxic effect by BES. Only the cytotoxic analog, BES, had affected growth and cell cycling prior to 48 h. The latter resulted in a significant loss in S phase cells by 24 h with a concomitant increase in G_1. Interestingly, it also binds to tRNA with a different regioselectivity than that of SPM or BMS. Thus, the effect on cell cycle by BES implies that there is some distinct gain or loss of biological action for this analog compared to SPM and BMS. One possibility has been demonstrated here that results from the different binding mode of BES to tRNA which could lead to a lack of fidelity in the translation mechanism, cell cycle arrest, and ultimately to a cytotoxic effect.

Experimental

^{1}*H-NMR determinations*

$tRNA^{Phe}$ from brewers yeast was from Sigma. It was dissolved in 0.5 mL of a buffer containing 50 mM sodium cacodylate, 2 mM EDTA, 400 mM NaCl (pH 7.2), and dialyzed for 20 h against the same buffer in double-sided biodialysers (Sialomed). The resulting $tRNA^{Phe}$ solution was polyamine-free (assayed by HPLC). The isotopically enriched spermines (Fig. 1) were added to the dialyzed tRNA solution to give final, 1 mM $tRNA^{Phe}$, 8 mM polyamine mixtures. ^{1}H-NMR spectra were recorded at 750 MHz at 5 °C. The water signal was suppressed by the jump

Table 1. Polyamine analysis of MALME-3M cells treated with SPM, BMS, and BES.

Treatment		Polyamine Pools*			
(10 μM, 48 h)	% Control growth	Put	Spd	Spm	Analog
			(pmol/10^6 cells)		
Control	100	995	1835	2090	--
SPM	120	< 5	410	2730	--
BMS	70	< 5	< 5	65	5950
BES	37	< 5	< 5	100	6140

*Pool values were based on averages of at least three separate determinations with S.D. < 15%.

return read pulse. The Δ period was set to 2.85 ms, the mixing period T_{mix} (τ) was 300 ms and the δ period was 45.3 μs; which placed the maximum of excitations at 12.1 ppm. The relaxation delay D_1 was 1 s and the acquisition time was 0.182 s. The ^1H transmitter frequency was on-resonance for H_2O, and the ^{13}C transmitter frequency was placed at 41 ppm. The ^1H spectra was 22,522 Hz (30 ppm) and for each acquisition 2,048 complex data points were collected. The number of acquisitions for these spectra was 100,000 (41 h collection time). The solvent composition was 95% H_2O/5% ^2H_2O. All chemical shifts are referenced to internal DSS.

Cell growth

MALME-3M cells obtained from the American Type Culture Collection (Rockville, MD) were grown and maintained as described[14]. Cells were seeded 24 h prior to treatment with 10 μM of SPM and each analog for an additional 120 h. Cell numbers were determined electronically (Coulter Counter; Coulter Electronics, Hialeah, FL).

Flow cytometry

Cell analysis was performed on attached cells following treatments of 10 μM analog or SPM for 24 h intervals up to 96 h. Briefly, cells were stained using a propidium iodide buffer and 10,000 events were recorded by FACScan flow cytometer (Becton Dickinson, San Jose, CA) to generate DNA histograms as described[14].

Polyamine pool determinations

Following treatment of the MALME-3M cells for 48 h with 10 μM SPM, DMSPM, and DESPM, the intracellular polyamine and analog pools were determined on acid extracts using a high-performance liquid chromatography system described previously[27].

Materials

[5,8-$^{13}C_2$]-Spermine, [5,8-$^{13}C_2$]-N^1,N^{12}-bismethylspermine, and [5,8-$^{13}C_2$]-N^1,N^{12}-bisethylspermine were prepared by Prof. Keijiro Samejima (Josai University, Saitama, Japan) following published procedures[28]. N^1,N^{12}-bismethyl and N^1,N^{12}-bisethylspermines were prepared as follows according to our published procedures[3]. 1,4-Bis(mesitylenesulfonyloxy) butane was condensed with either N-methyl or N-ethyl-N'-(3-(mesitylenesulfonylamino)propyl)-mesitylene sulfonamide. Deprotection in acid media of the resulting tetramides gave the tetrahydrochlorides of either **2** or **3**.

Conclusions

A) When spermine (SPM), N^1,N^{12}-bismethylspermine (BMS) and N^1,N^{12}-bisethylspermine (BES) enriched with ^{13}C at 5-CH_2 and 8-CH_2 were added to $tRNA^{Phe}$ it was possible to detect NOE cross peaks connecting the ^1H-NMR resonances of the —$^{13}CH_2N^+H_2$ residues and several base paired imino protons of the $tRNA^{Phe}$.

B) While SPM and BMS were found to bind to the N(3)-H of the base pairs T54-m^{1}A58, U50-A64, and U52 A62, BES was found to bind only to the base pairs T54-m^{1}A58 and U50-A64. All these base pairs are located at the junction of the TΨC loop and D loop.

C) When the effects of SPM, BMS, and BES on the growth of human melanoma cancer cells (MALME-3M) were compared, it was found that SPM and BMS behaved in a similar manner. They were minimally growth inhibitory up to 48 h, they did not alter the cell cycle whose profiles remained similar to the control cells in both cases, and they were not cytotoxic. BES, on the contrary, was rapidly cytotoxic after 48 h and caused a distinct G_1/S cell cycle arrest. These BES effects were not attributed to specific decreases in the intracellular polyamine pools. We, therefore, propose that the growth effects of BES could be correlated with its binding to tRNA that differs from that of SPM, the natural polyamine involved in cell growth. The cytotoxic action of BES could be related to its interference with the fidelity or efficiency of the cellular translation process.

Acknowledgments

NMR studies were carried out with support from the NIH Biomedical Technology Program (RR02301) and funding from the University of Wisconsin, NSF Academic Infrastructure Program (BIR-9214394), NIH Shared Instrumentation Program (RR02781, RR08438), NSF Biological Instrumentation Program (DMB-8415048), and U.S. Department of Agriculture.

References

1. Cohen, S.S. *A Guide to the Polyamines*; Oxford University Press, Oxford, 1998.
2. Marton, L.J.; Pegg, A.E. *Annu. Rev. Pharmacol. Toxicol.* **1995**, *33*, 55.
3. Reddy, V.K.; Valasinas, A.; Sarkar, A.; Basu, H.S.; Marton, L.J.; Frydman, B. *J. Med. Chem.* **1998**, *41*, 4723; and references therein.
4. Tabor, C.W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749.
5. Sakai, T.T.; Cohen, S. *Progr. Nucl. Acid. Res. Mol. Biol.* **1975**, *17*, 15.
6. Feuerstein, B.G.; Marton, L.J. In *The Physiology of Polyamines*; Bachrach, U.; Heimer, Y.M., eds., CRC Press, Boca Raton, Florida, v. I, 109, 1989.
7. Frydman, B.; de los Santos, C.; Frydman, R.B. *J. Biol. Chem.* **1990**, *265*, 20874.
8. Feuerstein, B.G.; Williams, L.D.; Basu, H.S.; Marton, L.J. *J. Cell. Biochem.* **1991**, *46*, 37.

9. (a) Chen, H.-H.; Behe, M.J.; Rau, D.C. *Nucl. Acid. Res.* **1984**, *12*, 2381; (b) Russell, W.C.; Precious, B.; Martin S.R.; Bayley, P.M. *EMBO J.* **1983**, *2*, 1647.
10. Feuerstein, B.G.; Pattabiraman, N.; Marton, L.J. *Nucl. Acid. Res.* **1990**, *17*, 6883.
11. (a) Frydman, B.; Westler, W.M.; Samejima, K. *J. Org. Chem.* **1996**, *61*, 2588; (b) Fernandez, C.O.; Frydman, B.; Samejima, K. *Cell. Mol. Biol.* **1994**, *40*, 933; (c) Frydman, L.; Rossomando, P.C.; Frydman, V.; Fernandez, C.O.; Frydman, B.; Samejima, K. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9186.
12. Hou, Y.M.; Schimmel, P. *Biochemistry* **1992**, *31*, 4157.
13. (a) Bergeron, R.J.; Neims, A.H.; McMannis, J.S.; Hawthorne, T.R.; Vinson, J.R.T.; Bartell, R.; Ingeno, M.J. *J. Med. Chem.* **1988**, *31*, 1183; (b) Bergeron, R.J.; McMannis, J.S.; Liu, Ch. Z.; Feng, Y.; Weimar, W.R.; Luchetta, G.R.; Wu, Q.; Ortiz-Ocasio, J.; Vinson, J.R.T.; Kramer, D.; Porter, C. *J. Med. Chem.* **1994**, *37*, 3464; (c) Basu, H.S.; Pellarin, M.; Feuerstein, B.G.; Shirahata, A.; Samejima, K.; Deen, D.F.; Marton, L.J. *Cancer Res.* **1993**, *53*, 3948; (d) McClosky, D.E.; Casero, R.A., Jr.; Woster, P.M.; Davidson, N.E. *Cancer Res.* **1995**, *55*, 3233; (e) Li, Y.; Eiseman, J.L.; Sentz, D.L.; Rogers, F. A.; Pan, S.-S.; Hu, L.-T.; Egorin, M.J.; Callery, P.S. *J. Med. Chem.* **1996**, *39*, 339; (f) Huber, M.; Poulin, R. *Cancer Res.* **1995**, *55*, 934; (g) Aizencang, G.; Frydman, R.B.; Giorgeri, S.; Sambrotta, L.; Guerra, L.; Frydman, B. *J. Med. Chem.* **1996**, *38*, 4337; (h) Frydman, J.; Ruiz, O.; Robetto, E.; Dellacha, J.M.; Frydman, R.B. ; *Mol. Cell. Biochem.* **1991**, *100*, 9; (i) Moyano, N.; Frydman, B.; Buldain, G.; Ruiz, O.; Frydman, R.B. *J. Med. Chem.* **1990**, *33*, 1969; (j) Aizencang, G.; Harari, P.; Buldain, G.; Guerra, L.; Pickart, M.; Hernandez, P.; Frydman, B. *Cell. Mol. Biol.* **1998**, *44*, 615.
14. Kramer, D.L.; Fogel-Petrovic, M.; Diegelman, P.; Cooley, J.M.; Bernacki, R.J.; McMannis, J.S.; Bergeron, R.J.; and Porter, C.W. *Cancer Res.* **1997**, *57*, 5521.
15. Basu, H.S.; Wright, W.D.; Deen, D.F., Roti-Roti, J.; Marton, L.J. *Biochemistry* **1993**, *32*, 4073.
16. Kusama-Eguchi, K.; Irisawa, M.; Watanabe, S.I.; Watanabe, K.; Igarashi, K. Arch. *Biochem. Biophys.* **1991**, *288*, 495.
17. Snyder, R.D.; Edwards, M.L. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 1383.
18. McMurry, L.; Algranati, I.D. *Eur. J. Biochem.* **1986**, *155*, 383.
19. Lotfield, R.B.; Eigner, E.A.; Patuszyn, A. J. *Biol. Chem.* **1981**, *256*, 6729.
20. Algranati, I.D., Goldenberg, S.H. *Trends Biochem. Sci.* **1977**, *2*, 272.
21. Abraham, A.K.; Phil, A. *Trends Biochem. Sci.* **1981**, *6*, 106.
22. (a) Rould, M.A.; Perona, J.J.; Soll, D. and Steita, T.A. *Science* **1989**, *246*, 1135; (b) Ruff, M.; Krishnaswamy, S.; Boeglin, M.; Poterszman, A.; Mitschler, A.; Podjarny, A.; Rees, B.; Thiery, J.C. and Moras, D. *Science* **1991**, *252*, 1682.
23. Hyde, E.I.; Reid, B.R. *Biochemistry* **1985**, *24*, 4315.
24. Heerschap, A.; Walters, J.A.L.I.; Hilbers, C.W. *Nucl. Acids Res.* **1986**, *14*, 983.
25. Hilbers, C.W.; Heerschap, A.; Haasnot, C.A.G.; Walters, J.A.L.I. *J. Biomol. Struct. Dyn.* **1983**, *4*, 1983.
26. Dirheimer, G.; Keith, G.; Dumas, P.; Westhof, E. In *tRNA: Structure, Biosynthesis and Function*, Söll, D.; RajBhandary, U.L., eds., ASM Press, Washington, DC, 93, 1995.
27. Kramer, D.L.; Miller, J.T.; Bergeron, R.J.; Khomutov, R.; Khomutov, A.; Porter, C.W. *J. Cell. Physiol.* **1993**, *155*, 300.
28. Samejima, K.; Matsushima, N.; Niitsu, M.; Beppu, T.; Frydman, B. *Chem. Pharm. Bull.* **1995**, *43*, 2001.

Received: March 29, 1999