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Research Article

Biochemical effects and growth inhibition in MCF-7 cells caused by novel sulphonamido oxa-polyamine derivatives

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Abstract. The novel polyamine derivatives sulphonamido oxa-spermine (oxa-Spm) and sulphonamido oxa-spermidine (oxa-Spd) exhibited rapid cytotoxic action towards MCF-7 human breast cancer cells with IC_{50} values of 4.35 and 6.47 µM, respectively, after 24-h drug exposure. Neither compound is a substrate of serum amine oxidase. Both oxa-Spm and oxa-Spd caused cell shrinkage, as determined by phase-contrast microscopy. After incubation with 10 µM of either compound for 8 h, the cells underwent chromatin condensation and nuclear fragmentation. However, no clear DNA ladder was obtained by electrophoresis. The sulphonamido oxa-polyamine derivatives and especially oxa-Spd enhanced the activity of polyamine oxidase (PAO), an enzyme capable of oxidising N^1-acetylated spermine and spermidine to spermidine and putrescine, respectively, generating cytotoxic H_2O_2 and 3-acetamidopropanal as by-products. The intracellular polyamine content was only marginally reduced in response to drug treatment. In conclusion, our data show that these novel sulphonamido oxa-polyamine derivatives possess high cytotoxic activity against MCF-7 cells and indicate that induction of PAO may mediate their cytotoxicity via apoptosis.

Key words. Polyamine derivative; cytotoxicity; MCF-7 cell; apoptosis; polyamine oxidase; polyamine.

The natural polyamines spermidine (Spd) and spermine (Spm) and their precursor the diamine putrescine (Put) are essential for cell proliferation and differentiation [1, 2] and, thus, polyamine metabolism has been extensively investigated as a target for therapeutic intervention [2–4]. Several polyamine biosynthetic inhibitors with antiproliferative and antiparasitic properties have been designed, among which difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, the first and key enzyme in polyamine biosynthesis, has attracted much interest as a chemotherapeutic and chemopreventive agent [3, 4]. A more recent approach has been the development of different types of structural analogues of the natural polyamines with antiproliferative properties [4–8]. Several symmetrically and unsymmetrically alkyl-substituted polyamines have shown good in vitro and in vivo anticancer activity, which has led to their clinical evaluation [5]. Apart from the inhibition of polyamine biosynthesis, stimulation of polyamine catabolism and subsequent depletion of intracellular polyamine levels have been suggested to be at least partially responsible for the anticancer activity of these synthetic polyamines. In the course of their catabolic interconversion pathway, Spm and Spd are first acetylated by Spd/Spm N^1-acetyl-

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transferase (SSAT) and then oxidatively deaminated by the action of the flavin adenine dinucleotide (FAD)-dependent polyamine oxidase (PAO) to Spd and Put, respectively [9]. Cytotoxic 3-acetamidopropanal and hydrogen peroxide (H_2O_2) are also generated as by-products in N^1-acetylpolyamine degradation by PAO [9]. H_2O_2 produced as a result of polyamine analogue-stimulated catabolic polyamine interconversion has recently been suggested to act as an apoptotic inducer in vitro [10]. In addition, the very recent cloning and characterization of a PAO inducible by the polyamine analogue N^1, N^{11}-bis(ethyl)norspermine [11] has renewed interest in the physiological functions of this enzyme and its role as a trigger of apoptosis.

For a number of years, our group has been involved in the design and synthesis of novel sulphonamido polyamine derivatives with aminooxy functionality within the Spd and Spm molecules, which we have called oxa-polyamines [12–15]. We have shown some biochemical effects of these oxa-polyamines in Swiss 3T3 cells [16] and in collaboration with the NCI, we have obtained preliminary data on the anticancer potential of sulphonamido oxa-polyamine derivatives against a broad spectrum of human cancer cells [17, 18]. The NCI COMPARE analysis [19] conducted with our most active compounds has shown no correlation with the mechanism of action of any of the standard categories of compounds in the NCI database, thus suggesting a novel mechanism of action and/or the involvement of novel biological targets. To gain further insight into the mechanisms underlying the growth inhibitory activity of the sulphonamido oxa-polyamine derivatives, oxa-Spm and oxa-Spd (fig. 1), we report in this paper, the antiproliferative properties of these compounds, their ability to induce apoptosis and their effects on PAO activity and polyamine levels in MCF-7 human breast cancer cells.

Materials and methods

Compounds and cell culture

Oxa-Spm and oxa-Spd were synthesised as described previously [13–15]. For all experiments, stock 40 mM solutions of the compounds were prepared in distilled water and stored at –20 °C. Prior to use, the stock solutions were diluted with medium to the desired concentrations. MCF-7 cells were maintained in minimal essential medium (MEM) with Earl's balanced salts (Labtech Int., UK), supplemented with 10% fetal calf serum (Labtech Int), 2 mM L-glutamine (Sigma), 1% non-essential amino acids (Sigma), 100 IU/ml penicillin (Sigma) and 100 µg/ml streptomycin (Sigma). The cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere and subcultured every 5 days.

Growth inhibition assay

Exponentially growing cells were plated at a seeding density of 2×10^4 cells/cm^2 in 96-well plates. After a 24-h period of attachment, the cells were treated with several drug concentrations. The growth inhibitory effects of the sulphonamido oxa-polyamines were measured using a standard tetrazolium MTT assay [20] with minor modifications. Briefly, after drug exposure at 37 °C, the medium was removed and 200 µl of MTT reagent (0.5 mg/ml) in serum-free medium was added to each well. The plates were incubated at 37 °C for an additional 4 h. At the end of incubation, the medium was removed and the formazan crystals formed as a result of MTT metabolism were solu-

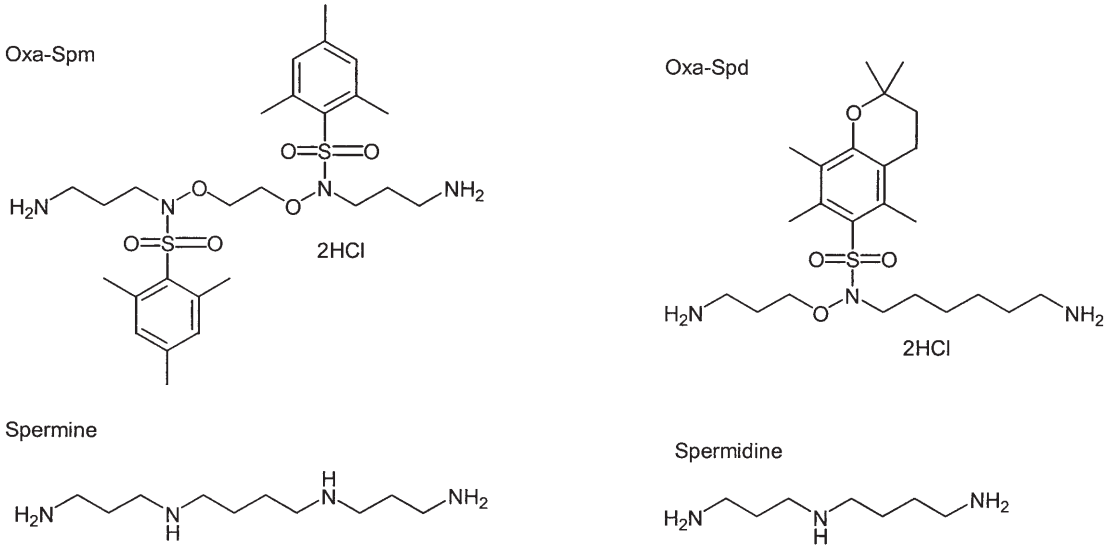

Figure 1. Structures of the sulphonamido oxa-polyamine derivatives oxa-Spm and oxa-Spd together with those of the natural polyamines spermine and spermidine.

bilized by the addition of 200 µl of dimethylsulphoxide to each well. The cellular metabolism of MTT was then quantified by reading the absorbance of the solubilized product at 560 nm on a Microplate reader (Dynex Technologies, USA). IC_{50} values are defined as the drug concentrations needed to inhibit cell growth to 50% of controls.

Assessment of cell shape and nuclear morphology

Cells were monitored by phase-contrast microscopy. Treated and untreated (control) cells were viewed using an inverted phase-contrast microscope model (PIM-II; World Precision Instruments, USA) and photographed using a Nikon camera.

To study nuclear morphology, exponentially growing cells were trypsinised and inoculated at a density of 2×10^4 cells/cm^2 onto sterile coverslips in 60-mm dishes (Nunc). After attachment, the cells were incubated in the presence or absence of 10 µM sulphonamido oxa-polyamines for different time intervals. At the desired time, the medium was aspirated and the cultures were rinsed with phosphate-buffered saline (PBS) before adding methanol to fix the cells for 5 min. The fixed cells were stained with either Hoechst dye 33342 (Sigma) (0.1 mg/ml solution in PBS) or Giemsa (BDH, UK) (10% solution in Sörensen phosphate buffer, pH 6.8). The coverslips were mounted onto microscope slides using liquid paraffin and PPX (BDH), respectively. Hoechst- (under UV excitation) and Giemsa-stained nuclei were visualised and photographed using a Leica DMRB microscope (Leica Microsystems Holdings, Germany).

Detection of DNA ladder by agarose gel electrophoresis

Exponentially growing cultures of MCF-7 cells were plated at a density of $2–5 \times 10^4$ cells/cm^2. After attachment, the cultures were exposed to either oxa-Spm or oxa-Spd derivatives in the concentration range 1–10 µM for different time periods. The isolation of DNA from treated and untreated cells was carried out using the Suicide-Track DNA Ladder Isolation Kit (Oncogene), according to the manufacturer's instructions using a procedure that ensured the prevention of contamination of DNA ladder fragments with high-molecular-weight DNA. Briefly, after drug exposure, the DNA was extracted from the cells with a lysis buffer. After RNA degradation, the DNA was isolated by propanol precipitation. The DNA pellets were washed with ethanol, resuspended with buffer and loaded onto a 1.5% agarose gel containing 0.1 g/l ethidium bromide. After electrophoresis, the DNA was visualised under UV illumination. A positive control (HL-60 cells treated with 0.5 µg/ml of actinomycin D for 19 h) supplied in the Oncogene kit was used regularly in parallel to the experiments to assess the efficacy of the isolation procedure and to validate the electrophoretic conditions.

Enzyme assays

Exponentially growing cells were treated with either 5 µM oxa-Spm or 7.5 µM oxa-Spd for 24 h. The medium was aspirated and the cells (treated and control) were rinsed with PBS. The cells were scraped and homogenised in 2 mM borate buffer (pH 7.4). PAO activity was determined in the cell extracts by the method of Suzuki et al. [21]. The level of H_2O_2 produced in the PAO-catalysed oxidation of N^1-acetylSpm was determined spectrofluorimetrically after reaction with homovanillic acid to form a fluorescent product.

The ability of the serum amine oxidase (SAO) present in fetal calf serum to oxidise the synthetic sulphonamido oxa- and natural polyamines was studied by the spectrofluorometric method of Snyder and Hendley [22].

Analysis of intracellular polyamine levels

Exponentially growing cultures of MCF-7 cells in 60-mm dishes (Nunc) were incubated in the presence or absence of either 5 µM oxa-Spm or 7.5 µM oxa-Spd for 24 h. After incubation, the medium was gently removed and the cells (treated and control) were rinsed with pre-warmed PBS. The cells were scraped from the dishes and homogenised in 2% perchloric acid (PCA) in distilled water (final concentration) containing 1 mM 1,7-diaminoheptane (an internal standard for polyamine analysis).

The polyamine concentrations were measured using the reverse-phase high-performance liquid chromatography procedure described by Seiler and Knödgen [23]. Briefly, the polyamines were separated using a µBondapac C18 column and derivatised post-column by the O-phthalaldehyde method. A fluorescence detector was used at 345-nm excitation and 455-nm emission.

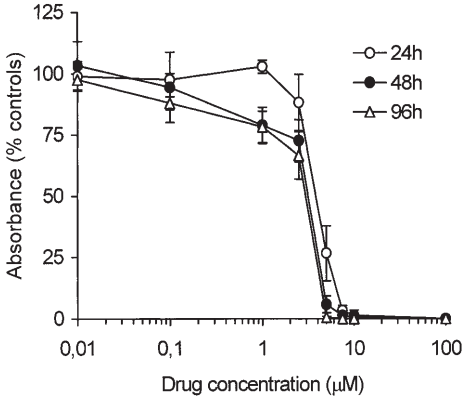

Figure 2. Dose-dependent growth inhibitory effects of the suphonamido oxa-polyamine derivative oxa-Spm in MCF-7 cells. Cultures were exposed to oxa-Spm at a range of concentrations (0–100 µM) for 24, 48 and 96 h. Results are means ± SD from at least three separate experiments with no less than three replicates per experiment.

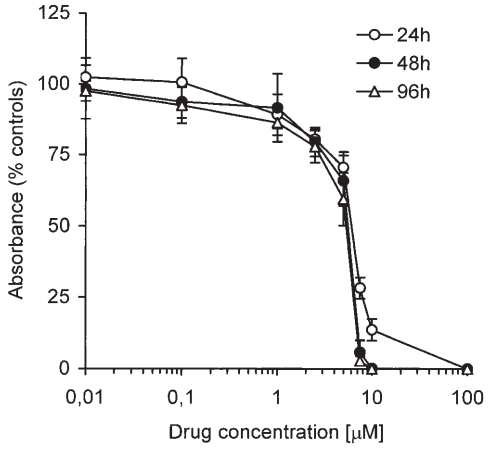

Figure 3. Dose-dependent growth inhibitory effects of the sulphonamido oxa-polyamine derivative oxa-Spd in MCF-7 cells. Cultures were exposed to oxa-Spd at a range of concentrations (0–100 μM) for 24, 48 and 96 h. Results are means ± SD from at least three separate experiments with no less than three replicates per experiment.

Protein determination

Protein was measured by the method of Bradford [24], using bovine serum albumin as standard.

Results

Growth inhibition of MCF-7 cells by sulphonamido oxa-polyamines

The effects of the sulphonamido oxa-polyamines oxa-Spm and oxa-Spd on the growth of MCF-7 cells were assessed using the MTT assay. The assay was performed after 24, 48 and 96 h of drug exposure. The dose-response curves presented in figs. 2 and 3 demonstrate that oxa-Spm and oxa-Spd caused a substantial inhibition in cell growth after 24-h drug exposure, with IC_{50} values determined at 4.35 and 6.47 μM, respectively. Longer incubation times did not lead to any major changes in the dose-response curves. For example, IC_{50} values after 96-h incubation were found to be 3.13 μM for oxa-Spm and 5.42 μM for oxa-Spd.

Phase-contrast monitoring of cells following sulphonamido oxa-polyamine treatment

Morphological changes in drug-treated cells were obvious when compared to the untreated (control) cells (fig. 4). The oxa-Spm- and oxa-Spd-treated cells (fig. 4 B, C) shrank and gradually detached from the dishes, a clear indication that the compounds were cytotoxic. Cell shrinkage, which may be indicative of apoptosis, occurred in treated cells but not in the controls (fig. 4 A).

SAO activity inhibition and determination

The extracellular, copper/quinone-containing SAO has been shown to oxidise preferentially Spm and Spd at the primary amino groups as well as some aliphatic and aromatic monoamines, generating cytotoxic aldehydes, H_2O_2 and ammonia [25, 26]. SAO activity has been shown to be high in ruminant sera such as bovine serum, and is usually a component of cell culture media. Since both oxa-Spd and oxa-Spm contain terminal (primary) amino groups, these compounds might be substrates for SAO. Thus, the eventual ‘primary’ degradation of the

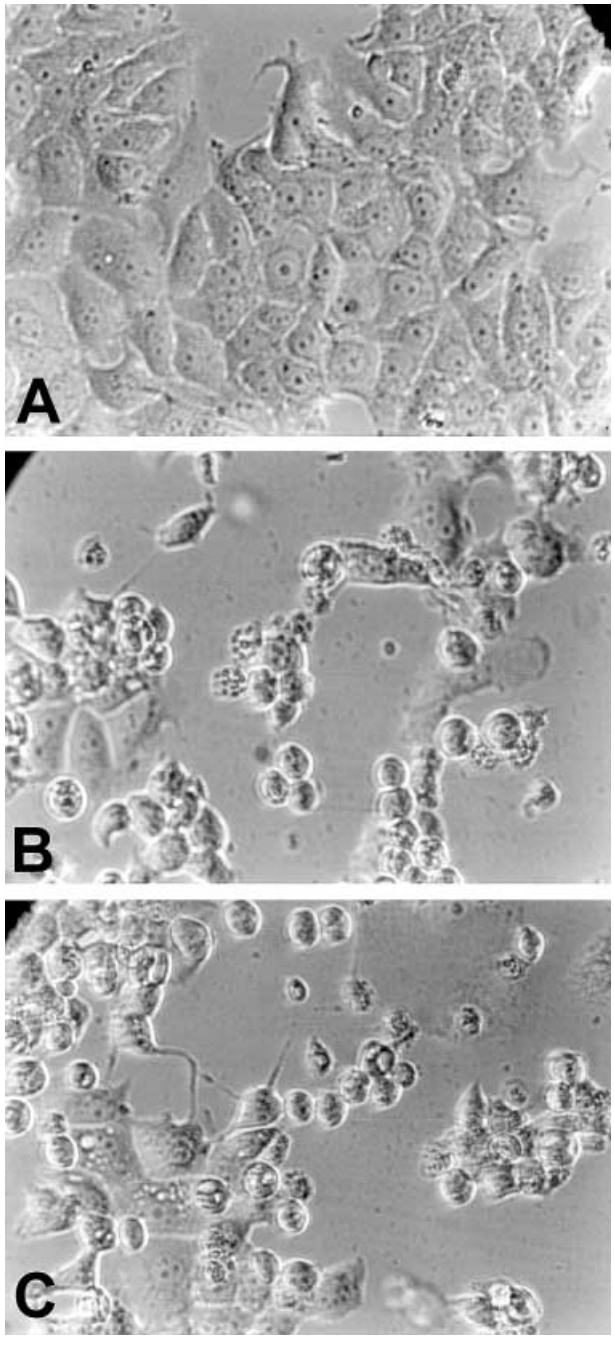

Figure 4. Phase-contrast photomicrographs of MCF-7 cells. Cells were untreated (*A*) or treated with 10 μM oxa-Spm (*B*) or oxa-Spd (*C*) for 8 h.

sulphonamido oxa-polyamines by SAO present in fetal calf serum could modulate or decrease their final growth inhibitory effects. On the other hand, the cytotoxic properties of sulphonamido oxa-polyamines may be due to the cytotoxic products generated as a result of the oxidation of the sulphonamido oxa-polyamine derivatives themselves, catalysed by SAO. To study these possibilities, we performed two types of experiment. In the first series of experiments, 1 mM of aminoguanidine, a potent SAO inhibitor was added to the MCF-7 cultures together with the sulphonamido oxa-polyamines. In these experiments, no statistically significant changes in the dose-response curve points were observed following the co-treatment with sulphonamido oxa-polyamines (oxa-Spm and oxa-Spd) and aminoguanidine (data not shown). The SAO inhibitor, i.e. aminoguanidine, itself had no effect on cell growth at 1 mM concentration.

In the second series of experiments, we tested the oxidation of sulphonamido oxa-polyamines and natural polyamines by SAO present in fetal calf serum. The rate of oxidation of the natural polyamines, using fetal calf serum as an enzyme source, is generally verified by the substrate specificity of SAO. Thus, the highest SAO activity was found when Spm (0.5 mM) was used as a substrate. The value of this activity (35.0 ± 3.8 pmol H_2O_2/min per milligram protein) was taken as 100%. The SAO activities found when Spd and Put were used as substrates (0.5 mM) were 92.8% and 9.4%, respectively. At the same substrate concentration (0.5 mM), H_2O_2 production was not detectable when oxa-Spm was used as the substrate, and was negligible (1%) in the case of oxa-Spd, thus indicating that these compounds are not substrates for SAO.

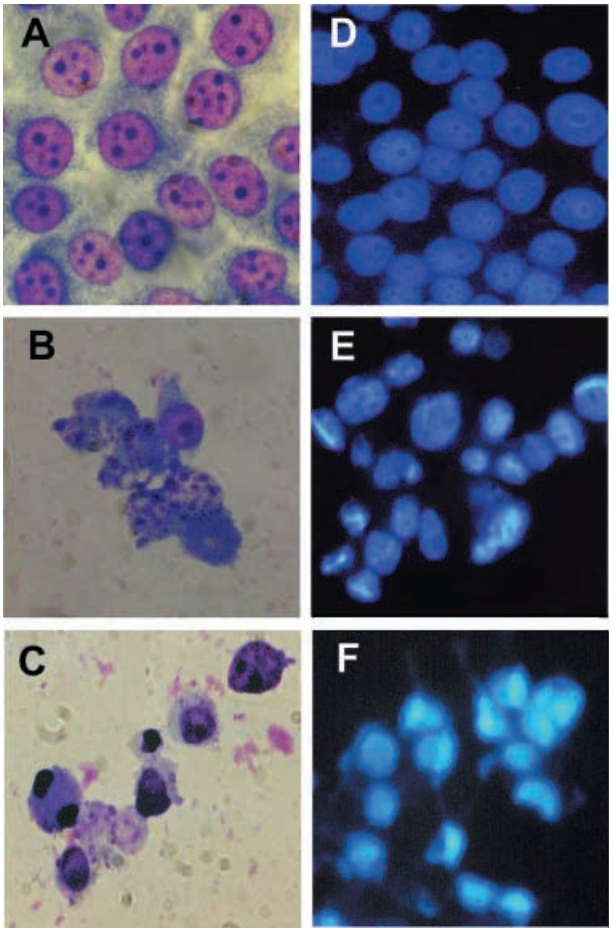

Figure 5. Morphological changes in MCF-7 cells following treatment with sulphonamido oxa-polyamine derivatives. Cells were untreated (*A, D*) or treated with 10 µM oxa-Spm (*B, E*) or oxa-Spd (*C, F*) for 8 h. Control and treated cells were stained with Giemsa dye (*A–C*) or Hoechst 33342 dye (*D–F*), visualised and photographed using a Leica microscope.

Changes in nuclear morphology in response to sulphonamido oxa-polyamine treatment

Since cell shrinkage was observed by phase-contrast microscopy in drug-treated cultures, we decided to investigate further the ability of the sulphonamido oxa-polyamine derivatives to induce apoptosis in MCF-7 cells. Apoptosis has been recognised to be of major importance for normal growth and development, as well as for the mechanism of death in cancer cells treated with chemotherapeutic drugs [27, 28]. The characteristic morphological features of apoptosis include cytoplasmic shrinkage, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies [27, 29]. Morphological examination of MCF-7 cells after staining nuclei with either Hoechst 33342 or Giemsa showed the presence of numerous cells with dense, pyknotic and fragmented nuclei in cultures treated with oxa-Spm and oxa-Spd (fig. 5B, C, E, F), but not in the controls (fig. 5A, D). These morphological features [dense nuclei, pyknosis and karyorrhexis (or nuclear fragmentation)] are all hallmarks of apoptosis. Of note is that such alterations in the chromatin and nuclei could be observed after incubations with oxa-Spm and oxa-Spd as short as 4 h.

DNA fragmentation in response to sulphonamido oxa-polyamine treatment

The morphological changes in cells undergoing apoptosis are usually accompanied by internucleosomal cleavage of genomic DNA, known as DNA laddering [29]. To determine if treatment with sulphonamido oxa-polyamines could cause DNA laddering, we processed the DNA isolated from treated and untreated (control) MCF-7 cells by agarose gel electrophoresis. Cells were monitored by phase-contrast microscopy for the presence of morphological changes before the beginning of the DNA isolation procedure. Figure 6 shows DNA lanes obtained after treatment with 5 µM oxa-Spm and 7.5 µM oxa-Spd for 16 h. Although the difference between the DNA lanes from treated and control cells was obvious (fig. 6) with a

Table 1. Effects of sulphonamido oxa-polyamine derivatives on PAO activity and polyamine concentrations in MCF-7 cells.

Drug treatment	PAO activity ($pmolH_2O_2$/min per mg protein	Polyamine concentration (nmol/mg protein)		
		Put	Spd	Spm
Control	140.4 ± 16.6	ND	12.29 ± 0.81	13.39 ± 1.16
Oxa-Spm	210.1 ± 19.9	1.82 ± 0.24	8.40 ± 0.90	9.81 ± 0.91
Oxa-Spd	580.3 ± 48.7	1.47 ± 0.35	8.16 ± 0.96	9.67 ± 1.46

Results are means ± SD from three independent experiments.

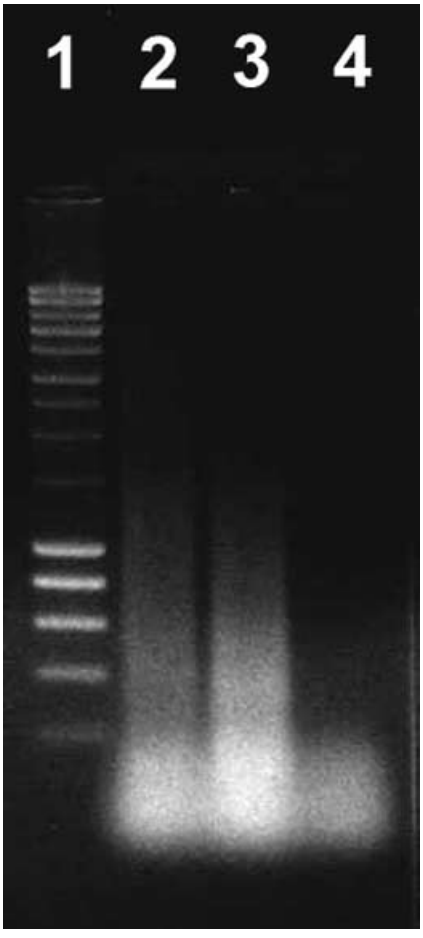

Figure 6. Effects of sulphonamido oxa-polyamine derivatives (16-h exposure) on oligonucleosomal DNA fragmentation in MCF-7 cells. Isolated DNA from treated and control cells was analysed using agarose gel electrophoresis. Lane 1, DNA ladder marker (Promega); lane 2, DNA from cells treated with oxa-Spd derivative (7.5 µM); lane 3, DNA from cells treated with oxa-Spm derivative (5 µM); lane 4, DNA from untreated (control) cells.

small amount of DNA fragmentation and a smearing of the DNA obtained from the treated cultures, the classical DNA fragmentation ladder pattern, typical of apoptosis, was not observed.

Effects of sulphonamido oxa-polyamines on polyamine levels and PAO activity

The induction of polyamine catabolism by some terminally alkyl substituted polyamine derivatives, leading to the generation of increased levels of H_2O_2, has been linked to their cytotoxicity and their ability to induce apoptosis [11]. The consequent depletion of polyamine levels following treatment with these derivatives has also been thought to be responsible for their cytotoxic effects [11]. We therefore investigated the ability of our sulphonamido oxa-polyamines to affect PAO activity and polyamine levels in MCF-7 cells. Although the levels of PAO activity were increased in both the cultures treated with oxa-Spm and oxa-Spd compared to the control values, the oxa-Spd analogue appeared to be a better inducer of PAO activity (table 1). Treatment with both oxa-Spm and oxa-Spd decreased Spd and Spm concentrations in the cultures equally (table 1), whereas Put was detected in cultures treated with sulphonamido oxa-polyamine analogues but not in the control cultures.

Discussion

Breast cancer is a common neoplastic disease. Therefore, the search for and evaluation of drugs with novel mechanisms of action, capable of effectively inhibiting the growth of breast cancer cells represent an important aspect of anticancer drug development. MCF-7 cells have been used to evaluate the biochemical properties of many compounds with anticancer activity, including polyamine derivatives [30–32]. We examined the effects of oxa-Spm and oxa-Spd derivatives, representatives of a new type of antiproliferative polyamine derivative, in MCF-7 cells. Since the preliminary data for the anticancer activity of our sulphonamido oxa-polyamines [18, 19] were only obtained after 48 h drug exposure using a standard in vitro toxicology assay based on the sulphorhodamine B dye

(SRB), we decided to carry out a time-detailed study of the antiproliferative effects of the oxa-polyamines, using another appropriate method, the MTT assay. Further details of the cytotoxicity data obtained by the NCI screening for the sulphonamido oxa-polyamines (NSC 703114-N, NSC 703120-T) as well as data for related molecules without the sulphonamido moieties (NSC 693218-H, NSC 694523-Y) can be obtained by accessing the NCI website (http://dtp.nci.nih.gov/docs/cancer_search.html). There are reports on unsymmetrical [30] and symmetrical [31] polyamine analogues or derivatives which inhibit growth of MCF-7 cells with IC_{50} values in the low micromolar range after a very long term exposure of 120 h. The results from our study demonstrate that despite their structural differences, both oxa-Spm and oxa-Spd derivatives are cytotoxic against MCF-7 cells, with IC_{50} values of 4.35 µM and 6. 47 µM obtained after only 24 h of drug exposure. Furthermore, drug treatment for 48 h and 96 h did not lead to markedly enhanced cell growth inhibition. These observations are also in contrast to data for the in vitro growth inhibitory properties of several polyamine analogues against other cancer cell types, which showed that IC_{50} values decrease considerably as drug exposure time increases from 48 to 96 h [33]. Although the intracellular metabolism of sulphonamido oxa-polyamines is not yet known, the possibility that they are transformed into less toxic compounds could be further explored by finding an explanation for the lack of correlation between the cytotoxicity and the duration of incubation.

Extracellular SAO seems to play no role in modulating the cytotoxic effects of sulphonamido oxa-polyamines in MCF-7 cells, since neither co-treatment with the SAO inhibitor aminoguanidine nor enzyme activity determination with sulphonamido oxa-polyamines as substrates indicate such a possibility. We showed that neither derivative is oxidised by the enzyme, thus suggesting their stability in complete serum-containing media. The presence of the sulphonamido groups (two Mts groups in the structure of oxa-Spm and a Pmc group in the structure of oxa-Spd) and/or oxygen in the structures of both oxa-Spm and oxa-Spd would appear to contribute to the higher stability of these compounds when compared with natural polyamines. Therefore, there was no requirement either to use serum-free medium or to add aminoguanidine in the incubations with sulphonamido oxa-polyamines. It is interesting to note that although Put has been demonstrated to be a very poor substrate for SAO [26], the pattern of oxidation of natural polyamines in fetal calf serum showed 9.4% H_2O_2 production with Put, which seems a little higher than expected. This may be due to the fact that in addition to SAO, fetal calf serum contains another amine oxidase that resembles the properties of tissue diamine oxidase [34]. While SAO and this amine oxidase are both inhibited by aminoguanidine, the substrate properties of the enzymes are different, i.e. in contrast to SAO, the other enzyme is capable of oxidizing Put.

Using MCF-7 and other hormone-dependent and -independent human breast cancer cell lines, McCloskey and colleagues [30] provided the first evidence that polyamine analogue treatment can induce apoptosis. Our results demonstrated that treatment of MCF-7 cells with both oxa-Spm and oxa-Spd caused morphological changes typical of apoptosis, such as cell shrinkage, nuclear condensation and fragmentation. Some cells are known to show apparent morphological induction of apoptosis in the absence of oligonucleosomal DNA fragmentation as a late event in the apoptotic process. There are some reports of MCF-7 cells producing a DNA ladder, but others have demonstrated that MCF-7 cells do not undergo apoptotic DNA fragmentation. In an attempt to clarify this conflicting information, Gooch and Yee [35] demonstrated recently that the ability of MCF-7 cells to undergo DNA laddering appears to be strain specific. In view of these findings, our failure to obtain typical DNA laddering after treating MCF-7 cells with sulphonamido oxa-polyamines may be due to the use of an MCF-7 strain which does not possess the capability to undergo oligonucleosomal DNA fragmentation. However, high-molecular-weight-DNA fragmentation (which is also typical of apoptosis) may occur in MCF-7 cells treated with sulphonamido oxa-polyamines. Therefore, based on morphological observations and despite the lack of characteristic DNA ladders, the cytotoxicity of both oxa-Spm and oxa-Spd in MCF-7 cells may involve induction of apoptosis.

The cytotoxicity of several terminally alkylated polyamine analogues has been linked to their ability to cause a manifold induction of SSAT, with subsequent generation of reactive oxygen species [5]. The second enzyme in the polyamine breakdown mechanism, PAO, has recently attracted much interest as a possible mediator of the cytotoxic and apoptotic effects of polyamine analogues [10, 11]. The suggestion has been made that agents which increase PAO activity may be better cytotoxic drugs than direct inducers of SSAT [36]. The inhibition of PAO with the specific inhibitor MDL-72,527 [N^1, N^4-bis(2,3-butadienyl)-1,4-butanediamine] has been shown to reduce the reactive oxygen levels and consequently reduces apoptosis in non-small-cell lung carcinoma cell line NCI H157 [10]. However, Dai and colleagues [37] have surprisingly shown that MDL-72,527 itself is capable of causing cytotoxic and apoptotic effects. Thus, we investigated the effect of our sulphonamido oxa-derivatives on PAO activity in MCF-7 cells by direct measurement of the enzyme activity rather than relying on a PAO inhibitor. Our results show that both sulphonamido oxa-polyamines, and particularly oxa-Spd, can stimulate PAO activity. Since an increase in the levels of cytotoxic H_2O_2 and 3-acetamidopropanal might be expected as a result of

PAO induction, this may contribute to the cytotoxicity and the apoptotic induction caused by both compounds. However, sulphonamido oxa-polyamine cytotoxicity is unlikely to depend solely on PAO induction, since the higher PAO stimulation by oxa-Spd was not accompanied by a higher degree of cell growth inhibition. In addition to the very recent finding of Wang et al. [11] that PAO is an enzyme inducible by certain polyamine analogues in the non-small-cell lung carcinoma cell line NCI H157, our study also demonstrates the inducibility of PAO in MCF-7 cells by other polyamine derivatives. Thus, the stimulation of PAO by anticancer polyamine analogues/derivatives extends knowledge on the inducibility of the enzyme shown by us [38, 39] and others [40] in in vivo normal physiological models following hormonal treatment. While, however, PAO induction in polyamine-analogue-treated cancer cells might be involved in the mechanisms of cell death, enhanced PAO activity following hormonal treatment may play a role in supporting cell growth [38].

Polyamines are known to bind to DNA, increasing the stability of chromatin and affecting gene expression. Thus changes in optimal polyamine concentrations might subsequently lead to structural and functional DNA changes. The increase in PAO activity in sulphonamido-oxa-polyamine-treated cells was consistently accompanied by a decrease in Spd and Spm concentrations. This effect of sulphonamido oxa-polyamines was not unexpected, because it has been shown with other polyamine analogues in MCF-7 cells [30, 31] and may suggest a role for decreased polyamine levels in the mediation of the cytotoxic and apoptotic effects of sulphonamido oxa-polyamines.

Binding to important anionic sites such as DNA is proposed as an important functional feature of some bis(ethyl) polyamine analogues [2, 3]. However, in previous studies, we have shown that the sulphonamido oxa-polyamine derivatives studied here have no effects on the thermal stability of calf thymus DNA [18]. Therefore, these sulphonamido oxa-polyamines are unlikely to displace the natural polyamines from the DNA of MCF-7 cells, and hence are unlikely to affect polyamine-controlled functions or the susceptibility of DNA to the endonucleases responsible for the oligonucleosomal DNA fragmentation.

Wallace and colleagues [41] have recently suggested that drugs inducing PAO activity might be a novel approach for effectively killing cancer cells. In view of this suggestion, the abilities of oxa-Spm and oxa-Spd derivatives to induce PAO activity and to cause morphological changes typical for apoptosis in MCF-7 breast cancer cells show promise for their further development as novel therapeutic agents.

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