ELSEVIER

Biochemical Pharmacology 64 (2002) 649–655

Biochemical Pharmacology

Insufficiently charged isosteric analogue of spermine: interaction with polyamine uptake, and effect on Caco-2 cell growth

Lyudmila Turchanowa[a], Alexander S. Shvetsov[b], Alexander V. Demin[c], Alex R. Khomutov[d], Heather M. Wallace[e], Jürgen Stein[a], Vladan Milovic[a,*]

[a]*Second Department of Medicine, Johann Wolfgang Goethe University, Theodor Stern Kai 7, D-60590 Frankfurt am Main, Germany*
[b]*Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia*
[c]*Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia*
[d]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*
[e]*Department of Medicine and Therapeutics and Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland, UK*

Received 21 February 2002; accepted 16 May 2002

Abstract

We characterised a novel, charge-insufficient isosteric analogue of spermine, 11-[(amino)oxy]-4,9-diaza-1-aminoundecane (AOSPM). This analogue was synthesised by displacing aminopropyl group by aminooxyethyl group, the latter having pK_a of about 5. Charge deficiency of the AOSPM molecule was fixed at a definite atom, while pK_a of the rest nitrogen was similar to the parent polyamine. AOSPM competed with putrescine, spermidine and spermine for the uptake into the cell, and was accumulated in the cells in high amounts when exogenous polyamine synthesis was impaired. It was not recognised by the cells as growth-promoting polyamine, since it was unable to restore growth arrest due to polyamine deprivation. Like natural spermine, this polyamine analogue prevented oxidative DNA damage. AOSPM could be used not only as a tool to study polyamine homeostasis in the cell, but may have distinct applications either as radiation protector, a stable and non-toxic inhibitor of polyamine uptake or, as an appropriate vector, to enhance the uptake of impermeable compounds into the cell.

Keywords: Aminooxyspermine; Polyamine transport; Putrescine; Spermidine; Spermine

1. Introduction

The polyamines putrescine, spermidine and spermine are aliphatic organic cations necessary for cell growth. The cells slow their proliferation rate and eventually stop dividing when deprived of their polyamines, and resume growth after reconstitution of normal polyamine levels [1]. Intracellular polyamine homeostasis is regulated by complex feedback mechanisms, controlling polyamine biosynthesis (by ODC and SAMDC), degradation (by spermidine/spermine N^1-acetyltransferase—SSAT), and, equally important, uptake from the extracellular space [2,3]. Polyamine uptake in mammalian cells substitutes for impaired synthesis in maintaining growth, and is up-regulated by mitogens or hormones [4–6], or by polyamine deprivation [7]. Exogenous polyamines, originating from food or synthesised by colonic bacteria, are readily absorbed in the gut and taken up by neoplastic cells throughout the body [8,9].

Biological activities of polyamines result from geometry of their molecules (spatial localisation of positively charged nitrogens), and from the degree of their charges. At physiological pH polyamines are fully protonated, and exert their growth-related actions by binding to all negatively charged molecules in the cell, including DNA, proteins and phospholipids [10].

Substances structurally similar to polyamines and recognised by the mammalian polyamine transporter as polyamines are accumulated in proliferating cells instead of

* Corresponding author. Tel.: +49-69-6301-5326; fax: +49-69-6301-6246.
E-mail address: milovic@em.uni-frankfurt.de (V. Milovic).

Abbreviations: AMA, *S*-(5′-deoxy-5′-adenosyl)-methylthioethyl-hydroxylamine; AOSPM, aminooxyspermine, 11-[(amino)oxy]-4,9-diaza-1-aminoundecane; APAPA, 1-aminooxy-3-*N*-[3-aminopropyl]-aminopropane; AOEPU, *N*-[2-aminooxyethyl]-1,4-diaminobutane; APA, 1-aminooxy-3-aminopropane; ODC, ornithine decarboxylase; PBS, phosphate-buffered saline; ROS, radical oxidative species; SAMDC, *S*-adenosylmethionine decarboxylase; SSAT, spermine/spermidine-N^1-acetyltransferase.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01225-X

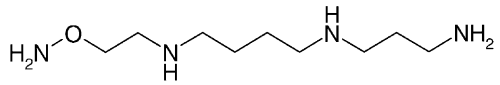

Fig. 1. Structure of 11-[(amino)oxy]-4,9-diaza-1-aminoundecane (aminooxysperine).

natural polyamines. If metabolically active, these substances can influence the feedback mechanisms controlling polyamine homeostasis. Because polyamines are essential for neoplastic cell growth [11,12], most efforts were focused on the synthesis of polyamine analogues aimed to deplete intracellular polyamine pools and impair cancer growth. Furthermore, polyamine analogues are used as radiation protectors [13], or vectors to enhance transport of nucleic acids into the cell [14,15].

Here we describe biological effects of a novel isosteric analogue of spermine, 11-[(amino)oxy]-4,9-diaza-1-aminoundecane (aminooxyspermine, Fig. 1). This analogue was designed in order to modify charges of the parent spermine molecule but to preserve its geometry as close as possible. This approach has been described earlier, and it involves substitution of the terminal aminopropyl group of the polyamine molecule by the aminooxyethyl group [16–18]. The pK_a of aminooxyethyl group is about 5 and, therefore, the polyamine analogues which contain the aminooxyethyl group are more than 1000-fold less protonated in comparison to natural polyamines [18]. Charge deficiency is here fixed to a definite atom, while pK_a of the remaining nitrogens is about the same as of the parent polyamine. Like natural amines, O-substituted hydroxylamines (aminooxy compounds) form stable salts with acids and participate in electrostatic interactions. Isosteric and charge-insufficient analogues of spermidine, APAPA and AOEPU, have been characterised in detail at enzymatic [19] and cellular level [20]. Both substances inhibited putrescine but not spermidine uptake; they were non-toxic, and moderately inhibited cell proliferation in a dose-dependent manner.

Our results indicate that the charge-insufficient isosteric analogue of spermine is stable and non-toxic; that it has no marked effect on polyamine-metabolising enzymes; that it competes with polyamines for the uptake in the cell, and, although it does not influence cell growth, that it does not reverse the polyamine deprivation-induced growth arrest.

2. Methods

2.1. Chemicals

Polyamines, *o*-phthalaldehyde and general biochemicals were purchased from Sigma Chemie. The D,L-[1-^{14}C]-ornithine (specific activity 43.8 mCi/mmol) was from Du PontNEN.*S*-adenosyl(carboxyl-^{14}C)-L-methionine(specific activity 59 mCi/mmol), [^{3}H]-acetyl-CoA (specific activity 220 mCi/mmol), [^{14}C]-labelled putrescine, spermidine and spermine (specific activities 122, 116 and 124 mCi/mmol, respectively) and L-[1-^{14}C]-leucine (specific activity 53 mCi/mmol) were from Amersham Buchler. Cell culture media and supplements were purchased from Gibco BRL. Enzyme inhibitors APA and AMA were synthesised as described in [16,17]. The AOSPM was synthesised as described before [18].

2.2. Cell culture

Caco-2 cells were obtained from the European Animal Cell Culture Collection, and used for experiments within five passages (39–44) in culture. The cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L glucose and 25 mM Hepes, supplemented with 10% foetal calf serum, 100 U/mL benzylpenicillin and 10 μg/mL streptomycin, 1% non-essential amino acids and 1% pyruvate. The medium was changed every second day. The cells were checked for Mycoplasma in monthly intervals.

For experiments, the cells were expanded in tissue culture flasks (75 cm^2 growth area), detached by treatment with 0.5 g/L trypsin and 0.2 g/L EDTA in PBS, and, as dictated by the design of each particular experiment, reseeded to 6- or 24-well tissue culture plates at a density indicated on Tables and Figures. Cell morphology was routinely examined by phase contrast microscopy. Additions of AOSPM were made from a frozen stock of 100 mM in serum-free DMEM.

2.3. Proliferation assay

Caco-2 cells were seeded at 3×10^4 cells per well in 2 cm^2 microplates and allowed to adhere overnight in growth medium. Together with 0.1 or 1 mM AOSPM, [^{3}H]thymidine (1 μCi/mL) and [^{14}C]leucine (0.1 μCi/mL) were added. The cells were incubated for desired time periods, rinsed twice with PBS, solubilised with 1 N NaOH, and neutralised with 1 N HCl. NaOH-extractable radioactivity was measured by liquid scintillation counting. In add-back experiments, the cells were allowed to attach overnight, and then either not treated or incubated with ODC and SAMDC inhibitors, APA and AMA (0.1 mM each) for 24 hr; AOSPM (0.1 mM), spermidine (0.1 mM) and [^{3}H]thymidine were added thereafter.

2.4. Polyamine uptake studies

The uptake studies were initiated by adding 10 or 100 μM of each polyamine (0.1 μCi/mL) and increasing concentrations (0–200 μM) AOSPM to the cell culture wells. After 60 min incubation at 37° the uptake was terminated by washing the cell monolayers twice with cold PBS to remove non-absorbed radioactivity from the cell surface. The cells were then solubilised with 1 N NaOH, neutralised with 1 N HCl and the radioactivity was measured by standard liquid scintillation counting. Each uptake data point presented in this paper is

mean ± SEM (pmol/10^6 cells/hr) from three independent experiments performed on duplicate plates.

2.5. Analytical procedures

The cells were harvested by scraping and permeabilised with 200 mM perchloric acid. Polyamines and AOSPM were analysed in the medium and in the cells simultaneously by HPLC, using the method of Hyvonen *et al*. [21]. The polyamine content of foetal calf serum, used for maintaining the cells in culture, when analysed by HPLC was found to be below the limit of detection.

ODC and SAMDC activity in Caco-2 cells and in cell homogenates were assessed by measuring the amount of $^{14}CO_2$ liberated from L-1-^{14}C-ornithine and *S*-adenosyl-(carboxyl-^{14}C)-L-methionine, respectively, as described [22]. The SSAT activity was measured by monitoring the production of [^{3}H]acetylspermidine in an assay mixture which contained 3 mM spermidine and 16 μM [^{3}H]acetyl-CoA (80 nCi per assay) according to Wallace and Evans [23]. Protein was measured by Coomassie blue assay. Specific activities of ODC and SAMDC were expressed in picomoles released CO_2 per milligram protein, and for SSAT in femtomoles of acetylated spermidine per minute per milligram protein.

2.6. Assay to DNA strand breaks

To evaluate the effect of AOSPM on DNA, assay for DNA strand breaks was done by the conversion of 200 ng of supercoiled pUC19 plasmid to open circular and linear forms in the result of exposition to the ROS-generating system consisting of 30 μM H_2O_2 and 10 μM $CuCl_2$ in PBS (pH 7.4) in a final volume of 30 μL, with PBS alone used as control. Incubation in the presence of 1 mM AOSPM or 1 mM spermine or 1 mM spermidine in PBS (pH 7.4) was used to protect DNA. The samples were analysed by electrophoresis in a 1% agarose gel containing 40 mM Tris–acetate and 1 mM EDTA in a horizontal slab gel apparatus using Tris–acetate gel buffer. The gel was stained with ethidium bromide (2 μg/mL) for 10 min, followed by destaining in water for 10 min, and then photographed by UV translumination.

2.7. Statistics

All data presented here are mean values from 3 to 6 different experiments ± SEM. One-way ANOVA was used to compare means; $P < 0.05$ was considered to be significant.

3. Results

In the initial studies the stability of AOSPM in cell culture was investigated. For this purpose, AOSPM (1 mM) was added to cell culture medium, kept at 37°, and its stability was measured by HPLC over 1 week. The substance was not degraded under these conditions even in the presence of 10% foetal calf serum, the values not falling below 0.95 mM throughout the time studied. This principally distinguishes AOSPM from other hydroxylamine-containing analogues of putrescine (APA) and spermidine (AOEPU and APAPA), whose half-life in the presence of serum was less than 12 hr [19–21,24]. Because these data imply that AOSPM is resistant to serum amine oxidases, it was subsequently used in cell culture experiments without addition of aminoguanidine as inhibitor of amine oxidases.

Table 1
Intracellular AOSPM concentrations in control and polyamine-depleted proliferating Caco-2 cells

	Duration of treatment (hr)		
	24	48	72
AOSPM			
Control	7.2 ± 0.7	7.0 ± 0.2	3.6 ± 0.2
Polyamine-deprived	11.5 ± 0.7**	10.1 ± 0.1**	7.4 ± 1.0**

AOSPM concentration was measured in Caco-2 cells during log phase of growth (<72 hr in culture). The cells were exposed to 10 nmol per well (100 μM) AOSPM alone, or to AOSPM and ODC and SAMDC inhibitors, APA and AMA (100 μM each), in concentrations shown to reduce intracellular polyamine content to non-detectable levels within 24 hr. Intracellular AOSPM concentration was expressed in pmol/mg cellular protein. Mean ± SEM, N = 4.
** $P < 0.01$.

As shown on Table 1, AOSPM was rapidly accumulated in Caco-2 cells during the first 48 hr in culture when the cells most extensively proliferate, and its accumulation was enhanced after polyamine deprivation with ODC and SAMDC inhibitors, APA and AMA. This set of data raised a need to investigate whether AOSPM is accumulated into the cells by utilisation of the polyamine transport system. To study this, Caco-2 cells were exposed to physiological concentrations of polyamines, in the presence of increasing concentrations of AOSPM. For this experiment the cells were kept until confluence, because at this point they become fully polarised and therefore they then resemble the conditions normally occurring in the intestinal/colonic lumen *in vivo*. Under these conditions, AOSPM competed for the uptake with all three polyamines (Fig. 2). K_i values, calculated after transformation of data according to Dixon, were 18.7 ± 1.3 μM for putrescine, 9.0 ± 1.1 μM for spermidine and 8.1 ± 0.2 μM for spermine.

AOSPM was readily accumulated in the cell after polyamine deprivation, and it was taken up via the polyamine transport system. This spermine analogue should, therefore, have at least some polyamine-like effects on polyamine homeostasis, or exert some actions similar to polyamines in mammalian cells. As to the first assumption, treatment with AOSPM significantly enhanced ODC and SAMDC activity during the initial 24 hr of treatment (ODC: 464 ± 22 pmol released CO_2 per mg protein in non-treated cells, vs. 901 ± 65 in AOSPM-treated cells,

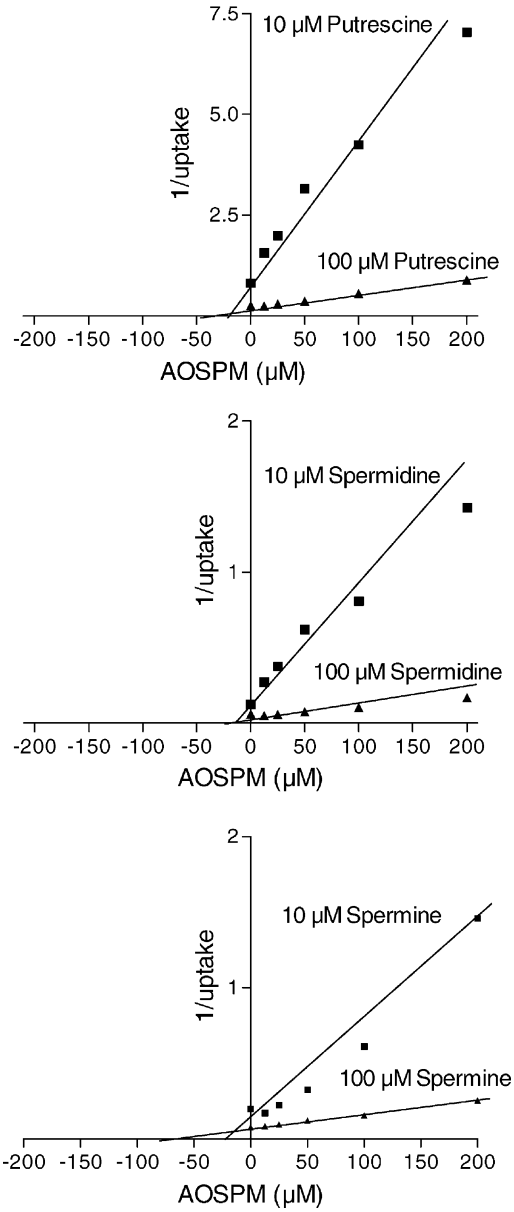

Fig. 2. Effect of AOSPM on polyamine uptake in confluent Caco-2 cells. To achieve polarisation, the cells were grown until confluence, and then exposed to increasing concentrations of AOSPM. Uptake of ^{14}C-polyamines was done as described in Section 2.

$P < 0.01$; SAMDC: 132 ± 5 pmol released CO_2 per mg protein in non-treated cells, vs. 254 ± 20 in AOSPM-treated cells, $P < 0.01$). After 24 hr in culture, however, the activities of the two polyamine-synthesising enzymes rapidly returned to baseline, and were not statistically different from control (data not shown). Intracellular polyamine concentrations followed this increase in the activities of polyamine-synthesising enzymes just to a minor extent, the values for putrescine, spermidine and spermine being increased only marginally and non-significantly (basal: putrescine 3.7 ± 0.8, spermidine 28.9 ± 1.5 and spermine 16.5 ± 0.1 nmol/mg protein; AOSPM-treated: putrescine 4.5 ± 0.4, spermidine 34.2 ± 2.3, and spermine 13.8 ± 1.1 nmol/mg protein; mean $\pm$ SEM, N = 4). To explore whether a transitory effect of AOSPM on ODC and SAMDC activities may be a consequence of a direct effect of the compound to the enzyme, ODC, SAMDC and SSAT activities were further measured in crude enzyme fraction obtained from homogenates of non-treated Caco-2 cells. Under such conditions AOSPM did not influence ODC and SAMDC activities (data not shown). However, in the case of SSAT a moderate increase of enzyme activity was observed (405 ± 18 fmol of acetylated spermidine per minute, vs. 333 ± 13 in controls, $P < 0.05$). To investigate this further, in paired experiments we measured SSAT activity in cell homogenates by replacing increasing concentrations (0–5 mM) spermidine in the incubation medium with equimolar concentrations of AOSPM. With K_m of 2.2 mM, AOSPM was found to be a substrate for SSAT. And indeed, when AOSPM concentration in Caco-2 cells was measured over time, the formation of increasing amount of a single AOSPM metabolite was observed, which presumably represented acetylated AOSPM.

Consistent with the finding that treatment of Caco-2 cells with AOSPM over 72 hr resulted in the absence of significant changes in intracellular polyamine levels, AOSPM (1 mM) had little effect on DNA or protein synthesis in Caco-2 cells over 72 hr in culture (Fig. 3). However, this isosteric spermine analogue could emulate at least one cellular function of its parent polyamine: in concentrations similar to those of the natural spermine,

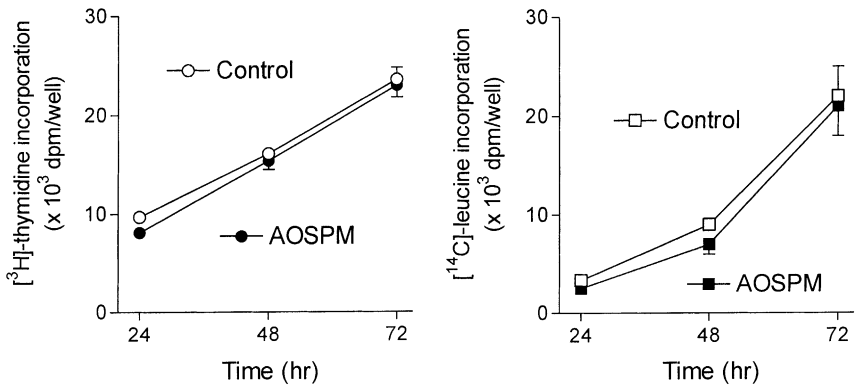

Fig. 3. Effect of AOSPM on Caco-2 cell proliferation. The cells were treated with 1 mM AOSPM. Mean $\pm$ SEM, N = 4.

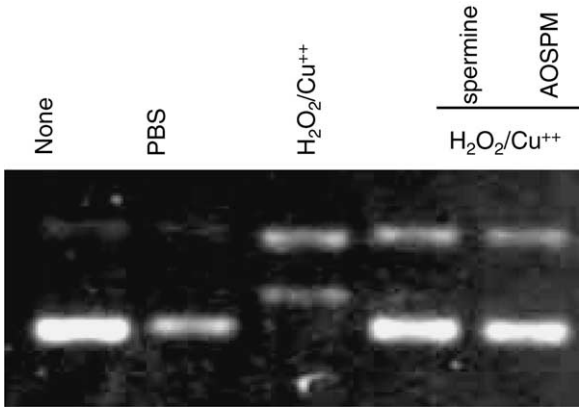

Fig. 4. Effect of AOSPM on oxidative radical-induced DNA damage. Supercoiled pUC19 plasmid DNA (200 ng) was incubated in the ROS-generating system for 60 min at 37°. AOSPM (1 mM) protected DNA from damage induced by oxidative radicals, similar to natural spermine (1 mM).

AOSPM protected isolated DNA exposed to ROS-generating system *in vitro*, to an extent similar to this of natural spermine (Fig. 4).

To test whether the cells recognise this isosteric spermine analogue as a growth-supporting polyamine, we cultured Caco-2 cells in the presence of APA (ODC inhibitor) and AMA (an irreversible inhibitor of SAMDC). Treatment of Caco-2 cells with APA and AMA for 24 hr inhibits the ODC and SAMDC activities to non-detectable levels and results in a growth arrest which could be reversed only by supra-physiological concentrations of exogenous spermidine [25]. As shown on Fig. 5, in polyamine-deprived Caco-2 cells AOSPM was unable to restore growth. And further, if spermidine was added together with AOSPM in concentrations sufficient to restore proliferation, AOSPM prevented spermidine-induced growth restoration after polyamine deprivation.

4. Discussion

These studies were designed to investigate whether a novel insufficiently charged isosteric analogue of spermine, AOSPM, may influence polyamine homeostasis in mammalian cells, or have an impact on known cellular functions of polyamines. Initial assessment of the stability of AOSPM in cell culture showed, first, that the analogue was stable in cell culture medium for at least 7 days. Second, Caco-2 cells rapidly accumulated this compound, by a mechanism that involves competition for the uptake with natural polyamines. This finding is unlikely to be due to tricationic structure of AOSPM at physiological pH, but rather indicates that its aminooxy group may be recognised by the polyamine transporter as aminomethylene group, in spite of the differences in their pK_a in solution. Co-operativity (zip-effect), together with the ability of aminooxy group to participate in electrostatic interactions, may be the driving force for transport of AOSPM across the cell membrane.

In Caco-2 cells, treatment with AOSPM alone had an impact neither on intracellular polyamine levels, nor on cell proliferation. Initial evaluation of the compound [26] showed that, in regard to cell growth, AOSPM behaved differently in rapidly proliferating L1210 cells, and in non-transformed and slowly proliferating BHK cells: while in rapidly proliferating neoplastic cells it inhibited growth

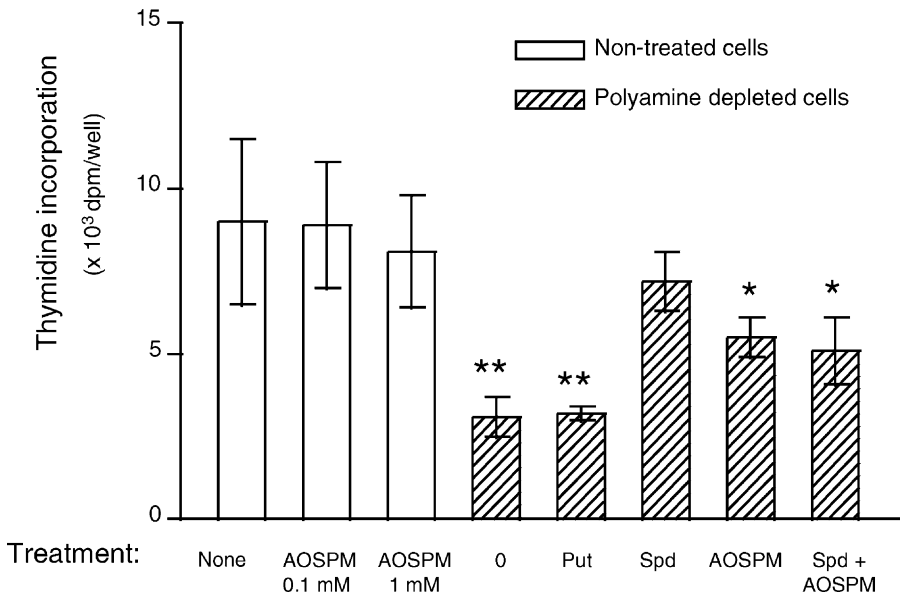

Fig. 5. Effect of AOSPM on growth arrest induced by polyamine deprivation. The cells were treated with 0.1 or 1 mM AOSPM, or with 0.1 mM APA (ODC inhibitor) and 0.1 mM AMA (SAMDC inhibitor). Treatment with APA + AMA reduces intracellular polyamines to non-detectable levels. In add-back experiments, the cells were pre-treated with APA and AMA as above; putrescine, spermidine and AOSPM were then added in concentrations of 0.1 mM. (*) $P < 0.05$; (**) $P < 0.02$. Mean ± SEM, N = 4. *P*-values were calculated in comparison to non-treated cells.

mildly but significantly, in BHK cells it had no effect. Caco-2 cells are of neoplastic origin, but still do possess characteristics of immature colonocytes rather than of true cancer cells [27], and divide relatively slowly [28]. It may be therefore possible that the effect of AOSPM may be cell type-specific, or, rather, dependent on the basal cell proliferation rate. Nevertheless, AOSPM prevented spermidine-induced growth restoration after polyamine deprivation. The latter observation seems to be important, indicating either competition between spermidine and AOSPM for transport, or competition for cellular receptors, essential for the integral effects of spermidine on cell growth.

Our data suggest that AOSPM is a substrate for SSAT and, as such, it was acetylated in the cells. In homogenates obtained from non-treated Caco-2 cells, the K_m value of AOSPM was 2.2 mM. In Caco-2 cells treated with AOSPM we detected one metabolite which, at present, remained unidentified as either N^1- or N^{11}-substituted derivative, because the corresponding mono-acetylated AOSPM derivatives which must be used as a reference have not yet been synthesised. Substrate properties of AOSPM in SSAT reaction not only underline its functional similarity with spermine, but also may be taken into consideration for the design of novel inhibitors of SSAT.

Exposure of the cell to unsymmetrically substituted polyamine analogues, inducers of SSAT, leads to an increased production of ROS and, as a result, cytotoxicity [29,30]. Polyamines are acetylated by SSAT and then subjected to the action of polyamine oxidase, which, besides initiating polyamine catabolism, may produce ROS that in turn trigger modifications in subcellular compartments such as mitochondria [31]. Spermine, but not putrescine or spermidine, reduces oxidative damage of isolated DNA by acting as a free-radical scavenger [32]. In our study AOSPM behaved similar to natural spermine in protecting isolated DNA from oxidative radical-induced damage. There are at least two possible mechanisms of this effect. First, the association of AOSPM with DNA might lead to conformational changes in DNA, making it less susceptible to oxidative damage. This mechanism is less likely, because of insufficient charge of the AOSPM molecule itself: due to the presence of its aminooxy group, the AOSPM molecule has pK_a about 5, and, in solution, it behaves like a trication such as spermidine. Since AOSPM was as efficient as spermine (a tetracation) in protecting DNA from ROS-induced damage, it seems more likely that scavenging of free radicals occurs not by the AOSPM molecule itself, but by the complex of AOSPM with DNA. Such complexes might be similar to those of DNA and spermine, due to the ability of O-substituted hydroxylamines to form stable salts with acids and to participate in electrostatic interactions. It should be noted that many radioprotectants, presumably acting by similar mechanisms, have structures closely related to the polyamines, and utilise the polyamine transport system to be transported into the cell [33].

In conclusion, the described actions of AOSPM point out to the importance of designing compounds for the control of cellular polyamine levels by utilising the polyamine transport system of the mammalian cells. Absence of any detectable effect of AOSPM on cell growth, its inability to restore growth after polyamine depletion, and its spermine-like action in preventing oxidative DNA damage, may indicate that this compound can be used not only as a tool to study polyamine homeostasis in the cell, but that it may have distinct applications, either as radiation protector, as a stable and metabolically neutral inhibitor of polyamine uptake or, in a form of an appropriate vector, in enhancing the uptake of otherwise impermeable compounds into the cell.

Acknowledgments

The authors are indebted to Dr. Patrick Brachet for critical discussions. This study was supported by grants of Arthur & Margarete Ebert Foundation (to V.M.), RFFI (grant No. 00-04-48244, to A.R.Kh.), and by Else Kröner-Fresenius Foundation.

References

[1] Dandrifosse G, Deloyer P, Grandfils C, Loret S, Peulen O, Dandrifosse AC. Statement on polyamines. Rev Med Liege 1999;54:175–83.
[2] Viguera E, Trelles O, Urdiales JL, Mates JM, Sanchez-Jimenez F. Mammalian L-amino acid decarboxylases producing 1,4-diamines: analogies among differences. Trends Biochem Sci 1994;19:318–9.
[3] Urdiales JL, Medina MA, Sanchez-Jimenez F. Polyamine metabolism revisited. Eur J Gastroenterol Hepatol 2001;13:1015–9.
[4] Buts JP, De Keyser N, Romain N, Dandrifosse G, Sokal E, Nsengiyumva T. Response of rat immature enterocytes to insulin: regulation by receptor binding and endoluminal polyamine uptake. Gastroenterology 1994;106:49–59.
[5] Milovic V, Deubner C, Zeuzem S, Piiper A, Caspary WF, Stein J. EGF stimulates polyamine uptake in Caco-2 cells. Biochem Biophys Res Commun 1995;206:962–8.
[6] Milovic V, Stein J, Odera G, Gilani S, Murphy GM. Low-dose deoxycholic acid stimulates putrescine uptake in colon cancer cells (Caco-2). Cancer Lett 2000;154:195–200.
[7] Kramer DL, Miller JT, Bergeron RJ, Khomutov R, Khomutov A, Porter CW. Regulation of polyamine transport by polyamines and polyamine analogs. J Cell Physiol 1993;155:399–407.
[8] Murphy GM. Polyamines in the human gut. Eur J Gastroenterol Hepatol 2001;13:1011–4.
[9] Milovic V. Polyamines in the gut lumen: bioavailability and biodistribution. Eur J Gastroenterol Hepatol 2001;13:1021–5.
[10] Wallace HM. The physiological role of the polyamines. Eur J Clin Invest 2000;30:1–3.
[11] Medina MA, Quesada AR, Nunez de Castro I, Sanchez-Jimenez F. Histamine, polyamines, and cancer. Biochem Pharmacol 1999;57:1341–4.
[12] Wallace HM, Caslake R. Polyamines and colon cancer. Eur J Gastroenterol Hepatol 2001;13:1033–9.
[13] Chiu S, Oleinick NL. Radioprotection of cellular chromatin by the polyamines spermine and putrescine: preferential action against formation of DNA–protein crosslinks. Radiat Res 1998;149:543–9.
[14] Gangamani BP, Kumar VA, Ganesh KN. Spermine conjugated peptide nucleic acids (spPNA): UV and fluorescence studies of PNA–DNA

hybrids with improved stability. Biochem Biophys Res Commun 1997;240:778–82.
[15] Antony T, Thomas T, Shirahata A, Thomas TJ. Selectivity of polyamines on the stability of RNA–DNA hybrids containing phosphodiester and phosphorothioate oligodeoxyribonucleotides. Biochemistry 1999;38: 10775–84.
[16] Khomutov AR, Khomutov RM. Sulphur-containing O-substituted hydroxylamines for inhibition of enzymes and modification of nucleic acids. Bioorgan Khim 1986;12:1662–74.
[17] Khomutov AR, Khomutov RM. Synthesis of putrescine and spermidine aminooxy analogues. Bioorgan Khim 1989;15:698–703.
[18] Khomutov AR, Vepsalainen JJ, Shvetsov AS, Hyvonen T, Keinanen TA, Pustobaev VN, Eloranta TO, Khomutov RM. Synthesis of hydroxylamine analogues of polyamines. Tetrahedron 1996;52:13751–66.
[19] Eloranta TO, Khomutov AR, Khomutov RM, Hyvonen T. Aminooxy analogues of spermidine as inhibitors of spermine synthase and substrates of hepatic polyamine acetylating activity. J Biochem 1990;108: 593–8.
[20] Hyvonen T, Keinanen TA, Khomutov AR, Khomutov RM, Eloranta TO. Aminooxy analogues of spermidine evidence the divergent roles of the charged amino nitrogens in the cellular physiology of spermidine. Life Sci 1995;56:349–60.
[21] Hyvonen T, Keinanen TA, Khomutov AR, Khomutov RM, Eloranta TO. Monitoring of the uptake and metabolism of aminooxy analogues of polyamines in cultured cells by high-performance liquid chromatography. J Chromatogr 1992;574:17–21.
[22] Milovic V, Turchanowa L, Fares FA, Lerner A, Caspary WF, Stein J. *S*-Adenosylmethionine decarboxylase activity and utilization of exogenous putrescine are enhanced in colon cancer cells stimulated to grow by EGF. Z Gastroenterol 1998;36:947–54.
[23] Wallace HM, Evans DM. Measurement of spermidine/spermine N^1-acetyltransferase activity. Methods Mol Biol 1998;79:59–68.
[24] Hyvonen T, Alakuijala L, Andersson L, Khomutov AR, Khomutov RM, Eloranta TO. 1-Aminooxy-3-aminopropane reversibly prevents the proliferation of cultured baby hamster kidney cells by interfering with polyamine synthesis. J Biol Chem 1988;263:11138–44.
[25] Milovic V, Turchanowa L, Khomutov AR, Khomutov RM, Caspary WF, Stein J. Hydroxylamine-containing inhibitors of polyamine biosynthesis and impairment of colon cancer cell growth. Biochem Pharmacol 2001;61:199–206.
[26] Khomutov AR, Shvetsov AS, Vepsalainen J, Kramer DL, Hyvonen T, Keinanen T, Eloranta TO, Porter CW, Khomutov RM. New aminooxy analogs of biogenic polyamines. Bioorgan Khim 1996;22:557–9.
[27] Engle MJ, Goetz GS, Alpers DH. Caco-2 cells express a combination of colonocyte and enterocyte phenotypes. J Cell Physiol 1998;174: 362–9.
[28] Rousset M, Chevalier G, Rousset JP, Dussaulx E, Zweibaum A. Presence and cell growth-related variations of glycogen in human colorectal adenocarcinoma cell lines in culture. Cancer Res 1979;39: 531–4.
[29] Chopra S, Wallace HM. Induction of spermidine/spermine N^1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen species. Biochem Pharmacol 1998;55:1119–23.
[30] Nairn LM, Lindsay GS, Woster PM, Wallace HM. Cytotoxicity of novel unsymmetrically substituted inhibitors of polyamine biosynthesis in human cancer cells. J Cell Physiol 2000;182:209–13.
[31] Bordin L, Vargiu C, Clari G, Brunati AM, Colombatto S, Salvi M, Grillo MA, Toninello A. Phosphorylation of recombinant human spermidine/spermine N^1-acetyltransferase by CK1 and modulation of its binding to mitochondria: a comparison with CK2. Biochem Biophys Res Commun 2002;290:463–8.
[32] Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA. The natural polyamine spermine functions directly as a free radical scavenger. Proc Natl Acad Sci USA 1998;95:11140–5.
[33] Mitchell JL, Judd GG, Diveley Jr RR, Choe CY, Leyser A. Involvement of the polyamine transport system in cellular uptake of the radioprotectants WR-1065 and WR-33278. Carcinogenesis 1995;16: 3063–8.