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Polyamine Analogues of Propanediamine Series Inhibit Prostate Tumor Cell Growth and Activate the Polyamine Catabolic Pathway

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Abstract. *Background/Aim. Polyamines are important for the growth of eukaryotic cells. At high levels, they promote proliferation, invasion and migration of tumour cells. Polyamine metabolism is an important new target for anticancer therapy. Some polyamine analogues can have an inhibitory effect on tumour cells. The aim of this study was to explore the potential of certain butylated derivatives of propanediamine for prostate cancer chemotherapy. Materials and Methods. Human prostate cancer cells, LNCaP, were used for the evaluation of the antiproliferative activity of polyamine analogs and their influence on spermine oxidase. Results. Tetrabutyl propanediamine and two new polyamine analogues inhibited the growth of LNCaP cells. At the same time, a strong activation of spermine oxidase was observed. Conclusion. The investigated compounds demonstrated their potential value in the therapy of human prostate cancer. Their effect might be attributed to the activation of the polyamine catabolic pathway.*

Polyamines (PA) are found in all living organisms (animals, plants, algae, fungi, bacteria and viruses). Tissues and biological fluids of animals contain spermine (Spm) and spermidine (Spd) in millimolar concentrations while putrescine (Put) is present only at low concentrations (nanomoles). The content in Put is higher than that of Spd in prokaryotes, and there is no Spm in most bacteria, with the exception of certain species (1).

PAs are organic cations involved in an unexplored series of cellular reactions. However, their exact functions in metabolism and specific interactions with cellular components remain largely unexplained. Pharmacological experiments have convincingly demonstrated that certain levels of these compounds are necessary for cell proliferation (2, 3).

Tissues with a high rate of protein synthesis have a high molecular ratio of Spd/Spm. In differentiated and aged tissues accumulation of Spm and the lowest ratio Spd/Spm (0.3 to 0.5) are typically observed. The differences in distribution of PAs in cells at various stages of the cell cycle might be associated with differences in their biological functions (4, 5). These organic poly-cations perform unique cellular functions that cannot be replaced by inorganic cations such as Zn^{2+}, Mg^{2+} in macromolecular synthesis or in cell growth (6). PAs are essential for the growth of eukaryotic cells, as these molecules are involved in many key processes, including gene transcription, protein function regulation and cell membrane stability (7, 8). The PA levels in rapidly proliferating tissues including tumors are much higher than those in normal tissues. PAs are involved in proliferation and migration of malignant cells. They have also been shown to promote cancer invasion (5, 9). Conversely, PA depletion through blocking their synthesis results in tumor cell growth inhibition (2). The PA degradation includes acetylation combined with consequent oxidative deamination for both Spd and Spm or only direct oxidation in case of Spm (10). In clinical cancers the PA oxidation is often lowered and does not follow the acetylation step which results in increased levels of acetylated PA in blood serum (11). Thereby the products of

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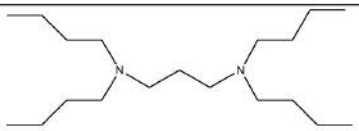
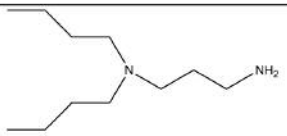
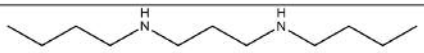
Code	Structure	Name
TBP		N,N,N′,N′-tetrabutylpropane-1,3-diamine
1,1DBP		N,N(1,1)-dibutylpropane-1,3 diamine
1,3DBP		NN′(1,3)-dibutylpropane-1,3-diamine

Figure 1. *The structures of the tested compounds.*

PA oxidation are cytotoxic and can cause apoptosis (3, 10). Thus, PA metabolism is considered an important novel target for anticancer drug design (2, 10).

Chemical compounds that are similar to PA in molecular structure or contain fragments resembling PA are defined as analogues of PA. A number of such agents has been synthesized and described. Some of them have an inhibitory effect on tumor cells, disrupting the metabolism of their own PAs (10, 12). Tetrabutyl propanediamine (TBP) is a newly developed analog of Put that has previously been shown to inhibit the growth and migration of hepatocellular carcinoma of human HepG2 and osteosarcoma MG-63 cells by inducing of apoptosis (13). Similar data have been found regarding leukemic K562 cells (14). The results showed that the use of TBP significantly reduced the proliferation of K562 cells and caused an arrest at the G_0/G_1 phase of the cell cycle. At the same time TBP was able to increase the activity of PA catabolic enzymes – spermine oxidase (SMO) and acetylpolyamine oxidase (APAO) (14).

The purpose of the current study was to explore the potential value of TBP and its 2 other derivatives for chemotherapy of human prostate cancer on the basis of studying the effects of these substances on prostate cancer LNCaP cell clone FGC (ESAS 89110211).

Materials and Methods

The studied compounds. The PA analog TBP (IUPAC name N,N,N',N'-tetrabutylpropane-1,3-diamine) as well as NN'(1,3)-dibutylpropane-1,3-diamine (1,3DBP) and N,N(1,1)-dibutylpropane-1,3-diamine (1,1DBP) were synthesized in "Polichem" (Moscow, Russia) according to the method described by Yang *et al.* (12). The structures of the tested compounds are presented in Figure 1.

Cell culture. The FGC clone (ESASS 89110211) (Sigma-Aldrich (Worldwide) Culture Collections, Public Health England, Porton Down Salisbury, SP4 0JG UK) of LNCaP cells was grown in RPMI-1640 medium containing 10% calf serum, 2 mM glutamine, 1,0 mM sodium pyruvate, 100 μg/ml streptomycin and 100 U/ml penicillin in moistened 5% CO_2 and 95% air incubator at 37˚C.

Analysis of cell proliferation. LNCaP cells were cultivated in 96-well plates: 2000 cells/well in 150 μl of standard medium RPMI-1640 + 10% calf serum + 2 mM glutamine + 1,0 mM sodium pyruvate + antibiotics were incubated with different concentrations (0, 20, 40, 60, 80, 100, 120 and 140 μmol/l) of PA analogues. After 48 hours of exposure to PA analogues, a standard MTT test was performed as described earlier by Wang *et al.* (14). Fifty μl of 3-(4,5-dimethylthiazole)-2,5-diphenyltetrazolium bromide (MTT) solution (250 μg/ml in RPMI-1640 medium) were added to each well, and the cells were incubated at 37˚C for 4 h. The plates were then centrifuged at 380xg for 5 min, the supernatant was removed and 200 μl of dimethyl sulfoxide were added to each well. After 20 min, the optical density (D) was measured at a wavelength of 490 nm. The cell survival rate was calculated using the following formula: Cell survival (%)=D test/D control ×100.

Determination of enzymatic activity of PA oxidase (PAO). SMO activity in cell lysates after 24 h exposure to different concentrations of PA analogues was determined by measuring the formation of hydrogen peroxide during the oxidation of Spm by SMO, as described earlier (14). The enzymatic activity was analyzed in a glycine buffer, pH 8.0, containing 5 nmol of luminol, 20 μg/ml horseradish peroxidase, a cocktail of inhibitors [0.2 mM 2-bromethylamine (catalase inhibitor), 15 μM deprenyl (copper-containing PA oxidase inhibitor), 0.15 μM clorgylin (mitochondrial oxidase inhibitor)], and 250 μM Spm as a substrate. All reagents, except the substrate were mixed in a volume of 250 μl and incubated for 2 min at 37˚C, then Spm was added and the chemiluminescence was measured in a luminometer "Chemiluminometer CHL-003" (Lumitester-C110). The enzyme activity was expressed as relative light units (RLU/μg of protein•min).

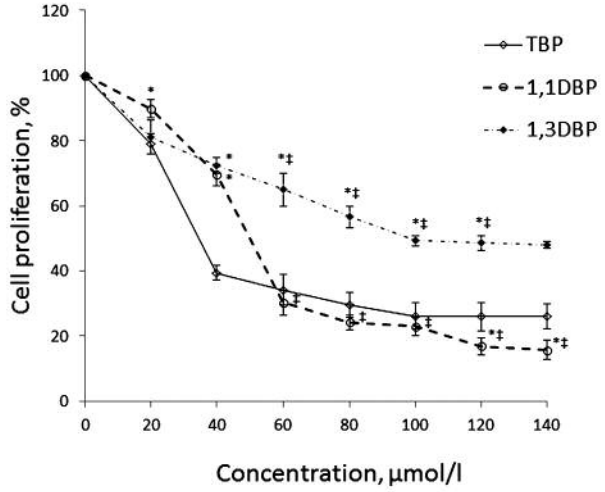


Figure 2. *The inhibitory effect of PA analogues on LNCaP cell proliferation (as a percentage of control). Each bar exhibits the mean±standard deviation (n=10). *p<0.05 in comparison with the cells treated with TBP and ‡p<0.05 for the differences between 1,1DBP and 1,3DBP groups at the same concentration (t-test). MTT-test was performed after 48 h of incubation with PA analogues.*

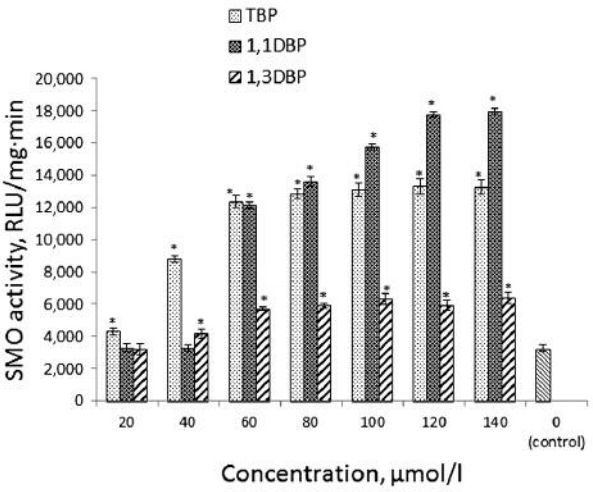


Figure 3. *SMO activity in LNCaP cells in the presence of PA analogues. Each bar exhibits the mean±standard deviation (n=10). *p<0.05 in comparison with the cells treated with TBP at the same concentration (t-test). PA analogues induce the SMO activity in a dose-dependent manner. The assay was conducted after 24 h incubation.*

Results and Discussion

TBP was shown to inhibit growth of the FGC (EAC 89110211) clone of LNCaP cells. A preliminary study of the TBP effect on LNCaP cell proliferation at a maximum concentration of 100 μM during 24, 48, 72 and 96 h revealed that TBP had the maximum inhibitory action on LNCaP cells on the second day of incubation. Longer periods had virtually no effect on the inhibitory activity. Therefore, the effect of analogues of PAs only was examined after 48-h incubation. Next, we examined whether the inhibitory effect of PA analogues was dependent on their concentration. The experiments were repeated 10 times (n=10). The results are shown in Figure 2.

As it can be seen in Figure 2, TBP inhibits the growth of tumor cells giving almost a sigmoid curve. Its inhibitory effect was significant at all tested concentrations. The analogues 1,1DBP and 1,3DBP also significantly inhibited the growth of LNCaP cells in all applied concentrations, but the profile of their effect differed markedly. At low concentrations (20 and 40 μM), 1,1DBP was less effective than TBP, but at concentrations 60, 80 and 100 μM had similar efficacy to TBP. At the concentrations of 120 and 140 μM, 1,1DBP was more cytotoxic than TBP. A comparison of the antiproliferative action of 1,3DBP indicated a significant inhibitory effect at all used concentrations against the control, but compared to the other two PA analogues this derivative showed a significantly lower inhibitory effect. At the maximum concentration of 140 μM it was almost 2 times and 3 times less effective than TBP and 1,1DBP, respectively.

The above results showed that PA analogues inhibit the proliferation of LNCaP cells. In order to investigate the mechanism underlying these effects, their effect on SMO activity was investigated. SMO and APAO are key enzymes in the PA catabolic pathway. SMO catalyzes the decomposition of Spm and APAO catalyzes the catabolism of acetyl-Spd and acetyl-Spm. Both these enzymes produce hydrogen peroxide (H_2O_2) as a by-product of PA degradation that is an important factor for induction of cell apoptosis (15-21). As SMO is considered the primary source of cytotoxic reactive oxygen species (ROS) (16), the effect of PA analogs on its activity was examined. We determined the activity of SMO in LNCaP cells treated with different concentrations of PA analogues for 24 h. Each experiment was repeated 10 times (n=10). The results presented in Figure 3 show that PA analogues have different profiles of enhancing SMO activity in LNCaP cells. TBP stimulated the activity of SMO at much lower doses compared with the dibutyl analogues. Treatment of LNCaP cells with 60 μM TBP for 24 h resulted in an about 3.8-fold (up to 12360 RUL/mg•min) induction in SMO activity. A further increase in the concentration of TBP did not lead to a significant increase in the activity of SMO. On the contrary, the ability of 1,1DBP to activate SMO was manifested only at concentrations above 60 μM and reached the maximum at 120 μM (17950 RUL/mg•min). Further increase in the concentration up to 140 μM did not lead to significantly stronger activation. However, the total activating effect of this analogue was higher than that of TBP, and more than 550% of the activity in the control group. Similarly, 1,3DBP significantly activated Spm oxidation

at concentrations higher than 60 μM, but this effect was less pronounced in comparison with other tested propanediamines (TBP and 1,1DBP). This compound increased the initial SMO activity by only ~75% (up to 5730 RUL/mg•min) compared to control at the concentration of 60 μM, and by 95% (up to 6375 RUL/mg•min) at 140 μM.

A strong reverse correlation (R^2=–0.929, p<0.001) was revealed between SMO activity and the corresponding inhibition of cell proliferation at different concentrations of the tested compounds. It is worth noticing that the maximum cytotoxic effect of the tested substances appeared after 48 h of incubation as revealed by the MTT-test and was preceded by an increase in SMO activity after 24 h of incubation. Taken together these results suggest that the antiproliferative effect of the studied butylated propanediamine derivatives can be attributed to the induction of SMO with consequent ROS formation. Thus, the antiproliferative action of PA analogues can be attributed to the activation of the PA catabolic pathway.

Conclusion

Our study found that the known PA analogue TBP can inhibit the growth of LNCaP prostate cancer cells, activating a key PA catabolic enzyme – SMO, and thus may have value for clinical therapy of human prostate cancer. The other two PA analogues - dibutylated propanediamine derivatives – can also inhibit the growth of LNCaP prostate cancer cells and activate SMO to varying degrees. Therefore, the antiproliferative action of these derivatives can be attributed to activation of the PA catabolic pathway. The antiproliferative activity of 1,1DBP exceeded that of TBP causing a stronger activation of Spm oxidation. The disadvantage of this analogue is that it is effective in higher concentrations.

The results of this study encourage further search for effective PA analogues with anticancer action among this group of chemicals, as well as research on the combination of PA analogues with other anticancer drugs.

Conflicts of Interest

All the Authors declare no conflicts of interest.

Authors' Contributions

The idea, study design and the interpretation of results was developed by M.V. Ploskonos. Literature search and analysis was conducted by S.P. Syatkin and E.V. Neborak. All other Authors contributed equally to experimental work.

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References

1 Sánchez-Jiménez F, Medina MÁ, Villalobos-Rueda L and Urdiales JL: Polyamines in mammalian pathophysiology. Cell Mol Life Sci *76(20)*: 3987-4008, 2019. PMID: 31227845. DOI: 10.1007/s00018-019-03196-0

2 Gamble LD, Hogarty MD, Liu X, Scu Y and Muy Y: Polyamine pathway inhibition as a novel therapeutic approach to treating neuroblastoma. Front Oncol *23(11)*: 162-172, 2012. PMID: 23181218. DOI: 10.3389/fonc.2012.00162

3 Damiani E and Wallace HM: Polyamines and Cancer. Methods Mol Biol *1694*: 469-488, 2018. PMID: 29080189. DOI: 10.1007/978-1-4939-7398-9_39

4 Persson L: Polyamine homeostasis. Essays Biochem *46*: 11-24, 2009. PMID: 16406751. DOI: 10.1042/bse0460002

5 Provenzano B, Lentini A, Tatti R, De Martino A, Borromeo I, Mischiati C, Feriotto G, Forni C, Tabolacci C and Beninati S: Evaluation of polyamines as marker of melanoma cell proliferation and differentiation by an improved high-performance liquid chromatographic method. Amino Acids *51(10-12)*: 1623-1631, 2019. PMID: 31617109. DOI: 10.1007/s00726-019-02799-y

6 Ramani D, De Bandt JP and Cynober L: Aliphatic polyamines in physiology and diseases. Clin Nutr *33(1)*: 14-22, 2014. PMID: 24144912. DOI: 10.1016/j.clnu.2013.09.019

7 Soda K, Kano Y, Chiba F, Soda A and Popov S: Increased polyamine intake inhibits age-associated alteration in global DNA methylation and 1,2-dimethylhydrazine-induced tumorigenesis. PLoS One *8*: 64357, 2013. PMID: 23696883. DOI: 10.1371/journal.pone.0064357

8 Coburn RF: Polyamine effects on cell function: possible central role of plasma membrane PI(4,5)P2. J Cell Physiol *221*: 544-551, 2009. PMID: 19746419. DOI: 10.1002/jcp.21899

9 Soda K: The mechanisms by which polyamines accelerate tumor spread. J Exp Clin Cancer Res *30(1)*: 95-103, 2011. PMID: 21988863. DOI: 10.1186/1756-9966-30-95

10 Murray-Stewart TR, Woster PM and Casero RA Jr: Targeting polyamine metabolism for cancer therapy and prevention. Biochem J *473(19)*: 2937-2953, 2016. PMID: 27679855. DOI: 10.1042/BCJ20160383

11 Venäläinen MK, Roine AN, HäkkinenMR, Vepsäläinen JJ, Kumpulainen PS, Kiviniemi MS, lehtimäki T, Oksala NK and Rantanen TK: Altered polyamine profiles in colorectal cancer. Anticancer Res *38(6)*: 3601-3607, 2018. PMID: 29848716. DOI:10.21873/anticanres.12634

12 Li M, Wang Y, Ge C, Chang L, Wang C, Tian Z, Wang S, Dai F, Zhao L and Xie S: Synthesis and biological evaluation of novel alkylated polyamine analogues as potential anticancer agents. Eur J Med Chem *143*: 1732-1743, 2018. PMID: 29133040. DOI: 10.1016/j.ejmech.2017.10.069

13 Yang JL, Han Y, Wang YL, Gu YE, Qian Q, Yu SF and Zhang J: Polyamine analogues tetrabutyl propanediamine inhibits proliferation, invasion and migration of human liver cell line HepG2 cells in vitro. China J Lab Diagn *16*: 1350-1354, 2012.

14 Wang Q, Wang YL, Wang K, Yan J and Cao CY: Polyamine analog TBP inhibits proliferation of human K562 chronic myelogenous leukemia cells by induced apoptosis. Oncol Lett *9(1)*: 278-282, 2015. PMID: 25435975. DOI: 10.3892/ol.2014.2615

15 Zhang HJ, Wang K, Wang YL and Cao CY: Effects of polyamine analogues tetrabutyl propanediamine on proliferation, apoptosis and migration of human MG63 myeloma cells. Chinese

Pharmacological Bulletin *28(3)*: 974-977, 2012. DOI: 10.3969/j.issn.1001-1978.2012.07.021

16 Pledgie A, Huang Y, Hacker A, Zhang Z, Woster PM, Davidson NE and Casero RA Jr: Spermine oxidase SMO(PAOh1), Not N1-acetylpolyamine oxidase PAO, is the primary source of cytotoxic H_2O_2 in polyamine analogue-treated human breast cancer cell lines. J Biol Chem *280(48)*: 39843-39851, 2005. PMID: 16207710. DOI: 10.1074/jbc.M508177200

17 Ploskonos MV: Externalization of phosphatidylserine on the membrane surface spermatozoa under the influence of oxidative stress. Russian Immunological J *9(18)*: 156-157, 2015.

18 Hilal A, Ploskonos MV, Terentyev AA, Syatkin SP, Neborak EV, Blagonravov ML, Protasov A, Kaitova Z and Chibisov SM: Regulation of apoptosis of human immunocompetent cells under the effect of polyamines. FEBS Open Bio *8(S1)*: 234, 2018. DOI: 10.1002/2211-5463.12453

19 Ploskonos M: The study of proteins expression as a marker of Fas and FasL apoptosis in human spermatozoa. Reprod Probl *21(2)*: 94-97, 2015. DOI: 10.17116/repro201521294-97

20 Sungrapova KY, Ploskonos MV, Terentyev AA, Syatkin SP, Blagonravov ML, Neborak EV, Protasov A and Kaitova Z: Evaluation of apoptogenic effect of a cytostatic agent on male gametes. FEBS Open Bio T *8(S1)*: 234, 2018. DOI: 10.1002/2211-5463.12453

21 Ploskonos MV: The role of markers of apoptosis Fas and FasL in spermatogenesis. Urology *1*: 77-80, 2012. PMID: 22646009.

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