ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Influence of Chemical Polyamines Analogs, Decarboxylated Ornithine and S-(Adenosyl)-Methionine on the Polyamine Synthesis Velocity in Test-Systems from Tissues With High Proliferation.

SP Syatkin*, IP Smirnova, OM Kuznetsova, ML Blagonravov, TA Lobaeva, EA Ryskina, VI Ivanova-Radkevich, RI Sokuyev, and NA Shevkun.

Russian Peoples' Friendship University, Miklukho-Maklaya str., 8, Moscow, 117198, Russia.

ABSTRACT

The aliphatic (amino)- and (oxy)-derivatives of polyamines effect on the rate of polyamines (Putrescine, Spermidine and Spermine) synthesis in cell-free test-systems from rat's regenerating liver and Hepatoma tissues was examined. The common known inhibitors of polyamines synthesis were also used for the analysis. The research data confirm that cell-free model systems function adequately to applied tests. The effectiveness of anti-proliferate action of chemical compounds was valued by the inhibition degree of Putrescine and polyamines synthesis. In addition, the coefficient of ratio Spermidine (moles)/ Spermine (moles) and the value of total Polyamines pool after 1 h of test-systems incubation with chemical compound were calculated. From the tested compounds 1-aminooxy-3-aminopropan (**I**), S-(5'-desoxyadenosile)-S-methyl-β-thioethyl-hydroxylamine (**IV**) and ethylenediamine (**V**) did satisfied the requirements to a considerable degree.

Keywords: polyamines, Putrescine, Spermidine, Spermine, oxidative deamination, Difluoromethylornithine, Ornithine decarboxylase.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

During the experiments with the extracts of L-1210 cells [1] the inhibitory effect α-difluoromethylornithine (DFMO) on the activity of ornithine decarboxylase (ODC) in the absence of such an effect for N^1, N^8 bis(ethyl) spermidine is demonstrated. However, both of these substances equally inhibit the growth of L-cells and reduce the biosynthesis of protein, RNA and DNA growth. This allows us to consider the use of these compound analogs as the regulators of ODC activity as an alternative path for the search of antiproliferative agents acting on PA biosynthesis without direct ODC inhibition. This determined the specificity of this stage of work.

The aim of this work was the effect of chemical analogs of polyamines, decarboxylate ornithine and S-adenosylmethionine study on the rate of putrescine and polyamine formation in the test systems of the tissues with an enhanced cell proliferation.

MATERIALS AND METHODS

O-substituted hydroxylamines (I-IV, Table 1), the analogues of the enzymatic decarboxylation of ornithine and S-adenosylmethionine products were synthesized at the Institute of Organic Chemistry named after N.D. Zelinsky (RAS). The chemical compounds (V-X, table 1), the chemical PA analogs were synthesized at the Institute of Physical Chemistry (RAS).

Table 1: Aliphatic amine and polyamine oxyderivatives

Chemical analogs of decarboxylated ornithine and S-adenosylmethionine		
Name	Index	Structure
α -diftormetilornitin	DFMO	CHF_2 | $H_2NCH_2CH_2CH_2CHCOOH$ | NH_2
1-aminooxy-3-aminopropane	I	$2HCl{\cdot}NH_2OCH_2CH_2CH_2NH_2$
1,2-diaminooxyethane	II	$2HCl{\cdot}NH_2OCH_2CH_2ONH_2$
S-(5′-dezoxyadenozil)-S-tioethylhydroxylamin	III	$H_2SO_4{\cdot}NH_2OCH_2CH_2SAde$
S-(5′-dezoxyadenozil)-S-methyl-β -tioethylhydroxylamin	IV	$2H_2SO_4NH_2OCH_2CH_2S^+Ade$ | CH_3
Chemical analogues of polyamines		
Name	Index	Structure
Ethylenediamine	V	$H_2NCH_2CH_2NH_2$
1,3-diaminopropane	VI	$H_2NCH_2CH_2CH_2NH_2$
1,5-diamino-3-azapentan	VII	$H_2N(CH_2)_2NH(CH_2)NH_2$
1,9-diamino-3,6-diazanonan	VIII	$H_2N(CH_2)_2NH(CH_2)NH(CH_2)_3NH_2$
1,9-diamino-3,7-diazanonan	IX	$H_2N(CH_2)_2NH(CH_2)_3NH(CH_2)_2NH_2$
Methylglyoxal bis (guanilhydrazon)	MGBG	NH | | $H_3C\text{-}C\text{=}N\text{-}NH\text{-}C\text{-}NH_2$ | $HC\text{=}N\text{-}NH\text{-}C\text{-}NH_2$ | | NH

In order to determine the rate of putrescine and polyamine synthesis in biological samples the method was used modified by us [2].

The white nonpedigreed male rats at the weight of 100 - 130 g were used in experiments. They were held on a standard RUDN vivarium diet with free access to water.

The partial hepatectomy (about 70% of tissue) was performed according to Higgins, Anderson method [3] under the ether anesthesia. In order to obtain the regenerating tissue of the animal liver were sacrificed by decapitation after 12 hours of surgery and took out small lobes of liver.

The hepatoma G-27 strain was obtained from the Cancer Research Center (RAMS) and subcultured on outbred white rats as it was described earlier by Shvemberger [4].

The cell-free test system consisted of cytosolic fraction (20,000 g x 20 min at 4 °C) of 33% homogenate of regenerating liver or hepatoma tissue with the addition of the necessary components for the presentation of ODC activity. The amount of protein in the samples of the examined tissues was determined by the method of Lowry (1951) in the modification of S.P. Syatkin (1981) [5].

The authenticity of mean values differences according to the experimental groups of the obtained results was performed using the Student's t-criterion (Afifi, Eisen, 1982) [6].

RESULTS

The efficacy and the nature of the actions listed in the table of chemical compounds on the formation of the studied amines was compared. The first group consisted of oxyamino analogs for decarboxylated ornithine and S-adenosylmethionine. The second group was formed from aliphatic homologues of putrescine (Put), spermidine (Sd) and spermine (Sm). The results of these studies are presented in the Table 2 and 3.

The efficacy of chemical compound antiproliferative activity was evaluated by the degree of Put and PA synthesis inhibition, and the coefficient of spermidine to spermine (Cd/Cm) molar ratio was calculated additionally and the value of the total PA pool (∑PA) was calculated after one hour of incubation. The indicator Sd/Sm is positively correlated with the speed of proliferation both for normal and tumor tissue [7].

The compounds I and IV in the first group of test compounds were the most active ones. They have the similar effective action, and by some values even surpassed DFMO and MGBG (tab. 2 and 3).

Table 2: The rate of putrescine and PA formation against the background of synthetic analog of the substrates and decarboxylation reaction products of ornithine, S-adenosylmethionine in a cell-free test system from regenerating rat liver

Substance	ODC	Spermidine synthesis	Spermine synthesis
-	2.7±0.1	1.29±0.04	0.48±0.01
DFMO	1.7±0.1*	0.87±0.02*	0.21±0.01*
MGBG	2.7±0.1	0.9±0.02*	0.31±0.01*
I	1.1±0.1*	0.57±0.01*	0.1±0.01*
II	2.2±0.1*	0.88±0.06*	0.25±0.08*
III	3.7±0.2*	0.92±0.03*	0.24±0.07*
IV	1.7±0.1*	0.63±0.02*	0.26±0.07*
V	2.3±0.1*	0.98±0.02*	0.32±0.09
VI	2.8±0.1	1.0±0.02*	0.19±0.04*
VII	2.5±0.1	0.74±0.02*	0.2±0.05*
VIII	2.4±0.1	0.78±0.04*	0.18±0.04*
IX	2.5±0.1	0.63±0.02*	0.28±0.06*
X	1.7±0.1*	0.9±0.2*	0.5±0.06

Note. *The rate of putrescine and PA biosynthesis is represented in mccat per 1 kg of protein. The results are given as M±m for 6 parallel measurements. * - significant differences from the control, P≤0.05.*

The second group compounds inhibited the PA synthesis in both systems with different degrees of effectiveness. In the tumor model it led to a dramatic, nearly double increase of the Put fraction. The exception was produced by ethylenediamine (V). It showed strong antiproliferative properties of all selected indicators (Sd/Sm, ∑PA and the level of Sd), particularly in relation to the tumor tissue.

Table 3: The rate of putrescine and PA formation against the background of synthetic analog of the substrates and decarboxylation reaction products of ornithine, S-adenosylmethionine in a cell-free test system from the rat hepatoma G-27

Substance	ODC	Spermidine synthesis	Spermine synthesis
-	2.6±0.2	1.1±0.04	0.4±0.01
DFMO	2.0±0.1*	1.1±0.1	0.2±0.01*
MGBG	2.7±0.2	0.97±0.02*	0.35±0.02*
I	2.0±0.1*	1.2±0.04	0.35±0.02*
II	2.3±0.3	1.0±0.2	0.4±0.01*
III	2.4±0.3*	0.9±0.1	0.3±0.04*
IV	1.5±.01*	0.8±0.01*	0.4±0.01*
V	1.6±0.1*	0.45±0.01*	0.2±0.03*
VI	4.3±0.5*	0.6±0.02*	0.16±0.02*
VII	4.8±0.4*	1.0±0.2	0.2±0.02*
VIII	3.3±0.2*	0.8±0.1*	0.4±0.02
IX	3.5±0.1*	1.2±0.1	0.45±0.03
X	3.4±0.2*	0.9±0.1	0.2±0.05*

Note. *The rate of putrescine and PA biosynthesis is represented in mccat per 1 kg of protein. The results are given as M±m for 6 parallel measurements. * - reliable differences from the control, P≤0.05.*

DISCUSSION

The situation when the substance structurally similar to polyamines or containing spermidine chain produce opposite effects, probably can be explained by a dual character of action and the polyamines themselves. So, depending on the concentration, the latter provide either stimulating or inhibitory effects on various biochemical and physiological processes.

Virtually identical baseline Put and PA synthesis levels used in two model systems have facilitated a comparative analysis of the test compound regulatory properties. DFMO and MGBG were selected as known inhibitors of ODC and adenosylmethionine decarboxylase (AMD) to assess the sensitivity of the test systems to the regulatory effects of chemicals on the process of Put and PA synthesis. Both known inhibitors showed a significant and specific effect (Table 2 and 3) with the preliminary intended trend as in the experiments with the tumor tissue, so as with the regenerating tissue. This suggests that these cell-free model systems are functioning adequately according to the stated objectives.

Various effectiveness and the selectivity of an action for the test substances on the rate of putrescine and PA formation within the systems of regenerating and tumor tissues indicate the imbalances in the tumor tissue of the PA phase synthesis and the changes in the structure and catalytic properties of the enzymes synthesizing PA. The compounds of the first and the second group differed in chemical structure and possible mechanism of action. The reactivity of aminooxy compounds I and IV is conditioned by the reaction of oximes pyridoxal-5'-phosphate formation [8]. The homologous series of the second group compounds varied in the length of the hydrocarbon chain separated by different location of the imino group into di- and trimethylene fragments. As the chemical analogues of Put and PA they probably could act by the feedback inhibition type or by competitive mechanism.

The trend of all 12 studied substances action is mostly the same in both systems. However, the depth of inhibition within PA biosynthesis process in the model of the regenerating tissue was greater. This indicates the greater sensitivity of the system in general and, in particular, the polyaminsynthesis enzymes to chemical impact. Since during the action of carcinogens the multiple forms of ODC are developed, this may be due to the existence of different structural and functional properties and ODC and AMDK forms in transformed and untransformed tissues, as well as with the lack of balance in the tumor tissue of a gradual PA biosynthesis process. More drastic changes in the rate of PA synthesis within the model of the regenerating liver tissue may be also associated with the simultaneous occurrence of the oxidative deamination process, as the activity of Put and PA oxidases is kept in this tissue near at normal level, but in the tumor tissue it is almost equal to zero. The absence of synchronization in ODC and AMDK action within tumor tissue and the presence of high

baseline Put level and decarboxylated S-adenosylmethionine points to the need of these two enzyme inhibitors and the activators of oxidative PA deamination process combined use.

The comparative analysis of the potential regulatory action effectiveness for the two groups of test compounds conducted in two model cell-free systems with different biologic, but with equally high proliferative properties showed that the potentially strong antiproliferative agents should simultaneously significantly reduce ∑PA, Sd/Sm ratio and the level of Sd to a critical value (70%). Among the studied compounds these requirements satisfy more 1-aminooxy-3-aminopropane (I), S-(5'-dezoxyadenozil)-S-methyl-ß-tioethylhydroxyilamin (IV) and ethylenediamine (V).

REFERENCES

[1] *Porter C.W., Sufrin J.R.* Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy // Anticancer Res. - 1986. - V.6 - P.525-542.

[2] Syatkin S.P., Berezov T.T., Gridina N.Ya. and others. Polyamines as a biochemical markers of antiproliferative inhibitor effect of polyamine and putrescine biosynthesis enzymes in the culture of L-cells // Medical chemistry issues. - 1991-V.37, №6.- P. 77-81.

[3] *Higgins G.M., Anderson R.M.* Experimantal pathology of liver: restoration of liver of white rat following partial surgical removal // Arch. Path. - 1931. - V. 12. - P. 186-202.

[4] Shvemberger I.N. Transplantable rat hepatoma strain G-27 // Cytology. - 1970 - V. 12. - P.1057-1059.

[5] Syatkin S.P. Modified method for the determination of protein in the samples with the increased content of lipo and glycoproteins // Medical chemistry issues. 1981.- V. 27, № 1.- P. 136-138

[6] Afifi A., Eisen S. Statistical analysis. Approach with the use of a computer. - M.: Mir. 1982. - 488 p.

[7] *Morgan DM*. Polyamines. An overview. // Mol Biotechnol. - 1999. – V. 11, N3. – P. 229-50.

[8] Homutov A.R., Homutov R.M. Synthesis of putrescine and spermidine aminooxyanalogs // Bioorg. chemistry. - 1989. - V. 15, №5. - p. 698-703.