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Impact of the Chemical Analogues of Decarboxylated Ornithine and S-Adenosylmethionine on the Rate of the L-Cells Growth in the Tissue Culture.

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ABSTRACT

The impact of the aliphatic amino- and oxy derivatives decarboxylated ornithine and S-adenosylmethionine on the rate of the L-cells growth in a tissue culture has been investigated. The known specific inhibitors of the polyamine synthesis enzymes (DFMO and MGBG) were used for control and comparison of efficacy of the substances tested. The nature of action of 1-aminooxy-3-aminopropane (APA) and S-(5'-deoxyadenosile)-S-methyl-β-thioethylhydroxylamine (AMA) used separately or combined one with another was similar to the effect of DFMO and MGBG though less expressed. The degree of inhibition of the L-cells growth as of the 4th day made 50-60%. By the 7th day the tendency to not only stabilization of the growth rate but also to the recovery thereof manifested itself. This points to the reversible nature of action of the APA and AMA combinations and constitutes the difference from the effect DFMO and MGBG.

Keywords: polyamines, putrescine, spermidine, spermine, L-cells, difluoromethylornithine (DFMO), methylglyoxal-bis (guanylhydrazone) (MGBG)

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INTRODUCTION

The issue of the analysis and development of approaches to the directed impact on the mechanisms of regulation the cell proliferation and differentiation are currently remaining the central issue of the fundamental and applied research in the biology and medicine.

It is well known that biogenic polyamines (PA) – spermine and spermidine as well as their precursor putrescine – take active participation on the process of regulation of gene activation and are the markers of the tumor cells proliferation [1, 2].

The use of the PA biosynthesis system as the basis for the development of the synthetic analogues of PA and inhibitors of the polyamine-producing enzymes resulted in the discovery of a wide range of the artificial chemical compounds with the potential antitumor, antiproliferative and even antiparasitic properties [3, 4].

The objective of this study was the analysis of impact of the aliphatic amino- and oxy derivatives decarboxylated ornithine and S-adenosylmethionine on the rate of the L-cells growth in a tissue culture.

MATERIALS AND TECHNIQUES

Quantitative measurement of the PA content

The quantitative content of PA in the animal tissues was determined with the use of the fluorescent reagent N, N-1-dimethylaminonaphthalene-5-sulphochloride (DANS-C1) produced by "Schuchardt" (Federal Republic of Germany) and "Koch-Light" (England) involving the primary and secondary amine groups. A tissue sample for analysis and dansylation reaction was prepared according to the method [5[5]]. The PA products by "Schuchardt" and "Serva" (Federal Republic of Germany) were used as the tracking substances. The HPLC was performed with the use of the chromatographer APC/GPC-204 ("Water-milipore", USA) with the fluorescent detector type 420 at the excitation wavelength of 365 nm and emission 495. The chromatographic column of the Bondapak C_{18} (3.9x300 mm) with the pre-column of 3.9x20 mm was used. The chromatography was performed during 10 minutes in the linear methanol gradient (75-95%). The incubation mixture was preliminarily passed through the column C_{18} Sep-Pak ("Waters", USA), washed with 5 ml of methanol. The dansyl derivatives of amines were eluted with 2 ml of 100% methanol and used for analysis by means of HPLC [6].

Table 1: Chemical analogues of the decarboxylated ornithine and S-adenosylmethionine

Name	*Index*	*Structure*
α - Difluoromethylornithine	DFMO	CHF_2 | $H_2NCH_2CH_2CH_2CHCOOH$ | NH_2
1- aminooxy-3-aminopropane	APA	$2HCl{\cdot}NH_2OCH_2CH_2CH_2NH_2$
S-(5′- desoxyadenosile)-S-methyl -β - thioethylhydroxylamine	AMA	$2H_2SO_4{\cdot}NH_2OCH_2CH_2S^+Ade$ | CH_3
Methylglyoxal-bis (guanylhydrazone)	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

Fibroblasts of rats of the C3H line

The cells of the L line were grown on the Eagle nutrient medium with glutamine and 10% bovine blood serum. In each bottle we introduced 8000 cells per 2 ml of the nutrient medium. 105 bottles were used.

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15 bottles were allocated for each of the 4 experimental groups. The first group was the control one. The remaining three groups were exposed to DFMO, MGBG and DFMO combined with MGBG, correspondingly. The finite concentration of the investigated compounds in the culture medium made 10^{-4} M. The first change of the culture medium and introduction of the investigated substances to the new culture medium were performed on the 4th day after the initial inoculation. One day after that 3 bottles were taken from each group and the number of cells was calculated in the Goryaev chamber, the cell suspension was centrifuged at 1500 rpm and the suspend was centrifuged for determination of Put and PA by means of HPLC. In all the remaining bottles the culture media was changed and the specified substances in the reference quantity were introduced. During the next 2 days the same actions were performed. After the 4th day the cultures of the L-cell tissue grew for another 3 days but without the change of the media and adding the chemical compounds.

The statistical processing of the findings of the study was performed with the use of the software package Statistica 6,0 (StatSoft, USA). The differences between the two samples were considered to be statistically significant at $p \leq 0,05$ [7].

Aliphatic amino- and oxy derivatives of PA.

O-substituted hydroxylamines (Table 1), analogues of the products of enzymic decarboxylation of ornithine and S-adenosylmethionine were synthesized at the Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences.

FINDINGS AND DISCUSSION

The analysis of the role of intracellular PA store in the realization of the antiproliferative effect of inhibitors of polyamine-producing enzymes was carried out with the use of the o-substituted derivatives of hydroxylamine (AMA and APA compounds) that have demonstrated the maximum regulatory activity in the model test-systems [8].

The schema of analysis of impact of 1-aminooxy-3-aminopropane (APA) and (5′-deoxyadenosile)-5′-methyl-β-thioethylhydroxylamine (AMA) on the rate of the L-cells growth in the tissue culture and intracelullar content of PA was similar to the experiments with DFMO and MGBG (ref. Materials and techniques). The results of this series of experiments are presented in the Fig. 1 and 2.

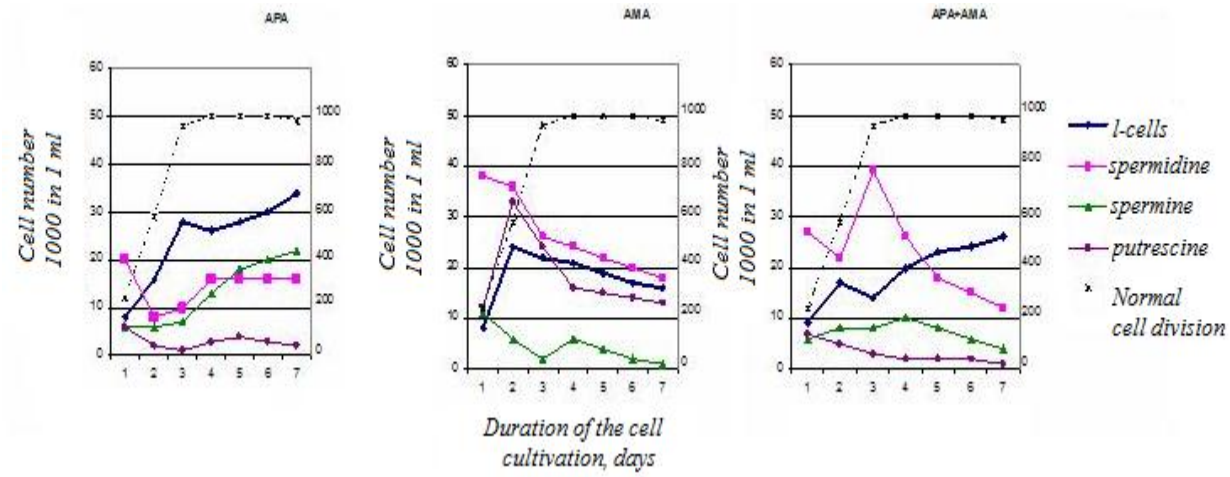

Figure 1: Impact of 1-aminooxy-3-aminopropane (APA) and 5′-deoxyadenosile)-5′-methyl-thioethylhydroxylamine (AMA) on the intracellular PA levels and growth of L-cells in the tissue culture.

Along the abscissa axis the period of cultivation of the L-cells (in days) is indicated, along the ordinate axis: at the left – number of L-cells (10^3 per 1 ml of the culture medium), at the right – polycationic content (pM per 10^6 cells). 1 - L-cells, 2 – Sd, 3 – Sm and 4 – putrescine.

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The nature of action APA and AMA used separately or combined one with another was similar to the effect of DFMO and MGBG though less expressed. Thus, the degree of inhibition of the L-cells growth as of the 4th day made 50-60%, correspondingly (Fig. 1). By the 7th day the tendency to not only stabilization of the growth rate but also to the recovery thereof manifested itself (Fig.2). This points to the reversible nature of action of the APA and AMA combinations and constitutes the difference from the effect DFMO and MGBG.

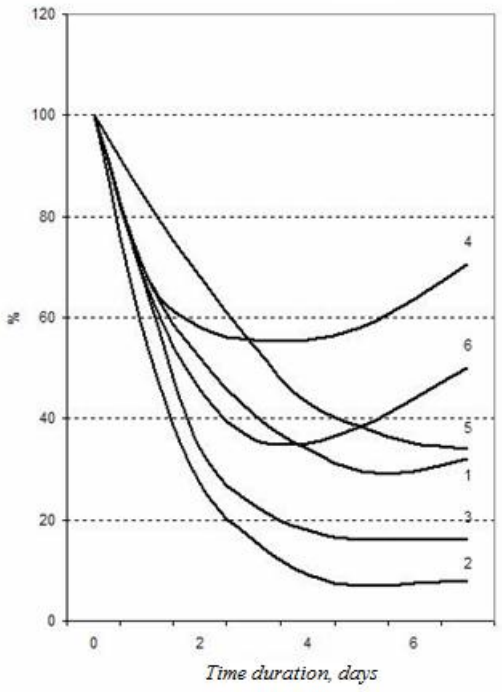

Figure 2: Impact of DFMO, MGBG, APA and AMA on the rate of the L-cells growth in the tissue culture.

Along the abscissa axis the period of cultivation of the L-cells (in days) is indicated, along the ordinate axis – the ratio of the cell quantity to the experimental and control group (%). 1 – DFMO, 2 – MGBG, 3 – DFMO+MGBG, 4 – APA, 5 – AMA, 6 – APA+AMA.

The coefficient of correlation between the degree of inhibition by the investigated compounds of the rate of formation of polycations in the cell-free test systems from the tissues with a high mitotic index [8] and level of inhibition of the L-cells growth in the tissue culture by the same substances equals to 0.87. This points to the presence of the close positive relation between the processes of the PA synthesis and proliferation of the L-cells.

Moreover, the changes in the PA content in the L-cells preceded and advanced by 2 days the changes in the rate of growth of these cells in the tissue culture. Such “independence” of the cell growth during the first two days against the use of the PA-synthesis inhibitors may be provided by that portion of the cell pool that had entered the mitosis cycle prior to the stage when the process of division becomes irreversible and does not depend on the changes in the PA concentration. Probably, in a single-cell culture with the synchronized cell division cycle the antiproliferative activity of inhibitors of the polyamine-producing enzymes will be higher.

The results obtained also show that inhibition of the spermidine affects the cell growth rather than the inhibition of ODC. This is consistent with the general concept of the PA metabolism system being programmed to maintain the optimum level of spermidine according to the proliferative activity of a cell. The APA and AMA compounds demonstrated high activity as inhibitors of the PA synthesis in the cell-free test systems [8] and growth of the L-cells in the tissue culture. This confirms the prospectivity of the further investigations of this group of oxy-amino-derivatives as the new potential antitumor substances. The amino-oxy-analogue of putrescine: 1-aminooxy-3-aminopropane (APA) inhibits the activity of the three enzymes of the PA biosynthesis: ODC, AMDK and spermine-synthase [9] and like the related substances may be used as antitumor agents [10].

The studies concerning the Put and PA content in the L-cells during the period of growth in the tissue culture affected by the inhibitors of the key enzymes for biosynthesis of these substances revealed the two specific features. Thus, changes in the polycationes concentration in the L-cells and the rate of growth of these cells in the tissue culture may be considered as two closely interrelated processes. Moreover, changes in the Put and PA concentration are likely to be of primary nature against the L-cells growth rate since they are ahead of them in terms of time as was shown by the experiments with inhibitors of the key polyamine-producing enzymes [6].

REFERENCES

[1] Bordo L. I., Yakhimovich L. V., Cherny V. A., et al. Detection of the biogenic amines in the tumors for diagnostic purposes // Archive for Pathology.-1976.-Vol.38. -№ 9.- P.33-38

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

[3] Wallace HM, Fraser AV. Polyamine analogues as anticancer drugs. // Biochem Soc Trans. -2003. V-31, N2.-P.393-6.

[4] Seiler N. Thirty years of polyamine-related approaches to cancer therapy. Retrospect and prospect. Part 1. Selective enzyme inhibitors.//Curr Drug Targets. -2003.-V.4, N7.-P.537-64.

[5] Seiler N., Wiechman M. Zum nachweis von aminen in 10^{-10} M masstab. Trennung von l-dimethylaminophtalin-5-sulfonsaureamiden auf dunnschi-chtchrcmatogrammen // Experientia. - 1965. - V.21. - P.203-204.

[6] Syatkin S. P., Berezov T. T., Gridina N. Y., et al. Polyamines as biochemical markers of the antiproliferaive effect of inhibitors of enzymes for the polyamines and putrescine synthesis in the L-cells culture // The issues of medical chemistry. - 1991.-Vol. 37, №6. - P. 77-81.

[7] Fadeev V. V. Editorial materials // The issues of endocrinology – 2002. – Vol. 48, № 3. – P. 47-48.

[8] Syatkin S. P., Fedoronchuk T. V., Gridina N. Y., et al. THE IMPACT OF THE CHEMICAL ANALOGUES OF DECARBOXYLATED ORNITHINE AND S-ADENOSYLMETHIONINE ON THE RATE OF THE POLYAMINES SYNTHESIS IN THE TEST SYSTEMS FROM THE TISSUES WITH INCREASED PROLIFERATION. // The Bulletin of PFUR, series Medicine.- 2010. - № 3. PP. 9-14.

[9] T.O. Eloranta, A.R. Khomutov, R.M. Khomutov et al. Aminooxy Analogues of Spermidine as Inhibitors of Spermine Synthase and Substrates of Hepatic Polyamine Acetylating Activity **//** J. Biochem.-1990.-V.108.-P.593 - 598.

[10] DL Kramer, RM Khomutov, YV Bukin et al. Cellular characterization of a new irreversible inhibitor of S-adenosylmethionine decarboxylase and its use in determining the relative abilities of individual polyamines to sustain growth and viability of L1210 cells // Biochem J.-1989.-V.259, N2.-P.325-31.