Amine oxidase-mediated cytotoxicity of spermine and epinephrine to human myelogenous leukemia K562 cells*

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The effects of spermine and β-adrenoceptor agonists (epinephrine, terbutaline and orciprenaline) in the presence and in the absence of fetal bovine serum (FBS) on human myelogenous leukemia K562 cells viability (V) and survival (N/Nc) were examined. Spermine-FBS significantly decreased both V and N/Nc of K562 cells. Aminoguanidine (AG), an amine oxidase inhibitor, and reduced form of glutathione abolished this effect demonstrating that the spermine-FBS action was amine oxidase-mediated. Epinephrine expressed a strong cytotoxicity to K562 cells which was abolished by pargyline, a specific monoamine oxidase (MAO) inhibitor, as well as by reduced form of glutathione. Terbutaline and orciprenaline exerted no cytotoxic activity to K562 cells cultured in FBS-supplemented medium, independently on the presence of spermine. However, terbutaline at concentrations of over 1 mmol strongly inhibited the cytotoxic effect on spermine-FBS. The relationship between cytotoxicity and chemical structure of β-adrenoceptor agonists was discussed especially with respect to their stability toward oxidation.

Key words: K562 cells, leukemia, cytotoxicity, spermine, epinephrine, β-adrenoceptor agonists.

Spermine, a naturally occurring aliphatic polyamine is synthesized by all eukaryotic cells. Its intracellular concentrations appear to correlate with enhanced cell proliferation which is directly connected to its role to stabilize and protect DNA and RNA from denaturation and nuclease attack [15]. However, although indispensable for cell growth, extrinsic spermine greatly suppresses cell growth and differentiation [4], as well as proliferation of several cell lines in vitro [2, 3, 9]. These inhibitory effects were ascribed to the cytotoxic action of oxidative products (aminoaldehydes, acrolein, H_2O_2 and ammonia) converted from polyamines by specific amine oxidases present in ruminant sera [1, 6, 8] and thus in FBS frequently used as a constituent of cell culture media.

On the other hand, it has been demonstrated that β-adrenergic receptor agonists stimulate several enzymes included in polyamine metabolism, such as ornithine decarboxylase [16] and diamine oxidase [5, 13, 14].

In the present work, cytotoxic activity of varying spermine concentrations on V and N/Nc of human myelogenous leukemia K562 cells was determined in the absence and in the presence of FBS as a source of amine oxidases. At the same time, we examined whether epinephrine, terbutaline and orciprenaline, well known β-adrenoceptor agonists, acting through cAMP accumulation and influencing numerous enzymes including those regulating polyamine metabolism would affect the activity of amine oxidases and contribute to cytotoxicity of spermine in the presence of FBS.

Materials and methods

Cell culture. Human myelogenous leukemia K562 cells used throughout this work were grown as a suspension culture in RPMI 1640 medium (5×10^4 cells/ml) supplemented with *L*-glutamine (2 mmol), streptomycin and garamycin (100 μg/ml each) with or without 10% heat-inactivated FBS. The cells were maintained by twice weekly subculture at 37°C in a humidified air atmosphere containing 5% CO_2.

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Treatment of K562 cells. Spermine was applied at different concentrations upon the transfer of the cells into a fresh medium. It was dissolved in culture medium containing 10% FBS immediately before the onset of experiment and diluted with the same medium to the desired spermine concentration.

Freshly prepared solutions of β-adrenoceptor agonists were added to the cell cultures and their effects on N/Nc and V were examined 24 h (epinephrine, concentration range 4 – 180 μmol and orciprenaline, concentration range 20 – 380 μmol), or 48 h (terbutaline, concentration range 20 – 1200 μmol) later. When combined action of β-adrenergic agonists and spermine was studied, spermine (final concentration 50 μmol) was introduced into the cell cultures 30 min upon addition of epinephrine, terbutaline and orciprenaline.

Amine oxidase inhibitors AG, pargyline and reduced form of glutathione were applied at final concentration of 1 mmol at the onset of experiment, 40 min before addition of cytotoxic concentrations of spermine or epinephrine.

Counting of viable K562 cells. Cell viability was assessed by trypan blue dye exclusion test. The number of viable and dead cells was counted using Fux-Rosenthal chambers and a Carl Zeiss, Jena microscope. Viability (V) was expressed as the number of viable cells per 100 cells of either treated or control culture. Cell survival was expressed as the number of surviving cells exposed to either of the above mentioned agents or their combinations in 1.0 ml of cell suspension per number of surviving cells in 1.0 ml of control culture × 100 (N/Nc).

Cell line, medium and chemicals. K562 a human myelogenous leukemia cell line without Ph chromosome was obtained from the American Type Culture Collection (Rockville, Md, USA).

RPMI 1640 cell culture medium and FBS were Gibco products. Epinephrine (4-[1-hydroxy-2-(methylamino) ethyl]-1,2-benzenediol), terbutaline (2-*t*-butylamino-1-[3,5--dihydroxyphenyl]ethanol), orciprenaline (3,5-dihydroxy--alpha-[(isopropylamino)methyl]-benzyl alcohol) and reduced form of glutathione were purchased from Sigma Chemicals, St. Louis, Mo, USA. Pargyline (*N*-methyl--*N*-propargylbenzylamine) was produced by Aldrich Chem. Comp., Inc., Wi, USA and aminoguanidine (AG) was obtained from ICN Biomedicals Ltd., Irvine, Ca, USA.

Data analysis. For the statistical evaluation of the data Student's *t*-test was used. A *p*-value of less than 0.001 was considered statistically significant.

Results

The effects of different spermine concentrations on cell survival (N/Nc •100) and viability (V) is shown in Fig. 1 (graphs a and b, respectively). In the presence of 10% FBS, spermine led to a conspicuous decrease of both N/Nc and V.

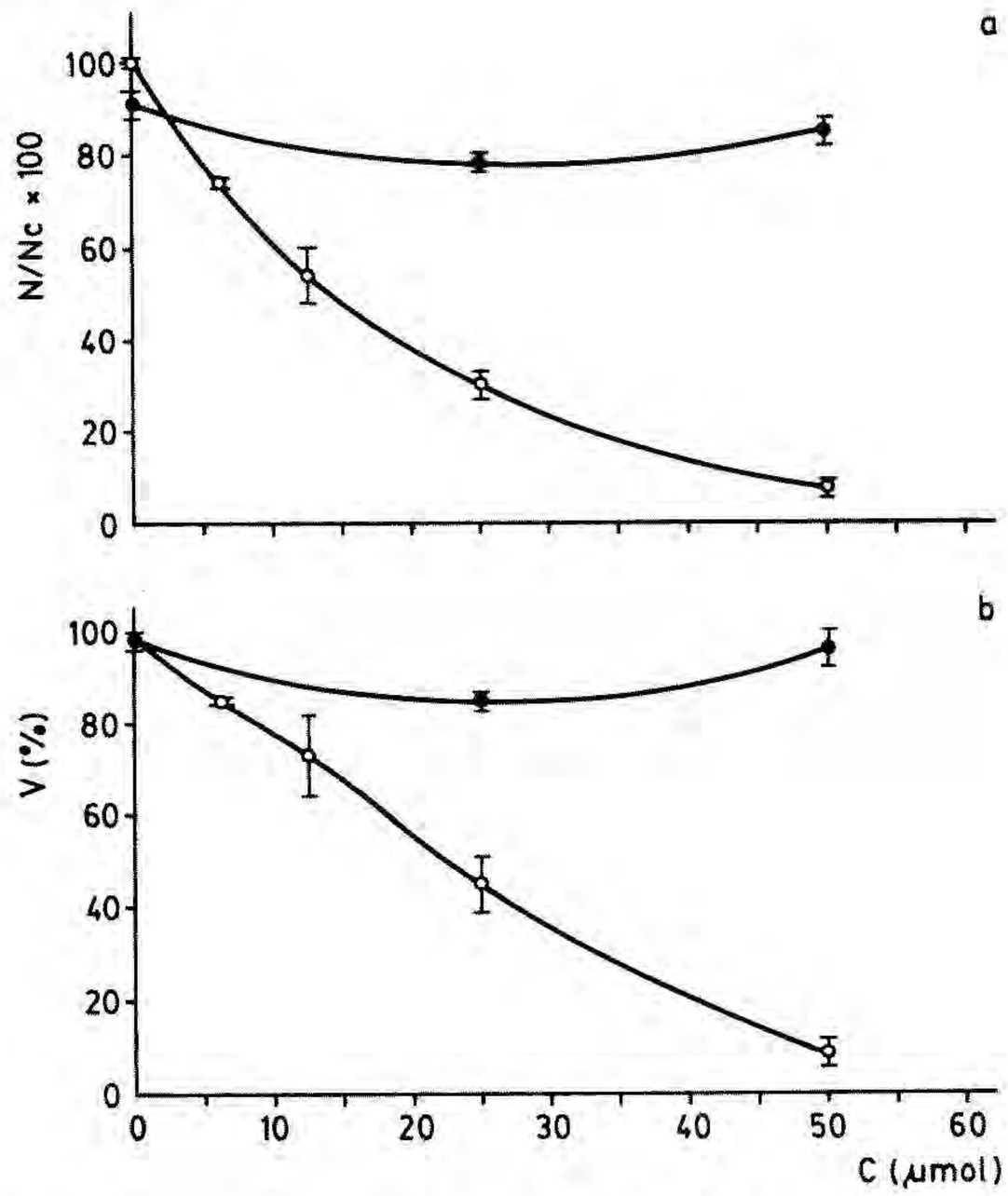

Fig. 1. Cytotoxic activity of spermine-FBS to K562 human myelogenous leukemia cells. Varying spermine concentrations were introduced into the culture upon cell transfer into a fresh FBS-supplemented RPMI 1640 medium. Cell survival (graph a, open circles) and viability (graph b, open circles) were examined 24 h later. Aminoguanidine was applied 40 min before addition of spermine (graphs a and b, full circles). Each point represents mean ± S.D. from five experiments.

The addition of AG abolished cytotoxic spermine-FBS effects, thus proving that they were mediated by amine oxidases present in FBS (Fig. 1). This was confirmed by experiments when K562 cells were transferred to FBS--free medium and treated with spermine (final concentration 50 μmol). In this case both N/Nc and V were at the control level.

The effects of different epinephrine concentrations (4 – 180 μmol) on N/Nc and V of K562 cells are presented in Fig. 2 (graphs a and b, respectively).

Epinephrine expressed a strong cytotoxicity toward K562 cells and at 180 μmol of epinephrine no surviving cells were found (Fig. 2). This effect could be ascribed to epinephrine oxidation products evolved by MAO action, since it was abolished by pargyline (1 mmol), a specific MAO inhibitor (Table 1). AG did not influence epinephrine cytotoxicity even at reduced epinephrine concentration (41 μmol) which did not significantly change N/Nc and V in comparison with the control (Table 2).

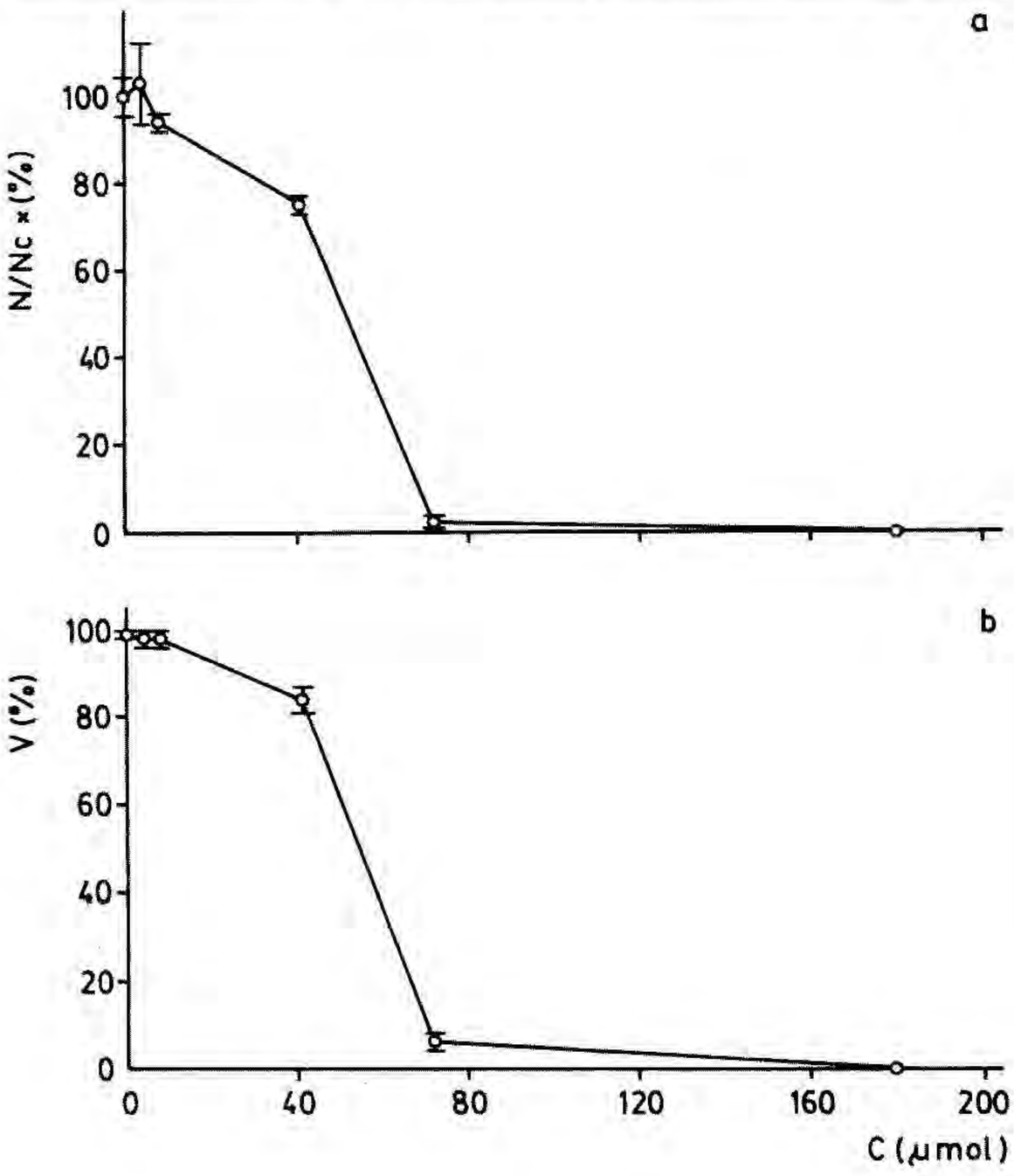

Fig. 2. Epinephrine cytotoxicity to K562 human myelogenous leukemia cells. Varying epinephrine concentrations were introduced upon the transfer of the cells into a fresh FBS-supplemented RPMI 1640 medium. Cell survival (graph a) and viability (graph b) were determined after a 24 h exposure to epinephrine. Each point represents mean ± S.D. from five experiments.

Table 1. The effect of pargyline on epinephrine cytotoxicity to K562 cells

Treatment	N/Nc . 100	V (%)
Control	100 ± 4	99 ± 1
Epinephrine	0	0
Pargyline	36 ± 4	96 ± 3
Epinephrine + pargyline	38 ± 12*	94 ± 3*

Epinephrine (final concentration 180 μmol) was introduced upon the transfer of the cells into a fresh FBS-supplemented RPMI 1640 medium. Cell survival and viability were determined 24 h later. Pargyline (final concentration 1 mmol) was applied 40 min before the addition of cytotoxic epinephrine concentration and their combined effects were examined after 24 h. The results are means ± S.D. from five experiments. *$p < 0.001$.

Varying terbutaline concentrations (20 – 1200 μmol) expressed no cytotoxic activity toward K562 cells cultured either in RPMI 1640 FBS-free or FBS-supplemented medium, independently on the presence of spermine. However, when applied at concentrations of over 1 mmol together with cytotoxic spermine level (50 μmol), terbutaline strongly inhibited spermine-FBS cytotoxicity (Table 3).

Table 2. The influence of aminoguanidine on epinephrine cytotoxicity to K562 cells

Treatment	N/Nc . 100	V (%)
Control	100 ± 4	99 ± 1
Epinephrine	75 ± 1	84 ± 3
Aminoguanidine	100 ± 3	99 ± 1
Epinephrine + aminoguanidine	77 ± 14	87 ± 6

Epinephrine (41 μmol) was introduced upon transferring the cells into a fresh FBS-supplemented RPMI 1640 medium. Cell survival and viability were determined 24 h later. Aminoguanidine (1 mmol) was added 40 min before epinephrine and their combined effects were observed after 24 h. The results are means ± S.D. from five experiments.

Table 3. Combined effect of spermine and terbutaline on survival and viability of K562 cells

Treatment	N/Nc . 100	V (%)
Control (FBS only)	100 ± 7	99 ± 1
FBS-terbutaline	102 ± 2	99 ± 1
FBS + spermine	0	0
FBS + terbutaline + spermine	45 ± 6*	95 ± 4*

FBS was applied at a final concentration of 10%. Terbutaline (final concentration 1.16 mmol) was added upon transfer of the cells into a fresh RPMI 1640 medium, or when applied with spermine (final concentration 50 μmol) 40 min before addition of polyamine. Effects on cell survival and viability were determined 48 h later. The results are means ± S.D. from five experiments. *$p < 0.001$.

Orciprenaline, although an efficient β-adrenoceptor agonist, acting through the activation of adenylate cyclase, exerted neither cytotoxic activity toward K562 cells grown in FBS-free or FBS-supplemented media, nor suppressed spermine-FBS cytotoxicity.

Reduced form of glutathione, applied as a nonspecific amine oxidase inhibitor, as well as reducing agent for spermine-evoled oxidation products, led to a decrease of N/Nc, but V was preserved and remained at the control level (Table 4).

However, when applied with spermine-FBS or epinephrine, glutathione strongly suppressed their cytotoxicity.

Discussion

Inhibition of growth and differentiation of various cells by spermine and spermidine has been observed by numerous authors and the necessity of amine oxidases in culture media has been repeatedly reported [3, 4, 9, 11, 12]. This

Table 4. The effect of reduced form of glutathione on spermine-FBS and epinephrine cytotoxicity to K562 cells

Treatment	N/Nc . 100	V (%)
Control (FBS only)	100 ± 4	99 ± 1
Spermine + FBS	0	0
Glutathione + FBS	42 ± 65	95 ± 5
Spermine + glutathione + FBS	41 ± 11*	84 ± 4*
Epinephrine + FBS	0	0
Epinephrine + glutathione + FBS	34 ± 5*	73 ± 13*

FBS was applied at a final concentration of 10%. Reduced form of glutathione (final concentration 1 mmol) was introduced upon transfer of the cells into a fresh RPMI 1640 medium 40 min before addition of spermine (50 μmol) or epinephrine (180 μmol) and the cells were examined 24 h later. * $p < 0.001$.

inhibitory effect of polyamines was ascribed to their metabolic products formed by the action of amine oxidases present in the ruminant sera [1, 6, 8]. HENLE et al. [7] reported cytotoxic effects of spermidine on CHO cells in culture in the presence of 10% FBS. PARCHMENT and PEARCE [10] reported that murine embryonic limb extracts which exerted polyamine oxidase activity expressed cytotoxic effects on melanoma cells by the above mentioned mechanism.

Our results demonstrated cytotoxic effects of spermine on human myelogenous leukemia K562 cells in the presence of 10% FBS in culture medium. These effects were amine oxidase-mediated and abolished by AG. Cytotoxic action of epinephrine on K562 cells was also observed, cytotoxic concentrations being much lower than those expressing cytotoxicity toward HeLa, L929 or RFL cells [18]. Epinephrine cytotoxicity was not influenced by AG, but it was abolished by pargyline, a specific MAO inhibitor, suggesting that the effects of epinephrine on K562 cells were MAO-mediated. Epinephrine cytotoxicity probably originates from some of its oxidation products, since both its phenolic and amino groups are known to be very susceptible to oxidation. This is supported by the data showing that reduced form of glutathione suppressed epinephrine cytotoxicity.These results could be connected to the data of YAMADA et al. [18, 19] who studied the response of several mammalian cell lines to epinephrine and its binding to chromatin components.

Terbutaline, a strong β-adrenoceptor agonist and a potent amine oxidase inducer, exerted no cytotoxicity to K562 cells, while orciprenaline, another β-adrenergic receptor agonist, neither expressed cytotoxic effects on K562 cells, nor inhibited spermine-FBS cytotoxicity. The different action of the three β-adrenoceptor agonists on K562 cells viability and susceptibility toward amine oxidase action could be explained in terms of their different chemical structure. The epinephrine molecule with a readily oxidizing catechol -OH group and -NH_2 group susceptible to oxidative deamination represents a suitable substrate to both amine oxidases and nonspecific oxidases. Our results obtained with epinephrine are in accordance with the data of WICK [17] who observed a suppression of malignant B-16 melanoma in vivo upon application of dopamine, another catecholamine, extremely susceptible to oxidation. Terbutaline and orciprenaline, agents lacking catecholamine moiety and much more resistant to enzyme-mediated or unspecific oxidation, did not express cytotoxic activity to K562 cells. Terbutaline, applied at concentrations of over 1 mmol strongly inhibited spermine-FBS cytotoxicity, but at present we have no appropriate explanation for this phenomenon. It could be only hypothesized that terbutaline acted as an inducer of compounds more susceptible to oxidase action than spermine and whose oxidation products are either inert or less cytotoxic than those evolved from spermine. Another possibility could be that terbutaline induced the synthesis of strong reducing agents, thus influencing cytotoxicity of spermine oxidation products.

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