To the mechanism of spermine-FBS cytotoxicity toward K562 human myelogenous leukemia cells

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The effects of several compounds acting through adenylate cyclase system and/or influencing prostaglandin biosynthesis on spermine-FBS cytotoxicity to human myelogenous leukemia K562 cells were studied.

Salbutamol, a β_2-adrenoceptor agonist inhibited to a certain extent spermine-FBS cytotoxic action to K562 cells, and propranolol, a β_2-adrenoceptor antagonist, did not affect this inhibition. Aminophylline, an inhibitor of cyclic nucleotide phosphodiesterase, acted suppressing spermine-FBS cytotoxicity to K562 cells. Pretreatment of the cells with dexamethasone did not significantly alter salbutamol-related inhibition of spermine-FBS cytotoxicity. Indomethacin, an inhibitor of cyclooxygenases directly involved in prostaglandin biosynthesis, did not interfere with protective terbutaline effects against spermine-FBS cytotoxicity to K562 cells during the 24-hour period.

Key words: K562 cells, leukemia, cytotoxicity, spermine, salbutamol, indomethacin, prostaglandins.

Although indispensable for normal cell functioning [7, 21, 22], exogenously added polyamines spermine and spermidine strongly inhibit cell growth and differentiation, as well as proliferation of several tumor cell lines, and immunity parameters in vitro in the presence of ruminant sera [4, 6, 11, 15]. Since these inhibitory effects disappear under ruminant sera-free conditions, it was generally accepted that most of them result from cytotoxic action of oxidative metabolites evolved from polyamines by Cu^{2+} – dependent amine oxidases present in ruminant sera [1, 2]. However, the inhibitory effects of polyamines on immune response in the absence of ruminant sera were also observed and demonstrated to depend on intrinsic polyamine oxidase activity, though in this case higher polyamine concentrations are necessary than when ruminant sera were present [11, 20].

On the other hand, it was shown that several adrenoceptor agonists and antagonists acting through adenylate cyclase system, but also expressing numerous side-effects, affect lymphocyte proliferation after stimulation, secretion of lymphokines [10, 17, 18], as well as different enzyme activities involved in biosynthesis and metabolism of polyamines [5, 8, 9, 23] and prostaglandins [19]. In addition, our recent results demonstrated that terbutaline, a potent β_2-adrenoceptor agonist, strongly inhibits spermine-fetal bovine serum (FBS) cytotoxicity to human myelogenous leukemia K562 cells [13]. All these data together with the observation that inhibitors of glutathione synthesis intensify cytotoxic polyamine-FBS effects [12], while reduced form of glutathione conspicuously attenuates them [13], suggest that the mechanism of spermine-FBS suppression of normal cell growth and differentiation is far more complex and that numerous molecules unrelated to polyamine oxidative products may influence in different ways the action of polyamines. It was recently shown that treatment of rat renal mesangial cells with agents activating adenylate cyclase, including salbutamol, led to an increase of phospholipase A_2 activity, an enzyme initiating arachidonic acid release from membrane phospholipids, directly related to prostaglandin synthesis [19]. This prompted us to examine the effects of the following compounds on spermine-FBS cytotoxicity to human myelogenous leukemia K562 cells: salbutamol (a β_2-adrenoceptor agonist), propranolol (a β_2-adrenoceptor antagonist), aminophylline (an inhibitor of cyclic nucleotide phosphodiesterase), dexamethasone (an in-

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hibitor of phospholipase A_2 activity) and indomethacin (an inhibitor of cyclooxygenases and thus of prostaglandin biosynthesis).

Material and methods

Cell culture. Human myelogenous leukemia K562 cells were grown as a suspension culture in RPMI 1640 medium (1–10 × 10^4 cell ml^{-1}) supplemented with *L*-glutamine (2 mmol), streptomycin and garamycin (100 μg/ml, each) and 10% heat-inactivated FBS. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air by twice weekly subculture.

Treatment of K562 cells. Spermine was employed in the cultures upon the transfer of the cells into fresh medium. It was dissolved in culture medium containing 10% FBS immediately before the start of experiments and diluted with the same medium to the desired concentration.

Freshly prepared solution of salbutamol (1 mmol) or aminophylline (1 mmol) was added to the cell cultures. Their effects on survival (N/Nc) and viability (V) were examined 24 h later. When combined action of either of these two compounds and spermine was studied, spermine was introduced into the cell cultures 40 min upon addition of salbutamol or aminophylline.

Dexamethasone (258 μmol) or indomethacin (35 μmol) were added 10 min prior to salbutamol, i.e. 50 min before spermine.

Counting of viable K562 cells. Cell viability was assessed by trypan blue dye exclusion test. Number of viable and dead cells was counted using Fuchs-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 survived cells exposed either to individual aforementioned agents or their combinations in 1.0 ml of cell suspension per number of survived cells in 1.0 ml of control culture × 100 (N/Nc).

Cell line, medium and chemicals. K562 human myelogenous leukemia cell line without Ph chromosome originated from American Type Culture Collection (Rockville, MD, USA).

RPMI 1640 cell culture medium and FBS were GIBCO (Paisly, Scotland, U.K.) products. Salbutamol (α[*t*-butylamino)methyl]-Δ-hydroxy-*m*-xylene-α,α'-diol), terbutaline (2-*t*-butylamino-1-[3,5-dihydroxyphenyl]ethanol) and propranolol (1-[isopropylamino]-3-[1-naphtyloxy]-2-propanol) were supplied by Sigma Chemicals (St. Louis, MO, USA). Aminophylline ([theophylline]$_2$-ethylenediamine) and dexamethasone (9α-fluoro-16α-methyl-prednisolone phosphate sodium salt) were obtained from ICN Biomedicals Ltd. (Irvine, CA, USA). Indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid) was purchased from Lek Pharmaceutical Works (Ljubljana, Slovenia). Spermine tetrachloride was a Calbiochem (San Diego, CA, USA) product.

Data analysis. For statistical evaluation of the data Student's *t*-test was used. A p value of less than 0.05 was considered statistically significant. Values obtained from samples pretreated with the agent(s) and spermine were compared with the values determined from the corresponding samples devoid of specific agent.

Results

The data on the effect of salbutamol on spermine-FBS cytotoxicity to K562 human myelogenous leukemia cells are listed in Table 1.

Table 1. The effect of salbutamol on cytotoxic spermine-FBS action to K562 human myelogenous leukemia cells

Treatment	N/Nc (%)	V (%)
Control	100 ± 9	99 ± 1
FBS + salbutamol	65 ± 15	99 ± 1
FBS + spermine	0	0
FBS + salbutamol + spermine	32 ± 16*	89 ± 2*

Salbutamol (final concentration 1 mmol) was added upon the transfer of the cells into fresh RPMI 1640 medium supplemented with 10% FBS, or when applied with spermine (final concentration 50 μmol) 40 min before the addition of the polyamine. Effects on cell survival (N/Nc) and viability (V) were determined 24 h later. The results are means ± S.D. of five replicates. * $p < 0.05$.

As seen, salbutamol inhibited somewhat spermine-FBS cytotoxic action to K562 cells.

Propranolol did not influence salbutamol-related suppression of cytotoxic spermine-FBS action either with regard to N/Nc or V value (Table 2). These experiments were performed with moderately cytotoxic spermine concentration in order to get a better insight into possible minute effects of propranolol on salbutamol inhibition of spermine-FBS cytotoxicity to K562 cells.

The effects of aminophylline on spermine-FBS suppression of both K562 cells survival and viability are summarized in Table 3. As shown, aminophylline acted as an inhibitor of spermine-FBS cytotoxicity.

Salbutamol-related suppression of cytotoxic spermine-FBS activity was not significantly altered by pretreatment of K562 cells with dexamethasone (Table 4).

Indomethacin did not influence protective terbutaline effect against spermine-FBS cytotoxicity to K562 cells during the 24-hour period (Table 5).

T a b l e 2. The effect of β_2-adrenoceptor agonist propranolol on salbutamol-induced suppression of spermine-FBS cytotoxicity to K562 cells

Treatment	N/Nc (%)	V (%)
Control	100 ± 12	99 ± 1
FBS + spermine	35 ± 4	67 ± 4
FBS + propranolol	88 ± 8	98 ± 2
FBS + propranolol + salbutamol + spermine	50 ± 11	93 ± 3
FBS + salbutamol + spermine	53 ± 5	94 ± 1
FBS + propranolol + salbutamol	70 ± 2	98 ± 2

Propranolol (final concentration 50 μmol), followed upon 10 min by salbutamol (final concentration 1 mmol), was added after the transfer of the cells into fresh RPMI 1640 medium supplemented with 10% FBS, or when applied with spermine (final concentration 20 μmol) 40 min before polyamine addition. Effects on cell survival (N/Nc) and viability (V) were observed 24 h later. The results are means ± S.D. of five replicates.

T a b l e 3. The influence of aminophylline on spermine-FBS cytotoxicity to K562 cells

Treatment	N/Nc (%)	V (%)
Control	100 ± 7	99 ± 1
FBS + aminophylline	64 ± 17	98 ± 1
FBS + spermine	17 ± 1	35 ± 17
FBS + aminophylline + spermine	59 ± 9*	99 ± 1*

Aminophylline (final concentration 1 mmol) was added upon the transfer of the cells into fresh RPMI 1640 medium containing 10% FBS, or when applied with spermine (final concentration 30 μmol) 40 min before the polyamine. Effects on cell survival (N/Nc) and viability (V) were determined 24 h later. The results represent means ± S.D. of four replicates. * $p < 0.05$.

T a b l e 4. Combined action of dexamethasone and salbutamol on spermine-FBS cytotoxicity to K562 cells

Treatment	N/Nc (%)	V (%)
Control	100 ± 10	99 ± 1
FBS + spermine	0	0
FBS + dexamethasone	93 ± 15	99 ± 1
FBS + dexamethasone + spermine	0	0
FBS + dexamethasone + salbutamol	53 ± 15	98 ± 2
FBS + salbutamol + spermine	57 ± 12	95 ± 4
FBS + dexamethasone + salbutamol + + spermine	45 ± 10	89 ± 7

Dexamethasone (final concentration 258 μmol), followed 10 min later by salbutamol (final concentration 1 mmol), was added upon the transfer of the cells into fresh RPMI 1640 medium supplemented with 10% FBS, or when applied with spermine (final concentration 50 μmol) 40 min before addition of the polyamine. Effects on cell survival (N/Nc) and

T a b l e 5. Combined action of indomethacin and terbutaline on spermine-FBS cytotoxicity to K562 cells

Treatment	N/Nc (%)	V (%)
Control	100 ± 20	98 ± 2
FBS + spermine	0	0
FBS + indomethacin	80 ± 27	100 ± 0
FBS + indomethacin + spermine	0	0
FBS + terbutaline	83 ± 12	99 ± 1
FBS + terbutaline + spermine	90 ± 23	96 ± 2
FBS + indomethacin + terbutaline + + spermine	82 ± 24	99 ± 1

Indomethacin (final concentration 35 μmol), followed 10 min later by terbutaline (final concentration 1 mmol), was added upon the transfer of the cells into fresh RPMI 1640 medium supplemented with 10% FBS, or when applied with spermine (final concentration 50 μmol) 40 min before addition of the polyamine. Effects on cell survival (N/Nc) and viability (V) were determined 24 h later. The results are means ± S.D. of three replicates.

However, dexamethasone and indomethacin pretreatment in the presence of salbutamol or terbutaline, respectively, and of spermine led to morphological changes seen as abortive mitoses in some 5–10% of the cells. In experiments performed with aminophylline and spermine, 4–6% of the cells showed morphological changes seen as appearance of droplet-shaped cells frequently transformed into the cells with cytoplasmatic protrusion(s) (data not shown). The number of such morphologically altered cells was increasing with time: It was higher 48 h than 24 h after the treatment.

Discussion

Enhanced levels of polyamines were recorded in different tissues including rapidly growing embryonic and malignant ones. Their amine oxidase-mediated cytotoxic action to different cell lines, e.g. fibroblasts [3], Chinese hamster ovary cells [12], human peripheral blood mononuclear cells, etc., has been repeatedly demonstrated. It is believed that lack of immunological control in malignant diseases is based on the suppression of immune response through an inhibition of lymphocyte function by oxidative products evolved from polyamines by amine oxidases action [1, 2].

The data obtained throughout the present study demonstrate that the agents acting as intracellular cAMP inducers (salbutamol and terbutaline, β_2-adrenoceptor agonists and aminophylline, an inhibitor of cyclic nucleotide phosphodiesterase activity) suppressed to a certain extent spermine-FBS cytotoxicity to human myelo-

genous leukemia K562 cells. This is in accordance with our previous observations concerning a rather strong terbutaline-related inhibition of spermine-FBS cytotoxic action to this cell line [13]. However, it seems that salbutamol-induced inhibition of spermine-FBS cytotoxicity is not directly connected to its interaction with β_2-adrenergic receptors and the resulting events, since propranolol, a strong β_2-adrenoceptor antagonist in a concentration applied did not affect the extent of salbutamol-related suppression of spermine-FBS action on K562 cells survival and viability. Similarly, dexamethasone (a synthetic glucocorticoid with numerous effects including the inhibition of phospholipase A_2, an enzyme initiating arachidonic acid release from cell membranes and enabling prostaglandin synthesis) did not influence salbutamol-induced suppression of spermine-FBS cytotoxicity to K562 cells. The same holds true for indomethacin, an inhibitor of cyclooxygenases, directly involved in prostaglandin biosynthesis.

However, in cell cultures pretreated with dexamethasone or indomethacin, and exposed to salbutamol or terbutaline prior to addition of spermine, 5–8% of the cells expressed morphological changes seen as abortive mitoses identical to those observed in murine leukemia cells treated with Vinca alkaloids [17]. These results suggest that prostaglandins act protecting K562 cells against harmful effects of spermine-FBS, as well as against morphological transformations.

Aminophylline, acting as an inhibitor of cyclic nucleotide phosphodiesterase [14] and tyrosinase stimulator [16] also suppressed cytotoxic action of spermine-FBS to K562 cells. It is worth mentioning that in aminophylline-spermine-treated cultures some 4–6% cells showed morphological changes seen as appearance of droplet-shaped forms frequently with a cytoplasmatic extension.

Morphological changes seen either as abortive mitoses or droplet-shaped forms could also result from alterations in the cell membrane permeability, i.e. in the cell membrane polarization.

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