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TERBUTALINE ABOLISHES SPERMINE-FBS SUPPRESSION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL VIABILITY AND MITOGENIC RESPONSIVENESS

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Summary: The effects of spermine and terbutaline on viability and mitogenic response of phytohaemagglutinin stimulated human peripheral blood mononuclear cells (PBMC) cultured in fetal bovine serum-supplemented RPMI 1640 medium were studied.

Spermine in the presence of fetal bovine serum (FBS) expressed cytotoxic activity toward human PBMC seen as a decrease of both cell viability and mitogenic responsiveness to phytohaemagglutinin (PHA) stimulation. This effect was significantly diminished by aminoguanidine (AG) thus demonstrating that metabolites converted from spermine through the action of amine oxidases present in FBS acted as cytotoxic agents.

Terbutaline, a potent β_2-adrenoceptor agonist and an inducer of diamine oxidases did not potentiate, but led to suppression of spermine-FBS cytotoxicity toward PBMC which was expressed to a similar extent as in the case of AG application, either with regard or to cell viability or mitogenic responsiveness to PHA-induced stimulation of PBMC.

Key words: terbutaline, spermine, human PBMC, FBS, amine oxidases.

Introduction

During the past decade the versatile polyamine role in cell viability and proliferation, dependent on the ratio of intra- and extracellular polyamine levels has been repeatedly demonstrated. Several authors suggested a certain relationship to exist between macromolecules involved in lymphocyte activation and molecules included in the biosynthetic pathways of polyamines, such as ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine synthesis. The elevation of ODC level 18 h after inception of mixed lymphocyte culture was found to be an early indicator of histocompatibility (1). Also, an increased rate of ODC synthesis in PHA-activated lymphocytes was observed (2), and recently, a marked increase in ODC mRNA expression upon IL-2 interaction with the corresponding lymphocyte receptors was reported (3). It has been also shown that the tissues known to fail in eliciting immunological response, such as fetal and neoplastic ones, contain high polyamine levels, and that the DNA synthetic response of stimulated murine spleen cells in the presence of fetal bovine serum (FBS) was inhibited by micromolar concentrations of spermine (4).

Spermine inhibition of mixed lymphocyte response to PHA stimulation and the induction, but not the expression of cytotoxic response, were observed in FBS-supplemented culture media. This suppression of immunological response *in vitro* was ascribed to the cytotoxic action of oxidative products (aminoaldehydes, acrolein, H_2O_2 and ammonia) converted from polyamines by copper-dependent amine oxidases present in ruminant sera (5-7) and thus, in FBS frequently used as a constituent of cell culture media.

The aim of the present work was to examine amine oxidase-mediated spermine cytotoxicity toward human PBMC and its effect on PHA-stimulated proliferation of these cells upon pretreatment with aminoguanidine (AG) a specific Cu^{2+} dependent amine oxidase inhibitor, or with the β_2-adrenoceptor agonist terbutaline acting through cAMP accumulation, known as a potent inducer of diamine oxidases (8).

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Experimental procedures

Culture medium and chemicals. RPMI 1640 culture medium and FBS were GIBCO, Paisly, Scotland, U.K. products; Lymphoprep™ was purchased from Nycomed (Oslo, Norway) and Heamaccel® from Jugoremedija (Zrenjanin, Yugoslavia). PHA was supplied by Wellcome Diagnostics (Dartford, England), spermine by Calbiochem Corp. (San Diego, CA, U.S.A.), terbutaline (2-*t*-butylamino-1-[3,5-dihydroxyphenylethanol) by Sigma Chemicals (St Louis, MO U.S.A.) and aminoguanidine by ICN Biochemicals Ltd. (Irvine, CA, U.S.A.).

Blood donors. Heparinized venous blood was obtained from healthy volunteers, age range 20-50 years.

Cell separation. Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood by Lymphoprep™ gradient centrifugation. Interface cells washed three times with Haemaccel^{R} aqueous solution (containing 145 mmol/L Na^+, 5.1 mmol/L K^+, 6.25 mmol/L Ca^{2+}, 145 mmol/L Cl^- and disintegrated gelatine polymers 35.0 g dm^{-3}), pH 7.4 were resuspended in RPMI 1640 medium with 25 mmol/L HEPES buffer, 2 mmol/L L-glutamine, 10% FBS, and 100 g of each garamycin and streptomycin to the density of $4 \cdot 10^6$ cells/mL medium.

Cell culture. PBMC were seeded in triplicate in 24 flat bottom wells (Costar) in the above medium ($53 \cdot 10^4$ cells/mL, *i.e.* $8 \cdot 10^5$ cells per well) with or without 1% PHA used to stimulate cell proliferation. The cultures were maintained at 37°C in 5% CO_2 humidified air atmosphere.

Cell treatment. Both PHA-stimulated and non-stimulated PBMC were treated with AG or terbutaline (1 mmol, each) for 40 min and then freshly prepared spermine solution was added to a final concentration of 50 μmol. Equivalent volumes of culture medium were added to the controls.

Counting of viable PBMC. Cell viability was assessed by trypan blue exclusion method 72 h upon inception of cell culture and exposure of the cells to above treatments. Number of viable and dead cells was counted using haemocytometer and viability was expressed as number of viable per 100 cells (V%).

Determination of PBMC mitogenic response to PHA-stimulation. Lymphocyte mitogenic response to stimulation with 1% PHA was determined 96 h after inception of the cell culture as previously described (9). The mitotic index was estimated by light microscopy using immersion oil upon Giemsa staining and was expressed as the number of mitoses per 1000 of total cells (TLT‰).

Data analysis. For the statistical evaluation of the data one-tailed Student's test was used. The differences were considered statistically significant when p was less than 0.02.

Results and discussion

The effect of aminoguanidine on FBS-spermine action to PBMC viability and mitogenic response. The data from experiments aimed to determine the effects of AG on FBS-spermine action to both the viability and the mitotic index of PHA-stimulated and control PBMC are summarized in Table I.

Table I: Effect of aminoguanidine on the cytotoxic action of FBS-spermine to human peripheral blood mononuclear cells.

Treatment	Viability (V%)	Mitotic index (TLT‰)
FBS	94±1	0
FBS+spermine	45±5	0
FBS+AG	91±1	0
FBS+AG+spermine	93±2*	0
FBS+PHA	74±11	56±12
FBS+PHA+spermine	33±11	0
FBS+PHA+AG	72±6	55±15
FBS+PHA+AG+spermine	72±11**	53±19**

Cells were cultured in FBS-supplemented RPMI 1640 medium. Stimulation to proliferation was achieved with 1% PHA. Aminoguanidine (1 mmol) was added to cell cultures 40 min before spermine (final concentration 50 μmol). Viability and mitotic index were determined 72 and 96 h later, respectively. The data are means ± SD obtained with monocytes isolated from peripheral venous blood of three healthy donors. Each sample was analyzed in triplicate. * $p < 0.001$; ** $p < 0.02$, in comparison with the corresponding value without inhibitor.

As seen, PBMC viability was twice reduced by FBS-spermine in comparison with the control cells grown in FBS-supplemented RPMI 1640 medium under spermine-free conditions. AG did not affect cell viability in the absence of spermine, but almost completely suppressed inhibitory effect of FBS-spermine. PHA slightly decreased cell viability and in the presence of FBS-spermine this value was significantly reduced comparing to the corresponding control, while mitotic index was zero. AG did not influence either cell viability or the mitotic index when added to PHA-stimulated cultures under spermine-free conditions, but strongly suppressed FBS-spermine cytotoxicity of PHA-stimulated PBMC cultures.

Suppression of FBS-spermine cytotoxicity to PBMC by terbutaline. Contrary to what expected, terbutaline, a potent β_2-adrenoceptor agonist and an inducer of diamine oxidases, expressed an inhibitory effect on FBS-spermine action with regard to both

PBMC viability and PHA-induced proliferation, as shown in Table II.

Table II: The effect of terbutaline on FBS-spermine cytotoxic action to human peripheral blood mononuclear cells.

Treatment	Viability (V%)	Mitotic index (TLT‰)
FBS	95±2	0
FBS+spermine	39±9	0
FBS+terbutaline	93±2	0
FBS+terbutaline+spermine	91±5*	0
FBS+PHA	76±12	61±18
FBS+PHA+spermine	32±10	0
FBS+PHA+terbutaline	73±12	58±12
FBS+PHA+terbutaline+spermine	68±8*	56±11**

For the details see legend to Table I. Instead of AG, the cells were pretreated with 1 mmol terbutaline for 40 min prior to addition of spermine. The data represent means ± SD of three healthy donors. Each sample was analyzed in triplicate. * $p < 0.02$; ** $p < 0.001$ in comparison with the corresponding value without terbutaline.

Terbutaline pretreatment of cells growing in FBS-supplemented medium did not affect cell viability. However, it led to the abolishment of FBS-spermine cytotoxicity toward the non-stimulated PBMC and the viability was at the control level. This agent also suppressed inhibitory action of FBS-spermine on mitogenic responsiveness of PHA-stimulated PBMC. It should be mentioned also that PBMC viability in PHA-stimulated cultures was somewhat lower than that of the non-stimulated cells, probably due to individual variations between blood donors.

The significance of polyamines for both cell growth and metabolic processes has been well recognized (10-12). The major role of common polyamines spermine and spermidine found in all animal cells is to stabilize and protect nucleic acids from denaturation and nuclease attack (11). They are essential for cell growth and inhibitors of ODC greatly suppress DNA synthesis (13). Paradoxically, extrinsic spermine and spermidine strongly inhibit cell growth and differentiation, as well as proliferation of several tumor cell lines *in vitro* (4,14,15). All these inhibitory effects disappear in cultures under ruminant sera-free conditions and most of them are considered to result from cytotoxic action of oxidative products converted from polyamines by Cu^{2+}-dependent amineoxidases present in ruminant sera (5,16).

However, the inhibitory effects of polyamines on immune response in the absence of ruminant sera were also reported and shown to depend on an intrinsic polyamine oxidase activity of lymphocytes, though in this case higher polyamine levels are necessary than when ruminant sera were present (7,17). Inhibition of bovine serum amine oxidases by AG and the resulting suppression of polyamine cytotoxicity have been observed long ago (18,19). Our results on the inhibitory effects of spermine on both PBMC viability and mitogenic response to PHA stimulation in FBS-supplemented medium were expected connected to the previously attained knowledge and numerous data of other authors.

On the other hand, during the past several years an increasing body of evidence has accumulated, demonstrating the effect of adrenergic receptor agonists and antagonists acting through adenylate cyclase system upon the interaction with specific membrane receptors, on lymphokines (20,21), as well as on different enzyme activities, including those involved in biosynthesis and metabolism of polyamines (22-24). The stimulatory capacity of adrenergic agonists and inhibitory action of the corresponding antagonists support the view on specific influence of catecholamines and adrenergic drugs on the lymphocyte function. So, it has been found that terbutaline, a potent β_2-adrenoceptor agonist acts as a strong inducer of renal diamine oxidase (8), the rate-limiting enzyme in the oxidative deamination of putrescine, a critical regulatory molecule for cell growth and precursor of higher amines (25-27). When designing the experiments described in the present paper, we expected terbutaline to add to spermine cytotoxicity through the products evolved from spermine by the action of terbutaline-induced diamine oxidases. Paradoxically, a suppression of cytotoxic FBS-spermine effects on PBMC viability and mitogenic responsiveness to PHA stimulation was observed. These results could be connected to data of Brunton et al. (28), who found that retroconversion pathway converting spermine and spermidine into their precursor putrescine expressed a cytoprotective action to baby-hamster kidney (BHK) cells. It could be also speculated that terbutaline acted as an inducer of compounds more susceptible to diamine oxidase action than spermine and that the resulting oxidation products were either inert or less cytotoxic than those evolved from spermine oxidation. Another possibility would be that terbutaline acted as an inducer of strong reducing agents, thus influencing cytotoxicity of spermine oxidation products converted from the polyamine by the action of copper-dependent amine oxidases present in the FBS.

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TERBUTALIN UKLANJA SUPRESIJU VIJABILNOSTI I MITOGENOG ODGOVORA MONONUKLEARNIH-ĆELIJA PERIFERNE KRVI LJUDI INDUKOVANU SPERMINOM U PRISUSTVU FBS

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Kratak sadržaj: U ovom radu ispitivani su efekti spermina i terbutalina na vijabilnost i na fitohemaglutinonom stimulisan mitogeni odgovor mononuklearnih ćelija periferne krvi ljudi (MĆPK) koje su uzgajane u RPMI 1640 hranljivom medijumu kome je dodat fetalni goveđi serum.

Spermin u prisustvu fetalnog goveđeg seruma (FBS) ispoljava citotoksičnu aktivnost prema MĆPK ljudi, što se vidi kao smanjenje kako vijabilnosti tako i mitogenog odgovora ispitivanih ćelija na stimulaciju fitohemaglutininom (PHA). Ovaj efekat bio je značajno umanjen aminoguanidinom (AG) što ukazuje da metaboliti spermina, nastali pod dejstvom aminooksidaza prisutnih u FBS, deluju kao citotoksični agensi.

Terbutalin (1 mmol), izrazit agonist β_2-adrenergičnih receptora i induktor diaminooksidaza ne potencira, već vodi do potiskivanja spermin-FBS citotoksičnosti prema MĆPK koja se ispoljava u sličnoj meri kao i u slučaju primene AG, kako u pogledu vijabilnosti ćelija tako i mitogenog odgovora na PHA-indukovanu stimulaciju MĆPK.

Ključne reči: Terbutalin, spermin, MĆPK ljudi, FBS, aminooksidaze

References

1. *Demetriou, A.A.; Tabor, C.W.; Tabor, H.:* Ornithine decarboxylase assay permitting early determination of histocompatibility in the mixed lymphocyte reaction; in: Collowick SP, Kaplan NO (Eds): Methods in Enzymol. New York, Academic Press, 1983, vol 94, pp.396-398.
2. *Korpela, H.; Hölttä, E.; Hovi, T.; Jänne, J.:* Response of enzymes involved in the metabolism of polyamines to phytohaemagglutinin-induced activation of human lymphocytes. Biochem. J. 196:733-738, 1981.
3. *Otani, S.; Matsui-Yuasa, I.; Yukioka, K.; Goto, H.; Morisawa, S.:* Regulation of the activity and expression of ornithine decarboxylase in lymphocytes; in Goldenberg SH, Algranati JD (Eds): Biology and Chemistry of Polyamines. Oxford, ISICU Press, Oxford University Press, 1989, pp 39-48.
4. *Bird, W.J.; Jacobs, D.M.; Amoss, M.S.:* Synthetic polyamines added to cultures containing bovine sera reversibly inhibit in vitro parameters of immunity. Nature 267:621-623, 1977.
5. *Alarcon, R.A.:* Acrolein, IV. Evidence for the formation of cytotoxic aldehyde acrolein from enzymatically oxidized spermine and spermidine. Arch. Biochem. Biophys. 137:365-372, 1970.
6. *Mondovi, B.; Riccio, P.; Agostinelli, E.:* The biological function of amine oxidases and their reaction products. An overview. Adv. Exp. Med. Biol. 250:147, 1989.
7. *Flescher, E.; Fossum, D.; Talal, N.:* Polyamine-dependent production of lymphotoxic levels of ammonia by human peripheral blood monocytes. Immunol. Lett. 28:85-90, 1991.
8. *Desiderio, M.A.; Sessa, A.:* Regulation of diamine oxidase expression by β_2-adrenoceptors in normal and hypertrophic rat kidney. Biochim. Biophys. Acta 845:463-468, 1985.
9. *Spužić, I.; Brankovan, V.; Garzičić, B.:* Modification du test de transormation des lymphocytes. Excerpta. Med. 24:235, 1971.
10. *Cohen, S.S.:* Introduction to the Polyamines. Englewood Cliffs, Prentice Hall, 1971.
11. *Tabor, C.W.; Tabor, H.:* Poluamines. Ann. Rev. Biochem. 53:749, 1984.
12. *Tabor, C.W.; Tabor, H.:* Polyamines in microorganisms. Microbiol. Rev. 49:81-99, 1985.
13. *Pegg, A.E.; McCann, P.P.:* Polyamine metabolism and function. Am. J. Physiol. 143:C212-C221, 1982.
14. *Allen, J.C.; Smith, C.J.; Hussain, J.I.; Thomas, J.M.; Gaugas, J.M.:* Inhibition of lymphocyte proliferation by polyamines requires ruminant plasma polyamine oxidase. Eur. J. Biochem. 102:153-158, 1979.
15. *Morgan, D.M.L.; Illei, G.:* Polyamine-polyamine oxidase interaction: Part of maternal protective mechanism against fetal rejection. Br. Med. J. 280:1295-1297, 1980.
16. *Allen, R.D.; Roberts, T.V.:* Role of spermine in the cytotoxic effects of seminal plasma. Am. J. Reprod. Immunol. Microbiol. 13:4-8, 1987.
17. *Smith, C.J.; Maschler, R.; Maurer, H.R.; Allen, J.C.:* Inhibition of cells in culture by polyamines does not depend on the presence of ruminant serum. Cell. Tissue. Kinet. 16:269-276, 1983.
18. Schuler, W.: Zur Hemmung der Diaminoöydase (Histaminase). Experientia 8:230, 1952.
19. *Gabl, W.A.; Pitot, H.C.:* Reversal by aminoguanidine of the inhibition of proliferation of human fibroblasts by spermidine and spermine. Chem. Biol. Interact. 22:91-98, 1978.

20. *Feldman, R.D.; Hunninghake, G.W.; McArdle, W.L.:* ß-Adrenergic receptor-mediated suppression of interleukin-2 receptors in human lymphocytes. J. Immunol. 139:3355-3559, 1987.

21. *Paeglow, I.; Werner, H.:* Influence of adrenergic agonists and antagonists on lymphokyne secretion in vitro. Int. J. Immunopharmacol. 9:761-768, 1987.

22. *Veldhuis, J.D.; Harrison, T.S.; Hammond, J.M.:* (1980). ß-2--adrenergic stimulation of ornithine decarboxylase activity in porcine granulosa cells in vitro. Biochim. Biophys. Acta. 627:123, 1985.

23. *Copeland, J.G.; Larson, D.F.; Roeske, W.R.; Russel, D.H.; Womble, J.R.:* β_2-Adrenoceptors regulate induction of myocardial ornithine decarboxylase in mice in vivo. Br. J. Pharmacol. 75:479-483, 1982.

24. *Bartolome, J.; Huguengard, J.; Slotkin, T.A.:* Role of ornithine decarboxylase in cardiac growth and hypertrophy. Science 210:793-794, 1980.

25. *Missala, K.; Sourkes, T.L.:* Putrescine catabolism in rats given heparin or aminoguanidine. Eur. J. Pharmacol. 64:307-311, 1980.

26. *Pegg, A.E.; Matsui, I.; Seely, J.E.; Pritchard, M.L.; Pösö, H.:* Formation of putrescine in rat liver. Med. Biol. 59:327-333, 1981.

27. *Jänne, J.; Pösö, H.; Raina, A.:* Polyamines in rapid growth and cancer. Biochim. Biophys. Acta 473:241-293, 1978.

28. *Brunton, V.G.; Grant, M.H.; Wallace, H.M.:* Mechanism of spermine toxicity in baby-hamster kidney (BHK) cells. Biochem. J. 280:183-198, 1991.