UDK 577.1:61

YU ISSN 0352-1311

Jugoslov. Med. Biohem. 12: 15 – 18, 1993

Originalni naučni rad
Original scientific paper

MODULATION OF EPINEPHRINE CYTOTOXICITY TO HUMAN MYELOGENOUS LEUKEMIA K562 CELLS BY INDOMETHACIN, AMINOPHYLLINE AND ASCORBIC ACID

Z. Juranić[1], J. Joksimović[2], I. Spužić[1] and I. Juranić[3]*

[1]Institute of Oncology and Radiology; [2]Institute for Biological Research; [3]Faculty for Chemistry, University of Belgrade, 11 000 Belgrade, Yugoslavia

Summary: The effects of indomethacin, aminophylline and ascorbic acid on epinephrine cytotoxicity to human myelogenous leukemia K562 cells were studied. Both indomethacin and aminophylline applied in the concentrations of 35 μmol/L and 1.0 mmol/L, respectively, significantly intensified epinephrine-induced decrease of the cell survival (N/Nc) and viability (V), while ascorbic acid (50 μmol/L) acted as a strong inhibitor of this cytotoxic epinephrine action during the 24 h of the treatment. In the cell cultures pretreated with indomethacin or aminophylline followed after 20 min by epinephrine, colour of the nutrient medium was changed into a yellowish-brown at the end of experiment. This could mean that both indomethacin and aminophylline stimulated tyrosinase activity, thus giving the rise to coloured epinephrine oxidation products expressing cytotoxic activity toward K562 cells. Protective role of ascorbic acid against epinephrine-induced decrease of both cell survival and viability could be explained in terms of its strong reducing potency and scavenging of toxic products evolved from epinephrine either by the action of amine oxidases present in fetal bovine serum used as a constituent of culture medium and/or through the tyrosinase-catalyzed metabolic pathway.

Key words: K562 cells, cytotoxicity, epinephrine, indomethacin, aminophylline, ascorbic acid.

Introduction

Cytoxicity of biogenic amines or metabolically related compounds to different cell lines including tumour cells, has been repeatedly demonstrated under *in vitro* conditions. Examinations of such a cytotoxic action are of great significance not only for the better understanding of the molecular events underlying the mechanism of selective cytotoxicity to certain compounds, but also for the design and synthesis of novel anti-tumour drugs which could be applied in the therapy of malignant diseases. It has been shown that epinephrine (1-3), L-DOPA (4-6), cyclic amines (7) and mercaptoamines (8) expressed growth inhibition of tumour cells grown in vitro, while dopamine prolonged the survival span of melanoma-bearing experimental animals (9). Many of these compounds have a characteristic catechol moiety which can be easily oxidized in o-quinones and free radicals, semiquinones, well known for their cytotoxic reactivity (10-12). In some cases the observed cytotoxicity was supposed to be mediated through the action of amine oxidases present in ruminant sera and thus in fetal bovine serum, a common constituent of nutrient media used for *in vitro* cell culture (3, 6, 8). It was also shown that cytotoxic action of cyclic amines can be modulated by theophylline, a known stimulator of tyrosinase, especially in melanin-rich cells (7). On the other hand, it was observed that ascorbic acid selectively inhibits the growth of a number of human and murine melanoma cell lines *in vitro* (13). All these reports prompted us to examine possible modulations of epinephrine cytotoxicity to human myelogenous leukemia K562 cells by indomethacin (an inhibitor of prostaglandin synthesis), aminophylline (a stimulator of tyrosinase activity) and ascorbic acid (a very potent antioxidant and free radical scavenger).

* Address of correspondence:
Dr Jelena Joksimović
Inst. Biol. Res.
29 Novembra 142
11 060 Belgrade, Yugoslavia

Material and methods

Cell culture. Human myelogenous leukemia K562 cells were grown as a suspension culture in RPMI 1640 medium ($5x10^4$ cells mL^{-1}) supplemented with L-glutamine (2 mmol/L), streptomycin and garamycin (100 μg/mL, each) and 10% heat-inactivated fetal bovine serum, at 37°C, in 5% CO_2 humidified air atmosphere. The cells were maintained by twice weekly subculture.

Treatment of K562 cells. Epinephrine was applied in final concentration of 180 μmol/L^{-1}, which reduced cell viability (V) to about 60%, upon the transfer of the cells into a fresh culture medium.

Freshly prepared solutions of ascorbic acid (50 mmol/L), aminophylline (1.0 μmol/L) or indomethacin (35 μmol/L) were added to the cell culture and their effects on survival (N/Nc) and V were examined 24 h later. When combined action of either of these agents and epinephrine was studied, epinephrine was introduced into the cell cultures 20 min upon pretreatment of the cells with ascorbic acid, indomethacin or aminophylline, applied in afore-mentioned concentrations. Experiments were done in five replicates.

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 (N/Nc) was given as the number of survived cells exposed either to the individual agents or to their combinations with epinephrine in 1.0 mL of cell suspension per number of survivors in 1.0 mL of control culture x 100.

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

RPMI 1640 cell culture medium and fetal bovine serum were Gibco products. Epinephrine (4-/1-hydroxy-2-(methylamino)ethyl/-1,2-benzenediol) hydrochloride (2.67×10^{-2} stock in 154 mmol/L NaCl, stabilized with acetone sodium hydrogensulphite) was supplied by "Jugoremedija", Zrenjanin, Yugoslavia. Ascorbic acid was a Merck product. Indomethacin (/1-p-chlorobenzoyl/-5-metoxy-2-methylindole-3-acetic acid) and aminophylline (/theophylline/2-ethylenediamine) were obtained from "Lek", Ljubljana, Slovenia.

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.

Results and discussion

The data on K562 cell survival and viability upon treatment with ascorbic acid, indomethacin and aminophylline, alone or in combination with the epinephrine are listed in Table I.

Table I: The effect of ascorbic acid, aminophylline and indomethacin on epinephrine cytotoxicity to K562 human myelogenous leukemia cells

Treatment	N/Nc-100	V(%)
Control	100±14	99±1
Ascorbic acid	88±3	98±2
Aminophylline	73±6	100±0
Indomethacin	60±15	98±2
Epinephrine	51±13	67±6
Ascorbic acid + epinephrine	76±18*	99±1*
Aminophylline + epinephrine	33±18*	48±12*
Indomethacin + epinephrine	31±19*	47±12*

Epinephrine (final conc. 180 μmol/L) 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. Ascorbic acid (50 μmol/L), aminophylline (1.0 mmol/L) or indomethacin (35 μmol/L) were applied 20 min before the addition of epinephrine and their combined effects were examined after 24 h. In the latter case the culture exposed to epinephrine served as the control. The results are means ± S.D. from five repetitions.
*$p<0,05$.

As seen, aminophylline and indomethacin did not affect cell viability and only slightly affected the cell survival, when compared with the control grown in culture medium. However, when applied with epinephrine, both these agents significantly increased cytotoxic epinephrine action during the 24 hour-period in relation to N/Nc and V recorded in the cultures treated with epinephrine alone. It should be mentioned that epinephrine in final concentration of 180 μmol/L led to the death of some 40% cells. However, in our previous work (3) no survivors were recorded upon the application of the same epinephrine concentrations. This discrepancy can be explained by different source of this compound and the presence of a stabilizer in the preparation used throughout the present study. Also, the change of the nutrient medium colour to yellowish-brown was observed in the cultures exposed to the combination of epinephrine and indomethacin or aminophylline. Since theophylline acts as a stimulator of tyrosinase activity (7), it could be supposed that the enhanced epinephrine cytotoxicity in the presence of aminophylline is based on tyrosinase-catalyzed epinephrine oxidation into toxic intermediate products of melanin biosynthesis. Indomethacin-induced augmentation of epinephrine cytotoxic action to K562 cells could be partly explained by the same mechanism, since in the cultures treated with epinephrine and indomethacin the change of the nutrient medium colour into yellowish-brown was also seen, although

the mechanism of possible tyrosinase stimulation by this compound is not clear at present and there are no relevant data in the available literature. Our results on indomethacin-related increase of epinephrine cytotoxicity toward K562 cells could be connected with recent report of Maca (14) who showed that indomethacin acted enhancing cytotoxicity of a potent anticancer drug etopoxide (VP-17) to several murine and human tumour cell lines by a mechanism independent on indomethacin effect on prostaglandin biosynthesis.

Ascorbic acid, a potent antioxidant, greatly suppressed epinephrine cytotoxicity to K562 cells during the first 24 h of treatment. This finding, together with our previous results (3) showing that reduced form of glutathione (a non-specific reducing agent) suppressed epinephrine cytotoxicity to K562 cells and that aminoguanidine, an inhibitor of copper-dependent amine oxidases did not affect cytotoxic epinephrine action, strongly support the suggestions reported earlier (10, 11, 12, 15) that cytotoxicity of catecholamines is based on their oxidation into o-quinones and semiquinones, free radicals which could express cytotoxicity through the reaction with enzymes containing -SH groups, such as DNA-dependent DNA polymerase and topoisomerase II. The results given in the present work demonstrate modulations of epinephrine cytotoxicity to K562 human myelogenous leukemia cells by several agents from different chemical categories. The details on the mechanism(s) and the molecular events underlying aminophylline- and indomethacin-related increase of epinephrine cytotoxic action to K562 cells remain to be elucidated. Further examinations along this line are in progress.

Acknowledgement. This work was supported by Research Science Funds of Serbia, grants Nos. 13-03 (Z.J. and I.S.), 0316 (J.J.) and 11 (I.J.)

MODULIRANJE CITOTOKSIČNOSTI EPINEFRINA PREMA K562 ĆELIJAMA MIJELOGENE LEUKEMIJE ČOVEKA INDOMETACINOM, AMINOFILINOM I ASKORBINSKOM KISELINOM

Z. Juranić[1], J. Joksimović[2], I. Spužić[1] i I. Juranić[3]

[1]Institut za onkologiju i radiologiju; [2]Institut za biološka istraživanja; [3]Hemijski fakultet, Univerzitet u Beogradu, 11 000 Beograd, Jugoslavija

Kratak sadržaj: Proučavani su efekti indometacina, aminofilina i askorbinske kiseline na citotoksično delovanje epinefrina prema K562 ćelijama mijelogene leukemije čoveka. Indometacin (35 μmol/L) i aminofilin (1,0 mmol/L) su značajno pojačavali smanjenje preživljavanja (N/Nc) i vijabilnosti (V) ćelija izazvano epinefrinom, dok je askorbinska kiselina (50 μmol) delovala kao snažan inhibitor ovog citotoksičnog delovanja epinefrina tokom prva 24 h tretiranja. U kulturama ćelija izlaganih najpre delovanju indometacina ili aminofilina, a posle 20 min tretiranih epinefrinom, primećena je promena boje hranljivog medijuma u žućkasto-smeđu koja se pojačavala tokom eksperimenta. To bi moglo da znači da su i indometacin i aminofilin stimulisali aktivnost tirozinaze i da su kao rezultat delovanja ovog enzima na epinefrin nastali obojeni proizvodi, citotoksični prema K562 ćelijama. Protektivna uloga askorbinske kiseline i ublažavanje citotoksičnog efekta epinefrina, može se objasniti njenim visokim redukcionim potencijalom i redukcijom oksidacionih proizvoda nastalih delovanjem amino-oksidaza prisutnim u fetalnom govedem serumu korišćenom kao dodatak hranljivom medijumu i/ili dejstvom na metabolički put koji katalizuje tirozinaza.

Ključne reči: K562 ćelije, citotoksičnost, epinefrin, indometacin, aminofilin, askorbinska kiselina.

References

1. *Yamada, K., Murakami, H., Nishiguchi, H., Shirahata. S., Omura, H.:* Varying responses of cultured mammalian cell lines to the cellular DNA breaking activity of epinephrine. Agric. Biol. Chem. 43: 901-906, 1979.
2. *Yamada, K., Murakami, H., Shirahata, S., Shinohara, K., Omura, H.:* Binding of epinephrine to chromatin components. Agric. Biol. Chem. 48:2033-2038, 1984.
3. *Juranić, Z., Joksimović, J., Spužić, I., Juranić, I., Kidrič, M.:* Amine oxidase-mediated cytotoxicity of spermine and epinephrine to human myelogenous leukemia K562 cells. Neoplasma 39:271-275, 1992.
4. *Wick, M.M.:* An experimental approach to the chemotherapy of melanoma. J.Invest. Derm. 19:329-334, 1978.
5. *Yamada, I., Seki, S., Ito, S., Suzuki, S., Matsubara, O., Kasuka, T.:* The effect of L-dopa on the potentiation of radiation damage to human melanoma cells. Br. J. Canc. 62:32-36, 1990.
6. *Mena, M.A., Pardo, B., Casarejos, M.J., Fahn, S., de Yebenes, J.G.:* Neurotoxicity of levodopa on catecholamine-rich neurons. Movement Disorders 7:23-31, 1992.

7. *Noga, E.J., Barthalamus, G.T., Mitchell, M.K.:* Cyclic amines are selective cytotoxic agents for pigmented cells. Cell Biol. Int. Reports 10:239-247, 1986.

8. *Inoue, S., Ito, S., Wakamatsu, K., Jimbou, K., Fujita, K.:* Mechanism of growth inhibition of melanoma cells by 4-S-cysteaminylphenol and its analogues. Biochem. Pharmacol. 36:1077-1083, 1990.

9. *Wick, M.M.:* Dopamine a novel antitumour agent active against B-16 melanoma in vivo. J. Invest. Derm. 71:163-177, 1978,

10. *Powis, G.:* Metabolism and reactions of quinones. Free Rad. Biol. Med. 6: 63-101, 1989.

11. *Powis, G.:* Free radical formation by antitumorquinones. FreeRad. Biol. Med. 6: 63-101, 1989.

12. *O'Brien, P.J.:* Molecular mechanisms of quinone cytotoxicity. Chem.-Biol.Interactions 80:1-41, 1991.

13. *Bram, S., Broussard, P., Guichard, M., Jasmin, C., Augery, Y., Sinoussi-Barre, F., Wray, W.:* Vitamin C preferential toxicity for malignant melanoma cells. Nature 284:629-631, 1980.

14. *Maca, R.D.:* Enhancement of etopoxide and methotrexate sensitivity by indomethacin in vitro. Anticancer Drug Res. 6:453-466, 1991.

15. *Fitzgerald, C.B., Wick, M.M.:* 3,4-Dihydroxybenzylamine: An improved dopamine analog cytotoxic for melanoma cells in part through oxidation products inhibitory to DNA polymerase. J.Invest. Dermatol. 80:119-123, 1983.