PARGYLINE INCREASES THE SURVIVAL OF K562 HUMAN MYELOGENOUS LEUKEMIA CELLS COCULTURED WITH HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Summary: The influence of pargyline, a monoamine oxidase (MAO) inhibitor on the survival of K562 human myelogenous leukemia cells grown in a mixed culture with peripheral blood mononuclear cells (PBMC) was studied. Upon the separation, PBMC (effectors) were counted and seeded together with K562 cells (targets) in varying ratios of the effector to the target (E/T) cells. Culture medium RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mmol/L L-glutamine and 100 μg/mL of each garamycin and streptomycin in the presence or absence of 1 mmol/L pargyline was used. The cultures were maintained at 37 °C in 5% CO2 humidified air atmosphere. The survival of K562 cells was checked 22±2 h later, applying trypan blue dye exclusion method. The results obtained demonstrate a significant pargyline-related increase of K562 cells survival rate only when E/T ratios exceeded 20, i.e. pargyline acted protectively against the natural PBMC cytotoxicity. These data suggest that metabolic products of biogenic amines evolved by MAO action could be an important factor for the expression of PBMC cytotoxicity which is greatly suppressed by MAO inhibitors, such as pargyline.

Key words: K562 cells, human PBMC, pargyline, MAO, cytotoxicity.

Introduction

The incubation of human peripheral blood mononuclear cells (PBMC) with K562 human myelogenous leukemia cells in vitro, results in a decrease of K562 cells viability, due to a direct cytotoxic action of some PBMC subpopulations, known by their natural killer (NK) activity, as demonstrated by Herberman et al. (1). In a number of papers, the basis for this cytotoxicity was attributed to numerous cytotoxic factors such as perforin, lymphotoxin, tumor necrosis factor, NK cytotoxic factors, etc., released from the effector cells (2–4).

Monoamine oxidase [EC 1.4.3.4; amine:oxygen oxidoreductase (deaminating, flavine-containing); MAO], catalyzing the oxidative deamination of monoamines, represents an integral protein of the outer mitochondrial membrane. Based on different substrate specificity and various sensitivity toward inhibitors, two subgroups of MAO isoenzymes have been identified so far (5). It was shown that serotonin represents a favourite substrate for the catalytic action of MAO A form of the enzyme, while 2-phenylethylamine, benzylamine and acetylputrescine are preferent substrates for MAO B form. Dopamine, epinephrine and norepinephrine are the substrates susceptible to the action of both MAO forms, but their affinities differ greatly for each of these compounds (6–10). MAO A form predominates in human placenta and MAO B in human platelets, while both forms occur in human liver and brain (11, 12).

In the present work, the effect of pargyline, a known MAO inhibitor, on the survival of K562 human myelogenous leukemia cells cocultured with human PBMC in vitro was studied. The main goal of these examinations was to find out whether the metabolism of the biogenic amines catalyzed by MAO and suppressed by pargyline would influence, at least partially, the expression of cytotoxic PBMC activity toward K562 cells.

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Material and methods

Culture medium and chemicals

RPMI 1640 culture medium and FBS were GIBCO, Paisley, Scotland, U.K., products; Lymphoprep™ was purchased from Nycomed (Oslo, Norway) and Haemaccel® from Jugoremedija (Zrenjanin, Yugoslavia). Pargyline (N-methyl-N-propargylbenzylamine) was a product of Aldrich Chem. Comp. Inc., WI, 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 venous blood by Lymphoprep™ gradient centrifugation. Interface cells, washed three times with Haemaccel® aqueous solution containing 145 mmol/L Na⁺, 5.1 mmol/L K⁺, 6.25 mmol/L Ca²⁺, 145 mmol/L Cl⁻ and disintegrated gelatine polymers (35.0 g dm⁻³), pH 7.4 were counted. They were further resuspended in RPMI 1640 culture medium enriched with 2 mmol/L L-glutamine, 10% heat inactivated FBS, garamycin and streptomycin (100 μg/mL, each), pH 7.2 (adjusted with a sodium bicarbonate solution).

Cell culture

PBMC prepared as described above were seeded in triplicate in 48 flat-bottom wells (Costar) together with K562 human myelogenous leukemia cells in various effector to target (E/T) ratios in the culture medium in the presence or absence of 1 mmol/L pargyline. The cultures were maintained at 37°C in 5% humidified air atmosphere.

Counting of survived K562 cells

Number of morphologically intact K562 cells with bright halo was assessed by trypan blue dye exclusion method using Fux-Rosenthal chambers and a Carl Zeiss, Jena, microscope, 22±2 h after the inception of mixed cell culture and exposure of the cells to the above treatment. Number of survived K562 cells in the corresponding well was assigned as N.

Data analysis

For the statistical evaluation of the data, one-tailed Student’s t test was used. The differences were considered statistically significant when p was less than 0.05. The results were expressed as the means from at least three experiments done in triplicate±S.D.

Results

Changes in the number of survived K562 cells (N) after 22±2 h coincubation with human PBMC in various effector to target (E/T) ratios in RPMI 1640 culture medium containing 10% heat inactivated FBS, 2 μmol/L L-glutamine and antibiotics (garamycin and streptomycin, 100 μg/mL each). After 22±2 h incubation (37°C, 5% humidified air atmosphere), K562 survivors were counted in both the cultures grown in the presence and absence of 1 mmol/L pargyline. Data are from representative experiments, each performed with PBMC prepared from the blood of one healthy volunteer. Each point represents the mean ± S.D. of three (A) or four (B) replicates. *P<0.001; **P<0.02; †P<0.03; ††P<0.05. Full line – pargyline present, dashed line – pargyline absent.
Fig. 2. Maximum difference in number of K562 cells, $N_{\text{max}}$, grown in mixed culture with human PBMC in the presence and absence of pargyline, as a function of the number of seeded K562 cells, $N_0$.

Survivors cocultured with PBMC at the E/T ratios over 20 was significantly higher in the presence of pargyline than in the controls devoid of this compound, i.e. pargyline expressed a protective effect against natural killing activity of some PBMC subpopulations.

Maximum difference in the survival rate of K562 cells was proportional to the gross number of seeded K562 cells, as seen from Fig. 2. It is worth mentioning that human platelets did not abate the survival of the investigated K562 cell line (data not shown).

The results of an alternate approach of measuring MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction by K562 survivors (13) in the presence of pargyline and PBMC originating from the blood of five healthy volunteers, led to the same conclusion concerning protective effect of this compound against PBMC cytotoxicity, as above.

**Discussion**

At present, there are some controversial reports on the role of monoamine oxidases. For example, Benedetti and Dostert (5) suggested that brain ageing could be attributed to toxic products such as NH$_3$, aldehydes and H$_2$O$_2$, evolved by MAO action on several substrates. Fritz et al. (14), however, reported possible detoxifying amine activity of MAO from both endogenous and exogeneous sources. It is well documented that pargyline acts primarily inhibiting MAO B form of the enzyme, but it also inhibits to a certain extent MAO A form, although with a rather low specificity. Acetylprescine, epinephrine, norepinephrine and dopamine represent some of the substrates susceptible to MAO B action. The importance of polyamines for cytotoxic activity of the lymphocytes, as well as the potentiation of natural killer activity and tumor immunity by diacetylprescine were emphasized in a series of papers by Bowlin et al. (15-17). In addition, it was also shown that the synthetic prescine homologues containing 2 or 3 carbon atoms in the molecule could restore impaired immune functions in animals treated with inhibitors of polyamine synthesis (18). These results, together with the data obtained throughout the present work, demonstrating a protective pargyline action against natural killer activity of PBMC subpopulations toward K562 cells, open the question on the importance of the preserved activity of MAO-catalyzed processes in both natural killer cells and target K562 human myelogenous leukemia cells for the expression of PBMC cytotoxic effects. However, it should be emphasized that some other MAO activity-independent pargyline actions can not be ruled out by the approach applied for the design of our examinations. The presence of the compounds with MAO inhibitor-like activity detected in human blood plasma (19, 20), makes the forthcoming investigations related to the modulation of the observed natural killing PBMC activity even more interesting.

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PARGILIN POVEĆAVA PREŽIVLJAVANJE K562 ĆELIJA HUMANE MIJEOLOGNE LEUKEMIJE GAJENIH U KULTURI SA MONONUKLEARNIM ĆELIJAMA PERIFERNIH KRVI ČOVEKA

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Kratko sadržaj: Proučavano je delovanje pargilina, inhibitora monoamin oksidaza (MAO) na preživljavanje K562 Ćelija humane mijelogene leukemije gajenih u kulturi sa mononuklearnim ĉelijama perifernih krvi čoveka (PBMC). Posle izdvajanja PBMC efektori su brojne i zasebavane zajedno sa ĉelijama K562 (target-ĉelije) u različitim odnosima efekatora prema target-ĉelijama (E/T). Korišćen je medijum za kulturu RPMI 1640 obogaćen 10% šećernog seruma goveđa (FBS) koji je inaktiviran izlaganjem površinoj temperaturi, L-glutaminom (2 µmok/L), garaminicom i streptomycinom (po 100 µg/mL). Ove mešovite kulture su gađene u prisustvu ili odsustvu 1 µmok/L pargilina, na 37 °C u atmosferi vazduha koji je sadržao 5% CO2 i pri određenoj vlažnosti. Preživljavanje ĉelija K562 je proveravano posle 22±2h, primenom testa izbicanja bojetripan plavo. Dobijeni rezultati pokazuju značajno povećanje preživljavanja u prisustvu pargilina samo kada je odnos E/T bio veći od 20, odnos pargilina je stiglo u ĉelije K562 od prilog citotoksčnosti PBMC. Ovi podaci sugerišu da metabolički proizvodi biogenih amina, nastali delovanjem MAO mogu biti važni faktor za ispoljavanje citotoksčnosti PBMC, koja je izrazito smanjena MAO inhibitorima, kakav je pargilin.

Ključne reči: K562 ĉelije, PBMC čoveka, pargilin, MAO, citotoksičnost.

References


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