Cytoprotective activity of Phenovine in mitogen-activated Jurkat cells

L. Ratiani, I. Chkhikvishvili, *M. Dgebuadze, T. Ratiani, G. Sharashenidze, N. Intskirveli, T. Sanikidze

Department of Anatomy, Tbilisi State Medical University
33, Vaja Pshavela Street, 0177, Tbilisi, Georgia

*Corresponding author e-mail: illusion_ia2001@yahoo.com
Manuscript received February 19, 20012; revised April 03, 2012

Abstract

The aim of the study: to establish the cytoprotective ability of the application of Phenovine to mitogen-activated Jurkat cells' modeling systems. Experiments were conducted on intact and PHA-stimulated Jurkat cells and in conditions of coincubation of intact and PHA-stimulated cells. Cytoprotectiv activity of Phenovine was established according to coefficients of viability of the cells and apoptosis intensity, calculated on the basis of the data from the MTT test. Based on the analysis of experimental data, it was concluded that the viability of Jurkat cells decreases (apoptosis intensity increases), while the rising ratio of activated and nonactivated cells in the incubation medium ($[Jurkat]_{PHA}/[Jurkat]_{intact}$). Therefore, Phenovin increases viability of PHA-stimulated Jurkat cells and prevents their apoptosis.

Key words: Phenovine, mitogen-activated Jurkat cells.

Цитопротекторная активность феновина в митоген-активированных клетках Jurkat

Целью работы явилось исследование цитопротекторной активности феновина на митоген-активированных клетках Jurkat. Эксперименты проводились на интактных, PHA-стимулированных клетках Jurkat и в условиях совместной инкубации интактных и стимулированных клеток. Цитопротекторная активность феновина определялась по значению коэффициентов жизнеспособности клеток и интенсивности их апоптоза, которые рассчитывались из данных MTT теста. На основании анализа результатов исследования сделано заключеиие, что жизнеспособность клеток Jurkat уменьшается, интенсивность апоптоза увеличивается, с увеличением соотношения PHA-стимулированных и не стимулированных клеток Jurkat в среде инкубации ($[Jurkat]_{PHA}/[Jurkat]_{интактные}$). Феновин увеличивает жизнеспособность PHA-стимулированных клеток Jurkat и предотвращает апоптоз.

Ключевые слова: феновин, митоген-активированные клетки Jurkat.

Introduction

Presently, much interest is focused on the autoimmune mechanism of atherosclerosis. It is known that infiltration of atherosclerotic plaques which is activated by T lymphocytes, occurs at an early stage and cytokines produced by those lymphocytes are actively involved in the inflammatory process [3, 6, 7, 8, 12]. Balance between pro- and anti-inflammatory cytokines may play crucial role in the progression of atherosclerosis. Pro-inflammatory cytokines are, hence, considered to bepro-atherogenic, anti-inflammatory-anti-atherogenic cytokines; and reduced production of anti-inflammatory cytokines results in impaired clearance of apoptotic cell debris. It also should be mentioned that the over abundance of reactive oxygen species (ROS) may induce apoptosis or necrosis in many cell types, which can cause tissue injury [1]. Apoptosis in activated T lymphocytes occurs at the same time as the over abundance of ROS and activation of MAP (mitogen-activated protein) kinases; apoptotic microparticles accumulate in the lipid core of atherosclerotic plaques, as a result of reduced capacities to clear of apoptotic cells with foam macrophages in an oxidant-rich environment [2].

The intensively proliferated Jurkat leukemic T cell line (human leukemic cell culture) is widely used in scientific and therapeutic research, as the human T lymphocyte pattern [4, 5, 9, 13]. Jurkat line has been extensively studied as a model system for understanding the conditions involving T cell activation. The latter is commonly determined by IL-2 secretion, which promotes T cell proliferation. The Jurkat T cell line, stimulated by phytohemaglutinine or Con A, is capable to produce 100 to 300 times as much IL-2 as lectin-stimulated normal human peripheral blood lymphocytes. The Jurkat cell derived from IL-2 has the capability to induce proliferation of antigen-stimulated human effector cells in vitro. Therefore, the Jurkat cell model system is a valuable reagent for researchers interested in proliferation T clonal human lymphocytes of various antigen and effector specificity, and in particular, the effect of potentially anti-atherogenic medications on cytokines and atherosclerosis-associated auto-antigenes such as oxidized LDL, Heat Shock protein, cardiolipine, and b-2-glycoproteine.

The autoimmune concept of atherosclerosis pathogenesis has been followed by a novel and promising strategy of atherosclerosis treatment and prevention, which occurs in the suppression of proatherogenic immune factors and the activation of atheroprotective immune factors. Consequently, the preparations capable of stabilizing immune cells may

only be effective for the treatment and prevention of atherosclerosis.

An extensive volume of basic clinical and experimental research proves the beneficial effects of the antioxidant, immunomodulatory therapy of atherosclerosis. In recent years in modern medicine great attention has been paid to natural antioxidant compounds taken from vegetables. The protective effect of poliphenols in cardiovascular diseases is due to the antioxidant and antiradical properties of these compounds [4], their ability to act as chelates for metals with alternating valency, to have an effect on the activity of the cellular detoxing system (superoxide dismutase (SOD), catalase, glutathione peroxidase), and to inhibit ROS-generating enzymes (xanthine oxidase, NADPH-oxidase). Protective effect of red vine poliphenol, rezveratrol against oxLDL toxicity in endothelial cells is a well known phenomenon [11]. Though many aspects of phenol activity are still to be specified and elucidated. The application of Phenovin (registration[1] P-000268, "Pharmipecs" Ltd for i/p "Irakli Chkhikvishvili – ITA"), synthesized in Georgia from bioactive phenol compounds (flavonoids, phenilpropanoids, stable phytoalexin from grape peel reserves?), is characterized by antioxidant, hypolipidemic, thrombolitic, and fibrinolitic properties.

The aim of the study: to establish the cytoprotective ability of the application of Phenovine on mitogen-activated Jurkat cells' modeling systems.

Material and Methods

Cell culture: research was conducted on human leukemic mature T cells (Jurkat cells) (DSMZ-Deutshe Sammulung von Mikroorganismen und Zellkulturen (Germany). Cells were proliferated in the bioactive medium RPMI 1640 (GIBSO), inactivated embrionic bovine serum (Sigma), L-glutamine (4mM), penicillin (100un/ml) and streptomycin (100un/ml) contained in a medium at 37^0C T and 5% CO_2 moisture. Experiments were carried out on cell at 0.3 – 0,6 x 10^6 concentration in 1ml of medium. The following series of experiments were implemented:

1) Jurkat cells were pre-stimulated through incubation with 50, ug/ml PHA at 37^0 for 5 min;
2) PHA-prestimulated Jurkat cells cultured for 24 hours alone;
3) PHA-prestimulated Jurkat cells cultured for 24 hours mixed with non-stimulated Jurkat cells;
4) PHA-prestimulated Jurkat cells cultured for 24 hours mixed with non-stimulated Jurkat cells and Phenovine (LTD Irakli Chkikvishvili) in a dose of 0.2 μg/100 microlitre suspensions.

Modelling of Activation-induced cell death

Jurkat cells pre-stimulated with phytohaemagglutinin (PHA) induced the death of non-activated Jurkat cells. Time-course analysis revealed that PHA-stimulated Jurkat cells quickly release a toxic molecule in the medium following a biphasic pattern, with maximal cytotoxic activities [10]. This data indicates the activation-induced cell death (AICD) in Jurkat cells.

The Jurkat cells (4 x 10^5 cells/ml) were pre-stimulated through incubation with 50, ug/ml PHA at 37^0 for 5 min. PHA was then removed by brief centrifugation in cells that had been washed three times with RPMI-1640, re-suspended in a complete medium, seeded in flat-bottom 96-well plates (25000 cells/well) and cultured for 24 hours alone or mixed with different concentration of non-stimulated Jurkat cells and Phenovine. We tested the toxicity of non-activated Jurkat cells with the MTT method.

The assessment MTT test of cells proliferation activity (viability)

We incubated cell suspended (2 x 10^6 cell/ml) in a growth medium (PRMI-1640 + 10% calf embryonic serum (sigma) + 1% penicillin+streptomycin) (together with hydrogen peroxide and antioxidant preparations) for 37°C in 5% CO_2 atmosphere. After this, the incubation period suspension was centrifugated at 1500 G for 5 minutes. MTT (3-(4.5-dimethyltiazol -2)-2.5- diphenyltetrazolium bromide) the (Sigma) solution was added to sedemented cells (30 microliter for 100 microlitre suspension; MMT was diluted in buffer) (140 mM NaCl, 5mM HEPES, pH 7.4) in dose of 2.5 mg for each MTT 300 microliter buffer) and incubated for 4 hours at 37°C in a 5% CO_2 condition. After the incubation we carefully took supernatant and added a 100% dimethyl sulfoxide (DMSO) dilutant to a 100 microliter.

Absorption was measured at spectrophotometer at a 570 nm wave-length. Through the research of suspension we determined that the average rate of measurement, divided by the average rate of control (separately for soil) calculates the coefficient of proliferation through the following formula:

$$K = A_{trial}/A_{control}$$

In cytotoxicity tests using the MTT detection method, target cell death was calculated as follows (10):

$$\% \text{ of target cell death} = (A_{550\ (effectors + targets)} - A_{550\ (effectors)})/ A_{550\ (targets)} \times 100$$

We determined the rate of absorbance of effector or target cells as 550 nm (A550) through separate cultures containing either effector or target cells alone.

We conducted statistic processing of the obtained results according to the SPSS 11.0 program. A student's t-test was used for the analysis of differences between means and thechange of the p value < 0.05 was considered to be statistically significant.

Results and Discussion

In table 1 the research results show the Jurkat cells' viability (MMT test) of both PHA-stimulated Jurkat cells alone and intact Jurkat cells incubated with PHA-stimulated Jurkat cells. As it was revealed, the viability of PHA-stimulated Jurkat cells is decreased statistically significant in comparison with viability of deactivated cells; in the case of co-incubation of intact Jurcat cells with PHA-stimulated Jurkat cells, there viability decreases with the rising ratio of activated and deactivated cells $[Jurkat]_{PHAact}/[Jurkat]_{intact}$.

Table 1

Jurkat cells viability (MMT test)

		24 hour	
Area		0.10 ± 0.02	K
Jurkat		0.52 ± 0.06	1
Jurkat+PHA		0,27 ± 0,04	0.41*
Jurkat+PHA	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 0,11 (10%/90%)	0.47 ± 0.09	0.88
	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 0,25 (20%/80%)	0. 46 ± 0.05	0.86
	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 0,43 (30%+70%)	0.41 ± 0.05	0.73*
	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 0,67 (40%+60%)	0.43 ± 0.04	0.79*
	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 1 (50%+50%)	0.37 ± 0.06	0.64*
	$[Jurkat]_{PHAact}/[Jurkat]_{intact}$ = 1,5 (60%+40%)	0.39 ± 0.054	0.69*

* – Statistical significancy according intact Jurkat cells.

Table 2 there shows the data of the influence of Phenovine on the viability of PHA-stimulated and coincubated intact + PHA-stimulated Jurkat cells (MMT test results). It was revealed, that Phenovine increases the viability of intact cells (by 22%) and PHA-stimulated Jurkat cells (by 60%).

In case of coincubation of intact Jurkat cells with PHA-stimulated Jurkat cells, Phenovine increases the viability 100%. In contrast, the viability decreases with the rising ratio of activated and non-activated cells $[Jurkat]_{PHA}/[Jurkat]_{intact}$.

Table 3 shows the data of the established influence of Phenovine on the intensity of apoptosis in the intact Jurkat cells mixed with PHA-stimulated Jurkat cells. As shown in table 3, prestimulated Jurkat cells induced the death of their non-activated counterparts. The percentage of cell death increased with the effector to target ratio. Phenovine prevents cell apoptosis by 20%-s.

Table 2

Influence of Phenovine on the viability of PHA-stimulated and coincubated intact+ PHA-stimulated Jurkat cells (MMT test results)

	Intact		Phenovine	
	I	K	I	K
Area	0.10 ± 0.02			
Jurkat	0.524 ± 0.062	1	0.617 ± 0.037	1.22**
Jurkat+PHA	0.273 ± 0.040	0.41*	0.447 ± 0.058	2.01** / 0.67***
$[Jurkat]_{PHA}/[Jurkat]_{intact}$ = 3/2	0.394 ± 0.054	0.69*	0.624 ± 0.039	1.78** / 1.0
$[Jurkat]_{PHA}/[Jurkat]_{intact}$ = 2/3	0.434 ± 0.044	0,79*	0.657 ± 0.023	1.67** / 1,1

* – Statistical significancy according intact Jurkat cells.

** – Statistical significancy between Jurkat cells bioviability with/without Phenovine.

*** – Statistical significancy according intact Jurkat cells + Phenovine.

Table 3

Influence of Phenovine on the intensity of apoptosis intact Jurkat cells mixed with of PHA-stimulated Jurkat cells (MMT test results)

	Intact %	Phenovine %
$[Jurkat]_{PHA}/[Jurkat]_{intact}$ = 2/3 (40% + 60%)	60%	40%
$[Jurkat]_{PHA}/[Jurkat]_{intact}$ = 3/2 (60% + 40%)	50%	28%

Conclusion

The viability of Jurkat cells decreases (apoptosis intensity increases) with the rising ratio of activated and nonactivated cells $[Jurkat]_{PHA}/[Jurkat]_{intact}$.

P – Phenovin increases the viability of PHA-stimulated Jurkat cells and prevents their apoptosis.

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Acknowledgment

Study was financed by Grant 383 of Rustaveli Scientific Foundation, Georgia.

Scorurile prognostice în contextul pneumoniilor gripale severe 2009 AH_1N_1

V. Botnaru, *D. Chesov, O. Munteanu, D. Rusu, S. Popa

[1]Department of Internal Medicine, Medical Clinic No 2
Nicolae Testemitanu State Medical and Pharmaceutical University
29, Nicolae Testemitanu Street, Chisinau, Republic of Moldova

*Corresponding author: +373 22 899044, chesov.dumitru@gmail.com
Manuscript received December 23, 2011; revised February 02, 2012

Prognostic scores in the context of severe 2009 AH_1N_1 influenza pneumonias

In this paper we analyzed the applicability of the classical prognostic scores – PSI, CURB65, CRB65, and ADROP used for evaluating community acquired pneumonia in patients during 2009 with AH_1N_1 influenza complicated with severe pneumonia. The analyzed scores were retrospectively applied to a prospective cohort of 75 fatal cases with requirement for invasive mechanical ventilation (IMV). All scores showed a very low sensitivity in scores' classes corresponding to severe pneumonia, when used for prediction of deathfatal cases (PSI > IV – 20%; CURB65 > 3 – 33.3%; CRB65 > 2 – 53.3; ADROP > 4 – 6.7%), as well as the need for IMV (PSI > IV – 17.2%; CURB65 > 3 – 44.8%; CRB65 > 2 – 10.3%; ADROP >4 – 3.4%). Thus, we concluded by analyzing the scores that the probability of death and the need for IMV was underestimated in patients with severe influenza pneumonia.

Key words: influenza pneumonia**,** prognostic scores, CUBR65, CRB65, ADROP, PSI.

Прогностические шкалы в контексте гриппозных пневмоний 2009 AH_1N_1 с тяжелым течением

В представленной работе анализируется применимость классических прогностических шкал (PSI, CURB65, CRB65, ADROP, используемых при оценке тяжести внебольничной пневмонии) у пациентов с гриппом 2009 AH_1N_1 осложненным пневмонией с тяжелым течением. Проанализированные шкалы были ретроспективно применены к проспективно сформированной когорте, состоящей из 75 случаев тяжелых больных, 2009 AH_1N_1 гриппозных пневмоний, для оценки вероятности наступления смерти или необходимости применения инвазивной вентиляции легких (ИВЛ). Все шкалы показали очень низкую чувствительность, в классах соответствующих тяжелой пневмонии, при использовании для оценивания вероятности смерти (PSI > IV – 20%; CURB65 > 3 – 33,3%; CRB65 > 2 – 53,3; ADROP > 4 – 6,7%), или необходимость ИВЛ (PSI > IV – 17,2%; CURB65 > 3 – 44,8%; CRB65 > 2 – 10,3%; ADROP > 4 – 3,4%). Полученные результаты доказывают недооценивание проанализированными шкалами вероятности смерти и необходимости применения ИВЛ у больных с тяжелой гриппозной пневмонией.

Ключевые слова: гриппозная пневмония, прогностические шкалы, CUBR65, CRB65, ADROP, PSI.

Introducere

Virusul gripal 2009 AH_1N_1 continuă să fie cel mai frecvent subtip viral izolat pe parcursul ultimelor sezoane gripale [1]. Posibilitatea evoluției nefaste, adesea fulminante, a infecției gripale 2009 AH_1N_1, suprapusă afluxului masiv de pacienți în perioadele de epidemie, impune necesitatea aplicării unor instrumente prognostice ce ar permite diferențierea promptă a pacienților cu un risc sporit pentru evoluția nefastă a gripei. Deși scorurile prognostice clasice aplicate în cazul pneumoniilor comunitare (PC) au fost elaborate și validate pentru pneumoniile bacteriene, aplicarea acestora pare a fi argumentată și la pacienții cu pneumonii gripale.

În prezenta lucrare ne-am propus să analizăm aplicabilitatea scorurilor prognostice clasice de evaluare a pneumoniilor