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Cytotoxic effect of Pregabalin on U937 and Molt-4 Leukemic Cells in Vitro

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ABSTRACT

Background: Pregabalin, a selective inhibitor of voltage dependent calcium channels has been used for treatment of epilepsy, fibromyalgia, generalized anxiety disorder and especially neuropathic pains. The anti-inflammatory properties of pregabalin in some pathologic conditions like orofacial pain and streptozotocin-induced diabetic mice have been reported. Effectiveness of pregabalin in treatment of pain in some cancer patients has been shown. The aim of this study was to investigate the cytotoxic effect of pregabalin on U937 and Molt-4 leukemic cells in vitro.

Methods: Human leukemic monocyte (U937) and T cell (MOLT-4) were cultured in Roswell Park Memorial Institute (RPMI)-1640 complete medium. Next, different concentrations of pregabalin (1, 10, 50, 100, 500 and 1000 µg/ml) were add to cultured U937 and MOLT-4 cells and incubated for 24, 48 and 72 hours. The cytotoxic effect of pregabalin was assessed by MTT (3-[4, 5 dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) test.

Results: Pregabalin significantly reduced U937 and Molt4 cells viability at 1000 µg/ml (6280.26 µM) concentration after 24 hours treatment ($P < 0.001$, $P = 0.029$, respectively). In addition, pregabalin also decreased U937 and Molt-4 cells viability at ≥ 500 µg/ml (≥ 3140.13 µM) concentrations after 48 hours incubation ($P < 0.001$). Besides, pregabalin significantly reduced leukemic U937 cells viability at ≥ 100 µg/ml (628.02 µM) ($P < 0.005$) after 72 hours incubation. Whereas, pregabalin significantly diminished Molt-4 cells viability at ≥ 1 µg/ml (6.28 µM, $P < 0.001$) after 72 hours incubation.

Conclusion: Our findings demonstrated that pregabalin was cytotoxic for U937 and Molt-4 cells in a time and dose dependent pattern. So Pregabalin might be a useful candidate as a therapeutic substance for treatment of leukemic patients.

Introduction

Leukemia, as one of the most important malignant leukocytosis is characterized by uncontrolled proliferation of myeloid or lymphoid lineage cells in blood and/or bone marrow.¹ Leukemia is accompanied by serious life threatening clinical manifestations such as systemic and specific organ involvement.² Nowadays, the approved treatments for leukemia are chemotherapy, monoclonal antibody therapy, radiotherapy and stem cell transplantation.³ However, the efficacy of leukemia

treatment due to pharmacogenetics differences is variable and the serious side effects of these treatments such as fatigue, hair loss, increased risk of infectious and bleeding lead to more death in leukemic patients.³

Cancer pain is known as a critical complication of malignant diseases that affects on quality of life in cancer patients and its treatment requires a multidisciplinary approach including systemic pharmacotherapy, psychosocial and physical therapy.⁴ Neuropathic pains are distinct clinical entities of cancer pains resulting from

conditions affect the somatosensory nervous system and are associated with high economic burden.⁵

Pregabalin is an anti-anxiety agent with widely medical applications in voltage dependent calcium channels dependent situations such as neuropathic pain, epilepsy, fibromyalgia and generalized anxiety disorder that mediate its function via inhibiting certain calcium channels.⁶ Administration of pregabalin solely or in combination with other agents such as opioids has been effective in treatment of neuropathic pain in cancer patients.⁷⁻⁹

Since pregabalin is widely used in cancer patients suffer from neuropathic pains, assessment of its cytotoxicity is necessary. The aim of this study, was to evaluate cytotoxic effect of pregabalin on monocyte-macrophage Leukemic U937 cells and T-lymphocyte leukemic MOLT-4 cells in vitro.

Materials and Methods

Reagents

Roswell Park Memorial Institute (RPMI) 1640 complete medium, streptomycin and penicillin were obtained from sigma company (USA). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetra-zolium bromide (MTT) was purchased from Sigma-Aldrich (USA). Fetal bovine serum was provided from Gibco (USA). Pregabalin was obtained from Iran Darou Co (Tehran, Iran). Microtiter plates, tubes and flasks were purchased from Nunc Company (Falcon, USA).

Cell Lines

Human leukemic monocyte [U937 (NCBI C130)] and T cell [Molt-4 (NCBI C149)] were obtained from National Cell Bank of Iran, Pasteur Institute of Iran, Tehran (NCBI).

Preparation of Pregabalin

Pregabalin was dissolved in RPMI-1640 to prepare the stock and stored at -20°C until experimental use. In order to prepare different concentrations of pregabalin, the stock was diluted in RPMI-1640 complete medium before experiments.

Cell Culture and Treatment

The detail of cell culture and treatment protocol has

been described by Hajighasemi et al before.¹⁰ In brief, leukemic cells were cultured in RPMI-1640 complete medium with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5 % CO₂ and were passaged several times in order to obtain required cell number for treatment. Each of U937 and Molt-4 cells were divided in 96 wells plates at 3×10^4 cells/well and treated with different concentrations of pregabalin: 1, 10, 50, 100, 500 and 1000 µg/ml (6.28- 6280.26 µM) for 24, 48 and 72 hours. Cell viability was evaluated by MTT assay.

Cell Viability Assay

The effects of pregabalin on viability of the leukemic cells were investigated by using MTT assay. This method based on reduction of yellow water soluble MTT by mitochondrial dehydrogenase of intact cells to blue insoluble formazan crystals. The MTT assay was performed after 24, 48 and 72 hours treatment. After incubation times, 20 µL of MTT solution (5 mg/ml) was added to each well and incubated for 4 hours at 37 °C. Then, 100 µL of Isopropanol hydrochloride solution was added to each well and was shaken to dissolve the formazan crystals. The optical densities (OD) were measured by using an ELISA microplate reader at 492 nm wavelength.

IC₅₀ Determination

The half inhibitory concentration (IC₅₀) of pregabalin was defined a concentration of drug which inhibits half of leukemic cells viability in comparison to untreated control cells. The IC₅₀ value of pregabalin was determined by drawing a dose-response curve based on MTT test data.

Statistical Analysis

The software SPSS 24 package (SPSS Inc, Chicago, IL) was used for statistical calculations and data analysis. The effect of pregabalin on leukemic cells viability was determined in 5 independent trials. The normality of data was assessed by Kolmogorov-Smirnov Z-test and then one way analysis of variance (ANOVA) and Tukey post hoc tests were used to determine the statistical significance and multiple comparisons between groups. Data are expressed as mean±standard error of the mean (SEM). Statistical significance considered when P value was <0.05.

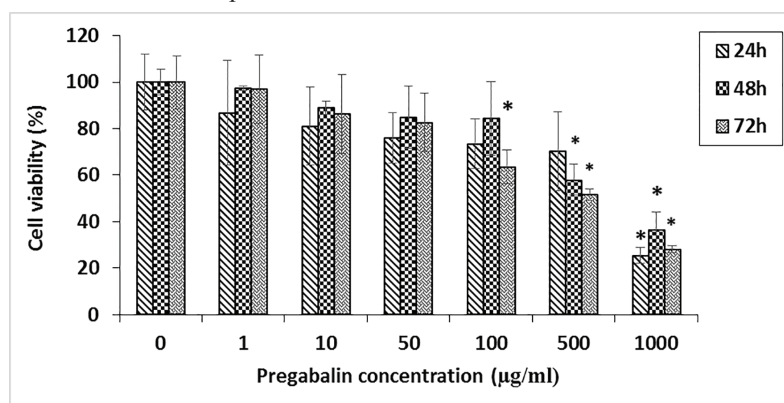


Figure 1: Effect of pregabalin on viability of U937 cells. U937 cells (3×10^4) were treated with different concentrations of pregabalin (1-1000 µg/ml) for 24, 48 and 72 hours. Cell viability was determined using MTT assay. Data are expressed as mean±SEM. * P<0.05 was considered as statistical significant.

Results

Figures 1&3 present the effect of different concentrations of pregabalin on U937 and Molt-4 cells in three time intervals. Figures 2&4 show the IC₅₀ value of pregabalin on U937 and Molt-4 cells in three time intervals. Pregabalin had an inhibitory effect on viability of U937 and Molt-4 cells with a time and concentration dependent manner.

Pregabalin Effect on Viability of U937 Cells

Pregabalin decreased the U937 cells viability in all three time intervals as was depicted in Figure 1. After 24 hours treatment of U937 cells with pregabalin, the cells viability was significantly reduced at 1000 µg/ml (6280.26 µM) concentration of the drug ($P<0.001$) (Figure 1). Moreover, after 48 hours incubation time, pregabalin

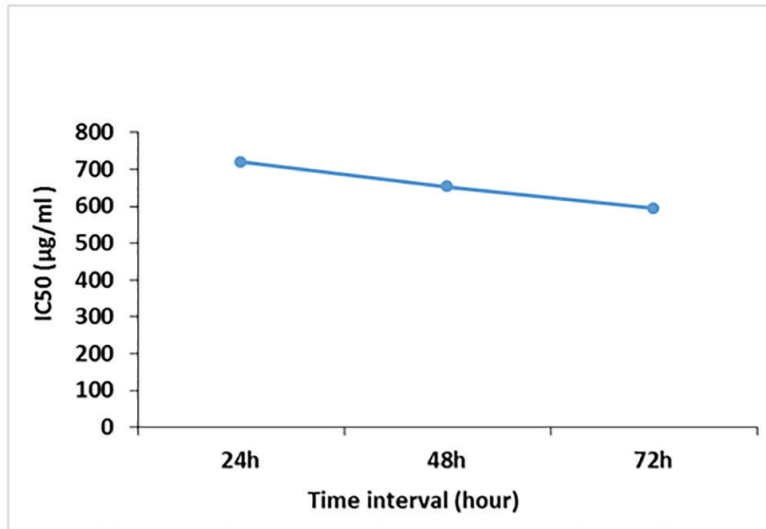


Figure 2: The IC₅₀ value of pregabalin in different time intervals on human U937 cells using a dose response curve based on MTT test data.

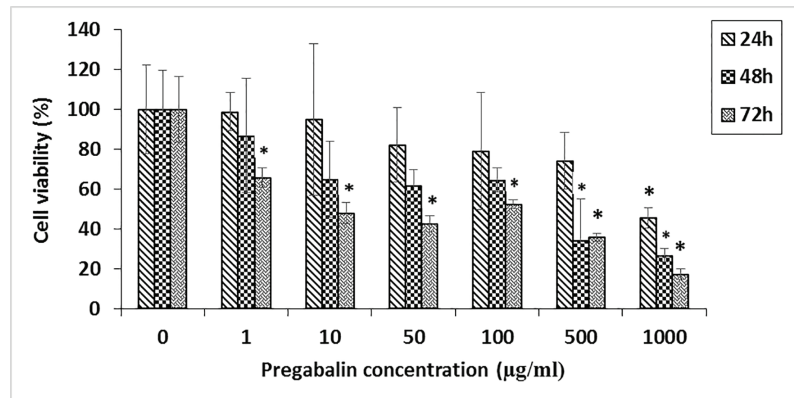


Figure 3: Effect of pregabalin on viability of Molt-4 cells. Molt-4 cells (3×10^4) were treated with different concentrations of pregabalin (1-1000 µg/ml) for 24, 48 and 72 hours. Cell viability was determined by using MTT assay. Data are expressed as mean $\pm$ SEM. * $P<0.05$ was considered as statistical significant.

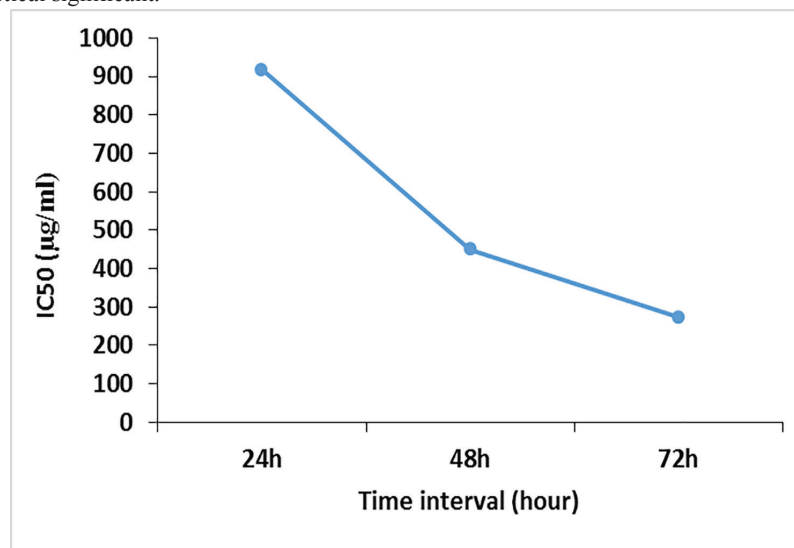


Figure 4: The IC₅₀ value of pregabalin in different time intervals on human MOLT-4 cells using a dose response curve based on MTT test data.

significantly declined the U937 cells viability at ≥ 500 $\mu\text{g/ml}$ (≥ 3140.13 μM) concentrations ($P < 0.001$) (Figure 1). Furthermore, after 72 hours incubation, pregabalin significantly decreased the U937 cells viability at ≥ 100 $\mu\text{g/ml}$ (≥ 628.02 μM) concentrations ($P < 0.005$) (Figure 1).

The IC₅₀ value of pregabalin for U937 cells at each incubation time is depicted in figure 2. The IC₅₀ values of drug for U937 cells after 24, 48 and 72 hours treatment were obtained 719.294, 652.433 and 594.291 $\mu\text{g/ml}$ respectively using a dose-response curve based on MTT test data.

Pregabalin Effect on Viability of Molt-4 Cells

Pregabalin also decreased the Molt-4 cells viability in all time intervals as was depicted in figure 3. After 24 hours treatment of Molt-4 cells with pregabalin, the cells viability was significantly reduced at 1000 $\mu\text{g/ml}$ (6280.26 μM) concentration of the drug ($P < 0.05$) (Figure 3). Moreover, after 48 hours treatment, pregabalin significantly declined the Molt-4 cells viability at ≥ 500 $\mu\text{g/ml}$ (≥ 3140.13 μM) concentrations ($P < 0.001$) (Figure 3). Furthermore, after 72 hours incubation, pregabalin decreased the Molt-4 cells viability at ≥ 1 $\mu\text{g/ml}$ ($P < 0.001$) (Figure 3).

The IC₅₀ value of pregabalin for Molt-4 cells at each incubation time is depicted in Figure 4. The IC₅₀ values of pregabalin for Molt-4 cells after 24, 48 and 72 hours treatment were obtained 916.919, 449.587 and 273.085 $\mu\text{g/ml}$ respectively using a dose-response curve based on MTT test data (Figure 4).

Discussion

In the present study, we assessed the cytotoxic effect of pregabalin on human U937 and Molt-4 cells. We observed the cytotoxicity of pregabalin on both mentioned cell lines in a time and concentration dependent manner. This cytotoxic effect was observed in both U937 and Molt-4 cells after 24 hours incubation. The cytotoxic effect of pregabalin on U937 cells was detected at 1000, ≥ 500 and ≥ 100 $\mu\text{g/ml}$ concentrations of drug after 24, 48 and 72 hours incubation, respectively. Moreover, cytotoxicity of pregabalin on Molt-4 cells was observed at 1000, ≥ 500 and ≥ 1 $\mu\text{g/ml}$ concentrations of drug after 24, 48 and 72 hours treatment, respectively. These results revealed that U937 and Molt-4 cells present different sensitivity to pregabalin after 72 hours incubation. Since, pregabalin significantly inhibited the U937 cells viability at ≥ 100 $\mu\text{g/ml}$ concentrations of drug after 72 hours incubation, while inhibited Molt-4 cells viability at ≥ 1 $\mu\text{g/ml}$ concentrations in the same time. However, it seems that these cells present similar sensitivity to pregabalin in shorter time intervals. In addition, the IC₅₀ values of pregabalin for U937 cells were 719.294, 652.433, and 594.291 $\mu\text{g/ml}$ after 24, 48 and 72 hours incubation respectively, while for Molt-4 cells were 916.919, 449.587 and 273.085 $\mu\text{g/ml}$ after 24, 48 and 72 hours treatment, respectively. These findings indicate more sensitivity of Molt-4 cells to pregabalin compared to U937 cells which may approve MTT data regard to higher sensitivity of Molt-4 cells to pregabalin related to U937 cells after 72 hours treatment suggesting that various cell

types exhibit different sensitivity pregabalin.

Similar to us, Salat et al studied the cytotoxic effect of pregabalin on in HepG2 and 3T3-L1 cell lines.¹¹ They observed that pregabalin does not have cytotoxicity effects on HepG2 and 3T3-L1 cells at 1-100 μM concentrations after 20 minutes incubation. This finding is in line with our results showed that pregabalin was not cytotoxic for leukemic cells at 1-100 μM concentrations. Jang et al have investigated the immunomodulatory effects of pregabalin on spleen cells in neuropathic mice.¹² They assessed NK cell activity and splenocyte proliferation from isolated spleen cells at different concentrations of pregabalin (3, 10, and 30 $\mu\text{g/mL}$) and observed that splenocytes proliferation and NK cell activity were suppressed at ≥ 10 $\mu\text{g/ml}$ concentrations of drug after 24 hours incubation time, while in our study, pregabalin was cytotoxic for leukemic cells at ≥ 1000 $\mu\text{g/ml}$ concentrations. The discrepancy between Jang et al. study and us may be due to the different sensitivity of splenocytes and leukemic cells to pregabalin. Adding to this, in Jang's study¹², cytotoxicity of pregabalin was assessed by using Brdu assay with enzyme linked immunosorbent assay method in presence of Phytohaemagglutinin (PHA) as a stimulator, while we assessed pregabalin cytotoxicity via MTT assay in absence of PHA.

The sensitivity of human U937 and Molt-4 cells to other drugs such as beta blockers has been studied.^{13,14} Cheng et al revealed that carvedilol induced programmed cell death in U937 cells with no significant cytotoxicity at ≥ 4.06 $\mu\text{g/ml}$ concentrations after 24h treatment, while in our study pregabalin showed significant cytotoxicity for leukemic cells at 1000 $\mu\text{g/ml}$ (6280.26 μM) concentration after 24 hours incubation. In Cheng et al study, the U937 cells were treated with 5×10^5 cells/ml with carvedilol and cytotoxicity was assessed by MTT assay and trypan blue dye exclusion method. Herein, we used 3×10^4 U937 cells/ml treated with pregabalin and MTT assay was used for cytotoxicity assessment. This discrepancy between our results and Cheng et al study may due to the number of facts including the number of used cells, type of drug and kind of assays. This discrepancy may also be due to the potent anti-proliferative property of carvedilol.^{13,15} In other similar study, Hajighasemi et al in an in vitro investigation showed the cytotoxicity of propranolol on human leukemic cells (Molt-4, Jurkat and U937) at $\geq 0.2\text{mM}$ (≥ 51.86 $\mu\text{g/ml}$) concentrations after 12 hours incubation. Here, we revealed the cytotoxicity of pregabalin on human leukemic Molt-4 and U937 cells in higher concentrations of pregabalin after 24, 48 and 72 hours treatment. This controversy could be explained by the fact that propranolol is a non-selective beta blocker which can potentially suppresses cancer cell viability.^{14,15} In addition we used pregabalin while Hajighasemi et al. used a different drug (propranolol).

Present study, for the first time provides evidences favoring cytotoxic effect of pregabalin on human leukemic U937 and Molt-4 cells. This cytotoxicity was time and dose dependent. Thus it seems that pregabalin along with its beneficial effects in cure of neuropathic pains, could have probable implication in treatment of

leukemic patients. However assessment of pregabalin cytotoxicity on normal cells as well as other cancer cells and its anti-tumoral properties in vitro and in vivo are warranted.

Conflict of Interest: None declared.

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