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Sensitivity of human leukemic cells to carvedilol

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Abstract

Background and Objective: Carvedilol, a non-specific β-blocker, has been used for treatment of hypertension, stroke and congestive heart failure. The therapeutic effects of β blockers in cancer patients have been shown. Carvedilol has considerable anti-inflammatory, anti-tumor and anti-angiogenic properties. In this study, the effects of carvedilol on proliferation of human U937 and Molt-4 leukemic cells were studied in vitro.

Materials and Methods: Human leukemic T cells [Molt-4] and monocytes [U937] were cultured in Roswell Park Memorial Institute (RPMI) 1640 complete medium and were treated with different concentrations of carvedilol (1, 5, 10, 20, 50 and 100 µg/ml) for 24, 48 and 72 hours. The cytotoxicity of carvedilol on U937 and Molt-4 cells was determined using MTT (3-[4, 5 dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay.

Results: Carvedilol significantly decreased human U937 and Molt-4 leukemic cells proliferation, concentration- and time-dependently in comparison with control cells.

Conclusion: According to our results, carvedilol has anti-proliferative effect on U937 and Molt-4 leukemic cells in a concentration- and time-dependent manner. Thus, carvedilol might be a useful candidate for treatment of leukemic patients as well as other cancers.

Key words: Carvedilol, U937, Molt-4, Sensitivity

1. Introduction

Cancer is one of the main causes of death in the world (1). After cardiovascular diseases, cancer is the second and third cause of mortality in developed and developing countries, respectively (2-4). Leukemia is a progressive malignant disease of leukocytes characterized by uncontrolled proliferation and increased number of immature leukocytes and their precursors in the blood and/or bone marrow (5). Leukemia accounts for approximately 8% of all cancers, has been observed in all age groups and causes significant mortality (5, 6). The conventional treatments for leukemia are chemotherapy, bone marrow transplantation, radiotherapy and monoclonal antibody therapy (7). However, these treatments usually have serious life-threatening adverse effects like anemia, increased susceptibility to infections, blood loss, secondary neoplasm, hair loss, extreme fatigue and heart disease (8).

Among the prevalent cardiovascular drugs, beta-blockers have notable utilization (9). Carvedilol is one of the non-specific β-blockers used clinically for treatment of hypertension, stroke, and congestive heart failure. Carvedilol improves these disorders not only by β-blocking activity but also by vascular dilatation, α1-blocking activity and anti-oxidant properties (10). Moreover, carvedilol is involved in inflammatory mediated diseases such as conjunctivitis, coxsackie virus B3-induced viral myocarditis and paraquat-induced lung injury (11-13). These studies have suggested that carvedilol modulates inflammation via inhibition and reduction of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, monocyte chemoattractant protein (MCP)-1, IL-8 and reduction in tissue

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remodeling factors like vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 (11-13).

Furthermore, improved survival and reduced tumor progression and mortality in several tumors such as breast, ovarian, lung and pancreatic cancer by use of β- blockers has been shown (14-19). Moreover, the anti-tumor effects of carvedilol are various and have been reported in several studies (19-21). In addition, it has been shown that carvedilol increases response to chemotherapy by direct anti-tumor and anti-angiogenic mechanisms in neuroblastoma (22). Also, suppression of IL-6 by carvedilol in hepatocellular carcinoma in rats has been revealed (23). The aim of this study was to evaluate the cytotoxic effect of carvedilol on human monocytic U937 and lymphocytic Molt-4 leukemic cells in vitro.

2. Material and Methods

2.1 Reagent

Roswell Park Memorial Institute (RPMI) 1640, penicillin and streptomycin were purchased from Sigma (USA). Dimethyl sulfoxide (DMSO) and MTT (3-[4, 5dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) were obtained from Merck (Germany). Fetal bovine serum (FBS) was obtained from Gibco (USA). Carvedilol was purchased from Pursina Pvt. Co. Ltd (Tehran, Iran). Microtiter plates, flasks and tubes were obtained from Nunc (Falcon, USA).

2.2. Cell lines

Human leukemic T cells [Molt-4 (NCBI C149)] and leukemic monocytes [U937 (NCBI C130)] were obtained from National Cell Bank of Iran, Pasteur Institute of Iran, Tehran (NCBI). RPMI-1640 complete medium was used for culture and maintenance of human leukemic cells at 37°C.

2.3. Preparation of carvedilol

Carvedilol was dissolved in DMSO and stored at -20°C until experimental use. This solvent was diluted in RPMI-1640 complete medium to prepare the needed concentrations of carvedilol before treating the cells.

2.4. Cell culture and treatment

The method has been described previously (24). Briefly, U937 and Molt-4 cells were cultured in RPMI-1640 complete medium. Cultured cells were seeded in 96 wells plates at 3×10^4 cells per well and then treated with different concentrations of carvedilol (1, 5, 10, 20, 50 and 100 µg/ml) for 24,48 and 72 hours. All assays were performed in five independent experiments.

2.5. MTT assay

In MTT assay, we assessed the conversion of yellow water soluble MTT to a blue insoluble formazan based on the method developed by Mosmann (25). At the end of incubation times (24, 48 and 72 h), the cell culture supernatant was substituted with fresh medium. Subsequently, 20 µl of MTT solution (5 mg/ml in PBS) was added to each well and then incubated at 37°C for 4 hours. Then, 100 µl of the acidic isopropanol solution (37% HCl), was added to each well and incubated at 37°C for 4 hours. The insoluble formazan derivative was dissolved and absorbance was measured at 492 nm using an ELISA microplate reader (Awareness Technology INC).

2.6. IC50 determination

The fifty percent inhibitory concentration (IC50) of carvedilol has been described as a concentration of drug which inhibited proliferation of the half leukemic cells. The IC50 value for carvedilol was determined by a dose-response curve based on MTT assay.

2.7. Statistical analysis

In the present study, the SPSS 24 package (SPSS Inc. Chicago, IL) software was used for data analysis and statistical calculation. The normal distribution of the continuous numerical variables was assessed by Kolmogorov-Smirnov Z-test. The effect of different concentrations of the drug on proliferation of leukemic cells was performed in five independent experiments (n=5) and the results were presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used to compare the cell proliferation between different groups. P value <0.05 was described as significant.

3. Results

The effects of different concentrations of carvedilol on proliferation of U937 and Molt-4 cells after 24, 48 and 72 hours of treatment are shown in Figures 1 and 3. DMSO (1%) was not cytotoxic neither for U937 nor for Molt-4 cells in all three-time intervals. So, DMSO as a solvent of carvedilol does not interfere with cells proliferation.

3.1. Effect of carvedilol on proliferation of U937 cells

As illustrated in Figure 1, carvedilol decreased the U937 cells proliferation in a time and concentration dependent manner. Accordingly, carvedilol significantly reduced the U937 cells proliferation at ≥100 µg/ml concentrations ($p<0.001$) and at ≥20 µg/ml concentrations ($p\leq0.001$) after 48 and 72 hours treatment, respectively (Figure 1).

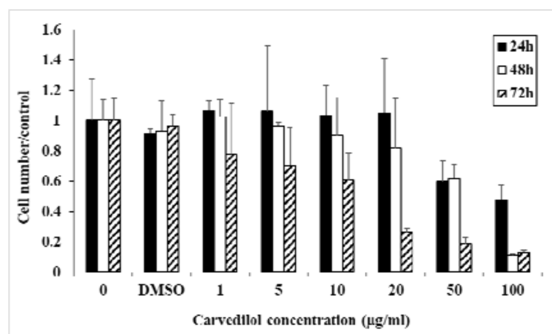

Figure 1. The effect of carvedilol on proliferation of U937 cells.

The U937 cells were treated with different concentrations of carvedilol (1-100 µg/ml) for 24, 48, and 72 hours. Cell proliferation was measured using MTT test. Data are presented as mean± SEM. * $p < 0.05$ was defined as statistical significance.

The IC50 of carvedilol on U937 cells after 24, 48 and 72 hours of treatment were obtained as 124.19, 57.93 and 36.24 µg/ml, respectively, based on a typical dose-response curve (Figure 2).

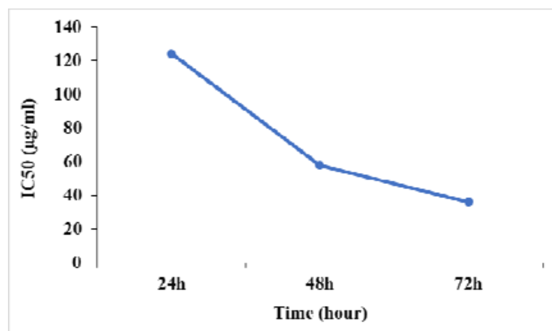

Figure 2. The IC50 of carvedilol in different time intervals on human U937 cells based on MTT data using a dose-response curve.

3.2. Effect of carvedilol on proliferation of Molt-4 cells

As shown in Figure 3, carvedilol decreased the Molt-4 cells proliferation dose and time dependency. This inhibitory effect was significant after 48 and 72 hours of treatment. After 48 hours of treatment, the anti-proliferative effect of carvedilol was significant at ≥20 µg/ml concentrations ($p<0.001$). Additionally, after 72 hours of treatment, the anti-proliferative effect of carvedilol was significant at ≥10 µg/ml concentrations ($p<0.001$) (Figure 3).

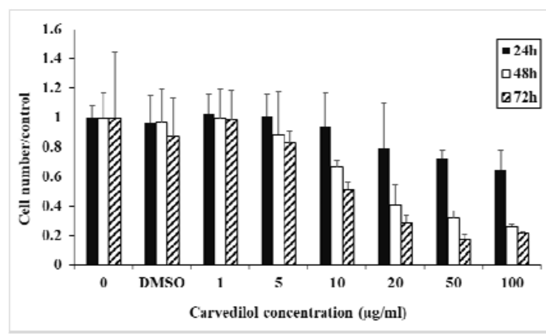

Figure 3. The effect of carvedilol on proliferation of Molt-4 cells.

The Molt-4 cells were treated with different concentrations of carvedilol (1-100 µg/ml) for 24, 48 and 72 hours. Cell proliferation was measured using MTT test. Data are presented as mean± SEM. * $p < 0.05$ was defined as statistical significance.

The IC50 of carvedilol on Molt-4 cells after 24, 48 and 72 hours of treatment were obtained as 88.5, 47.17 and 29.93 µg/ml, respectively, based on a typical dose-response curve (Figure 4).

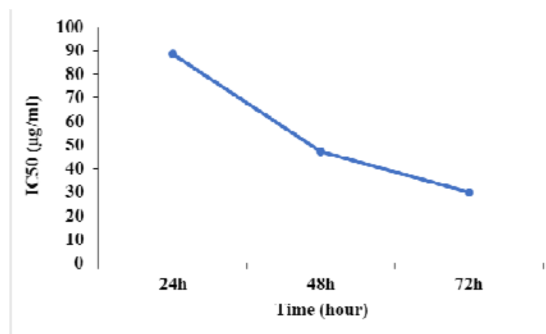

Figure 4. The IC50 of carvedilol in different time intervals on human Molt-4 cells based on MTT data using a dose-response curve.

4. Discussion

The results of the present study revealed that carvedilol has time and dose-dependent anti-proliferative effects on human leukemic U937 and Molt-4 cells. After 24 hours of incubation, carvedilol did not reduce proliferation of neither U937 nor Molt-4 cells, which is consistent with the IC50 of this drug at this time. The significant anti-proliferative effects of carvedilol for both U937 and Molt4 cells were shown after 48 hours of incubation. This cytotoxicity for U937 cells was shown at 100 µg/ml and ≥20 µg/ml concentrations of carvedilol after 48 and 72 hours of treatment, respectively. In addition, the anti-proliferative properties of carvedilol on Molt-4 cells was detected at ≥20 and ≥10 µg/ml concentrations of drug after 48 and 72 hours of incubation, respectively. Our data demonstrated that Molt-4 and U937 cells show different sensitivity to carvedilol. Accordingly, Molt-4 cells are more sensitive to carvedilol than

U937 cells. Anti-proliferative effects of carvedilol on Molt-4 and U937 cells were determined at ≥20 and =100 µg/ml concentrations after 48 h of incubation, respectively. Also, this cytotoxicity on Molt-4 and U937 cells was detected at ≥10 and ≥20 µg/ml concentrations, respectively, after 72 h of incubation. Furthermore, in our study, the IC50 of carvedilol for Molt-4 cells at 24, 48 and 72 h were 88.5, 47.17 and 29.93 µg/ml, respectively, while for U937 cells were 124.19, 57.93 and 36.24 µg/ml, respectively. Based on these data, the IC50 of carvedilol for Molt-4 was lower than U937 cells. This confirms that Molt-4 cells are more sensitive to carvedilol than U937 cells and once again demonstrates that different cells show different sensitivity to carvedilol. Consistent to our findings, Stanojkovic et al reported that different human malignant cells including breast tumor (MDA-MB-361), melanoma (Fem-x), cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562) showed different sensitivity to carvedilol (10).

Cheng et al studied the cytotoxic effect of carvedilol on human hepatoma cells (HA 59T) *in vitro* (26). They observed that carvedilol significantly decreased HA 59T cells viability at ≥1 µM (≥0.406 µg/ml) concentrations and also induced apoptosis in these cells at 30 µM (12.18 µg/ml) concentration after 24 hours of incubation. They reported the prolonged and uncontrolled levels of Ca^{2+} as the mechanism of apoptosis in these cells. But in our study, carvedilol did not show any cytotoxicity after 24 hours of treatment on U937 and Molt-4 cells. The difference between our results and Cheng et al study may be due to different used cell lines, different number of cells and different methods. Cheng et al used 10^4 cells/well and WST-1 reagents for determination of cell viability and flow cytometry assay for measurement of cell apoptosis while we used 3×10^4 cells/well and MTT method to evaluate the cytotoxic effect of carvedilol.

In another study, Cheng et al demonstrated that carvedilol can inhibit the granulocyte-macrophage colony-stimulating factor after (GM-CSF)-induced IL-10 production in U937 cells after 24 h of treatment (27). Also, Cheng et al found that carvedilol was cytotoxic at >10 µM (>4.06 µg/ml) concentrations. Moreover, in Cheng et al study, carvedilol induced apoptosis at 30-40 µM (12.18 - 16.24 µg/ml) concentrations. In the present study, carvedilol did not show any significant anti- proliferative effect on U937 cells at all concentrations after 24 h of treatment. This discrepancy between our study and Cheng et al may be partly due to the different-used methods and number of cells. Cheng et al used 5×10^5 cells/well and trypan blue dye exclusion method whereas we used 3×10^4 cells/well and MTT method.

In addition, increased response to chemotherapy by carvedilol in neuroblastoma has been reported (22). Pasquier et al investigated the anti-tumor and anti-angiogenic properties of carvedilol and other β-blockers alone and in combination with vincristine on BE (2)-C and SHEP neuroblastoma cell lines *in vitro* and *in vivo* (22). In Pasquier et al study, carvedilol reduced BE (2)-C and SHEP cells proliferation at 100 µM (40.6 µg/ml) concentration after 72 h of incubation. In our study, carvedilol showed cytotoxicity on U937 and Molt-4 at ≥20 and ≥10 µg/ml concentrations, respectively, after 72 hours. The difference between Pasquier et al study and our results may be partly due to a number of facts including cell types, used methods and number of cells. Pasquier et al used 3750 neuroblastoma cells/well and Alamar blue method for cytotoxicity evaluation (22) while we used 3×10^4 leukemic cells/well and MTT assay for cytotoxicity assessment.

In another study, Hajighasemi et al studied the sensitivity of human U937, Molt-4 and Jurkat leukemic cells to propranolol (24). They have shown that propranolol exhibited cytotoxic effect on U937, Molt-4 and Jurkat cells at ≥0.2 mM (≥50 µg/ml) concentrations after 12 hours. In Hajighasemi et al study (24), all three U937, Molt-4 and Jurkat cell lines presented similar sensitivity to propranolol. Conversely, in the present study, Molt-4 cells showed more sensitivity to carvedilol than U937 cells. This controversy may be somewhat due to the different investigated drugs. Hajighasemi et al (24) used propranolol while in the present study we used carvedilol which is more potent than propranolol (22).

The effect of beta-adreno receptor antagonists on proliferation, invasion and migration of malignant cells is well characterized (28-30). According to these studies, β-blockers are able to reduce cancer cells proliferation via induction of apoptosis, invasion and migration. The anti-tumoral properties of other β-blockers like metoprolol in cancer patients (18) maybe partly due to their direct cytotoxicity. Other similar studies have revealed that use of β-blockers is associated with reduced mortality, prolonged survival and more response to chemotherapy and subsequently better outcomes in different cancers including ovarian, breast, prostate, colorectal and etc (18, 22, 31-36). However, β-blockers do not seem to have an effect on cancer recurrence (31). In Chin et al study, carvedilol usage correlated with reduced risk of upper gastrointestinal tract and lung cancers (19).

Carvedilol, as a non-cardio selective β-blocker, and other β-blockers exert their anti-tumor and anti-angiogenic effects via targeting β-adrenoceptor (β-AR) 1&2 which are expressed by all types of cancers except neuroblastoma that only expresses β2-AR (37). According to a proposed model for β-AR signaling in cancer (38), catecholamines such as epinephrine and norepinephrine can act as an agonist for β-ARs and trigger activation of downstream effector systems like protein kinase A (PKA). Activated PKA is capable to active other mediators result in pathogenic processes including inflammation, angiogenesis, tissue invasion and apoptosis. It has also been shown that carvedilol can inhibit activation of cAMP/PKA-Src pathway in

MDA-231 cells and PKCδ-Src pathway in MCF-7 cells through blocking beta or alpha adrenergic receptors that lead to suppression of migration and invasion of malignant breast cells (21).

Taken together, our findings along with other investigations suggest that carvedilol with its β-blocking activity could be cytotoxic for tumor cells and different cancer cells have different sensitivity to carvedilol. Although anti-tumoral effects of carvedilol have been shown (21), its cytotoxicity on normal cells and its exact anti-tumor concentrations have not yet been determined. Therefore, it is necessary to investigate the cytotoxic effect of carvedilol on cancer as well as normal cells *in vivo*. Finally, our data suggest that carvedilol might be a putative useful drug for treatment of leukemic patients as well as other cancers.

Conclusion

The anti-proliferative effects of carvedilol in human leukemic U937 and Molt-4 cells are indicated in this study, offering promising therapeutic strategies to consider β-blockers as targets for leukemia treatment. The anti-tumoral effects of carvedilol shown by other investigators might be partly due to its anti-proliferative effects. Further studies on the anti-proliferative and cytotoxic effect of carvedilol in other cancerous as well as normal cells *in vivo* are required.

Conflict of interest

Authors declared that no conflict of interest exist.

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