**Effect of Probucol on Proliferation of Leukemia, Multiple Myeloma, Lymphoma, and Fibroblast Cells**

Probukolün lösemi, multipl miyeloma, lenfoma ve fibroblast hücre hatlarının proliferasyonu üzerindeki etkileri

**ABSTRACT**

**Objectives:** Probucol is a bisphenol antioxidant with antiinflammatory, antilipidemic and antidiabetic effect. Development and progression of cancer is closely related to chronic inflammation and oxidative stress. Agents that target these processes have been shown to modulate cancer cell proliferation. In this regard, the effect of probucol on proliferation of different cancer cell lines was investigated.

**Materials and Methods:** Different concentrations of probucol solutions were prepared and applied to the following cancer cell lines: K562S (imatinib sensitive) and K562R (imatinib resistant) chronic myeloid leukemia (CML) cells; U937 histiocytic lymphoma cells; HL60 acute myeloid leukemia cells; U266, H929, and RPMI8226 multiple myeloma cells; and L929 fibroblast cells. Cell viability was conducted by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

**Results:** Significant toxicity was not exhibited due to probucol treatment (0.1-10 μM) in K562S and K562R CML cells, U937 histiocytic lymphoma cells, HL60 acute myeloid leukemia cells, U266 multiple myeloma cells, and L929 fibroblast cells. However, probucol treatment significantly inhibited the viability of H929 and RPMI8226 multiple myeloma cells at the concentration of 0.5-10 μM and 5-10 μM, respectively.

**Conclusion:** Probucol treatment slightly inhibited the viability of other cancer cell lines, but significantly inhibited the viability of H929 and RPMI8226 multiple myeloma cells. However, its effect was not potent, since a 50% reduction in cell viability could not be achieved at the concentrations of probucol treatment administered.

**Key words:** Probucol, chronic myeloid leukemia, multiple myeloma, histiocytic lymphoma, acute myeloid leukemia

**ÖZ**

Amaç: Probukol, antiinflamatuar, antlipidemik ve antidiabetik aktivitelerine sahip bir antioksidan bir karasıdır. Karnar gelişimi ve ilerlemesi kronik infiamasyon ve oksidatif stres ile yakın bir ilişkidir ve bu süreçlerin hedeflenmiş olan kanser hücre proliferasyonunu modüle etkileri göstermiştir. Bu bağlamda, probukolün farklı kanser hücre hatlarının proliferasyonu üzerindeki etkileri araştırılmıştır.

Gereç ve Yöntemler: Probukolün farklı konsantrasyonları hazırlanarak uygulanan hücreler K562S (imatinib duyarlı) ve K562R (imatinib dirençli) kronik miyeloid lösemi (CML) hücreleri, U937 histiositik lenfoma hücreleri, HL60 akut miyeloid lösemi hücreleri, U266 multipl myeloma hücreleri ve L929 fibroblast hücreleri olarak ele alınmıştır.

Bulgular: Probukol uygulaması (0,1-10 μM) K562S ve K562R CML hücreleri, U937 histiositik lenfoma hücreleri, HL60 akut miyeloid lösemi hücreleri, U266 multipl myeloma hücreleri ve L929 fibroblast hücreleri üzerinde anlamlı etkiler sergilememiştir. Diğer taraftan, H929 ve RPMI8226 multipl myeloma hücreleri üzerinde sırasıyla 0,5-10 μM ve 5-10 μM konsantrasyon aralığında anlamlı olarak etkisizdir (p<0,05).

Sonuç: Probukol uygulaması H929 ve RPMI8226 multipl miyelom hücre hatlarının canlanması anlamlı olarak inhibe etmiştir. Fakat, probukolün bu etkisi potansiyel bulunmamıştır ve uygulanan konsantrasyon aralığındaki hücre canlanması %50 ve üzerinde azalma sergilemediği belirlenmiştir.

Anahtar kelimeler: Probukol, kronik miyeloid lösemi, multipl miyelom, histiositik lenfoma, akut miyeloid lösemi
INTRODUCTION
Probucol is a bisphenol, which was originally synthesised as an antioxidant compound with antilipemic effect. Probucol has been shown to inhibit the oxidation of low density lipoprotein (LDL) and lower the level of cholesterol in the bloodstream by increasing the rate of catabolism of LDL. Although probucol has been used extensively as a lipid-lowering drug in Japan, its reducing effect on serum high density lipoprotein cholesterol has been used extensively as a lipid-lowering drug in Japan, its pleiotropic functions and effects. According to these studies, probucol has been demonstrated to inhibit inflammation due to its antinflammatory effect in beta cells, as well as its inhibitory activity on nuclear Factor kappa B (NF-κB) in spinal cord inflammation.

Oxidative stress and chronic inflammation are among the characteristic features of neoplastic diseases. Overproduction of reactive oxygen species within the cell plays a major role in signaling pathways, leading to the initiation and progression of cancer and possible drug resistance. In contrast, some studies showed that increasing oxidative stress may aid the elimination of cancer. Type, stage, oxygen dependence, metastasis, and angiogenesis status under hypoxia are among the determinants of the modulation of cancer cell viability by oxidative stress. Therefore, targeting oxidative stress has a great potential in cancer therapy. Treatment of cancer cells with antioxidants or oxidative stress inducing agents have been widely examined in different studies. In literature, a wide range of antioxidant compounds, such as N-acetyl cysteine, vitamin E, epigallocatechin gallate, vitamin C, and curcumin have been examined for their potential effects in cancer prevention. Antioxidants, including ascorbic acid, naringenin, and curcumin, have been examined to induce apoptosis in K562 chronic myeloid leukemia (CML) and HL60 acute myeloid leukemia cells. In addition to the above mentioned antioxidant compounds, resveratrol has been shown to inhibit proliferation, migration, and invasion of U266 multiple myeloma cells; however, quercetin (an antioxidant and antiinflammatory compound) was found to inhibit RPMI8226 multiple myeloma cell proliferation by inducing cell cycle arrest and apoptosis.

Based on these findings, we aimed to analyze the effects of a known antioxidant and antiinflammatory agent (probucol) on viability of K562S and K562R CML cells, U937 histiocytic lymphoma cells, HL60 acute myeloid leukemia cells, U266 multiple myeloma cells, and L929 fibroblast cells.

MATERIALS AND METHODS

Cell culture
Human multiple myeloma cell lines [H929, U266, and RPMI8226 multiple myeloma; human CML (K562), human histiocytic lymphoma (U937), human acute myeloid leukemia (HL60), and mouse fibroblast (L929)] were cultured in RPMI-1640 (Sigma) cell culture medium including: 10% heat inactivated fetal bovine serum, L-glutamine (2 mmol/L), 100 μ/mL penicillin, and 100 μg/mL streptomycin. Cells were incubated at 37°C, 5% CO₂, and 95% humidity. At 80% confluence, adherent L929 cells were passaged with trypsinization.

Imatinib resistant K562 cell line
K562R cells, which are resistant to 5 μM imatinib, were used in our experiments. 0.6 μM imatinib resistant cells were kindly provided by Prof. Carlo Gambacorti-Passerini from University of Milano-Bicocca, Monza, Italy. The Imatinib resistant cells were increased to 5 μM by incubating the cells with increasing concentration of imatinib at time intervals.

Cell viability
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted to test the effect of probucol on the various human cancer cell lines and mouse fibroblast cells. For this purpose, cells (20,000 cells/well) were seeded onto 96-well plates. 20 mM stock solution of probucol was prepared by dissolving probucol in dimethyl sulfoxide (DMSO) and further dilutions were prepared with culture medium. Maximum concentration of probucol applied to cells was 10 μM, due to low solubility of the compound at higher concentrations. H929, U266, RPMI8226, U937, and HL60 cells were treated with 0.1-10 μM probucol for 72 h at 37°C. Control cells were incubated with the same concentration of DMSO as the probucol-treated cells and the DMSO concentration never exceeded 0.5%. For each cell line, the same protocol was followed. K562S (imatinib sensitive) and K562 R (imatinib resistant) cells were treated with probucol 0.1-10 μM, imatinib, and imatinib/probucol combination for 72 h.

Imatinib concentrations used for K562S and K562R cells were 0.5 μM and 20 μM, respectively. Since imatinib is a first line therapeutic option for CML, we determined its single effect or combination effect with probucol. The effect of probucol was also determined in non-cancerous L929 fibroblast cell line. After proper incubation, MTT solution (5 mg/mL) was prepared with phosphate buffer saline and cells were incubated with MTT solution for a period of four hours. Insoluble formazan crystals were dissolved by SDS-HCl solution. Absorbance was measured at 550 nm using a microplate reader (Molecular Devices-Spectra Max spectrophotometer, Sunnyvale, CA, USA).

Statistical analysis
One-way analysis of variance with Tukey post hoc test was performed with StatistiXL Software (Nedlands, Western Australia, 6009). Data were presented as mean ± standard deviation. P values less than 0.05 was considered statistically significant.

RESULTS
According to our experiments, probucol significantly decreased the viability of H929 and RPMI8226 cells at the concentrations of 0.5-10 μM and 5-10 μM, respectively. However, we did not
observe any antiproliferative effect on the other cell lines (Figure 1).

In our previous study, we administered 0.5 μM and 20 μM imatinib to K562S and K562 R cells, respectively, which are the known cytotoxic concentrations of imatinib in these cells. Treatment with imatinib alone significantly decreased the viability of K562S and K562R cells to 36.92±3.44% and 48.02±1.55%, respectively (p<0.001). On the other hand, probucol did not exhibit any antiproliferative effect on both cell lines either as a single agent or in combination with imatinib (Figure 2 and 3).

**DISCUSSION**

In literature, various studies have investigated the efficacy of probucol treatment in cancer. In one of these studies, directed nanoassembly formulation of probucol was shown to suppress lung metastasis of breast cancer and, in another study, it inhibited benzopyrene-induced lung tumorigenesis. In addition, probucol was reported to exhibit its antiproliferative effects via inhibition of cell cycle progression and inactivation of NF-κB and mitogen-activated protein kinase pathways in human ovarian cancer cells. Probucol was also reported to exert chemopreventive effect in kidney cancer and probucol treatment of H929 and RPMI8226 multiple myeloma cell lines, and L929 fibroblast cells. In contrast to studies that reported a reduction of cancer cell proliferation by probucol treatment, we did not observe any potent inhibition of cell viability at 0.1-10 μM probucol treatment for all the cell lines investigated. Probucol significantly inhibited the viability of H929 and RPMI8226 at the concentrations of 0.5-10 μM and 5-10 μM, respectively, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

**CONCLUSION**

Our results collectively showed that probucol was not effective in inducing cell death as a single agent in U937 histiocytic lymphoma cells, HL60 acute myeloid leukemia cells, and U266 multiple myeloma cells. Although probucol significantly inhibited H929 and RPMI8226 cell proliferation at particular doses, its effects were not potent. Probucol combined with imatinib did not alter the viability of K562S and K562R cells.
thus showing its ineffectiveness as a combinatorial therapeutic agent in CML.

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