

New indication for therapeutic potential of an old well-known drug (propranolol) for multiple myeloma

Ilknur Kozanoglu · Melis Kartal Yandim ·
Zeynep Birsu Cincin · Hakan Ozdogu ·
Bedia Cakmakoglu · Yusuf Baran

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Abstract

Purpose Propranolol, a non-selective β -adrenergic receptor blocker, has been used for the treatment of the patients with hypertension for more than 50 years. There are several in vitro and in vivo evidences that β -adrenergic receptor antagonists inhibit proliferation and angiogenesis and also increase apoptosis in breast, skin, and colon cancers. The aim of this study was to investigate the cytotoxic and apoptotic effects of propranolol and the genes involved in propranolol-induced apoptosis in multiple myeloma cells.

Methods Time-dependent antiproliferation and apoptotic effects of propranolol were subsequently determined by MTT cell proliferation assay, changes in caspase-3 activity, loss of mitochondrial membrane potential (MMP), and also the localization of phosphatidylserine in the plasma

membrane. Changes in expression levels of NF- κ B pathway were examined by qRT-PCR array.

Results IC50 values of propranolol on U266 cells were calculated as 141, 100, and 75 μ M after 24-, 48-, and 72-h propranolol exposure, respectively. There were significant increases in caspase-3 activity, loss of MMP, and increases in apoptotic cell population in response to propranolol in U266 cells in a time- and dose-dependent manner. There were increases in expression levels of BCL10, TRAF family members, interleukins, TLR1-4, TNFRSF10B, NF- κ B, and the inhibitors of NF- κ B genes, and significant decreases in expression levels of Bcl-2 in response to propranolol treatment were observed.

Conclusion These results revealed that propranolol has antiproliferative and apoptotic effects on multiple myeloma cells. Being supported with in vivo analyses, propranolol can be a good and economical way to treat multiple myeloma patients.

I. Kozanoglu
Adana Adult Bone Marrow Center, Cell Processing Unit,
Baskent University, Ankara, Turkey

I. Kozanoglu
Faculty of Medicine, Department of Physiology,
Baskent University, Ankara, Turkey

M. K. Yandim · Y. Baran (✉)
Department of Molecular Biology and Genetics,
Izmir Institute of Technology, 35430 Urla, Izmir, Turkey
e-mail: yusufbaran@iyte.edu.tr; iytecancer@gmail.com

Z. B. Cincin · B. Cakmakoglu
Department of Molecular Medicine, Institute of Experimental
Medical Research, Istanbul University, Capa, Istanbul, Turkey

H. Ozdogu
Bone Marrow Transplantation Unit, Faculty of Medicine,
Adana Teaching and Medical Research Center,
Baskent University, Adana, Turkey

Keywords Multiple myeloma · Propranolol · NF- κ B pathway · Apoptosis

Introduction

Propranolol was successfully developed as a first beta-blocker in 1960s (Emilien and Maloteaux 1998). Propranolol, a non-selective β -adrenergic blocking agent, is used predominantly for the treatment for hypertension, cardiac arrhythmias, coronary artery disease, thyrotoxicosis, migraine headache, and a number of other conditions such as psychiatric diseases (Emilien and Maloteaux 1998; Frohlich 1977; Featherstone 1983; Lee et al. 1982). In recent years, the data obtained from meta-analysis and in vitro and in vivo experimental studies showed that beta-

receptor antagonists inhibit tumor proliferation and metastasis in breast, stomach, skin, and colon cancers (Masur et al. 2001; Benjamin et al. 2010; Slotkin et al. 2000; Glasner et al. 2010; Benish et al. 2008; Algazi et al. 2004; Park et al. 1995). In vitro, the antiangiogenic biological activity of propranolol was also shown to inhibit human brain microvascular endothelial cell tubulogenesis (Annabi et al. 2009). Propranolol has recently been introduced as a pharmacologic treatment for infantile hemangiomas (Sidbury 2010; Storch and Hoeger 2010; Léauté-Labrèze et al. 2008; Sarialioglu et al. 2010; Sans et al. 2009; Sommers Smith and Smith 2002; Love and Sikka 2004; Buckmiller et al. 2010).

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the aberrant expansion of plasma cells within the bone marrow, as well as at extramedullary sites (Mahindra et al. 2010). MM accounts for 13.4 % of all hematologic malignancies diagnosed, 19 % of all deaths resulting from hematologic malignancies, and 2 % of all cancer deaths (Mahindra et al. 2010). MM is an incurable disease, although patient survival has increased with the availability of novel agents (Mahindra et al. 2010; Minnema et al. 2010). Both multiple myeloma and its therapies often affect the renal, immune, skeletal, hematologic, and nervous systems (Minnema et al. 2010; Richardson et al. 2007; Elouni et al. 2010). The resulting organ dysfunctions often impair the life quality of affected patients, complicate and limit subsequent therapies, and may result in significant mortality.

Our aim in this study was to investigate the in vitro effects of the non-selective β -adrenergic receptor blocker propranolol on U266 MM cells and to examine the mechanisms involved in propranolol-induced apoptosis.

Materials and methods

Cell line and culture conditions

Human U266 multiple myeloma cells were obtained from German Collection of Microorganisms and Cell Cultures (Germany). Propranolol was kindly provided by Baskent University, Department of Hematology (Sanofi Aventis, Istanbul, Turkey). 10 mM stock solutions of propranolol was prepared in dimethyl sulphoxide (DMSO) and stored at -20°C . U266 human multiple myeloma cells were cultured in RPMI-1640 growth medium containing 10 % fetal bovine serum and 1 % penicillin–streptomycin at 37°C in 5 % CO_2 .

Measurement of cell growth by MTT assay

Time-dependent IC₅₀ value (drug concentration inhibits cell growth by 50 %) of propranolol was determined by MTT cell proliferation assay. In short, 1×10^4 cells/well

were seeded into 96-well plates containing 100 μL of the growth medium in the absence or presence of increasing concentrations of propranolol and then incubated at 37°C in 5 % CO_2 for 24, 48 and 72 h. After incubation period, cells were treated with 20 μL MTT (5 mg/mL) for 4 h. Then, plates were centrifuged for 10 min at 1,800 rpm. After centrifugation, supernatants were removed from the plates and then the MTT crystals were homogenized by adding 100 μL DMSO into each well. In order to homogenize the pellets more efficiently, the plates were shaken for 5 min by shaker. Afterward, the plates were read under 570 nm wavelengths by Elisa reader (Thermo Electron Corporation Multiskan Spectrum, Finland). Finally, IC₅₀ value of propranolol was calculated according to the cell proliferation plots.

Analysis of caspase-3 enzyme activity

Changes in caspase-3 enzyme activity of the cells, as an important sign of apoptosis, were examined by caspase-3 colorimetric assay kit (BioVision Research Products, USA). This assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the labeled substrate DEVD-*p*NA that can be recognized by caspase-3. In short, the cells (1×10^6 cells/2 mL/well), induced to undergo apoptosis, were collected by centrifugation at 1,000 rpm for 10 min. The cells were lysed by adding 50 μL of chilled $1 \times$ Cell Lysis Buffer and incubated on ice for 10 min before centrifugation at 10,000g for 1 min. Supernatants were transferred to new Eppendorf tubes, and the reaction mixture was prepared in 96-well plates by adding 50 μL of $2 \times$ Reaction Buffer (containing 10 mM DTT), 50 μL of sample, and 5 μL of DEVD-*p*NA substrate and incubated for 2 h at 37°C in CO_2 incubator. At the end of this period, the plate was read under 405 nm wavelengths by Elisa reader (Thermo Electron Corporation Multiskan Spectrum, Finland). The absorbance values are normalized to protein concentrations determined by Bradford assay as described previously.

Determination of loss in mitochondrial membrane potential

We have also examined the loss of MMP, another important sign of apoptosis, in response to propranolol in U266 cells by JC-1 Mitochondrial Membrane Potential Detection Kit (Cayman Chemicals, USA). This kit uses JC-1, a unique cationic dye, to signal the loss of the MMP. JC-1 accumulates in the mitochondria which stain red in non-apoptotic cells, while in apoptotic cells, the MMP collapses, and thus the JC-1 remains in the cytoplasm as a monomer that stains green under fluorescent light. Briefly, the cells (1×10^6 cells/2 mL), induced to undergo

apoptosis, were collected by centrifugation at 1,000 rpm for 10 min. Supernatants were removed, pellets were homogenized by 300 μ L of medium, and 30 μ L of JC-1 dye was added onto the cells; then, the cells were incubated at 37 °C in 5 % CO₂ for 30 min. Then, they were centrifuged at 400g for 5 min, supernatants were removed, and 200 μ L of assay buffer was added onto the pellets and vortexed. Then, this step was repeated once more. Afterward, all pellets were re-suspended with 320 μ L assay buffer and 100 μ L from each of them was added into the 96-well plate as triplicates. In healthy cells, the aggregate red form has absorption/emission maxima of 560/595 nm, whereas in apoptotic cells, the monomeric green form has absorption/emission maxima of 485/535 nm. The plate was read in these wavelengths by fluorescence Elisa reader (Thermo Varioskan Spectrum, Finland). At the end, green/red (485/560) values were calculated to determine the changes in MMP.

Analysis of apoptotic cells by AnnexinV-FITC staining

In addition to the analysis of changes in caspase-3 enzyme activity and mitochondrial membrane potential as apoptotic markers, we have also determined the translocation of phosphatidylserine from the inner membrane to the outer cell membrane. Initially, 1×10^6 cells were treated with increasing concentrations of propranolol (50–200 μ M) for 24, 48 and 72 h. After the incubation periods, cells were washed twice with cold PBS and then re-suspended with 1 mL $1 \times$ binding buffer. Then, 100 μ L of this solution was added into glass tubes. 5 μ L of AnnexinV-FITC and 10 μ L of PI were added onto the solutions. These samples were vortexed gently and then incubated for 15 min at RT in the dark. Afterward, 400 μ L of $1 \times$ binding buffer was added to each tube, and then they were analyzed by flow cytometry (BD Facsanto Flowcytometry, Belgium) within 1 h.

Total RNA isolation from cells and reverse transcriptase-polymerase chain reaction NF- κ B TaqMan array (qRT-PCR TaqMan array)

The cells were incubated in the absence and presence of increasing concentrations of propranolol for 72 h, and total RNAs were isolated by using RNA Isolation Kit (High Pure Isolation Kit, Roche, USA). mRNAs from the total RNA population were reverse-transcribed into cDNA using reverse transcriptase enzyme (Transcriptor First Strand cDNA Synthesis Kit, Roche, USA). The resulting total cDNA was used in PCR to measure the mRNA levels of Human NF- κ B Pathway (TaqMan® Array Human NF- κ B Pathway, Applied Biosystems). This assay panel targets genes which encode the 5 proteins of the Rel/NF κ B family: NFKB1, NFKB2, REL A, REL B, and REL. Also, on

the panel, there are genes from families that include the I κ B kinase; I κ Bs (inhibitor of κ B); Toll-like receptor (TLR); tumor necrosis factor (TNF); tumor necrosis factor receptors (TNFR); and tumor necrosis factor receptor-associated factor (TRAF). Additional genes associated with NF- κ B function in apoptosis, immune/inflammation responses, as well as chemokines and cytokines are included. mRNA levels of HPRT and GSUB were used as endogenous positive control.

Results

Propranolol inhibited proliferation of U266 human multiple myeloma cells in a time- and dose-dependent manner

In order to determine antiproliferative effects of propranolol on human U266 MM cells, the cells were incubated with increasing concentrations of propranolol for 24, 48 and 72 h and MTT cell proliferation assay was conducted. The results of these assays showed that there were time- and dose-dependent decreases in cell proliferation as compared to untreated controls. Different exposure times resulted in two different IC₅₀ values. IC₅₀ values of propranolol for 24, 48 and 72 h were calculated from cell proliferation plots and found to be 100 and 75 μ M, respectively (Fig. 1).

Propranolol increases caspase-3 enzyme activity in a time- and dose-dependent manner

In order to determine apoptotic effects of propranolol on U266 MM cells, these cells were incubated with increasing concentrations of propranolol for 24, 48 and 72 h and changes in caspase-3 enzyme activities were analyzed.

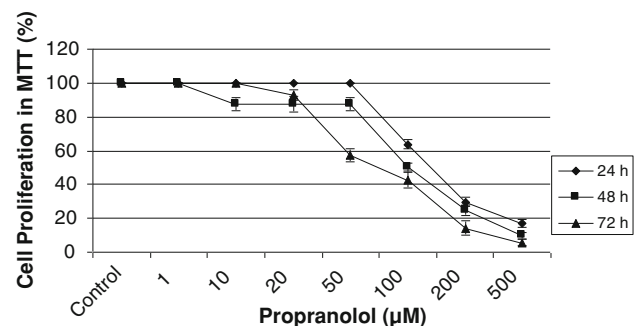


Fig. 1 Antiproliferative effects of propranolol on U266 cells. The IC₅₀ value of propranolol was calculated from cell proliferation plots. The MTT assays were performed using triplicate samples in at least two independent experiments. Statistical significance was determined using two-way analysis of variance, and $p < 0.05$ was considered significant

There were 1.10-, 1.18-, and 1.50-fold increases in caspase-3 activity in response to 48-h incubation with 50, 100, and 200 μM propranolol, respectively, as compared to untreated cells (Fig. 2). The same concentrations of propranolol increased caspase-3 activity 1.46-, 1.49-, and 1.55-fold after 72-h incubation, respectively (Fig. 2). Propranolol induced apoptosis in a dose-dependent manner that is also directly related with the caspase-3 activity.

Propranolol induces the loss of mitochondrial membrane potential in a time- and dose-dependent manner

In order to assess the loss of MMP, U266 cells were exposed to 50, 100, and 200 μM propranolol for 48 and 72 h and JC-1 MMP assay was conducted. The results of this assay revealed that there were 1.35-, 1.11-, and 82.5-fold increases in loss of MMP in response to 50, 100, and 200 μM propranolol for 48 h, respectively (Fig. 3). The same concentrations of propranolol induced loss of MMP for 1.73-, 2.10-, and 127.69-fold after 72-h incubation as compared to untreated control group, respectively (Fig. 3).

Propranolol causes the modulation of the cell membrane resulting in the translocation of PS from the inner leaflet to the outer one in a time- and dose-dependent manner

In order to confirm the results of caspase-3 activity and loss of MMP, FITC AnnexinV/PI double staining was conducted in U266 cells exposed to 50, 100, and 200 μM propranolol for 24, 48 and 72 h. The results demonstrated that 48-h incubation of U266 cells with these concentrations of propranolol increased apoptotic cell death by 14, 232, and 555 % as compared to untreated control group (Figs. 4, 5a), while 72-h incubation increased apoptosis by 48, 370, and 802 %, as compared with untreated controls, respectively (Figs. 4, 5b).

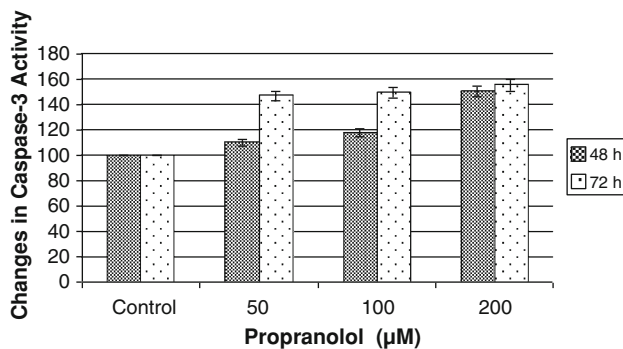


Fig. 2 Changes in caspase-3 enzyme activity in response to increasing concentrations of propranolol in U266 cells. The results are the means of two independent experiments. $p < 0.05$ was considered significant

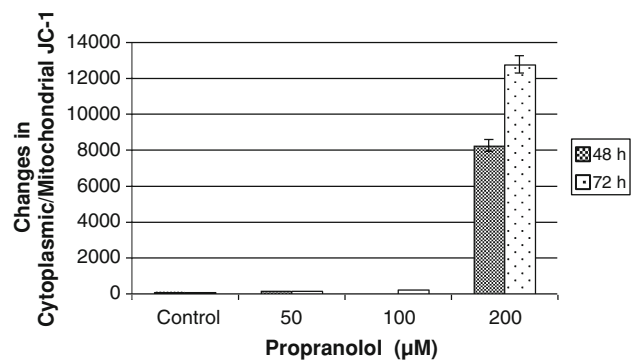


Fig. 3 Loss of mitochondrial membrane potential in response to increasing concentrations of propranolol in U266 cells. The results are the means of two independent experiments. $p < 0.05$ was considered significant

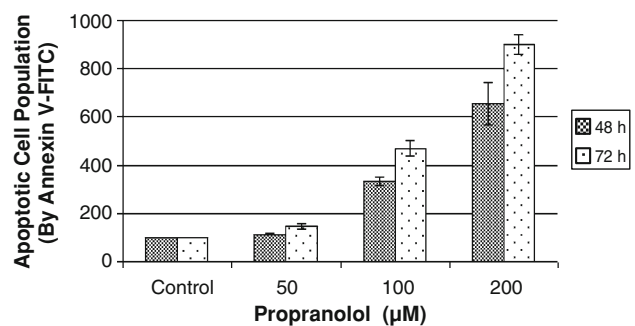
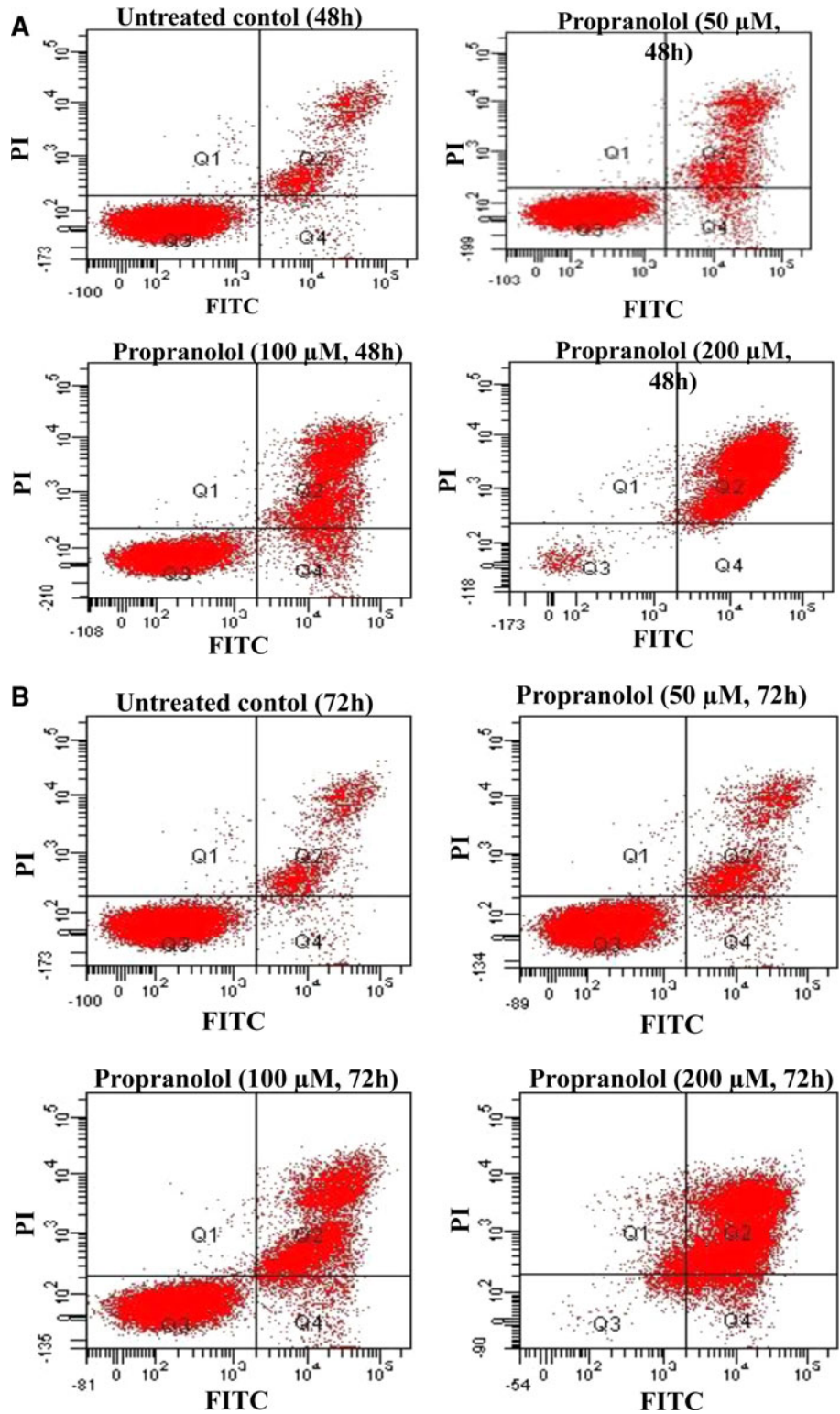


Fig. 4 FITC AnnexinV/PI double staining of U266 cells treated with increasing concentrations of propranolol. The results are the means of two independent experiments. $p < 0.05$ was considered significant

Changes in expression levels of NF- κB pathway genes in response to propranolol

In order to analyze the genes involved in NF- κB pathway regulated by propranolol treatment, U266 cells were treated with increasing concentrations of propranolol (20, 50, and 100 μM) and expression levels of approximately 80 genes involved in NF- κB pathway were determined by qRT-PCR array. According to the array results, the expression levels of some important genes were changed significantly. For instance, the expression levels of apoptotic Bcl-10 gene increased by approximately sevenfold in response to 50 μM propranolol. However, the expression levels of antiapoptotic Bcl-2 gene decreased around fivefold in response to the same concentration of propranolol. In addition, the expression levels of tumor necrosis factor receptor-associated factor (TRAF) genes increased in response to increasing concentrations of propranolol. These genes lead to antiapoptotic events by interacting with inhibitor of apoptosis proteins (IAPs). There were also increments and decrements in response to propranolol in expression levels of TLR family. Importantly, the expression levels of tumor necrosis factor receptor superfamily

Fig. 5 Flow cytometric analysis of apoptosis in U266 cells. Early apoptotic cells labelled with Annexin-V but not PI (shown in *lower right quadrant*) and apoptotic cells labelled with Annexin-V and PI were found in the *upper right quadrant* in flow cytometric graphics



member 10b (TNFRSF10B), an important transducer of apoptotic signals, increased significantly. There were 4.8-, 156.25-, and 700-fold increases in expression levels of TNFRSF10B in response to 20, 50, and 100 μM propranolol, respectively, as compared to control group.

Furthermore, there were 3.86- and 16.6-fold increases in IL-10 expression levels in response to increasing concentrations of propranolol. IL-10 is known to suppress NF-κB activity, and increase B-cell survival, proliferation, and antibody production. Treatment of U266 cells with

increasing concentrations of propranolol also caused increases in the expression levels of IL-6 and IL-8 genes. Moreover, there were significant changes in the expression levels of NF- κ B in response to propranolol. There were 2.1-, 2.28-, and 6-fold increases in a dose-dependent manner (10 and 50 μ M). Nevertheless, there were also increases in the expression levels of the inhibitors of NF- κ B. NF- κ BIA expression levels decreased approximately twofold, and NF- κ BIB expression increased by fivefold in response to 20 μ M propranolol. In addition, the expression levels of RIPK1 gene, which is involved in NF- κ B pathway and also cellular necrosis, were shown to be increased for 4.5- and 6.33-fold in 50 and 100 μ M propranolol-treated cells, respectively (Table 1).

Discussion

Propranolol is a non-selective β -adrenergic antagonist and is widely used clinically for various conditions including hypertension, anxiety, cardiac arrhythmias, and thyrotoxicosis (Emilien and Maloteaux 1998; Frohlich 1977; Featherstone 1983; Lee et al. 1982). Clinical benefits have been observed in combination with COX-2 inhibitors in postoperative cancer patients, in whom perioperative treatment resulted in improved immune competence and in reduced risk of tumor metastasis (Lee et al. 1982; Masur et al. 2001; Benjamin et al. 2010; Slotkin et al. 2000). It was therefore inferred that blockade of β -adrenergic receptor functions would affect tumor development, an effect that was confirmed by the inhibition of experimentally induced pulmonary adenocarcinoma development (Park et al. 1995). The contribution of β -adrenergic receptor functions to tumorigenesis was also reflected by the suggested antiangiogenic effects of β -blockers on a tumor-associated endothelial cell model (Park et al. 1995; Annabi et al. 2009; Sidbury 2010; Sommers Smith and Smith 2002).

Propranolol may revolutionize the treatment for problematic hemangiomas that cause imminent functional or cosmetic sequelae (Storch and Hoeger 2010; Léauté-Labrèze et al. 2008; Sarialioglu et al. 2010; Sans et al. 2009; Buckmiller et al. 2010). At therapeutic doses, propranolol is safe and effective in the majority of patients. Early effects (brightening of the hemangioma surface within 1–3 days after start of therapy) are attributable to vasoconstriction due to decreased release of nitric oxide. Intermediate effects are due to the blocking of proangiogenic signals (vascular endothelial growth factor, basic fibroblast growth factor, and matrix metalloproteinase 9) and result in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells and result in tumor regression

Table 1 Changes in expression levels of the genes in NF- κ B pathway

| Genes | Control | 20 | 50 | 100 |
|-----------|---------|--------|--------|--------|
| NFKB2 | 100 | 200 | 227 | 613 |
| RELB | 100 | 1,108 | 1,411 | 11,164 |
| NFKBIA | 100 | 72 | 69 | 47 |
| NFKBIB | 100 | 503 | 241 | 524 |
| NFKBIE | 100 | 171 | 297 | 789 |
| NKIRAS1 | 100 | 31 | 517 | 486 |
| NKIRAS2 | 100 | 0 | 164 | 453 |
| IKBKB | 100 | 131 | 50 | 256 |
| IKBKG | 100 | 0 | 245 | 919 |
| BTRC | 100 | 101 | 243 | 466 |
| CHUK | 100 | 119 | 236 | 204 |
| RIPK1 | 100 | 62 | 450 | 628 |
| TBK1 | 100 | 24 | 106 | 426 |
| IL10 | 100 | 366 | 1,611 | 0 |
| IL6 | 0 | 100 | 234 | 0 |
| IL8 | 100 | 104 | 882 | 1,204 |
| IRAK1BP1 | 100 | 255 | 71 | 0 |
| IRAK2 | 100 | 0 | 49,456 | 39,618 |
| TNF | 100 | 408 | 361 | 938 |
| TNFAIP3 | 100 | 121 | 93 | 68 |
| TNFRSF10B | 100 | 429 | 13,814 | 63,915 |
| TNFRSF1A | 100 | 293 | 62 | 153 |
| TNFSF15 | 100 | 3,406 | 246 | 42 |
| LTA | 100 | 378 | 711 | 1,851 |
| TLR1 | 100 | 517 | 10,990 | 0 |
| TLR2 | 0 | 100 | 0 | 4,784 |
| TLR3 | 100 | 2,476 | 0 | 2,710 |
| TLR6 | 100 | 11,536 | 2,581 | 0 |
| TLR9 | 100 | 238 | 0 | 45 |
| TRAF1 | 100 | 7 | 0 | 945 |
| TRAF2 | 100 | 281 | 201 | 406 |
| TRAF3 | 100 | 279 | 47 | 140 |
| TRAF4 | 100 | 489 | 373 | 1,336 |
| TRAF5 | 100 | 57 | 66 | 129 |
| ZNF675 | 100 | 0 | 203 | 57 |
| MAP3K1 | 100 | 138 | 486 | 664 |
| MAP3K14 | 100 | 72 | 235 | 524 |
| BCL10 | 100 | 0 | 682 | 318 |
| BCL2 | 100 | 46 | 22 | 56 |
| CD40 | 100 | 2,054 | 0 | 0 |
| CD83 | 100 | 34 | 57 | 48 |
| AKT1 | 100 | 30 | 103 | 0 |
| CREB1 | 100 | 126 | 168 | 193 |
| CREBBP | 100 | 0 | 453 | 150 |

(Storch and Hoeger 2010; Léauté-Labrèze et al. 2008; Sarialioglu et al. 2010; Sans et al. 2009; Buckmiller et al. 2010).

Our data in agreement with each other revealed that propranolol inhibited proliferation of U266 human multiple myeloma cells in a time- and dose-dependent manner. Propranolol increased caspase-3 enzyme activity, as an important sign of apoptosis, and it also induced the loss of mitochondrial membrane potential in a time- and dose-dependent manner. FITC AnnexinV/PI double staining by flow cytometry also confirmed time- and dose-dependent apoptotic effects of propranolol on multiple myeloma cells. When all the findings obtained are evaluated totally, it has been displayed that the propranolol has antiproliferative and apoptotic effects on U266 human multiple myeloma cells.

MM is a malignant plasma cell disorder and it was first described in 1873 by J. Von Rustizky (Buckmiller et al. 2010). Typical clinical and laboratory features in patients with MM include bone pain, lytic lesions, osteoporosis, anemia, renal failure, hypercalcaemia, and increased susceptibility of infections (Mahindra et al. 2010; Minnema et al. 2010). In conclusion, multiple myeloma is a complex disease having a number of complications and therapeutic challenges. Unfortunately, complete cure is not succeeded yet for multiple myeloma. Much progress has been made in the treatment of patients with MM. Therefore, MM is relatively resistant to conventional chemotherapeutic agents. The introduction of new drugs such as thalidomide, bortezomib, and lenalidomide has created more possibilities for patients than many years before (Minnema et al. 2010). Therefore, both multiple myeloma and these new therapies often affect the renal, immune, skeletal, hematologic, and nervous systems. We need new but less toxic drugs for patients with multiple myeloma. With 40 years of extensive clinical experience, there is no documented case of death or serious cardiovascular morbidity resulting directly from β -adrenergic receptor blocker exposure. However, several well-known side effects, such as bradycardia and hypotension, justify close monitoring at the onset of treatment (Emilien and Maloteaux 1998; Frohlich 1977; Featherstone 1983; Lee et al. 1982).

The results of this study showed that treatment of U266 human multiple myeloma cells with increasing concentrations of propranolol affected the expression levels of some important genes involved in upstream and downstream targets of NF- κ B signaling pathway which is the main targets of multiple myeloma treatment. BCL10 gene encoding B-cell lymphoma/leukemia 10 protein was upregulated in response to propranolol. BCL10 gene contributes to the proliferation of lymphocytes through activating NF- κ B signaling and induces apoptosis through the recruitment of caspases (Du and Isaccson 2002; Willis et al. 1999). B-cell lymphoma 2 (Bcl-2) gene encodes an anti-apoptotic protein, which is overexpressed in several types of cancer such as leukemia, breast cancer, melanoma,

and prostate cancer and its overexpression often results in drug resistance (Karnak and Xu 2010; Levine et al. 2008). In addition to the regulation of apoptosis, Bcl-2 also inhibits autophagy via forming a complex with Beclin-1 (Marquez and Xu 2012). In our study, decrements in expression levels of Bcl-2 in response propranolol treatment were observed. TRAFs communicate with signaling molecules such as NF- κ B and also with cell surface proteins. This protein family has six members, and all of them are known to regulate apoptotic events and stress-mediated cellular responses. Some of these proteins (TRAF-2, TRAF-5, and TRAF-6) are known to trigger NF- κ B signaling pathway (Bradley and Pober 2001). It is also known that TRAF-1/TRAF-2 complex formation causes the recruitment of anti-apoptotic signals via interacting with the proteins that are members of IAP family (Yoneda et al. 2000; Wajant et al. 2001). Our results demonstrated that propranolol treatment causes increments in expression levels of TRAF family members. Toll-like receptor family (TLRs) members, important regulators of innate immune system, recognize foreign substances and pathogenic particles, and then trigger the synthesis of appropriate cytokines, and also activate NF- κ B (Do et al. 2012). Davoodi et al. reported that TLR expression increases the activity of NF- κ B in colorectal cancer cells (Davoodi et al. 2012). In a recent study, it has been reported that TLRs can be potential targets for cancer therapy (Connolly and O'Neill 2012). Our results showed that there were significant increases in expression levels of TLR1-4. Tumor necrosis factor receptor superfamily member 10b (TNFRSF10B) gene includes a death domain inducing apoptotic events. It was previously reported that TNFRSF10B could be a tumor suppressor gene since it triggered p53-mediated apoptosis in cancer cells (Takimoto and El-Deiry 2000). In addition, another study also reported that treatment of K562 human chronic myeloid cells with an inhibitor of histone deacetylase, KBH-A42, caused TNFRSF10B overexpression and resulted in apoptosis (Kang et al. 2012). Our results are in consistent with the literature and revealed that propranolol treatment resulted in considerable increment in the expression levels of TNFRSF10B gene in a dose-dependent manner. Interleukins are the genes encoding different types of cytokines. IL-10 was reported to suppress the activity of NF- κ B and also to increase the survival and proliferation of B cells (Eskdale et al. 1997). Our results showed that propranolol treatment increased interleukin expression. In addition, while the expression levels of NF- κ B have increased in response to propranolol treatment, the inhibitors of NF- κ B genes were also highly overexpressed in response to propranolol in U266 cells.

In conclusion, taking together, all these data showed antiproliferative and apoptotic effects of propranolol on multiple myeloma cells. When we look at the final outcome

from the responses of the cells to propranolol, we can argue that both apoptotic and antiapoptotic, tumor suppressor, prosurvival, and also inflammatory genes are activated via propranolol treatment, but the expression of antiapoptotic and tumor suppressor genes may eliminate the effects of the other genes. On the other hand, propranolol has been well studied in adults and infants (Metry et al. 2012). The most common serious adverse effects of propranolol are bradycardia and hypotension (Metry et al. 2012; Zusman et al. 1987). Propranolol can be used in high doses but even these concentrations do not have serious adverse effects (Zusman et al. 1987). On the other hand, it is very well known that 320 mg/day propranolol was used in hypertension (Zusman et al. 1987). Therefore, the effective concentrations of propranolol as determined in this study can be accepted. But still effective dose of propranolol should be optimized for in vivo applications.

These results may open the way of the treatment for multiple myeloma for the treatment procedure of MM patients. Therefore, further research to evaluate propranolol application to multiple myeloma is needed.

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Conflict of interest We, the authors of the manuscript, do not have any conflict of interest.

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