European Review for Medical and Pharmacological Sciences 2018; 22: 2564-2571

BAY-11-7082 induces apoptosis of multiple myeloma U266 cells through inhibiting NF-κB pathway

Y. WANG[1], X.-L. ZHANG[2], C.-M. SUN[3]

[1]Department of Hematology, Yantai Yuhuangding Hospital, Yantai, China
[2]Department of Blood Laboratory, Yantai Yuhuangding Hospital, Yantai, China
[3]Department of Laboratory, Yantai Yuhuangding Hospital, Yantai, China

Yan Wang and *Xiaolu Zhang* contributed equally to this work

Abstract. – OBJECTIVE: To study the effects of BAY-11-7082 on proliferation and apoptosis of U266 cells and its mechanism of action.

MATERIALS AND METHODS: Multiple myelomas U266 cells were cultured and divided into control group and gradient-concentration BAY-11-7082 groups (1 μmol/L, 2 μmol/L, 4 μmol/L and 8 μmol/L). Cells in BAY-11-7082 groups were treated with drugs in different concentrations for 4 h, while those in control group were added with an equal volume of solvent. The cell viability was detected via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and lactate dehydrogenase (LDH) release assay was used to detect the cytotoxicity. Furthermore, cells in low-concentration and high-concentration BAY-11-7082 groups were compared with those in control group. The cell proliferation level was evaluated via cell cycle assay, the interleukin-6 (IL-6) level in cells was detected via enzyme-linked immunosorbent assay (ELISA), and the β-catenin protein expression was detected via Western blotting. Moreover, flow cytometry and Hoechst staining were performed to detect the number of apoptotic cells, and the apoptosis level was detected via caspase3 activity and apoptosis-related protein expression. Finally, the levels of p65, p50 and inhibitor kappa B kinase β (IKKβ) were detected via polymerase chain reaction (PCR), and the expressions and changes of phosphorylated (p)-p65 and p-IKKβ were detected via Western blotting.

RESULTS: BAY-11-7082 could reduce the U266 cell viability and increase the cytotoxic effect. Based on gradient concentration, 2 μmol/L was selected as the low concentration, while 4 μmol/L was selected as the high concentration. Compared with those in control group, the number of cells in the S and G2/M phases in drug administration groups was significantly decreased, but that in the G0/G1 phase was significantly increased. Besides, the secretion of IL-6 in cells in drug administration groups was significantly decreased compared with that in control group. The β-catenin protein expression was decreased in drug administration groups, and there was also a difference between high concentration group and low concentration group. Flow cytometry and Hoechst staining showed that the proportion of apoptotic cells in drug administration groups was significantly increased. Western blotting and detection of caspase3 activity revealed that the expression and activation of apoptosis-related protein were increased in drug administration groups. It was found in the detection of nuclear factor-κB (NF-κB) pathway that the NF-κB pathway was inhibited in drug administration groups, and there was also a statistically significant difference between high concentration group and low concentration group.

CONCLUSIONS: BAY-11-7082 inhibits the proliferation and induces the apoptosis of U266 cells through inhibiting NF-κB pathway.

Key Words:
Multiple myeloma, BAY-11-7082, NF-κB pathway, Apoptosis, Cell proliferation.

Introduction

Multiple myeloma (MM), a kind of malignant tumor, derived from terminally-differentiated B lymphocytes. MM is characterized by malignant clonal proliferation of plasma cells in bone marrow, and synthesis and secretion of many immunoglobulins[1]. Currently, the treatment means of MM include chemotherapy, radiotherapy, immunotherapy and hematopoietic stem cell transplantation[2]. In recent years, with the constant emergence of immunotherapy and other new drugs, the effective rate of MM treatment has been increased, and the remission rate of refrac-

Corresponding Author: Chengming Sun, MD; e-mail: wangyanyhd@sina.com

tory or recurrent MM has been also improved. However, due to toxic and side effects of drugs and the formation of multi-drug resistance, MM is still a refractory tumor of blood system. Therefore, exploring the new therapeutic drug and new therapy of MM has important clinical significance.

Nuclear factor-κB (NF-κB) family mainly consists of five members, RelA (p65), RelB, c-Rel, NF-κB1 (p105-p50), and NF-κB2 (p100-p52). Those factors play important roles in immune response, inflammatory response, cell proliferation, apoptosis, tumor formation, and other biological processes in the body[3]. Many stimulating factors, such as pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), reactive oxygen species (ROS), lipopolysaccharide (LPS), T cell and B cell activation antigens, ultraviolet radiation, and various colony stimulating factors, can activate NF-κB signaling pathway[4]. After activation of NF-κB pathway, various cytokines, chemical factors, adhesion molecules and cyclin D are expressed, promoting cell growth and survival[5]. The increased expressions of intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule-1 (VCAM-1) can stimulate increase of IL-6 secretion by stromal cells, which are closely related to the drug resistance of myeloma. In the pathogenesis of MM, NF-κB resists apoptosis *via* both pathways[6].

With the in-depth study on the etiology and pathogenesis of MM, much attention has been paid to the interaction between bone marrow microenvironment and tumor cells, signal transduction in tumor cells and cytokine network drug therapy. Now the treatment is no longer limited to the cytotoxic drugs to reduce tumor load, but the development of new drugs that can selectively kill tumor cells without damaging normal cells[7]. NF-κB inhibitor BAY-11-7082 inhibits proliferation and promotes apoptosis of a variety of tumor cells[8, 9]. However, there is a lack of relevant research in MM cells.

In this paper, the roles of BAY-11-7082 in the proliferation and apoptosis of MM U266 cells were preliminarily investigated, and its mechanism was further studied.

Materials and Methods

Cell and Experimental Materials

MM U266 cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). BAY-11-7082, IL-6 and caspase3 assay kits were purchased from Beyotime (Shanghai, China). β-catenin, phosphorylated (p)-p65, and p-inhibitor kappa B kinase β (IKKβ) antibodies were purchased from CST (Danvers, MA, USA), and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were bought from Gibco (Rockville, MD, USA). Flow cytometry kit, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) kit and lactate dehydrogenase (LDH) assay kit were bought from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China).

Cell Culture

MM U266 cells were cultured in a high-glucose DMEM containing 10% FBS in an incubator under saturated humidity and 5% CO_2 at 37°C. Cells showed single-layer adherent growth, and after cells covered the bottom of culture flask (usually after 2-3 d), the cell concentration was adjusted into 5×10^5/mL, followed by digestion with 0.25% trypsin and passage under aseptic conditions.

MTT (Methyl Thiazolyl Tetrazolium) Assay

Cells in the logarithmic growth phase were digested, collected and adjusted into cell suspension at a concentration of 1×10^5/mL. The suspension was inoculated into a 96-well plate (100 μL per well), 3 repeated wells were set in the experiment, and the blank control was also set. 20 μL MTT was added into each well, cells continued to be cultured at 37°C for 4 h, and the supernatant was carefully discarded. Then 150 μL dimethylsulfoxide (DMSO) was added into each well and mixed evenly for 10 min. The optical density (OD) value of each well was detected at a wavelength of 570 nm using a microplate reader. The experiment was repeated for 3 times. Proliferation inhibition was calculated according to the following formula: inhibition rate = $\text{OD value}_{\text{drug administration group}} / \text{OD value}_{\text{control group}} \times 100\%$.

LDH Assay

Cells in the logarithmic growth phase were digested, collected and adjusted into cell suspension at a concentration of 1×10^5/mL. The suspension was inoculated into the 96-well plate (100 μL per well), 3 repeated wells were set in the experiment, and the blank control was also set.

The supernatant was taken (20 μL per well) and added with the corresponding reagent according to instructions of the kit. The mixture was mixed evenly and placed at room temperature for 3 min, followed by zero setting using 440 nm double distilled water and detection of OD value using the microplate reader. Unit definition: After 1000 mL culture solution reacted with the substrate at 37°C for 15 min, 1 gmoL pyruvic acid produced in the reaction system was regarded as 1 unit. The LDH content in the medium was calculated using the formula.

Flow Cytometry

Cells were inoculated into a 6-well plate (5×10^5/mL) overnight. After treatment, cells were digested with trypsin and collected, followed by centrifugation at 1000 rpm and 4°C for 4 min. Then cells were collected, and the medium was abandoned. Centrifuged cells were washed twice with cold phosphate-buffered saline (PBS), and resuspended using 200 μL Binding Buffer at a concentration of approximately 1×10^6/mL. 10 μL Annexin V-fluorescein isothiocyanate (FITC) was added into the cell suspension and gently mixed evenly, followed by detection of apoptosis using a flow cytometer within 1 h. Data were obtained and analyzed using the Cellquest professional software.

After treatment, cells were digested with trypsin, collected and stained, followed by detection of cell cycle *via* flow cytometry.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum IL-6 and caspase3 activation levels were measured using the ELISA kit in strict accordance with instructions of the kit.

Western Blotting

Cells in each group were taken and washed twice with D-Hank's solution, and D-Hank's solution was sucked dry with absorbent paper. 150 μL pre-cooled Lysis Buffer was added in each group and cells were lysed on ice for 30 min. The protein in each group was collected using a cell scraper into an Eppendorf (EP) tube, followed by centrifugation at 12000 rpm and 4°C. The supernatant was taken and transferred into a new EP tube. After the protein concentration was determined using the bicinchoninic acid (BCA) method, 5 × loading buffer was added and mixed evenly, and the protein was heated at 100°C for 6 min. 30 μL protein was added into the loading wells of separation gel and spacer gel, followed by electrophoresis in the electrophoretic buffer solution under appropriate voltage. After electrophoresis, gel closely contacted with the polyvinylidene difluoride (PVDF) membrane, followed by membrane transfer in transfer buffer at 0°C under constant voltage at 100 V for 60 min. Then the PVDF membrane was sealed in 5% skim milk powder at room temperature for 1 h, cut according to the molecular weight and sealed in the primary antibody in a refrigerator at 4°C overnight. On the next day, the PVDF membrane was taken, rinsed with Tris-buffered saline with Tween-20 (TBS-T), and added with the secondary antibody immunoglobulin G (IgG) (1:5000) for incubation at room temperature for 1 h. After incubation, the membrane was rinsed again with TBST, and the image was developed using the Tannon 5200 immunofluorescence imaging system, followed by calculation of gray scale.

Polymerase Chain Reaction (PCR)

The reverse transcription reaction system was prepared on ice using PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan), and cDNA was obtained after the reaction completed. The miRNA quantitative PCR was performed according to the miScript SYBR Green PCR Kit manual. The volume of total reaction was 10 μL. PCR amplification conditions are pre-denaturation at 94°C for 5 min followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min 30 s. The primers used in this study were shown below: p65 (Forward) 5'-GTGGGGACTACGACCTGAATG-3', p65 (Reverse) 5'-GGGGCACGATTGTCAAAGATG-3'; p50 (Forward) 5'-AACAGAGAGGATTTCGTTTCCG-3', p50 (Reverse) 5'-TTTGACCTGAGGGTAAGACTTCT-3'; IKKβ (Forward) 5'-GTCTTTGCACATCATTCGTGGG-3', IKKβ (Reverse) 5'-GTGCCGAAGCTCCAGTAGTC-3'.

Statistical Analysis

Experimental data were presented as mean ± standard deviation (x±s). Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Comparison between groups was done using One-way ANOVA test followed by Post Hoc test (Least Significant Difference). $p<0.05$ suggested that the difference was statistically significant.

Results

Effect of BAY-11-7082 on the Viability of U266 Cells and Its Toxic Effect

The cell viability in control group and gradient-concentration BAY-11-7082 groups (1 μmol/L, 2 μmol/L, 4 μmol/L and 8 μmol/L) was detected *via* MTT (Table I). Results showed that there was no significant change in the cell viability at the drug concentration of 1 μmol/L ($p < 0.05$), but the cell viability was significantly reduced at the drug concentrations of 2 μmol/L, 4 μmol/L and 8 μmol/L compared with those in control group ($p < 0.05$). Moreover, the cell viability in 4 μmol/L and 8 μmol/L groups was further significantly decreased compared with that in 2 μmol/L group ($p < 0.05$), but there was no statistically significant difference between4 μmol/L and 8 μmol/L groups.

The cytotoxic effect in each group was detected *via* LDH release assay (Table II). The drug at the concentration of 1 μmol/L had no toxic effect on cells ($p < 0.05$). The LDH release in cells in 2 μmol/L, 4 μmol/L and 8μmol/L groups was significantly increased compared with that in control group ($p < 0.05$). Moreover, the LDH release in 4 μmol/L and 8 μmol/L groups was increased more obviously than that in 2 μmol/L group ($p < 0.05$), but there was no statistically significant difference between 4 μmol/L and 8 μmol/L groups, demonstrating that BAY-11-7082 has gradient-concentration effects on cell viability and toxicity.

Effects of BAY-11-7082 on Proliferation and Activity of U266 Cells

The cell cycles in control group and low- and high-concentration BAY-11-7082 groups (2 μmol/L and 4 μmol/L) were detected *via* flow cytometry. Results showed that compared with that in control group, 2 μmol/L and 4 μmol/L BAY-11-7082 decreased the proportion of cells in S and G2/M phases, and increased the proportion of cells in G0/G1 phase ($p < 0.05$). The proportion of cells in G0/G1 phase was further decreased in 4 μmol/L group compared with that in 2 μmol/L group ($p < 0.05$) (Figure 1A-B). The secretion level of IL-6 in each group of cells was detected *via* ELISA. Results showed that the secretion of IL-6 in low- and high-concentration BAY-11-7082 groups was significantly inhibited compared with that in control group, and the cell viability was reduced ($p < 0.05$). The secretion of IL-6 in 4 μmol/L group was further decreased compared with that in 2 μmol/L group ($p < 0.05$) (Figure 1C). Western blotting was used to detect the expression of proliferation-related protein β-catenin. Results showed that compared with that in control group, the expression of β-catenin was inhibited after addition of BAY-11-7082, and the inhibitory effect in high concentration group was more significant than that in low concentration group (Figure 1D-E), proving that BAY-11-7082 inhibits cell proliferation by regulating cell cycle.

Table I. Detection of cell viability in each group *via* MTT.

Group	Control group	BAY-11-7082 (μmol/L)			
		1	2	4	8
Percentage of viable cells (%)	99 ± 0.7	95 ± 1.1	84 ± 1.8*	48 ± 1.8**	46 ± 2.1**

*$p < 0.05$ *vs*. blank group, **$p < 0.05$ *vs*. 2 μmol/L BAY-11-7082 group.

Table II. Detection of cytotoxic effect in each group *via* LDH release.

Group	Control group	BAY-11-7082 (μmol/L)			
		1	2	4	8
LDH release (*vs*. blank group)	1	1 ± 0.01	2.2 ± 0.04*	4.2 ± 0.03**	4.2 ± 0.08**

*$p < 0.05$ *vs*. blank group, **$p < 0.05$ *vs*. 2 μmol/L BAY-11-7082 group.

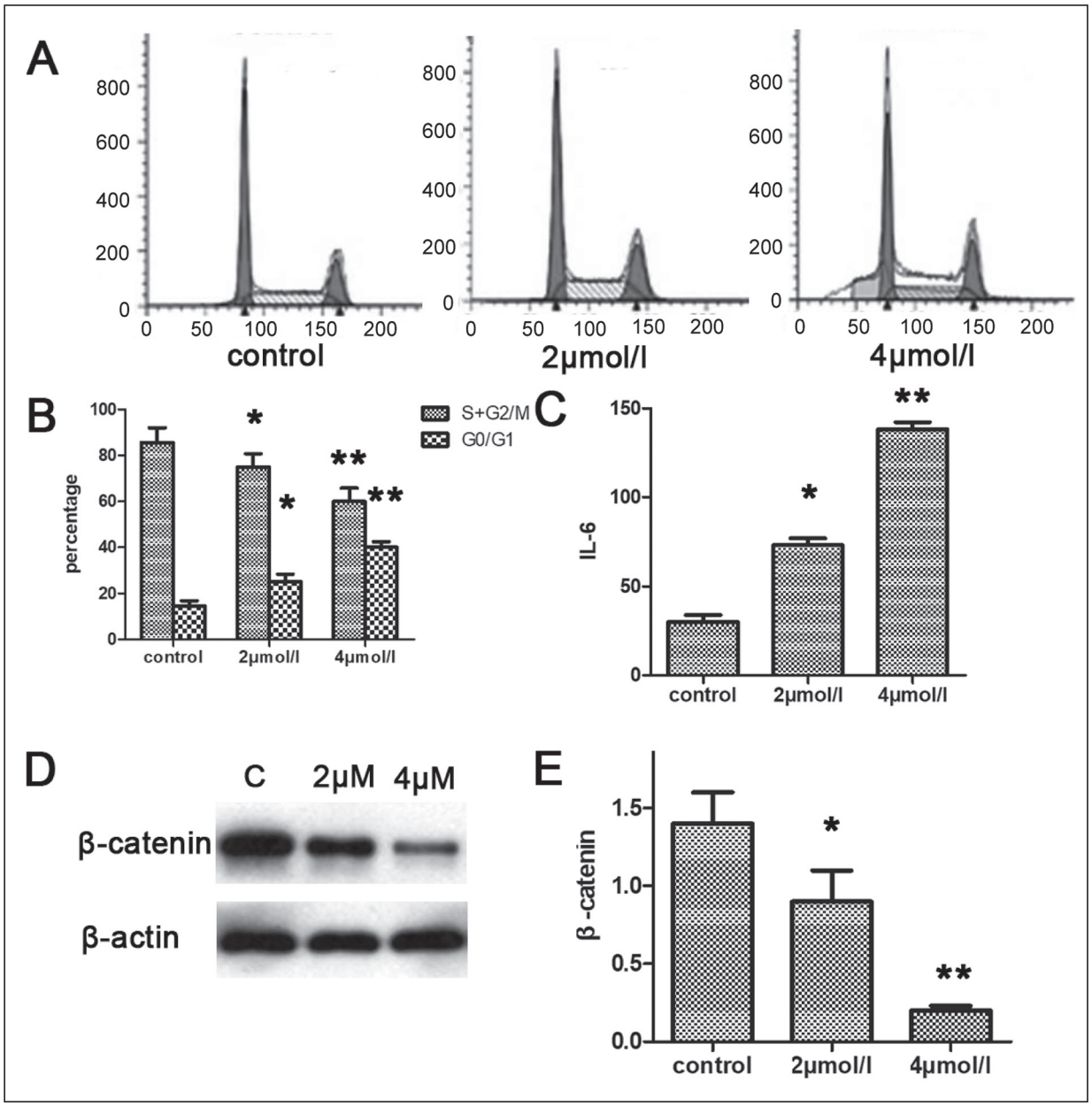

Figure 1. Effects of BAY-11-7082 on cell proliferation and activity levels. ***A,*** Detection of cell cycles in control group, low-concentration BAY-11-7082 group (2 μmol/L) and high-concentration BAY-11-7082 group (4 μmol/L) *via* flow cytometry. ***B,*** Statistical graph of cell cycle in each group ***C,*** Detection of IL-6 release level in each group *via* ELISA. ***D,*** Detection of expression of proliferation-related protein β-catenin in each group *via* Western blotting. ***E,*** Statistical graph of gray value in Western blotting. *$p < 0.05$ *vs.* blank group, **$p < 0.05$ *vs.* 2 μmol/L BAY-11-7082 group.

Effect of BAY-11-7082 on Apoptosis of U266 Cells

The proportions of apoptotic cells in control group and low- and high-concentration BAY-11-7082 groups (2 μmol/L and 4 μmol/L) were detected *via* flow cytometry. Results revealed that compared with that in control group, the proportion of apoptotic cells was increased after addition of BAY-11-7082 ($p < 0.05$), and the apoptosis induction in high concentration group was more significant than that in low concentration group ($p < 0.05$) (Figure 2A-B). The caspase3 activation level in each group of cells was detected *via* ELISA, and it was found that BAY-11-7082 significantly increased the activation level of caspase3 in cells, and there were statistically sig-

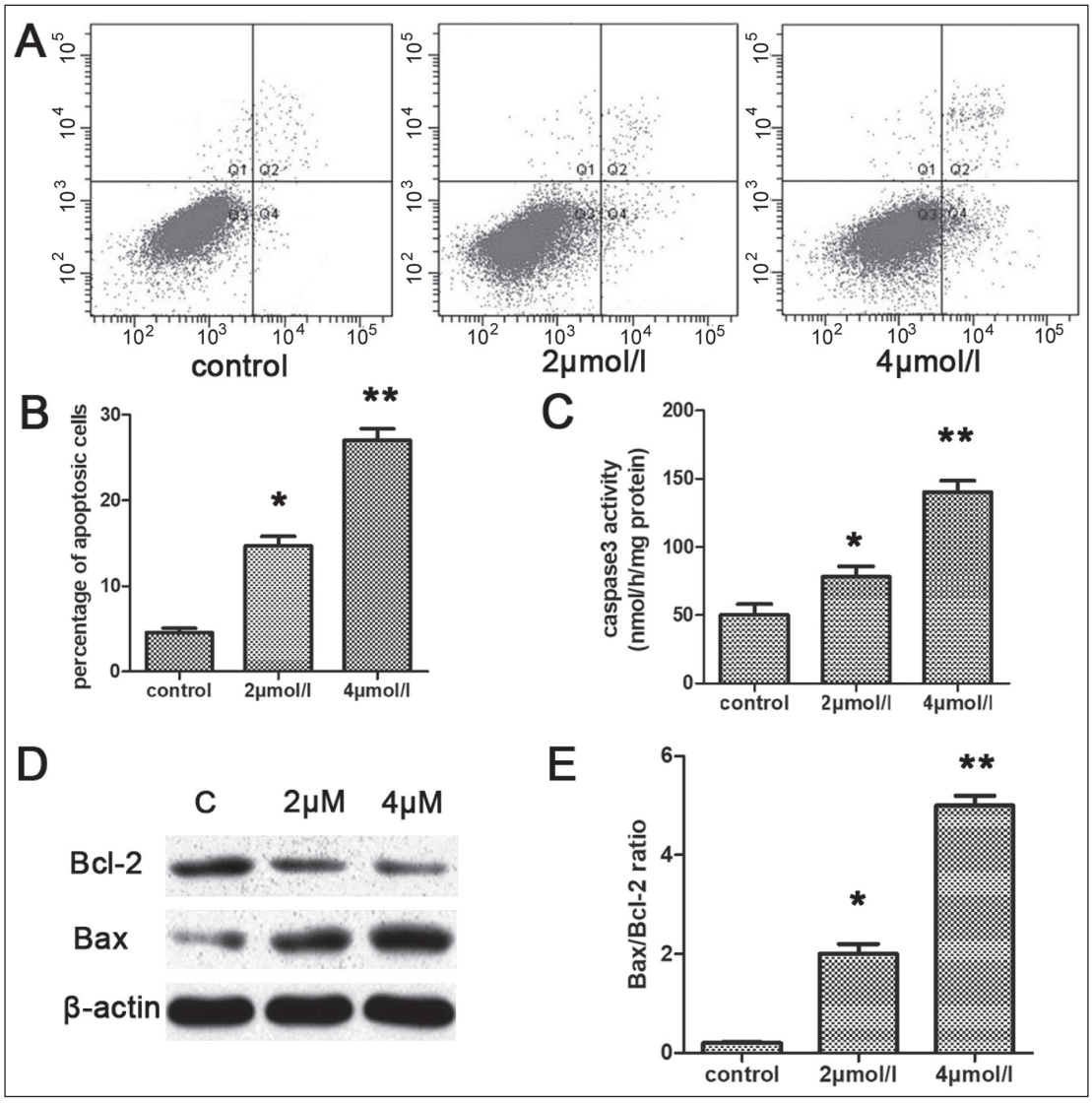

Figure 2. Effect of BAY-11-7082 on apoptosis level. ***A,*** Detection of proportions of apoptotic cells in control group, low-concentration BAY-11-7082 group (2 μmol/L) and high-concentration BAY-11-7082 group (4 μmol/L) *via* flow cytometry. ***B,*** Statistical results of proportion of apoptotic cells in each group. ***C,*** Detection of caspase3 activity in each group *via* ELISA. ***D,*** Detection of expression levels of apoptosis-related proteins (Bcl-2 and Bax) in each group *via* Western blotting. ***E,*** Statistical graph of gray value and Bax/Bcl-2 ratio in Western blotting. *$p < 0.05$ *vs.* blank group, **$p < 0.05$ *vs.* 2 μmol/L BAY-11-7082 group.

nificant differences compared with control group. Caspase3 activation was increased in high-concentration BAY-11-7082 group more significantly than that in low concentration group (Figure 2C). Expressions of apoptosis-related proteins [B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax)] were detected *via* Western blotting. The experimental results were similar to those before: BAY-11-7082 could reduce the expression of Bcl-2 and increase the expression of Bax. The effect in high concentration group was more significant than that in low concentration group (Figure 2D-E), proving that BAY-11-7082 can induce apoptosis.

Regulation of NF-κB Pathway in U266 Cells Via BAY-11-7082

The mRNA levels of p65, p50 and IKKβ in cells in control group and low- and high-concentration BAY-11-7082 groups (2 μmol/L and 4 μmol/L) were detected *via* PCR. Results revealed that mRNA expressions of p65, p50 and IKKβ

in BAY-11-7082 groups were significantly decreased compared with those in control group, and they were decreased more significantly in high concentration group ($p < 0.05$) (Figure 3A). The activated protein level, p65, p-p65, IKKβ and p-IKKβ expressions were further detected *via* Western blotting. Results revealed that the p-p65/p65 and p-IKKβ/IKKβ ratios were decreased significantly after addition of drug, and the protein activation level was inhibited. In 4 μmol/L group, the inhibitory level of NF-κB pathway protein activation was higher ($p < 0.05$) (Figure 3B & C), proving that BAY-11-7082 has a strong inhibitory effect on NF-κB activation in cells.

Discussion

The pathogenesis of MM is more complicated and has not yet been fully clarified. In recent years, the research has mainly focused on the cytogenetic abnormalities, the interaction between bone marrow microenvironment and myeloma cells, mechanism of drug resistance and so on[10,11]. In research of myeloma cell proliferation and apoptosis and drug resistance-related signaling pathways, abnormalities in NF-κB, Notch and other signaling pathways are most closely linked[12].

NF-κB is an important nuclear transcription factor *in vivo*. Studies have found that NF-κB can control cell proliferation and malignant transformation, regulate cell cycle and apoptosis, affect cell differentiation and promote tumor metastasis, which is related to multidrug resistance[13]. In most malignant tumors of blood system, NF-κB can persistently activate or present abnormal nuclear localization through genetic changes or signal transduction abnormalities. Activated NF-κB has multiple protective effects on tumors. NF-κB promotes proliferation: Regulating cell cycle and promoting cell proliferation are important mechanisms of NF-κB involved in malignant transformation. NF-κB inhibits apoptosis: Blocked apoptosis is also an important feature of tumor cell growth. NF-κB, as a nuclear transcription factor, can exert its anti-apoptotic effect through the regulation of downstream anti-apoptotic factors. Angiogenesis: Tumor growth is dependent on the neovascularization, and angiogenesis is

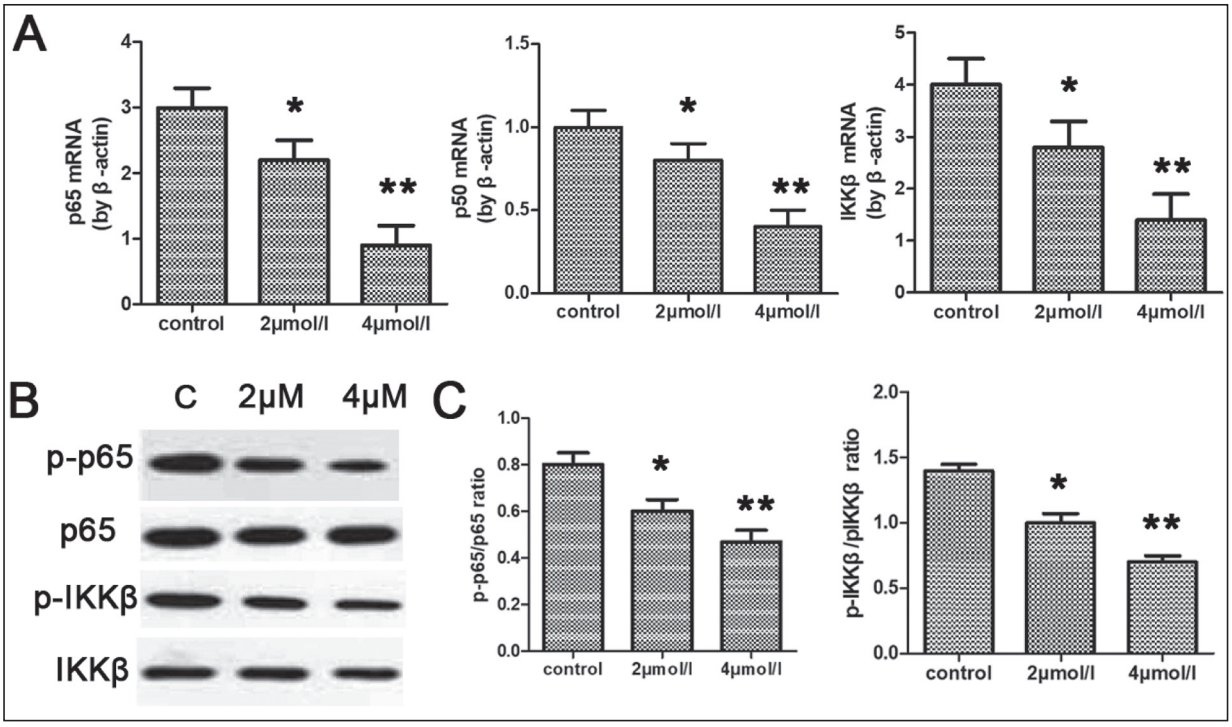

Figure 3. Effect of BAY-11-7082 on NF-κB pathway activation in cells. ***A,*** Detection of p65, p50 and IKKβ mRNA expressions in control group, low-concentration BAY-11-7082 group (2 μmol/L) and high-concentration BAY-11-7082 group (4 μmol/L) *via* PCR. ***B,*** Detection of expression levels of p65, p-p65, IKKβ and p-IKKβ in each group *via* Western blotting. ***C,*** Statistical graph of gray value, p-p65/p65 and p-IKKβ/IKKβ ratios in Western blotting. *$p < 0.05$ *vs.* blank group, **$p < 0.05$ *vs.* 2 μmol/L BAY-11-7082 group.

a prerequisite for tumor growth and metastasis. Activation of NF-κB is generally accompanied by the activation of angiogenic factors, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs)[14].

Sustained activation of NF-κB plays a decisive role in the pathogenetic process of MM. Targeted therapy on NF-κB and therapeutic drugs developed on this basis have received increasing attention[15]. The most important kind of drug is proteasome inhibitors, which stabilize IKB and inhibit NF-κB activity by inhibiting proteasome activity. On the one hand, they can reduce the secretion of IL-6, inhibit the mitogen-activated protein kinase (MAPK) growth signal and induce apoptosis. On the other hand, they can reduce the level of anti-apoptotic protein (such as Bcl-2) and promote apoptosis. BAY-11-7082 is an NF-κB pathway inhibitor that can inhibit phosphorylation of IKBα, prevent NF-κB activation, and block NF-κB conduction pathway, thereby inhibiting the effect of NF-κB[16].

Results in this paper indicate that, in the proliferation and apoptosis processes of MM U266 cells, BAY-11-7082 can be involved in the regulation *via* inhibiting NF-κB pathway. Thus, BAY-11-7082 can inhibit U266 cell proliferation, reduce cell viability and release IL-6, and can induce increased proportion of apoptosis and increased expression of apoptosis-related protein. In the regulation of NF-κB pathway, BAY-11-7082 can decrease p65, p50 and IKKβ mRNA expressions, and reduce p65 and p-IKKβ activation.

Conclusions

We showed that BAY-11-7082 can inhibit proliferation and activation, and promote apoptosis of MM U266 cells. These effects may be achieved through the regulation of NF-κB pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Manier S, Kawano Y, Bianchi G, Roccaro AM, Ghobrial IM. Cell autonomous and microenvironmental regulation of tumor progression in precursor states of multiple myeloma. Curr Opin Hematol 2016; 23: 426-433.
2) Landgren O, Iskander K. Modern multiple myeloma therapy: Deep, sustained treatment response and good clinical outcomes. J Intern Med 2017; 281: 365-382.
3) Rickert RC, Jellusova J, Miletic AV. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. Immunol Rev 2011; 244: 115-133.
4) Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer 2010; 102: 639-644.
5) Ledoux AC, Perkins ND. NF-kappaB and the cell cycle. Biochem Soc Trans 2014; 42: 76-81.
6) Matthews GM, de Matos SR, Dhimolea E, Sheffer M, Gandolfi S, Dashevsky O, Sorrell JD, Mitsiades CS. NF-kappaB dysregulation in multiple myeloma. Semin Cancer Biol 2016; 39: 68-76.
7) Ramakrishnan V, D'Souza A. Signaling pathways and emerging therapies in multiple myeloma. Curr Hematol Malig Rep 2016; 11: 156-164.
8) Chen L, Ruan Y, Wang X, Min L, Shen Z, Sun Y, Qin X. BAY 11-7082, a nuclear factor-kappaB inhibitor, induces apoptosis and S phase arrest in gastric cancer cells. J Gastroenterol 2014; 49: 864-874.
9) Pickering BM, de Mel S, Lee M, Howell M, Habens F, Dallman CL, Neville LA, Potter KN, Mann J, Mann DA, Johnson PW, Stevenson FK, Packham G. Pharmacological inhibitors of NF-kappaB accelerate apoptosis in chronic lymphocytic leukaemia cells. Oncogene 2007; 26: 1166-1177.
10) Wang J, Faict S, Maes K, De Bruyne E, Van Valckenborgh E, Schots R, Vanderkerken K, Menu E. Extracellular vesicle cross-talk in the bone marrow microenvironment: Implications in multiple myeloma. Oncotarget 2016; 7: 38927-38945.
11) Lu MH, Fan MF, Yu XD. NSD2 promotes osteosarcoma cell proliferation and metastasis by inhibiting E-cadherin expression. Eur Rev Med Pharmacol Sci 2017; 21: 928-936.
12) Abdi J, Chen G, Chang H. Drug resistance in multiple myeloma: Latest findings and new concepts on molecular mechanisms. Oncotarget 2013; 4: 2186-2207.
13) Prasad S, Ravindran J, Aggarwal BB. NF-kappaB and cancer: How intimate is this relationship. Mol Cell Biochem 2010; 336: 25-37.
14) Braun T, Carvalho G, Fabre C, Grosjean J, Fenaux P, Kroemer G. Targeting NF-kappaB in hematologic malignancies. Cell Death Differ 2006; 13: 748-758.
15) Fuchs O. Targeting of NF-kappaB signaling pathway, other signaling pathways and epigenetics in therapy of multiple myeloma. Cardiovasc Hematol Disord Drug Targets 2013; 13: 16-34.
16) Krishnan N, Bencze G, Cohen P, Tonks NK. The anti-inflammatory compound BAY-11-7082 is a potent inhibitor of protein tyrosine phosphatases. FEBS J 2013; 280: 2830-2841.