Journal of
Blood Disorders & Transfusion

Wang et al., J Blood Disord Transfus 2015, 6:3
DOI: 10.4172/2155-9864.1000281

Research Article Open Access

Detection of the Impact of Bisphosphonate on Multiple Myeloma using Lactadherin

Shuye Wang*, Xiaomin Zhang, Jinxiao Hou, Yafeng Chen and Yueyue Fu

Division of Hematology, The First Affiliated Hospital, Harbin Medical University, Harbin-150001, China

***Corresponding author:** Shuye Wang, MD, PhD, Division of Hematology, The First Affiliated Hospital Harbin Medical University, 23 Youzheng Street Nan Gang District, 150001 Harbin, China, Tel: 53643849; E-mail: wangshuye@126.com

Received date: Jun 05, 2014, **Accepted date:** Jun 02, 2015, **Publication date:** June 06, 2015

Abstract

Background: Explore the impact of second-generation bisphosphonate drugs pamidronate on proliferation and apoptosis of human multiple myeloma cell line in vitro. Verify the synergistic effect of combination of pamidronate and melphalan on apoptosis of myeloma cells and demonstrate that lactadherin is the most effective probe for apoptosis detection.

Method: (1) Myeloma cells were treated with pamidronate or combination of melphalan and pamidronate at different concentrations. The cells were divided into 12 groups and treated with the drugs at intervals of 24 hours for 48 hours. After 48 hours treatment, inhibition rate of cell growth in different group were measured by MTT. (2) Two groups were treated with PBS buffer. PS exposure of the cells treated with drugs was detected using FITC-Annexin-V and FITC-lactadherin by flow cytometry.

Result: The increase of the proliferation and effect of apoptosis by pamidronate on multiple myeloma cells was parallel as the increase of pamidronate dose. In combination with melphalan, the effect of apoptosis induction was stronger than single drug group. PS exposure detected by lactadherin was significantly increased as the increase of amidronate concentration. PS exposure in treated group is more than that of control group.

Conclusion: Pamidronate exerts effects of growth inhibition and apoptosis induction on multiple myeloma cell in a dose-dependent way; Combination therapy increases apoptosis and exerts a synergistic effect; Lactadherin is the most effective probe in detecting myeloma cell apoptosis.

Keywords: Myeloma cell line; Pamidronate; Melphalan; Lactadherin; Apoptosis

Introduction

Multiple myeloma (MM) is a malignancy of clonal proliferation of plasma a cell. Plasma cells clonally proliferate in bone marrow and induced osteolytic bone destruction. It accounts for 10% of the blood system tumors. MM patients are mostly 50 to 60 years old and only 2% of them are under 40 years old. In China, the incidence of myeloma is about 1/100000, lower than that of Western industrial countries (about 4/100000).

Since the low percentage of tumor cells and multi-drug resistance, treatment of MM is still very difficult. Bisphosphonates is a new drug used to treat MM in recent years which has the effect of inhibiting the activation of osteoclasts and inducing apoptosis of myeloma cells. In this study, Annexin-V and lactadherin were used as molecular probesto detect PS exposure of myeloma cells after bisphosphonates treatment [1,2].

This study aims to prove that bisphosphonate drugs can induce tumor cell apoptosis, which will establish a new way in prevention and treatment of myeloma. It also provides basis for developing new drugs.

Materials and Methods

Experimental cells

Human multiple myeloma cell lines RPMI8226: First Affiliated Hospital of Harbin Medical Center laboratories. Cells were frozen in liquid nitrogen before culture.

Drugs and reagents

Bisphosphonate injection drugs purchased from Jinan Chia Tai Biochemistry Co., Ltd., melphalan William purchased from Glaxo Inc., FITC labeled lactadherin presented by professor Shi Jialan, Annexin-V (Beijing Bao sai Biotechnology Co., Ltd.), RPMI1640 medium, PAA double-resistant (penicillin, streptomycin), fetal calf serum (purchased from the holly company)

Experimental methods

MTT assay

Cells were suspended in RPMI1640 medium containing 10% fetal calf serum and seeded on 96 well plates. Each well contains 200 μl suspensions of 1×10^4 cells. And then various concentrations of pamidronate (1, 10, 50, 100, 150 μmol/l), and pamidronate disodium

(1, 10, 50, 100, 150 μmol/l) and melphalan (15 μmol/l) parallel to each concentration of 3 holes. After 48 h culturing, 5 μL different concentrations of cell suspension were properly diluted to 50 μl. To count under the microscope, 5 μL cell suspensions were drawn and dropped to the board. Then obtained cell growth inhibition rate: inhibition rate=(1-concentration of experimental cells/concentration of control cells) × 100%.

Calcium-dependent annexin (Annexin-V method) method

Multiple myeloma cells of logarithmic growth phase were collected (1×10^6/ml). Control group, pamidronate (10, 100, 150 μmol/1) monotherapy group, pamidronate (10, 100, 150 μmol/l) and melphalan (15 μmol/l) combination group were set and each group repeated three times. Cells were collected after 48 hours culturing and washed twice with PBS. 100 ul binding buffer and FITC-Annexin-V (20 ug/ml) 10 ul were added, incubated at room temperature for 30 min in dark, and then added 5 ul PI (50 ug/ml).

After reacting 5 mins in dark, it added 400 ul binding buffer and detected immediately by FACScan flow cytometry (generally less than 1 h). The tube without FITC-AnnexinV and PI was negative control.

Calcium-independent lactadherin method

The method of collecting Multiple myeloma cells is as the same. Cells were collected after 48 hours culturing and washed twice with PBS. 100 ul binding buffer and FITC-lactadherin were added, incubated at room temperature for 30 min in dark,And then added 5 ul PI(50 ug/ml). After reacting 5 mins in dark, added 400 ul binding buffer and detected immediately by FACScan flow cytometry (generally less than 1 h). The tube without FITC-lactadherin and PI was negative control.

Statistical analysis

Data was expressed with (x ± s). Utilizing the SPSS software, statistical analysis of the data was done. Use ANOVA and comparative analysis for statistical analysis.

Results

Growth inhibition curve was made using MTT method

When the concentration of pamidronate was more than 100 μmol/L, growth of 80% cells was inhibited. Therefore, we did not increase the concentration of cell growth study any more. This result shows that cell survival rate was negatively correlated with drug concentration in a dose dependent manner.

The combination of two drugs for pamidronate (150 μmol/l) +melphalan (15 μmol/l) in the MTT cell growth inhibition was determined, the results show that the combination of two drugs inhibition better than single drug, about 90% more cell growth was inhibited, which indicates that the cell survival rate was negatively correlated with drug concentration, and there is obvious dose-dependent.

Apoptosis induction effect of pamidronate on multiple myeloma cells

Using calcium-dependent annexin (Annexin-V method) method and calcium-independent lactadherin method, apoptosis proportion ismeasured by flow cytometry. The results show that with, the increase of pamidronate concentration, myeloma cell apoptosis was significantly increased. Lactadherin can detect more apoptosis cells than Annexin-V and the sensitivity of lactadherin is higher than Annexin-V. PS exposure on cell surface of treated group was more than that of control cells.

Annexin-V method in apoptosis induction effect of pamidronate. Figure 1 shows that cells of treated group and control group were mainly distributed in the third quadrant (Figure 1A and 1B). When drug concentration is higher than 10 μmol/L (Figure 1C), cells in the first quadrant move upward first under the same voltage. When the concentrations of representations increase, cells move to the fourth quadrant (right).

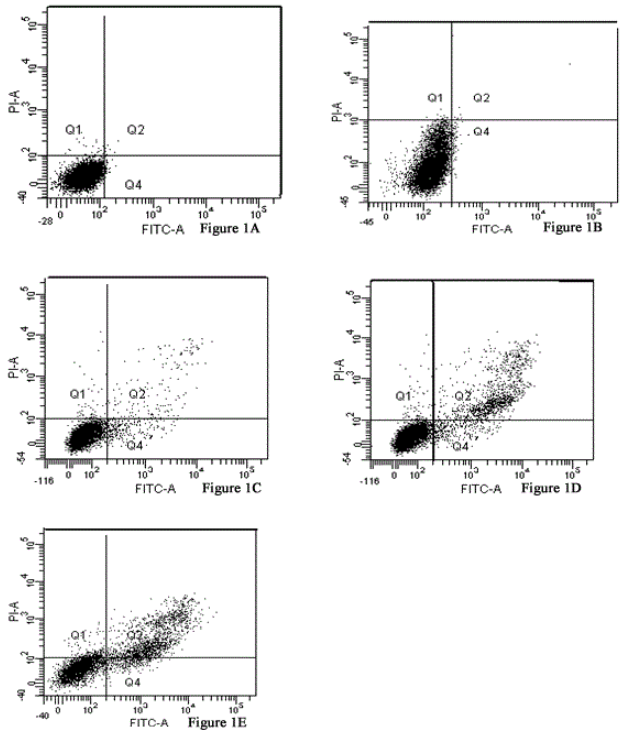

Figure 1: Flow cytometry analysis of different drug concentrations Pamidronate treatment of bone marrow tumor cell surface PS exposure.

FITC-lactadherin in apoptosis induction effect of pamidronate is shown in Figure 2. From the concentration of 10 μmol/L, FITC-lactadherin labeled cells in the FL1 channel began shifting to the right (Figure 2C).

This indicated PS has exposed to the outer surface of cell membrane. With the increase of drug concentration, the peak shifted to the right gradually, and reached maximum when the drug concentration turn to 150 μmol/L (Figure 2E). Cells dealt with medium and control cells have no significant right shift (Figure 2A and 2B). The treated group shifted to the right more than control group.

Flow cytometry analysis of different drug concentrations pamidronate treatment combined melphalan myeloma cell surface PS exposure with two methods

Annexin-V method: Figure 3 shows that cells of treated group and control group were mainly distributed in the third quadrant (Figure 3A and 3B). From drug concentration in combination with 10 μmol/L pamidronate and 15 μmol/L melphalan (Figure 3C), cells in the first quadrant move upward first under the same voltage. When the concentrations of representations increase, cells right move to the fourth quadrant.

FITC-lactadherin method: As shown in Figure 4, from the concentration of 10 μmol/l pamidronate and 15 μmol/L melphalan, FITC-lactadherin labeled cells in the FL1 channel began shifting to the right (Figure 4C). This indicates PS has exposed to the outer surface of cell membrane. With the increase of drug concentration, the peak shifted to the right gradually, and reached maximum when the drug concentration turn to 150 μmol/L (Figure 4E). Cells dealt with medium and control cells have no significant right shift (Figure 4A and 4B). The treated group shifted to the right more than control group. We can see after combination treatment, PS exposed cells are more than that of single agent.

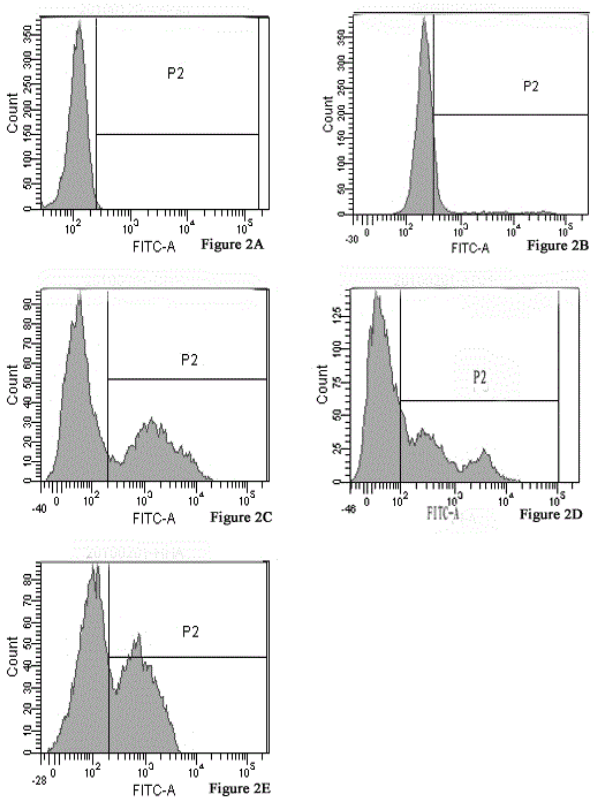

Figure 2: Histogram of FITC-lactadherin in FL1 channel.

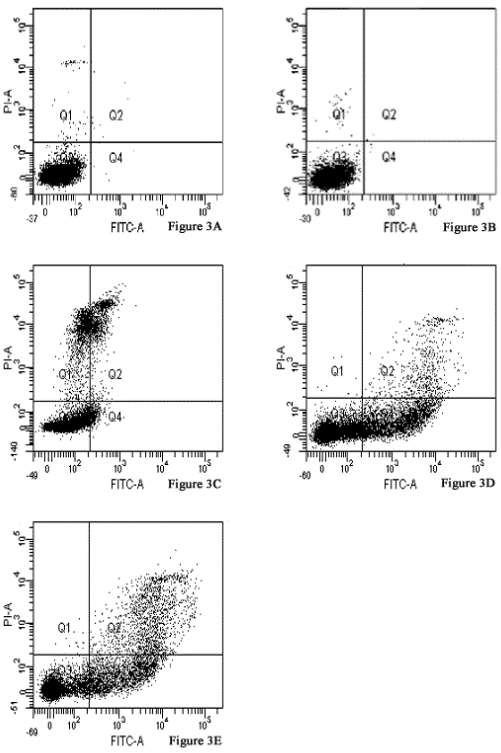

Figure 3: Flow cytometry analysis of different drug concentrations pamidronate treatment combined melphalan myeloma cell surface PS exposure.

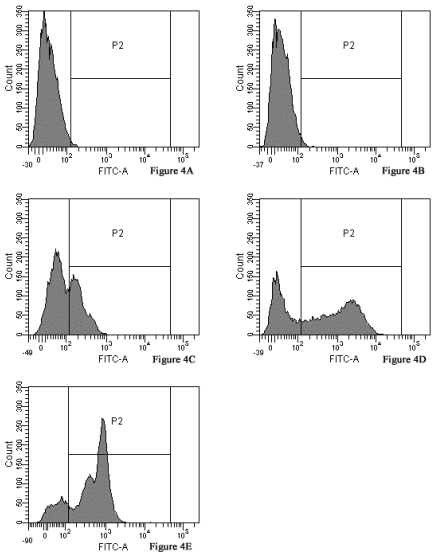

Figure 4: Histogram of FITC-lactadherin in FL1 channel.

Discussion

Multiple myeloma is caused by abnormal proliferation of plasma cells, final stage of B cell. Clinical characteristics are abnormal increase of plasma cells in bone marrow, which results in M proteins and osteolytic lesions. Despite of conventional and high-dose chemotherapy treatment, MM remains largely incurable. In multiple myeloma, bone marrow microenvironment plays an important supporting role in survival of malignant plasma cells. Bone marrow stromal cells can produce the key cytokine interleukin 6 (IL-6) to promote the growth of malignant plasma cells and inhibit their apoptosis [1,3]

The main pathological feature of multiple myeloma is abnormal amplified plasma cells in bone. The prominent clinical feature is bone destruction. A large survey showed that about 2/3 of MM patients come to the hospital for bone pain [4]. This is the main reason leading to bone destruction. In addition, infiltration of tumor cells can also lead to bone damage. The extent of bone destruction is related with OAF concentration in local micro-environment and does not fully reflect the tumor load.

Bisphosphonates can effectively inhibit osteoclast-mediated bone re-absorption. Nitrogen-containing bisphosphonates can function in mevalonate which is important in maintaining membrane stability and inducing phagocytosis of osteoclasts. In addition, bisphosphonates can be adsorbed on the surface of trabecular bone, forming a protective membrane and selective inhibiting osteoclast bone dissolution [5]. When combined with trabecular, bisphosphonates can block the combination of bone tumor cells and bone, which may prevent metastasis of malignant tumors. It has been found that bisphosphonates have direct anti-tumor effect. Some in vitro experiments show that bisphosphonates can inhibit the growth of various cancer cells. A large number of clinical studies have shown that bisphosphonates can decrease the complications caused by bone metastasis of cancer, and some clinical studies and vivo studies have provided several proof on the bisphosphonate anticancer activity in multiple myeloma [2-4]. But, the impact of bisphosphonate on multiple myeloma in virto is not fully understanded. Roelofs et al. study reveals that analogue of bisphosphonate induces apoptosis in human myeloma cells [5], and Mariani S's study reveals that bisphosphonate inhibits multiple myeloma through immunomodulatory effects [6,7].

In this study, the role of pamidronate in inhibiting cell growth and promoting apoptosis effects on multiple myeloma cell lines was tested by MTT, flow cytometry and other methods. Through MTT detection, we confirmed the inhibitory effect of pamidronate on multiple myeloma cells proliferation. This effect is correlated with drug concentration and reaction time. Flow cytometry detection confirmed the occurrence of apoptosis and pamidronate has proliferation and apoptosis induction effect on multiple myeloma cells.

This study demonstrated that bisphosphonates can inhibit the growth of multiple myeloma cells through inducing apoptosis. This may provide theoretical basis for clinical using bisphosphonate to induce apoptosis. However, because of the less drug amount, short medication time, less toxic side effects, no bone marrow suppression and good tolerance of patients of bisphosphonate drugs, the role they play in cancer therapy is more and more important. We applied two methods to detect apoptosis. The first detecting method of is correlated with free Annexin-V concentration, ratio of PS exposure and calcium concentration. So in early apoptosis, when PS exposure is limited, Annexin-V cannot be used as an effective probe. For the second method, lactadherin can closely bind with membrane in the presence of 0.5% PS and do not depend on the concentration of phosphatidylethanolamine (PE). Annexin-V needs to have the participation of physiological Ca2+ concentration, 2% PE, above 8% PS, or 10% PE and 2.5% PS to bind with membrane [1]. When PS concentration reached 8%, the force of lactadherin and Annexin-V binding with the membrane reaches its maximum and is not influenced by PE concentration. Therefore, lactadherin is more sensitive than Annexin-V in detecting PS exposure of cell surface.

We used two kinds of probes to prove that bisphosphonates can induce apoptosis of multiple myeloma cells. Also, we proved that new calcium-independent apoptosis probe – lactadherin is more effective than calcium-dependent annexin V. These two conclusions obtained from the experiments can give effective evidence for future clinical development of new drugs and effective experimental determination of apoptosis.

References

1. Miwa A, Takezako N, Hayakawa H, Hayakawa M, Tominaga S, et al. (2012) YM175 induces apoptosis of human native monocytelineage cells via inhibition of prenylation. Am J Hematol 87: 1084-1088.
2. Terpos E (2012) Bisphosphonate anticancer activity in multiple myeloma. Anticancer Agents Med Chem 12: 123-128.
3. Morgan GJ, Child JA, Gregory WM, Szubert AJ, Cocks K, et al. (2011) Effects of zoledronic acid versus clodronic acid on skeletal morbidity in patients with newly diagnosed multiple myeloma (MRC Myeloma IX): secondary outcomes from a randomised controlled trial. Lancet Oncol 12: 743-752.
4. Guenther A, Gordon S, Tiemann M, Burger R, Bakker F, et al. (2010) The bisphosphonate zoledronic acid has antimyeloma activity in vivo by inhibition of protein prenylation. Int J Cancer 126: 239-246.
5. Roelofs AJ, Hulley PA, Meijer A, Ebetino FH, Russell RG, et al. (2006) Selective inhibition of Rab prenylation by a phosphonocarboxylate analogue of risedronate induces apoptosis, but not Sphase arrest, in human myeloma cells. Int J Cancer 119: 1254-1261.
6. Mariani S, Muraro M, Pantaleoni F, Fiore F, Nuschak B, et al. (2005) Effector gammadelta T cells and tumor cells as immune targets of zoledronic acid in multiple myeloma. Leukemia 19: 664-670.
7. Roodman GD (2010) Pathogenesis of myeloma bone disease. J Cell Biochem 109: 283-291.