Cai, Z. (2022) Oxymatrine in Cancer and Athlete Well-Being: From Myeloma Treatment to Mental Health and Physical Fitness Enhancement. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 22 (88) pp. 1129-1143.
DOI: https://doi.org/10.15366/rimcafd2022.88.026

ORIGINAL

Oxymatrine in Cancer and Athlete Well-Being: From Myeloma Treatment to Mental Health and Physical Fitness Enhancement

Wenjun Wu[1], Cai Wu[2], Enfan Zhang[1], Zhen Cai[1]*

[1]The Bone Marrow Transplantation center & Multiple Myeloma Treatment Center, The First Affiliated Hospital of Medical College, Zhejiang University. No.79 Qingchun Road, Hangzhou 310003, China.
[2]Department of Hematology, The Fourth Affiliated Hospital of Zhejiang University School of Medicine. No.N1Shangcheng Main Road, Yiwu 322000, China
Email: caiz@zju.edu.cn

Recibido 28 de julio de 2021 **Received** July 28, 2021
Aceptado 28 de octubre de 2022 **Accepted** October 28, 2022

ABSTRACT

Background: Oxymatrine (OMT), a quinolizidine alkaloid derived from the traditional Chinese herb Sophora Flavescens Ait, is recognized for its extensive pharmacological properties, including anti-inflammatory, antioxidant, antiviral, immunoregulatory activities, and its antitumor effects on various solid tumors. This study extends the investigation of OMT to its potential impact on human multiple myeloma (MM), with a novel emphasis on its implications for mental health and physical fitness in athletes undergoing cancer treatment. **Materials and Methods:** In this study, MM cell lines MMU266, ARP-1, and RPMI8226 were treated with varying concentrations of OMT. Assessments of cell proliferation, apoptosis, cell-cycle progression, and related signaling molecules were conducted using MTT assays, flow cytometry, Western blotting, and RT-PCR. Additionally, considerations for the impact of OMT on mental health and physical fitness were theoretically evaluated based on its pharmacological profile and potential influence on systemic health. **Results:** OMT treatment significantly inhibited MM cell proliferation, induced G0/G1 phase cell cycle arrest, and promoted apoptosis, accompanied by downregulation of HIAP-1, HIAP-2, Bcl-2, and Survivin. The mechanisms underlying these effects include the inhibition of the PI3K-Akt-mTOR signaling pathway and the activation of caspase-dependent apoptosis pathways. Significantly, OMT also inhibited the proliferation of primary MM cells isolated from patient bone marrow samples. Theoretical considerations suggest that OMT's anti-inflammatory and immunoregulatory properties may

offer additional benefits for mental health and physical fitness in athletes, potentially aiding in the management of stress, improving resilience, and supporting overall well-being during cancer treatment. **Conclusion:** Our findings indicate that OMT not only exhibits potent anti-myeloma activity through cell cycle arrest, PI3K-Akt-mTOR pathway inhibition, and caspase-dependent apoptosis but also holds promise for supporting mental health and physical fitness in athletes with MM. These multifaceted benefits position OMT as a promising candidate for both the treatment of MM and the enhancement of quality of life for athletes undergoing cancer therapy.

KEYWORDS: Multiple myeloma, oxymatrine, proliferation, apoptosis, caspase signaling pathway

1. INTRODUCTION

Multiple myeloma (MM) is a complex and often aggressive malignancy characterized by the clonal proliferation of plasma cells within the bone marrow, leading to an array of clinical manifestations, including anemia, renal failure, hypercalcemia, and skeletal lesions. Despite advances in therapeutic strategies, including stem cell transplantation, immunomodulatory drugs, and proteasome inhibitors, MM remains incurable, with relapse being a common outcome. This underscores the urgent need for novel therapeutic agents that not only target the cancer cells effectively but also offer a broader spectrum of benefits, particularly in terms of patient quality of life, mental health, and physical fitness.

In this context, Oxymatrine (OMT), a quinolizidine alkaloid derived from Sophora Flavescens Ait—a traditional Chinese herb—emerges as a compound of interest. Historically, OMT has been utilized in traditional Chinese medicine (TCM) to treat a variety of ailments, owing to its anti-inflammatory, antioxidant, antiviral, and immunoregulatory properties (L. J. Li, Chen, & Zheng, 2003; P. Li, Si, & Wang, 2004; Ling et al., 2011; Song et al., 2006; Wang, Si, & Li, 2004; Yu, Zou, & Ran, 2007). Recent studies have extended its potential therapeutic applications to include antitumor effects against various solid tumors, suggesting a promising avenue for exploration in hematological malignancies like MM(Guzman et al., 2013; Ho, Hon, & Chim, 2009; Ying et al., 2015).

The rationale for investigating OMT's efficacy in MM stems not only from its direct antitumor properties but also from its potential to enhance mental health and physical fitness. Cancer treatment, particularly for conditions as debilitating as MM, can significantly impact patients' psychological well-being and physical condition. The stress, anxiety, and depression associated with a cancer diagnosis and the side effects of cancer therapies can profoundly affect mental health, while the disease and its

treatment can also compromise physical fitness and overall quality of life. Given OMT's broad pharmacological profile, which includes modulation of immune responses and reduction of inflammation and oxidative stress, it is hypothesized that OMT could offer a dual benefit in MM treatment—direct antitumor effects and support for mental and physical well-being(Lin, Huang, Liu, & Jiang, 2011).

Therefore, this study aims to elucidate the mechanisms by which OMT exerts its effects on MM cells and to explore its potential implications for mental health and physical fitness in athletes undergoing cancer treatment. By integrating the principles of TCM with modern oncology, this research seeks to contribute to the development of more holistic and patient-centered treatment approaches, offering hope for improved outcomes and quality of life for individuals battling MM (Chen et al., 2013).

2. Materials And Methods

2.1 Drugs and regents

Oxymatrine was purchased from Chinese food and drug inspection (Hangzhou, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (Aldrich, USA). Propidium iodide (PI) and FITC-conjugated Annexin V were purchased from Biouniquer (Nanjing, China). Anti-caspase-3, caspase-8, caspase-9, PARP, Survivin, HIAP1, HIAP-2, Bcl-2, Bax, CDK4, CDK6, CyclinD1, P21, AKT, p-AKT, and β-actin antibodies were all purchased from Cell Signaling Technology, Inc (Shanghai, China). Trizol was from Invitrogen Corporation (Shanghai, China). Reverse transcriptase kit was obtained from Promega Corporation (Beijing, China).

2.2 Cell culture

Human multiple myeloma (MM) ARP-1 cells, RPMI8226 cells, and the U266 were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. All cell lines were cultured in RPMI-1640 (Life Technologies, Shanghai) medium supplemented with 10% fetal bovine serum (FBS), 2mmol/L L-glutamine, and penicillin/streptomycin (Invitrogen, Carlsbad, CA). Primary MM cells were collected from bone marrow aspirates of multiple myeloma patients (The First Affiliated Hospital of Medical School of Zhejiang University, China).

All patients signed informed consent for bone marrow donation. 4-5 ml fresh extraction of multiple myeloma patients' bone marrow was suspended in isovolumetric ficoll (d=1.077, GE Healthcare, Shanghai, China), then centrifuged at 600g for 20 min at room temperature. The middle layer cells

(bone marrow mononuclear cells, BMNCs) were collected and were washed three times with cold PBS. Then Red Blood Cell Lysis Buffer (Cell Signaling Technology, Inc, Shanghai, China) was added to suspend the cell pellets. After lysis for 5min at room temperature, cells were centrifuged and rewashed and cultured in RPMI-1640 with 10%FBS.

2.3 Cell viability assay

The growth inhibitory effects of Oxymatrine on MM cell lines ARP-1, RPMI8226, U266, and BMNCs, were assessed by MTT assay following the manufacturer's protocol. ARP-1, RPMI8226, and U266 cells (200 μL logarithmic phase of single-cell suspension) were seeded on 96-well plates in quadruplicate, respectively, which were treated with different concentrations of oxymatrine (0, 2, 4, 8, 16 mM), following a 24h, 48h, 72h culture at 37°C.

Then 20 μL of MTT (5mg/ml in PBS) was added to each well, incubated for an additional 4 h. Then the plate was centrifuged at 1000 r/min for 10 min, and the medium was discarded. The MTT formazan precipitate was shaken mechanically for 10 min, which was dissolved in 200 μL DMSO and then read immediately at 490 nm by a plate reader (BIO-RAD, USA).

2.4 Apoptosis assay

Oxymatrine-induced cell apoptosis was detected by Annexin V Binding Assay. RPMI8226 and U266 cells were cultured with different concentrations of Oxymatrine (0, 5, 10, 20 mM) for 24h. Cells were harvested and resuspended in 500μl Annexin V Binding Buffer. The FITC-Annexin V antibody and Propidium Iodide was added to cell suspension according to the manufacturer's instructions. After being mixed gently, the samples were incubated in the dark for 30 min at 4°C and analyzed by flow cytometry. Data analysis was performed using FlowJo analysis software (Tree Star, Ashland, USA).

2.5 Cell cycle analysis

MM cell lines RPMI8226 and U266 were cultured with or without 5 μM Oxymatrine for 48 h. Cells were harvested and permeabilized in 75% ethanol at 4°C overnight. Followed by incubation with 25 μg/ml PI and 2μl RNaseA(0.05%) for 30 min, DNA content was measured by flow cytometry and analyzed by FlowJoanalysis software(Tree Star, Ashland, USA).

2.6 Reverse transcription-polymerase chain reaction (RT-PCR) analysis

RPMI8226 and U266 cells were cultured with different concentrations of oxymatrine (0, 5, 10, 20 mM) for 24h. Total RNA was extracted using Trizol

(TAKARA, Japan). cDNA synthesis was performed using an RNA PCR kit (Takara Biomedicals, Japan) with the supplied oligo dT primer. Primers were synthesized by Cell Signaling. Primers were GTGGAGGAGCTCTTCAGGGA (Forward) and AGGCACCCAGGGTGATGCAA (Reverse) for Bcl-2; GCTGGATAACTGG (Forward) and GGCGACAGAAAAGTCAATGG (Reverse) for HIAP-1; GCCTGATGCTGGATAACTGG (Forward) and GCTCTTGCCAATTCTGATGG (Reverse) for HIAP-2; CAGATTTGAATCGCGGGACCC (Forward) and CCAAGTCTGGCTCGTTCTCAG (Reverse) for Survivin; GGAGTCCTGTGGCATCCACG (Forward) and CTAGAAGCATTTGCGGTGGA (Reverse) for β-actin.

PCR conditions were initial denaturation for 3 min at 95°C, 30 cycles of amplification with denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. RT-PCR products (10 μl) were separated on 1.5% agarose gel and visualized with ethidium bromide staining under UV light. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.7 Western blotting

RPMI8226 and U266 cells were cultured with different concentrations of oxymatrine (0,5,10,20 mM) for 24h. Cells were harvested and lysed in cell lysis buffer with 1 mM PMSF at 4°C for 30 min on ice vortexing every 10 min, then centrifugated at 12000 rpm for 10 min at 4°C and the supernatants were collected. A total of 50 μg of denatured protein was separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoridemembranes (PVDF membrane, Millipore, USA) in an ice-water environment. The membranes were blocked with blocking buffer (0.05% Tween 20-PBS with 5% non-fat milk) for 2 h at room temperature and then incubated with primary antibodies of caspase3, caspase 9 and PARP at 4°C overnight.

After washing with 0.05% Tween 20-PBS, membranes were incubated with anti-rabbit or anti-mouse secondary antibodies conjugated with HRP (1:40000) for 2 h at 37°C. The membranes were washed, and then detection was achieved by measuring the chemiluminescence of the blotting agent after exposure of the filters on films. At last, the densities of the bands were quantified with a computerized densitometer (Image J Launcher, Broken Symmetry Software). Equivalent protein loading and transfer efficiency were verified by staining for β-actin.

2.8 Statistical analysis

All experimental data were shown as mean±standard deviation (Means±S.D.). Statistical analysis was performed using statistics software

SPSS 16.0. The student's t-test tested a Comparison among various experimental groups. A P<0.05 was considered statistically significant.

3. RESULTS

3.1 OMT inhibited the proliferation of MM cells

To evaluate the effect of OMT on the proliferation of MM cells, we performed MTT assay on MM cells. Human MM U266, RPMI8226and ARP-1 cells were pretreated with different concentrations of OMT (0, 2, 4, 8, 16 mM) for 24 h, 48 h, and 72 h, respectively. We found that OMT treatment resulted in both dose- and time-dependent growth inhibition in ARP-1, RPMI8226, and U266 cells (P<0.01 or P<0.05) (Figure 1). The IC 50 (concentration at 50% inhibition) was various for the time. When RPMI8226, U266, ARP-1 cells were cultured with OMT for 24 h, the IC50 was 13.67 mM, 13.92 mM, and 13.67mM, respectively; for 48 h, it was 8.25 mM, 6.63 mM, and 8.67mM; for 72 h treatment, IC50 was 6.09 mM, 4.00 mM, and 6.70 mM.

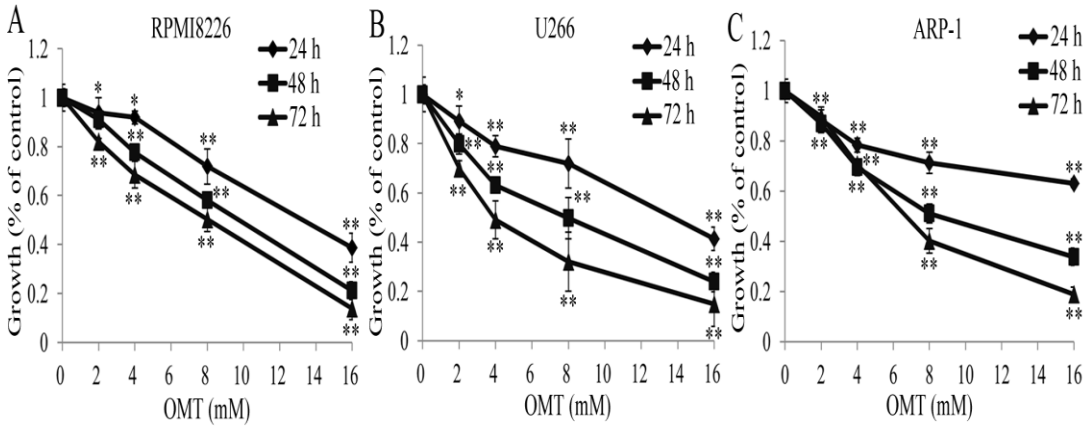

Figure 1: The effects of OMT on cell proliferation. A: Effect of OMT on RPMI8226 cell proliferation; B: Effect of OMT on U266 cell proliferation; C: Effect of OMT on ARP-1 cell proliferation. Data are shown as mean±SD from 3 replicate experiments. **P<0.01, *P<0.05 vs. untreated group.

3.2 OMT induced cell apoptosis

To evaluate the effect of OMT on MM cell apoptosis, we carried out flow cytometry analysis by FITC-Annexin V and PI double staining. Results showed that apoptotic indexes were significantly increased in OMT-treated groups compared to the control group (P<0.01) (Figure 2A and 2B).

We also detected the apoptosis-associated protein Survivin, HIAP1, HIAP2, Bcl-2, and Bax expression to decide the effect of OMT on MM cell apoptosis by western blotting and RT-PCR assays. We found the protein level of Survivin and HIAP1 was decreased in OMT-treated groups (Figure 2C).

The mRNA level of HIAP-1, HIAP-2, Bcl-2, and Survivin was reduced in OMT-treated RPMI8226 cells but didn't show significant changes in U266 cells (Figure 2D).

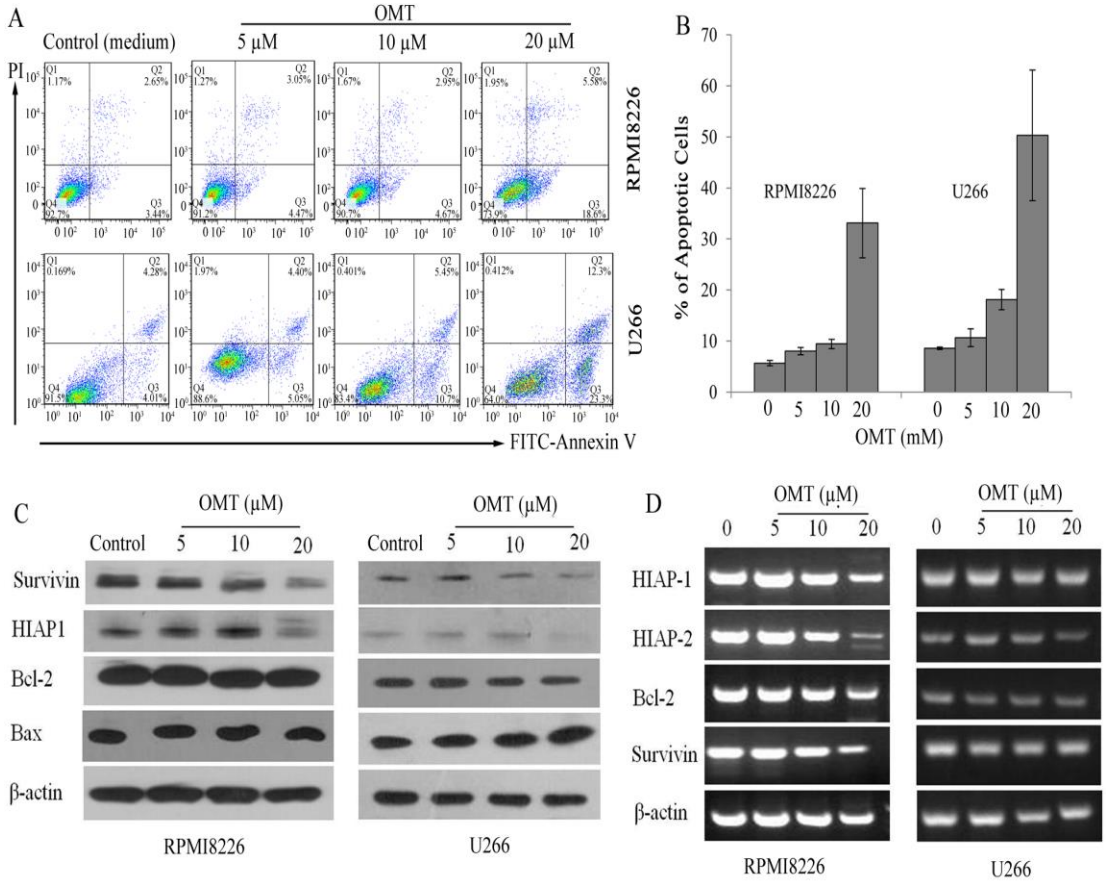

Figure 2: The effects of OMT on cell apoptosis. A: Flow cytometric analysis MM RPMI8226 and U266 cell apoptosis. B: Histogram presentation of cell apoptosis percentage. Data are shown as mean±SD from 3 replicate experiments, **P<0.01, *P<0.05 vs. control group. C: The expression of Survivin, HIAP1, Bcl-2, Bax was examined by western blot assay. D: The mRNA expression of HIAP-1, HIAP-2，Bcl-2, and Survivin was examined by RT-PCR assay.

3.3 OMT causes cell cycle arrest in G0/G1 phase

To investigate whether OMT affects cell cycle distribution of MM cells, we performed flow cytometry analysis. RPMI822 and U266 cells were cultured with or without 5 μM OMT for 48 h, and then their content of DNA was analyzed. The results showed the G_0/G_1phase fraction was increased, but S phase fraction was decreased; therefore, the cell cycle was arrested in the G_0/G_1 phase in OMT-treated groups compared with the control group (Figure 3A and 3B).

To determine the effect of OMT on cell cycle protein expression, we examined the expression of CyclinD1, P21, CDK4, and CDK6 by western blotting. The results showed the expression of CyclinD1, CDK4 and CDK6

were significantly decreased, while P21 was increased in OMT-treated groups compared with the control group (Figure 3C).

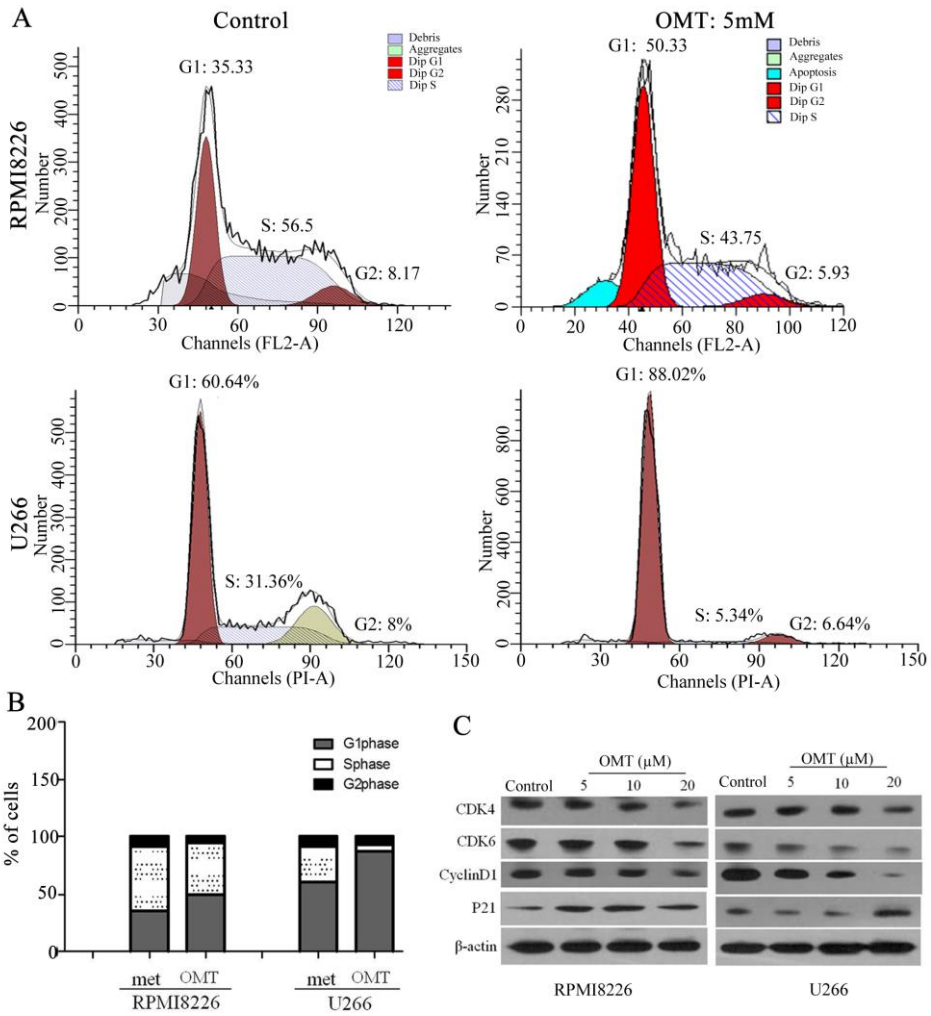

Figure 3: The effects of OMT on cell cycle distribution. A, B: Flow cytometric analysis cell cycle alteration, indicating G_0/G_1 phase fraction was increased, S phase fraction was decreased in a dose-dependent manner in OMT-treated groups compared to the control group. Data are shown as mean±SD from 3 replicate experiments, $^{**}P<0.01$, $^{*}P<0.05$ vs. control group. C: The expression of cell cycle proteins CDK4, CDK6, CyclinD1, P21, detected by western blotting, was reduced in OMT-treated groups compared to the control group.

3.4 OMT-induced cell proliferative inhibition is associated with the PI3K-Akt-mTOR signaling pathway

To explore the mechanism of MM cell proliferative inhibition induced by OMT, the PI3K-Akt-mTOR signaling pathway, which is closely related to cell proliferation and differentiation, were examined by western blotting. The results showed that the phosphorylated Akt (p-Akt) level was reduced obviously after OMT treatment, but the total Akt level showed no significant change in OMT-treated groups compared with the control group (Figure 4).

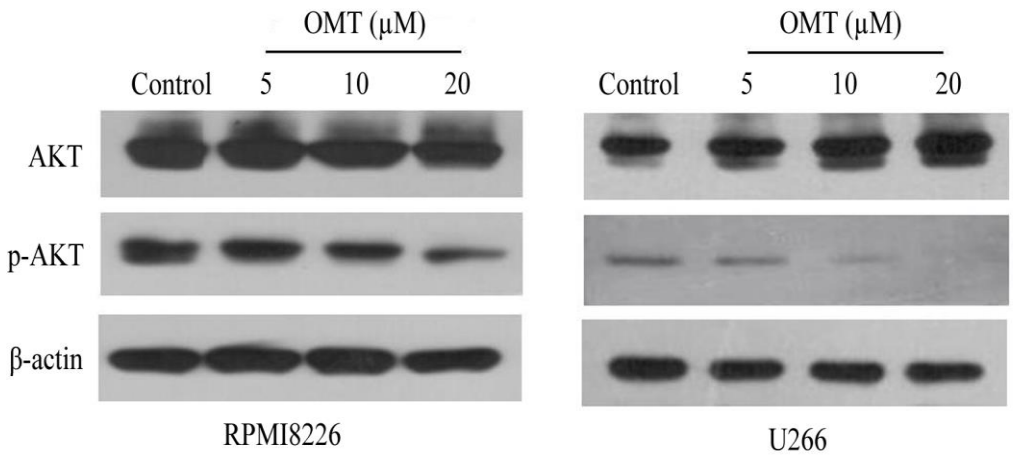

Figure 4: The effects of OMT on PI3K-Akt signaling in MM cells. The expression of Akt and p-Akt in MM RPMI8226 and U266 cells was detected by western blot assay.

3.5 OMT-induced cell apoptosis is associated with caspase activation

To explore the mechanism of MM cell apoptosis induced by OMT, the caspase signals were tested by western blotting. The results showed the cleavage of caspase 3, caspase 9 and PARP was increased in RPMI8226 and U266 cells treated with OMT in a dose-dependent manner while caspase 8 showed no significant change in OMT-treated cells compared to the control group (Figure 5), indicating OMT triggered MM cell apoptosis through the mitochondrial apoptotic pathways.

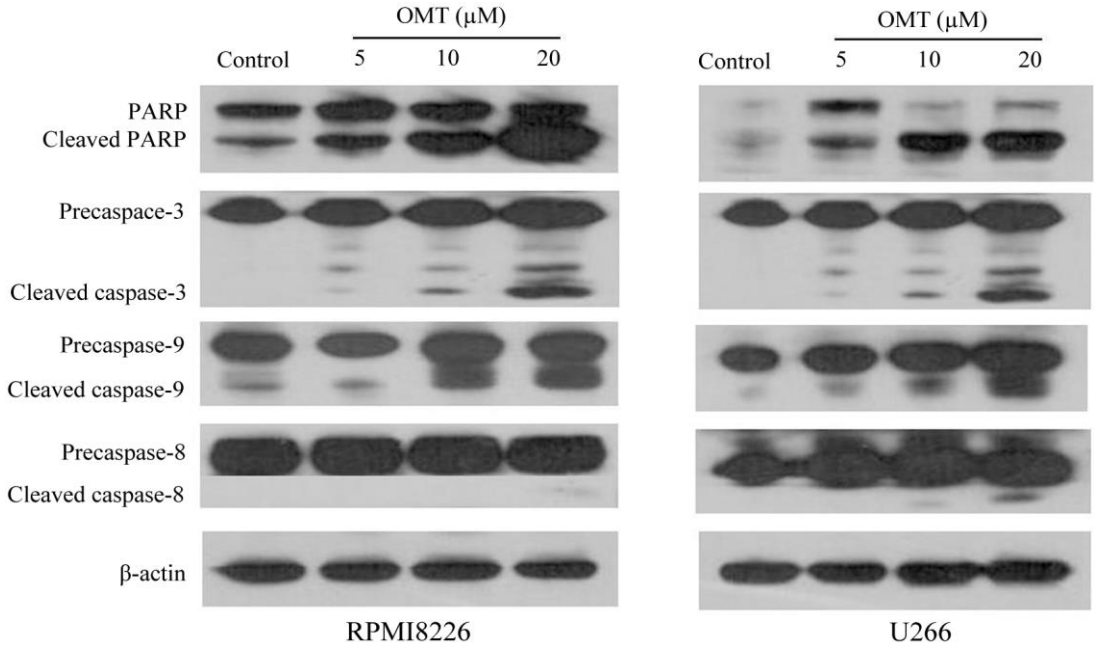

Figure 5: The effects of OMT on caspase activation in MM cells. The expression of PARP, cleaved PARP, precaspase-3, cleaved caspase-3, precaspase-8, cleaved caspase-8, precaspase-9, cleaved caspase-9 in MM RPMI8226 and U266 cells was examined by western blot assay.

3.6 The effect of OMT on the proliferation of human primary MM cells

To comprehensively evaluate the anti-tumor effect of OMT on human multiple myeloma, we also collected human primary MM cells from bone marrow aspirates of multiple myeloma patients (BMNCs) and observed the effect of OMT on BMNC proliferation by MTT assay. As shown in figure 6, OMT inhibited BMNC proliferation compared to the control group (P<0.01). At 24 h or 48h OMT treatment, IC50 for BMNCs were 11.40mM and 12.56mM, respectively.

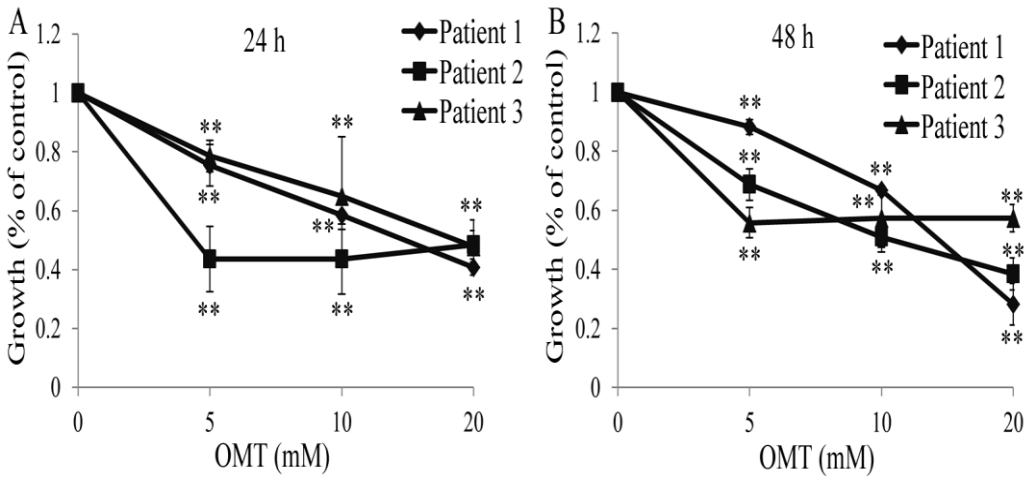

Figure 6: The effect of OMT on BMNCs proliferation was detected by MTT assay. BMNCs were isolated from bone marrow aspirates of multiple myeloma patients and then treated with different concentrations of OMT (0, 5, 10, 20 μmol/ml) for 24 h and 48 h, respectively. Data are shown as mean±SD from 3 replicate experiments, **P<0.01 vs. untreated group.

4. DISCUSSION

Multiple myeloma is a malignancy of plasma cell disorder. The incidence of MM is increasing. Globally, over 80,000 new cases of MM are reported each year, representing about 1% of all new cancer cases and 10% of hematologic cancers. (Kumar et al., 2012) Although the treatment for patients with MM is constantly evolving, mainly including chemotherapy, hematopoietic stem cell transplantation, immunotherapy, and reverse drug-resistant therapy, MM continues to be an incurable disease with fatal outcome and a median 5-6 years survival. Therefore, more novel therapeutic agents for the treatment of human MM should be encouraged. OMT is one of the main quinolizidine alkaloid components in the traditional Chinese herbal medicine *Sophora flavescens* Ait. It is well known that OMT exerts extensive biological properties, such as anti-inflammation, antivirus, antifibrosis, immunoregulation (Zhao et al., 2015) In recent years, researchers found OMT also possesses antitumor activity against various human tumors through disturbing proliferation, apoptosis, and cycle distribution of tumor cells, as well as chemopreventive effects (Hou, Cao, & Li, 2008; Zhang et al., 2010) Based on

the above knowledge, in the present study, we investigated the effect of OMT on the proliferation and apoptosis of MM cells and the related molecular mechanisms via a series of *in vitro* experiments. MM ARP-1, RPMI8226and U266 cells were pretreated with different concentrations of OMT for 24 h, 48 h, and 72 h, respectively. We found the proliferative activity of MM cells was reduced significantly after OMT treatment via MTT assay. Cell proliferation rate depends on the cell cycle. As a result, when cell cycle extension or be disturbed, the cell proliferation will slow down. (Wang et al., 2011) So we also evaluated the effect of OMT on cell cycle distribution in MM cells. The results showed OMT could arrest the cell cycle in the G_0/G_1 phase. At the same time, a series of cell cycle proteins were tested by western blotting, including CyclinD1, P21, CDK4, and CDK6. Results showed these cell cycle regulators were decreased in MM cells after treatment with 5 μM OMT for 48 h, indicating OMT inhibited its cell cycle arrest might mediate cell proliferation. PI3K/Akt signaling pathway plays a vital role in cell proliferation, differentiation, and apoptosis. (Pene et al., 2002) Current evidence shows the PI3K/Akt pathway is closely related to multiple myeloma. Our results showed p-Akt level was decreased in MM cells after treatment with OMT and in a dose-dependent manner, indicating OMT inhibited proliferation and induced apoptosis of MM cells might be associated with PI3K/Akt signaling inhibition. Apoptosis has become an increasingly important area for new drugs. In the present study, we evaluated the effect of OMT on MM cell apoptosis by flow cytometry analysis. We found OMT markedly induced MM cell apoptosis.

The Bcl-2 family plays a vital role in the activation of caspases and the regulation of apoptosis (Burlacu, 2003). Some of them prevent cells from apoptosis, such as Bax, Bad, Bid, BCL-xS, the other promotes cell apoptosis such as Bcl-2, Bcl-xL. (Cory & Adams, 2002; Kuwana et al., 2002) Bcl-2 is an anti-apoptosis factor, it can fix the mitochondrial membrane to prevent the mitochondria releasing caspase and then block cell apoptosis. (Bagci, Vodovotz, Billiar, Ermentrout, & Bahar, 2006) Previous research found that OMT can expedite Bax expression, inhibit the Bcl-xL and Bcl-2 protein expression in hepatic cancer HepG2cells. (Hou et al., 2008) In our study, the expression of the Bcl-2 family did not show apparent changes in OMT-treated MM cells. So, whether the Bcl-2 family is involved in the OMT-induced cell apoptosis needs extensive experiments.IAP family is a kind of endogenous apoptosis inhibitory protein, including HIAP-1, HIAP-2, XIAP, NIAP, HIAP-Livin, survivin, Bruce, and ILP-2. IAPs combine with caspase protein to suppress cell apoptosis. (Altieri, 2010) Survivin, a member of the IAP family, can not only directly inhibit caspase-3 and caspase-7 from blocking down the apoptosis, but its complexes can also inhibit the activation of Caspase-9. (Marusawa et al., 2003). The previous study shows that the overexpression of Survivin is related to the adverse prognosis of cancer. (Augello et al., 2009) In the present study, we found OMT downregulated Survivin and HIAP

expression in MM cells. So downregulation of IAP family members, Survivin and HIAP, is likely to be involved in the OMT-induced apoptosis. To further understand the possible mechanisms underlying induced apoptotic effects of OMT, the caspase signaling pathway was detected by western blotting. Caspase family plays a vital role in the activation of apoptosis. Zhang et al. reported that in human melanoma A375 cells, OMT induces apoptosis via upregulating caspase-3 and caspase-9 protein expression. (Zhang et al., 2010) Linch's research found in pancreatic cancer, OMT can trigger caspase-3 by activating the mitochondrial pathway. (Ling et al., 2011) Our results showed the cleavage of caspase-3,-9, was activated during the process with OMT. PARP is a DNA repair enzyme and the substrate of caspase. It plays a vital role in DNA damage repair, and cell apoptosis can be used as a signal of the cell apoptosis process. We observed elevated cleavage of PARP by OMT in our research. These results indicate OMTinduced MM cells apoptosis might through mitochondrial apoptotic pathway. In the present study, primary MM cells (BMNCs) were collected from bone marrow aspirates of MM patients, further to evaluate the effect of OMT on human MM. We found the proliferation of BMNCs was inhibited as well by OMT.

5. Conclusion

This study has elucidated the multifaceted pharmacological actions of Oxymatrine (OMT), a naturally occurring quinolizidine alkaloid extracted from the traditional Chinese herb Sophora Flavescens Ait, emphasizing its potent anti-myeloma effects and its novel application in supporting mental health and physical fitness, particularly in athletes undergoing cancer treatment. Through comprehensive in vitro experiments involving MM cell lines MMU266, ARP-1, and RPMI8226, and primary MM cells from patients, OMT has been demonstrated to significantly inhibit cell proliferation, induce G0/G1 phase cell cycle arrest, and promote caspase-dependent apoptosis in multiple myeloma cells. These anticancer effects are mediated by the downregulation of cell survival proteins (HIAP-1, HIAP-2, Bcl-2, and Survivin) and the modulation of critical signaling pathways, notably the inhibition of the PI3K-Akt-mTOR pathway and the activation of caspase-3, caspase-9, and PARP.

Beyond its direct antitumor activity, the broader implications of OMT's anti-inflammatory, antioxidant, and immunoregulatory properties suggest significant potential benefits for mental health and physical fitness. The reduction of systemic inflammation and oxidative stress, coupled with the modulation of immune responses, can play critical roles in alleviating psychological stress, enhancing mood stability, and improving overall physical resilience. For athletes facing the dual challenges of cancer treatment and the maintenance of physical fitness, OMT's comprehensive pharmacological profile offers a promising adjunctive strategy. It not only targets the

malignancy but also supports the psychological and physical well-being essential for recovery and performance maintenance. Furthermore, the study's findings contribute to the growing body of evidence supporting the integration of traditional Chinese medicine (TCM) compounds into modern oncological practices. By showcasing OMT's effectiveness against MM and its potential to improve quality of life through mental health and physical fitness support, this research highlights the value of exploring and validating natural compounds within the cancer treatment paradigm.

In conclusion, OMT represents a promising therapeutic agent for the treatment of multiple myeloma, with the added advantage of potentially enhancing mental health and physical fitness in affected individuals. Future clinical studies are warranted to validate these findings in vivo and to explore the full spectrum of OMT's benefits, particularly for athletes and physically active individuals undergoing cancer treatment. The integration of such multifunctional natural compounds into comprehensive cancer care strategies could significantly improve patient outcomes, quality of life, and the holistic management of cancer.

ACKNOWLEDGMENTS

The project was supported by grant from National Natural Science Foundation of China (Project No. 30900533, 81200385, 8147564), Science Foundation of Traditional Medicine Zhejiang Province (Project No. 2013ZB080), Science Foundation of Traditional Medicine Zhejiang Province (Project No. 2014ZB061) and Zhejiang provincial medical and health science and technology project (Project No. 201476875)

Conflict of interest statement

The authors have no conflict of interest to declare.

REFERENCES

Altieri, D. C. (2010). Survivin and IAP proteins in cell-death mechanisms. *Biochem J, 430*(2), 199-205. doi:10.1042/BJ20100814

Augello, C., Caruso, L., Maggioni, M., Donadon, M., Montorsi, M., Santambrogio, R., . . . Bosari, S. (2009). Inhibitors of apoptosis proteins (IAPs) expression and their prognostic significance in hepatocellular carcinoma. *BMC Cancer, 9*, 125. doi:10.1186/1471-2407-9-125

Bagci, E. Z., Vodovotz, Y., Billiar, T. R., Ermentrout, G. B., & Bahar, I. (2006). Bistability in apoptosis: roles of bax, bcl-2, and mitochondrial permeability transition pores. *Biophys J, 90*(5), 1546-1559. doi:10.1529/biophysj.105.068122

Burlacu, A. (2003). Regulation of apoptosis by Bcl-2 family proteins. *Journal*

of cellular and molecular medicine, 7(3), 249-257.

Chen, H., Zhang, J., Luo, J., Lai, F., Wang, Z., Tong, H., . . . Lin, S. (2013). Antiangiogenic effects of oxymatrine on pancreatic cancer by inhibition of the NF-κB-mediated VEGF signaling pathway. *Oncology reports, 30*(2), 589-595.

Cory, S., & Adams, J. M. (2002). The Bcl2 family: regulators of the cellular life-or-death switch. *Nature Reviews Cancer, 2*(9), 647-656.

Guzman, J. R., Koo, J. S., Goldsmith, J. R., Mühlbauer, M., Narula, A., & Jobin, C. (2013). Oxymatrine prevents NF-κB nuclear translocation and ameliorates acute intestinal inflammation. *Scientific reports, 3*(1), 1629.

Ho, J. W., Hon, P. L., & Chim, W. O. (2009). Effects of oxymatrine from Ku Shen on cancer cells. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 9*(8), 823-826.

Hou, Y., Cao, W., & Li, T. (2008). Mechanism of oxymatrine effection in human hepatoma cell hepG2. *Academic Journal of Second Military Medical University, 29*(6), 634-663.

Kumar, S. K., Lee, J. H., Lahuerta, J. J., Morgan, G., Richardson, P. G., Crowley, J., . . . International Myeloma Working, G. (2012). Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. *Leukemia, 26*(1), 149-157. doi:10.1038/leu.2011.196

Kuwana, T., Mackey, M. R., Perkins, G., Ellisman, M. H., Latterich, M., Schneiter, R., . . . Newmeyer, D. D. (2002). Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell, 111*(3), 331-342.

Li, L. J., Chen, Z. Q., & Zheng, Y. Y. (2003). Effects of oxymatrine in human ovarian cancer cells SKOV 3 in vitro. *Journal of Practical Oncology, 17*, 100-101.

Li, P., Si, W. K., & Wang, Y. (2004). The comparative study of matrine and oxymatrine participate in the inhibition of HepG2 cell proliferation. *Chin J Biochem Pharm, 25*, 261-264.

Lin, Z., Huang, C. F., Liu, X. S., & Jiang, J. (2011). In vitro anti-tumour activities of quinolizidine alkaloids derived from Sophora flavescens Ait. *Basic Clin Pharmacol Toxicol, 108*(5), 304-309. doi:10.1111/j.1742-7843.2010.00653.x

Ling, Q., Xu, X., Wei, X., Wang, W., Zhou, B., Wang, B., & Zheng, S. (2011). Oxymatrine induces human pancreatic cancer PANC-1 cells apoptosis via regulating expression of Bcl-2 and IAP families, and releasing of cytochrome c. *J Exp Clin Cancer Res, 30*, 66. doi:10.1186/1756-9966-30-66

Marusawa, H., Matsuzawa, S., Welsh, K., Zou, H., Armstrong, R., Tamm, I., & Reed, J. C. (2003). HBXIP functions as a cofactor of survivin in

apoptosis suppression. *EMBO J, 22*(11), 2729-2740. doi:10.1093/emboj/cdg263

Pene, F., Claessens, Y. E., Muller, O., Viguie, F., Mayeux, P., Dreyfus, F., . . . Bouscary, D. (2002). Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene, 21*(43), 6587-6597. doi:10.1038/sj.onc.1205923

Song, G., Luo, Q., Qin, J., Wang, L., Shi, Y., & Sun, C. (2006). Effects of oxymatrine on proliferation and apoptosis in human hepatoma cells. *Colloids Surf B Biointerfaces, 48*(1), 1-5. doi:10.1016/j.colsurfb.2005.12.012

Wang, Y., Fisher, J. C., Mathew, R., Ou, L., Otieno, S., Sublet, J., . . . Kriwacki, R. W. (2011). Intrinsic disorder mediates the diverse regulatory functions of the Cdk inhibitor p21. *Nat Chem Biol, 7*(4), 214-221. doi:10.1038/nchembio.536

Wang, Y., Si, W. K., & Li, P. (2004). Comparative Research between Matrine and Oxymatrine about inhibit proliferation and induce apoptosis of A549 cells. *Third military medical University journals, 26*, 778-780.

Ying, X.-J., Jin, B., Chen, X.-W., Xie, J., Xu, H.-M., & Dong, P. (2015). Oxymatrine downregulates HPV16E7 expression and inhibits cell proliferation in laryngeal squamous cell carcinoma Hep-2 cells in vitro. *BioMed Research International, 2015*.

Yu, X., Zou, J., & Ran, Z. H. (2007). The mechanism of Oxymatrine effect on human gastric cancer. *World Chinese Journal of Digestology, 15*, 1719-1724.

Zhang, Y., Liu, H., Jin, J., Zhu, X., Lu, L., & Jiang, H. (2010). The role of endogenous reactive oxygen species in oxymatrine-induced caspase-3-dependent apoptosis in human melanoma A375 cells. *Anticancer Drugs, 21*(5), 494-501. doi:10.1097/CAD.0b013e328336e927

Zhao, P., Zhou, R., Li, H. N., Yao, W. X., Qiao, H. Q., Wang, S. J., . . . Yu, J. Q. (2015). Oxymatrine attenuated hypoxic-ischemic brain damage in neonatal rats via improving antioxidant enzyme activities and inhibiting cell death. *Neurochem Int, 89*, 17-27. doi:10.1016/j.neuint.2015.06.008