

Omacetaxine mepesuccinate induces apoptosis and cell cycle arrest, promotes cell differentiation, and reduces telomerase activity in diffuse large B-cell lymphoma cells

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Abstract. Clinical studies have demonstrated that omacetaxine mepesuccinate exerts beneficial effects on acute myelogenous leukemia. It has been suggested that omacetaxine mepesuccinate, used alone or with interferon- α or cytarabine, induces remission in patients with chronic myelogenous leukemia. These effects are possibly mediated by its ability to induce apoptosis of leukemia cells and inhibit the activity of telomerase. To determine whether omacetaxine mepesuccinate is beneficial in diffuse large B-cell lymphoma (DLBCL), two DLBCL cell lines [a germinal center B cell-like subtype (GCB) and an activated B cell-like subtype (ABC)] were treated with omacetaxine mepesuccinate at various concentrations for different durations. The present study indicated that omacetaxine mepesuccinate exerts proapoptotic effects in the two cell types in a dose- and time-dependent manner. The ABC subtype demonstrated increased sensitivity compared with the GCB subtype. At 40 ng/ml, omacetaxine mepesuccinate exhibited a marked proapoptotic effect on DLBCL cells compared with the other tumor cells investigated. Furthermore, omacetaxine mepesuccinate induced cell cycle arrest at G₀/G₁ phase, and promoted cell terminal differentiation of pro-B cells. The present study also demonstrated that omacetaxine mepesuccinate exerted its antitumor effect by

reducing telomerase activity. In conclusion, the present study demonstrated that omacetaxine mepesuccinate may induce apoptosis and cell cycle arrest, promote cell differentiation, and reduce telomerase activity in DLBCL cells, thus aiding the development of omacetaxine mepesuccinate-based DLBCL therapeutic strategies.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of adult non-Hodgkin's lymphoma, a group of highly invasive and heterogeneous cancers, accounting for 30-40% of cases (1). According to the immune phenotype, DLBCL is divided into three subtypes, namely, germinal center B cell-like subtype (GCB), activated B cell-like subtype (ABC), and type III diffuse large B-cell lymphoma (2). Immunological therapy using rituximab, a monoclonal antibody against B cell surface protein cluster of differentiation (CD)20 (3), combined with traditional therapeutic strategies, have notably improved the rate of complete remission and disease-free survival in patients with DLBCL (4,5). However, a marked number of patients are resistant to these advanced therapies for reasons that remain to be elucidated (6,7). Previous studies have suggested that the prognosis of GCB subtype is better compared with that of the other two subtypes (8,9).

Omacetaxine mepesuccinate is a plant alkaloid extracted from the total alkaloids of *Cephalotaxaceae*. Omacetaxine mepesuccinate has been used as an antitumor therapeutic agent to treat acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML) and myelodysplastic syndrome (10-12). Furthermore, previous studies have reported that omacetaxine mepesuccinate may be useful in treating lymphoma (13-15); however, it remains to be elucidated how omacetaxine mepesuccinate exerts its therapeutic effects on this type of cancer. The present study examined the cellular effect of omacetaxine mepesuccinate on two human DLBCL cell lines and demonstrated that omacetaxine mepesuccinate induces apoptosis and regulates cell cycling, differentiation and telomerase activity in the ABC and GCB subtype of human DLBCL cells. Notably, the efficacy of omacetaxine mepesuccinate was higher in the ABC compared with in the GCB subtype. The present study provides evidence regarding the development of omacetaxine

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Abbreviations: Omacetaxine mepesuccinate, homoharringtonine; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell-like subtype; ABC, activated B cell-like subtype; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; SU-4, SU-DHL-4; LY3, OCI-LY3; CD19, B-lymphocyte antigen CD19; CDK, cyclin-dependent kinase

Key words: homoharringtonine, diffuse large B-cell lymphoma, apoptosis, cell cycle, cell differentiation, telomerase

mepesuccinate into a potential therapeutic agent for the treatment of DLBCL.

Materials and methods

Cell lines and reagents. Human DLBCL cell lines SU-DHL-4 (SU-4; GCB subtype) and OCI-LY3 (LY3; ABC subtype) were provided by the Shanghai Institute of Hematology, Ruijin Hospital (Shanghai, China). These cell lines together with Kasumi-1 human AML cell line, K562 human CML cell line, MCF-7 human breast cancer cell line, and SGC-7901 human gastric cancer cell line (provided by Tianjin Institute of Hematology, Tianjin, China) were maintained for use in the present study. SU-DHL-4, OCI-LY3, K562, SGC-7901 and Kasumi-1 cells were cultured in RPMI 1640 medium (Hyclone; GE Healthcare Life Sciences, Chalfont, UK or Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.). MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS. These cultures were maintained at 37°C in a humidified incubator supplied with 5% CO₂.

Omacetaxine mepesuccinate (Hangzhou Minsheng Pharmaceutical Group Co., Ltd., Hangzhou, China) was dissolved at a concentration of 4 ng/ μ l in culture medium without FBS according to the cell type and maintained at 4°C prior to use. Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit and Cell Cycle Detection kit were purchased from BestBio Company (Shanghai, China). Telomeric repeat amplification protocol (TRAP)-silver staining telomerase detection kit was purchased from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). CD19 allophycocyanin (APC) antibodies (cat. no. 302211), CD138 phycoerythrin (PE) antibodies (cat. no. 352305), immunoglobulin (Ig) D FITC antibodies (cat. no. 348205), IgM APC antibodies (cat. no. 314509) and mouse IgG antibodies (cat. no. 409305) were purchased from BioLegend, Inc. (San Diego, CA, USA). Protein standards were purchased from Biomed Mechnikov (Moscow, Russia) and an RNA inhibitor was purchased from Promega Corporation (Madison, WI, USA).

Omacetaxine mepesuccinate treatment. To determine whether omacetaxine mepesuccinate-induced apoptosis was dose-dependent, LY3 cells and SU-4 cells were incubated with omacetaxine mepesuccinate at various concentrations (5, 10, 20, 40 and 100 ng/ml), or with a vehicle (0 ng/ml) serving as a control, for 48 h. To determine the time-dependent effects of omacetaxine mepesuccinate, LY3 and SU-4 cells were incubated with 40 ng/ml omacetaxine mepesuccinate for 0, 4, 8, 24, 48 or 72 h. To compare the differences between the various cancer cells, MCF-7, SGC-7901, Kasumi-1 and K562 cells were incubated with 40 ng/ml omacetaxine mepesuccinate for 0, 24 or 48 h. For cellular morphological analysis, LY3 and SU-4 cells were treated with 40 ng/ml omacetaxine mepesuccinate for 0 or 24 h. To determine the stage of the cell cycle, cell differentiation status, and telomerase activity, LY3 cells and SU-4 cells were exposed to 20 ng/ml omacetaxine mepesuccinate for 0, 4, 8, 12, 24 and 48 h. To determine cell differentiation status, LY3 cells and SU-4 cells were exposed to 20 ng/ml omacetaxine

mepesuccinate for 0, 12, 24 and 48 h. All experiments were repeated at least three times.

Cell apoptosis analysis. Cells were plated in 12-well plates at a density of 5x10⁵ cells/well and exposed to omacetaxine mepesuccinate or a vehicle for the designated period of time. Cells were then collected and apoptosis analysis was conducted using Annexin V-FITC Apoptosis Detection kit, according to the manufacturer's protocol. In apoptotic cells, the phospholipid phosphatidylserine (PS) is translocated from the inner to the outer surface of the plasma membrane. While exposed to the external cellular space, PS is labeled by FITC-conjugated Annexin V, which binds PS with high affinity. Annexin V/FITC staining is used in conjunction with propidium iodide (PI), which is a vital dye that permeates damaged membranes of dead cells but is excluded by the intact membrane of healthy cells, for identification of early and late apoptotic cells. Viable cells are Annexin V and PI-negative, whereas early apoptotic cells are Annexin V-positive and PI-negative, and late apoptotic or dead cells are Annexin V and PI-positive. The number of cells with single or double staining was counted using a FACSCalibur™ flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). For cell morphological analysis, images of the cells were captured using a microscope (BX51; Olympus Corporation, Tokyo, Japan) following conventional Wright staining (Shanghai Hengyuan Biological Technology Co., Ltd., Shanghai, China).

Cell cycle detection. Cells were plated at 5x10⁵ cells/well in 12-well plates. Each well contained ~2 ml culture medium. In each well, omacetaxine mepesuccinate was first diluted in 10 μ l culture medium and then added into the well. Cultures were incubated at 37°C in an atmosphere containing 5% CO₂ until experimentation. Cells were collected and prepared for cell cycle detection using the Cell Cycle Detection kit, according to manufacturer's protocol. Cells were counted using a FACSCalibur™ flow cytometer. FCS Express 4 Plus Research version 4.0 (De Novo Software, Los Angeles, CA, USA) was used to analyze flow cytometry results.

Cell surface antigen detection. Cells were cultured and treated with omacetaxine mepesuccinate as described previously. The cells were collected, washed twice with cold 1X phosphate-buffered saline (PBS), and were adjusted to a concentration of 3-6x10⁴ cells/10 μ l. For each sample, four 10 μ l aliquots were taken. One aliquot was used as a blank control, whereas the other three aliquots were incubated with CD19 APC/CD138 PE, IgD FITC/IgM APC, or mouse IgG antibodies (all diluted 1:200). The four aliquots were incubated at 4°C for 30 min in the dark. Cells were subsequently washed twice with cold PBS and subjected to analysis using a FACSCalibur™ flow cytometer.

Cell telomerase activity measurement. Cells were maintained and treated with omacetaxine mepesuccinate as described previously, until ready for experimentation. Telomerase activity was measured using the TRAP-silver staining telomerase detection kit, according to the manufacturer's protocol. Briefly, pellets were resuspended in ice-cold lysis buffer containing 200 U/ml RNase inhibitor, 0.1 mM

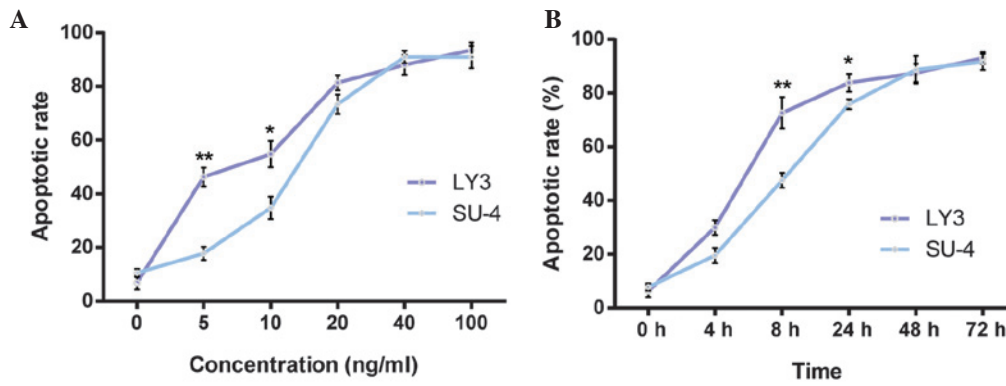


Figure 1. Omacetaxine mepesuccinate induced apoptosis in a dose- and time-dependent manner in DLBCL cells. (A) Graph presenting increased apoptotic rates in the LY3 and SU-4 DLBCL cells treated with omacetaxine mepesuccinate at various concentrations (0, 5, 10, 20, 40, or 100 ng/ml) for 24 h. (B) Graph presenting increased apoptotic rates in the two types of cells exposed to 40 ng/ml omacetaxine mepesuccinate for 0, 4, 8, 24, 48, or 72 h. The data are presented as the mean \pm standard error of the mean and were analyzed using Student's t-test; n=3. *P<0.05, **P<0.01 vs. SU-4 cells. LY3, OCI-LY3; SU-4, SU-DHL-4; DLBCL, diffuse large B-cell lymphoma.

benzamidine and 10 mM β -mercaptoethanol, and incubated on ice for 30 min with gentle rocking. After centrifugation at 12,000 \times g for 30 min, the supernatant containing telomerase was collected, aliquoted into a small volume to avoid freeze-thaw cycles, measured for total protein concentration and stored at -70°C . The protein concentration was adjusted to 10-750 ng/ μl prior to telomerase extension and PCR amplification. A master mix was prepared in an RNase-free PCR tube by mixing the following: 39.5 μl diethylpyrocarbonate H₂O, 5 μl 10X TRAP buffer, 1 μl dNTP, 1 μl TS primer, 1 μl TRAP primer mix, 0.5 μl Taq-DNA polymerase and 2 μl telomerase extract. PCR amplification was performed using the following thermal cycling conditions: 30 $^{\circ}\text{C}$ For 30 min (telomerase extension reaction), 95 $^{\circ}\text{C}$ for 5 min, and 30-33 cycles at 94 $^{\circ}\text{C}$ for 30 sec and 59 $^{\circ}\text{C}$ for 30 sec. The PCR products then underwent polymerase chain reaction amplification and separation by 14% non-denaturing polyacrylamide gel electrophoresis. Immediately after the completion of gel electrophoresis, the gel was placed in 500 ml fixative solution containing 10% ethanol and 0.5% acetic acid in deionized water for 10-20 min. The gel was then transferred into staining solution containing 0.2% silver nitrate, 10% ethanol and 0.5% acetic acid, and stained for 20-30 min. Following rinsing with water for 5-10 sec, the gel was developed for 15-30 min, rinsed in water for 5-10 min and photographed for analysis.

Statistical analysis. Data are presented as the mean \pm standard error of the mean. Statistical analyses were conducted using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was determined by Student's t-test for pairwise comparisons or by analysis of variance (ANOVA) with Bonferroni's multiple comparisons test. P<0.05 was considered to indicate a statistically significant difference.

Results

Induction of apoptosis by omacetaxine mepesuccinate. Apoptotic cells were detected by Annexin V/FITC and PI double staining. Early apoptotic cells were labeled with Annexin V-FITC but not PI, whereas late apoptotic or dead

cells were indicated by double staining with Annexin V-FITC and PI. The number of apoptotic cells was detected using flow cytometry. The number of total apoptotic cells was calculated as the sum of early apoptotic cells and late apoptotic or dead cells. As presented in Fig. 1, LY3 and SU-4 cells exhibited increased rates of apoptosis following treatment with omacetaxine mepesuccinate. Increased apoptosis was particularly noticeable in the cells treated with omacetaxine mepesuccinate at 20 ng/ml or higher for 24 h, in which the apoptotic rates were >60% (Fig. 1A). Notably, the apoptotic rates were significantly higher (P<0.004 and P<0.044, respectively) in LY3 cells compared with in SU-4 cells following exposure to 5 or 10 ng/ml omacetaxine mepesuccinate for 24 h (Fig. 1A). Consistently, the apoptotic rates were significantly higher in LY3 cells compared with in SU-4 cells when these cells were treated with 40 ng/ml omacetaxine mepesuccinate for 8 and 24 h (P<0.007 and P<0.013, respectively; Fig. 1B). These results suggest that omacetaxine mepesuccinate induces apoptosis in DLBCL cells in a dose- and time-dependent manner, and that ABC subtype cells (LY3) are more vulnerable than GCB subtype cells (SU-4).

Consistent with omacetaxine mepesuccinate-mediated apoptotic effects, cell morphology was altered following exposure to 40 ng/ml omacetaxine mepesuccinate for 24 h. Compared with vehicle treatment, omacetaxine mepesuccinate induced shrinkage, karyopyknosis and an increased number of apoptotic bodies (Fig. 2). A greater number of morphologically irregular apoptotic cells were observed in LY3 cells than in SU-4 cells (data not shown), which is consistent with the flow cytometry results demonstrating that the ABC subtype exhibits increased sensitivity to omacetaxine mepesuccinate than the GCB subtype.

To determine whether omacetaxine mepesuccinate results in a different apoptotic effect in different types of cancer, omacetaxine mepesuccinate (40 ng/ml) was administered to different cell lines, including MCF-7 human breast cancer cell line, SGC-7901 human gastric cancer cell line, Kasumi-1 AML cell line, and K562 human CML cells for 0, 24 or 48 h. As presented in Table I and Fig. 3, omacetaxine mepesuccinate application resulted in a significant increase in the apoptotic rates of Kasumi-1 AML cells and K562 human CML cells, but

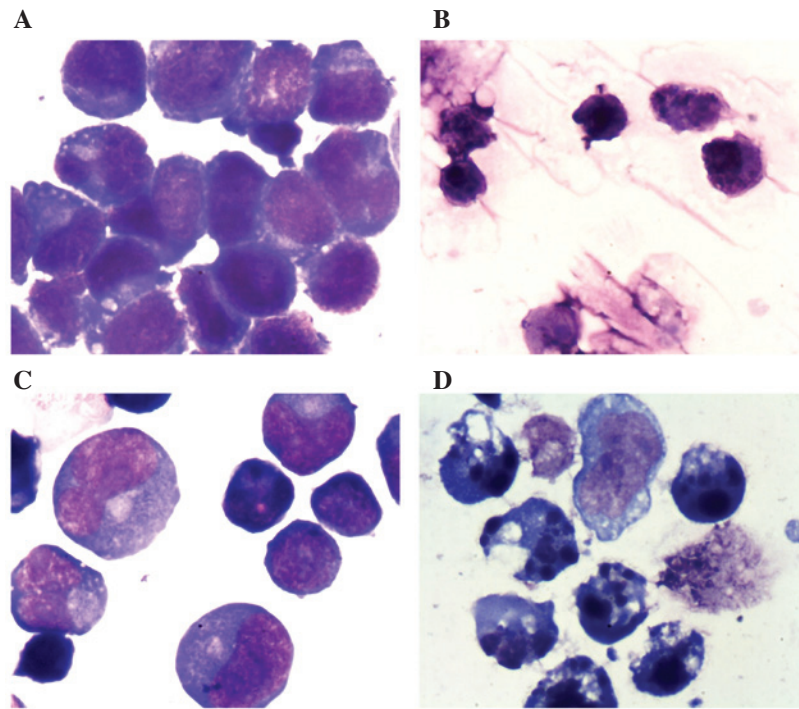


Figure 2. Omacetaxine mepesuccinate-induced cell morphological changes in cells undergoing apoptosis. Wright staining in (A) LY3 cells treated with vehicle control, (B) LY3 cells treated with 40 ng/ml omacetaxine mepesuccinate, (C) SU-4 cells treated with vehicle or (D) SU-4 cells treated with 40 ng/ml omacetaxine mepesuccinate for 24 h. Magnification x100. LY3, OCI-LY3; SU-4, SU-DHL-4.

not in the MCF-7 human breast cancer and SGC-7901 human gastric cancer cells. These results indicate that omacetaxine mepesuccinate-induced apoptosis is specific to the type of tumor.

Cell cycle detection. The present study demonstrated that cell cycle distribution of LY3 and SU-4 cells was altered by omacetaxine mepesuccinate exposure for a selected period of time. LY3 and SU-4 cells exposed to omacetaxine mepesuccinate demonstrated a typical subdiploid apoptotic peak prior to G_0/G_1 phase (Fig. 4A and B). Prior to omacetaxine mepesuccinate treatment, there were more cells in S phase than in G_0/G_1 phase in the LY3 and SU-4 cells (Fig. 4C and D). Upon omacetaxine mepesuccinate exposure, the majority of LY3 and SU-4 cells were in G_0/G_1 phase. Following treatment with omacetaxine mepesuccinate for 24 h or longer, more than half of the cells were arrested in G_0/G_1 phase in the two cell lines (Fig. 4C and D).

Cell surface antigen detection. The effect of omacetaxine mepesuccinate on cell differentiation in DLBCL cells was investigated by detecting changes in the expression of B lymphocyte antigen, CD19 and plasma cell antigen, CD138. As presented in Fig. 5A, the percentage of CD19⁺/CD138⁺ cells increased gradually in the LY3 and SU-4 cells following treatment with omacetaxine mepesuccinate. Omacetaxine mepesuccinate-mediated differentiation into B lymphocytes was more effective in LY3 cells than in SU-4 cells ($P < 0.01$, according to two-way ANOVA). Similarly, upon omacetaxine mepesuccinate exposure, the percentage of LY3 cells expressing IgM and IgD (IgM⁺/IgD⁺, mature B lymphocytes) increased steadily, whereas most of the untreated LY3 control cells

expressed IgM but not IgD (IgM⁺/IgD⁻; Fig. 5B). Conversely, the majority of untreated SU-4 control cells were IgM⁻/IgD⁻. However, the percentage of IgM⁺ SU-4 cells was significantly increased ($P < 0.01$ according to one-way ANOVA; data not shown) upon omacetaxine mepesuccinate exposure (Fig. 5C). These results demonstrate that omacetaxine mepesuccinate application in DLBCL cells promotes cell differentiation and maturation.

Omacetaxine mepesuccinate reduces cell telomerase activity in DLBCL cells. LY3 cells and SU-4 cells were incubated in 20 ng/ml omacetaxine mepesuccinate for 4, 8, 12, 24 or 48 h, and were then subjected to measurement of telomerase activity using TRAP-silver staining. DNA strips indicated a 6 bp ladder gap in non-denaturing polyacrylamide gel electrophoresis, where the number and depth of the DNA strips represented telomerase activity. Untreated control cells (0 h) served as positive controls. As presented in Fig. 6, telomerase activity in the LY3 and SU-4 cells was not significantly altered following treatment with omacetaxine mepesuccinate for 4, 8, 12 and 24 h compared with the activity of the control. However, the two types of cells demonstrated a marked decrease in telomerase activity following exposure to omacetaxine mepesuccinate for 48 h. This change is particularly clear in LY3 cells, in which telomerase activity was almost completely absent.

Discussion

It has previously been demonstrated that omacetaxine mepesuccinate results in the apoptosis of various types of cancer cells, including leukemia cells (16,17), certain types of solid carcinoma cells (18-21), and selected types of lymphocytes (13-15).

Table I. Omacetaxine mepesuccinate-induced apoptosis in various cancer cell lines.

Cell line	Time (h)					
	0		24		48	
	Apoptotic rate	P-value	Apoptotic rate	P-value	Apoptotic rate	P-value
MCF-7	6.4967±2.3099	0.934	4.3200±0.8007	0.001	4.6500±1.1805	0.001
SGC-7901	6.4667±0.6623	0.915	4.6567±1.1621	0.001	5.9833±0.7514	0.001
Kasumi-1	7.3267±1.3540	0.657	59.4867±1.4009	0.011	72.9900±3.8071	0.071
K562	4.5867±1.0151	0.305	9.0333±1.5047	0.001	46.0767±3.7792	0.009

P-values were determined by comparing omacetaxine mepesuccinate-induced apoptosis rates between each type of cell in the table and the OCI-LY3 cells (in Fig. 1) under the same conditions.

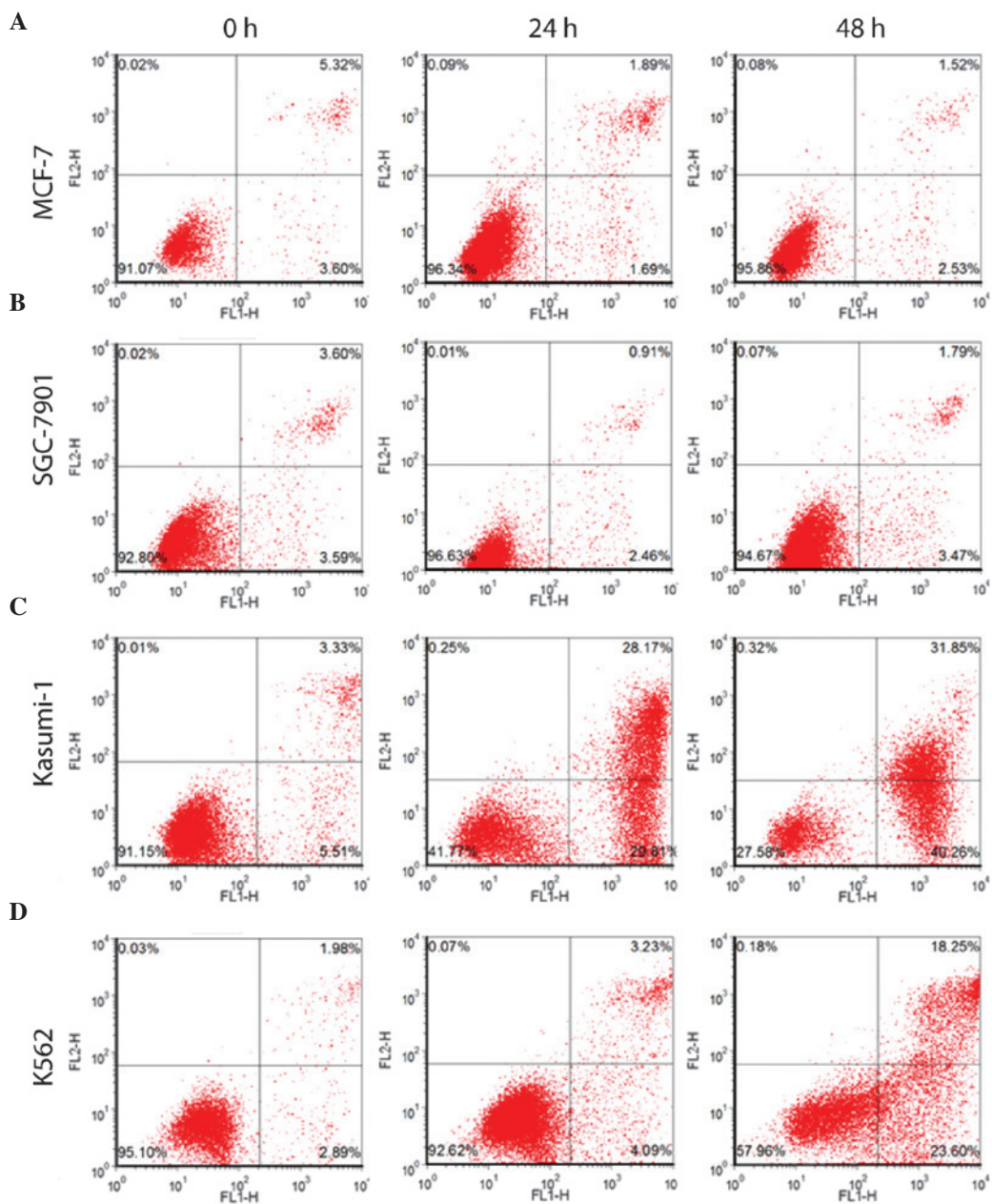


Figure 3. Omacetaxine mepesuccinate-induced apoptosis in different tumor cell lines. (A) MCF-7, (B) SGC-7901, (C) Kasumi-1, and (D) K562 cells were treated with 40 ng/ml omacetaxine mepesuccinate for 0, 24 or 48 h as indicated and then subjected to cell apoptosis detection and flow cytometry to count apoptotic cells. Data were quantified and summarized in Table I.

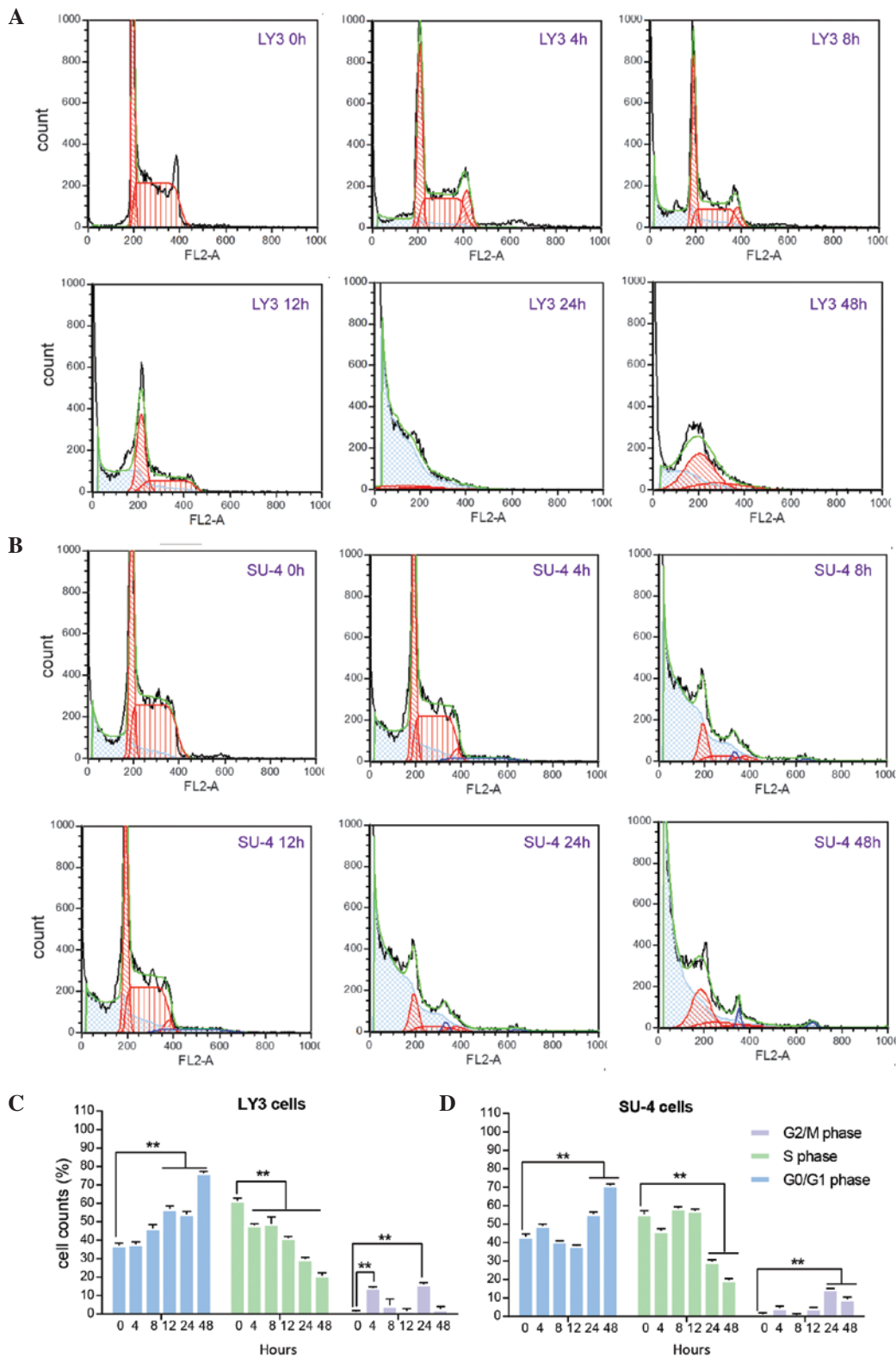


Figure 4. Effect of Omacetaxine mepesuccinate on the cell cycle. (A and B) Flow cytometric analysis of the cell cycle in LY3 and SU-4 cells exposed to omacetaxine mepesuccinate. (C and D) Graphs demonstrating altered cell cycle by omacetaxine mepesuccinate application in (C) LY3 cells and (D) SU-4 cells. Data are presented as the mean \pm standard error of the mean. Compared with the control cells, the percentage of LY3 or SU-4 cells in G₂/G₁ phase was not significantly altered following treatment with omacetaxine mepesuccinate for 4 and 8 h, however it was significantly different following treatment with omacetaxine mepesuccinate for >12 h, or 24 h, respectively. **P<0.01. Experiments were repeated 3 times.

However, omacetaxine mepesuccinate-mediated cell death has not yet, to the best of our knowledge, been reported in DLBCL cells. The present study is the first, to the best of our knowledge,

to indicate that omacetaxine mepesuccinate induces apoptosis in DLBCL cells in a dose- and time-dependent manner. The ABC subtype was demonstrated to be more sensitive to

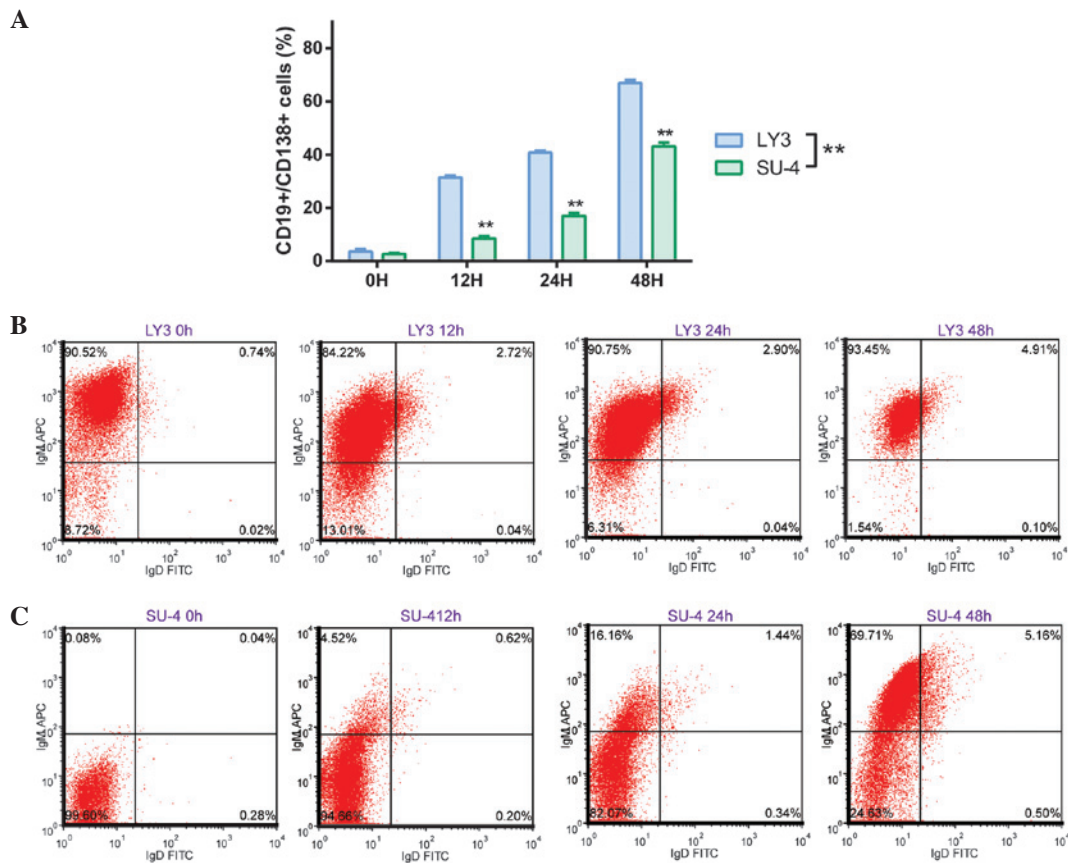


Figure 5. Omacetaxine mepesuccinate promotes B lymphocyte differentiation in cultured DLBCL cells. (A) Graph indicating that the percentage of CD19⁺/CD138⁺ cells was significantly increased following treatment with omacetaxine mepesuccinate. Data are presented as the mean \pm standard error of the mean and analyzed by Student's t-test for pairwise comparison or two-way ANOVA; n=3. (B and C) Flow cytometric analysis demonstrating the expression of mature B-lymphocytes surface antigen IgD in (B) LY3 cells or (C) SU-4 cells incubated with omacetaxine mepesuccinate for the indicated period of time. **P<0.01 vs. OH. DLBCL, diffuse large B-cell lymphoma; LY3, OCI-LY3; SU, SU-DHL-4; CD, cluster of differentiation; Ig, immunoglobulin.

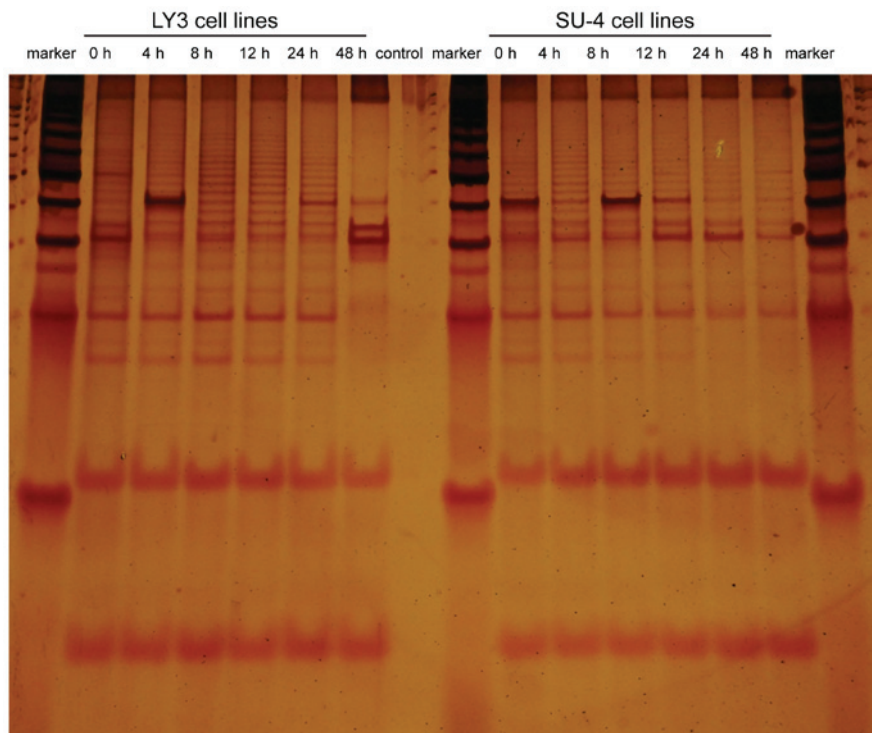


Figure 6. Effect of Omacetaxine mepesuccinate on cell telomerase activity. LY3 and SU-4 cells were treated with 20 ng/ml omacetaxine mepesuccinate for a series of time durations as indicated. Cell lysates were then prepared and subjected to the determination of telomerase activity, represented by number and density of the DNA strips, using telomerase repeat amplification protocol-silver staining. LY3, OCI-LY3; SU, SU-DHL-4.

omacetaxine mepesuccinate-induced apoptosis than the GCB subtype. In addition, omacetaxine mepesuccinate was shown to induce cell cycle arrest, promote cell differentiation and maturation, and reduce telomerase activity. Consistent with a previous study (18), omacetaxine mepesuccinate application also induced apoptosis in K562 cells (CML) and Kasumi-1 cells (AML), but not in MCF-7 cells (breast cancer) or SGC-7901 cells (gastric cancer). These results suggested that omacetaxine mepesuccinate-mediated apoptosis is cancer type-specific.

An efficient anticancer therapeutic agent is often evaluated for its ability to induce apoptosis and cell cycle arrest. It has been demonstrated that omacetaxine mepesuccinate increases the expression levels of cyclin-dependent kinase (CDK) inhibitors, p27 and p21, which in turn bind to CDK or cyclin/CDK complexes to inhibit enzymatic activity, leading to the arrest of the cell cycle at G₁ phase (22,23). Consistently, the present study observed that more than half of DLBCL cells were arrested at G₀/G₁ phase following Omacetaxine mepesuccinate application for 24 and 48 h. Accordingly, the percentage of cells at S phase and G₂/M phase was significantly decreased. Consistent with this result, omacetaxine mepesuccinate promotes cell differentiation into mature B lymphocytes, as indicated by the increased number of cells expressing CD19/CD138 in addition to IgD/IgM, which are signs of cell differentiation and maturation of B lymphocytes.

Telomeres are considered the biological clock of cell ageing and cell life span/survival (24,25). Telomerase activity, which caps the ends of chromosomes to facilitate chromosome duplication and cell division, is inactivated or undetectable in normal somatic cells but present in germ cells with proliferative potential, embryonic stem cells and certain lymphocyte cells. However, during cancer development, telomerase activity is aberrantly increased, which allows cancer cells to divide continuously. Increased telomerase activity has been detected in human colon, lung, liver and breast cancer, in addition to leukemia and lymphoma (26-29). Therefore, telomerase may be used as a tumor marker, and may be investigated as a target for anticancer therapeutic agents in somatic tissues (30-32). Reducing telomerase activity may result in cell senescence, inhibition of cell proliferation, and programmed cell death, suggesting it may be an effective approach to treat cancer (33-37). Consistent with the results of a previous study (38), the present study demonstrated that the GCB and ABC subtype DLBCL cells exhibited high telomerase activity, as demonstrated using TRAP-silver staining. In addition, the present study demonstrated that the increased telomerase activity was suppressed by omacetaxine mepesuccinate application for 24 and 48 h, and that omacetaxine mepesuccinate-mediated inhibition is more effective in ABC subtype cells, as telomerase activity in LY3 cells was reduced to close to nothing upon omacetaxine mepesuccinate exposure for 48 h. Notably, the results of the present study indicated that omacetaxine mepesuccinate-induced apoptosis occurred earlier than omacetaxine mepesuccinate-mediated telomerase activity reduction, which has also been reported by previously published studies (29,39).

In conclusion, omacetaxine mepesuccinate induces apoptosis in DLBCL cells in a dose- and time-dependent manner.

The effect of omacetaxine mepesuccinate is more effective in ABC subtype (LY3 cells) than in GCB subtype (SU-4 cells) cells. Furthermore, omacetaxine mepesuccinate was able to arrest the cell cycle, promote cell differentiation/maturation, and reduce telomerase activity. The findings of the present study may provide valuable insight into the molecular mechanism underlying omacetaxine mepesuccinate-mediated apoptosis. Since it is already in use for the treatment of AML and CML, omacetaxine mepesuccinate may be further investigated for its therapeutic effects in DLBCL.

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