EFFECTS OF TAXOL ON THREE DIFFERENT TYPES OF LYMPHOMA CELL LINES

ZHOU Xiaoyan 周晓燕 [1], XU Liangzhong 许良中 [1], ZHU Weiping 朱伟萍 [1]
ZHANG Taiming 张泰明 [1], LI Xiaomei 李小妹 [1], JIN Aiping 金爱萍 [1]
HE Kailing 何开玲 [2]

[1] *Department of Pathology, Cancer Hospital, Shanghai Medical University, Shanghai 200032, China*
[2] *Department of Biochemistry, Shanghai Medical University, Shanghai 200032, China*

ABSTRACT

Objective: To observe the effects of the new anticancer drug taxol on different types of lymphoma cells and explore the value of its clinical application. Methods: Inverted microscopy, light microscopy, electron microscopy, flow cytometry (FCM) assay and DNA gel electrophoresis were used to observe the effects taxol on three different types of lymphoma cell lines, i.e., Jurkat (T cell lymphoma), BJAB (B cell lymphoma, EBV negative) and Raji (B cell lymphoma, EBV positive). Results: Taxol was able to inhibit the growth of and induce apoptosis in all of the three cell lines. Jurkat cells were the most sensitive, apoptosis being the main effect; BJAB was the second sensitive showing G2/M arrest first and then apoptosis; Raji was the least, showing G2/M arrest in most of the cells and entering apoptosis in only a few of them. Conclusion: Taxol is a valuable chemotherapeutic agent for lymphoma therapy. The senstivity to the agent may vary with the tumor type, the existence of EBV infection or not and the extent of induced apoptosis.

Key words: Lymphoma cells, Taxol, Apoptosis

Lymphoma is a common malilgnant tumor. Relapse and refractory cases are often seen during routine therapy. It is necessary to search for potent drugs and to study their mechanisms. Taxol (Paclitaxel) is a new anticancer agent,[1] which is effective in a number of cases in tumor therapy, such as advanced ovarian and breast cancer, prostate cancer and non-small cell lung cancer. However, little is known in lymphomas. It has been reported that taxol is more effective in relapse and middle grade lymphomas than in other types of the tumor.[2] It can obviously inhibit the syngenic lymphoma transplanted in mice and induce apoptosis.[3] Distinct heterogeneity exits indifferent types of lymphomas. The effects of taxol on different types of lymphomas and its mechanism have not been reported. Non-Hodgkin's lymphomas can be classified into two types, i.e., T and B cell lymphoma. EBV infection is an important cause of lymphoma. In this study, three different types of lymphoma cell lines were chosen to observe the effects of taxol on them.

MATERIALS AND METHODS

Cell Lines

Three different types of lymphoma cell lines were used. i.e., Jurkat (T cell lymphoma cell line, provided by Shanghai Institute of Hematology, Ruijin Hospital, Shanghai Second Medical University), BJAB (B cell lymphoma cell line, EBV negative, supplied by Department of Pathology, Osaka University Medical School, Japan) and Raji (B cell lymphoma cell line, EBV positive, purchased from cell bank, Shanghai Institute of Cell Biology, Chinese Academy of Science).

Cell Culture and Taxol Treatment

Jurkat, BJAB and Raji cells were cultured in fresh RMPI 1640 medium (GIBCO-BRL, Grand Island, NY), supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified

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Correspondence to: XU Liangzhong, Department of Pathology, Cancer Hospital, Shanghai Medical University, No. 309 Ling-ling Road, Shanghai 200032, China; Phone: (086-021)-64175590 Ext.357; Fax:(0086-021)-64174774; E-mail: zxy65@hotmail. com

atmosphere of 95% air/5% CO_2, and passed on every 2~3 days. Cells in the logarithmic growth phase at concentration of 3×10^5/ml were inoculated into sterile flasks or 24 well plates. After culturing for 12 hours, taxol [Bristol-Myers Squibb Company (Princeton, NJ)] at various concentrations was added to the cells which then were incubated for a certain period of time to observe the growth of the cells. Then the cells were harvested by mixing with an equal volume of phosphate-buffered saline (PBS) containing 0.4% trypan blue dye and manually counted. The percentage was calculated as: Cell inhibition was determined by the trypan blue exclusion method. Cell inhibition % = (control group – experimental group) /control groups × 100%.

Morphological Observation

Under inverted microscope, morphological changes were observed and cell membrane permeability was determined by the trypan blue exclusion method.

Cells were fixed on slides with acetone for ten minutes, air dried, stained with hematoxyin and eosin and observed under light microscope.

A total of 5 $\times10^5$ cells were harvested, routinely processed and observed under JEM-1200 EX .transmission electron microscope.

Flow Cytometry (FCM) Analysis

A total of 2×10^6 cells were washed three times with 0.1 MPBS, centrifuged, and fixed with 70% cold ethanol for 30 minutes. After fixation, the pellet cells were treated with 1% triton x-100 10 ml for 10 minutes, digested with 0.01% RNAase 1 ml for 10 minutes, and then incubated with 0.25% propidium iodide (PI) for 20 minutes. Cells were analyzed on a Becton Dickinson FACS Cablibur flow cytometer with the incident beam at 488 nm.

DNA Fragmentation Assay

A total of 2×10^6 cells were washed with 0.1 MPBS three times and centrifuged. The pellet was resuspended using 60 μl 0.1M PBS, and mixed thoroughly with an equal volume of lysis buffer (50 mM Tris, 40 mM EDTA, 1.6% Sarcoyl), RNAase was added at a final concentration of 100 μg/ml and incubated for 30 minutes. Then the cells were digested with proteinase K at a final concentration of 1 mg/ml at 50°C for 4~24 hours. The digested lysates were heated at 70°C for 10 minutes. 30 μl lysates mixed with 6 μl 6×loading buffer were analyzed by electrophoresis on 1.5% agarose gel for 4 hours at 40V. The gel was visualized by transillumination with ultraviolet (UV) light and photographed.

RESULTS

Growth Inhibition and Induction of Apoptosis by Taxol in the Three Lymphoma Cell Lines

Taxol could obviously inhibit the growth of the three lymphoma cell lines. Jurkat cells were the most sensitive, showing growth inhibition with 1 μg/ml taxol treatment for only 3 hours. In BJAB and Raji cells higher concentration of taxol and longer incubation were needed. Within a certain range of dose and incubation time, the three lymphoma cell lines showed apoptosis and G_2/M arrest in different extent. The growth inhibition increased with the increase of dose and incubation time (Figure 1). The ranges were as follows, Jurkat: 1~200 ng/ml, 3~24 hours; BJAB and Raji: 40~4000 ng/ml, 12~36 hours. Over the high limit, cells showed necrosis characterized with cell membrane disruption and cell disintegration.

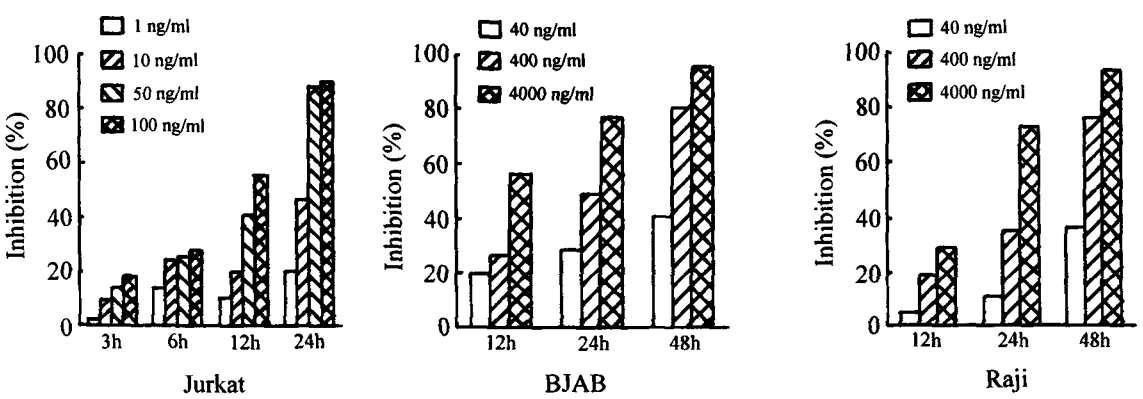

Fig. 1. Growth inhibition of Jurkat, BJAB and Raji lymphoma cells treated with taxol *in vitro*

Morphological Changes

Under inverted microscope, part of Jurkat and BJAB cells deformed after taxol treatment, that is, cells become long with surface blebbing and gradually separated to form apoptotic bodies. Raji Cells after taxol treatment mainly showed decreased transparency and intracellular granules. Trypan blue dye staining in all the cell lines was negative, suggesting the cell membrane was intact and the permeability was not obviously changed.

Light and electron microscopical observation showed that Jurkat cells had the most obvious changes. With only 1 ng/ml taxol treatment for 3 hours, the cells were characterized with nuclear condensation, chromatin packed densely against the cell's periphery like a new moon. The nucleus, cell membrane and cytoplasm separated to form apoptostic bodies (Figure 2). This kind of cells increased with the increase of dose and incubation time (Figure 3). Only a small part of karyokinetic phase cells could be seen. BJAB cells after taxol treatment for 12 hours mainly showed karyokinesis. Cells with nuclear condensation and margination increased with time and gradually fragmented into apoptotic bodies (Figure 4). However, Raji cells after taxol treatment mainly showed karyokinesis. Only a small part of cells entered apoptosis characterized with chromatin condensation and fragmentation into condensed bodies (Figure 5).

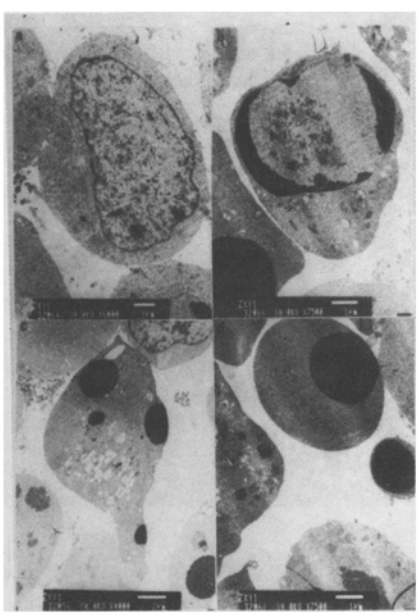

Fig. 2. Elecrton microscopy of Jurkat cells. a: With no treatment (large nuclear, small cytoplasm); b,c,d: 100ng/ml taxol treatment for 16 hours; b: Nuclear condensation, chromatin packed densely against the cell's periphery like a new moon; c: Nuclear separated in the cell; d: Nucelar separated with cell membrane and cytoplasm into apoptotic bodies.

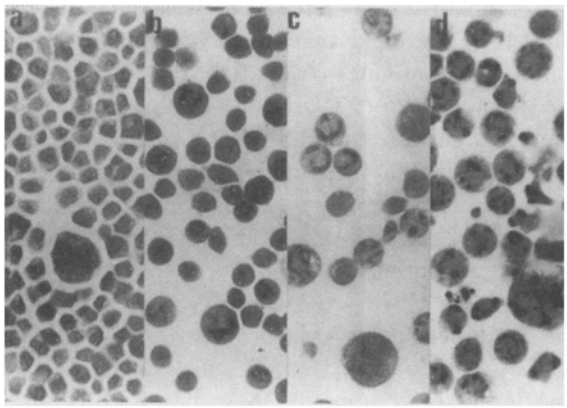

Fig. 3. Light microscopic morphology of Jurkat cells. .With no treatment (a) and 100ng/ml taxol treatment for 6h(b), 12h(c), 24h(d). Apoptotic cells are increased with incubation time (HE, × 100)

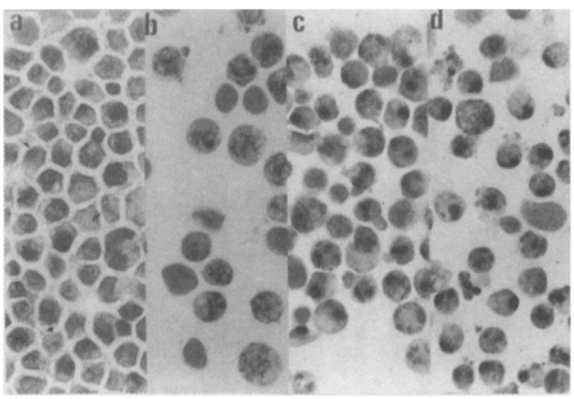

Fig. 4. Light microscopic morphology of BJAB. With no treatment (a) and 1000ng/ml taxol treatment for 12h(b), 24h(c), 36h(d). b: most cells arrest in karyokinesis; c: apoptosis is induced; d: most cells show apoptosis (HE, ×100)

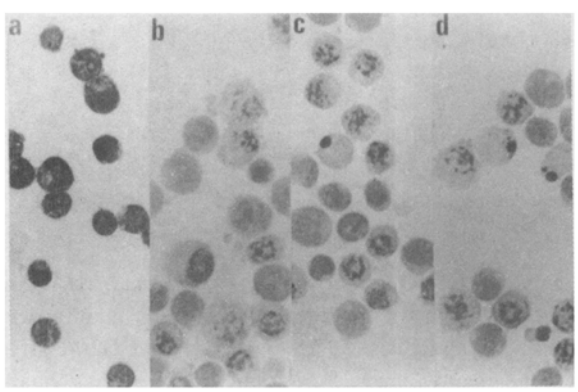

Fig. 5. Light microscopic morphology of Raji cells. with no treatment (a) and 1000 ng/ml taxol treatment for 12h(b), 24h(c), 36h(d); b: most cells arrest in karyokinesis; c,d: small amount of cells show apoptosis (HE ×100)

Flow Cytometry (FCM) Analysis

A total of 2×10^6 cells were washed three times with 0.1M PBS, centrifuged, and fixed with 70% cold ethanol for 30 minutes. After fixation, the pellet cells were treated with 1% triton ×–100 10 ml for 10 minutes, digested with 0.01% RNAase 1 ml for 10 minutes, and then incubated with 0.25% propidium iodide (PI) for 20 minutes. Cells were analyzed on a Becton Dickinson FACS Cablibur flow cytometer with the incident beam at 488 nm.

DNA Fragmentation Assay

After taxol treatment DNA of the cells on gel electrophoresis showed typical DNA ladder pattern (Figure 7), demonstrating that DNA degraded into oligonuleosomal sized fragments. Of the three cell lines, Jurkat had most obvious changes.

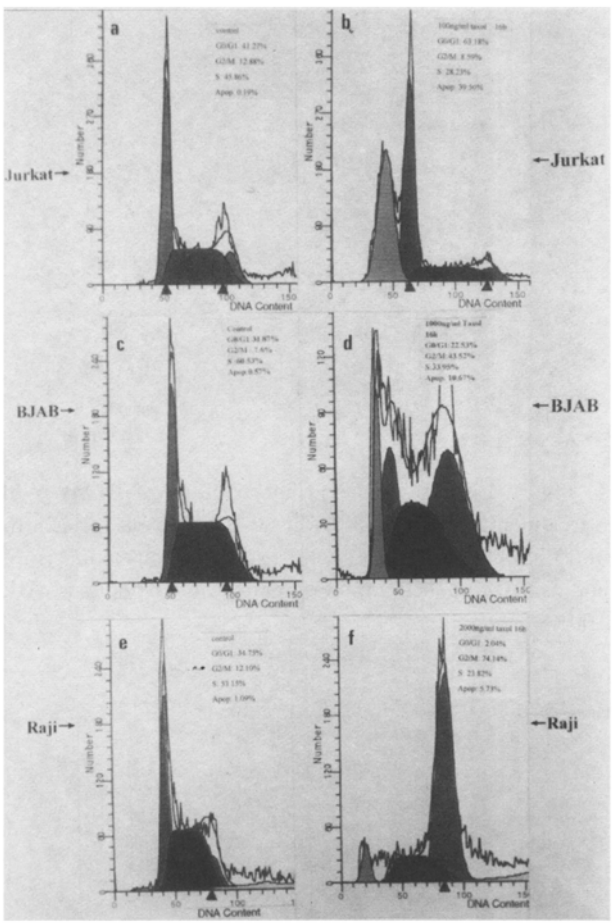

Fig. 6. Flow cytometry analysis of Jurkat, BJAB and Raji cells with taxol treatment. Jurkat cells mainly occur AP showing apoptosis, BJAB occur AP and G2/M arrest, Raji cells mainly occur G2/M and AP is always smaller than 10%.

DISCUSSION

Apoptosis is an important mode of cell death. It is an active, regulated process under some physiologic or pathologic condition. It occurs not only in embryogenesis and growth development, but also associates with tumorgenesis and tumor therapy. It has been reported that apoptotic sensitivity may be an important determinant of therapeutical effectiveness and apoptotic inhibition is associated with multi-drug resistance.[4] Apoptosis is thought to be one of the standards of anticancer agents screening. Anticancer strategy now is aiming at how to induce apoptosis.

It is reported that apoptosis is characterized with morphologically endoplasmic reticulum and cell membrane blebbing, cell shrinkage, chromatin condensation and margination like a new moon, and nuclear fragmentation with membrane and cytoplasm into apoptotic body. DNA electrophoresis produces the characteristic DNA ladder pattern because of the activation of endogenous nuclease and genomic DNA degradation into oligonucleosomal sized DNA fragments. Degraded DNA penetrates easily through cell membrane leading to the reduction of intracellular DNA and the decrease specific fluorescence staining. FCM analysis shows apoptotic peak before G1 peak.[4,5] The present study demonstrated that taxol induced apoptotic changes as mentioned above in all the three cell lines though their sensitivities were different.

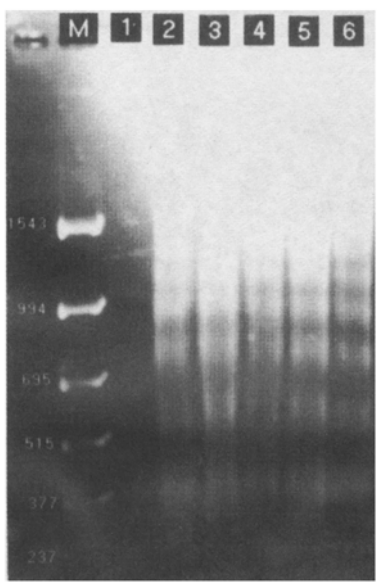

Fig. 7. DNA electrophresis of Jurkat cells. With no treatment (1) and 1 ng/ml (2), 10 ng/ml(3), 50 ng/ml(4), 100 ng/ml(5), 200 ng/ml respectively for 30h.

Taxol, as a new anticancer drug, has been studied in breast, ovarian, prostate and other cancers. It can combine with microtubules, stabilize tubulin and inhibit its degradation, increasing the fraction of cells

in the G2 or M phase of the cell cycle.[6,7] Taxol can also induce apoptosis.[8,9] However, little is known in lymphoma. This study showed that taxol produced effects of apoptosis and G2/M arrest in lymhmas, indicating taxol is a valuable drug for lymphoma therapy.

The sensitivity and level of apoptosis is different in different cell lines. The reasons may be as follows: 1. Apoptosis is associated with cell phenotype and intracellular genes status, such as p53, bcl-2 family and c-myc.[10] It has been reported that haematopoietic tumor cells are more sensitive to apoptosis induced by anticancer agents than epithelia tumor cells.[4] Wild-p53 cells are more sensitive to apoptosis than mutant-p53 cells.[11] This study shows Jurkat is more sensitive than BJAB and Raji. Is apoptosis easier to be induced in T cell lymphoma? Do different mechanisms exist in different cell lines? Further studies are needed. 2. Epstein-Barr virus infection may be a factor affecting taxol-induced apoptosis. BHRF-1, LMP-1 encoded by Epstein-Barr virus or Mcl-1 and bcl-2 expression induced by them has been reported to inhibit apoptosis.[12,13] So Epstein-Barr virus infection may be a major reason why Raji cells were more resistant to apoptosis than BJAB which is Epstein-Barr virus negative. Since Epstein-Barr virus infection is an important cause for the genesis of lymphoma and apoptosis is associated with the inhibiting affect of anticanceragent, the tumor type and Epstein-Barr infection should be taken into consideration.

Another effect of taxol on lymphoma cells is G2/M arrest that always occurred before apoptosis, suggesting G2/M arrest might be a factor of taxol-induced apoptosis. Most Jurkat cells entered apoptosis shortly after G2/M arrest, however, Raji cells mainly showed G2/M arrest and only a small part of cells entered apoptosis. So there are differences in the time of entering G2/M, the time from G2/M to apoptosis and the ability to induce apoptosis in different cells. It has been reported that both G2/M cells and G0/G1 transition cells synchronously initiate similar apoptotic DNA fragmentation within 20 hours of taxol treatment.[14] It indicates that G2/M arrest is one of the effects of taxol on lymphoma cells, and may be associated with apoptotic induction, but it is not a requisite to initiate cell death, and apoptosis is not the inevitable sequel to G2/M arrest yet. A study *in vivo* with mice has showed that not mitosis block but apoptosis is concerned with tumor cells growth inhibition. The ability of induced apoptosis is a useful parameter of treatment efficiency.[3] However, the cells in division stage are more sensitive to radiation than the cells in other stage, taxol treatment following radiation may obtain better efficiency.

Apoptosis is usually induced within a certain dose and time. Lower than the limit, apoptosis can not be induced, however, over the limit, necrosis will be the result. The results suggest how to use taxol safely and effectively for lymphoma therapy. High dose of chemotherapeutic agents always has serious clinical side effect. Induced apoptosis by lower dose is similarly an irreversible change, which is a programmed cell death regulated by genes. To induce apoptosis through gene regulation mechanism may be an important strategy for cancer therapy.

The present study indicates that apoptosis is an important mechanism of taxol on lymphoma cells. The level of apoptosis is different in different type of lymphoma cells. It will provide a theoretical basis for clinical treatment of lymphoma and a good model for further study apoptotic gene modulation in lymphoma cells.

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