Altered maturation of dendritic cells by taxol, an anticancer drug

Hong-Gu Joo

Department of Veterinary Medicine, Cheju National University, Jeju 690-756, Korea

Taxol is a clinically useful anticancer drug against a variety of cancers. Although it has been known that taxol induces the apoptosis of cancer cells through cytochrome C release and the activation of caspases, the effect of taxol on dendritic cells (DCs) has not been studied. In this study, taxol enhanced the expression of MHC class II on DCs, compared to medium-treated immature DCs. Surprisingly, the viability of DCs was not decreased by taxol, whereas that of cancer cells was. It was confirmed that taxol did not induce the apoptosis of DCs based on annexin V-FITC/propidium iodide (PI) staining assay. Since previous study demonstrated that taxol induced the production of nitric oxide (NO) related to the viability of DCs, the level of NO from taxol-treated DCs was determined. Any significant amount of NO was not detected. Although taxol enhanced the expression of a maturation marker, MHC class II molecules, it strikingly inhibited the proliferation of splenic T lymphocytes activated by DCs. Taken together, this study demonstrated that taxol induced an altered maturation of DCs, the increase of MHC class II molecule but the inhibition of proliferation of splenic T lymphocytes. It is suggested that taxol may induce the immunosuppression in patients with cancer by the inhibition of DC-activated T cell proliferation, but not by the direct killing of DCs.

Key words: dendritic cells, taxol, maturation

Introduction

Taxol is a clinically effective anticancer drug against a variety of cancers including breast cancer. Taxol binds to tubulin, retards microtubule depolymerization, impairs mitosis, blocks cell cycle, and facilitates apoptosis [15]. Although the effect of taxol on tumor cells has been studied, the effect of taxol on various immune cells remains unclear. Recent studies demonstrated that taxol bound to CD11c/CD18 in concert with CD14 and Toll-like receptor (TLR) 4 to elicit taxol-inducible gene expression in macrophages [14] and enhanced the production of IL-12 in macrophages of tumor-bearing host through nitric oxide [12].

Immunosuppression including myelosuppression is one of major side effects in cancer patients treated with chemotherapeutic agents. Since a variety of immune cells of bone marrow are in proliferating status, most anticancer drugs can attack normal immune cells as well as cancer cells, resulting in myelosuppression [11]. Tumor burden induces the immunosuppression in patients with advanced cancer and chemotherapy escalates it. Recent study demonstrated that the presence of tumor-derived soluble factor, vascular endothelial growth factor was closely associated with the decrease of DC number in the peripheral blood of cancer patients [1].

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and play a critical role in host immune system [16]. DCs originated from bone marrow migrate to peripheral tissue and organ. DCs take up, process antigen, and present antigenic peptides to naive T lymphocytes, stimulating their proliferation. Although taxol is widely used as an anticancer drug against cancers, the effect of taxol on DCs has not been studied yet. Based on the fact taxol shares receptors with LPS to bind macrophages and enhances the production of IL-12, taxol was expected to induce maturation of DCs and further enhance the proliferation of T lymphocytes. However, it was demonstrated in this study that taxol enhanced the expression of MHC class II molecules, as a marker of DC maturation, but decreased the proliferation of T lymphocytes activated by DCs.

It is thus suggested that taxol may induce an altered maturation of DCs. This study first demonstrated the effect of taxol on DCs and thus may provide new insight of the chemotherapy using taxol for cancer patients.

Materials and Methods

Animals and reagents

C57BL/6 and Balb/c mice were purchased from Japan
SLC (Shizuoka, Japan) and maintained in the lab animal facility for breeding. 7- to 10-week-old female mice were used for experiments. Purified anti-mouse CD8, CD19, Gr-1 monoclonal antibodies (mAbs, BD PharMingen, San Diego, CA) were used for the detection of CD8+ T lymphocytes, B lymphocytes, granulocytes in bone marrow-derived DCs. Cells were stained with trypan blue solution (Sigma, St. Louis, MO) and counted for viable and dead cells.

Preparation of DCs

DCs were cultured from bone marrow of mice using a general method that was initially established by Inaba et al. [6]. Briefly, bone marrow cells were harvested from tibia and femur of mice by flushing with PBS. Cells were cultured at a concentration of 2 × 10^6 cells/ml in 6-well culture plates. RPMI-1640 medium containing 5% fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin (all from Life Technologies Inc, Gaithersburg, MD), and 10 ng/ml mouse GM-CSF (Biosource International, Camarillo, CA) were used. The culture medium was replaced with fresh medium at every two days. To increase the purity of CD11c+ DCs, floating cells including T, B lymphocytes, and granulocytes were thoroughly removed at 2 and 4 day of culture. At 6-10 day of culture, 70% (v/v) of the medium was replaced by fresh medium and floating cells were used as DCs for experiments. DCs in this study were over 85% CD11c+ DCs based on FACS analysis.

T cell preparation and proliferation assay

Spleen cells from Balb/c mouse were prepared by mechanical disruption and hypotonic lysis of red blood cells as described in previous report [7]. The non-adherent cells were washed twice with Hanks balanced saline solution (HBSS) and used for allogeneic T cell proliferation assay. 2 × 10^6 cells/well T cells were cultured with 1 × 10^6 cells/well DCs in 96-well culture plate. Before experiments, DCs were treated with taxol (Paclitaxel®, Sigma) or LPS for 48 hrs. Cell debris was removed by centrifugation at 10,000 rpm for 30 sec. The nitrite levels were determined using modified Griess reagent (Sigma) following the manufacturer's manual. Briefly, 50 µl culture supernatant of DCs was mixed with 50 µl Griess reagent at a final concentration of 40 mg/ml. The O.D. of mixture was measured at 570 nm after 15 min. A serial dilution of NaNO_2 was used as standard.

Determination of nitric oxide production

To analyze nitric oxide (NO) release, culture supernatants were harvested after incubation of DCs in the absence or presence of taxol or LPS for 48 hrs. Cell debris was removed by centrifugation at 10,000 rpm for 30 sec. The nitrite levels were determined using modified Griess reagent (Sigma) following the manufacturer’s manual. Briefly, 50 µl culture supernatant of DCs was mixed with 50 µl Griess reagent at a final concentration of 40 mg/ml. The O.D. of mixture was measured at 570 nm after 15 min. A serial dilution of NaNO_2 was used as standard.

Flow cytometry analysis

To block Fc receptors, cells were incubated with purified anti-mouse CD16/CD32 mAb (BD PharMingen) at a concentration of 1 µg/100 µl/10^6 cells for 15 min at 4°C. Cells were incubated with each mAb at a concentration of 1 µg/100 µl for 30 min at 4°C and washed twice with HBSS containing 5% FBS and 0.1% sodium azide. Fluorescein isothiocyanate (FITC)-labeled anti-mouse I-A^b mAb, phycoerythrin (PE)-labeled anti-mouse CD11c mAb (BD PharMingen) were used for direct staining. FITC- or PE-labeled isotype-matched mAb (BD PharMingen) was used as control, respectively. Cells were stained with 2 µl/sample annexin V-FITC (Biosource International) and propidium iodide (PI, Sigma) at 4°C for measuring apoptosis of cells. After staining, cells were analyzed with FACSCaliber flow cytometer (Becton Dickinson, Mountain View, CA) and CellQuest software.

Statistical analysis

In MTT and T cell proliferation assay, the result of each sample is mean ± standard deviation (SD) from three independent wells. Most of data are the representative of three individual experiments with similar results. The statistical significance of experimental data was evaluated by the Student’s t-test. P < 0.05 was considered as statistically significant.

Results

The expression of MHC class II on DCs was enhanced by taxol

DCs were cultured from bone marrow cells by using 10 ng/ml GM-CSF. Cells were characterized by FACS analysis using anti-CD11c mAb as a DC marker. To investigate if taxol affects the maturation of DCs, the
Taxol induces the maturation of DCs

Taxol enhances the expression of MHC class II on DCs. After 6-8 day culture, DCs were seeded at a concentration of 5x10^5 cells/ml in 24-well culture plate. Cells were incubated with taxol for 48 hr. After washing twice with HBSS, the expression of MHC class II molecules was analyzed by using flow cytometry. LPS was used as a maturing agent for DCs. Result is a representative of three individual experiments.

No change in the viability of DCs by taxol, an anticancer drug

MTT assay was performed for measuring the viability of DCs. Cells were cultured in 96-well culture plate and treated with taxol in 3-fold serial diluted concentration. The optimal concentration of taxol was determined based on its biological activity on other immune cells including macrophages in previous studies [12,14]. Surprisingly, taxol did not decrease the viability of DCs (Fig. 2). To confirm the cytotoxicity of taxol, B16F10 melanoma cells were used as positive control cells. Taxol decreased the viability of B16F10 melanoma cells in a concentration-dependent manner. This data suggest that taxol may differentially act on DCs compared to cancer cells.

Taxol did not induce the cell death of DCs

Annexin V-FITC staining was performed to check if
232 Hong-Gu Joo

Annexin V is a 35-36 kDa calcium-dependent phospholipid binding protein with high affinity for phosphatidylinerine, which found in outer cell membrane beginning early in the process of apoptosis [10]. In preliminary experiments, the duration of taxol treatment was determined for annexin V-FITC staining since annexin V specifically binds to apoptotic cells at early stage of apoptosis (data not shown). In addition, cells were stained by propidium iodide for the detection of DC necrosis. Annexin V-positive/PI-negative, annexin V-positive/PI-positive, annexin V-negative/PI-positive cells represent cells in early apoptosis, late apoptosis, necrosis, respectively. Taxol did not significantly increase the cell death, apoptosis and necrosis, of DCs in any concentration (Fig. 3). This result is consistent to that of MTT assay as in Fig. 2. It is strongly suggested that an anticancer drug, taxol may not kill DCs.

**Fig. 3.** Taxol did not induce the apoptosis of DCs. As described in Fig. 1, DCs were seeded and treated. Cells were stained with annexin V-FITC/PI and analyzed by using flow cytometry. An anticancer drug, mitomycin C was used as positive control for the apoptosis of DCs. Result is a representative of three individual experiments.

**Fig. 4.** Taxol failed to produce significant amount of NO. DCs were treated with taxol at a range of concentration for 48 hrs. Supernatants of DCs were harvested and used for the determination of NO levels. NO concentration was divided by cell number to calculate NO concentration/10^6 DCs. LPS was used as a positive control for the production of NO. Results are representative of three experiments.

No detection of NO in the supernatant of taxol-treated DCs

Previous report demonstrated that taxol induced the production of NO in macrophage [12]. NO is well known to induce the apoptosis of DCs and inhibit the proliferation of T lymphocytes activated by DCs [8]. The level of NO was determined by using Griess reagent. Indeed, there was no detectable amount of NO in the supernatants of DCs treated with taxol at a range of concentration (1-10 µM).

LPS, as a positive control, produced significant amount of NO under same condition (Fig. 4). This result suggested that taxol may differentially act in DCs compared to other cell types including macrophage.
Taxol induces the maturation of DCs

Taxol-treated DCs strongly inhibited the proliferation of T lymphocytes

To investigate if taxol may affect the APC function of DCs, T cell proliferation assay was performed. After the treatment of taxol for 48 hr, DCs were washed twice with HBSS and cultured with allogeneic T lymphocytes for 5 days. The number and viability of T lymphocytes activated with DCs were determined by trypan blue exclusion test. Taxol significantly inhibited the proliferation and viability of T lymphocytes activated by taxol-treated DCs (Fig. 5A). To verify the direct effect of taxol on the interaction between DCs and T lymphocytes, taxol was added into the culture of no pretreated DCs and T lymphocytes at a range of concentration (100 nM-10 µM). Taxol significantly inhibited the proliferation and viability of T lymphocytes at 1 µM and 10 µM, but not 100 nM (Fig. 5B). It is suggested that taxol may inhibit the APC function of DCs.

Discussion

A plant-derived diterpenoid, taxol has been recognized as a potent inhibitor of cell cycle progression, resulting in cell cycle arrest and death of cancer cells [9]. Taxol demonstrated significant anti-cancer efficacy in human clinical trials and became a representative chemotherapeutic agent for the treatment of breast, ovarian, and non-small cell lung cancer [4,5]. In addition to its well-characterized anti-cancer activity, taxol induces the activation of macrophage in host [12]. Taxol and LPS share some receptors, CD11b/CD18, CD14, and TLR4, to transduce signals in macrophages [14]. Taxol has LPS-mimetic capabilities, the production of NO, IL-1 beta, IL-12, TNF-alpha and through TNF-alpha and NO production, taxol enhances the cytotoxicity of cancer cells [3]. Although the mechanism of taxol has been well characterized in tumor cells, the effect of taxol on immune cells remains unclear.

This study demonstrated that taxol did not kill DCs, the most potent APCs in immune system, based on MTT assay and annexin V-FITC/PI staining. Since taxol has already well known efficiently to kill cancer cells, this data...
suggests that taxol may remove cancer cells, but not DCs in host upon application. Furthermore, taxol enhanced the expression of MHC class II molecules, a representative maturation marker, on DCs. Since previous reports demonstrated that the maturation process tranduced survival signals in DCs, there is a possibility that taxol may provide DCs with survival signal through maturation process to protect taxol-induced cytotoxicity of DCs. The signal tranduction of taxol in DCs can be a valuable topic for further study.

To investigate the effect of taxol on antigen-presenting capability, DCs were pretreated with taxol and incubated with allogeneic T lymphocytes. Interestingly, taxol inhibited the proliferation of T lymphocytes activated by pre-treated DCs even though it enhanced the expression of MHC class II molecules. Furthermore, taxol only marginally inhibited the proliferation of T lymphocytes activated by non-treated DCs when it was directly added into the co-culture. These data strongly suggest that taxol may negatively change the APC function of DCs. It should be valuable in future study to investigate the production of immunosuppressive molecules including IL-10 from DCs treated with taxol [2,13]. As a candidate molecule, the level of NO was determined in the supernatants of DCs treated by taxol, since previous study demonstrated that NO induced the apoptosis of DCs, inhibited the proliferation of CD4+ T lymphocytes activated by DCs, and furthermore taxol produced NO in macrophage [8,12]. Taxol did not produce any detectable amount of NO in DCs, suggesting that taxol may have unique effector molecules or regulatory mechanism in DCs.

Taken together, it was in this study demonstrated that taxol did not kill DCs, further induced an altered maturation of DCs, the enhanced expression of MHC class II but the inhibition of T cell proliferation. This study may provide clinical trials using taxol with new insights to develop more effective therapy.

Acknowledgment

This work was supported by Korea Research Foundation Grant (KRF-2003-003-E00243).

References

1. Almand, B., Resser, J. R., Lindman, B., Nadaf, S., Clark, J. L., Kwon, E. D., Carbone, D. P. and Gabrilovich, D. I. Clinical significance of defective dendritic cell differentiation in cancer. Clin. Cancer Res. 2000, 6, 1755-1766.
2. Dieckmann, D., Ploettner, H., Berchtold, S., Berger, T. and Schuler, G. Ex vivo isolation and characterization of CD44+CD25+ T cells with regulatory properties from human blood. J. Exp. Med. 2001, 193, 1303-1310.
3. Ding, A. H., Porteu, F., Sanchez, E. and Nathan, C. F. Shared actions of endotoxin and taxol on TNF receptors and TNF release. Science 1990, 248, 370-372.
4. Eisenhauer, E. A. and Vermorken, J. B. The taxoids. Comparative clinical pharmacology and therapeutic potential. Drugs 1998, 55, 5-30.
5. Ettinger, D. S. Taxol in the treatment of lung cancer. J. Natl. Cancer Inst. Monogr 1993, 15, 177-179.
6. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. and Steinman, R. M. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 1992, 176, 1693-1702.
7. Joo, H. -G., Goedegebuure, P. S., Sadanaga, N., Nagoshi, M., Bernstorff, W. and Eberlein, T. J. Expression and function of galectin-3, a beta-galactoside-binding protein in activated T lymphocytes. J. Leukocyte Biol. 2001, 69, 555-564.
8. Lu, L., Bonham, C. A., Chambers, F. G., Watkins, S. C., Hoffman, R. A., Simmons, R. L. and Thomson, A. W. Induction of nitric oxide synthase in mouse dendritic cells by IFN-gamma, endotoxin, and interaction with allogeneic T cells: nitric oxide production is associated with dendritic cell apoptosis. J. Immunol. 1996, 157, 3577-3586.
9. MacKeigan, J. P., Collins, T. S. and Ting, J. P. MEK inhibition enhances paclitaxel-induced tumor apoptosis. J. Biol. Chem. 2000, 275, 38953-38956.
10. Martin, S. J., Reutelingsperger, C. P., McGahan, A. J., Rader, J. A., van Schie, R. C., LaFace, D. M. and Green, D. R. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J. Exp. Med. 1995, 182, 1545-1556.
11. Morstyn, G. and Burgess, A. W. Hematopoietic growth factors: a review. Cancer Res. 1988, 48, 5624-5637.
12. Mullins, D. W., Burger, C. J. and Elgert, K. D. Paclitaxel enhances macrophage IL-12 production in tumor-bearing hosts through nitric oxide. J. Immunol. 1999, 162, 6811-6818.
13. Munn, D. H., Sharma, M. D., Lee, J. R., Jhaver, K. G., Johnson, T. S., Keskin, D. B., Marshall, B., Chandler, P., Antonia, S. J., Burgess, R., Slingluff, C. L. Jr. and Mellor, A. L. Potential regulatory function of human dendritic cells expressing indoleamine 2, 3-dioxygenase. Science 2002, 297, 1867-1870.
14. Perera, P. Y., Mayadas, T. N., Takeuchi, O., Akira, S., Zaks-Zilberman, M., Goyert, S. M. and Vogel, S. N. CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. J. Immunol. 2001, 166, 574-581.
15. Schiff, P. B., Fant, J. and Horwitz S. B. Promotion of microtubule assembly in vitro by taxol. Nature 1979, 277, 665-667.
16. Steinman, R. M. and Nussenzweig, M. C. Avoiding horror autotoxocus : the importance of dendritic cells in peripheral T cell tolerance. Proc. Natl. Acad. Sci. USA. 2002, 99, 351-358.