Paclitaxel Inhibits Natural Killer Cell Binding to Target Cells by Down-Regulating Adhesion Molecule Expression

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Abstract. Background: Chemotherapy with paclitaxel is associated with impaired natural killer (NK) cell function. The purpose of this study was to determine the effect of paclitaxel treatment on NK cell adherence to target cells. Methods: Human NK-like YT cells or NK-sensitive K562 cells were exposed to submaximal cytotoxic concentrations (EC₁₀ and EC₃₀) of paclitaxel. The ability of surviving YT or K562 cells to adhere to untreated K562 cells or YT cells, respectively, was assessed in a conjugation assay. The effect of paclitaxel on adhesion molecule expression was determined by flow cytometry. Results: Paclitaxel treatment resulted in decreased conjugate formation, as well as decreased α₄β₇, α₅β₇, and β₂ integrin expression by YT cells and decreased ICAM-1 expression by K562 cells. Conclusions: Paclitaxel inhibition of adhesion molecule expression resulted in impaired NK cell binding to target cells, which may have a negative impact on immune surveillance.

Paclitaxel (taxol) is a potent chemotherapeutic agent that was originally isolated from the bark of the Pacific yew, Taxus brevifolia (1). Human cancers that respond to paclitaxel-based chemotherapy include refractory breast and ovarian carcinomas and advanced non-small cell lung cancer (2-4). Paclitaxel alters microtubule dynamics by promoting microtubule assembly and inhibiting microtubule depolymerization (5, 6), thus preventing cancer cells from traversing the G2/M boundary of the cell cycle (7). The result is growth arrest and the induction of caspase-dependent apoptosis that has recently been shown to require Fas-associated death domain protein signaling that is independent of cell-surface death receptors (8).

Unfortunately, in many cases cancer cells ultimately develop resistance to paclitaxel (9). In addition to its anti-cancer activity, paclitaxel has been reported to have both immunostimulatory and immunosuppressive properties (10). In mice, paclitaxel is a lipopolysaccharide mimic that induces the synthesis of pro-inflammatory interleukin (IL)-12 by macrophages via an autocrine signaling pathway involving macrophage-derived nitric oxide (11). Paclitaxel, in combination with interferon-γ, also induces nitric oxide-dependent killing of P815 mastocytoma cells by murine macrophages (12). In addition, paclitaxel causes increased secretion of pro-inflammatory IL-1β by unprimed human monocytes (13). Collectively, these findings suggest that paclitaxel-mediated enhancement of certain immune effector mechanisms may contribute to the anti-neoplastic activity of paclitaxel. However, there is also evidence that paclitaxel suppresses the function of other immune effector cells, which may result in impaired immune surveillance. The proliferative capacity of T cells from normal and tumor-bearing animals is reduced by paclitaxel, although the inhibitory effect is reversed by exogenous IL-12 (14). Paclitaxel also inhibits IL-2 synthesis and the induction of cytotoxic T cells in response to alloantigen, leading to prolonged survival of rat cardiac allografts (15). Interestingly, paclitaxel treatment of dendritic cells results in impaired dendritic cell-activated proliferation of mouse T lymphocytes (16), suggesting that paclitaxel exerts an inhibitory effect on T cell function at the level of dendritic cell-T cell interactions. In vitro exposure to paclitaxel also impairs the cytotoxic function of major histocompatibility complex unrestricted T cells and natural killer (NK) cells, as well as their activation by IL-2 (17, 18, 19). In addition, a transient decrease in NK cell activity has been reported for patients with non-small cell lung cancer undergoing weekly paclitaxel therapy (20).

Little is known about the mechanism by which paclitaxel causes decreased NK cell function. In this study, we determined whether prior exposure of a human NK cell line (YT cells) or K562 target cells to submaximal cytotoxic...
concentrations of paclitaxel (EC\text{10} and EC\text{30}) had an inhibitory effect on the recognition/adhesion stage of NK cell-mediated cytotoxicity. It is well established that conjugate formation between NK cells and target cells is an important prerequisite for target cell killing by NK cells (21). Adhesion molecules that participate in NK cell binding to target cells include LFA-1 (\alpha\text{L}\beta\text{2}) and its ligand ICAM-1. We reasoned that an inhibitory effect by paclitaxel on the expression or function of adhesion molecules such as LFA-1 and ICAM-1 might account for paclitaxel-mediated inhibition of NK cell activity.

Materials and Methods

Cell lines and reagents. K562 chronic myeloid leukemia cells were from the American Type Culture Collection (Manassas, VA, USA). The continuous human NK-like YT cell line (22) was a generous gift from Dr. M. Abdelhaleem ( Sick Children’s Hospital, Toronto, ON, USA). Cell lines were maintained in RPMI 1640 medium (Sigma-Aldrich, Mississauga, ON, USA) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 Ìg/ml streptomycin, 5 mM HEPES buffer (pH 7.4), and 5% (K562) or 10% (YT) heat-inactivated fetal bovine serum (all from Invitrogen Canada, Burlington, ON, USA). Mouse anti-human anti-\alpha\text{4}, anti-\alpha\text{5}, anti-\alpha\text{L}, anti-\alpha\text{1}, anti-\beta\text{1}, anti-\beta\text{2}, anti-ICAM-1, anti-VCAM-1, and anti-fibronectin monoclonal antibodies (mAb) were purchased from BD PharMingen (Mississauga, ON, USA). Mouse IgG used as an isotype control was from Bio/Can Scientific (Mississauga, ON, USA). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody was from Cedarlane Laboratories (Hornby, ON, USA). Dimethyl sulfoxide (DMSO) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were from Sigma Aldrich. Paclitaxel was purchased from Calbiochem (San Diego, CA, USA). A stock solution of paclitaxel was prepared at 25 mg/ml in DMSO, aliquoted, and stored at -20°C.

MTT assay. Paclitaxel cytotoxicity against YT and K562 cells was assessed by MTT assay, which measures the activity of mitochondrial dehydrogenases (23). YT or K562 cells were cultured for 43 h at 37°C in a 5% humidified CO_2 atmosphere (1 x 10^6 cells/ml in quadruplicate wells of a 96-well U-bottom microtiter plate) in the presence of various concentrations of paclitaxel or the drug vehicle. MTT (final concentration 500 ìg/ml) was then added to each well and 5 h later cells were sedimented by centrifugation and the supernatant discarded. Cell pellets were then solubilized in DMSO and transferred to a 96-well flat-bottom microtiter plate. Absorbance was measured at 490 nm using a Biotek microplate reader (Highland Park, VT, USA).

Conjugate formation assay. K562 cells were stained with neutral red dye (1 mg/ml in RPMI 1640 medium for 15 min). Stained K562 cells and YT cells were washed thoroughly after all treatments, counted, and resuspended in complete RPMI 1640 medium at a concentration of 5 x 10^6 viable (by trypan blue dye exclusion) cells/ml. YT cells were combined in a 1:1 ratio with K562 cells in a final volume of 0.2 ml in round-bottom polystyrene tubes, centrifuged for 5 min at 100 g, and incubated for 45 min at 37°C in a 5% humidified CO_2 atmosphere. The tubes were then placed on ice and the cell pellet gently resuspended using a pipetman. Unbound YT cells and YT cells that had conjugated with K562 cells were enumerated by microscopic examination using a hemocytometer. At least 100 YT cells in quadruplicate samples were counted. The percentage of YT cells bound to K562 cells was then calculated.

Flow cytometry. YT cells and K562 cells were washed thoroughly and resuspended in immunofluorescence (IF) buffer (phosphate buffered saline with 1% bovine serum albumin and 0.2% sodium azide) containing 10 ìg/ml mouse anti-human anti-\alpha\text{4}, anti-\alpha\text{5}, anti-\alpha\text{L}, anti-\alpha\text{1}, anti-\beta\text{1}, anti-\beta\text{2}, anti-ICAM-1, anti-VCAM-1, or anti-fibronectin mAb (K562 cells), or mouse IgG (YT and K562 cells). Following a 30-min incubation at
4°C, cells were washed twice with IF buffer and resuspended in fresh IF buffer containing fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody (10 μg/ml). After an additional 30 min incubation in the dark at 4°C, cells were washed with IF buffer, resuspended in a 1% paraformaldehyde solution and analyzed with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and FCS Express software (De Novo Software).

**Results**

To determine *in vitro* sensitivity to paclitaxel, NK-like YT cells or K562 cells were cultured for 48 h in the absence or presence of different concentrations of the drug. An MTT assay was then used to measure the effect of paclitaxel on cell viability. The approximate EC10 and EC30 concentrations of paclitaxel for YT cells were 0.01 and 0.05 μg/ml, respectively (Figure 1A), whereas the approximate EC10 and EC30 concentrations of paclitaxel for K562 cells were 0.1 and 5.0 μg/ml, respectively (Figure 1B). K562 cells were therefore significantly less sensitive than YT cells to the cytotoxic effect of paclitaxel.

We next examined the effect of prior exposure to EC10 or EC30 concentrations of paclitaxel on the binding of NK-like YT cells to K562 target cells during the recognition/adhesion phase of cytolysis. As shown in Figure 2, YT cells that survived 48 h of culture in the presence of 0.01 or 0.05 μg/ml paclitaxel exhibited a dramatic reduction in their ability to bind to untreated K562 target cells. K562 cells that survived 48 h of culture in the presence of 0.1 or 5.0 μg/ml paclitaxel showed a similar reduction in their ability to form conjugates with untreated YT cells. Prior exposure of YT cells or K562 cells to the drug vehicle (DMSO) did not affect conjugate formation.

Flow cytometric analysis revealed that culture for 48 h in the presence of submaximal cytotoxic concentrations of paclitaxel caused a reduction in adhesion molecule expression by surviving NK-like YT cells and K562 target cells. YT cells expressed α4, αL, and β7 integrins, but not of α5 or β1 integrins (Figure 3). EC10 and EC30 concentrations of paclitaxel caused reduced expression of α4, αL, and β7 integrins by surviving YT cells. K562 cells expressed ICAM-1 but not VCAM-1 or cell-surface fibronectin (Figure 4). EC10 and EC30 concentrations of paclitaxel caused reduced expression of ICAM-1 by surviving K562 cells.

To determine whether reduced LFA-1 (αLβ2) expression by paclitaxel-treated YT cells and/or reduced expression of the LFA-1 ligand ICAM-1 by paclitaxel-treated K562 cells might account for impaired conjugate formation, we tested the effect of α4 or ICAM-1 blockade with specific mAb on the subsequent adhesion of YT cells to K562 cells. As shown in Figure 5, pretreatment of YT cells with anti-α4 mAb or K562 cells with anti-ICAM-1 mAb had an inhibitory effect on conjugate formation that was comparable to that achieved by pretreatment with EC10 or EC30 concentrations of paclitaxel.

**Discussion**

Paclitaxel is a potent anti-neoplastic agent with demonstrated effectiveness against metastatic breast and ovarian cancers, as well as advanced non-small cell lung...
Figure 3. Prior exposure to paclitaxel results in decreased α4, α5, and β1 expression by YT NK cells. YT cells were cultured for 48 h in the presence of the drug vehicle (DMSO) or the indicated concentrations of paclitaxel. YT cells were then washed extensively (to recover viable cells), resuspended in IF buffer, and stained with mAb specific for the indicated integrins. Cytosfluorometric profiles for unstained isotype controls (filled peaks) and YT cells stained with anti-α4, anti-α5, anti-αL, anti-β1, or anti-β2 mAb (open peaks) are shown. Data are from one experiment and are representative of 3 independent experiments.

Figure 4. Prior exposure to paclitaxel results in decreased ICAM-1 expression by K562 leukemia cells. K562 cells were cultured for 48 h in the presence of the drug vehicle (DMSO) or the indicated concentrations of paclitaxel. K562 cells were then washed extensively (to recover viable cells), resuspended in IF buffer, and stained with mAb specific ICAM-1, VCAM-1, or cell-surface fibronectin. Cytosfluorometric profiles for unstained isotype controls (filled peaks) and K-562 cells stained with anti-ICAM-1, anti-VCAM-1, or anti-fibronectin mAb (open peaks) are shown. Data are from one experiment and are representative of 3 independent experiments.
Paclitaxel has also been shown to enhance the tumoricidal activity of macrophages (11, 12), which may contribute to the drug’s overall anti-cancer activity. However, there is mounting evidence that paclitaxel also inhibits T lymphocyte and NK cell function (14-20). Here, we show that a 48-h exposure to EC10 or EC30 concentrations of paclitaxel interfered with the ability of surviving human NK-like YT cells to adhere to K562 target cells. A similar inhibitory effect on conjugate formation was obtained when K562 cells were cultured for 48 h in the presence of EC10 or EC30 concentrations of paclitaxel, and surviving K562 cells were then allowed to adhere to YT cells. NK-like YT cells that survived treatment with paclitaxel showed reduced expression of α4, α3, and β7 integrins, whereas K562 cells that survived paclitaxel treatment showed reduced ICAM-1 expression. These data suggested that exposure to paclitaxel resulted in impaired LFA-1 (αLβ2) and LPAM-1 (α4β7) expression by human NK cells, as well as impaired ICAM-1 expression by leukemic cells that serve as target cells for human NK cells. We have previously reported that short-term culture in the presence of paclitaxel or vinblastine results in decreased LFA-1 and ICAM-1 expression by mouse cytotoxic T lymphocytes and P815 mastocytoma cells, respectively (24). Short-term culture in the presence of paclitaxel also causes decreased LFA-1, VLA-4, VLA-5, and LPAM-1 expression by the Jurkat human T cell line (25). A similar inhibitory effect on LFA-1 and L-selectin surface expression by human T lymphocytes, as well as ICAM-1 and E-selectin surface expression by human umbilical vein endothelial cells was noted following exposure to colchicine (26), which, like paclitaxel, is an antimicrotubule agent (27). Taken together, these findings indicate that paclitaxel and other antimicrotubule agents down-regulate adhesion molecule expression by human NK cells and T cells, as well as by potential target cells and antigen-presenting cells. This drug-induced decrease in adhesion molecule expression may have a negative impact on certain adhesion molecule-dependent cell-cell interactions.

Binding of LFA-1 on NK cells to ICAM-1 on target cells is an important step in the recognition/adhesion phase of NK cell-mediated cytotoxicity (21). Thus, antibody blockade of LFA-1 on NK-like YT cells or ICAM-1 on K562 cells had a strong inhibitory effect on conjugate formation between YT cells and K562 cells, which was similar in magnitude to the inhibition caused by paclitaxel treatment of YT cells or K562 cells. Prior exposure to submaximal cytotoxic concentrations of paclitaxel therefore interfered with conjugate formation between NK-like YT cells and K562 target cells by a mechanism that most likely involved a reduction in LFA-1-ICAM-1 interactions due to decreased surface expression of LFA-1 and ICAM-1. Since a diminished capacity of NK cells to form conjugates with target cells is predicted to result in decreased lysis of target cells, it is surprising that pretreatment of K562 cells with paclitaxel has been reported to increase their sensitivity to killing by NK cells (28). This apparently contradictory finding may be explained by the observation that paclitaxel sensitizes different human tumor cells to Fas-mediated apoptosis (29, 30), which is a major mechanism by which human NK cells mediate cytotoxicity.

Figure 5. Inhibition of YT NK cell adhesion to K562 target cells by antibody blockade of α4 or ICAM-1. YT cells or K562 cells were pretreated with the indicated mAb (10 µg/ml), washed extensively, and combined at a 1:1 ratio with untreated K562 cells or YT cells, respectively. Following a 45 min incubation at 37°C, the YT cell:K562 cell conjugates were visualized by microscopy. Results from a representative experiment (n=3) are expressed as mean percent conjugated YT cells of quadruplicate samples (± SD).
(31). Enhanced sensitivity of paclitaxel-treated K562 cells to Fas-mediated killing by NK cells might more than offset the impact of decreased conjugate formation on overall cytotoxicity. However, it is important to note that pretreatment with paclitaxel would be expected to cause tumor cells that are refractory to Fas-mediated apoptosis to be less sensitive to NK cell-mediated cytotoxicity.

Activated NK cells are crucial for the elimination of virus-infected cells during the early stages of viral infection (32) and are also involved in the maturation of antigen-presenting dendritic cells (33). In addition, NK cell recognition and cytolysis of tumor target cells is critical for the induction of tumor-specific cytotoxic T lymphocytes (34). Since adhesion interactions between LFA-1 and ICAM-1 are an essential initial step in activating the cytolytic machinery of NK cells (21), paclitaxel-induced reductions in LFA-1 and ICAM-1 expression are likely to at least contribute to decreased NK cell function following paclitaxel treatment (17-20). Our data also suggest that paclitaxel therapy may result in impaired immune surveillance and increased susceptibility of cancer patients to viral infections. Moreover, cancer patients who are receiving paclitaxel may have a diminished capacity to generate tumor-specific cytotoxic T lymphocytes in response to therapeutic cancer vaccines. The inhibitory effect of paclitaxel on NK cell function should therefore be taken into account when designing immune-based cancer therapies that will be employed concurrently with paclitaxel-based chemotherapy.

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References


