Effect of Docetaxel on the Surgical Tumor Microenvironment of Head and Neck Cancer in Murine Models

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Objectives: To identify the antitumor activity and wound-healing effect of docetaxel delivered in the surgical tumor microenvironment of head and neck squamous cell carcinoma (HNSCC).

Design: Control and experimental series.

Setting: Academic medical center.

Subjects: BALB/c and severe combined immunodeficiency mice.

Intervention: Intrawound (IW) docetaxel therapy was tested in 3 HNSCC xenograft and 2 taxane-resistant models. Intratumoral (IT) docetaxel therapy was further tested in the 2 taxane-resistant models.

Main Outcome Measures: Tumor size, survival, and wound toxic effects were measured. The effect of docetaxel on various factors involved in wound healing and tumor growth within the surgical tumor microenvironment was also analyzed.

Results: In a pilot study using BALB/c mice, IW docetaxel therapy was not associated with problems in wound healing. Using the HN6, HN12, and HN30 HNSCC xenograft model, IW docetaxel prevented tumor growth and improved survival when compared with controls. No local or systemic toxic effect or wound-healing problem was noted. Using taxane-resistant xenograft lung cancer (H460/T800) and syngeneic salivary cancer (BALB/c mucoepidermoid carcinoma) models, IW therapy did not delay tumor growth. An antitumor effect was detected with repeated docetaxel injections in the H460/T800 taxane-resistant model but not in the BALB/c mucoepidermoid carcinoma model. Docetaxel inhibited the expression of growth factors and receptors in tumor cells; however, it did not inhibit the level of wound-healing growth factors in the surgical tumor microenvironment.

Conclusions: These preclinical results support further testing of IW docetaxel treatment in HNSCC. Docetaxel appears to exert antitumor activity without affecting factors involved in wound healing in the tumor microenvironment.


The American Cancer Society estimates that approximately 45,660 new cases of head and neck squamous cell carcinoma (HNSCC) will have been diagnosed in the United States, and 11,210 Americans will have died of this disease in 2007. Worldwide, HNSCC is the sixth most common malignancy, with an incidence of 644,000 new cases a year. In advanced HNSCC, the 5-year survival rate is less than 40%. Although most advanced and resectable HNSCCs were treated with surgery followed by postoperative radiotherapy in the past, nonsurgical therapy is now more commonly used. Cisplatin-based chemoradiotherapy compared with radiotherapy alone after surgical resection has demonstrated a benefit in overall survival in the European Organization for Research and Treatment of Cancer trial (53% vs 40%; P = .02). However, results from the US Intergroup phase 3 trial demonstrated an improvement only in disease-free survival (P = .04) and did not confirm a benefit in overall survival (P = .19).

Taxanes promote tubulin assembly in microtubules, inhibit depolymerization, act as a poison to mitotic spindle cells, and induce a mitotic block in proliferating cells. Furthermore, taxanes induce G2M phase arrest and p53-independent apoptosis. Taxanes (paclitaxel and docetaxel) also have been shown to improve survival in breast, lung, ovarian, and many other cancers. Although intravenous taxanes in combination with 1 or more agents (cis-
platin, fluorouracil, and/or leucovorin calcium) have demonstrated high clinical response rates in newly diagnosed HNSCC (75%-100%).\textsuperscript{13,24} Response rates using taxanes alone or in combination in recurrent disease have been disappointing (13%-77%).\textsuperscript{15} Recently, 3 phase 3 trials have demonstrated improvement in overall survival when taxane was added to the standard induction chemotherapy regimen of cisplatin and fluorouracil in locally advanced unresectable head and neck cancers.\textsuperscript{16-18}

Head and neck squamous cell carcinoma is a significant health problem, with extremely poor outcomes and significant morbidity if patients have a disease recurrence at the locoregional site. After surgical resection, microscopic cancer cells may still be left behind in the margins and wound of the surgical resection bed and may increase the likelihood of local failures. Leaving microscopic cancer cells behind may occur even when margin findings are negative based on results of frozen section analysis. Tumor cells harboring mutations of the TP53 gene (Genbank NP_000537) have been detected in histopathologically negative surgical margins.\textsuperscript{19} The immediate postoperative period may be a time of maximum growth stimulus for any residual tumor cells. Cytoreduction by surgery may remove growth inhibition (via contact inhibition, removal of growth inhibitors, etc), resulting in a rapid increase in proliferation of residual tumor cells.\textsuperscript{20-22} Furthermore, the process of wound repair after surgical extirpation in the surgical wound may involve the secretion of many factors that can promote tumor cell growth.\textsuperscript{20} Thus, despite aggressive treatment involving surgery and postoperative adjuvant radiotherapy and/or chemotherapy, local recurrence rates of 25% to 40% still exist.\textsuperscript{25-27}

Intratumoral (IT) and intrawound (IW) injection of chemotherapeutic agents have been described as novel approaches to drug therapy that allow delivery of high therapeutic doses of chemotherapeutic agents locally while limiting systemic toxic effects.\textsuperscript{26-28} Injection of a supra-therapeutic dose of a chemotherapeutic agent such as docetaxel directly into the surgical tumor microenvironment immediately after surgical extirpation of the tumor may improve local cancer control by effectively eradicating any microscopic residual disease left behind in the surgical resection bed. However, after surgical manipulation, the tumor microenvironment is a complex milieu of microscopic cancer cells, inflammatory and immune cells, fibroblasts, growth factors, cytokines, and extracellular matrix proteins.\textsuperscript{29} These cells and molecules interact in a complex fashion to allow angiogenesis, wound repair, and possibly promotion of tumor growth. Tissue repair and tumor formation share some of the molecular mechanisms such as angiogenesis that can be ascribed to inflammation.\textsuperscript{30-32} Because tumor growth and wound healing are activated by many of the same growth factors and share many of the same molecular pathways,\textsuperscript{30-32} there is a concern that, although IT and IW docetaxel therapy can effectively target tumor regrowth, it may also adversely affect wound healing in the surgical wound.

In this study, we sought to assess the effect of IW docetaxel therapy on antitumor activity and local wound healing in the surgical tumor microenvironment. We also examined the activity of many of the growth factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β), that are involved in promoting wound healing and tumor growth.

### METHODS

#### PILOT STUDIES USING IW DOCETAXEL INJECTIONS IN BALB/c MICE

All mice studies were performed in compliance with and with the approval of the Wayne State University Institutional Animal Care and Use Committee, Division of Laboratory Animal Resources. After BALB/c mice (10 weeks old) were anesthetized by intraperitoneal injection of ketamine hydrochloride (67 mg/kg) and xylazine hydrochloride (10 mg/kg), their backs were shaved and prepared with ethanol. A 1-cm skin incision was then made, and IW injections of docetaxel or diluents (5% dextrose) were performed. Docetaxel (Sanofi-Aventis Pharmaceuticals, Paris, France) in concentrated form (20 mg per 0.5 mL) was initially reconstituted using 1.5 mL of a 13% ethanol solution, and the reconstituted docetaxel solution (10 mg/mL) was stored at -20°C. Immediately before use, the reconstituted docetaxel solution was diluted using 5% dextrose to make a final desired docetaxel concentration of 0.75 mg/mL. The final ethanol concentration was less than 0.05% by volume. Using a 1-mL syringe attached to a 25-gauge needle (5/8-in) Precision Glide needle; Becton Dickinson and Co, Franklin Lakes, New Jersey), 4 injections of 0.1 mL of docetaxel solution were placed approximately 3 mm apart over the 1-cm skin incision at the level of the dermis and subcutaneous tissue. The wound was closed using wound clips and covered with bacitracin. Wound clips were removed 7 days later. Toxic effects in the wound after IW injections were measured and recorded twice weekly for 3 weeks.

#### IMPLANTATION OF CELL LINES IN SEVERE COMBINED IMMUNODEFICIENCY DISORDER MICE

Three HNSCC cell lines, HN6, HN12, and HN30, have been previously described.\textsuperscript{33,34} The H460 cells are a non–small cell lung cancer line with a wild-type TP53 gene,\textsuperscript{35} and the H460 xenograft model is sensitive to taxanes.\textsuperscript{36} A taxane-resistant subline (H460/T800)\textsuperscript{37} was obtained. The mouse BALB/c mucosidermoid carcinoma (BMEC) cell line was derived from a mucosidermoid carcinoma spontaneously arising from a parotid gland.\textsuperscript{38} The BMEC cell line overexpresses p53 protein and bears a mutation in codon 129. The BMEC cells formed rapidly growing tumor nodules after subcutaneous injection into syngeneic BALB/c mice and severe combined immunodeficiency disorder (SCID) mice. The cell lines were grown in Dulbecco Modified Eagle Medium with 10% fetal calf serum. Cells were washed twice in phosphate-buffered saline solution and suspended in a 0.1-mL phosphate-buffered saline solution and a 0.1-mL basement membrane matrix (Matrigel; Becton Dickinson, Bedford, Massachusetts) for a total volume of 0.2 mL per injection site. After female CB17 SCID mice aged 6 to 11 weeks (Harlan, Madison, Wisconsin) were anesthetized by intraperitoneal injection of ketamine hydrochloride (67 mg/kg) and xylazine hydrochloride (10 mg/kg), their backs were shaved and prepared with ethanol. A 1-cm skin incision was then made and IW injections of HN6 (15 × 10⁶), HN12 (15 × 10⁶), HN30 (15 × 10⁶), H460/T800 (5 × 10⁶), and BMEC (1 × 10⁶) cells were performed using a 1-mL syringe attached to a 25-gauge 3/8-in Precision Glide needle. Four injections of 0.05 mL of cells were...
placed approximately 3 mm apart over the 1-cm skin incision at the level of the dermis and subcutaneous tissue.

**SINGLE INJECTION OF DOCETAXEL IN THE MICE MODELS**

Immediately after tumor cell implantation, IW docetaxel or diluent injections were performed at the same level of the dermis and subcutaneous tissue. Again, using a 1-mL syringe attached to a 25-gauge 5⁄8-in Precision Glide needle, 4 injections of 0.1-mL docetaxel solution were placed approximately 3 mm apart over the 1-cm skin incision at the level of the dermis and subcutaneous tissue. The wound was closed using wound clips and covered with bacitracin. Wound clips were removed 7 days later. The formation of tumor nodules and toxic effects in surgical wounds after IW injections were measured for 6 weeks.

**MULTIPLE IT INJECTIONS OF DOCETAXEL IN TAXANE-RESISTANT MODELS**

Approximately 14 to 21 days after the IW implantation, tumor nodules ranging from 7 to 10 mm formed at the site of implantation. At this point, IT docetaxel injections were performed along with control injections (diluent alone). The IT injections of docetaxel (15 mg/kg per injection) twice a week (days 1 and 3) were performed every week for a 6-week cycle. After IT therapy was completed, mice with a complete or a partial regression of tumor were observed 6 more weeks until day 85. At day 95, all surviving mice were humanely killed. At the time when no tumor was noted, IT therapy was discontinued and mice were observed until day 95. Weight, tumor size, survival, and toxic effects were monitored throughout the course of this study.

**DOCETAXEL EFFECT ON TUMOR SIZE AND SURVIVAL AND TOXIC EFFECTS IN THE WOUND**

The primary study endpoint was tumor size. The secondary end points were overall and disease-free survival and toxic effects in the wound between the experimental groups and control groups. Animals were humanely killed by means of carbon dioxide narcosis and cervical dislocation when tumors reached 1500 mg or 10% of the animal's body weight or when animals displayed any open lesion or weight loss of more than 20% of body weight. Tumor nodules were weighed directly at the time mice were humanely killed; otherwise, tumor weight was estimated when mice are alive using the formula [length × width (in square centimeters)] × 0.5 × 1 g/cm².

Calipers were used to measure tumors 3 times a week in the experimental and control groups. Animals were also observed for weight loss, scuff appearance, listlessness, uncongregated movements, inability to grasp a pencil, or play-footed walking.

**WESTERN BLOT ANALYSIS**

The HN12 and HN30 cells (1.2 × 10⁶ cells) were plated (100 m²) in 6 mL of defined keratinocyte–serum free medium. After 48 hours of incubation with the viruses, the cells were washed with phosphate-buffered saline solution. Total cell lysates were prepared by sonicating and incubating the cells in radioimmuno-precipitation assay buffer (150mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.4% sodium dodecyl sulfate [SDS], 20mM EDTA, and 50mM Tris buffer [pH, 7.5]) and incubated with the primary antibodies. Primary antibodies (epidermal growth factor receptor [EGFR], phosphorylated EGFR [pEGFR], and basic fibroblast growth factor [bFGF]) were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, California). A secondary antibody, horseradish peroxidase–conjugated IgG, was incubated with membranes and developed according to an enhanced chemiluminescence protocol (SuperSignal West Pico; Pierce Biotechnology Inc, Rockford, Illinois).

**ENZYME-LINKED IMMUNOSORBENT ASSAY**

The concentration of VEGF was determined by means of an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, Minnesota). The HN12 and HN30 cells (3 × 10⁵ cells) were placed in 6-well plates for each experiment in a final culture medium volume of 3 mL. During logarithmic growth, cells were analyzed at 24 and 48 hours after 70% to 80% confluence was reached. The supernatant (500 µL) of each well was then collected and stored in the refrigerator. Each sample was diluted with the calibration diluent in a 1:4 ratio. Serial dilutions were prepared with the VEGF standard to yield an 8-point standard curve from 0 to 1000 pg/mL. The colorimetric ELISA was performed in a 96-well plate. The intensity of the color developed was measured at a wavelength equal to 450 nm by means of a microplate reader (Molecular Devices Corporation, Sunnyvale, California), and a corrective reading, owing to optical imperfections in the plate, was performed at a wavelength equal to 570 nm and subtracted from the reading at 450 nm. The data were analyzed by means of a microplate reader software program (SoftMax; Molecular Devices Corporation). The logarithm of VEGF standard concentrations was graphed against that of the optical density of experimental concentrations. Average concentrations of VEGF secreted by the cells were determined. All analyses were performed in triplicate.

**IMMUNOHISTOCHEMICAL EXPRESSION ANALYSIS OF WOUND GROWTH FACTORS**

To determine whether IW docetaxel can modulate the surgical tumor microenvironment, the expression of growth factors that regulate wound healing and tumorigenesis were measured. Because tumor induction was not required for these experiments, BALB/c mice were used instead of SCID mice. At 24 hours after docetaxel injections, the mice were humanely killed, and the wound with a 2-cm cuff of tissue was harvested and embedded in paraffin. Tissue was rinsed in isotonic sodium chloride solution, sliced, and fixed in 10% neutral buffered formalin. The samples were then embedded in paraffin using standard histochemical techniques. The tissue samples were sectioned (3-4 µm) and stained for the biomarkers keratinocyte growth factor (KGF), VEGF, bFGF, PDGF-β, TGF-β, and epidermal growth factor (EGF) (Santa Cruz Biotechnology, Inc). Once stained, the slides were evaluated by a board-certified pathologist (F.L.). The level and intensity of gene expression in treated mice were compared with those of the control groups. The level of staining was scored as follows: 0 indicates no cells stained; 1, no more than 10% of the cells stained; 2, 10% to 40% of the cells stained; and 3, 40% or more of the cells stained. The intensity of staining was scored on a progressive and discontinuous scale (3+, strong staining; 2+, moderate staining; 1+, weak staining; and 0+, no staining).
RESULTS

IW DOCETAXEL THERAPY IN BALB/c MICE MODEL

After docetaxel (15 mg/kg) or diluent was injected into the surgically created open wound, the 6 BALB/c mice were monitored for evidence of toxic effects in the wound for 3 weeks. A No wound-healing complications or infections were noted in any of the BALB/c mice, and all the incisions (arrows) healed well. B, Close-up view of a single mouse. Arrow indicates the healed incision.

Figure 1. After docetaxel (n=3) (docetaxel group) or diluent (n=3) (control group) was injected into the surgically created open wound, the 6 BALB/c mice were monitored for evidence of toxic effects in the wound for 3 weeks. A, No wound-healing complications or infections were noted in any of the BALB/c mice, and all the incisions (arrows) healed well. B, Close-up view of a single mouse. Arrow indicates the healed incision.

IW DOCETAXEL THERAPY IN THE 3 HNSCC CELL LINES IN SCID MICE

Given the lack of toxic effects demonstrated in the wound in the previous pilot study, further study was undertaken to evaluate the antitumor effect of IW docetaxel therapy. The SCID mice (n=48) were divided into 3 groups, with each group (n=16) receiving intrawound (IW) implantation of cancer cells from 1 of the 3 cell lines (HN6 [A], HN12 [B], and HN30 [C]). After IW implantation of cancer cells, half of the mice (n=8) in each group received an IW docetaxel injection (docetaxel group) and the other half received an IW diluent injection (control group). Tumor weight was then measured and recorded. Error bars indicate standard deviation.

Figure 2. The severe combined immunodeficiency mice (n=48) were divided into 3 groups, with each group (n=16) receiving intrawound (IW) implantation of cancer cells from 1 of the 3 cell lines (HN6 [A], HN12 [B], and HN30 [C]). After IW implantation of cancer cells, half of the mice (n=8) in each group received an IW docetaxel injection (docetaxel group) and the other half received an IW diluent injection (control group). Tumor weight was then measured and recorded. Error bars indicate standard deviation.

RESULTS

IW DOCETAXEL THERAPY IN BALB/c MICE MODEL

After docetaxel (15 mg/kg) or diluent was injected into the surgically created open wound, the 6 BALB/c mice were monitored for 3 weeks for evidence of toxic effects in the wound. The incision in all 6 mice healed well without any evidence of infection, skin breakdown, or alopecia (Figure 1).

IW DOCETAXEL THERAPY IN THE 3 HNSCC CELL LINES IN SCID MICE

The average tumor weight in all 3 HNSCC models was significantly less in the docetaxel-treated groups compared with the diluent-treated control groups (Table 1). In the control groups of the HN6 and HN12 HSNCC models, all of the mice had to be humanely killed because of excess tumor burden, whereas 100% of the docetaxel-treated mice were alive. Although all of the mice in the HN30 control groups were alive at the end of the study, all 8 of these mice (100%) had tumor as opposed to 3 of the mice that received IW docetaxel (38%). In the IW-treated group of the HN6 and HN12 models, 2 mice and 1 mouse (25% and 13%), respectively, had small tumor nodules present at the end of the study.

LOCAL WOUND-HEALING PROBLEMS AND SYSTEMIC TOXIC EFFECTS IN THE SCID MOUSE MODEL

Throughout the study, no SCID mice in the IW docetaxel-treated groups displayed a scruffy appearance, listlessness, uncoordinated movements, inability to grasp a pencil, or splayfooted walking. A broad measurement of systemic toxic effect, weight loss, was not significantly different between the experimental and control groups. No local toxic effects (skin breakdown and alopecia) or wound-healing problems in any of the groups were noted.
In the H460/T800 model (Figure 3A), no mice survived past 21 days in the control group or past 29 days in the docetaxel-treated group owing to rapid tumor growth (Table 2). The IW injection of docetaxel also did not inhibit BMEC tumor growth (Figure 3B), and all groups (docetaxel- and diluent-treated) had to be humanely killed 24 days later because of the large tumor burden (Table 2).

**IT DOCETAXEL THERAPY AND OVERALL SURVIVAL**

Because a single injection may not be sufficient for antitumor activity, repeated tumor exposure to docetaxel was tested using IT injections. The H460/T800 xenograft model was partially resistant to biweekly IT docetaxel injections (Figure 4A). After 52 days of initiating therapy in the IT docetaxel-treated group (n=8), 2 mice had died of excessive tumor burden, 4 mice had no tumor, and 2 mice were alive with tumor. The mean (SD) tumor weight was 403 (413) mg. All mice in the control group (n=8) had to be humanely killed by day 37 (Table 2).

In the BMEC model (Figure 4B), nodules grew rapidly in the control and treatment groups, and all of the mice had to be humanely killed after 28 days. Although slower tumor growth was observed in the treated group (mean [SD] tumor weight, 1338 [274] vs 2550 [543] mg) at day 28, no benefit in survival was noted (Table 2).

**EXPRESSION OF TUMOR CELL AND WOUND-HEALING GROWTH FACTORS**

To further elucidate the biological effect of IW docetaxel therapy on cancer and wound healing in the surgical tumor microenvironment, expression levels of growth factors involved in cancer growth and wound healing were measured. Docetaxel decreased EGFR expression and VEGF secretion in the HN12 and HN30 cells, whereas pEGFR and bFGF expression was lowered only in the HN30 and HN12 cells, respectively (Figure 5). To determine whether IW docetaxel treatment affects wound healing in the surgical tumor microenvironment, the expression levels of growth factors (KGF, VEGF, bFGF, PDGF-β, TGF-β, and EGF) involved in wound healing were measured using standard immunohistochemical techniques. The level and intensity of expression of these growth factors (except for TGF-β) were found to be the same between the docetaxel- and diluent-treated wounds.

**COMMENT**

Nonsurgical options, using a combination of chemotherapy and radiotherapy, are often used in the treatment of advanced oropharyngeal, hypopharyngeal, and...
laryngeal cancers. Surgery, however, remains an important first-line treatment in certain types of HNSCC (such as oral cavity cancer) and a salvage treatment after failure of radiotherapy with or without chemotherapy. Despite the best effort of surgeons, negative surgical resection margins may sometimes be unattainable. Even when the finding of a negative margin is obtained on the basis of frozen section and final permanent section analysis results, the possibility of leaving behind microscopic cancer cells still exists. Thus, despite aggressive treatment involving surgery and postoperative adjuvant radiotherapy and/or chemotherapy, the local recurrence rate may still be 25% to 40%.23-25

Table 2. Improvement of Overall Survival in H460/T800 and BMEC Models After IT Docetaxel Therapy

<table>
<thead>
<tr>
<th>Therapy Delivery</th>
<th>Mouse Model</th>
<th>Treatment</th>
<th>Mean (SD) Tumor Weight, mg</th>
<th>Mice With Tumor Present, No. (%)</th>
<th>Mice Surviving at End of Study, No. (%)</th>
</tr>
</thead>
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<tr>
<td>IW</td>
<td>BMEC</td>
<td>Docetaxel (n=8)</td>
<td>3441 (2060)</td>
<td>8 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>H460/T800</td>
<td>Docetaxel (n=8)</td>
<td>3438 (2617)</td>
<td>8 (100)</td>
<td>0</td>
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<tr>
<td>IT</td>
<td>BMEC</td>
<td>Docetaxel (n=8)</td>
<td>2241 (519)</td>
<td>8 (100)</td>
<td>0</td>
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<tr>
<td></td>
<td>H460/T800</td>
<td>Docetaxel (n=8)</td>
<td>403 (413)</td>
<td>4 (50)</td>
<td>6 (75)</td>
</tr>
</tbody>
</table>

Abbreviations: BMEC, BALB/c mucoepidermoid carcinoma; IT, intratumoral; IW, intrawound.

Figure 4. Intratumoral (IT) treatment in taxane-resistant models. After implantation of severe combined immunodeficiency mice with the H460/T800 (n=16) (A) and BALB/c mucoepidermoid carcinoma (BMEC) (n=16) (B) cell lines, half of the mice (n=8) in each group received a biweekly IT injection of docetaxel (15 mg/kg per injection) (docetaxel group), and the other half received an IT diluent injection (control group). Error bars indicate standard deviation.

Figure 5. Effect of docetaxel treatment on the expression level of endothelial growth factor receptor (EGFR), phosphorylated EGFR (pEGFR), and basic fibroblast growth factor (bFGF) in HN30 and HN12 cells as measured by Western blot analysis (A) and vascular endothelial growth factor (VEGF) secretion in HN30 (B) and HN12 (C) cells as measured by enzyme-linked immunosorbent assay.
resent a novel and effective strategy to reduce local recurrence. The use of high drug concentrations at the site of microscopic disease increased dose-related cell killing and reduced systemic toxic effects. The chemotherapy-induced cytotoxic effects in tumor cells are proportional to the drug concentration. An antitumor effect of supra-dosing of IT docetaxel has been shown. However, such a strategy has raised concern regarding the possible adverse effect of the chemotherapeutic agent on wound healing because many of the same growth factors and share many of the same molecular pathways. This class of chemotherapeutic agent acts by promoting tubulin assembly in microtubules, inhibiting depolymerization, poisoning mitotic spindles, and inducing G2M phase arrest and thus blocking mitosis in proliferating cells. These agents have also been shown to improve survival in breast, lung, ovarian, and many other cancers. In this study, we wished to assess the effect of IW docetaxel on antitumor activity and local wound healing in the surgical tumor microenvironment. In the HN6, HN12, and HN30 SCID mouse models, IW docetaxel therapy was associated with significant suppression of tumor growth (lower average tumor weight) and improved survival compared with control groups (100% vs 33%). We also wanted to determine the antitumor activity of a supratherapeutic dose of docetaxel in known taxane-resistant cancer cell lines. We have previously demonstrated in our pharmacokinetic studies that IT therapy can increase peak concentrations of docetaxel in tumors by 1000-fold over the conventional intravenous dose used clinically. In both the taxane-resistant H460/T800 and BMEC mouse models, IW docetaxel therapy failed to suppress tumor growth and improve survival. However, the use of biweekly IT docetaxel injection improved overall survival and slowed tumor progression in the H460/T800 models, possibly by partially overcoming taxane resistance. On the other hand, biweekly IT docetaxel injection failed to suppress tumor growth and improve survival in the BMEC models. The mechanism that allows a supratherapeutic dose of docetaxel to partially overcome taxane resistance in H460/T800 cells but not in BMEC cells is interesting and needs to be investigated further.

Finally, we showed that IW or IT docetaxel therapy did not interfere with normal wound healing in the BALB/c and SCID mouse models. All incisions healed well without any evidence of infection, skin breakdown, or alopecia. Our result agrees with those of other studies, which showed the use of perioperative chemotherapeutic injections (cisplatin and bleomycin sulfate) in surgical wounds after resection of cutaneous cancers improved local control without compromising wound healing in equine and feline models.

To further elucidate the biological effect of IW docetaxel therapy in the surgical tumor microenvironment, expression levels of growth factors involved in cancer growth and wound healing were measured. After treatment with docetaxel, there is decreased expression of EGFR and bFGF as well as secretion of VEGF in HN12 cells. In HN30 cells, docetaxel treatment was associated with a decrease in EGFR, pEGFR, and VEGF levels. On the other hand, the level and intensity of expression of growth factors such as KGF, VEGF, bFGF, PDGF-B, and EGF involved in wound healing were found to be the same between the docetaxel- and diluent-treated wounds. Thus, it appears that docetaxel inhibited the expression of growth factors and receptors in tumor cells but not the growth factors involved in wound healing in the surgical tumor microenvironment. Although docetaxel decreased the secretion of VEGF from tumor cells, it did not alter the level of VEGF in the wound tissue. Thus, although docetaxel has antiangiogenic properties, potential wound-healing problems or infections were not observed. Consistent with our findings, Myoung et al showed that paclitaxel has an inhibitory effect on the growth of transplanted human oral squamous cell carcinoma and reduced the immunohistochemical expression of VEGF. Finally, Xie et al also showed that intratumoral injection of paclitaxel against laryngeal SCC in nude mice was associated with decreased expression of bFGF and VEGF within the tumor on immunohistochemical studies.

In conclusion, our data showed that IW docetaxel can suppress tumor growth and improve survival without adversely affecting wound healing in these HNSCC surgical tumor microenvironment models. These preclinical results are promising and support further testing of IW docetaxel injections into the surgical microenvironment in HNSCC to improve local cancer control.

Submitted for Publication: August 13, 2007; final revision received September 14, 2007; accepted October 15, 2007.

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Author Contributions: All of the authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Yoo, Ensley, Kucuk, Tulunay, Lonardo, and Kim. Acquisition of data: Subramanian, Lonardo, Won, Stevens, and Lin. Analysis and interpretation of data: Yoo, Subramanian, Piechocki, Ensley, Kucuk, Tulunay, Lonardo, Kim, Won, Stevens, and Lin. Drafting of the manuscript: Yoo, Subramanian, Piechocki, Ensley, Kucuk, Tulunay, Lonardo, Kim, and Lin. Critical revision of the manuscript for important intellectual content: Yoo, Subramanian, Piechocki, Ensley, Kucuk, Tulunay, Lonardo, Kim, Won, Stevens, and Lin. Obtained funding: Yoo. Administrative, technical, and material support: Yoo, Subramanian, Piechocki, Ensley, Kucuk, Tulunay, Lonardo, Kim, Won, Stevens, and Lin. Study supervision: Yoo, Piechocki, Ensley, Kucuk, and Lin.

Financial Disclosure: None reported.

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2. Ragaz J. Comments on kinetics and biology of the residual cancer, and on relevant therapeutic strategies based on these phenomena. Prog Clin Biol Res. 1990;354B:117-139.


