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Research Article

Effect of Combination Therapy of Desmopressin and Docetaxel on Prostate Cancer Cell (DU145) Proliferation, Migration and Tumor Growth

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Abstract

Background: This study was designed to assess the efficacy of the combination of Desmopressin and Docetaxel for prostate cancer. Desmopressin has been demonstrated to inhibit tumor progression and metastasis in *in vitro* and *in vivo* models of breast cancer. Docetaxel, an anti-mitotic chemotherapeutic agent, is widely used for the treatment of castration resistant prostate cancer. However, it is associated with adverse effects and eventual drug resistance. This is the first report on the effect of combining Desmopressin and Docetaxel in DU145 prostate cancer cells, both *in vitro* and *in vivo*.

Methods: An established castrate resistant prostate cancer cell line DU145 was used. Cellular proliferation was determined using the MTS assay. The migratory inhibition potential of Desmopressin alone and in combination with Docetaxel was accessed using the wound healing assay. *In vivo* evaluation was performed on a prostate cancer xenograft model using athymic nude mouse. Treatment was administered bi-weekly and tumor volumes were measured throughout the treatment period. Following a six-week treatment period, tumors were excised and tumor volume measured.

Results: A combination therapy of 1 μ M Desmopressin with 100nM Docetaxel resulted in dramatic inhibition of proliferation of DU145 cells 72 hours post treatment compared to either agent alone ($p < 0.05$). Wound healing assay revealed inhibition of cellular migration as well ($p < 0.05$). The use of a xenograft mouse model followed by treatment with 5 mg/kg Docetaxel intraperitoneally with concomitant 2 μ g/ml/kg Desmopressin administered intravenously 30 minutes before administering chemotherapy and 24 hours after, resulted in a significant decrease in tumor volume ($P < 0.05$), while not impacting body weight.

Conclusions: Desmopressin significantly enhanced the anti-proliferative and anti-migratory potential of Docetaxel. Combination treatment had no additional effect on body weight or mortality. These studies could potentially demonstrate an enhancement of the efficacy of Docetaxel-based chemotherapy treatment for castrate resistant prostate cancer.

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Introduction

Desmopressin is a well-known synthetic analogue of the antidiuretic hormone vasopressin. It has recently been demonstrated to inhibit tumor progression and metastasis both *in vitro* and *in vivo* models in breast cancer [1]. Docetaxel, a well established anti-mitotic and chemotherapeutic agent, acts by reversibly binding microtubules. It is widely used as first line treatment for castration resistant prostate cancer [2,3], however, this treatment has significant adverse effects and drug resistance eventually develops. Research to improve Docetaxel antitumor effects is ongoing [4]. Previous studies published by our group demonstrated this combination to have an additive effect by inhibiting growth, migration and invasion when tested on PC3 and LNCaP prostate cancer cells [5].

In this study, we aimed to further investigate and validate the anti-tumor effect of Desmopressin in combination with Docetaxel using additional prostate cancer cell line, DU145, *in vitro* and *in vivo*.

Materials and Methods

Cell culture and chemicals

Castrate resistant prostate cancer cell line DU145 were obtained from the American Type culture collection (Rockville, MD, USA). Cell culture procedures were followed according to previously described procedures [5,6]. Docetaxel was purchased from Sigma-Aldrich ©, prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich, MO, USA) and diluted with cell culture medium at a final concentration of 0.01% for cell culture treatments. Desmopressin was purchased as Octostim (15 µg/ mL per ampoule).

Cell proliferation assay

Cell proliferation was determined using MTS assay as previously described [5,6]. DU145 cells were plated in 96-well plates at a density of 4000 cells per well. Cells were left to adhere for 24 hours and then dose standardization was performed using various

concentrations of Docetaxel (1 nM, 10nM, 100nM and 1µM) and Desmopressin (1nM, 10 nM, 100 nM and 1 µM). Measurements were obtained at 24, 48 and 72 hours post treatment. Based on the results, cell proliferation assays were compared for combination treatments of 10 nM and 100 nM Docetaxel and 1 µM Desmopressin. Results were analysed using two-sided student t-test with significance level of $p < 0.05$.

Wound healing assay

The motility inhibitory potential of Desmopressin alone and in combination with Docetaxel was assessed using a wound healing assay according to previously described protocols [5,7]. DU145 cells were plated on 24-well plate at a density of 50,000 cells per well and were allowed to grow until reaching 90-100% confluence. After incubating the cells with 1 mg/L Mitomycin C (Sigma®) for 1 hour, a vertical scratch was made with a 10 µl pipette tip, followed by two washes with PBS. Cell media or media with treatment agents were added, and images were obtained at zero-time point and 24 hours following treatment. A computer based microscopy imaging system with 200X magnification (Axiovision®) was used to access cell migration. Each experiment was carried out in duplicate wells and experiments were repeated three times. Results were analysed using two-sided student t-test with significance level of $p < 0.05$.

In vivo studies with xenograft model

All procedures were done in accordance with the Canadian Council of Animal Care (CCAC) regulations and local animal research ethics board procedures and approval. Six-week old male athymic nude mice (Charles river, QC, Canada) were used to evaluate the effect of combination therapy on DU145 tumor growth *in vivo*. Mice were housed and maintained in laminar flow cabinets under pathogen-free conditions. Following acclimatization, of housing, 1×10^6 DU145 cells per animal in 100 µL matrigel solution (BD Bioscience, CA, USA) were inoculated subcutaneously in the left lower flank, according to procedure described by Fridman et al. [8]. After 14 days, body weight and tumor size were measured, and mice were randomly assigned to four treatment groups. Groups included control (sham

Figure 1: Cell Proliferation and wound healing as depicted at 72 and 24 hours, respectively

A: MTS cell proliferation results

B: Wound Healing assay results

C: Wound healing assay representative pictures

* $P < 0.05$ for combination of 100nM Docetaxel and 1µM Desmopressin compared to 100nM Docetaxel

** $P < 0.05$ for Wound closure measurement when treating with 1µM Desmopressin compared to 1µM Desmopressin + 10nM Docetaxel

$P < 0.05$ for 10nM and 100nM Docetaxel or in combination with 1µM Desmopressin compared to control (0.01% DMSO solution in media)

treatments), 5 mg/kg Docetaxel intraperitoneally, 2 µg/ml/kg body weight Desmopressin intravenously 30 minutes prior to Docetaxel administration and 24 hours after, or combination therapy (5 animals per group). Each group received treatments bi-weekly starting 14 days post inoculation, for a total of 3 treatments. Animal weight and tumor measurements were assessed twice weekly. Tumor volume (V) was calculated by measurement of tumor length (L) and width (W) with a caliper according to the following formula: $V = (L \times W^2) (\pi/6)$. Two weeks following the third and treatment, mice were euthanized and tumors were excised and measured directly with a caliper. Tumors were sent for histopathological analysis. Tumor volumes during the treatment period were compared using repeated measures one-way ANOVA test (SPSS®). Final tumor sizes and body weight of animals were separately compared using one-way ANOVA test.

Results

Combining desmopressin with docetaxel inhibits cellular proliferation *in vitro*

The MTS cell proliferation assay was carried out and cell growth assessed at 24, 48 and 72-hour time points, and with different monotherapy concentrations (1 nM, 10 nM, 100 nM and 1 µM of Desmopressin or Docetaxel). After determining optimal concentrations, combination therapy of 10 nM and 100 nM of Docetaxel and 1 µM Desmopressin were selected. Combining 100 nM Docetaxel with 1 µM Desmopressin resulted in a significant inhibition of cell proliferation 72 hours post treatment ($p < 0.05$) (Figure 1A). Similar analysis was completed for earlier time points (24 and 48 hours), and with a concentration of 10 nM Docetaxel combined with 1 µM Desmopressin, revealing no statistical significant significance when compared to Docetaxel treatment alone (data not shown).

Desmopressin enhances the anti-migratory effect of Docetaxel

Cell migration studies were conducted to determine whether combining Desmopressin and Docetaxel could further enhance the anti migratory effect of Docetaxel.

Using DU145 cells, we determined if inhibition of wound closure occurred using a combination of 10 µM Docetaxel with 1 µM Desmopressin compared to either treatment alone. Results revealed that combination therapy reduces wound closure compared to Docetaxel treatment alone (24.8% vs 48.9%, $p < 0.05$, two-tailed student's *t*-test) (Figure 1B). Monotherapy using 10 nM Docetaxel or 1 µM Desmopressin had the potential to inhibit migration when compared to control (Figure 1B).

We also evaluated combination therapy using 1 µM Desmopressin and 100 nM Docetaxel. Significant cell death was observed at these concentrations; therefore, no reliable measurement could be drawn. Representative pictures as captured by the Axiovision® imaging system are presented in Figure 1C.

Combining Desmopressin with Docetaxel inhibits tumor growth in male athymic nude mice

A combination of 5 mg/kg Docetaxel Intraperitoneally and 2 µg/ml/kg body weight Desmopressin Intravenously 30 minutes before chemotherapy and 24 hours after resulted in a significant decrease in tumor volume, while not impacting weight of animals (Figure 2). Commencing from day 42 post tumor cell inoculation, significant difference in tumor volumes were noted. Difference was noted in the group that received a combination of Docetaxel with Desmopressin when compared to Docetaxel alone. While Docetaxel alone reduced tumor volume compared to control, Desmopressin alone did not. Final tumor measurements on day 55 post inoculation were recorded after tumors were excised. Average tumor volume in four groups were as follows: Control - $2049 \pm 520 \text{ mm}^3$, Desmopressin - $1597 \pm 681 \text{ mm}^3$, Docetaxel - $1330 \pm 550 \text{ mm}^3$, Combination treatment - $773 \pm 314 \text{ mm}^3$ ($P < 0.05$ for combination treatment VS Docetaxel therapy, and for combination treatment and Desmopressin VS Control). Representative photographs of animals and tumors post excision are depicted in Figure 3.

Discussion

In 2015, we published the observation that Desmopressin enhances the effect of Docetaxel on

Figure 2: Tumor volume measurements over time following treatment with Desmopressin and Docetaxel in a xenograft model of prostate cancer

Nude mice were subcutaneously inoculated with 1×10^6 DU145 cells per mouse. The tumor volumes were measured twice per week.

* $P < 0.05$ for combination therapy of 2 mg/kg Desmopressin intravenous + 5 mg/kg Docetaxel intraperitoneal compared to 5 mg/kg Docetaxel

prostate cancer cell proliferation and migration [5]. We have now demonstrated decrease proliferative and migratory potential of DU-145 cells both *in vitro* as well as in a xenograft model of prostate cancer. In previous studies, Desmopressin has been shown to increase cancer cell apoptosis directly in MCF-7 human breast carcinoma [9], and other prostate cancer cell lines [5]. The DU145 cell line is derived from brain metastasis, is not hormone-sensitive and does not express prostate-specific antigen [10]. We showed previously *in vivo* tumor volume reduction in response to Desmopressin and Docetaxel with PC3 cells [5]. PC3 cells are isolated from bone metastasis and are considered poorly differentiated cells with higher metastatic potential compared to DU145 [11]. Despite these differences, we noted similar responses to combination treatment.

In our present study using the xenograft model,

Desmopressin alone reduced tumor volume as well as when given concomitantly with Docetaxel. Desmopressin treatment was administered by two intravenous injections covering a 24-hour time period following chemotherapy treatment. We attribute the tumor volume reduction to apoptosis induction. It could also be related to reduced angiogenesis, as previously demonstrated with breast cancer cells [1]. Desmopressin has been known to stimulate endothelial cells to release Von Willebrand factor (VWF) [12]. Recent studies have suggested that VWF plays a more complex role as a regulator of angiogenesis, tumor metastasis and as an inducer of apoptosis in cancer cells [13]. Tumor size reduction *in vivo* is likely the result of several mechanisms. In our xenograft model, we demonstrated that adding Desmopressin to Docetaxel was only able to delay tumor growth (Figure 2) of this aggressive form of Castrate resistant prostate

Figure 3: Representative photograph of tumors from each group depicting reduction in tumour volume with the various treatments

cancer cell line. Combining Desmopressin during chemotherapy for Castrate resistant prostate cancer could increase the efficacy of Docetaxel.

The anti-tumor effect of this combination treatment was previously shown by our group to be associated with down-regulation of both urokinase-type plasminogen activator (uPA) and matrix metalloproteinase (MMP-2 and MMP-9) in prostate cancer cell lines PC3 and LNCaP [5]. Desmopressin stimulates tumor-mediated production of angiostatin, a strong angiogenesis inhibitor. The compound induces secretion of soluble uPA, favoring angiostatin generation by the proteolytic cleavage of plasminogen. At the same time, Desmopressin activates endothelial release of VWF by exocytosis. VWF plays a protective role against tumor cell dissemination and may cause apoptosis of micro metastatic foci. Data supporting this was collected from breast cancer *in vitro* and *in vivo* models, supporting anti angiogenic as well as cytostatic effects [1,14], and reduction of cancer cells in distant metastasis [14]. Endothelial V2 receptor activation could contribute to tumor reduction via angiogenesis inhibition and reduced VWF levels in tumor microenvironment. Garona and Alonso [15] suggested that vasopressin receptor V2 activation in endothelial and cancer cells enhances cancer cell apoptosis and migration, and

supports the rationale for combining Desmopressin and Docetaxel. Research attempting to reduce cancer cell proliferation and metastasis via induction of V2 receptor activation by various vasopressin analogues is ongoing, mainly in the field of breast cancer [9].

Garona and Alonso [15] suggested that Desmopressin triggers V2 receptor agonist signaling in both tumor and endothelial cells, causing adenylate cyclase activation followed by cAMP-dependent PKA activation. However, V2 receptors are not expressed in DU145 cells [16,17]. We attribute the anti proliferative and anti migratory effect of vasopressin observed in V2 negative prostate cancer cell lines *in vitro* to reduced pro- uPA and active uPA expression, thus attenuating uPA activity on the cell surface. uPA down-regulation by Desmopressin inhibits invasion and migration of PC3 and LNCaP cancer cells [5], thus Desmopressin is acting to inhibit uPA activity in prostate cancer cells not only by V2 receptor activation (Figure 4).

A limitation of this study is that metastatic spread was not evaluated directly in the xenograft model. This could be evaluated using a transgenic model. Giron et al [18] demonstrated that Desmopressin was capable of inhibiting lung and axillary lymph node metastasis following mechanical manipulation in a mouse

Figure 4: Putative mechanism of the desmopressin treatment

As indicated in previous published work, Desmopressin reduces uPA expression on tumor cells, inducing migration and invasion of prostate cancer cells via uPA-MMP pathway. Desmopressin may also increase secretion of tPA and intravascular fibrinolysis, helping dissolve the protective fibrin shield of circulating tumor cells [5].

mammary carcinoma model. Although not representing a spontaneous metastatic dissemination mechanism, these results could be attributed to impaired cell migration and reduced VWF production.

The authors have no conflict of interest to declare.

Conclusions

Combining Desmopressin and Docetaxel reduces proliferation and migration in castrate resistant prostate cancer cells *in vitro*, and tumor size in an *in vivo* model, compared to either treatment alone. This could be indicative of enhanced efficacy of Docetaxel based chemotherapy treatment for castrate resistant prostate cancer.

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Conflict of interests

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