Noscapine Inhibits Human Prostate Cancer Progression and Metastasis in a Mouse Model

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Abstract. *Background: Noscapine, a non-toxic alkaloid and common constituent of cough medicine, stabilises tubulin. It inhibits the growth of several human and murine neoplasms, with no significant toxicity. Its effect on prostate cancer has not been evaluated. Materials and Methods: Noscapine was administered orally (300 mg/kg per day) for 56 days to PC3 human prostate cancer-bearing immunodeficient mice (n=10). Immunodeficient control mice (n=10) received only diluent in an identical regimen. Results: Mean total tumour weight was 0.42±0.23 g and 0.97±0.31 g (p<0.001) in the noscapine-treated group and the control group, respectively, without evidence of toxicity. Metastases occurred less frequently in the treatment than the control group (30% vs. 90% ; p<0.05). Conclusion: Oral administration of noscapine limited tumour growth and lymphatic metastasis of PC3 human prostate cancer in this mouse model, supporting its therapeutic potential as a nontoxic and easily administered treatment for metastatic cancer.*

Despite significant advances in early detection and diagnosis, and a better understanding of the biology of the disease, metastatic prostate cancer remains largely untreatable. Noscapine, an opium-derived alkaloid originally isolated in 1817 (1), was initially used to prevent and treat malaria (2). Its application as an antimalarial was discontinued after 1930 and noscapine is now widely used as an antitussive in cough medicines (3, 4). The anticancer effect of noscapine was first observed in cell-culture studies performed in the 1950s (5, 6) and was rediscovered in 1997 by Ye *et al*. (7) during a search for microtubule-inhibiting compounds. It was subsequently demonstrated that noscapine has an antitubulin effect, which causes mitotic arrest in rapidly growing cells (7, 8), and that it induces apoptosis in many cell types (7). Moreover, noscapine was shown to have potent antitumour activity against a variety of solid murine lymphoid tumours (9) and against human breast (8) and bladder tumours (7) implanted in nude mice. Because noscapine is easily absorbed after oral administration and demonstrates low toxicity to normal tissues (10), its chemotherapeutic potential in human cancer generated interest in further evaluation. The anticancer effects of noscapine have been evaluated in a number of cancer models, including ovarian cancer (11), malignant melanoma (12), bladder cancer (7) and glioblastoma (13). These studies demonstrated that noscapine given orally in high doses produced significant benefits with minimal toxicity.

The potential of noscapine to limit the growth of prostate cancer has not been evaluated. As a first step in this endeavour, we determined whether oral noscapine would limit tumour formation, growth and metastasis of PC3 prostate cancer cells transplanted into immunodeficient (nude) mice.

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Key Words: Prostate cancer therapy, noscapine, tumour inhibition.

Materials and Methods

Cell culture. PC3 human prostate cells (ATCC, Rockville, MD, USA) were incubated in RPMI-1640 (Gibco-BRL, Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS; Gibco-BRL, Life Technologies, Inc.). Cells were incubated at 37°C in 5% CO_2/95% air. Before inoculation of the mice, the cells were passaged twice. Cell viability was determined by Trypan blue exclusion.

Animals. Male, athymic, Sim (NCr) nude mice, 5-6 weeks of age, were obtained from Nxgen Bio Sciences (Sorrento Valley, San Diego, CA, USA). The animals were housed in cages in a HEPA-filtered environment; food and bedding were sterilized by irradiation and autoclaving. They were maintained on chow obtained from Newco Distributions, Inc. (Rancho Cucamonga, CA, USA). Drinking water was autoclaved and chlorinated, however, no antibiotic was added. All protocols involving these mice were subject to guidelines established by the institutional Animal Care Committee in accordance with national regulations concerning the use of animals in scientific experimentation.

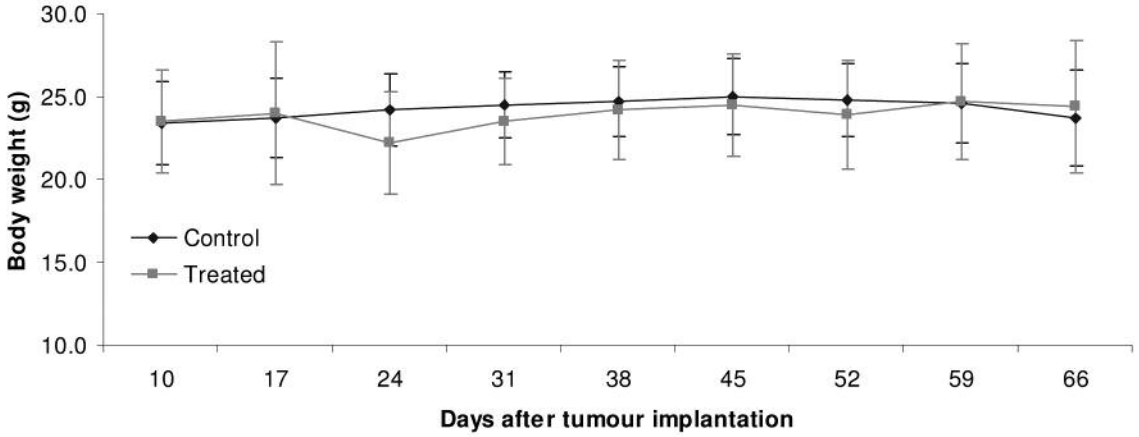

Figure 1. *Mean body weights of noscapine-treated mice in comparison to control mice during the course of treatment. Animals were weighed before treatment and twice weekly after the initiation of treatment [noscapine, 300 mg/kg, p.o. or acidified deionised water (control)] as described in Materials and Methods. Results shown are the mean±SD of 10 animals in each group over the course of treatment.*

Inoculation of tumour cells and evaluation of primary tumour growth and metastasis. Suspensions of 1×10^6 PC3 human prostate cancer cells in 0.2 ml of phosphate-buffered saline (PBS) were inoculated subcutaneously into the right posterior flank within 40 min of harvesting the cells from culture. The inoculated mice were randomly divided into two groups of 10 animals. Each mouse was marked by ear-cut for identification during the course of follow-up. Treatment commenced on day 10 after PC3 cell inoculation, when palpable tumours were present at the inoculation site. One group of 10 mice was treated daily with noscapine (300 mg/kg, obtained from Netzah Israel Pharmacy, Tel Aviv, Israel) diluted in acidified deionised water (pH 4.0), administered by gavage. Control mice received acidified deionised water only by gavage, on a daily basis. Treatments were continued for 56 days. Body weight was determined and tumours were measured before treatment and twice weekly after the initiation of treatment. Tumour volumes were determined by measuring two perpendicular diameters using callipers; the volume was then calculated using the standard formula $V=(L\times W^2)/2$.

All animals were checked on a daily basis for mortality or signs of morbidity during the treatment and observation period. Fifty-six days after initiation of treatment, the mice were euthanised and primary tumours, lymph nodes, and lungs were examined. Whole-body photographs of each animal were acquired. The primary tumours and lymphatic lesions were removed and then direct images of tumour and lymphatic lesion(s) of each animal were taken. All images were acquired with a Canon digital camera. The final weight from the primary tumour and metastases was acquired after each animal was sacrificed at the termination of the study. The tumour growth inhibition rate (IR) was calculated according to the following formula: $IR\ (\%)=(1-TW_t/TW_c)\times100$, in which TW_t and TW_c are the mean tumour weight of the treated group and that of the control group, respectively.

Histological assessment of primary tumours and confirmation of metastasis. Tissue samples of the primary tumour, metastatic lymph nodes and lungs were removed and processed with 10% formalin for haematoxylin and eosin (H&E) staining and subsequent microscopic examination.

Statistical analysis. Statistical analyses were performed with the aid of Microsoft Excel™ and two-tailed Student's t-tests. *P*-values <0.05 were considered indicators of statistical significance. Data are reported as mean±SD of values measured in 10 mice in each group.

Results

Body weight. None of the mice in either treatment group died during the course of the experiment. There were also no apparent changes in body weight (Figure 1) and no significant differences between the control and noscapine-treated groups in the endpoint mean body weight (23.7±2.9 g and 24.4±4.0 g, respectively $p>0.05$). The observed similarity in body weights and morbidity between the control and the noscapine-treated groups during the course of the experiment suggested that noscapine had no significant toxicity and appeared to be well tolerated at high doses.

Primary tumour growth. Tumour growth curves for noscapine-treated and control mice are shown in Figure 2. The endpoint mean values of primary tumour volumes were 1047.6± 288.2 mm^3 in the control group and 421.2±259.9 mm^3 in the noscapine-treated group, respectively ($p<0.01$). At sacrifice, the mean total tumour weight was 0.72±0.24 g in the control group and 0.37±0.22 g in the noscapine-treated group ($p<0.01$; Table I). The primary and overall tumour (including primary tumour and metastases) inhibition rates were calculated as follows:

Primary tumour inhibition rate by volume:

$$IR=(1-421.2/1047.6)\times100=60\%$$

Overall tumour inhibition rate (primary tumour + metastatic tumours) by weight:

$$IR=(1-0.42/0.97)\times100=57\%$$

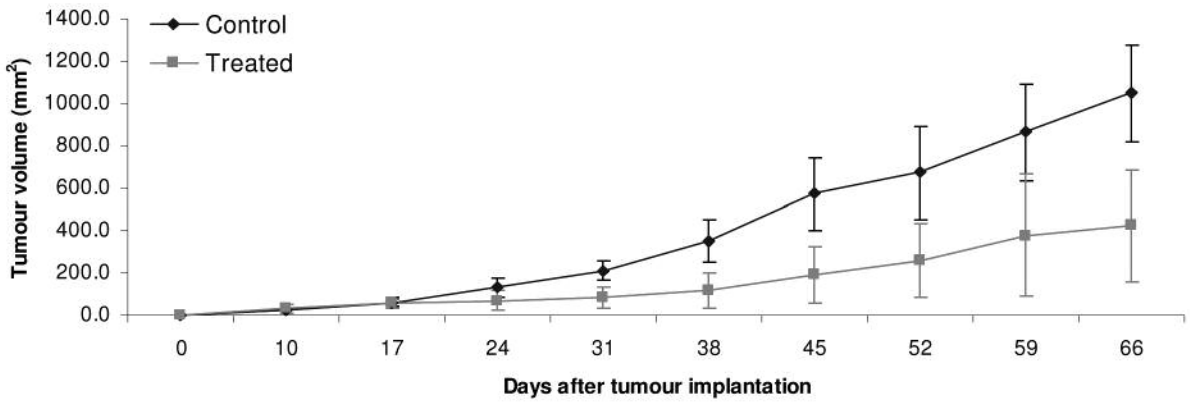

Figure 2. *Changes in tumour volume in noscapine-treated and control mice during the course of treatment. Tumour volumes were measured with callipers and calculated at the intervals shown in the figure and as described in Materials and Methods. Results shown are the mean± SD of 10 mice in each treatment group.*

Incidence of metastases. Lymphatic metastases were observed in 90% of the animals in the control group and 30% of the animals in the treated group ($p<0.05$). This finding suggests that daily oral noscapine (300 mg/kg) may reduce or forestall the metastasis of prostate cancer to lymph nodes. However, the incidence of metastases to the lung was not significantly lower in the treated group than in the control group (treated 20% *vs*. control 30%). Nevertheless, total metastatic tumour weights were significantly lower in noscapine-treated mice (0.05±0.11 g) compared to control mice (0.25±0.25 g) ($p<0.05$) as shown in Table I.

Table I. *Average end-point tumour weights in noscapine-treated and control mice.*

Parameter	Tumour weight, g (Mean±SD)		*p*-Value
	Noscapine-treated (n=10)	Control (n=10)	
Total tumour	0.42±0.23	0.97±0.31	<0.001
Total primary tumour	0.37±0.22	0.72±0.24	<0.01
Total metastatic tumour	0.05±0.11	0.25±0.25	<0.05

Discussion

Earlier studies demonstrated that noscapine, a common antitussive in over-the-counter cough medicines, also has properties that limit the growth of certain types of cancer both *in vitro* and *in vivo*. This inhibition of growth has been ascribed to the antitubulin activity of noscapine, as well as its ability to induce apoptosis in transformed cells (7, 11, 12). As such, noscapine functions similarly to taxanes, which are currently used to treat prostate cancer refractory to hormonal therapy. However, in contrast to taxanes, which may induce leucopenia, diarrhoea, alopecia and peripheral neuropathy, noscapine demonstrates little or no toxicity to normal tissues. We demonstrated in this study, for the first time, that oral administration of noscapine at a daily dose of 300 mg/kg was effective in reducing primary tumour growth and lymphatic metastasis of PC3 human prostate cancer cells transplanted into immunodeficient nude mice, without visible signs of toxicity to other tissues or induction of additional morbidity. This suggests that orally administered noscapine may be a potential therapeutic agent for safe and efficacious treatment of prostate cancer in humans and a desirable alternative to currently used therapeutic agents that have serious side-effects.

Acknowledgements

The authors acknowledge with gratitude the generosity of Mr. Edmund Gann, which enabled us to conduct this study.

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Received April 8, 2008
Revised June 24, 2008
Accepted July 1, 2008