Noscapine Alters Microtubule Dynamics in Living Cells and Inhibits the Progression of Melanoma

Jaren W. Landen, Roland Lang, Steve J. McMahon, Nasser M. Rusan, Anne-Marie Yvon, Ashley W. Adams, Mia D. Soricelli, Ross Campbell, Paola Bonaccorsi, John C. Ansel, David R. Archer, Patricia Wadsworth, Cheryl A. Armstrong, and Harish C. Joshi


ABSTRACT

Cellular microtubules, polymers of tubulin, alternate relentlessly between phases of growth and shortening. We now show that noscapine, a tubulin-binding agent, increases the time that cellular microtubules spend idle in a paused state. As a result, most mammalian cell types observed arrest in mitosis in the presence of noscapine. We demonstrate that noscapine-treated murine melanoma B16.S9 cells do not arrest in mitosis but rather become polyploid followed by cell death, whereas primary melanocytes reversibly arrest in mitosis and resume a normal cell cycle after noscapine removal. Furthermore, in a syngeneic murine model of established s.c. melanoma, noscapine treatment resulted in an 85% inhibition of tumor volume on day 17 when delivered by gavage compared with untreated animals (P < 0.01), without evidence of toxicity to the spleen, liver, duodenum, bone marrow, or peripheral blood. This inhibition was greater than that seen in vivo by paclitaxel (Taxol) alone and similar to the inhibition of tumor volume observed when noscapine was combined with paclitaxel. Importantly, noscapine also demonstrated the ability to significantly inhibit melanoma progression by 83% on day 18 when delivered in drinking water (P < 0.01) and conferred a significant survival advantage (P < 0.01). Our results demonstrate that p.o.-administered noscapine significantly inhibits the progression of melanoma cells through alterations in microtubule dynamics, with no detected toxicity to the host. Consequently, noscapine could be a valuable chemotherapeutic agent, alone or in combination, for the treatment of advanced melanoma.

INTRODUCTION

The dynamic nature of microtubules was first predicted by observations of the effects of antimitotic agents on the mitotic spindle in living cells (1, 2). Examples of these natural compounds include colchicine, Vinca alkaloids, and paclitaxel. When incubated with living cells, these agents either eliminate cellular microtubules or cause excessive polymerization and bundling of microtubules within a relatively short period of time (2). The exquisite sensitivity of the mitotic checkpoint mechanisms to extremely minor fluctuations of microtubule dynamics was recently revealed by the demonstration that these microtubule agents can block the onset of anaphase at very low substoichiometric concentrations that were insufficient to cause cellular inactivity in living cells. This provides an explanation for why mammalian cells arrest in mitosis when treated with noscapine. Furthermore, we show that murine melanoma B16.S9 cells do not arrest in mitosis but become polyploid and then subsequently die. In contrast, primary melanocytes arrest reversibly in mitosis when treated with noscapine for limited exposure times. In addition, when administered p.o., noscapine effectively inhibits melanoma progression and significantly increases animal survival in a syngeneic murine model of melanoma. We do not observe any significant toxic effects in tissues with active cell proliferation such as bone marrow, spleen, and intestine.

MATERIALS AND METHODS

Cell Lines. Murine B16.S9 melanoma cells were grown as described previously (8). Primary melanocytes were isolated from the Emory Skin Diseases Research Center and maintained in melanocyte basal medium supplemented with melanocyte growth medium with Single Quots (Clonetics, San Diego, CA). All cells were grown at 37°C in a 5% CO2/95% air atmosphere. Cell viability was determined by trypan blue exclusion analysis.

Analysis of Microtubule Dynamics. Analysis of microtubule behavior was performed in mammalian rat kangaroo endothelial Ptk2 cells stably transfected with green fluorescent protein-tagged α-tubulin (9). These cells are ideal for microtubule dynamics studies because they have sufficiently flat, peripheral cell margins that enabled us to visualize individual microtubules. In addition, Ptk2 cell fluorescence was not quenched due to the absence of pigmented granules. Cells were treated with 25, 50, or 250 μM noscapine (Aldrich Chemical Co., Milwaukee, WI) in vehicle solution (DMSO) for 60 min before imaging. Control cells received vehicle solution alone. Image sequences of the peripheral regions were acquired at 2-s intervals with a Nikon Eclipse 300 microscope using a ×100, 1.3 numerical aperture objective lens and a micromax interline transfer cooled charge-coupled device camera (Roper Scientific) as described previously (9). To quantify the behavior of individual microtubules, the positions of the microtubule ends were followed using the “track points” function of Metamorph software (Universal Imaging Corp., Downingtown, PA). For each microtubule, a life history plot was generated, and the phases of growth, shortening, and pause were selected; only changes >0.5 μm were considered growth or shortening events. The duration, distance, and rate of growth and shortening events were determined using Microsoft Excel software. The frequency of catastrophe was determined by dividing the sum of

Received 11/5/01; accepted 5/16/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by grants from the NIH, American Cancer Society, and the University Research Committee (to H. C. J.); a grant from the American Skin Association (to C. A. A.); a grant from the Beat Leukemia Jill Andrews Fund (to D. R. A.); and a grant from the American Skin Association University Research Committee (to H. C. J.); a grant from the American Skin Association.

2 J. W. L. and R. L. contributed equally to this work.

3 To whom requests for reprints should be addressed, at Department of Cell Biology, Emory University School of Medicine, 1648 Pierce Drive, Room 045, Atlanta, GA 30322. Phone: (404) 727-6445; Fax: (404) 727-6256; E-mail: joshi@cellbio.emory.edu.

administered to patients with advanced melanoma (6). These observations prompted us to screen for additional natural tubulin-binding compounds. We based our screen upon structural similarities among some microtubule depolymerizing agents that can engage mitotic checkpoint without causing gross deformations of cellular microtubules even at high concentrations. From our initial screen, we identified the opium alkaloid noscapine as a tubulin ligand that alters the autofluorescence and circular dichroism spectrum of tubulin, arrests a variety of cell types in mitosis, and inhibits the growth of several human and murine neoplasms including lymphoma, thymoma, and breast cancer (7). We now provide evidence showing that noscapine alters microtubule dynamics apparently without altering cellular microtubule arrays, thus providing an explanation for the mitotic arrest observed (7). We demonstrate that noscapine treatment substantially increases the amount of time cellular microtubules spend idle, pausing in inactivity in living cells. This provides an explanation for why mammalian cells arrest in mitosis when treated with noscapine. Furthermore, we show that murine melanoma B16.S9 cells do not arrest in mitosis but become polyploid and then subsequently die. In contrast, primary melanocytes arrest reversibly in mitosis when treated with noscapine for limited exposure times. In addition, when administered p.o., noscapine effectively inhibits melanoma progression and significantly increases animal survival in a syngeneic murine model of melanoma. We do not observe any significant toxic effects in tissues with active cell proliferation such as bone marrow, spleen, and intestine.
the number of transitions from growth to shortening and from pause to shortening by the sum of the duration of growth and shortening; thus, the position of their plus ends changed significantly over 30 s (fixed pixel locations are marked with arrowheads). In contrast, noscapine markedly suppressed microtubule dynamics in cells, as indicated by the unaltered position of their plus ends (bar = 0.5 μm). Quantitative parameters of microtubule dynamics are listed in B. Values are the mean ± SE. Dynamicity represents the sum of changes in length (growth or shrinkage) over the life time of a microtubule. *, P ≤ 0.01.

**Fig. 1.** Noscapine increases the average time cellular microtubules remain inactive (pause duration). A, a gallery of video frames, 10 s apart, showing the plus ends of several microtubules in a control cell and a noscapine-treated cell. As expected in control cells, microtubules alternated between phases of growth and shortening; thus, the position of their plus ends changed significantly over 30 s (fixed pixel locations are marked with arrowheads). In contrast, noscapine markedly suppressed microtubule dynamics in cells, as indicated by the unaltered position of their plus ends (bar = 0.5 μm). Quantitative parameters of microtubule dynamics are listed in B. Values are the mean ± SE. Dynamicity represents the sum of changes in length (growth or shrinkage) over the life time of a microtubule. *, P ≤ 0.01.

<table>
<thead>
<tr>
<th>Dynamic Parameters</th>
<th>Control</th>
<th>25μM</th>
<th>250μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>37 M Ts</td>
<td>45 M Ts</td>
<td>44 M Ts</td>
</tr>
<tr>
<td>Rate (min/μm)</td>
<td>9.89 ±0.78</td>
<td>9.29 ± 1.05</td>
<td>6.34 ± 0.72</td>
</tr>
<tr>
<td>Distance (μm)</td>
<td>1.16 ±0.09</td>
<td>0.97 ±0.10</td>
<td>0.75 ±0.03*</td>
</tr>
<tr>
<td>Duration (sec)</td>
<td>9.62 ±0.95</td>
<td>8.30 ±1.44</td>
<td>8.94 ±0.74</td>
</tr>
<tr>
<td>Shrink</td>
<td>n=78</td>
<td>n=79</td>
<td>n=19</td>
</tr>
<tr>
<td>Rate (min/μm)</td>
<td>11.39 ±0.81</td>
<td>9.06 ±0.76*</td>
<td>8.89 ±1.19*</td>
</tr>
<tr>
<td>Distance (μm)</td>
<td>1.60 ±0.30</td>
<td>1.04 ±0.09</td>
<td>1.26 ±0.36</td>
</tr>
<tr>
<td>Duration (sec)</td>
<td>8.75 ±1.26</td>
<td>8.06 ±0.58</td>
<td>9.62 ±2.13</td>
</tr>
<tr>
<td>Average Pause Duration (sec)</td>
<td>22.54 ±2.18</td>
<td>34.66 ±3.27*</td>
<td>77.60 ±5.68*</td>
</tr>
<tr>
<td>% Time per Phase</td>
<td>22.71/8:60:5</td>
<td>12.3/10:87:6.9</td>
<td>2.03/394:7</td>
</tr>
<tr>
<td>Rescure Frequency (sec⁻¹)</td>
<td>0.147 ±0.02</td>
<td>0.128 ±0.01</td>
<td>0.138 ±0.03</td>
</tr>
<tr>
<td>Catastrophe Frequency (sec⁻¹)</td>
<td>0.029 ±0.004</td>
<td>0.028 ±0.004</td>
<td>0.007±0.001*</td>
</tr>
<tr>
<td>Dynamicity (m/min)</td>
<td>102.70 ±12.94</td>
<td>57.56±7.22*</td>
<td>10.40 ±2.83*</td>
</tr>
</tbody>
</table>

4 The abbreviations used are: PI, propidium iodide; CBC, complete blood count; Cdk, cyclin-dependent kinase.

**Cell Viability Assay.** Cells (1000 cells/well) were plated in 96-well microtiter plates in 0.2 ml of culture medium. After 12 h, cells were incubated with 0, 0.1, 1, 2, 50, 100, or 1000 μM noscapine for 0, 12, 24, 48, 72, or 96 h. Staurosporine (100 nM) was used as a positive control. In an independent study, cells were treated with noscapine at the dosages and durations specified above and washed three times with PBS at 37°C, fresh media replaced, and cells were allowed to recover for 96 h. Cells from both experiments were then washed twice in ice-cold PBS, fixed overnight in 70% ethanol at −20°C, and centrifuged at 1000 x g for 10 min. Cells were resuspended in 30 μl of phosphate/citrate buffer [0.2 M Na2HPO4/0.1 M citric acid (pH 7.5)] and incubated with PI (20 μg/ml) and RNase A (20 μg/ml) in PBS for 30 min. The PI fluorescence was measured using a Becton Dickinson flow cytometer. Data were analyzed using Winlist software (Verity Software House, Topsham, ME).

**Visualization of Microtubules and Chromosomes.** B16LS9 melanoma cells and primary melanocytes were cultured on poly-γ-ornithine-coated glass coverslips and incubated at 37°C with 25, 50, 250, 500, or 1000 μM noscapine for 0, 12, 24, 48, 72, or 96 h. Staurosporine (100 nM) was used as a positive control for cell death. Five h before the end of the specified incubation period, the colorimetric reagent WST-1 (Roche Diagnostics Corp., Indianapolis, IN) was added, and cell viability was determined at the end of the incubation period.

**Flow Cytometric Analysis.** Cellular DNA content was determined by staining with PI followed by flow cytometry. Thirty thousand cells were plated in 10-cm dishes and incubated for 24 h before the addition of 50, 250, 500, or 1000 μM noscapine in DMSO or an equivalent volume of DMSO alone for 0, 6, 12, 24, 48, 72, or 96 h. Staurosporine (100 nM) was used as a positive control. In an independent study, cells were treated with noscapine at the dosages and durations specified above and washed three times with PBS at 37°C, fresh media replaced, and cells were allowed to recover for 96 h. Cells from both experiments were then washed twice in ice-cold PBS, fixed overnight in 70% ethanol at −20°C, and centrifuged at 1000 x g for 10 min. Cells were resuspended in 30 μl of phosphate/citrate buffer [0.2 M Na2HPO4/0.1 M citric acid (pH 7.5)] and incubated with PI (20 μg/ml) and RNase A (20 μg/ml) in PBS for 30 min. The PI fluorescence was measured using a Becton Dickinson flow cytometer. Data were analyzed using Winlist software (Verity Software House, Topsham, ME).

**In Vivo Analysis of Melanoma Progression.** Pathogen-free 8–10-week-old female C57BL/6 mice were obtained from Jackson Animal Laboratories (Bar Harbor, ME) and housed in the Emory University Animal Care Facility. Suspensions of 1 x 10^6 syngeneic B16LS9 murine melanoma cells in 0.2 ml of PBS were inoculated s.c. into the anterior flank. Treatment was initiated at 4–6 days, when s.c. tumors were palpable. To determine the ability of p.o.-delivered noscapine to affect melanoma progression, studies were conducted in which mice (n = 15 mice/group) were treated with 300 mg/kg noscapine in acidified deionized water (pH 4.0) administered by daily gavage. Untreated mice received daily gavage of acidified water only. To determine the effectiveness of noscapine in drinking water, studies were conducted in which animals (n = 9 animals/group) were treated with 3.0 mg/ml noscapine in drinking water (pH 4.0), and control animals were treated with acidified...
drinking water alone (pH 4.0). To compare noscapine with the established antimicrotubule agent, paclitaxel, animals were treated when tumors became palpable with 300 mg/kg noscapine in deionized water (pH 4.0) administered by gavage daily (n = 11); 25 mg/kg paclitaxel (Bristol-Myers Squibb, Syracuse, NY) was delivered as a 0.2-ml i.p. injection on days 4, 6, 10, 12, 14, and 16 (n = 10; Ref. 10), or combination therapy with 300 mg/kg noscapine daily by gavage plus 25 mg/kg paclitaxel i.p. on the dose schedule indicated above (n = 10) was administered. Because paclitaxel cannot be given by the oral route, control animals in these studies received either 0.2 ml of acidified water by gavage daily (n = 11) or 0.2 ml of PBS i.p. on alternate days (n = 7). Paclitaxel was supplied as a concentrated sterile solution (6 mg/ml in a 5-ml ampule) in 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol. Paclitaxel was further diluted in PBS before administration.

Tumor volumes for all studies were determined on alternate days by measuring tumors in three perpendicular diameters using calipers, and the volume was calculated as 1/2 (length \times width \times height) (Ref. 11). In all animal tumor studies, mice were euthanized when tumors ulcerated, or when animals exhibited other criteria of morbidity as defined by our Institutional Animal Care and Use Committee guidelines. The rapid growth of s.c. injected B16LS9 melanoma cells required that untreated animals be euthanized at day 17–18. Accordingly, this end point was used to compare tumor size in untreated mice with those receiving p.o.-administered noscapine. For survival studies, it is thoroughly evaluate how noscapine affects tumor progression, we defined survival as the date on which the animals were euthanized.

**Histopathological Analyses.** On day 17, melanoma-bearing mice treated with 300 mg/kg noscapine by oral gavage daily and untreated melanoma-bearing mice were given an overdose (0.2 ml) of 3.5% chloral hydrate, blood was taken from the heart, and CBC analysis was performed using a CBC instrument (CDC Technologies, Oxford, CT). Next, animals were perfused with 3% paraformaldehyde and 2% glutaraldehyde mixture in PBS (pH 7.4), and brain, duodenum, sciatic nerve, liver, lungs, thymus, and tumor were removed and processed for histopathological analysis. Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Bone marrow was removed before fixation from the femur and tibia bones and analyzed by flow cytometry with antibody lineage markers CD3 (T cells), B220 (B cells), MAC-1 (macrophages), and Gr-1 (granulocytes; PharMingen, San Diego, CA). Cells were also incubated with 20 \mu g/ml PI to determine the percentage of cell death.

**RESULTS AND DISCUSSION**

We have previously shown that noscapine binds to the microtubule subunit, tubulin, and arrests mammalian cells in mitosis despite the presence of intact cellular microtubule arrays (7). To determine which dynamic parameters of cellular microtubules are affected by noscapine, we followed the life history of the plus ends of noscapine-treated cellular microtubules by fluorescence microscopy using stably transfected mammalian cells (PtK2) with green fluorescent protein-tagged tubulin. These cells are specifically suited for visualizing individual microtubules due to their flat, peripheral cell margins. Also, PtK2 cells lack pigmented granules, thus preventing the quenching of tubulin fluorescence. Fig. 1A shows a gallery of video frames, 10 s apart, of the plus ends of several microtubules in control and noscapine-treated cells. As expected in control cells, microtubules alternated between phases of growth, shortening, and pause, as determined by noting the position of the plus ends over time (in seconds). Control cells show different positions of microtubule plus ends in a 30-s time period. In contrast, noscapine markedly suppressed microtubule dynamics in cells as indicated by unaltered position of their plus ends (Fig. 1A, arrowheads).

Results from a detailed analysis of microtubule growth, microtubule shortening, the frequencies of catastrophe and rescue, and the average duration of pause are shown in Fig. 1B. The most significant
change that noscapine induced was an increased pause duration from 54% (at 25 μM noscapine) to 244% (at 250 μM noscapine) and the rate of microtubule shortening decreased by 20% (at 25 μM noscapine) and 22% (at 250 μM noscapine). Perhaps the best measure of overall dynamics is dynamicity, which represents the summed gain and loss (exchange) of tubulin subunits at microtubule ends (12). Noscapine significantly reduced microtubule dynamicity by 44% (at 25 μM noscapine) and by 90% (at 250 μM noscapine). We conclude that noscapine highly decreases the number of dynamic events in the life history of a microtubule without affecting its long-term existence. Whereas other possibilities of a microtubule-independent effect on mitotic checkpoint cannot be ruled out, our data are consistent with the simplest hypothesis that minor perturbations in microtubule dynamics can engage a mitotic checkpoint, arresting cells in mitosis.

Mutations that inactivate mitotic checkpoints have recently been defined in several types of human cancers (13–15). In fact, a mitotic stress checkpoint gene Chfr (checkpoint with FHA and ring finger domain) was found to become inactivated in cells due to a lack of expression or by mutation in half of the human cancers examined (16). Therefore, the loss of mitotic checkpoints in tumor cells might, in fact, be a very common occurrence associated with cancer. Cells that lack mitotic checkpoints are highly sensitive to mitotic stress caused by microtubule-targeting agents, leading to the appreciation that checkpoint loss might contribute positively toward chemotherapeutic outcome of some cancers (13–15, 17–19). Typically, drugs that interfere with microtubule dynamics arrest cells in mitosis for variable durations ranging from a few hours up to 10 h (20). After mitotic arrest, normal cells enter a G1-like state with 4 N DNA content and do not enter further rounds of S phase (18). This is a result of the tight regulation of the G1–S transition controlled by proteins such as Cdk4/Cdk6 and of the tumor suppressor proteins p16INK4a and p53 strongly implicated in the regulation of Cdns, thus affecting the phosphorylation of the retinoblastoma family of tumor suppressor proteins and entry into the subsequent S-phase (18, 21–22). Furthermore, in addition to its involvement in the G1–S checkpoint, the presence of the intact tumor suppressor gene, p53, is also required for preventing the next round of DNA replication (23). Due to mutations in gene encoding, many of these proteins have been found to confer susceptibility to a wide spectrum of melanoma in mice (21, 22) including germ-line mutations in CDKN2A found in more than one-fourth of melanomas (24). In fact the parental cell line B16 from which the murine melanoma cell line B16LS9 was derived has been associated with altered expression of p53 and retinoblastoma (25).

The emerging fact that many types of cancer cells lack intact mitotic checkpoint mechanisms combined with the unique properties of noscapine, including the substantial oral bioavailability, reported low toxicity, a short half-life in humans and animals, and a minimal point loss might contribute positively toward chemotherapeutic outcome of some cancers (13–15, 17–19). Typically, drugs that interfere with microtubule dynamics arrest cells in mitosis for variable durations ranging from a few hours up to 10 h (20). After mitotic arrest, normal cells enter a G1-like state with 4 N DNA content and do not enter further rounds of S phase (18). This is a result of the tight regulation of the G1–S transition controlled by proteins such as Cdk4/Cdk6 and of the tumor suppressor proteins p16INK4a and p53 strongly implicated in the regulation of Cdns, thus affecting the phosphorylation of the retinoblastoma family of tumor suppressor proteins and entry into the subsequent S-phase (18, 21–22). Furthermore, in addition to its involvement in the G1–S checkpoint, the presence of the intact tumor suppressor gene, p53, is also required for preventing the next round of DNA replication (23). Due to mutations in gene encoding, many of these proteins have been found to confer susceptibility to a wide spectrum of melanoma in mice (21, 22) including germ-line mutations in CDKN2A found in more than one-fourth of melanomas (24). In fact the parental cell line B16 from which the murine melanoma cell line B16LS9 was derived has been associated with altered expression of p53 and retinoblastoma (25).

Fig. 3. Noscapine treatment inhibits tumor progression in vivo. Palpable tumors were established 4–6 days after injecting 10^6 B16LS9 melanoma cells s.c. in mice. A, mice were treated beginning on day 6 with 300 mg/kg noscapine in acidified water by gavage (□), whereas the control group received acidified water alone by gavage (□). Tumor volume measurements showed a significant reduction as early as day 14 (92% tumor reduction compared with untreated animals. On day 17, the reduction in tumor volume was 85%, B, in an independent experiment, animals received 300 mg/kg noscapine alone by gavage daily, 25 mg/kg i.p. paclitaxel on alternate days, or a combination of noscapine + paclitaxel administered by gavage and i.p. injection, respectively. All groups receiving noscapine alone or in combination with an established antimitotubule agent had a significant reduction in tumor volume at day 17. C, mice were treated beginning on day 4 with noscapine delivered in drinking water instead of gavage (□), whereas the control group received acidified drinking water alone (□). D, noscapine treatment delivered in the drinking water conferred a significant survival advantage. Tumor volume shown is ± SE. *, P ≤ 0.01; #, P ≤ 0.05 (Mann-Whitney test, A–C; log-rank test, D).

B16LS9 melanoma cells showed large, abnormal, multilobed nuclei and dramatically increased cell size (Fig. 2F). Mitotic figures, when observed, had an abnormal appearance with improperly aligned chromosomes in B16LS9 melanoma cells (Fig. 2F, inset). As expected, primary cultures of melanocytes showed an increase in the number of cells with 4 N DNA content when treated continuously with noscapine for 48 h, but did not show >4 N DNA content (Fig. 2F). We did not detect
abnormal morphological changes in the nuclei of primary melanocytes (Fig. 2L, inset), in agreement with previously reported in vivo results in other nontransformed cells (28). At noscapine doses ranging from 50–250 μM for 48 h, we found B16LS9 melanoma cells with abnormal nuclei and improperly aligned mitotic figures; however, at doses <250 μM, these events were attenuated. Consistent with the effect of noscapine on microtubule dynamics, we did observe minor changes in the microtubule arrays of B16LS9 melanoma cells and primary melanocytes (Fig. 2, F and L). We also observed that normal cell division is resumed in primary melanocytes but not in B16LS9 melanoma cells after continuous incubation with noscapine for 6 h or less, removal of noscapine from the culture medium, and 96 h of recovery (Fig. 2, E and K). This suggests that the effect of noscapine on microtubule dynamics is reversible.

To examine whether noscapine can inhibit melanoma progression in vivo, we injected syngeneic mice s.c. with 1 × 10^6 murine B16LS9 melanoma cells. Six days later, when tumors were palpable, mice received 300 mg/kg body weight noscapine as an aqueous solution (30 mg/ml) by gavage daily. Fig. 3A shows that 11 days of noscapine administration via gavage after 6 days of tumor establishment inhibited the progression of tumor volume by 85% compared with animals receiving only acidified water by gavage (2687 ± 752 and 409 ± 159 mm³, average tumor volume ± SE for untreated and noscapine-treated groups, respectively).

Next, we compared the melanoma-inhibitory activity of noscapine with that of the antimicrotubule agent, paclitaxel. As shown in Fig. 3B, there was a clear antitumor advantage on day 17 in the groups receiving noscapine alone or the noscapine + paclitaxel combination group compared with untreated animals. Animals receiving paclitaxel, including animals receiving combination therapy, developed observable limb motor deficits suggestive of peripheral neuropathy beginning on day 12. No observable side effects were apparent in mice treated with noscapine alone. By day 17, all animals in the control groups had to be euthanized due to tumor ulcerations or extreme morbidity. Tumor volume for the
NOSCAPINE FOR TREATMENT OF MURINE MELANOMA

was 4759 required euthanasia by day 22, at which time the average tumor volume in each animal until euthanasia was determined to evaluate the effect of noscapine in drinking water over an extended time period, tumor volume was measured in each animal until euthanasia.

To further determine the effect of noscapine in drinking water over an extended time period, tumor volume was measured in each animal until euthanasia was required (see criteria in “Materials and Methods”). All untreated mice required euthanasia by day 22, at which time the average tumor volume was 4759 ± 389 mm³ in the remaining mice (Fig. 3, C and D). Noscapine-treated animals displayed a significantly lower average tumor volume on day 22 (1188 ± 433 mm³), and Kaplan-Meier analysis showed a significantly increased median survival time from 18 days to 28 days (P ≤ 0.01, log-rank test) with noscapine-treated animals surviving until day 38 (Fig. 3D). The continued growth of s.c. melanomas in noscapine-treated mice from day 22 until day 38 indicates that this agent inhibits tumor progression but does not arrest it.

To determine whether noscapine treatment results in toxicity to tissues with frequently proliferating cells, we analyzed the peripheral blood, bone marrow, spleen, duodenum, liver, and thymus. Surprisingly, we did not detect any differences in the cellular architecture of treated and untreated animals in the histopathological examination of duodenum, spleen, thymus, or liver (data not shown) as revealed by H&E staining (Fig. 4A). A CBC analysis revealed no significant differences in the number of RBCs, basophils, eosinophils, monocytes, lymphocytes, neutrophils, and total WBCs. In addition, no differences were detected in mean platelet volumes, mean corpuscular volumes, and hemoglobin concentrations (Fig. 4B). Furthermore, flow cytometric analysis of bone marrow and spleen tissue, after staining with antibodies specific for cell lineage markers CD3 for T cells, B220 for B cells, MAC-1 for macrophages, and Gr-1 for granulocytes, revealed no significant differences in these cell populations of noscapine-treated or untreated mice (Fig. 4C).

Therefore, no toxic effect from noscapine treatment was detected.

Why noscapine does not affect normally dividing tissues in mice is unclear. The most likely explanation seems to be that noscapine can activate the mitotic checkpoint. This would cause mitotic arrest in healthy cells, whereas B16LS9 melanoma cells would not arrest and would accumulate a toxic polyplid amount of DNA, resulting in cell death. Because noscapine only affects microtubule dynamics, cellular functions that do not require exquisite control of microtubule dynamics may not be interrupted. Furthermore, noscapine levels rise only transiently in plasma.

Pharmacokinetic studies in mice and humans reveal peak concentration at 3 h after oral ingestion and a relatively fast clearance thereafter (reported half-life ranges from 1.7–4.5 h; Ref. 28). Therefore, it is conceivable that normal cells resume cell division after the noscapine concentration decreases below the threshold level in a few hours.

Whatever the precise mechanism of the tumor-specific toxicity of noscapine is, it is clearly effective in selectively inhibiting tumor growth. It will be necessary to continue to examine the effect of noscapine on a variety of animal and human neoplasms. Additionally, because immune cells do not appear to be depleted by noscapine treatment, and because noscapine treatment is well tolerated, noscapine may also offer the possibility of combining chemotherapy with immunotherapeutic regimens for the management of melanoma.

ACKNOWLEDGMENTS

We thank the members of the Joshi laboratory, Joyce Yao and Jun Zhou, for carefully reading the manuscript and helping with the figures. We also thank the Emory Skin Diseases Research Center for isolation of primary melanocytes and Dr. Dirk Dillahay, a veterinary pathologist, for blindly evaluating animal tissue sections.

REFERENCES


6. Noscapine on a variety of animal and human neoplasms. Additionally, the use of noscapine as an adjunct to anti-mitotic agents in the treatment of cancer is under investigation.


Noscapine Alters Microtubule Dynamics in Living Cells and Inhibits the Progression of Melanoma


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/14/4109

Cited articles
This article cites 27 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/14/4109.full.html#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
/content/62/14/4109.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.