Inhibition of Tumor Growth, Angiogenesis, and Tumor Cell Proliferation by a Small Molecule Inhibitor of c-Jun N-terminal Kinase

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ABSTRACT

c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase family, and its function is critical for signal transduction in tumor and endothelial cells. JNK is a serine/threonine protein kinase that phosphorylates c-Jun, a component of the activator protein-1 transcription factor complex. We hypothesize that inhibiting JNK will lead to the inhibition of tumor growth; therefore, we evaluated the efficacy of the recently described JNK inhibitor SP600125 [anthra[1,9-cd]pyrazol-6 (2H)-one]. SP600125 is an anthrapyrazole that is a reversible, ATP-competitive inhibitor of JNK1/2. SP600125 exhibited broad-based antiproliferative activity in human endothelial and tumor cell lines. SP600125 affects proliferation by arresting cells in the G2/M phase of the cell cycle. SP600125 also acts to inhibit endothelial cell migration. In cell lines, a correlation of cell growth inhibition with reduced JNK activity was observed. The systemic administration of SP600125 resulted in the inhibition of DU145 human prostate carcinoma xenografts and murine Lewis lung carcinoma. SP600125 also enhanced the potency of cyclophosphamide in the inhibition of Lewis lung tumor growth. These data indicate the therapeutic antitumor potential of small molecule inhibitors that act to block the cellular activity of JNK.

The c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) family. The MAPK family phosphorylates distinct groups of substrates and regulates many cellular activities, such as proliferation, tumorigenesis, differentiation, and apoptosis (Whitmarsh and Davis, 1996; Davis, 2000). JNKs are serine/threonine protein kinases that phosphorylate serine 63 and 73 at the N-terminal domain of c-Jun, thus activating the transcriptional activity of activator protein-1 (Karin et al., 1997). c-Jun plays a pivotal role in mediating cellular responses to external stimuli. c-Jun is required for progression through the G1 phase of the cell cycle, and it protects cells from UV-induced apoptosis (Wisdom et al., 1999). There are three JNK genes each encoding JNK1, JNK2, and JNK3 (Gupta et al., 1996). JNK1 and JNK2 genes are expressed ubiquitously, whereas JNK3 is expressed only in the brain and testis (Davis, 2000). Ten JNK isoforms can result from the alternative splicing of the three different JNK gene products (Gupta et al., 1996). JNK has been implicated in, if not required for, oncogenic transformation (Raitano et al., 1995; Xu et al., 1996; Davis, 2000), apoptosis (van Dam et al., 1995; Xia et al., 1995; Tournier et al., 2000; Vivo et al., 2003), and cell proliferation (Xu et al., 1996). JNK is involved in the maintenance of cellular adhesions required for cell migration (Huang et al., 2003). JNK2 knockout mice have been used to determine that JNK2 is critical for 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion (Chen et al., 2001). The use of characterized antisense oligonucleotides complementary to JNK has shown that the resulting inhibition of JNK expression will cause growth arrest and apoptosis in tumor cells (Bost et al., 1999; Potapova et al., 2000). A recent study using systemically administered antisense oligonucleotides also resulted in the inhibition of PC3 prostate carcinoma xenografts, indicating that JNK expression is required for the growth of prostate carcinoma (Yang et al., 2003). JNK is activated by a variety of DNA-damaging agents such as UV (Derijard et al., 1994), cisplatin (Potapova et al., 1997), and...
camptothecin (Gjerset et al., 1999). Activation of the JNK pathway following DNA damage is required for DNA repair, thus indicating an essential role for JNK in the DNA repair process (Potapova et al., 1997, 2001).

The importance of JNK in the control of cell proliferation and apoptosis makes it an obvious target for therapeutic intervention. We hypothesize that the inhibition of the JNK pathway will lead to the inhibition of tumor growth. To test this hypothesis, we determined and evaluated the ability of the recently described JNK inhibitor SP600125 (Bennett et al., 2001; Han et al., 2001) to inhibit in vitro angiogenesis, tumor cell proliferation, and tumor growth. SP600125 is an anthrapyrazole with an IC50 value of 0.04 μM against JNK1 and JNK2, as determined by biochemical assay. This kinase inhibitor is a reversible, ATP-competitive inhibitor of JNK and has been shown to exhibit a selectivity of >20-fold relative to other tested kinases (Bennett et al., 2001). Numerous studies have indicated the in vitro and in vivo effectiveness and specificity of SP600125; thus, it has become the small molecule inhibitor of choice in studying the role of JNK in cellular processes (Brint et al., 2002; Reuther-Madrid et al., 2002; Zhang et al., 2002; Javelaud et al., 2003; Vivo et al., 2003; Masamune et al., 2004). The evaluation of JNK function in cell proliferation and tumor growth previously has relied on using genetic approaches (Gjerset et al., 1999; Chen et al., 2001; Potapova et al., 2001; Barr et al., 2002). We now show that SP600125 inhibits cancer cell proliferation, endothelial cell migration and proliferation, and tumor growth in mice. Our data indicate the utility of this compound for evaluating the role of JNK in cancer and the potential for similar inhibitors as therapeutic agents in cancer treatment.

Materials and Methods

Reagents. SP600125 (mol. wt., 220.23) is a novel JNK inhibitor synthesized at Celgene Corporation (San Diego, CA). Its structure has been presented previously (Bennett et al., 2001; Han et al., 2001). Tumor necrosis factor-α (TNF-α) was obtained from R&D Systems (Minneapolis, MN). The following antibodies were obtained from Cell Signaling Technology Inc. (Beverly, MA): phospho-JNK (T183/Y185, 9251), total JNK (9252), phospho-c-Jun (Ser73, 9164), total c-Jun (9162), and phospho-mitogen-activated kinase kinase (MKK) 4 (Ser80, 9155). Antibodies obtained from Santa Cruz Biotechnology were phospho-JNK (Ser63, 9164), and phospho-c-Jun (Ser73, 9164). Mouse monoclonal β-actin (A5441) was obtained from Sigma-Aldrich (St. Louis, MO).

Cell Lines. Tumor cell lines were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) and 50 μg/ml gentamicin. Human microvascular endothelial cells (HMVECs; Cambrex Bio Science Walkersville, Walkersville, MD) were cultured in media according to supplier recommendations, i.e., endothelial cell basal medium (Cambrex Bio Science Walkersville) supplemented with 10 ng/ml epidermal growth factor, 1 μg/ml hydrocortisone, 50 μg/ml gentamicin, 3 μg/ml bovine brain extract, and 10% fetal bovine serum. All cell lines were maintained in a humidified atmosphere at 37°C/5% CO2. Cells used in tumor studies were verified pathogen-free (University of Missouri, Research and Investigative Laboratory, Columbia, MO).

Cell Proliferation Assay. Cells were plated into quadruplicate wells of 24-well culture plates, 1 × 105 cells/well. The next day, SP600125 dissolved in dimethyl sulfoxide (DMSO; 0.1% final concentration), or DMSO alone was added to quadruplicate wells. After 5 days of culture (4 days of treatment), cells were washed with phosphate-buffered saline (PBS), harvested using trypsin, and counted with an automated cell counter (Beckman Coulter, Fullerton, CA). Cell number data were normalized to the percentage of vehicle-treated control and then graphed. IC50 values were determined by graphing cell number data as percentage of control versus compound concentration, and the IC50 was the point on the graph where 50% growth inhibition occurred. To assess cytotoxicity, GI curves were generated in which the percentage of growth (PG) was determined and plotted against compound concentration. PG was obtained using the formula PG = 100 × (T – T0)/(C – T0), where T = mean cell number after 4 days of treatment, T0 = mean cell number at time 0, and C = mean cell number after 4 days of exposure to 0.1% DMSO. GI50 values can be determined at the concentration of SP600125 where PG = 50%, and these values emphasize the correction for cell count at time 0 (i.e., cell number at the time compounds are added).

Endothelial Cell Migration Assay. HMVECs growing under normal culture conditions were harvested with trypsin, and 50,000 cells/well were loaded in quadruplicate into the lower wells of 48-well Boyden chambers (Neuro Probe, Gaithersburg, MD). Chambers were then inverted, and the cells were allowed to attach for 2 h at 37°C to polycarbonate chemotaxis membranes (8.0-μm pore size) that had been soaked overnight in 0.1% gelatin and dried. Culture media containing 10% serum and SP600125 were placed into the top wells of upright chambers, and incubation continued for an additional 2 h. Membranes were removed, and migrated cells were fixed, stained (Diff-Quick; VWR, West Chester, PA), and then counted by objective magnification (three fields/well).

Flow Cytometry. Cells were added to six-well plates (1 × 106 cells/well) and the next day were treated with SP600125 under normal culture conditions. After 24 h, compound-treated cells were harvested with trypsin, stained with propidium iodide, and acquired with a Beckman Coulter Epics flow cytometer running Expo 32 software (Beckman Coulter). Cell cycle parameters were determined using ModFit software (Verity Software House, Topsham, ME).

Western Blot Analysis. Cells were seeded 1 × 106 cells/100-mm dish in 10 ml of normal growth medium. The next day, the medium was replaced with that containing 0.5% fetal bovine serum. Twenty-one hours later, cells were treated with SP600125 in DMSO for 4 h. Then 10 ng/ml of TNF-α was added for 15 min, and the cells were subsequently washed with PBS and lysed with radioimmunoprecipitation assay lysis buffer [PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, phosphatase inhibitor mixture 2 (Sigma-Aldrich), and one protease inhibitor mixture tablet (Roche Diagnostics, Basel, Switzerland) per 20 ml of buffer volume]. Lysates were collected into microtubes, incubated on ice for 15 min, and centrifuged at 15,000 rpm for 20 min. Supernatants were collected, and protein concentration was determined by the Bradford assay. Sixty micrograms of protein were run on 4 to 20% Tris-Glycine gradient gels (Invitrogen) and transferred onto nitrocellulose membranes (Amersham Biosciences Inc., Piscataway, NJ). Immunoblot analysis was performed using PBS + 0.1% Tween 20 and Odyssey blocking buffer (LI-COR Bioscience, Lincoln, NE), and primary antibodies were incubated overnight at 4°C. Secondary antibodies, conjugated to Alexa-Fluor 680 dye (Molecular Probes, Eugene, OR) or IRDye 800 (Rockland, Gilbertsville, PA), were incubated with the blot for 1 h at room temperature, and images were obtained using the Odyssey (LI-COR Bioscience).

Tumor Studies. Tumor cells were cultured under normal conditions, harvested with trypsin, washed with PBS, and then resuspended at 1 × 107 cells/ml in Matrigel (Collaborative Research, Bedford, MA). C57BL/6 or C3H10T1/2 mice (female, 18–20 g; Charles River Laboratories, Inc., Wilmington, MA) were lightly anesthetized using isoflurane and inoculated s.c. under the right flank with 0.1 ml of Matrigel containing 1 × 106 tumor cells. Mice were randomized into treatment groups immediately after cell inoculation. For treatment, mice were injected i.p. once per day with 60 mg/kg SP600125 or with the vehicle alone, DMSO (100%) was used as a vehicle in C57BL/6 mice, and N3PS (5% N-methyl-pyrolidone,
30% polyethylene glycol 400, 25% polyethylene glycol 200, 20% propylene glycol, and 20% saline) was used in severely compromised immunodeficient (SCID) mice. Tumor volumes were determined by caliper measurement, and tumor volume was calculated by the formula \( V = \frac{L \times W^2}{2} \). Animals were allowed to acclimate for 7 days before the start of studies. SCID mice were housed under pathogen-free conditions, and all animals were given water and chow ad libitum. Animal care and use were in accordance with Celgene's Institutional Animal Care and Use Committee requirements and guidelines.

Results

SP600125 Inhibits Proliferation of Cells in Culture. The effects of SP600125 on cell proliferation were determined in numerous cell lines representing various cancer types. Cells were exposed to a concentration range of SP600125 in normal growth medium for 4 days. The IC50 concentration at which 50% of a cell population was inhibited (IC50) relative to vehicle control was determined (Table 1). Fifteen cell lines were assayed, and SP600125 exerted a growth inhibitory effect on all of the lines. IC50 values ranged from 1.5 to 10.5 \( \mu \text{M} \).

SP600125 Is a Noncytotoxic Inhibitor of Cell Proliferation. In an effort to assess whether SP600125 inhibition of cancer cell proliferation was a cytotoxic or cytostatic effect, a separate set of proliferation studies was carried out on selected cell lines from which GI curves were generated (Fig. 1). GI50 is the concentration of test compound at which the percentage of growth of cells, relative to control cell number at the time of compound addition, is +50. GI values less than zero indicate cytotoxicity (i.e., cell numbers less than those at the start of treatment). None of the five cell lines exposed to SP600125 yielded a GI value less than zero. That is, the inhibition of cell proliferation induced by SP600125 did not fall below the initial density at the time of compound addition, suggesting that SP600125 inhibits cell proliferation by mechanisms other than nonspecific cytotoxicity.

SP600125 Inhibits the Cellular Activity of JNK. The kinase specificity and JNK selectivity of SP600125 have been demonstrated previously (Bennett et al., 2001). Considering the antiproliferative effects seen in the present study, we wished to demonstrate the inhibition of cellular JNK activity by SP600125. As seen in Fig. 2, SP600125 inhibited the cellular activity of JNK (i.e., the phosphorylation of c-Jun). There was a concentration-dependent reduction of TNF-\( \alpha \)-stimulated JNK activity in DU145 human prostate carcinoma cells, murine Lewis lung carcinoma (LLC) cells, and HMVECs. Western analysis of total c-Jun indicated the decrease of phospho-c-Jun. The levels of total JNK and phospho-JNK were not affected by SP600125 except in DU145 cells, in which the level of phospho-JNK was decreased at higher SP600125 concentrations. In HMVECs, but not LLC or DU145 cells, SP600125 had an effect on phospho-MKK4, the kinase directly upstream from JNK. There was no effect on the levels of total MKK4. The inhibition of JNK activity also has been observed in MDA435 human breast carcinoma cells and MiaPaCa human pancreatic carcinoma cells growing in normal culture conditions (i.e., without TNF-\( \alpha \) stimulation; data not shown).

SP600125 Blocks Cells in the G2 Phase of the Cell Cycle. We next considered whether exposure to SP600125 would alter the cell cycle of tumor and endothelial cells. DU145, LLC cells, and HMVECs were exposed to increasing concentrations of SP600125 for 24 h under normal culture conditions and then processed for flow cytometric analysis. The data shown in Fig. 3 represent three separate experiments. A concentration-dependent block in the G2/M phase of the cell cycle was observed in all of the cell lines. The greatest effect was seen in LLC cells, and the least effect was seen in HMVECs. At the highest concentration, 30 \( \mu \)M SP600125, 91% of LLC, 54% of DU145 cells, and 33% of HMVECs were blocked in the G2/M phase.

TABLE 1

IC50 values (micromolar) for SP600125 effects on cancer cell line proliferation

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Breast</th>
<th>Prostate</th>
<th>Pancreas</th>
<th>Leukemia</th>
<th>Colon</th>
<th>Marine</th>
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<td>6.5</td>
<td>8.9</td>
<td>3.0</td>
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<td>7.0</td>
<td>6.0</td>
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<tr>
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<td>7.0</td>
<td>10.0</td>
<td>10.0</td>
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</tr>
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</tr>
<tr>
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</table>
SP600125 Inhibits HMVEC Proliferation. Considering that SP600125 inhibits JNK activity in HMVECs and also perturbs the cell cycle to block these cells in the G2 phase, we studied the effects of SP600125 on endothelial cell proliferation. HMVECs were exposed to a concentration range of SP600125 during 4 days of growth in culture, and the cell number then was determined (Fig. 4A). SP600125 inhibited HMVEC cell proliferation in a concentration-dependent manner. The concentration of SP600125 at which a 50% growth inhibition occurred was 2.5 μM. In a further experiment, HMVECs were exposed to a single concentration of SP600125 (3 μM) during 10 days of growth in culture (Fig. 4B). The proliferation of endothelial cells was effectively inhibited throughout the duration of exposure. During this time, cell number did not decrease below the initial plating density, suggesting a cytostatic rather than a cytotoxic effect. At the end of the experiment, the control cell number was increasing, whereas cells treated with SP600125 showed only a small increase in number by day 8, with no increase after this time.

SP600125 Inhibits Endothelial Cell Migration. The process of tumor angiogenesis requires endothelial cell migration and proliferation. Having determined that SP600125 effectively inhibits endothelial cell proliferation, we assessed effects on endothelial cell migration (Fig. 5). The results indicate that SP600125 inhibits HMVEC migration that is stimulated by growth medium in a concentration-dependent manner. At the highest concentration, the number of migrated cells was equivalent to that in the absence of chemoattractant (i.e., growth medium). This inhibitory effect of SP600125 on endothelial cell migration was verified using a migration assay in which the fluorescence intensity of migrated cells is measured (FluorBlok; BD Biosciences, San Jose, CA) rather than cell counts. The same inhibitory effect of SP600125 on endothelial cell migration was seen using the FluorBlok assay (data not shown).

SP600125 Inhibits Tumor Growth. Considering that the in vitro antiproliferative properties of SP600125 indicate its potential for antitumor activity, SCID mice bearing DU145 human prostate carcinoma xenografts were treated with SP600125 (Fig. 6). The JNK inhibitor was efficacious at inhibiting the growth of DU145 tumors during a growth period of 32 days. The greatest antitumor efficacy was seen at the end of the study when treatment with SP600125 resulted in a 54% growth inhibition relative to vehicle-treated controls. Throughout the treatment period, mouse body weights were monitored, and the mean body weight of treated mice never decreased below that of vehicle-treated control mice. Control mice underwent a mean weight loss of 12% versus a loss of 7.8% in SP600125-treated mice (data not shown).

The ability of systemically administered SP600125 to inhibit tumor growth also was tested in a murine model system. Mice bearing LLC were treated daily with SP600125 (Fig. 7). Just as with DU145 tumors, LLC growth was inhibited. Tumor growth inhibition was observed as early as 8 days of tumor growth, and this growth inhibition continued throughout the study. After 15 days of tumor growth at the end of the study, a growth inhibition of 59% relative to vehicle control was observed (Fig. 7). Mouse body weight was not affected as a result of SP600125 treatment. After 1 week of treatment, both treatment groups (SP600125 and vehicle control) lost approximately 10% body weight. As treatment
continued, however, all body weights remained stable and constant throughout the study (data not shown).

We considered whether the antitumor effects observed using SP600125 in monotherapy also would occur if SP600125 was administered in combination with a chemotherapeutic drug. Mice bearing LLC were treated daily with SP600125 or cyclophosphamide (CTX) alone or in combination (Fig. 8). As before, the systemic administration of SP600125 resulted in the inhibition of tumor growth. When SP600125 and CTX were combined, growth inhibition was greater than when either of the two treatments were given alone. At day 10 of tumor growth and thereafter, tumor inhibition resulting from combination treatment was significantly greater than that resulting from treatment with CTX alone. This difference continued until the experiment’s end on day 17, when combination treatment resulted in a 77% growth inhibition.

**Discussion**

JNK is an important regulatory kinase whose signal transduction pathway is integral for survival as cells respond to stress occurring in the tumor environment. Inhibitors of the JNK pathway should be effective antitumor therapeutics because of their ability to disrupt the cellular response to this stress. We have shown that the JNK inhibitor SP600125 is an inhibitor of tumor and endothelial cell proliferation, and this translates to inhibition of tumor growth upon systemic administration. The in vitro effects of SP600125 provide insight into its antitumor activity and its potential for therapeutic activity. SP600125 inhibited all of the tumor cell lines tested. Cell proliferation was inhibited after 4 days of exposure, and this inhibition does not seem to be a result of a nonspecific cytotoxic mechanism. Cell cycle studies indicated an effect on cell cycle progression as early as 24 h after exposure to SP600125. Among the many in vitro assays used to study SP600125 effects, IC$_{50}$ values varied. This is not unexpected considering that functionally different assay readouts were carried out using acute and chronic exposure to the compound. It also is probable that the effect of JNK in cell cycle progression will vary among cell lines and cell types. However, the differing degree of SP600125 effects is probably because of inhibition of JNK. Western blot biomarker analysis indicates that SP600125 is acting to inhibit JNK activity in cells. The selectivity of SP600125 for JNK inhibition has been demonstrated previously (Bennett et al., 2001; Han et al., 2001). An expanded panel of enzymes was assayed for the inhibitory effects of SP600125. The inhibition of M KK4 occurred with 10-fold selectivity; a 25-fold selectivity was observed for M KK3, M KK6, Akt, and protein kinase C-γ. All other kinases were affected by SP600125 with $>100$-fold selectivity relative to JNK (Bennett et al., 2001). Other studies also have shown no significant inhibitory activity against 18 inflammatory enzymes with diverse catalytic activities (Han et al., 2001). In previous studies using cell-based assays, SP600125 inhibited c-Jun phosphorylation in Jurkat T cells with an IC$_{50}$ of 5 to 10 μM (Bennett et al., 2001). The data show that SP600125 blocked JNK and not other inflammatory signaling cascades with at least a 10-fold selectivity for the JNK pathway. Higher IC$_{50}$ values seen in cell-based assays, relative to biochemical assay, probably reflect the higher ATP concentration in cells than that used in biochemical assay. A more recent study (Bain et al., 2003) examining the biochemical selectivity of SP600125 against various kinases in vitro provided data to indicate less selectivity than reported by others. Direct comparison between these studies...
is difficult because JNK activity was assayed under different conditions, in particular, different ATP concentrations. However, the more important question remains as to SP600125 selectivity in cellular systems under conditions in which JNK is activated. There is little doubt that, in addition to JNK, SP600125 inhibits other kinases in the cell. However, we can be certain from our biomarker assays that cellular JNK is inhibited and that this inhibition likely plays a central role in the cellular responses measured in this study. This is supported by a number of studies using biological reagents to block JNK activity, resulting in inhibition of tumor cell proliferation and growth (Bost et al., 1999; Potapova et al., 2000; Yang et al., 2003).

In addition to cancer cell lines, SP600125 also inhibited the proliferation of microvascular endothelial cells. This inhibition occurred in a concentration- and time-dependent manner during a 10-day period after a single addition of compound. Therefore, a substantial sensitivity of endothelial cells to SP600125 is indicated. In addition to proliferation, endothelial cell migration also is a defining characteristic of tumor angiogenesis (Trochon et al., 1996; Yoshida et al., 1996; Vernon and Sage, 1999; Auerbach et al., 2003). SP600125 was effective in the inhibition of HMVEC migration. Considering also that SP600125 inhibits JNK activity in HMVECs and it affects the HMVEC cell cycle to cause a...
blockade in the G2 phase, one can conclude that SP600125 exhibits antiangiogenic properties.

The fact that SP600125 inhibits tumor cell proliferation and is antiangiogenic indicates the likelihood that a dual antitumor effect is occurring in vivo. SP600125 is not only inhibiting tumor growth by affecting the proliferation of tumor cells, it is also interfering with tumor angiogenesis, a process essential for tumor growth (Ferrara, 2002; Folkman, 2002). In the present study, SP600125 effectively inhibited LLC and DU145 tumor growth. These tumors exhibit different growth rates. Slow- and fast-growing tumors were inhibited to the same extent relative to vehicle-treated controls (i.e., 54 and 59%). It is intriguing to speculate that the antiangiogenic properties of SP600125 were the consistent factor normalizing the contrasting growth rates for these tumors.

The dual antiproliferative and antiangiogenic effect of SP600125 indicates the therapeutic potential of JNK inhibitors. Future studies are planned to address the effect of SP600125 on large established tumors.

This therapeutic potential may be enhanced by combining JNK inhibitors with traditional chemotherapy. In the present study, the data indicate an additive antitumor effect when SP600125 and CTX were used together in the treatment of mice bearing LLC. Because the antitumor effect of CTX probably results from the induction of apoptosis, one can consider the role of JNK as a stress-activated kinase in protecting against the apoptotic effect of CTX (or other chemotherapeutics). It is possible to speculate that JNK could be activated as a result of stress induced by treatment with chemotherapeutic agents. In the absence of inhibitor, JNK would function to protect cells from this chemo-induced stress. If JNK is inhibited, such as with SP600125, the effect of a chemotherapeutic would be enhanced as a result of neutralizing the stress response to the chemo that is facilitated by an activated JNK.

In summary, our data confirm that JNK plays a protective/causative role in various tumors and during treatment with certain chemotherapeutic reagents. Therefore, a small molecule JNK inhibitor may have clinical utility as an anticancer agent as a monotherapy or in combination with other anti-tumor agents.

**References**


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