Preclinical Development

SCH 2047069, a Novel Oral Kinesin Spindle Protein Inhibitor, Shows Single-Agent Antitumor Activity and Enhances the Efficacy of Chemotherapeutics

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Abstract

Kinesin spindle protein (KSP) is a mitotic kinesin required for the formation of the bipolar mitotic spindle, and inhibition of this motor protein results in mitotic arrest and cell death. KSP inhibitors show preclinical antitumor activity and are currently undergoing testing in clinical trials. These agents have been dosed intravenously using various dosing schedules. We sought to identify a KSP inhibitor that could be delivered orally and thus provide convenience of dosing as well as the ability to achieve more continuous exposure via the use of dose-dense administration. We discovered SCH 2047069, a potent KSP inhibitor with oral bioavailability across species and the ability to cross the blood-brain barrier. The compound induces mitotic arrest characterized by a monaster spindle and is associated with an increase in histone H3 and mitotic protein monoclonal 2 phosphorylation both in vitro and in vivo. SCH 2047069 showed antitumor activity in a variety of preclinical models as a single agent and in combination with paclitaxel, gemcitabine, or vincristine. Mol Cancer Ther; 9(11); 2993–3002. ©2010 AACR.

Introduction

Kinesin spindle protein (KSP) is a plus-end directed motor protein that uses ATP hydrolysis to drive antiparallel sliding of microtubules (1, 2). This mitotic kinesin is required for separation of duplicated spindle poles in prometaphase and thus critical for the formation of a bipolar mitotic spindle (3). Inhibition of KSP, by small molecule or antibody microinjection, results in mitotic arrest characterized by a misformed mitotic spindle (4–6). This arrest results in a ring of condensed chromosomes circling unseparated spindle poles, commonly referred to as a monaster spindle with DNA in a rosette configuration. High levels of mitotic markers, phosphorylation of histone H3 and mitotic protein monoclonal 2 (MPM2), are observed following KSP inhibition. As a result of the mitotic defect, KSP inhibition induces cell death (7).

Unlike antimitotic agents that target tubulin, KSP inhibitors are expected to be specific to dividing cells. Anti-mitotic agents that target tubulin are clinically validated, including microtubule stabilizers (e.g., taxanes) and microtubule destabilizers (e.g., Vinca alkaloids). However, tubulin also functions in postmitotic nerve cells, and thus, treatment with agents that stabilize or destabilize tubulin is associated with peripheral neuropathy (8). KSP represents a novel mitotic target that is not present in postmitotic neurons (9, 10) and offers a potential advantage of negligible peripheral neuropathy. Due to their distinct mechanism of action, KSP inhibitors have potential for utility in taxane-sensitive disease indications [breast, prostate, ovarian, and non–small cell lung cancer (NSCLC)], and preclinical data suggest that KSP inhibitors may have activity in patients who are refractory to or have relapsed from taxane-based therapy (11). Additionally, KSP inhibitors show preclinical activity in disease indications that are not sensitive to taxanes clinically (e.g., leukemia; ref. 12). There also exists the potential of combining KSP inhibitors and taxanes.

KSP inhibitors show antitumor activity in a variety of preclinical models, and several small-molecule KSP inhibitors are currently undergoing testing in clinical trials (http://clinicaltrials.gov). Thus far, some clinical responses have been seen with ispinesib in breast cancer and Arry-520 and AZD-4877 in acute myelogenous leukemia (AML). Early clinical trials done with ispinesib and MK-0731 used dosing schedules of once every 3 weeks. Some more recent clinical studies are using every 2 weeks, weekly, and multiple times a week scheduling. The optimal clinical schedule for these agents has yet to be identified. Furthermore, all of the inhibitors tested
clinically thus far have been dosed intravenously. Recently, 4SC-205, a novel oral agent, is entering clinical studies.

We set out to identify a potent KSP inhibitor that could be delivered orally, thus providing convenience of dosing as well as the ability to achieve more continuous exposure via the use of dose-dense administration. We discovered SCH 2047069, a potent KSP inhibitor with oral bioavailability across species and the ability to cross the blood-brain barrier. The compound induces mitotic arrest characterized by a monaster spindle and is associated with an increase in histone H3 and MPM2 phosphorylation both in vitro and in vivo. SCH 2047069 showed antitumor activity in a variety of preclinical models as a single agent and in combination with paclitaxel, gemcitabine, or vincristine.

Materials and Methods

Materials

SCH 2047069 (synthesized at Schering-Plough; ref. 13) was dissolved in DMSO or 20% hydroxypropyl B cyclo-dextrin (HPBCD) for in vitro and in vivo studies, respectively. For in vitro studies, paclitaxel (Bristol-Myers Squibb), gemcitabine (Eli Lilly), and vincristine (Hospira) were dissolved in 20% HPBCD.

Biochemical assays

The assay consisted of 10 nmol/L KSP motor domain (amino acid 15–368; ref. 14), 20 μmol/L paclitaxel (Cytoskeleton), 0.02 mg/mL microtubules [reconstituted in 80 mmol/L Na-Pipes (pH 6.9), 1 mmol/L EGTA, 1 mmol/L MgCl2, 100 μL of 2 mmol/L paclitaxel], 100 μmol/L ATP (Roche), and kinesin buffer [20 mmol/L ACES (pH 7.0), 1 mmol/L EGTA, 1 mmol/L MgCl2, 25 mmol/L KCl, 1 mmol/L DTT]. Kinesin counterscreens were done similarly using microtubules as substrate; myosin counterscreen was measured using actin as a substrate. Compound was added and the reaction started by the addition of 5 μL ATP. After 1 hour, the reaction was stopped by adding 50 μL Biomol Green (Biomol International). After an additional 30 minutes, absorbance at 620 nm was measured using an Envision (Perkin-Elmer) microplate reader. To generate IC50 values, the concentration-response curves were fitted to a standard sigmoidal curve and IC50 values were derived by nonlinear regression analysis (Microsoft XL-fit).

Thermal shift assay (temperature-dependent fluorescence)

The assay consisted of 3 μmol/L KSP protein (14), 25 mmol/L HEPEs (pH 7.5), 150 mmol/L NaCl, 1 mmol/L DTT, Sypro Orange dye (5 μmol/L final concentration; Sigma-Aldrich), and 100 μmol/L ADP and was conducted in the 96-well–based CHROMO-4 real-time fluorescence plate reader (Bio-Rad). The sample plate was heated from 25°C to 90°C with thermal ramping rate of 1°C/min. Sypro Orange was used to monitor the protein folding-unfolding transition. Protein-compound binding dissociation constant was calculated based on the change in the unfolding transition temperature (ΔTv) in the presence of compound compared with protein alone. The fluorescence signals were acquired with excitation and emission wavelengths centered at 490 and 560 nm, respectively. Binding affinity constant was calculated as described previously (Mayhood, 2005).

Cell culture

Human tumor cell lines were grown in DMEM/F12 medium supplemented with 2 mmol/L glutamine, 50 units/mL penicillin, and 50 units/mL streptomycin with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen) at 37°C with 5% CO2. Cells were obtained from the American Type Culture Collection (ATCC) and used within 6 months of receipt, with the exception of SNB19, SNB87, U251 (National Cancer Institute tumor repository), EOL-1 (European Collection of Animal Cell Cultures), and A2780 (kindly provided by Dr. Janet Price, M.D. Anderson). The five cell lines received from sources other than ATCC have not been authenticated in our lab.

Immunofluorescent assays

HCT-116 cells were plated at 15,000 per well in poly-d-lysine–coated black microtissue culture plates (Greiner). Cells were treated for 16 hours with compound (0.1% final DMSO concentration) in triplicate wells. Cells were fixed with Prefer fixation solution (Anatech) plus 1,000 nmol/L Hoechst 33342 dye and incubated for 30 minutes at room temperature. The fixation solution was removed, and cells were washed with PBS. Cells were permeabilized with 0.2% Triton X-100 in PBS and incubated for 10 minutes. Cells were washed with PBS and incubated with PBS containing 3% FBS for 30 minutes. Cells were then stained overnight at 4°C with phospho-histone H3 (Ser10)–Alexa Fluor 488 conjugate (Cell Signaling) and phospho-MPM2–Texas red conjugate (Millipore) antibody solution in PBS plus 3% FBS. Cells were washed with PBS, and then immunofluorescent images were captured at 10× using the Pathway 855 automated fluorescent microscope (BD Biosciences). Percent positive cells were quantitated using Hoechst 33342 staining for total cell number using Attovision software (BD Biosciences). To generate IC50 values, the concentration-response curves were then fitted to a standard sigmoidal curve and IC50 values were derived by nonlinear regression analysis (Microsoft XL-fit). Anti-tubulin (Sigma) and anti-pericentrin (Abcam) antibody staining was done similarly with the additional last step of 1-hour incubation with Alexa Fluor 647–conjugated anti-rabbit IgG and Alexa Fluor 488–conjugated anti-mouse IgG, respectively. These images were captured at 40×.

Cell cycle analysis

HCT-116 cells were plated using 2 × 106 cells per 10-cm tissue culture dish. The next day, cells were treated with compound (0.1% final DMSO concentration) and,
16 hours later, collected and centrifuged for 1 minute at 1,000 rpm. Cell pellet was resuspended in 0.5 mL PBS, and the solution was transferred to cold 70% methanol and incubated at −20°C for 30 minutes. Cells were then centrifuged for 1 minute at 1,000 rpm, the supernatant was removed, and the pellet was washed with 2 mL PBS and centrifuged. Cells were resuspended in 0.5 mL propidium iodine stain (0.1 mmol/L EDTA, 0.05 mg/mL RNase, 50 μg/mL propidium iodine), transferred to a filter cap tube, and read by fluorescence-activated cell sorting (FACS).

Cell growth

Cells were plated at a cell density ranging from 625 to 3,750 per well depending on rate of cell growth in poly-D-lysine–coated black microclear 384-well tissue culture plates (Greiner). Cells were treated in triplicate wells with compound (0.1% final DMSO concentration). A plate was stained at the start of the study (0 hour), and a second plate was incubated for 72 hours at 37°C and then stained. Cells were fixed with Prefer fixation solution plus 1,000 nmol/L Hoechst 33342 dye and incubated for 30 minutes. The fixation solution was removed, and cells were washed twice with PBS. Then, immunofluorescent images were captured at 10× using the Pathway 855 automated fluorescent microscope. The difference in cell number between 0 and 72 hours was plotted against compound concentration, and the concentration-response curves were fitted to a standard sigmoidal curve and IC₅₀ values were derived by nonlinear regression analysis (Microsoft XL-fit).

Poly(ADP-ribose) polymerase Western blots

Cells were treated with compounds for the indicated time, lysed in radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl, 50 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 1 mmol/L EDTA, 2.5 mmol/L Na₃VO₄, 20 mmol/L β-glycerol phosphate, and Complete protease inhibitor (Roche)], and cleared by centrifugation. Protein concentration was determined using bicinchoninic acid reagent (Pierce Chemical Co.). Samples were separated by 8% SDS-PAGE (Invitrogen), transferred to polyvinylidene difluoride membrane, immunoblotted with anti-p85 poly(ADP-ribose) polymerase (PARP; Promega), and detected by chemiluminescence using the enhanced chemiluminescence detection reagents (Amersham).

Immunohistochemistry

Female nu/nu mice (5–7 weeks of age; Charles River) were injected s.c. with 5 × 10⁶ cells per mouse in the right flank. When tumor cells reached ~200 mm³, mice were randomly grouped into treatment groups (n = 10). Tumor volumes and body weights were measured twice a week throughout the study using calipers and calculated by the formula (width × length × height) × 4/π. For EOL-1 leukemia and DOHH2 lymphoma models, severe combined immunodeficient mice were injected through the tail vein with 10 × 10⁶ cells per mouse. The next day, mice were randomized into treatment groups (n = 10). Mice were monitored daily until death or sacrifice based on humane end points. Statistically significant differences were determined by the multiple comparison one-way ANOVA (Turkey's test) at the 95% confidence level using GraphPad Prism. All animal studies were carried out in accordance with institutional guidelines and the NIH Guide for the Care and Use of Laboratory Animals.

Results

SCH 2047069 is a novel oral KSP inhibitor with the ability to cross the blood-brain barrier

We identified SCH 2047069 as a novel inhibitor of KSP (Supplementary Fig. S1A). The biochemical activity of SCH 2047069 was determined by assessing the ability to inhibit the ATPase activity of KSP using microtubules as a substrate. SCH 2047069 potently inhibited KSP ATPase activity with an IC₅₀ of ≤5 nmol/L (assay detection limit; Supplementary Fig. S1B). To better assess the in vitro potency of SCH 2047069, we used temperature-dependent fluorescence (TdF) to measure the binding affinity to KSP. TdF measurements yielded a K₅ value of 0.5 nmol/L (Supplementary Fig. S1C).

Counterscreening assays showed that SCH 2047069 was selective and did not inhibit the ATPase activity of other motor proteins. When SCH 2047069 was tested on November 6, 2017. © 2010 American Association for Cancer Research.

Published OnlineFirst October 26, 2010; DOI: 10.1158/1535-7163.MCT-10-0548
against 13 other kinesin proteins, the IC50 values were >10 μmol/L (Supplementary Fig. S1D). Similarly, it did not inhibit the motor protein myosin (IC50 >10 μmol/L; Supplementary Fig. S1D).

We evaluated the pharmacokinetic properties of SCH 2047069 in mice, rats, dogs, and monkeys (Supplementary Tables S1 and S2). Oral bioavailability of the compound was 88%, 64%, 44%, and 50% in mice, rat, dog, and monkey, respectively. Oral dosing of 7.5 mg/kg in mice and 5 mg/kg in rats, dogs, and monkeys yielded area under the curve (AUC) values of 4.1, 2.6, 6.9, and 4.8 μmol/L·h. SCH 2047069 dosed orally at 5 mg/kg in the mice and rats showed significant brain uptake, with brain/plasma AUC ratio of 4 (Supplementary Fig. S2).

**SCH 2047069 displayed potent mechanism-based activity**

SCH 2047069 potently induced mitotic arrest. Exposure to SCH 2047069 concentrations as low as 6.25 nmol/L for 16 hours resulted in significant increase in HCT-116 cells in mitosis. At this concentration, 73% of cells were found to have 4N DNA content (Fig. 1A). SCH 2047069-induced mitotic arrest was associated with increased markers of mitosis, including phosphorylation of histone H3 (Ser10) and MPM2. Significant induction of these markers was seen following 16-hour treatment of HCT-116 colon cancer cells. For example, 6.25 nmol/L SCH 2047069 increased the percentage of cells with histone H3 phosphorylation from 5% to 69% (Fig. 1B and C).

The mitotic arrest associated with SCH 2047069 was further characterized using more detailed immunofluorescence analysis (Fig. 1D). HCT-116 cell were treated with increasing concentrations of SCH 2047069 for 16 hours, fixed, and stained with Hoechst (DNA), tubulin, and pericentrin antibodies. The majority of cells in the vehicle control groups were in interphase with uncondensed DNA in the nucleus along with a minor population of mitotic cells, which were characterized by spindle poles at opposite ends of the cell, DNA aligning at the metaphase plate, and microtubules pulling the DNA toward the poles. On treatment with SCH 2047069, pericentrin stain was localized at the center of the cell, indicating colocalization of both spindle poles. The pericentrin staining was surrounded by a ring of microtubules, commonly referred to as a monastral spindle formation. DNA staining was observed as an outer ring surrounding the microtubule staining, a pattern commonly referred to a rosette formation.

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**Figure 1.** SCH 2047069 induced mitotic arrest. A, HCT-116 cells were treated with increasing concentrations of compound for 16 h and cell cycle profile was analyzed by FACS. B and C, HCT-116 cells were treated with increasing concentrations of compound for 16 h, and phosphorylation of histone H3 (HH3; green) and MPM2 (red) was measured by immunofluorescence. D, HCT-116 cells were treated with DMSO or 12.5 nmol/L SCH 2047069 for 16 h and DNA (yellow), tubulin (blue), and pericentrin (red) were visualized by immunofluorescence.
SCH 2047069 inhibited a broad panel of tumor cell lines

We evaluated the activity of SCH 2047069 across a panel of 57 tumor cell lines from different tissue origin (colon, ovarian, prostate, breast, pancreatic, melanoma, brain, NSCLC, renal, gastric, leukemia, and cervical carcinoma). We found that all cells tested were growth inhibited, with IC$_{50}$ values of <30 nmol/L; 51 of the 57 cells had IC$_{50}$ values of <10 nmol/L (Supplementary Table S3).

SCH 2047069 activity was maintained in multidrug-resistant cells expressing high levels of P-glycoprotein (PgP) protein (Supplementary Table S3). We found that concentrations required to inhibit cells that have high levels of PgP were similar to those required for cells that have low PgP expression. For example, HCT-15 cells, which have significant PgP expression, are growth inhibited with an IC$_{50}$ of 0.7 nmol/L. Consistent with these results, the apical to basolateral, and basolateral to apical permeabilities observed in Caco-2 cells at 0.5 μmol/L were 106 and 115 nm/s, respectively, suggesting that SCH 2047069 was not a substrate for efflux transporters.

Several experiments were done to determine the optimal exposure time required for SCH 2047069 to induce mitotic arrest and death in cells. We treated cells with 10 nmol/L SCH 2047069 for 24 hours continuously or for shorter periods of time followed by a washout period. At the end of 24 hours, mitotic arrest was measured, and at 48 hours after treatment, PARP cleavage was assessed. Some cell lines (e.g., HCT-116 colon cancer and A2780 ovarian cancer) underwent mitotic arrest at 24 hours after treatment following exposure to compound for as little as 1 hour (Fig. 2A and B). In contrast, PA-1 ovarian cancer cells required continuous exposure to compound for 24 hours to undergo complete mitotic arrest (Fig. 2C). Similarly, we found that HCT-116 and A2780 cells underwent apoptosis at 48 hours after treatment if cells were exposed to compound for 1 hour, whereas PA-1 cells required 24-hour exposure to elicit an apoptotic response 48 hours after treatment (Fig. 2D).

We explored the effect of exposure time using a larger panel of tumor cells using either 10 or 20 nmol/L SCH 2047069 (depending on the cell growth IC$_{50}$ value; Supplementary Table S1). We found that 25 of the 57 cell lines required short exposure (4 hours) to induce mitotic arrest. Most the cell lines that required 4 hours to induce mitotic arrest also showed PARP cleavage following a 4-hour exposure. The exceptions were U87-MG and HL-60, which died following SCH 2047069 treatment but failed to show PARP cleavage by Western blot.

![Graphs showing exposure time effects](image-url)
Of the remaining 32 cell lines, 30 underwent mitotic arrest following a 24-hour exposure to SCH 2047069. The exceptions were BxPC3 and NCI-226, which failed to show a significant mitotic arrest 24 hours after treatment. These results suggest that a subset of cell lines requires 4 hours of drug exposure for cell kill, whereas other cell lines require 24 hours of exposure. The reason for this difference remains to be determined. We found no correlation to genetic background, including p53 status, or mitotic index of the cell lines.

Contrary to the findings with SCH 2047069, most cells showed PARP cleavage following a 4-hour exposure to taxanes (paclitaxel and docetaxel), suggesting that the mechanism of action and optimal schedule for a KSP inhibitor may differ from that of a taxane (data not shown).

**SCH 2047069 induced pharmacodynamic markers in vivo**

Mechanism of action biomarkers for KSP inhibition include induction of histone H3 and MPM2 phosphorylation as well as monaster spindle/rosette formation (Fig. 1). In A2780 ovarian tumor xenografts grown in mice, we found that these markers increased in a time- and dose-dependent manner following a single oral dose of SCH 2047069. Both biomarkers increased over a 16-hour period after dose (7.5 mg/kg) compared with vehicle-treated animals (Fig. 3A). Treatment with SCH 2047069 induced a time-dependent increase in phospho-histone H3 levels in the tumor compared with vehicle-treated animals. We found that phospho-histone H3 gradually increased over time after SCH 2047069 treatment by 2-, 4-, and 5.5-fold at 6, 12, and 16 hours, respectively (Fig. 3B). At later times, the signal decreased and returned to background levels by 36 hours after dose. Increasing doses of SCH 2047069 (2, 4.5, and 7.5 mg/kg) lead to increased histone H3 phosphorylation; no further increase was observed at 15 mg/kg (Fig. 3C).

Additionally, treatment with SCH 2047069 led to rosette formation in tumor tissue, further verifying the evidence for KSP inhibition. These were easily observed by H&E staining (Fig. 3A) in compound-treated animals. Few normal mitotic cells were observed in tumor tissue from vehicle-treated animals. In these cells, the DNA appeared condensed and either at the metaphase plate or separating from the metaphase plate.

**SCH 2047069 showed antitumor activity**

The antitumor activity of SCH 2047069 was initially evaluated in mice bearing established A2780 ovarian tumor xenografts. Doses of 5, 7.5, or 20 mg/kg were administered on a schedule of three consecutive days, every third day, or every seventh day, respectively, based on...
pilot maximal tolerated dose studies. All three SCH 2047069 treatment schedules showed tumor growth inhibition (TGI); however, the degree of activity was schedule dependent, with the best efficacy achieved on the q3d schedule (79% TGI versus 53–66% TGI; P < 0.05; Fig. 4A).

Three additional solid tumor xenograft models were tested using the q3d schedule (Fig. 4). In each of these models, we observed a dose-dependent antitumor activity when SCH 2047069 was dosed orally. SCH 2047069 induced regressions in Colo-205 colon tumor xenografts when dosed at 3.75 mg/kg (116% TGI) and 7.5 mg/kg (126% TGI; P < 0.05; Fig. 4B). In U373 glioblastoma model, 5 and 7.5 mg/kg induced 29% and 75% TGI, respectively (P > 0.05; Fig. 4C). The fourth model tested was HCT-116 colon tumor xenografts (Fig. 4D). Doses of 5 and 7.5 mg/kg q3d in this model resulted in 53% and 97% TGI, respectively (P < 0.05). In all studies, SCH 2047069 was well tolerated and there was no significant body weight loss or lethality.

We next evaluated the activity of SCH 2047069 in leukemia and lymphoma models (Fig. 5). In EOL-1 AML model, cancer cells are engrafted in the bone marrow of inoculated mice. These mice progress from paresis to paralysis to mortality; paresis is used as a humane end point. Vehicle-treated animals had a median survival of 19 days and were all sacrificed by day 21. Treatment with 3.75 and 7.5 mg/kg orally q3d (dosed to day 26) extended median survival 13 and 26 days, respectively (P < 0.05; Fig. 5A). Additionally, we found that SCH 2047069 enhanced the antitumor activity of paclitaxel, gemcitabine, and vincristine

We evaluated the combination of SCH 2047069 and paclitaxel, gemcitabine, or vincristine in the A2780 ovarian tumor xenograft model. In all studies, single-agent and combination treatments were well tolerated and

Figure 4. Single-agent efficacy of SCH 2047069 in solid tumor xenograft models. Mice bearing A2780 (A), Colo-205 (B), U373 (C), and HCT-116 (D) xenograft tumors were dosed orally with vehicle or SCH 2047069. Tumor volume was measured throughout the study.
there was no significant body weight loss or lethality. We first evaluated the combination of a single dose of paclitaxel (20 mg/kg i.p.) and SCH 2047069 dosed on a q3d schedule (days 0, 3, 6, and 9; Fig. 6A). As single agents, we observed 43% and 90% TGI, respectively ($P > 0.05$ and $P < 0.05$, respectively). The combination of the two compounds resulted in a significant enhancement of activity, 98% TGI ($P < 0.05$ versus vehicle and single agent).

We also explored weekly dosing of SCH 2047069 and paclitaxel (Fig. 6B). SCH 2047069 dosed orally weekly at 10 mg/kg resulted in 48% TGI ($P > 0.05$), and paclitaxel dosed weekly at 20 mg/kg i.p. inhibited tumor growth by 36% ($P > 0.05$). Similar to the enhanced antitumor activity in the above schedule, the combination of the two compounds dosed weekly resulted in a significant enhancement of activity, 89% TGI ($P < 0.05$ versus vehicle and single agent).

Similar to the combination study with paclitaxel, we evaluated the combination of a single dose of gemcitabine (100 mg/kg i.p.) and SCH 2047069 on a q3d schedule (days 0, 3, 6, and 9; Fig. 6C). As single agents, we observed 80 and 74% TGI, respectively ($P < 0.05$). The combination of the two compounds resulted in a significant enhancement of activity, 97% TGI ($P < 0.05$ versus vehicle and single agent).

We also studied the effect of combining SCH 2047069 with vincristine (Fig. 6D). SCH 2047069 dosed orally weekly at 10 mg/kg resulted in 41% TGI ($P > 0.05$), and vincristine dosed weekly at 0.75 mg/kg i.p. inhibited tumor growth by 39% ($P > 0.05$). The combination of the two compounds dosed weekly resulted in a significant enhancement of activity, 76% TGI ($P < 0.05$ versus vehicle and single agent).

For the above four experiments, we found that altering the combination by dosing SCH 2047069 before or after the cytotoxic agents had little effect on enhancement, as similar antitumor activity was observed in all combination arms (data not shown).

**Discussion**

We identified SCH 2047069 as a novel KSP inhibitor with oral bioavailability and the ability to cross the blood-brain barrier. The ability to dose the compound orally provides convenience of dosing as well as the ability to achieve more continuous exposure via the use of dose-dense administration.

Here, we describe the biological properties of SCH 2047069. The compound was found to be a subnanomolar inhibitor with an excellent selectivity profile that showed potent mechanism-based activity in cell culture. SCH 2047069 inhibited proliferation of a panel of 57 cell lines, including PgP-overexpressing cells (e.g., HCT-15). The effect of SCH 2047069 was irreversible, in that exposure for 24 hours or less following a washout period led to proliferation inhibition of the entire cell panel at the end of 72 hours. Interestingly, we found that some cells required exposure to drug for 24 hours to induce optimal effect, whereas other cell lines were...
on an irreversible path to cell death following 4-hour exposure. This contrasts the findings with KSP-1A in HCT-116 cells, where 12-hour exposure resulted in reversible mitotic arrest and prolonged exposure was required for induction of apoptosis (7). Further, Tao et al. found that HT-29 cells were resistant to KSP-1A and failed to undergo apoptosis following compound treatment. However, we found that treatment of HT-29 cells with SCH 2047069 led to mitotic arrest, PARP cleavage, and inhibition of proliferation. These discrepancies may be accounted for by differences in potency, binding affinity, and other properties of the compounds.

In vivo, SCH 2047069 treatment of mice bearing tumor xenografts induced MPM2 and histone H3 phosphorylation in tumors. We found the pharmacodynamic effect to be dose and time dependent. The time dependence and peak at 16 hours is reminiscent of the response seen with taxanes preclinically. The pharmacodynamic marker reached maximal induction at 7.5 mg/kg and could not be further increased by higher doses, suggesting that at any given point in time, there is a fixed saturable amount of cells that can be affected by SCH 2047069. Theoretically, these would be cells in mitosis. These data suggest that repeat dosing at later times would be required to reach cells not yet in mitosis.

SCH 2047069 showed significant antitumor activity as a single agent in several solid tumor xenograft models, including A2780 ovarian, Colo-205 colon, U373 glioblastoma, and HCT-116 colon. An important finding in our studies was the schedule dependency of SCH 2047069 antitumor efficacy. Optimal antitumor activity was observed using the maximally tolerated dose of 7.5 mg/kg on a q3d schedule. Depending on the model, activity of SCH 2047069 ranged from 75% TGI to regressions. Dosing on the q3d schedule was more efficacious than administering maximum tolerated doses on a continuous or less frequent schedule. It is important to note that the q3d schedule we established as optimal for efficacy in xenograft tumor models may not be applicable to human studies given the difference in tumor growth rates and the potential differences in pharmacokinetics between rodents and humans. Optimal dose and schedule will need to be evaluated for SCH 2047069 in clinical studies and may also require modification for different indications (e.g., hematopoietic tumors versus solid tumor indications where the tumor doubling times are different). Nevertheless, these data suggest that dosing schedule is an important factor in attaining maximal tumor cell kill and clinical benefit with SCH 2047069 and possibly other KSP inhibitors. Specifically, these findings support the evaluation of more dose-dense schedules such as the weekly and multiple times a week scheduling being tested with newer agents. Interestingly, limited activity was observed in early clinical trials, where other KSP inhibitors such as ispinesib or MK-0731 were dosed once every 3 weeks.

SCH 2047069 was also active in EOL-1 leukemia and DoHH2 lymphoma models and significantly extended survival of these animals. In the EOL-1 model, we also found that leukemic disease in the bone marrow was...
Reduced by SCH 2047069 treatment. Hematologic diseases where cells are rapidly dividing provide a potential indication where KSP inhibitors could be quite useful.

Few published studies have explored combination therapy preclinically using KSP inhibitors. Enhanced apoptosis has been shown for the KSP inhibitor dimethyltriazenostron with agents that abrogate Hsp70 upregulation (Hsp70 sRNA, FTI-277, farnesyltransferase inhibitor FTI-277, and phosphatidylinositool 3-kinase inhibitor LY294002; ref. 15). In addition, enhanced apoptosis was shown for the KSP inhibitor SB-715992 and genistein (16). Contrary to our findings with SCH 2047069 and paclitaxel, combination studies reported previously with KSP inhibition and paclitaxel are reported to be antagonistic. Treatment of LNCap and PC3 prostate tumor xenograft models with KSP antisense was reported to be antagonistic when combined with paclitaxel (17). Combination of the KSP inhibitor HR22C16-A1 with paclitaxel in 1A9 cells resulted in antagonistic antiproliferative and anti-mitotic effect (11). We found that SCH 2047069 used in combination enhanced the antitumor activity of paclitaxel, gemcitabine, and vincristine in A2780 xenograft models.

These combinations were well tolerated and provide support for the clinical evaluation of these combinations.

In conclusion, we have characterized SCH 2047069, a novel oral KSP inhibitor that shows potent mechanism-based activity both in vitro and in vivo. The compound showed antitumor activity both as a single agent and in combination with other chemotherapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Drs. W. Robert Bishop, N-Y. Shih, and Hung Le for their advice and support; Drs. Mohamed Ladha and Johanna Horowitz for their scientific discussions, ideas, and encouragement; and Eugene Maxwell, the entire KSP team, and the in vivo dosing team for their work.

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Received 06/09/2010; revised 08/25/2010; accepted 09/02/2010; published OnlineFirst 10/26/2010.

References


Mol Cancer Ther; 9(11) November 2010 Molecular Cancer Therapeutics
Molecular Cancer Therapeutics

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